



المؤتمر الدولي السادس للتقانات الحديثة في العلوم الزراعية

19-18 كانون الاول 2024

**6th International Conference of Modern
Technologies in Agricultural Sciences**

Conference

18-19 December 2024, Kufa, Najaf, Iraq.

[Publishing House Al-Kunooze Scientific Journal, Al-Kunooze University, Basrah, Iraq.](#)

<https://kunscij.kunoozu.edu.iq/index.php/Al-Kunooze/>

عنوان الكتاب :

المؤتمر الدولي السادس للتقانات الحديثة في العلوم الزراعية

6th International Conference of Modern Technologies in Agricultural Sciences

المؤلف : كلية الزراعة / جامعة الكوفة / العراق

سنة الطبع : 2025

عدد صفحات الكتاب : 984

حجم الكتاب : B5

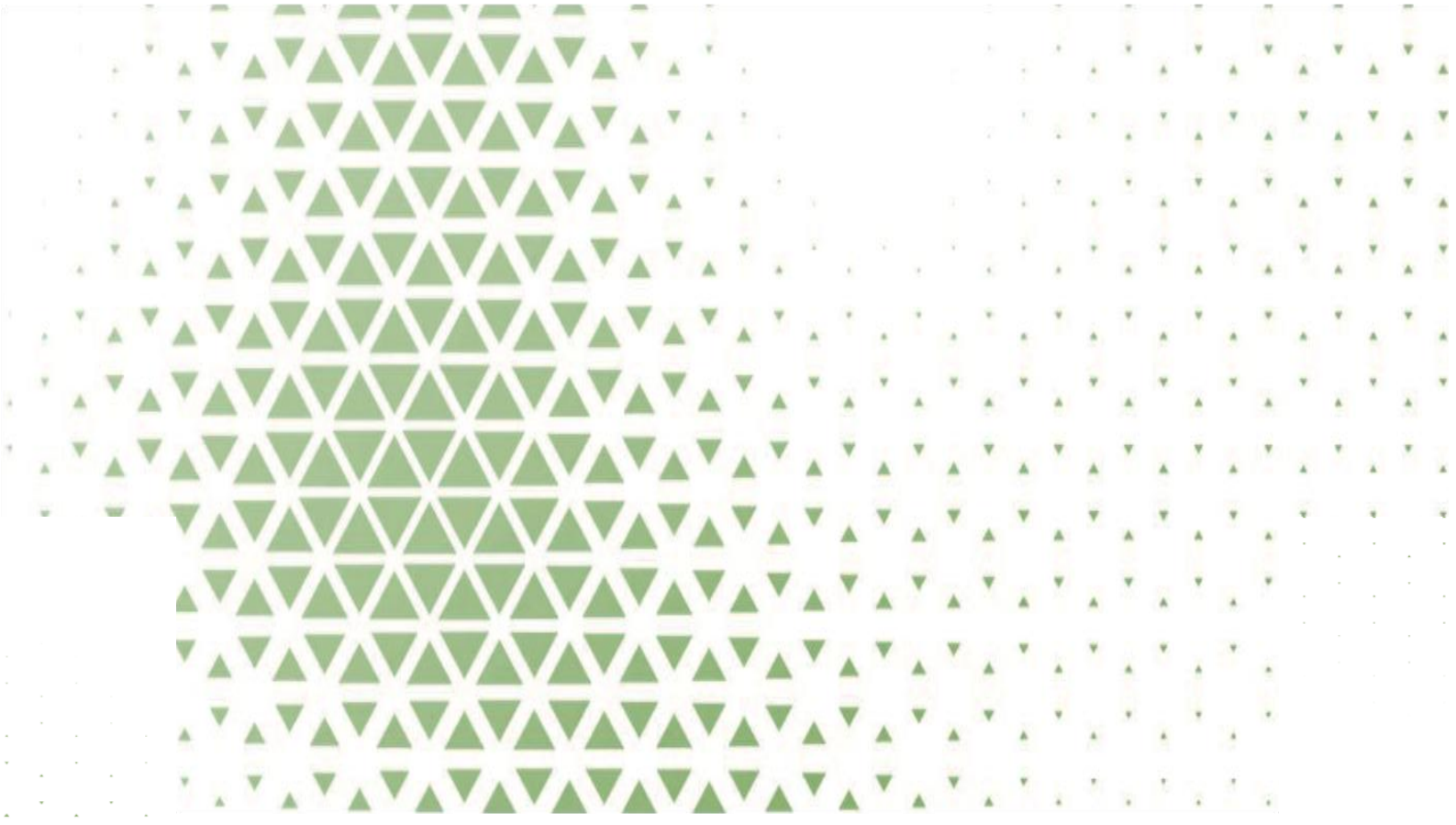
الطبعة الاولى

جميع الحقوق محفوظة . لا يسمح بإعادة اصدار هذا الكتاب او اي جزء منه او تخزينه في نطاق استعادة المعلومات او نقله باي شكل من الاشكال بدون اذن مسبق من الناشر أو المؤلف.

جميع حقوق الطبعة محفوظة

International Standard Number (I. S. B. N)

978-9922-29-079-9



كتاب وقائع المؤتمر

Full Text Book

[Publishing House Al-Kunooze Scientific Journal, Al-Kunooze University, Basrah, Iraq.](https://kunsij.kunoozu.edu.iq/index.php/Al-Kunooze/)

<https://kunsij.kunoozu.edu.iq/index.php/Al-Kunooze/>



6th International Conference of Modern Technologies in Agricultural Sciences, 18-19 December 2024, Kufa, Najaf, Iraq

978-9922-29-079-9	ISBN	نظام الترميز الدولي لترقيم الكتاب
Prof. Dr. Alaa E. Hasan	Editor	المحرر
Assist.Prof. Dr. Mohammed Ahmed	Publication Coordinator	منسق النشر
18-19 December 2024	Conference date	تاريخ المؤتمر
13-5-2025	Printing date	تاريخ الطباعة
984	page	عدد الصفحات
	URL	رابط النشر

<https://kuns cij.kunoozu.edu.iq/index.php/Al-Kunooze>



6th International Conference of Modern Technologies in Agricultural Sciences, 18-19 December 2024, Kufa, Najaf, Iraq

SPONSORED





6th International Conference of Modern Technologies in Agricultural Sciences, 18-19 December 2024, Kufa, Najaf, Iraq

محاور المؤتمر

1. البيئة والتلوث
2. بيئة البستنة والغابات
3. وقاية النبات
4. المحاصيل الحقلية والنباتات الطبية
5. تكنولوجيا الأغذية واستدامة الأمن الغذائي
6. تقنيات الإنتاج الحيواني المستدامة
7. موارد التربة والمياه المستدامة
8. تقنيات المكننة الزراعية ومصادر الطاقة المتجددة
9. تقنيات العلوم الاقتصادية والاجتماعية الزراعية

Conference topics

1. Pollution and Ecology
2. Plant Protection
3. Horticulture and Forest Ecology
4. Agronomy and Medicinal Plants
5. Food Technology and Sustainability of Food Security
6. Sustainability of Animal Production Techniques
7. Sustainability of Soil and Water Resources
8. Agricultural Mechanization Techniques and Renewable Energy Resources
9. Techniques of Agricultural Economic and Social Sciences

Chairman of The Conference

<u>Name</u>	<u>E-mail</u>	<u>Affiliation</u>
Prof. Dr. Alaa E. Hasan	alaa.albdairi@uokufa.edu.iq	Dean of the Faculty of Agriculture / University of Kufa

Speakers

<u>Name</u>	<u>E-mail</u>	<u>Affiliation</u>
Prof. Dr. Ahmad Salih Al Mashadani	profahmad1958@yahoo.com	UN Habitat/ Iraq
Dr. Ian Oliver	i.oliver@keele.ac.uk	Keele University/UK



6th International Conference of Modern Technologies in Agricultural Sciences, 18-19 December 2024, Kufa, Najaf, Iraq

The Conference's Special Committees

Scientific Committee

<u>Name</u>	<u>Position</u>	<u>Affiliation</u>	<u>E-mail</u>
Prof. Dr. Laith J. Hussein	Chairman	University of Kufa / Iraq	laithj.hnoosh@uokufa.edu.iq
Prof. Dr. Jamal A. Abbas	member	University of Kufa / Iraq	jamal.selman@uokufa.edu.iq
Prof. Dr. Ameer Khalil Yasser	member	University of Kufa / Iraq	ameer.alrikabi@uokufa.edu.iq
Prof. Dr. Akeel Y. Abdulnabi	member	University of Kufa / Iraq	aqeel.alshukri@uokufa.edu.iq
Prof. Dr. Adnan W. Habeeb	member	University of Kufa / Iraq	adnan.almudhafar@uokufa.edu.iq
Prof. Dr. Ayad J. Jaber	member	University of Kufa / Iraq	iyad.alkhafaji@uokufa.edu.iq
Asst. Prof. Dr. Nasser M. Aljanabi	member	University of Kufa / Iraq	naseer.aljanabi@uokufa.edu.iq
Asst. Prof. Dr. Firas A. Alrikabi	member	University of Kufa / Iraq	firasa.alrekaby@uokufa.edu.iq
Prof. Dr. Gaber A. Bassuoni	member	University of Alexandria / Egypt	drgaber2000@yahoo.com
Prof. Dr. Oner Cetin	member	University of Dicle / Turkey	onercetin@dicle.edu.tr
Prof. Dr. Kourosh Vahdati	member	University of Tehran / Iran	kvahdati@ut.ac.ir
Prof. Dr. Mima H. Todorova	member	Trakia University/ Bulgaria	mtodorova@uni-sz.bg

Organizing Committee

<u>Name</u>	<u>Affiliation</u>	<u>E-mail</u>
Prof. Dr. Alaa E. Hasan	University of Kufa / Iraq	alaa.albdairi@uokufa.edu.iq
Prof. Dr. Ghalib B. Abboud	University of Kufa / Iraq	ghalib.alabbasi@uokufa.edu.iq
Prof. Dr. Karim M. Abbass	University of Kufa / Iraq	jamal.alsharmani@uokufa.edu.iq
Prof. Dr. Anmar A. Majeed	University of Kufa / Iraq	anmar.alwazeer@uokufa.edu.iq
Prof. Dr. Ayad Kadium Ali	University of Kufa / Iraq	ayadk.alhusseini@uokufa.edu.iq
Asst. Prof. Dr. Akram A. Mohammed	University of Kufa / Iraq	akrama.abodarb@uokufa.edu.iq
Asst. Prof. Dr. Akeel K. Hassan	University of Kufa / Iraq	aqeel.altufaili@uokufa.edu.iq
Asst. Prof. Dr. Maysaa A. Majeed	University of Kufa / Iraq	maysaa.alhar@uokufa.edu.iq
Asst. Prof. Dr. Khudheyer A. Aziz	University of Kufa / Iraq	khudhair.alnomani@uokufa.edu.iq
Asst. Prof. Dr. Mohanad A. Mhawi	University of Kufa / Iraq	mohanada.alhamid@uokufa.edu.iq



6th International Conference of Modern Technologies in Agricultural Sciences, 18-19 December 2024, Kufa, Najaf, Iraq

Media Committee

<u>Name</u>	<u>Position</u>
Asst. Prof. Dr. Hisham R. Muteeb	Chairman
Dr. Haider F. Abbas	member
Mr. Ammer Abbas Khudhair	member
Mr. Thaeer R. Humood	member
Mr. Ali A. Mahdi	member
Mrs. Nada A. Majeed	member

Financial affairs Committee

<u>Name</u>	<u>Position</u>
Mr. Wathiq Abdulhassan	Chairman
Mr. Latif R. Nief	member
Miss Nada M. Hassan	member



6th International Conference of Modern Technologies in Agricultural Sciences, 18-19 December 2024, Kufa, Najaf, Iraq

Index

No.	Article Information	Pages
1	Effect of Nutrient Solution and Cytokinin on some Chemical and Vegetative Traits of Local Orange Seedlings Grafted on Rangpur Rootstock (<i>Citrus sinensis</i> L.)	1-11
2	Evaluation of the Herbicide Tarzec Performance Under Different Application Time (Some Growth Traits of Wheat and Accompanied Weeds)	12-20
3	Evaluation of Small Agricultural Projects Provided by the Iraqi Institute for Development (IID) to the People of Bashiqa District / Nineveh Governorate / Iraq	21-31
4	Precipitation Efficiency Index Indicator of Climate Changes for Growth <i>Quercus eagilops.</i> and <i>Pistacia khinjuk</i> Trees in the Dohuk Region	32-42
5	Investigation of the Causal Agent of Wilt and Root Rot Disease <i>Fusarium</i> spp. on Pepper Plants in Certain Areas of Baghdad and Its Morphological and Molecular Identification	43-50
6	The Use of Alcoholic and Aqueous Extracts of E Co-Friendly and Safe Buckthorn Leaves Combating the Hairy Grain Beetle Insect <i>Trogoderma granarium</i> (Coleoptera : Dermestidae)	51-62
7	Effect of Crop Type and Soil on the Population Density of <i>Bacillus subtilius</i> and <i>Pseudomonas putida</i> in Some Soil Properties	63-73
8	An Economic and Econometric Study of the Gross Domestic Product in Iraq Through the Oil, Industrial and Agricultural Sectors	74-83
9	Economic Analysis of the Impact of Some Economic Variables on Investment in the Iraqi Agricultural Sector for the Period (1990-2022)	84-102
10	Okra <i>Abelmoschus esculentus</i> is a New Host of Cowpea Mild Mottle Virus <i>Carlavirus vignae</i> in Iraq	103-107
11	Estimating the Percentage of Vitamins, Gibberellin and Indole for Different Soil Textures in the Root Zone	108-121
12	Effect of Two Hybrids of Cherry Tomato (<i>Solanum Lycopersicum</i> L.) Grown in Greenhouse and Sprayed with Calcium and Iron on Yield and its Components	122-134
13	<i>Tetranychus urticae</i> Koch Mites Severity Response in Some Eggplant Varieties Treated with Salicylic Acid Concentrations and Their Relationship to Some Anatomical Characteristics of Plant Leaves Fieldally	135-145
14	Adopting Thermodynamic Criteria to Evaluate Potassium Available in Saline Soils Under Different Temperatures	146-159
15	Effect Type of Nutrient Medium and Cytokinin on the Production and Development of the Embryonic Callus of <i>Phoenix dactylifera</i> L., Albarhi date Palm Variety, in Vitro	160-170
16	Effect of Using Different Levels of Active Biochar on Increasing Soil Fertility and Radish Crop Growth	171-176
17	Efficiency of the RAPD in Determining the Genetic Dimension of Genotypes of Cotton	177-183
18	The Role of Thyme Oil on the Enzymatic Activity and Histological Changes in Common Carp (<i>Cyprinus carpio</i>) Exposed to Cadmium Chloride	184-206
19	Water Quality Index Estimation Al-Hawija Irrigation Project, Kirkuk, Iraq	207-218
20	Polycyclic Aromatic Hydrocarbons (PAHs) in Meat Cooked by Charcoal Grilled	219-226



6th International Conference of Modern Technologies in Agricultural Sciences, 18-19 December 2024, Kufa, Najaf, Iraq

21	Effect of Seed Priming and Foliar Spray with Seaweed Extract on Some Growth Characteristics of Two Sunflower <i>Helianthus annuus</i> L. Cultivar	227-240
22	Effect of Seaweed Extract and Bio-Health on the Growth of Statis Plant (<i>Limonium sinuatum</i>)	241-249
23	HACCP Application in Next-Generation Sequencing in Assuring the Safety of Mosul Factories	250-258
24	Effect of DCI in Pumpkin Seed Alcoholic Extract on Type 2 Diabetic Rats Fed a Fatty Diet with Histological Study of Pancreas	259-270
25	Effect of Nitrogen Fertilizer Fractionation and Adding Bio-Organic Fertilizer on the Content of Maize Leaves of Macronutrients and Chlorophyll Index	271-280
26	Effect of the Type and Concentration of Salt Ions on the Growth of <i>Bacillus</i> Bacteria and Some Soil Traits	281-292
27	Effect of Different Levels of Cadmium and Bacterial Inoculum on Barley Growth, Soil Availability and Cadmium Content	293-301
28	Requirements for Implementing Total Quality Management in Training Extension Centers in Iraq	302-316
29	Assessing the Effectiveness of Inoculating Mycorrhiza and <i>Trichoderma</i> Fungus, Adding Seaweed Extract and Interfering with Their Growth in Olive Seedlings	317-324
30	Effect of Mycorrhizae Fungus, Seaweed Extract Spray, Amino Acid Arginine and Their Interaction on Vegetative Growth Characteristics of Sweet Corn	325-331
31	The Impact of Foliar Nutrition and Plant Waste on Yield Characteristics of Three Potato Cultivars (<i>Solanum tuberosum</i> L.)	332-345
32	Sustainability of Potato Farming under Drought Conditions	346-353
33	An Investigation of the Type of Bacteria Found in the Dust of Iraq's Al-Diwaniyah City	354-370
34	Studying the Possibility of Growing the Reishi Mushroom, <i>Ganoderma</i> spp. , on Agricultural Waste and Testing its Effect in Protecting Cucumber Plants from Some Pathogenic Fungi	371-382
35	Bioaccumulation of the Concentration of some Heavy Metals in, Spring Water in Al-Shinafiyah District in Al-Diwaniyah Governorate - Iraq	383-395
36	Improved Efficiency of Actara and Movento Insecticidal by Silica Oxide Nanoparticles to Control <i>Bemisia tabaci</i>	396-405
37	Susceptibility of Two Cucumber Hybrids to Infection with Whitefly <i>Bemisia tabaci</i> (Gennadius) (Homoptera: Aleyrididae)	406-414
38	The Effect of NPK Nano Elements and Amino Spray on some Physiological, Chemical and Production Characteristics of Date Palm Fruits <i>Phoenix dactylifera</i> L. Sayre Cultivar	415-422
39	Producing Single Hybrids with Early Flowering of Maize that are Suitable for Fall Season (Agronomic Traits)	423-435
40	Studying of Some Chemical Properties of the Soil and Evaluating Its Suitability for Growing Potatoes, Sustaining the Soil in the Lands of Ibrahimiyya District/Babylon Governorate Using Geospatial Technologies	436-448
41	The Role of Organic Matter on Phenolic Compounds in Vegetable Plants	449-460
42	Performance Evaluation of the Varieties of <i>Sorghum Bicolor</i> L. Moench and the Estimation of Some Genetic Indicators Based on the Effect of NPK Nanofertilizer	461-474
43	Evaluation of some Vegetative Growth Traits of Tomato Lines and Their Individual Hybrids Under the Effect of Spraying with Nano-Calcium	475-488



6th International Conference of Modern Technologies in Agricultural Sciences, 18-19 December 2024, Kufa, Najaf, Iraq

44	Effect of Ordinary and Nano Calcium on some Growth Characteristics of Tomato Lines and Their Individual Hybrids	489-500
45	Measuring Radioactive Pollution by ²³⁸ U, ²³² Th, ⁴⁰ K in Table Salt Using (HPGe)	501-508
46	Molecular Diagnosis and Genetic Divergence of Some Flax Genotypes (<i>Linum usitatissimum</i> L.)	509-516
47	Effect of Water Stress on the Growth of Several Flax Cultivars	517-526
48	Efficiency of Treatment Plant and Drinking Water Quality Assessment in Al-Numaniyah City, Iraq	527-537
49	Comparison Between the Growth Trait Performance of Awassi and Karakul Sheep	538-552
50	Effect of Natural Products of C3 and C4 Weeds on Yield and its Component of Some Bread Wheat Varieties (<i>Triticum aestivum</i> L.)	553-566
51	Some Factors Limiting Rural Women's Participation in the Development and Sustainability of the Family Farm	567-577
52	Effect of Nano and Organic Fertilizers on the Vegetative Traits of Local Orange Seedlings Grafted on Two Different Rootstocks	578-594
53	Effect of Organic and Bio Fertilizer and Spraying with Nano Zinc on the Vegetative Growth of Aswd Diyala Fig Seedlings	595-606
54	Effect of Adding Gum Arabic to the Dietary on the Productive Characteristics of Quail Birds	607-613
55	Effect of Adding Gum Arabic to the Quails Diet on the Qualitative Characteristics of Stored Eggs	614-619
56	Effect of Using Algae Oil (Sources of Omega-3) on the Productive Performance of Broiler	620-625
57	Effect of Different Concentrations of Floratone Growth Regulator and Spray Dates on the Growth Traits of Two Types of Wheat <i>Triticum aestivum</i> L.	626-637
58	Importance of the Azotobacter and Mineral Fertilizers in Plant Growth	638-649
59	Production of Lettuce Under Low Levels of Mineral Fertilizers in the Presence of Mycorrhizal Fungi and Azospirillum Bacteria and Spraying Organic Nutrients and Glutamic Acid	650-667
60	Effect of Reducing Mineral Fertilizers, Using Biofertilizers and Spraying Organic Nutrients and Glutamic Acid on Some Vegetative Traits of Lettuce	668-687
61	The Impact of Potassium Fertilizer Levels and the Method of Addition on the Growth and Yield of Cauliflower Plants <i>Brassica Oleracea</i> var. <i>Botrytis</i>	688-694
62	Detection the Active Compounds of the Ethanol Extract of <i>Cordia myxa</i> L. Leaves Using GC Mass Technology	695-704
63	Estimating the Effectiveness of some Types of Sugars on Oxidative Stress Indicators and Kidney Function in Animals with Induced Diabetes and Obesity	705-716
64	Extension Needs for Sheep Breeders in Nimrud District / Nineveh Governorate	717-727
65	Molecular Diagnosis of Armyworm in <i>Zea mays</i> L. Fields in Iraq	728-733
66	Antioxidants and Their Important Role in Resisting Stresses in Plants: A Review	734-744
67	Influence of Calcium Spraying and Packaging Type on Some Anatomical Characteristics of Seedless Pomegranate Fruits Rawa cv.	745-755



6th International Conference of Modern Technologies in Agricultural Sciences, 18-19 December 2024, Kufa, Najaf, Iraq

68	Effect of Spraying with Arginine and Indole Acetic Acid on Growth and Flowering of Marigold <i>Tagetes erecta</i> L.	756-771
69	Effect of Cultivation Methods, Adding Organic Matter and Spraying Nano-Fertilizers on Some Vegetative Growth Traits of Broad Bean Plant	772-782
70	Effect of Seed Stimulation by Alpha-Amylase Enzyme, Stimulus Temperature Degree and Sowing Dates on Viability and Vigour of Sorghum Seeds	783-789
71	Study of Variation in Characteristics of Clay Minerals in Soils North and Southe Al-Handia Dam - Middle Euphrates / Iraq	790-801
72	Effect of Planting Date on Vegetative Growth Traits, Yield and its Components of Sweet Corn Hybrids	802-811
73	Dosing with an Aqueous Extract of Watercress Seeds for Iraqi Lambs and Its Effect on Reproductive and Antioxidants Parameters	812-820
74	Impact of Watercress Seed Aqueous Extract Dosing on Productive Traits and Testicle Dimensions of Iraqi Lambs	821-831
75	Study the Effect of Adding (<i>Punica granatum</i>) Extract to the Tris Extender on the Characteristics of Goats Sperm Epididymal Under Cooling Conditions	832-839
76	Effects of Salt Stress and TiO ₂ NPs on the <i>vicia faba</i> L. Yield Characteristics	840-849
77	Response of Roselle Genotype to Spraying with Glutamic and Potassium and Their Effect on the Yield and Active Compounds	850-859
78	Role of Biofertilization on the Growth and Yield of Strawberry Plants (<i>Fragaria x ananassa</i> Duch.) cv.Camarosa	860-869
79	Effect of Vermicompost Fertilizer and Spraying with Salicylic Acid and Gibberellin on <i>camellia japonica</i> Plant	870-881
80	Knowledge Guidance Needs of Rural Women in the Field of Raising Domestic Chickens in Al-Elm District / Salah Al-Din Governorate	882-891
81	Isolation and Identification of <i>Bifidobacterium</i> Sp. Bacteria in the Stomach of Honey Bees	892-901
82	A micro Morphological Study of Pollen Grains and Male Reproductive Organs of Two Species of the Genus <i>Senna</i> in Iraq	902-915
83	Evaluating the Impact of Farm Ownership on Agricultural Production by Estimating the Superior Efficiency of Baghdad Governorate Farms	916-923
84	An analytical Economic Study of the Relationship Between Individual Food Security and Possession Security in Baghdad Governorate	924-934
85	Effect of Planting Distances Between Pits and Lines on the Growth and Yield of Broad Bean (<i>vicia faba</i> L.)	935-942
86	Algal Antimicrobial Power: Investigating the Influence of <i>Spirogyra varians</i> Extract on <i>Staphylococcus aureus</i> Biofilm Gene Expression	943-953
87	Effect of Soaking with Glycine Betaine Acid on some Physiological Qualities of Sesame Under the Influence of Salt Stress	954-961
88	Effect of Partial Replacement of Dietary Corn by Soaked Barley with Water or Supplemental Enzymes on the Productive Characteristics of Broilers	962-970
89	The Effect of Spraying Thiamine and Nano-Potassium in the Growth and Flowering of <i>Catharanthus roseus</i>	971-976
90	Evaluation of the Diffusion Results of the Dairy Industry Techniques in Ninevah Province	977-984

Effect of Nutrient Solution and Cytokinin on some Chemical and Vegetative Traits of Local Orange Seedlings Grafted on Rangpur Rootstock (*Citrus sinensis* L.)

**Reem Mohammed Noman Al-Awady¹, Hadi Kazim Hussein Al-Jabouri² and
Basem Halim Kashash Al-Jawdhari³**

¹⁻³Department of Horticulture and Landscape gardening, College of Agriculture,
Al-Qasim Green University, Babylon, Iraq.

¹E-mail: Reem.mohamed@agre.uoqasim.edu.iq

²E-mail: hadi.kadum@agre.uoqasim.edu.iq

Abstract. The study was conducted in the western Khaikan area, south of Babylon province, during the study period from 9/1/2023 to 6/1/2024 on orange seedlings grafted on Rangpur rootstock at the age of 6 months to study the effect of spraying with nano-nutrient solution at concentrations (2, 1, 0 g/L) and traditional (1 g/L) as well as spraying with the growth regulator cytokinin type (benzyladenine) and their interactions on the chemical and vegetative characteristics of seedlings. The results showed that spraying seedlings with single and joint study treatments led to a significant increase in (leaf chlorophyll content, percentage of total carbohydrates, percentage of nitrogen, percentage of phosphorus, leaf iron content, leaf zinc content, seedling height rate, average number of branches, number of leaves) where the treatment with nutrient solution 1 g/L with a cytokinin concentration of 200 mg/L excelled on the leaf content of Chlorophyll, percentage of carbohydrates, seedling height rate, number of branches rate and number of leaves obtained the highest rates compared to control treatment. As for the major and minor nutrients, the concentration of 2 g/L of nutrient solution with a concentration of 200 mg/L cytokinin exceeded the highest rates compared to control treatment.

Keywords. Rangpur rootstock, Cytokinin (benzyl adenine), Nutrient solution.

1. Introduction

Citrus trees (local orange *Citrus sinensis* L.) belong to the Rutaceae family, which is characterized by the presence of oil glands with an aromatic smell in most parts of the plant that distinguishes it from other types of fruits. Citrus is considered one of the most important groups of plants as it grows and bears fruit in tropical and subtropical regions extending from the southeast and from there it extended to Europe and Africa [1,2]. The majority of citrus varieties in

Iraq are found in the country's central and southern areas. Because of the nutritional, economic, medicinal, aesthetic, and environmental value of their fruits—which are rich in mineral salts like potassium, calcium, iron, magnesium, sodium, sulfur, and phosphorus—as well as vitamin C and significant amounts of vitamins A, B1, and B—citrus trees hold a special place among fruit trees [3]. Citrus fruits are important in the world and Iraq, as they rank second after grapes in the world in terms of production, and the area planted with citrus fruits in the world is estimated at about (512) million hectares, and the global production of fruits amounts to (97,310,000) tons [4]. With an estimated 6,383,881 productive trees, an area and output of around (142,717) tons, and an average production per tree of about 22.4 kg, oranges are regarded as one of the key fruit trees for local consumption in Iraq. Diyala Province ranks first in terms of cultivated area and production amount [5]. The majority of citrus fruit varieties are grown through grafting on various citrus rootstocks, such as Rangpur rootstock, which is regarded as one of the rootstocks that can withstand high soil salinity and drought because of its deep root system, resistance to heavy soil conditions, and ability to fend off gummosis disease (*Phytophthora citrophthora*). Grafted citrus trees require nutrients in order to grow and develop properly. These nutrients are essential for many metabolic processes that the trees participate in, so their lack results in a physiological imbalance that impacts the growth of the rootstock and scion as well as the productivity of the grafted plants in the future. [6]. The implementation of nanomaterials and nanotechnology techniques to enhance agricultural practices and increase food production is known as nanotechnology in agriculture. In order to optimise soil quality. A healthier environment and reduced health hazards for producers and consumers are the results of a decrease in the use of chemical pesticides and fertilizers [7]. Nanotechnology has the potential to improve water efficiency in agriculture by facilitating the absorption and retention of water by vegetation. - Greater food security and more resilient crops as a consequence of enhanced resistance to environmental stressors, including extreme temperatures and drought periods. In general, nanotechnology has the potential to significantly transform agriculture and resolve the challenges associated with supporting a growing global population in a sustainable manner [8]. The significant increase in growth characteristics and chemical content when using nano fertilizers may be due to the unique properties of nano fertilizers such as small particle size (1-100 nm) and increased surface area of interaction, which facilitates their penetration. Therefore, the research aims, through the use of both the nutrient solution and cytokinin, to improve vegetative and root growth, accelerate the growth of orange seedlings, reach the appropriate size, shorten the time required to reach the fruiting stage, overcome the slow growth of seedlings, and determine the best concentration of study factors [9,10].

2. Materials and Methods

Six-month-age orange seedlings grafted on Rangpur rootstock were selected as homogeneous in their growth as possible from the certified citrus production nursery of the Iraqi Ministry of Agriculture / General Directorate of Horticulture and Forestry in Karbala Province / Hindiya District. The seedlings were transferred to the study site and placed in a shade covered with Saran, and were planted in plastic bags with a capacity of (2 kg) transferred to anvils with a capacity of 7 kg on 10/1/2023 and filled with soil (5 soil: 1 peat moss) and all service operations were carried out from irrigation and fertilization, to study the effect of spraying with nano and traditional fertilizer and cytokinin. The experiment was carried out using a randomized block design as a complete factorial experiment (R.C.B.D) (4×4). The experiment included (16) treatments with three replicates and the experimental unit included 5 seedlings. The results were analyzed using the statistical analysis program, and the averages were compared using the least difference test at a probability level of 0.05 (Al-Rawi and Khalaf, 2000). The use of nano

fertilizer in powder form is a foliar fertilizer produced using the nano technology Nono NPK at a concentration of (2,1,0) g/L of Indian origin and the regular foliar fertilizer at a concentration of (1) g/L of American origin. As for cytokinin, it was also used in powder form at a concentration of (200,100,50,0) mg/L, which is a powder of the type benzyl adenine (N6-Benzyladenine) produced by the company DIREVO Industrial Biotechnology.

2.1. The Studied Traits

2.1.1. Leaf Chlorophyll Content (Spad)

The total chlorophyll content of the leaves was measured using the Spad device. 5 fully expanded leaves were randomly selected from the bottom, middle and top of the seedling for all seedlings in the experimental unit and then the average values for each seedling were calculated [11].

2.1.2. Total Carbohydrate Percentage (%)

After the leaves are ground and dried, the proportion of carbs in them is determined. The dried sample is then taken in 0.250 g portions, added to an ampoule, and filled with 10 ml of distilled water. Following ten minutes in a centrifuge, the samples are filtered and finished with ten milliliters of distilled water. Next, 1 milliliter of the filtrate is collected, and 5 milliliters of sulfuric acid (concentrated at 80%) and 1 milliliter of phenol reagent (concentrated at 5%) are added to it. There is a 25-minute cooling period for the solution. according to the method [12]. Using a spectrophotometer with a wavelength of 490 nanometers.

2.1.3. Percentage of Nitrogen and Phosphorus in Leaves (%)

Samples of fully developed orange seedlings from the area were gathered, cleaned with distilled water, allowed to air dry, then sealed in perforated paper bags and baked at 65°C in an electric oven until the weight remained constant. Following drying, the leaf samples were pulverized in an electric mill and digested in a 4:1 ratio with concentrated sulfuric acid H₂SO₄ and perchloric acid HCL₄, as mentioned by [13] to obtain a colorless extract ready for mineral determination. After completing the digestion process, the following elements were estimated:

2.1.3.1. Nitrogen (%)

The percentage of total nitrogen was calculated using the Kjeldahl apparatus and according to the method mentioned by [14] in the Field Crops Laboratory / Al-Qasim Green University.

2.1.3.2. Phosphorus (%)

Phosphorus was determined by the molybdate-ammonium vanadate blue method, which was prepared by dissolving 22.5 g of ammonium molybdate in 400 ml of distilled water (solution A) and 1.25 g of ammonium vanadate in 300 ml of hot distilled water (solution B). Then solution B was added to solution A in a 1-liter flask, then left to cool at room temperature and 250 ml of concentrated nitric acid was added to it. When it cools, the volume is completed to 1 ml.

Measurement:

5 ml of the digested sample were taken and 5 ml of molybdate-ammonium vanadate solution was added to it, and the volume was completed to 50 ml. Then the sample was left for 30 minutes, after which the light absorption intensity was measured in a spectrophotometer at a wavelength of 410 nm according to the method of [15]. Blank was prepared in the same way, but without adding the sample. The standard curve was prepared using potassium phosphate dibasic Hydrogen (KH₂PO₄).

2.1.4. Leaf Content of Nutrients Zinc and Iron (mg/kg dry weight)

These elements were estimated by Automic Absorption Spectrophotometer according to the method proposed by [16] in the U-science scientific laboratory / Diwaniyah.

2.1.5. Seedling Height Rate (cm)

The seedling height was measured from the grafting area to the top of the seedling using a metric tape measure for all seedlings in the experimental unit and the average was calculated for them.

2.1.6. Total Number of Branches Rate (branch/seedling)

The number of total lateral branches on the main stem of each seedling was calculated and the average was extracted.

2.1.7. Number of Leaves (leaf/seedling)

All leaves on the seedling and for all plants in the experimental unit were counted and the average was calculated for them.

3. Results and Discussion

3.1. Total Chlorophyll Content of Leaves (Spad)

Table 1 shows that applying a nano-nutrient solution to orange seedlings in the area has a notable impact on the relative amount of total chlorophyll in the leaves. Specifically, when comparing the concentration of 1 g/L nano, the highest rate of chlorophyll was obtained, reaching 55.90Spad, while the lowest rate was 49.61Spad. Regarding cytokinin, the same table's data demonstrated a considerable impact: at 200 mg/L treatment, the maximum percentage of total chlorophyll reached (25. Spad55), whereas the control treatment produced the lowest chlorophyll rate of (50.92 (Spad). The relative chlorophyll content in the leaves was significantly influenced by the interaction between the research components, with the interaction treatment (2 g/L nano-nutrient solution and 200 mg/L cytokinin) producing the maximum chlorophyll content.

Table 1. Effect of spraying the traditional and nano-nutrient solution and cytokinin and their interactions on the total chlorophyll content of the leaves of local orange seedlings (Spad).

Cytokinin concentration mg/L	Nutrient solution concentration g/L				Average
	0	1traditional	Nano 1	Nano 2	
0	46.30	48.51	54.37	54.50	50.92
50	49.07	53.36	56.97	54.30	53.42
100	49.67	54.77	55.01	54.60	53.51
200	53.40	52.81	57.25	57.55	55.25
average	49.61	52.36	55.90	55.23	
LSD 0.05	Nutrient solution concentration 1.366		Cytokinin concentration 1.366		interaction 2.732

The nutrients in the nutrient solution play a part in stimulating and activating the cell's essential processes, which in turn increases the cell's vital processes and other chemical contents like chlorophyll. This is the cause of the increase in the relative content of total chlorophyll. It was discovered that the nitrogen element is crucial for the synthesis of the chlorophyll pigment. [17]. Spraying cytokinin may have caused an increase because it delays the aging process of leaves by

preventing the quick and rapid decline in cell concentrations of protein, RNA, and chlorophyll [18].

3.2. Percentage of Total Carbohydrates (%)

The results in Table 2 demonstrate that the percentage rate of carbohydrates was significantly affected by nutrient solution spraying. When treated with a concentration of 1 g/L nano-fertilizer, the greatest rate was seen (22.33%), which was higher than the lowest rate in the control, which was recorded (17.17%). When the seedlings were treated with 200 mg/L of cytokinin, the percentage of carbohydrates increased significantly; it reached a maximum of 23.79%, whereas the control treatment's lowest value was 15.61%). The percentage rate of carbohydrates was significantly impacted by the interaction between the study factors in the same table. When the factors were treated with a concentration of 1) g/L nano-fertilizer and 200 mg/L cytokinin, the interaction between them reached the highest value of 26.64%, while the rate for the control treatment was the lowest, at 13.10 percent. The components' involvement might be the cause of the rise in the percentage of carbs. By activating the starch synthesis enzyme, food plays a role in the production of food materials, including potassium. This has a positive effect on increasing the percentage of manufactured materials because the ineffectiveness of this enzyme lowers the amount of ready energy in the plant as a whole [18,19].

Table 2. The effect of spraying the traditional and nano nutrient solution and cytokinin and their interactions on the percentage of carbohydrates for local orange seedlings %.

Cytokinin concentration mg/L	Nutrient solution concentration g/L				Average
	0	1traditional	Nano 1	Nano 2	
0	13.10	15.23	21.53	16.58	15.61
50	17.75	17.54	20.66	18.37	18.08
100	18.34	20.66	24.50	21.42	21.23
200	21.51	22.28	26.64	24.73	23.79
average	17.17	18.92	22.33	20.27	
LSD 0.05	Nutrient solution concentration 1.03		Cytokinin concentration 1.03		interaction 2.06

3.3. Percentage of Nitrogen in Leaves (%)

Table 3's results showed that applying the nutrient solution to local orange seedlings had a substantial impact. The maximum rate (3.6135%) was obtained when the plants were treated with a concentration of 2 g/L nano fertilizer, while the lowest value (1.769%) was obtained when the plants were treated with the comparison. Regarding the outcomes in the same table after cytokinin treatment, they had a noteworthy impact, with the concentration of 200 mg/L yielding the greatest value and outperforming the other concentrations, amounting to (2.770%), whereas 2.096% was the lowest percentage obtained from the comparison. The nutritional solution and cytokinin interaction in the same table showed a substantial impact; when treated (2 g/L nutrient solution and 200 mg/L cytokinin), the interaction produced the greatest percentage (3.820%), while the comparison gave the lowest percentage (1.187%).

The cause of the rise in nutritional content in the leaves is because The concentration and accumulation of nutrients within the plant are enhanced by the nutrition solution. The enhanced effectiveness of the photosynthetic process or the direct absorption of these elements might be the cause of the rise in nitrogen. [20] The reason for the increase in nitrogen in the leaves as a result

of treatment with cytokinin is due to its stimulating role in the absorption and transport of nutrients in the plant [21].

Table 3. The effect of spraying the traditional and nano-nutrient solution and cytokinin and their interactions on the percentage of nitrogen in the leaves of local orange seedlings (%).

Cytokinin concentration mg/L	Nutrient solution concentration g/L				Average
	0	1traditional	Nano 1	Nano 2	
0	1.1 87	1.853	2.107	3.240	2.096
50	1.6 27	2.157	2.337	3.737	2.4 64
100	2.137	2.223	2.833	3.657	2.7125
200	2.1 25	2.477	2.560	3.820	2.770
average	1.769	2.177	2.459	3.6135	
LSD 0.05	Nutrient solution concentration 0.462	Cytokinin concentration 0.231		Interaction 0.231	

3.4. The Percentage of Phosphorus in the Leaves (%)

The nutrient solution treatment, which produced the highest rate of 0.68% when treated with a concentration of 2 g/L nano-fertilizer, was found to have a significant impact on the seedlings (Table 4). Similarly, the cytokinin spray treatment, which produced the highest rate of 0.50% when treated with a concentration of 200 mg/L, was found to have a significant impact on the seedlings (Control treatment, 0.36%). The greatest rate of bi-interaction between the research components was 0.73%, indicating a considerable impact on the phosphorus content in the leaves. when spraying with a concentration of 2 g/L nano-fertilizer with 200 mg/L cytokinin, while control treatment recorded the lowest rate of 0.21%. The reason for the significant increase in phosphorus may be due to the role of the nutrient solution, as it contains elements that play a role in increasing the effectiveness of the plant. Phosphorus enters into the formation of amino acids, which are the units of protein formation and plant hormones, including auxins and cytokinins, and thus stimulates cell division. The notable impact of benzyl adenine on elevating the phosphorus concentration in the leaves of treated plants promotes vegetative growth, which in turn speeds up photosynthesis to produce carbohydrates, a source of energy needed for the plant's growth and development. This thus causes the plant to absorb more phosphorus and increases the amount of the mineral in the plant's tissues, which is in line with what was previously stated by [22].

Table 4. Effect of spraying the traditional and nano nutrient solution and cytokinins and their interactions on the percentage of phosphorus in the leaves of local orange seedlings (%).

Cytokinin concentration mg/L	Nutrient solution concentration g/L				Average
	0	1traditional	Nano 1	Nano 2	
0	0.21	0.32	0.3 4	0.59	0.36
50	0.25	0.34	0.52	0.70	0.45
100	0.22	0.47	0.5 6	0.71	0.49
200	0.21	0.51	0.56	0.7 3	0.50
average	0.22	0.41	0.49	0.68	
LSD 0.05	Nutrient solution concentration 0.07	Cytokinin concentration 0.07		Interaction 0.08	

3.5. Iron Content of Leaves (mg/kg dry weight)

Table 5's findings demonstrate that, at a concentration of 2 g/L nano-fertilizer, spraying with the nutrient solution significantly affected the iron content in the leaves, resulting in the highest rate of 55.5 mg/kg dry weight as opposed to the lowest rate of (.748 mg/kg dry weight) in the comparison. In relation to the cytokinin treatment, it was possible to attain the greatest rate of 57.65 mg/kg dry weight at a concentration of 200 mg/L cytokinin, which was significantly higher than the lowest concentration of 45.69 mg/kg dry weight attained by the other therapy.. In the same table, the interaction between the study factors showed a significant effect on the iron percentage at a concentration of (2 g/L nano-fertilizer with 200 mg/L cytokinin) of (60.14 mg/kg dry weight), while control treatment recorded the lowest concentration of (41.34 mg/kg dry weight).

Table 5. Effect of the traditional and nano-nutrient solution and cytokinin and their interactions on the iron content of the leaves of local orange seedlings (mg/kg dry weight).

Cytokinin concentration mg/L	Nutrient solution concentration g/L				Average
	0	traditional	Nano 1	Nano 2	
0	41.34	44.11	47.28	50.05	45.69
50	46.13	51.21	53.17	54.34	51.21
100	52.73	55.42	56.41	57.47	55.50
200	54.60	57.21	58.66	60.14	57.65
average	48.7	51.9875	53.88	55.5	
LSD 0.05	Nutrient solution concentration 1.90		Cytokinin concentration 1.90		interaction 3.79

The increase in iron content in the leaves may be due to the increase in vegetative growth in the plant, which resulted in increased iron absorption to meet the plant's need, in addition to the nutrient solution containing iron, which contributed to increasing its concentration inside the plant, which includes iron in the composition of the mineral flavoprotein, which has an effective role in vital oxidation processes [23].

3.6. Zinc Content of Leaves (mg/kg dry weight)

When compared to the control treatment, which produced the lowest concentration of zinc (31.34 mg/kg dry weight), Table 6's results demonstrate that treating the seedlings with the nutrient solution had a significant impact on the zinc content of the leaves. The zinc content of the leaves reached the highest rate (43.99 mg/kg dry weight) at a concentration of (2 g/L nano-fertilizer). Regarding the cytokinin therapy, it was shown to have a noteworthy impact, as evidenced by its greatest rate of 43.57 mg/kg dry weight, in contrast to the lowest rate seen in the control treatment, which was 32.87 mg/kg dry weight. As for the data in the same table, the interaction between the study factors showed a significant effect, as it gave the highest concentration of (50.30 mg/kg dry weight) at a concentration of (2 g/L nano-fertilizer with 200 mg/L cytokinin) compared to control treatment, which gave the lowest concentration of (25.34 mg/kg dry weight).

Table 6. Effect of spraying the nutrient solution and cytokinin and the interaction between them on the rate of leaf content of zinc (mg/kg dry weight).

Cytokinin concentration mg/L	Nutrient solution concentration g/L				Average
	0	1traditional	Nano 1	Nano 2	
0	25.34	33.21	35.84	37.11	32.87
50	30.41	37.43	40.41	42.41	37.66
100	33.97	42.43	44.97	46.17	41.88
200	35.67	43.02	45.31	50.30	43.57
average	31.34	39.02	41.63	43.99	
LSD 0.05	Nutrient solution concentration 2.201	Cytokinin concentration 2.201			Interaction 4.523

The effect of growth regulator on increasing nutrients in leaves, as cytokinins increase the opening of the stomata by inhibiting ABA acid [24] and as a result of this expansion, the rate of transpiration increases and the absorption and transfer of mineral ions resulting from transpiration increases [20].

3.7. Seedling Height (cm)

The results of Table (7) make it evident that spraying seedlings with nano fertilizer increased the rate of seedling height significantly. The seedlings treated with a concentration of 1 g/liter of nano fertilizer reached the highest rate of height (64.32 cm), compared to the lowest height (53.41 cm) in the control treatment. Additionally, spraying cytokinin on seedlings significantly increased the pace at which seedlings grew in height; the maximum height (62.55 cm) was recorded in seedlings treated with a 200 mg/L concentration, compared to the lowest rates (57.16 cm) in comparison seedlings.. As for the interaction between the concentrations of the nutrient solution and cytokinin, it was significant, as seedlings treated with a concentration of 1 g/L of nano-fertilizer with 200 mg/L of cytokinin excelled by obtaining the highest rate of this trait, which reached (68.09 cm) compared to the lowest rates (48.42 cm) in control treatment.

Table 7. Effect of spraying the nutrient solution and cytokinin and their interaction on the rate of seedling height / cm.

Cytokinin concentration mg/L	Nutrient solution concentration g/L				Average
	0	1traditional	Nano 1	Nano 2	
0	48.42	55.30	63.70	61.25	57.16
50	51.42	54.47	62.01	61.64	57.38
100	55.60	59.79	63.50	62.50	60.34
200	58.21	60.52	68.09	63.39	62.55
average	53.41	58.52	64.32	62.19	
LSD 0.05	Nutrient solution concentration 3.823	Cytokinin concentration 3.823			Interaction 7.646

The reason for the increase in the rate of seedling height is due to the effect of the nutrient solution in meeting the plant's need for mineral elements necessary for photosynthesis, respiration and various metabolic processes due to what the nutrient solution contains of primary elements NPK and trace elements (Mn, B, Cu, Zn, Fe) in balanced quantities in the process of cell division and elongation [5] Its role in producing proteins and enzymes unique to the process of cell division and expansion may have contributed to the reason for the increase in seedling height. This, in turn, increased the osmotic pressure inside the plant and allowed for the absorption of

more water and nutrients, which in turn led to an increase in plant growth indicators, including seedling height [23].

3.8. Average Number of Leaves (leaf/seedling)

Table 8's results show that the nutrient solution treatment had a significant impact on the average number of leaves. The treatment with 1 g/liter of nano-fertilizer was superior, yielding the highest rate of leaves per seedling (64.01), while the control treatment produced the lowest rate of leaves per seedling (50.02). The same table also shows that cytokinin therapy significantly affected the average number of leaves on the seedlings, with the maximum rate being attained when the treatment was administered at a dosage of 200 mg/liter (66.43 leaves/seedling). while control treatment gave the lowest rate for this trait (47.67 leaves/seedling). The results of the interaction between the study factors had a significant effect on this trait, as the seedlings treated with a concentration of 1 g/L of nano-fertilizer with a concentration of 200 mg/L of cytokinin excelled by obtaining the highest rate of leaf number (76.37 leaves/seedling) compared to the lowest rates (37.18 leaves/seedling) in control treatment.

Table 8. Effect of spraying the nutrient solution and cytokinin and the interaction between them on the rate of leaf number (leaf/seedling).

Cytokinin concentration mg/L	Nutrient solution concentration g/L				Average
	0	1 traditional	Nano 1	Nano 2	
0	37.18	47.37	55.01	51.14	47.67
50	49.80	55.56	63.36	60.11	57.20
100	54.63	57.04	61.32	61.36	58.58
200	58.44	64.43	76.37	66.5	66.43
average	50.02	56.10	64.01	59.77	
LSD 0.05	Nutrient solution concentration 9.74		Cytokinin concentration 9.74		Interaction 19.48

The reason for the increase in the number of leaves as a result of spraying the nutrient solution and cytokinin is due to the same reasons that explained the increase in the height of the seedlings, the number of branches, as the increase in these characteristics increases the number of leaves.

3.9. Average Number of Branches (branch/plant)

We notice from the results of Table (9) that spraying with the nutrient solution has a significant effect on the average number of branches, as the 1 g/L nano-fertilizer treatment excelled by giving the highest rate of (6.86 branches/seedling), while control treatment gave the lowest rate of (5.45 branches/seedling). Also, spraying with cytokinin had a significant effect on the average number of branches, as the highest rate reached the treatment with a concentration of 200 mg/liter to (9.01 branches/seedling), while control treatment gave the lowest rate for this characteristic, which reached (4.75 branches/seedling). It is noted that the interaction of the study factors had a significant effect on this trait and the highest rate of the number of branches was reached when using 1 gm / liter of nano-fertilizer with 200 mg / liter of cytokinin, which reached (9.89 branches / seedling) compared to the lowest rate (4.27 branches / plant) in control treatment.

Table 9. The effect of spraying the nutrient solution and cytokinin and the interaction between them on the rate of the number of branches.

Cytokinin concentration mg/L	Nutrient solution concentration g/L				Average
	0	1 traditional	Nano 1	Nano 2	
0	4.27	4.33	5.27	5.15	4.75
50	5.12	6.17	6.57	6.11	5.99

100	5.39	6.01	6.73	6.42	6.13
002	7.03	7.48	98.8	8.67	8.01
average	5.45	5.99	6.86	6.58	
LSD 0.05	Nutrient solution concentration 1.384	Cytokinin concentration 1.384		Interaction 2.669	

The reason for the increase in the number of main branches as a result of using the nutrient solution may be attributed to its role in containing many nutrients that have a positive effect on the vital processes within the plant and increasing the process of photosynthesis, which is reflected in increasing the number of branches [5] As for the reason for the increase in the number of branches in seedlings treated with cytokinin, it is due to its role in increasing division, expansion and increasing the number of branches [9].

Conclusions

Spraying seedlings with single and joint study treatments led to a significant increase in (leaf chlorophyll content, percentage of total carbohydrates, percentage of nitrogen, percentage of phosphorus, leaf iron content, leaf zinc content, seedling height rate, average number of branches, number of leaves).

References

- [1] Wu, G.A.; Terol, J.; Ibanez, V.; Lopez-Garcia, A.; Perez-Roman, E.; Borreda, C.; Domingo, C.; Tadeo, F.R.; Carbonell-Caballero, J.; Alonso, R.; et al. 2018. Genomics of the origin and evolution of Citrus. *Nature*, 554, 311–316.
- [2] Hasan, A.M., T.J. Mohamed Ali., D.K.A. Al-Taey (2019). Effects of Winter Foliar Fertilizing and Plant Growth Promoters on Element and Carbohydrate Contents on the Shoot of Navel Orange Sapling. *International Journal of Fruit Science*, 19(1)1-10. <https://doi.org/10.1080/15538362.2019.1668331>
- [3] Ibrahim, Alaa Suhail, Ishraq Sulaiman Ali, Raed Nazih Subaih, Rafiq Ali Abboud, and Fadel Sulaiman Al-Qaim. 2014. Effect of seven citrus rootstocks on the growth and yield of Abu Navel orange cultivar Washington Navel 141). *Syrian Journal of Agricultural Research*, 1(1): 59- 69.
- [4] FAO 2020. Global Network on Integrated soil. Management.
- [5] Central Statistical Organization, 2020. Ministry of Planning, Directorate of Agricultural Statistics
- [6] Boughalleb, F.; M. Mahmoud and H.Hajlaoui . 2010. Response of young citrus trees to NPK fertilization under greenhouse and field conditions. *Agricultural Journal*, 6 (3): 66-73.
- [7] Al-Taey, D.K.A. and Z.J. M. Al-Musawi.(2022). The Impact Of Nano Fertilization And Salicylic Acid On Growth, Yield And Anti-Oxidant Contents In Rocket Plant Under Salt Stress. *Agraarteadus*. 32 (1) :43-47. <https://dx.doi.org/10.15159/jas.21.30>
- [8] AL-Taey D.K.A. , K.M.N.AL-Dirbil and S.S.M.AL-Azawi.(2021). Study The Effect of Water Quality, Bio Booster (Zytonic F) and Nano NPK fertilizer on Tomato Growth and Yield. *IOP Conf. Series: Earth and Environmental Science* 910 (2021) 012063. doi:10.1088/1755-1315/910/1/012063
- [9] Al-Rawi, Khashea Mahmoud and Khalaf Allah Abdul Aziz Mohammed. 2000. Design and analysis of agricultural experiments.
- [10] Joslyn, M.A. 1970. Method in Food Analysis, Physical, Chemical and Instrumental Methods of Analysis. 2nd ed. Academic Press ,New York and London.
- [11] Johnson, C. M. and A. Ullrich. 1959. Analytical Methods for Use in Plant Analysis. *Bull. Calif. Agric. No.* 766.
- [12] Al-Sahhaf, Fadhel Hussein . 1989. Natural Plant Nutrition. Higher Education Press in Mosul, Bayt Al-Hikma for Publishing, Translation and Distribution. University of Baghdad-Ministry of Higher Education and Scientific Research. Iraq.



- [13] Al-Mawsili, Ahmed. Dawoud.2018. Complete in fertilizers and fertilization (Soil, plant and water analysis (. Dar Al-Kotob Al-Ilmiyah, Beirut, Lebanon.
- [14] Chapman, H. D and P. F. Pratt. 1961. Method of Analysis for Soils.
- [15] Agbede, T. M.; S. O. Ojeniyi and A. J. Adeyemo. 2008. Effect of poultry manure on soil physical and chemical properties, growth and grain yield of sorghum in southern Nijeria. Amer. Eurasian J. Sustainable Agric., 2: 72-77.
- [16] Groshe, Kamel Hassan Mohamed. (2020). Growth regulators and plant physiology. University of Mentouri. Ministry of Higher Education and Scientific Research. People's Republic of Algeria.
- [17] Davies, P. J. 2010. The plant hormones: their nature, occurrence, and functions. In: Plant Hormones (pp. 1-15). Springer, Dordrecht.
- [18] Xia, Z., He, Y., Yu, L., Miao, J., Korpelainen, H. and Li, C. (2020). Root traits and rhizosphere processes reflect differential phosphorus acquisition strategies in contrasting Populus clones. Forest Ecology and Management, 457, 117-140.
- [19] Mohamed Mohamed Yaqoub, Salah El-Din Mustafa Abu Gharsa. 2022. Fundamentals of Plant Nutrition, First Revised Edition, National Library, Omar Al-Mukhtar University, Libya
- [20] Abd-Alfatah, G.H. (1995). Physiological studies on rose. cv. Tea Rose. M. Sc. Thesis, Fac. Agric. Moshtohor, Zagazig. Univ. Egypt.
- [21] Nour El-Din, Shawqi Ali. 2012. Guide to Plant Nutrition. University of Baghdad. Ministry of Higher Education and Scientific Research. Iraq.
- [22] Radhi, Mustafa Mohamed. (2021) . Plant Growth Regulators. Ministry of Higher Education and Scientific Research. Fayoum University. Arab Republic of Egypt
- [23] Awad, S. M. & A. R. Atawia 1995. Effect of foliar sprays with some micronutrients on "Le-Conte" pear trees. 1-Tree growth, flowering & leaf mineral contents. Annals Agric. Sci, Ain Shams Univ. 40(1): 359-367.
- [24] Al-Naimi, Saad Allah Najm Abdullah. 2000. Fertilizers and Soil Fertility, Second Revised Edition, Dar Al-Kutub for Printing and Publishing, University of Mosul, Iraq.

Evaluation of the Herbicide Tarzec Performance Under Different Application Time (Some Growth Traits of Wheat and Accompanied Weeds)

Suhad Mathkooor A. Safi¹, Nesoora Adil Jassem² and Watheq Falhi Hammood³

^{1,3}College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.

²Agricultural Protection Department, Ministry of Agriculture, Baghdad, Iraq.

¹E-mail: suhad.mathkor@coagri.uobaghdad.edu.iq

³E-mail: wathiq.filhye@coagri.uobaghdad.edu.iq

Abstract. A field experiment was conducted during the winter season 2023-2024 at the research stations affiliated with the General Department for Crop Protection in Abu Ghraib to investigate the effect of the Tarzec herbicide and different application dates on the weeds accompanying crop and their impact on some growth traits of the wheat, variety (Buhuth 22). The experiment was implemented following the split-plot arrangement according to the randomized complete block design (RCBD) with three replicates. The main plots included the treatments of application Tarzec @ three periods after planting (3, 5, and 7 weeks). The subplots comprised weed control treatments involving: 1) Tarzec herbicide @ 75 g h⁻¹ (h₁), 2) Tarzec herbicide @ 90 g h⁻¹ (h₂), 3) Weedy treatment (h₀), and 4) Weed-free treatment. The results showed that the second period of application herbicides, i.e. 5 weeks after planting, was superior in affecting the weeds accompanying the wheat crop and improving some wheat growth traits, achieving the best values, including the lowest weed density and weed dry weight, highest percentage of control and inhibition, highest height, dry weight and the number of tillers m⁻² of wheat plants after 90 days of application, which attained to 29.17 plants m⁻², 106.2 g m⁻², 75.00%, 75.00%, 79.62 cm, 318.90 g m⁻², and 310.92 tillers m⁻² for the traits mentioned above, respectively, compared to the two other periods after planting (3 and 7 weeks), which recorded lower averages. The results also showed the efficiency of the herbicide Tarzec @ 75 and 90 g h⁻¹ in controlling weeds accompanying the wheat crop and improving some growth traits. It is noted that the rate @ 90 g h⁻¹ achieved high averages coming after the averages of the weed-free treatment and achieved the lowest weed density after 90 days of application, reaching 2.44 plants m⁻² and the lowest dry weight of weeds, reaching 13.64 g m⁻², thus achieving the highest percentage of control and inhibition, average of plant height, dry weight of wheat plants, number of tillers, recording 97.97%, 96.80%, 81.7 cm, and 315.40 g m⁻², 325.89 tillers m⁻², respectively with insignificant differences from the rate @ 75 g h⁻¹ in weed density and dry weights, compared to the weedy treatment, which recorded the lowest averages for the traits above. The application @ 75 and 90 g ha⁻¹ at the three periods achieved the best averages for the traits of weed density, dry weights, percentage of control and inhibition, dry weight of wheat plants, and number of their tillers compared to the two periods of 3 and 7 weeks after planting, confirming the role of application periods after planting in improving the ability of the herbicide to eliminate weeds and thus enhancing the growth traits of wheat plants, compared to the weedy treatment at the same periods.

Keyword: Tarzec herbicide, application time, Growth parameters, application rate, Bread Wheat.

1. Introduction

Wheat (*Triticum aestivum* L.) ranks primarily among the strategic field crops worldwide and for Iraq due to its importance as a major food source and its role in economic and social development. Despite the strategic importance of this crop in Iraq, the average yield of its grains per unit area is much lower than the global production rate. Weeds are among the most important agricultural pests due to the loss in production they cause for their direct competition with the crop or their indirect effects. Weeds compete with crop plants efficiently for the most vital growth requirements, including water, light, and nutrients, especially in the early stages of crop growth. As a result of this competition, crop growth, especially wheat, would be weak, leading to a decrease in yield. The spread of various weed species in wheat fields in Iraq, especially in irrigated areas, and their intense competition with this crop is one of the most important reasons for the crop production decline since weeds compete with crop plants for water, food, light, and other agents that limit growth, hence all of which lead to a decrease in the yield in terms of quantity and quality. Wheat is considered a weak competitor to weeds compared to other crops, so the growth of both types of weeds, narrow and broad-leaved, in wheat fields is one of the most critical challenges facing this crop and leads to a decrease in its productivity and deterioration of its quality [1]. One of the most essential losses caused by weeds is yield reduction, at various rates ranging from 30 to 50% and sometimes reaching 70% depending on the prevailing weed species and density [2-4]. Weeds accompanying the wheat crop in Iraq were classified into more than 16 species of broad leaf weeds, such as *Silybum marianum* (L) Gaertn, *Polygonum aviculare* L., *Malva rolandifolia* L., and *Secale cereal* L., and more than 12 species of narrow leaf weeds, such as *Avena fatua* L., *Phalaris minor* L., *Lolium temulentum* L., and *Lolium rigidum* L. These weeds impact crop performance and reduce its productivity [5]. The severe damage caused by these weed plants is due to their high ability to compete with crop plants for the essential growth elements, in addition to the allelopathic effect of some of these weeds for secreting inhibitory compounds from their roots that inhibit the crop growth, as it was found in wild oats [6], *Schismus arabicus*, L. and darnel (*Lolium temulentum* L.) [7]. Therefore, researchers have turned to controlling these weeds and reducing their harm by using various means, the most crucial of which is relying on chemical herbicides, which have achieved dramatical results in reducing the weed damage [8-11]. These studies have displayed the efficiency and effectiveness of these herbicides in controlling weeds accompanying wheat. The effectiveness of using chemical control to manage weeds depends on several factors, including the efficiency of the herbicides used, their selection, as well as the soil and environmental conditions during the application process. [12] reported that using the herbicides Pallas 4.5%OD (at 125ml Dunam⁻¹), Spotlight (at 75%), DWG (at 5g Donum⁻¹), and Clodex100EC (at 150 ml Dunam⁻¹) reduced the dry weight of narrow and broad leaf weeds and achieving the highest percentage of weight inhibition and thus improving the growth traits of the crop plants as these herbicides achieved a high plant height (79.69, 72.4 and 75.46 cm) and number of tillers (646.6, 549.67 and 565.83 tiller m⁻²) for the mentioned herbicides respectively. [13,14] stated that using the herbicides reduced the dry weight of weeds and increased the dry weight inhibition percentage compared to the weedy treatment. [15-18] reported that using chemical herbicides increased the plant height and number of tillers m⁻² in wheat. The herbicide Atlantis reduced the number of weeds 90 days after application to 2.6 plants m⁻², compared to the weedy treatment, exhibiting the highest average weed density of 168.7 plants m⁻². Additionally, Atlantis reduced the weed dry weight to 5.7 g m⁻² and inhibited 97.18%

of the weed dry weight. In comparison, the weedy treatment had a weed dry weight of 223.8 g m⁻² and no inhibition. The herbicide also achieved a wheat plant height of 97.40 cm and a number of tillers of 367.30 tillers m⁻² compared to the weedy treatment, which recorded 89.07 cm and 178.60 tillers m⁻², respectively [19,20]. [21] reported that using the YAMABIC 30 EC herbicide @ 640 ml ha⁻¹, added at the 2-4 leaf stage of wheat (stage 2 tillers) reduced the dry weight of the narrow and broad-leaved weeds accompanying the crop and increased the inhibition percentage of the weed weight compared to the herbicide Axial 50 EC, which recorded higher averages for the weed dry weight. Hence, this research was implemented with the aim of investigating the effect of Tarzec herbicide on controlling the weeds accompanying the wheat crop and in the crop growth traits.

2. Materials and Methods

A field experiment was conducted during the winter season 2023-2024 at the research stations affiliated with the General Department for Crop Protection in Abu Ghraib, to determine the effect of the herbicide Tarzec at different application dates on the weeds accompanying wheat crop and on some growth traits of the crop (variety Buhuth 22). The experiment was implemented within a split-plot arrangement based on the randomized complete block design (RCBD) with three replicates. The main plots involved the treatments of three periods for application Tarzec herbicide after planting (3, 5, and 7 weeks). The sub-plots included the weed control treatments of 1) Tarzec herbicide @ 75 g h⁻¹ (h₁) 2) Tarzec herbicide @ 90 g h⁻¹ (h₂), 3) Weedy treatment (h₀) (i.e. leaving the weeds to compete with the crop until the end of the season), and 4) Weed free treatment, which is the complete elimination of all weeds and preventing them from competing with the wheat crop relying on manually weeding.

The experimental land was prepared by plowing the soil using a moldboard plow, loosened with disc harrows, and then leveled. After that, the experiment land was divided into three replicates. Wheat seeds of the variety Buhuth 22 were planted on 11/23/2023 at a seed rate of 120 kg ha⁻¹ in rows distanced by 20 cm from one another. The experimental soil was fertilized with triple superphosphate fertilizer (46% P₂O₅) @ of 100 kg ha⁻¹ before planting. Urea fertilizer (46% N) was added to the soil @ 200 kg N ha⁻¹ [22] divided into three batches. The first batch was added at the beginning of the tillering stage, the second at the elongation stage initiation (two nodes on the main stem), and the third at the boot stage. The herbicide was applied according to the experimental treatments on the shoot of the crop and the weeds using water as an application solution @ 400 liters of water ha⁻¹, using a 2-liter sprinkler.

2.1. Tarzec Herbicide

It is a selective systemic herbicide for controlling broad-leaf and narrow-leaf weeds in the post-emergence stage in wheat fields. It is applied as a foliar spray on the weeds and transmitted to the whole plant through the leaves. It leaves a limited residual in the soil and can eliminate young weeds. This herbicide contains two active ingredients: Pyroxsulam which belongs to the triazole-pyrimidine family, which inhibits the action of the ALS (Acetolactase Synthetase) enzyme. The herbicide is easily transferred within the targeted weeds, thus preventing the synthesis of amino acids responsible for cell division in the meristematic tissue, which hinders growth within hours of application. The herbicide is easily transferred within the targeted weeds, thus preventing the synthesis of amino acids responsible for cell division in the meristematic tissue, which hinders growth within hours of application. The second ingredient is halauxifen-methyl, belonging to the new Picolinic acid family, which controls stubborn broadleaf weeds. The herbicide is sprayed from the stage of 3 true-leaves emergence to the stage of two nodes in the crop. The actual time taken for the symptoms of the effect to appear and weed death varies according to the weed

species and weather conditions. In some cases, the symptoms may be delayed for about four weeks. The herbicide controls the weeds of *L. Avena fatua*, *Bromus tectorum* L., *Hordeum spp.*, *Lolium spp.*, *Phalaris spp.*, *Centaurea pallescens* L., *Galium aparine* L., *Malva spp.*, *Convolvulus arvensis* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., *Sonchus oleraceus* L., *Stellaria media* L., *Silybum marianum* L. Gaertn. [23].

2.1.1. The weeds Accompanying Wheats

2.1.1.1. Weed Species and Density

To identify the degree of weed spread in each experimental unit the species and density of weeds were recorded 90 days after application depending on the square method using a square dimensioned 50 x 50 cm for an area of one square meter of each experimental unit [24].

2.1.1.2. Weed Dry Weight ($g\ m^{-2}$) at Harvesting

The weeds were cut randomly at the soil surface level from an area of $1\ m^2$, placed in perforated bags, and then dried in an oven at $70\ ^\circ C$ until the weight was stable. After that, the weed dry weight was calculated [25].

2.1.1.3. Inhibition Percentage Weed Dry Weight

The inhibition percentage of the weed dry weight for the different treatments was calculated according to the following equation [26]:

$$\text{Inhibition percentage (\%)} = 100 - (A/B \times 100)$$

Where:

A= Weed dry weight of weed control treatments

B= Weed dry weight of weedy treatment

2.2. Plant Height (cm)

It was calculated as the distance from the soil surface to the spike base on the main stem at harvest time as an average of ten plants for each experimental unit [27].

2.3. Number of Tillers m^{-2}

Tillers were counted at harvest for a square meter area of each experimental unit.

2.4. Dry Weight of Wheat Plant ($g\ m^{-2}$)

Wheat plants were harvested randomly at the soil surface level from an area of $1\ m^2$ and left to dry, then the dry weight of these plants was calculated.

2.5. Statistical Analysis

The data for the studied traits were collected and tabulated, then statistically analyzed based on the randomized complete block design using the software GENSTAT. The arithmetic means of the treatments were compared relying on the least significant difference (LSD) at a probability level of 0.05 [28].

3. Results and Discussion

3.1. Effect of Different Treatments on Weeds Accompanying Wheat

Identifying the weed species during the growing season revealed ten species. Broad-leaved weed plants constituted 9 of which were (Table 1). The most widespread of them were *Beta vulgaris* L., *Melilotus indicus* L., and *Malva rolundifolia* L., and there were small numbers of *Sonchus oleraceus* L., *Silybum marianum* L Gaertn, *Daucus carota* L., and *Lactuca serriola* L., in contrast, there were only narrow-leaved one species plants of *Phalaris minor* L.

Table 1. Weed species, grown in the experimental land during the winter season 2023-2024.

English name	Scientific name	Family name	Type	Life cycle
Canary grass	<i>Phalaris minor</i> L.	Poaceae	Narrow-leaf	Annual
sweet clover	<i>Melilotus indicus</i> L.	Fabaceae	Broad-leaf	Annual
Button weed ¹	<i>Malva rolundifolia</i> L.	Malvaceae	Broad-leaf	Annual
Prickly lettuce	<i>Lactuca serriola</i> L.	Campositeae	Broad-leaf	Annual
Wild carrot	<i>Daucus carota</i> L.	Umbellifereae	Broad-leaf	Annual
White goosefoot	<i>Chenopodium album</i> L.	Chenopodiaceae	Broad-leaf	Annual
Wild beets	<i>Beta vulgaris</i> L.	Chenopodiaceae	Broad-leaf	Annual
field bindweed	<i>Convolvulus arvensis</i> L.	Convolvulaceae	Broad-leaf	Annual
Milk thistle	<i>Silybum marianum</i> (L)Gaertn	Campositeae	Broad-leaf	Annual
common sow thistle	<i>Sonchus oleraceus</i> L.	Campositeae	Broad-leaf	Annual

3.2. Effect of Different Treatments in Some Weed Traits

3.2.1. Weed Density Weed Dry Weight, Control Percentage, and Inhibition Percentage of Weed dry Weight at 90 Days After Application

Table 2 demonstrates that the different treatments and their interaction significantly affected the weed plant density, control percentage, weed dry weight, and inhibition percentage @ 90 days after application. The effect of the application periods after planting (3, 5, and 7 weeks) was insignificant in the density of the weeds. However, the period of 5 weeks after planting achieved the lowest density of weeds, amounting to 29.17 plants m⁻², and the lowest weed dry weight, amounting to 106.20 g m⁻², thus achieving highest percentage of control and inhibition, reaching 75.00% for each of these two traits, compared to the periods of 3 and 7 weeks after planting, which recorded higher density and dry weight of these weeds, and lower percentages of control and inhibition, attaining 31.00 and 3.17 plants m⁻²; 115.08 and 120.17 g m⁻²; 73.28 , 73.25%; and 72.61 , 72.30% for the mentioned traits and the two periods, respectively, with insignificant differences after 90 days of application. This result was consistent with [21] , obtaining a high effectiveness of the herbicide applicate at different dates and its effect in dry weight of weeds and the inhibition percentage of the weeds accompanying the wheat crop. Application @ 90 g h⁻¹, after 90 days of application, achieved the lowest weed density and weed dry weight and the highest percentage of control and inhibition, recording 2.40 plants m⁻², 13.64 g m⁻², 97.97%, and 96.80%, differing insignificantly from the rate @ 75 g h⁻¹ which recorded averages of 3.10 plants m⁻² and 15.27 g m⁻² and a control rate of 97.41% and an inhibition rate of 96.42%, respectively. It is noticed that the averages were close between the two application rates; nevertheless, their achievement of high averages may be due to the role of Tarzec herbicide at the two application rates in eliminating weeds accompanying the wheat crop, inhibiting the action of the enzyme Acetolactase Synthetase ALS, thus preventing the synthesis of amino acids responsible for cell division in the meristematic tissue, and hence hindering weed growth within hours of application. This result is consistent with what [21] about the difference in the effect of the herbicide

according to the application rates used, and also consistent with [3,12,29,30,31,32] , reporting the role of herbicides in reducing the density of weeds and their dry weights, and thus increasing the percentage of weed control and the percentage of inhibition in the dry weight of these weeds.

Application the herbicide @ 75 and 90 g ha⁻¹ at the three periods (3, 5, and 7 weeks) after planting achieved the best averages for the traits mentioned above. The best of these averages were five weeks after planting for the application rates @ 75 and 90 g ha⁻¹, achieving equal averages for the two rates regarding the weed density and weed dry weight, recording 0.00 plant m⁻² and 0.00 g m⁻², respectively, and the percentage of control and inhibition recorded 100.00% for both rates, compared to the weedy treatment, which recorded the highest weed density and the highest dry weight of the weeds and, consequently, recorded the lowest percentage of inhibition at the three periods, which explains that the behavior of Tarzec herbicide at the two application rates was positive and at the three periods, especially after five weeks from planting.

3.3. Effect of Different Treatments on Some Wheat Growth Traits

3.3.1. Plant Height (cm) Dry Weight of Wheat Plants (g m⁻²), and Number of Tillers m⁻²

Data in Table 2 reveal a significant effect of the experiment factors and their interaction on the traits, the plant height, dry weight of wheat plants, and number of tillers m⁻². It is noticed that this difference was insignificant in the trait of plant height between the three periods; yet, the period of application five weeks after planting achieved the highest dry weight of wheat plants, reaching 318.90 g m⁻², and the highest number of tillers, reaching 310.92 tiller m⁻², compared to the other two periods (3 and 7 weeks after planting), which recorded lower averages of plant dry weight (300.00 and 280.70 g m⁻²) and number of tillers (305.17 and 297.08 tiller m⁻²) for the two periods, respectively. The superiority of the period of five weeks after planting may be due to the lowest weed density and weed dry weight achieved by the period after 90 days of application, resulting in increasing the percentage of control and inhibition, allowing the crop plants the opportunity to grow without competition and thus performing their vital activities regularly due to the absence of the competition factor.

The weed-free treatment achieved the highest averages for the traits mentioned above, followed by the application @ 90 g h⁻¹, which achieved a crop plant height of 81.77 cm, a dry weight of crop plants of 315.40 g m⁻², and a number of tillers of 325.89 tillers m⁻², followed by application @ 75 g h⁻¹, recording averages of 74.8 cm, 271.00 g m⁻², and 313.11 tiller m⁻², compared to the weedy treatment, which recorded the lowest averages of 69.00 cm, 217.30 g m⁻², and 234.67 tiller m⁻², respectively. The superiority of Tarzec herbicide at both application rates may be due to its role in reducing the number of weeds and their dry weights after 90 days of application, allowing the crop plants to grow and utilizing optimally the surrounding conditions, leading to an increase in the height of the plants and the number of their tillers, and thus increasing their dry weights, which in turn, is positively reflected in improving the crop plant interception for light and enhancing the absorption of water and nutrients, and thus boosting the growth traits mentioned above. This result is consistent with each of [9, 11, 12, 14, 21] referring to the effective role of herbicides in improving some growth traits of wheat crop.

The interaction effect was insignificant only in the trait of plant height (cm), while application @ 90 g h⁻¹ at the three periods after planting (3, 5, and 7 weeks), achieved the best averages for the traits of plant dry weight and number of tillers of wheat. However, the best averages were at the period of 5 weeks from planting, reaching 379.50 g m⁻² and number of tillers reaching 35.67 tiller m⁻², with an insignificant difference from the application @ 75 g h⁻¹ in the number of tillers, with an average of 330.33 tiller m⁻². It is noticed that the positive behavior of the two application rates

differs with the application period after planting compared to the weedy treatment, which recorded the lowest averages at the same application period.

Table 2. Effect of different treatments in some weed traits and some growth traits of wheat crop.

Traits / Treatments	Number of weeds m ⁻² after 90 days of Application	Control % after 90 days of Application	Weeds dry weight g.m ⁻²	Inhibition %	Plant height (cm)	dry weight of wheat plants (gm m ⁻²)	Number of tillers m ⁻²
A1	31.00	73.28	115.08	72.61	78.34	300.00	305.17
A2	29.17	75.00	106.22	75.00	79.62	318.90	310.92
A3	33.17	73.25	120.17	72.30	77.68	280.70	297.08
L.S.D 0.05	N.S	0.856	4.009	0.406	N.S	11.70	3.735
H1	3.11	97.41	15.27	96.42	74.88	271.00	313.11
H2	2.44	97.97	13.64	96.80	81.77	315.40	325.89
Weedy	118.89	0.00	426.38	0.00	69.00	217.30	234.67
Weedfree	0.00	100.00	0.00	100.00	88.55	395.80	343.89
L.S.D 0.05	1.648	0.428	1.856	0.220	4.028	13.23	5.522
H1	4.67	95.99	21.66	94.843	77.50	284.70	315.33
H2	3.33	97.13	18.37	95.623	79.55	300.30	324.00
A1 Weedy	116.00	0.00	420.30	0.000	68.76	221.20	235.33
weedfree	0.00	100.00	0.00	100.00	87.55	393.90	346.00
H1	0.00	100.00	0.00	100.00	75.24	282.50	330.33
H2	0.00	100.00	0.00	100.00	84.44	379.50	335.67
A2 Weedy	116.67	0.00	424.87	0.000	69.15	212.00	234.67
weedfree	0.00	100.00	0.00	100.00	89.66	401.50	343.00
H1	4.67	96.25	24.17	94.427	71.89	245.70	293.67
H2	4.00	96.78	22.54	94.803	81.31	266.30	318.00
A3 Weedy	124.00	0.00	433.97	0.000	69.08	218.60	234.00
weedfree	0.00	100.00	0.00	100.00	88.44	392.20	342.67
L.S.D 0.05	3.566	0.938	4.281	0.460	N.S	21.46	8.660

*A1: Herbicide application @ 3 weeks after planting

A2: Herbicide application @ 5 weeks after planting

A3: Herbicide application @ 7 weeks after planting

H1: Tarzac herbicide @ 75 g h⁻¹

H2: Tarzac herbicide @ 90 g h⁻¹

Conclusion

We conclude that using the herbicide Tarzac @ 75 and 90 g ha⁻¹ after 5 weeks of planting reduced the number of weeds accompanying the wheat crop after 90 days of application and decreased their dry weights, consequently causing a significant increase in the dry weight of wheat plants, the number of their tillers m⁻², and the height of their plants, compared to the two periods of 3 and 7 weeks after planting and to the weedy treatment that recorded the lowest averages of the traits for the same periods.

References

- [1] Al-Chalabi, F.T.2003. Biological response of wheat to weeds control with Diclofop-methyl herbicide sequentially with 2,4-D and its effect on grain yield. Iraqi Journal of Agricultural Sciences .34(1):89-100.
- [2] AL-Kafaji, M. J., S.M. A .Safi, & W. F. Hammood .2023 .Effect of herbicides on growth , grain yield and quality of Barley. Iraqi Journal of Agricultural Sciences. 54(4):1094-110.

- [3] Hammood, W.F. , Al-Khaldy, R.A.A., Safi, S.M.A. , Al-Azawi, N.M. 2023. Impact of some Herbicides on Growth Indicators of Some Wheat Cultivars and Weight of Companion weeds. IOP Conf. Series: Earth and Environmental Science. , 1158(6), 062026.
- [4] Heather , E.M; A Navabi ;B.I.Erick; T. Onavan and D.M. Spancer .2007. The Weedcompetitive of Canada western spring cultivars grown organic management. Crop Science. 47: 1167-1176 .
- [5] Al-Jubouri, B. A.K. 2002. Weed Science. Ministry of Higher Education and Scientific Research. Baghdad. Iraq. 320 pp.
- [6] Fay, P. K. and W. B. Duke. 1977. An assessment of allelopathic potential in Avena germ plasm .Weed Sci. 25:224–228.
- [7] Liebl ,R. and A.D. Worsham.1987. Interferon of Italin ryegrass (*Lolium multiflorum* L.) in wheat(*Triticum aestivum* L.) Weed Science .35(6):819-823.
- [8] Steven, Z.k .2010. Use of Herbicide -Tolerant Crops as part of an Integrated Weed Management Program. University of Nebraska –Lincoln Extension ,Institute of Agriculture and Natural Resources .Neb Guide. G 1484.
- [9] Said, I. A and D. M. A. Jaff. 2020. Evaluation of Chevalier WG and Atlantis OD herbicides to control weeds in winter wheat fields. Iraqi Journal of Agricultural Sciences. 51(Special Issue): 96-100.
- [10] EL-Taif, M. R. A . 2020. Effect of competitive ability of soft wheat varieties (*Triticum aestivum* L.) and chemical control of weeds/weeds accompanying wheat crop. Journal of Plant Protection Arabic. 38(4): 354-359.
- [11] Safi, S.M.A, R.A.A ,Al-Khaldy,. and W.F. Hammood . 2022. Evaluation of the efficiency of some herbicides in controlling weeds in wheat (*Triticum aestivum* L.). Research on Crops., 23(1): 21–25.
- [12] Khudur , A. S, T. Y.M. Al-Edany & L. A. Bnayan .2019. Evaluation of some Herbicides' Efficacy in Weed Control Accompanying some Wheat Cultivars and Their Effect on Yield and Its Components. Basrah Journal of Agriculture Science , 32(Spec. Issue 2):140-155.
- [13] Singh, D. P., R. S. Singh., R. Niwas., A. Kumar., B. N. Singh and G. Singh. 2020. Effect of Different Weed Management Practices on Weed Flora in Wheat (*Triticum aestivum* L.). International Journal of Current Microbiology and Applied Sciences .Special Issue-11: 464-471.
- [14] Al-Khaz'ali, A. J., N. A. Mutllag., M. N. Kadum and K. A. Salman. 2021. Assessment of the efficacy and impact of certain herbicides in the control of bread wheat narrow leaf weeds (*Triticum aestivum* L.) on yield components. international journal of agricultural and statistical sciences. 17(1): 1765-1770.
- [15] Mohammed, A.T. , Shati R.K. and Ali A.J. 2016. Evaluate the effectiveness of the weed Herbicide Atlantis WG for some varieties of wheat which be approved in Iraq and associated weed and its impact on the economic quotient. Anbar Journal of Agricultural Sciences, 41 (4):183-194.
- [16] Bari, A., M. S. Baloch., A. N. Shah., A. A. Khakwani., I. Hussain., J. Iqbal., A. Ali and M. Bukhari. 2020. Application of various herbicides on controlling large and narrow leaf weeds and their effects on physiological and agronomic traits of wheat. Planta daninha. 38(1):1-12.
- [17] Shivran, A. C., Sarita., J. Choudhary and J. S. Bamboriya. 2020. Effect of Different Herbicides on Growth and Yield of Wheat (*Triticum aestivum* L.). **International Journal of Current Microbiolog and Applied Sciences**, 9(4): 438-448.
- [18] Zahan, T., M.R. Islam., M.A. Hossain., M.F. Hossain., Q. Naher., S. Ishtiaque and M.A. Ali. 2020. Post -emergence weed control -of strip-planted wheat by herbicides. Bangladesh Agronomy Journal . 23(1): 91-99.
- [19] EL- Delfi ,M. R. B. 2023. Testing of Efficiency of some Herbicides and their impact on growth, yield and accompanied weeds in wheat. Master's Thesis, College of Agricultural Engineering Sciences, University of Baghdad, Iraq. P.60 .
- [20] El-Delfi, M. R. and S.M.A. Safi. 2023. Evaluating newer herbicides on the performance of wheat (*Triticum aestivum* L.) in Central Iraq . Research on Crops, 24(1): 8–14.

- [21] Hammood , W. F., .S.M. A .Safi, & M. J.AL-Kafaji .2024. Effect of Newer Herbicide in Controlling Weeds Associated with Bread Wheat. IOP Conf. Series: Earth and Environmental Science 1371 (2024) 052002. Doi:10.1088/1755-1315/1371/5/0520021.
- [22] Jadoua, K. A. and H. M. Saleh. 2012. Fertilization of wheat crop. Guidance Bulletin No. 2, Ministry of Agriculture, National Program for the Development of Wheat Cultivation in Iraq. Issue No. 11.
- [23] Specimen Label Revised 12-14-20. 2024. Tarzec Herbicide .COR_ Tarzec_121420 SpecLbl.indd. https://s3-us-west-1.amazonaws.com/agrian-cg-fs1-production/pdfs/Tarzec_Label1.pdf
- [24] Al-Khaldy, R.A.A., Hammood, W.F., Safi, S.M.A. 2022. Effects of chemical herbicides and Datura leaves extract on the companion weed of two barley cultivars (*Hordeum vulgare* L.), the yield and its components. Caspian Journal of Environmental Sciences, 20(2): 351–357.
- [25] Al-Chalabi, F.T .1988. Biological interaction between growth regulating substances and herbicides in weed control. Ph.D. Thesis, Uni. of Wales, U.K. PP. 204.
- [26] Ciba–Giegy Agrochemicals Division . 1975. Field Trial Manual. Ciba-Geigy, S.A., Basle, Switzerland.
- [27] Pedro, A., R. Savin, M.A.J. Parry, and G.A. Slafer. 2012. Selection for high grain number per unit stem length through four generations from mutants in a durum wheat population to increase yields of individual plants and crops. Field Crops Research. 129: 59–70.
- [28] Steel , R.G.D and J.H. Torrie .1980. Principles and Procedures of Statistics.2nd ed., McGraw-Hill Book Co., Inc., New York , PP: 485 .
- [29] Hammood, W.F., Al-Khafaji, M.J., Safi, S.M.A.2022. The effect of some herbicides on the companion weed to three cultivars of oats. International Journal of Agricultural and Statistical Sciences, 16 : 1527–1532.
- [30] Nemr, R.H., and , W.F. Hammood. 2023. Effect of Quizalofop-p-Tefuryl herbicide in combination with Johnson grass extract on weed control in flax crop (*Linum usitatissimum* L.).Research on Crops, 24(1): 89-98.
- [31] Safi, S.M.A., , W.F. Hammood, and R.A.A Al-Khaldy,. 2023. Response of Barley Cultivars to Weed Control with Herbicides and Datura Leaf Extract and Its Reflection on Growth Indicators .IOP Conference Series: Earth and Environmental Science,, 1158(6), 062018.
- [32] Al-Jourani ,A. Y. A. A. and W. F. Hammood.2024. Effect of some Elements of Integrated Weed Management on the Yield and Its Component of the Sunflower. IOP Conf. Series: Earth and Environmental Science .1371 (2024) 052016. Doi:10.1088/1755-1315/1371/5/052016 .

Evaluation of Small Agricultural Projects Provided by the Iraqi Institute for Development (IID) to the People of Bashiqa District / Nineveh Governorate / Iraq

Abdul Aziz Hameed Midhas¹, Hafsa Fattah Hadi² and Amina Zuhair Younis³

¹⁻³Department of Agricultural Extension and Technology Transfer, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq.

¹E-mail: Abdalazizhm@uomosul.edu.iq

²E-mail: hfshalnzy48@uomosul.edu.iq

³E-mail: aminazuhair@uomosul.edu.iq

Abstract. The study aimed to identify the institution's work in terms of providing services in general, identify its source of funding and its partners, identify the beneficiaries in the Bashiqa district of the services of this institution and identify the activities and services provided to them by it, what is the economic benefit they obtained from this Projects: The study included a case study of the Iraqi Foundation for Development in Bashiqa District / Nineveh Governorate. A questionnaire form was used for information about the institution. The results of the study were that the institution was rated good for providing projects and conducting development courses for beneficiaries in Bashiqa District / Nineveh Governorate used a questionnaire form for information about the institution, and the results of the study were that the institution was rated good for providing projects and conducting development courses for beneficiaries in the Bashiqa district / Nineveh Governorate, and it served the people to the fullest extent. The study concluded some conclusions, the most important of which is that the institution is an institution of Iraqi origin. Its work is subject to Iraqi laws. The number of beneficiaries of the organization's services from the people of Bashiqa district was (300) beneficiaries, males and females, at a rate of (50%) for each of them The opportunity for females in the work and data of the institution was the same as the opportunity for men, and there was no difference between them, males and females. Sectarianism and racism were rejected by the institution and its employees in their dealings with the beneficiaries and trainers. They were chosen from all spectrums of the people. Workers from all spectrums, races, and religions worked in it with all objectivity, science, and impartiality with the beneficiaries. It ran its courses. Training from the best trainers specialized in course topics from the University of Mosul, the Northern Technical University and other reputable universities. The study recommended several recommendations, the most important of which was that the Iraqi government should encourage organizations and institutions that work to serve society in Iraq and facilitate their tasks Giving sufficient time for civil society organizations to work in Iraq to finance projects and improve the level of services and develop the country's infrastructure. Ensuring the integrity and dependency of some organizations that enter Iraq. Conducting research and studies on the organizations' work and evaluating them by researchers from time to time. Increasing the number of beneficiaries of bids. Civil society organizations: Iraqi universities must provide civil society organizations with specialized professors who have solid information in their specializations Involving all sects, races, and religions in benefiting



from civil society organizations. Working to run courses from the best trainers specialized in course topics from reputable universities in Iraq. Universities allowing their members to work in civil society organizations to provide them with solid academic information. Organizations must work in all governorates, Iraq from north to south.

Keywords. Small agricultural projects, Bashiqa district, Agricultural extension.

1. Introduction

Government support is the main driver for civil society organizations in all their specializations and projects. These small projects, especially agricultural ones, and especially in the seventies and eighties of the twentieth century, were very great in interest, which was reflected in an increase in agricultural production in a positive way, as well as employing a large number of farmers and the unemployed. Iraq has tens of thousands of small and medium-sized agricultural projects, represented by the establishment of a large number of poultry fields and animal husbandry projects for cows, sheep, and fisheries, but agricultural production quickly deteriorated during the nineties of the last century due to the cessation of government support as a result of the economic blockade [1]. It became a significant role for civil society organizations, regardless of their specializations, to fill the service gap among rural people in particular. It was of great importance during the limited role of services due to the absence of the government role and its abandonment of many services and basic needs that are considered among the basics and necessities of life, until the role of organizations emerged to carry out. With this role and in all areas of knowledge, material, and services, especially in recent times, Iraq has witnessed a widespread demand for the work of organizations, to provide small projects to raise the level of poverty on the shoulders of the lower class of society members, who are described as destitute. These small projects, especially agricultural ones, were an urgent necessity to serve farmers and employ the unemployed. Work and its importance in increasing production on a limited basis at the level of one governorate or some of them [2]. Recently, interest in rural areas has increased in development programs and projects, because most of the natural resources are concentrated there, which led to organizations specialized in agricultural projects seeking to work in the countryside and provide services to them, but the state has resumed its support for small and medium enterprises after signing the memorandum of understanding with the United Nations. The United States financed it mainly through the Agricultural Bank, and as soon as it recovered, it relapsed again after the American occupation in 2003, which led to the cessation of thousands of small and medium-sized agricultural projects. After that, the dark era of ISIS came until the projects were stopped by the government and organizations, especially in Nineveh, Salah al-Din, and Anbar provinces. After the announcement of the elimination of these gangs, Iraq returned to health, especially Nineveh Governorate, which encouraged the return of civil society organizations to resume their work in supporting the people of the aforementioned governorates [3], Nineveh Governorate was not isolated from these projects, whether small or medium, especially agricultural ones, as it received many services provided by local civil society organizations and foreign organizations [4]. Bashiqa district was an essential pillar of support for these organizations and institutions, and it was the largest support at all levels, especially agricultural ones, which was the subject and subject of the study as it is the specialty of the researchers, and from here came the idea of this study to evaluate one of these organizations or institutions that worked in Nineveh Governorate and in Bashiqa District, so the choice was on the Iraqi Development Foundation, which provided its services to the people of Bashiqa District in Nineveh Governorate, where It provided many services and projects in this area of Nineveh

Governorate, for the purpose of reaching the facts and desired goals of the work of this institution, and knowing whether it provided its services in the desired manner?, and how many were its achievements?, and who are the beneficiaries?, and what is the extent of benefit from the projects presented to them? All these questions came for the purpose of achieving the objectives of the study, which were as follows:

1.1. Objectives of the Study

- The first goal: Evaluating the work of the Iraqi Development Corporation and its services provided to the people of Bashiqa district.
- The second goal: Identifying the training courses held by the organization in the Bashiqa district.
- The third goal: Identifying the small projects provided by the Iraqi Foundation for the people of Bashiqa district.
- The fourth goal: Problems faced the organization's work in the Bashiqa district.

1.2. The Importance of the Study

The importance of the study lies in the presence of civil society organizations, and their effective role in bringing about a change in the lives of the beneficiaries in their lifestyle and helping them change their living conditions, and civil society organizations have a major role in providing services that have a direct impact on people's lives, and the institution Al-Iraqiya is one of the institutions that provided great services to the people of Bashiqa district, and people benefited from it in a positive way [3]. Therefore, this study came to learn about the tasks of this institution, study its condition, evaluate its tasks provided to the citizens of Bashiqa, and come up with important recommendations for the stakeholders in this study.

1.3. Procedural Definitions

- The Iraqi Institution for Development (IID): is an Iraqi agricultural institution that has been operating in the Bashiqa district of Nineveh Governorate for 3 years, providing services, training courses, and financing small agricultural projects [2].
- Small projects: These are agricultural projects provided to the people of the Bashiqa region. These are agricultural and animal projects, and their cost does not exceed two million Iraqi dinars.

1.4. The Theoretical Framework of Study

1.4.1. The Concept of Small Projects

There is no agreement among researchers, scholars, and specialists about determining a specific definition for small projects. This is due to the difference in standards approved by the competent authorities, as well as the difference in economic circumstances and situations between one country and another, the degree of economic progress, and the extent of progress in the use of technology in industry. Therefore, the concept of small projects is considered one of the concepts Relative, not absolute [5], and among the criteria that can be relied upon to determine the concept of small projects (production standard, labor standard, volume and quality of energy used standard, capital standard [6]. In Iraq, small projects are defined as an establishment that is independent in management and ownership and has a share in the market. It is a company with a family connection. The president is at the top and is the owner of the project in partnership with friends and relatives who work for its success. Management is informal and directives are usually verbal, without the need for documentation.

- It can be established easily because it does not require large.
- The flexibility of its means of production and the speed of its response and adaptation to the changes and difficulties it faces during economic crises.
- Activating productive, manufacturing and marketing investment and exploiting available raw materials to produce goods that contribute to the tastes and needs of consumers, in addition to filling the local market's need for these goods, which reduces imports from abroad.
- These projects represent an important source for many large industries, which leads to expanding the local production base.
- Small projects contribute to reducing the unemployment rate and raising the standard of living for groups characterized by low educational and professional levels, dahesh, without mentioning years (6-7).
- It has a prominent role for women by empowering them and increasing their self-confidence, whether they work as owners of the project or workers in it [8].
- Decrease in the volume of investment in small projects.
- Easy to manage
- Reduced costs and potential losses resulting from fluctuations in prices, climate, diseases and pests that it these projects [9].

1.4.2. Establishing a Successful Small Agricultural Project

- Choosing a modern agricultural project that is compatible with current market requirements.
- Identifying the bodies and entities concerned with supporting small projects as charitable support or encouraging small agricultural projects
- Develop project plans and work strategies to clarify the stages that the project must go through to achieve its goals.
- Conducting an economic feasibility study for the project in all its aspects
- Identify the banks and financing institutions that provide loans and financial facilities to establish the project.
- The project owner must complete all legal papers and procedures related to establishing a small agricultural project and ensure that they are certified by the departments concerned with them.
- The project owner seeks the help of an experienced person in this field, a specialist, or an expert, which saves a lot of time and money [10].

1.4.3. Fields of Work of Civil Society Organizations

- The field of human rights: establishing specialized legal services to follow up on detainees' issues, defending workers' rights, participating in international campaigns against human rights violations, and preparing regular bulletins about violations etc...
- The field of economic development: developing or establishing small projects to reduce unemployment, raise the standard of living of poor families, provide agricultural loans, and establish centers for preserving livestock and agricultural wealth..
- The field of environmental conservation: reducing pollution, publishing agricultural publications, environmental education activities, developing small agricultural projects, developing modern agricultural models, and solving agricultural marketing problems..

- The field of youth and sports: such as establishing sports clubs, local sports committees, and sports federations that participate in international and Arab sports forums, and preparing young leaders by providing opportunities for qualification and training.
- The field of women and children: Preparing programs related to the rights of women and children, which also include women in communities, villages, and poor neighborhoods
- The field of health: It provides preventive and curative services, rehabilitation programs for the disabled, the establishment of physical therapy centers, awareness programs on the dangers of drugs, and mental health programs.
- The field of rehabilitation: holding vocational qualification courses, strengthening institutional capabilities, establishing kindergartens, establishing schools to teach arts to children, implementing institutional building and family planning programmes, training volunteers to eradicate illiteracy, preparing programs to educate mothers and train them on how to deal with their children and society as a whole, and prepare them for the labor market..... Mobile: The role of civil society organizations in reducing unemployment [11].

1.4.4. The Relation of Agricultural Civil Society Organizations with Agricultural Extension

Agricultural extension is an essential element of every agricultural system and plays a role in achieving agricultural and rural development [12], by exploiting all the natural and human resources and capabilities in the countryside, educating farmers, developing their abilities and skills, and changing their attitudes and way of thinking so that they can benefit from agricultural technological progress. Thus raising their standard of living and improving the standard of their communities [13]. Civil society organizations in general and organizations that provide agricultural extension services in particular play a very important role in improving the reality of rural society. The importance of this role is increasing, especially with the limited role played by government agricultural extension institutions. Its inability to perform all tasks and meet all basic needs [14], which necessitates the need for governmental agricultural extension institutions to work side by side with agricultural and non-agricultural non-governmental organizations and bodies. The matter also requires coordination and cooperation between the agricultural extension apparatus and these institutions. Development agencies to unify efforts, avoid repetition and preserve human and economic resources [8].

1.5. The Following are Proposals to Improve Agricultural Extension Services Provided by Civil Society Organizations in the Field of Agriculture

- Providing production requirements for farmers at reasonable prices
- Providing services on an ongoing basis
- Paying attention to the problems and needs of farmers
- Create extension fields.
- Working to provide modern irrigation methods for farmers.
- Increasing the number of associations working in the agricultural field.
- Providing markets to sell the crop at reasonable prices.
- There is communication and cooperation between these associations and the agricultural extension worker [15].
- Employees take into account ethical behavior when dealing with counselors.
- Providing counseling services to all counselors without discrimination.
- Providing good guidance services, regardless of the fact that they are free,
- Employees' sense of responsibility towards extension work.

- Workers' sense of responsibility towards rural natural resources.
- Providing extension services to small farmers and the destitute.
- Workers' sense of responsibility towards preserving the environment from pollution [16].
- Providing these associations with specialists in all agricultural fields.

1.6. Definition of the Iraqi Development Foundation (IID)

It is a civil society organization that works in Iraq in several governorates Baghdad, Erbil, Nineveh [17], and each of its branches in Nineveh Governorate has (10) employees working in the institution. Work began in Nineveh Governorate in Bashiqa district on 1/11/2021 and its work will end on 6/1 / 2025, meaning its work in Nineveh Governorate will continue for (3.5) years. The institution works in partnership with the International Rescue Committee (IRC) and is funded by the German Federal Ministry for Economic Cooperation and Development (BMZ). The institution has two types of courses, which are:

1.6.1. Agricultural Project Management Course (General) for Developing Agricultural Businesses

The Foundation holds (10) courses of this type per year. These courses are similar but for different beneficiaries, as each course consists of 15 people (50% females, 50% males). Their ages range between (18-30) years, and no academic qualifications are required for their acceptance. Each course lasts for (13) days, at a rate of (3) hours per day, and after the end of each course, questionnaire forms are distributed to the trainees, to evaluate the trainer, the scientific material, and recommendations. In addition to subjecting the trainees to a pre- and post-test, to estimate the extent to which they benefited from the course, where the results of the pre-test for the trainees for this course ranged between (20-40%), while the percentage of the post-test ranged between (60-80%), meaning that the knowledge gap was in percentage between (40%), and after the trainees are tested by the Supreme Committee from (Erbil and Baghdad), they select (7) people from the trainees only, to give them a financial grant ranging from (1,700,000) one million seven hundred thousand Iraqi dinars, to 2,250,000 two hundred and fifty thousand Iraqi dinars, according to Type of project to finance their small agricultural projects, where the beneficiary undertakes the implementation of his agricultural project, with all its expenses, while the institution follows up his project and estimates the economic return generated by the project, provided to the beneficiary by the institution.

1.6.2. Education in an Agricultural Profession or Craft a Development Course

It will be opened for the beneficiaries on the topic of their project, which will last (30) days. The beneficiaries will be divided into (5) groups, each group includes (10) trainees, at a rate of (50) people per course, and each group has a special trainer to train them on the specific topic of the course. The subject of the course consists of two aspects (practical and theoretical). This type of training does not grant the trainees financial grants, only transportation fees (10) thousand Iraqi dinars per day. Every period, the relevant authorities in the institution meet to develop a work plan that is binding on the employees working for it, and implement it within the agreed upon period. It has to do so, even if it requires employees to stay for hours outside their designated daily working hours.

1.7. Previous Studies

- Study [14], The role of civil society organizations in providing health services, a case study of the Kirkuk Castle Organization for Orphan Care.

- Study [15], on the quality of the extension organization and its impact on the quality of agricultural extension service in Assiut Governorate.
- Study [16], on the importance of government support in developing small and medium agricultural enterprises in Iraq

2. Research Methodology

The descriptive approach was used, which accurately describes the situation. Among the branches of the descriptive approach is the study of Hilla. In this research, the case study of the Iraqi Development Foundation was used to determine its conditions, services, and treatment of the people in the Bashiqa district.

2.1. Data Collection

Data was collected on the status of the institution, through the institution's records, for a period of three years of work in the Bashiqa district

3. Results and Discussion

3.1. The First Goal: Evaluation the Work and Services Provided to the People of Bashiqa District by the Iraqi Development Foundation

The results of the study showed that the work completed by the Iraqi Development Foundation in Bashiqa district It was as in Table (1).

Table 1. Shows the services provided by the Iraqi Development Foundation in Bashiqa district.

Categories	Years	Beneficiaries				Economic returns
		Female		male		
		Number	%	number	%	
financing agricultural projects	2021	57	38	42	28	17500000
Agricultural training courses	2022	48	32	53	35	112000000
Opening small agricultural projects (animal and plant)	2023	45	30	55	37	20500000
Total		150	%100	150	%100	50000000

Table (1) shows that the largest percentage of beneficiaries are males in small projects (37%), and the economic benefits were (20.5) million dinars in the year 2023. The next percentage is (38%), which is the percentage of female beneficiaries. Of financial amounts. As for training courses, the highest percentage of beneficiaries were males (35%). This means that males were the beneficiaries more than females, and this may be attributed to the fact that males are more active than females at work, so the evaluation of the institution was good and it provided great services to the people of Bashiqa district.

3.2. The Second Goal: Identifying the Training Courses Held by the Foundation in the Bashiqa District

The study showed that the training courses held by the Iraqi Development Foundation in Bashiqa district are of three types (animal husbandry, beekeeping, crop cultivation), as shown in the following table (2).

Table 2. Shows the training courses provided by the Iraqi Development Foundation in Bashiqa district.

Training courses	Participants	Years

	Number	Female		Male		
		Number	%	number	%	
Training course in animal husbandry	10	33	22	52	35	2021
A training course in beekeeping	10	48	32	55	37	2022
Training course in crop cultivation	10	62	41	50	33	2023
Total	30		150		150	

Table (2) shows that the number of training courses in each year was (10), so the total number of courses was (30) in three years. The highest percentage of trainees were females in the crop cultivation course, where their percentage reached (41%). The next percentage is males in the beekeeping course, where their percentage was (37%). This may be attributed to the fact that females are more willing to grow crops at home, and males are more involved in beekeeping and hobby.

3.3. The Third Goal: Identifying the Small Projects Provided by the Iraqi Foundation for the People of Bashiqa District

The study showed that the small projects provided by the Iraqi Development Foundation to the people of Bashiqa District are (70) small projects of seven types, which are projects (Cow breeding, sheep breeding, Goat breeding, poultry breeding, bees breeding, Cultivation of tarouz and olive pressing), and each type has (10) projects, and the number of beneficiaries reached Male and female (300 male and female beneficiary), as shown in Table No (2).

Table 3. Shows the types of small projects presented in Bashiqa district by the Iraqi Development Foundation.

Types of project	Number	Participants			
		Male		Female	
		number	%	Number	%
Cow breeding	10	14	9	32	21
sheep breeding	10	22	14	12	8
Goat breeding	10	25	17	16	11
Poultry breeding	10	22	15	18	12
Beekeeping	10	31	20	21	14
Cultivation of tarouz	10	28	18	18	12
Olive pressing	10	8	5	33	22
Total	70	150	100%	150	100%

Table (3) shows that the beneficiaries of small projects are (300), half of whom are male and the other half are female, and they entered training courses in (livestock, bees, and other small agricultural projects) for the three years (2021,2022,2023) and the highest percentage was (21%) in the cowbreeding project for females, followed by the second percentage (20%) in the bee-keeping project for men. This may be due to the fact that the cow-breeding project concerns women more than it does men, while the bee-keeping project concerns men more than it does women.

3.4. Fourth Objective: Obstacles Facing the Organization's work in Bashiqa District

- The trainees' educational level is very low, as it does not exceed the primary level (as their educational level ranges between (illiterate and primary)).
- Lack of agricultural information for trainees
- Trainees are sometimes chosen from those with special needs.
- The lack of trainers who specialize in teaching people with special needs.

- Embarrassment towards employees by trainees who did not receive small projects who were selected by the higher committees.
- Some people receive grants and do not implement the project.
- Beneficiaries buy unhealthy animals from the market, which leads to their death and the failure of the project.
- Purchasing unsound crop seeds, which led to the loss of the project.
- Lack of management by the beneficiaries of their project, which led to a decrease in yield and profit.

Conclusions

The institution is an Iraqi institution whose work is subject to Iraqi laws

- Work began in Nineveh Governorate in Bashiqa district on 1/11/2021, and its work will end on 6/1/2025, meaning its work in Nineveh Governorate will continue for (3.5) years.
- This institution was solid and greatly interested in serving the study area.
- The organization was evaluated as good because of its charitable work and training courses for the people of Bashiqa district.
- The number of beneficiaries of the Foundation's services from the people of Bashiqa district was (300) beneficiaries, male and female, at a rate of (50%) for each of them.
- The opportunity of females in the work and the institution's data was the same as that of men, and there was no difference for them, males and females.
- Renouncing sectarianism and racism by the institution and its employees in their dealings with beneficiaries and trainers, and they are chosen from all segments of the people.
- It employs people from all walks of life, races, and religions, and works objectively, scientifically, and impartially with the beneficiaries.
- Its training courses are run by the best trainers specialized in the course topics from the University of Mosul and the Technical University Northern and other solid universities.
- It operates in several Iraqi governorates, including (Baghdad, Erbil, and Nineveh).
- The organization works in partnership with the International Rescue Committee IRC.
- The Foundation is funded by the German Federal Ministry for Economic Cooperation and Development (BMZ).
- The organization works in partnership with the International Rescue Committee (IRC).
- It has two types of training courses (project management course for developing commercial and agricultural businesses and the education course in a profession or craft agricultura.
- Males benefited more than females due to their ability to work more than females, especially in projects that they specialize in, such as beekeeping And sheep farming..
- Females benefited more than males in projects related to females because of their ability to work on them, such as raising cows and pressing olives And others..
- The Foundation provided (70) small projects in (3) years of its work in Bashiqa, and most of the grantees benefited from financial returns from Their projects.
- One of the obstacles that hindered the Foundation's work is the lack of benefit from the project by the beneficiaries, and the disbursement of the funds granted to them For personal purposes other than the funded project.
- Beneficiaries purchased unsound animals and crop seeds, which led to the loss of the project.

Recommendations

- The Iraqi government must encourage organizations and institutions that work to serve society in Iraq and facilitate their tasks.
- Giving civil society organizations enough time to work in Iraq to finance projects, improve the level of services, and develop the country's infrastructure
- Ensuring the integrity and dependency of some organizations entering Iraq
- Increasing the number of beneficiaries of civil society organizations' tenders
- Iraqi universities should provide civil society organizations with specialized professors who possess solid information in their specializations.
- All civil society organizations must follow the example of the Iraqi Development Foundation in raising the spirit of tolerance and rejecting Ta'ism and justice among the people of The people.
- Involving all sects, races and religions in benefiting from civil society organizations.
- The best trainers specialized in course topics from reputable universities in Iraq work on running the courses.
- Universities allow their affiliates to work in civil society organizations to provide them with solid academic information.
- Organizations must work in all governorates of Iraq from north to south.
- Opening courses by organizations on modern and useful topics similar to developed countries.
- Involving both genders in the work of organizations, each within their specialty.
- Accommodating unemployed graduates with bachelor's and higher degrees and providing them with the opportunity to express their knowledge And their skills, each according to his specialty, to serve his country.
- Conducting projects in all service and educational fields, funded by organizations.
- Establishing scientific partnerships and cooperation between universities and organizations.
- Avoid fraud and manipulation of the beneficiaries of the submitted projects and deal with the funded organizations with all credibility and honesty.
- The security authorities must protect civil society organizations so that they can present their projects to beneficiaries in the country.
- The government should fund, encourage, and honor organizations that worked diligently, credibly, smoothly, and objectively in Iraq.

References

- [1] Ahmed, Abbas Ali (2014) Vegetable growers' attitudes towards some sustainable agricultural practices in the Nimrud district of Nineveh Governorate and their relationship to some variables, Al-Rafidain Agriculture Journal, Volume (42),No. (1).
- [2] Ajwad, Sari Alaa and Khaled Hussein Al-Marzouk (2022) The role of specialized banks in supporting small and medium enterprises in Babylon Governorate for the period (2005-2020) The Agricultural Bank as a Model, Journal of the College of Management and Economics for Economic, Administrative and Financial Studies, Volume (14), NO (3), 36 -54
- [3] Akkawi, Omar Mahmoud, Nadia Mahdi Abdel Qader and Alia Hussein Khalaf (2019) Financing small and medium enterprises and their role in achieving economic development, Dinar Magazine, NO(17)
- [4] Ali, Manal Fahmy Ibrahim and Asmaa Muhammad Abdel Samad Mandour Badawi (2022) The cooperative relations of the agricultural extension organization with rural organizations in Kafr El-Sheikh Governorate, Journal of Sustainable Agricultural Sciences, Volume (48), NO (2).

- [5] Al-Aktari, Youssef Maher Youssef (2017) The role of civil society organizations in reducing unemployment among university graduates (Master's thesis), Academic Administrative Leadership, administration and Politics, Al-Aqsa University.
- [6] Al-Awad, Ayman Abdel-Ghani Hamza (2023) The role of small projects in determining the severity of poverty and unemployment in Sudan for the period from 2014-2020 AD, an applied study (the case of the Ebdaa Microfinance Bank), Anbar University Journal of Economic and Administrative Sciences, Volume (15), Issue (2).
- [7] Al-Dujaili, Jaafar Baqir (2021) The role of financing small agricultural projects in increasing production and reducing the phenomenon of unemployment in Iraq for the period from 1985-2015, Journal of Management and Economics, College of Management and Economics, Baghdad, Iraq, Issue (130), pp. 120-129.
- [8] Shirwan, Jalil Ahmed and Hoshamhand Rafiq Ibrahim (2022) Towards directing agricultural graduates to small agricultural projects, Journal of Historical and Cultural Studies, Volume (13), Issue (56).
- [9] Dahesh, Fadel Jawad (without year) The importance of agricultural loans for small projects in visiting agricultural production in Iraq.
- [10] FAO /UNESCO (2003) Education for rural development, Roma, Italy.
- [11] Fathi, Arwa Moayed (2013) The level of knowledge of employees in the Nineveh Agriculture Directorate regarding the foundations of selecting and implementing agricultural extension methods, Al-Rafidain Agriculture Journal, Volume (41), N0 (4).
- [12] Food and Agriculture Organization of the United Nations (2022) Country Assessment by Gender in Agriculture and Rural Sector: Egypt, Gender Assessment Group I, East Jordan and North Africa.
- [13] Haneen, Samia Hanna, Hanaa Muhammad Hawari, and Salwa Muhammad Abdel-Gawad (2018) The quality of agricultural extension service provided by civil society organizations working in the agricultural field in some villages of Fayoum Governorate, Journal of the Scientific Society for Agricultural Extension
- [14] Rajab, Sawsan Ibrahim and Issam Salah al-Din al-Bayati (2022) The role of civil society organizations in providing health services, a case study of the Kirkuk Castle Organization for Orphan Care, Journal of Kut University College, a special issue of research at the fifteenth International Scientific Conference for Administrative and Economic Sciences (Towards Modern Trends and Advanced Management in... Building an economy that keeps pace with the times), for the period from (26-27) March, 583-593.
- [15] Abdel-Ghani, Mohamed Mohamed Mohamed, Omar Bahaa El-Din Ahmed El-Sherbasy and Asmaa Bakr Mohamed Bakr (2022) The quality of the extension organization and its impact on the quality of the agricultural extension service in Assiut Governorate, Journal of Agricultural Sciences, Assiut).
- [16] Anama, Salam, (2016) The importance of government support in developing small and medium agricultural enterprises in Iraq, Journal of the Baghdad University College of Economic Sciences - Baghdad, Issue (48), pp. (323-338) .

Precipitation Efficiency Index Indicator of Climate Changes for Growth *Quercus eagilops*. and *Pistacia khinjuk* Trees in the Dohuk Region

Shaymaa Dhayaa Al-Saygh¹ and Muzahim Saeed Al-Bek²

^{1,2}Department of Forestry, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq.

¹E-mail: Shaymaa_dhayaa@uomosul.edu.iq

²E-mail: Muzahim_saeed@uomosul.edu.iq

Abstract. Long-lived trees are one of the most important indicators in inferring the environmental factors that play a vital role in the growth and development of these trees. Therefore, random samples were taken in the sites of (Dohuk-Sarsank) for two types of trees (*Pistacia khinjuk* - *Quercus eagilops*) with two trees of *Pistacia khinjuk*, one in the Dohuk site and the other in the Sarsank site, as well as five *Quercus eagilops* in the Dohuk site and five others in the Sarsank site. From each of them, measurements were taken at the diameter at breast height(cm), total height (m), crown center height (m), crown width (m), as well as cross sections along the main stem of the tree until reaching the diameter at the diameter (7 cm). The Auto-CAT program was used in the computer to count the annual rings and measure their thickness (in mm), and rainfall data for the two sites. The data were analyzed, and a clear decrease in the rainfall rate was observed and the lack of Stability around the general average of these values and it was shown that there is a clear decrease in recent decades and this is what the standard deviation of rainfall indicated, and it was also shown that there is fluctuation in the relationship between rainfall and years, and according to the drought index (SPI) for the two study sites and it was shown from the estimated values of this index, that both sites do not provide the water needs of trees to grow optimally as (SPI) was between the highest and lowest (- 2.38230_ -2.39522) for the Dohuk site and also ((-2.37946_-2.39068) for the Sarsank site and these values indicate that these sites are dry sites and require higher humidity than that for trees to grow well and it showed that the values for the study years were negative, and that the Sarsank site gave higher (SPI) values than the Dohuk site and also less variation, meaning that the amount of fluctuation in Sarsank was low, which indicates somewhat an advantage in terms of tree growth than the Dohuk site Also, the Rainfall Efficiency Index (PEI) was estimated based on (SPI) for the two study sites for a period of time (1900-2022) AD, where we saw that the (PEI) values were low and fluctuating and indicated a significant decrease in rainfall, especially in the past two decades, where the drought period increased and the humidity decreased.

Keywords. Drought Index (SPI), Precipitation Efficiency Index (PEI), *Pistacia khinjuk*, *Quercus eagilops*.

1. Introduction

Rainfall is one of the environmental elements that control the growth and development of trees. This is evident in the spread of trees of different types according to their ecological requirements (IPCC 2007). Climate changes lead to differences in the growth and survival of trees and cause changes in the number of species spread and their density in the various locations where they grow. Tree growth has been particularly linked to fluctuating rainfall, which often causes a decrease in the growth of many plant species. Decreased rainfall leads to a decline in the growth of various plant covers, in addition to increased emissions of greenhouse gases emitted from the atmosphere resulting from various human activities that cause many environmental disturbances that occur naturally. Increases in temperatures on the Earth's surface lead to activity in plants in the event of moisture availability, and this rarely happens, especially in dry and semi-dry areas [1]. From the above, we note that climate elements play a fundamental role in the growth of living organisms, including woody trees, which we can see through the annual rings formed by these trees, as it was found that there is a relationship between the amount of rainfall and the thickness of the ring resulting in that period in which these trees grew. Many studies have indicated the relationship between rainfall and Rainfall in previous years with the growth of annual rings [2], and by knowing the growth that occurred in trees for past periods of time, we can read their previous growth by monitoring the growth that the main stem of the tree and taking cross-sections along the extensions of its main trunk and reading the cross-sections represented by the width of the annual rings of these sections, and this can be done by analyzing the annual rings of the sections taken from the tree, and from this we notice that we can use the Dendrochronology application on trees of large ages as it provides us with a long-term record of rainfall and various environmental factors that grew during the growth of that tree, so we can use the chronology of tree rings in the past and the environmental factors affecting their growth, as the Dendrochronology system provides the possibility of determining the time in which those rings grew and the amount of relative variation in the structure of those rings in different climatic seasons, so through these rings we can determine the environments in which they grew, whether they were wet or dry and the environmental variables affecting the growth of these trees (IPCC, 2007), and it has been shown that there is a relationship between tree growth and the index The drought index (SPI) and the rain efficiency index (PEI) from which we can predict the current and future environmental changes affecting the growth of these trees, especially rainfall. Therefore, choosing specific sites for the growth of long-lived woody trees and recording environmental variables is of utmost importance in knowing the climate records in the past in which these trees grew. Therefore, we took two types of forest trees with large ages and growing naturally in the (Dohuk - Sarsink) region, which is *Pistacia khinjuk* and *Quercus eagilops*, to study the drought index (SPI) and the rain efficiency index to predict the effect of rainfall on the growth of the study trees in the Dohuk and Sarsink sites.

2. Materials and Method

The study area is characterized by the climatic characteristics of the Mediterranean basin, which led to the spread of many species with special characteristics that can grow in dry and semi-dry areas [3]. The most important types of forest trees and shrubs spread in the study area are *Quercus eagilops*, *Pistacia khinjuk*, *Juniperus sp.*, *Crataegus sp.*, *Juglans sp.*, *Populus sp.*, *Salix sp.*, *Morus alba L.*, The coordinates of the site under study are at longitude (43 ° 4 '1.18") east and latitude (36 ° 50' 0.17") north (43 ° 18 '1.67") and (37 ° 1' 29.84") respectively, sites (Dohuk - Sarsink) are located within the mountainous geographical area represented by wide differences in slopes and elevations, which show very different geomorphology ranging between (0-87.9°), and their elevations range from (438) m in the flood plains to (1136) m in the mountainous area, while

the alluvial fans include a large area in the area under study, which are located at elevations ranging between (593.1 - 748.2) m [4], the climate of a site represents cases Long-term weather changes in meteorology, mainly represented by temperature and rainfall rates. The climate is affected by many other factors, the most important of which are topography, elevation above sea level, water bodies, and air currents [5]. Rainfall in arid and semi-arid regions is one of the most important natural factors affecting the growth, production, and plant distribution of various plant covers. Raindrops are formed from the evaporation of sea, river, and lake water due to heat. The hot air rises upwards carrying water vapor. When it reaches the upper layers of the atmosphere, its temperature decreases and begins to condense in the form of clouds. Raindrops start to form around the nuclei of condensation and accumulation of dust particles and others. The more condensation increases, the more water droplets increase, and they begin to stick together until their weight becomes heavy and they fall from the clouds to the Earth in the form of rain [6]. Therefore, I took the rainfall for the Dohuk and Sarsink sites for the years (1900-2022) AD. (Space source for rainfall by month and for a century from the year (1900-2022) <http://cru.uea.ac.uk> Climatic Research Unit – Groups and Centers for Sites (Sarsank-Dahuk). It was found that the maximum rainfall for the Dahuk region was (673.6) mm in the year (2018) AD, while we noticed a decrease in rainfall during the lowest period, which was (373.4) mm in the year (2021) AD. Also, the standard deviation in it has a high value (44.9102). This variance indicates fluctuations in rainfall for the site, rainfall for the Sarsink region was (907.8) mm in the year (2018) AD, while the decrease in rainfall at the lowest period was (787) mm in the year (2016) AD, and the standard deviation was (53.7352). The Standardized Precipitation Index (SPI) was calculated. SPI is based on measuring the extent to which actual rainfall differs from the expected normal rate in the area. SPI values are calculated statistically and can be positive or negative, with negative values indicating drought periods and positive values indicating flood periods. SPI is an important tool for analyzing climate phenomena such as drought and floods and helps in understanding the impacts of climate change on water resources, agriculture, and the environment. (SPI) can be used to identify potential drought periods and take the necessary measures to deal with them. It is also used in planning for water resources management, agriculture, and other sectors that depend on rainfall. (SPI) is estimated by converting rainfall values to a standard rainfall index to convert the average rainfall values to (zero) and convert the standard deviation of rainfall to (1). We convert the deviation in the data to (zero) and then achieve these goals. Then the rainfall index is standard and it is interpreted as an average of (zero) and a standard deviation of (1.0) [7]. The annual rainfall rate is calculated through the following relationship:

$$\text{Mean} = \bar{X}$$

$$S = \sqrt{(\sum(X - \bar{X})^2 / N)}$$

$$\text{Skew} = N / ((N-1)(N-2)) \times \sum_{i=1}^N ((X_i - \bar{X}) / S)^3$$

Then convert the rainfall to normal values and u-statistics, while the shape is calculated from the Gamma distribution constants.

$$\text{Log mean} = \ln(\bar{X})$$

$$u = \bar{X} \ln \left[- \left(\sum_{i=1}^N \ln \left(\frac{X_i}{\bar{X}} \right) \right) / N \right]$$

$$\beta = (1 + \sqrt{(1 + 44/3)}) / 44$$

$$\alpha = \bar{X} / \beta$$

The resulting constants are then used to find the cumulative probability observed during rainfall. The cumulative probability is given by the following relationship:

$$G(X) = \left(\int_0^X X^{(a-1)} e^{(-X)/B} dx \right) / \left(\beta^a \Gamma(a) \right)$$

The Gamma function (G) is unknown when the value of $X = 0$ and then the rainfall is (zero), and the cumulative probability becomes:

$$H(x) = q + (1-q)(G(x))$$

Then the possible q is (zero) and then the cumulative probability $H(x)$ is transformed into a standard normal variable (Z) with mean (zero) and variance (1). This is the SPI that was prepared by [8], (Bhagat et al. 2022) [9], and the standard transformation provided by [10] was used as an alternative to that.

$$Z = \text{SpI} = - \left(t - (c_0 + c_1 t + c_2 t^2) / (1 + d_1 t + d_2 t^2 + d_3 t^3) \right)$$

$$0 < H(x) \leq 0.5$$

$$Z = \text{SpI} = + \left(t - (c_0 + c_1 t + c_2 t^2) / (1 + d_1 t + d_2 t^2 + d_3 t^3) \right)$$

$$0.5 < H(x) < 1.0$$

$$t = \sqrt{\ln \left(\frac{1}{1 - (H(x))^2} \right)}$$

$$0 < H(x) \leq 0.5$$

$$t = \sqrt{\ln \left(\frac{1}{1 - (H(x))^2} \right)}$$

$$0.5 < H(x) < 1.0$$

$$C_0 = 2.515517, c_1 = 0.802583, c_2 = 0.010328, d_1 = 1.432788, d_2 = 0.182262, d_3 = 0.001308$$

The mathematical model constants for most of the (SPI) areas prepared by [8] are shown. The calculation of the standard index (standard) for rainfall (SPI) is presented for the area (Dohuk-Sarsink) for some time (1969-2007). The average rainfall (61.981) was modified to (SPI) (0.0111), the standard deviation (52.2187) to (0.99760), and the standard deviation in the data (Skew) was reduced from (2.2196) to (0.76644).

3. Results and Discussion

Rainfall is considered one of the most important sources of water availability necessary for tree growth, as it provides moisture to the soil, which trees benefit from through the absorption of water and nutrients by the roots [11], which contributes to the processes of photosynthesis and physiological processes within trees, in addition to the fact that rainfall works to maintain the environmental balance between trees and the atmosphere and that the low humidity rate, whether in the atmosphere or the soil, exposes trees to water stress and thus affects the growth and development of trees in the site [12] Therefore, rainfall data were taken for the sites (Dohuk - Sarsink) A graphical relationship has been prepared between the years and rainfall, as shown in Figures (1) and (2).

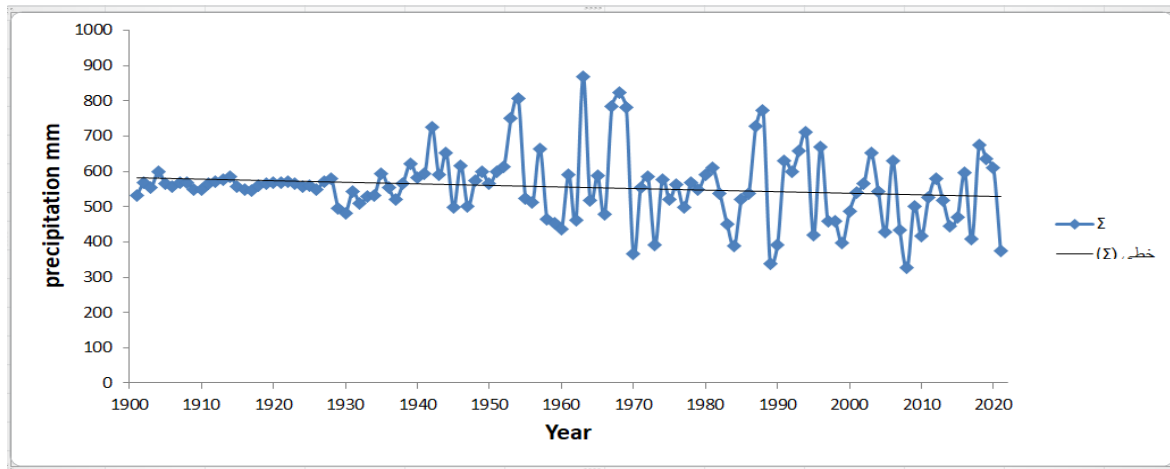


Figure 1. Rainfall for the years (1900-2022) AD for the Dohuk site.

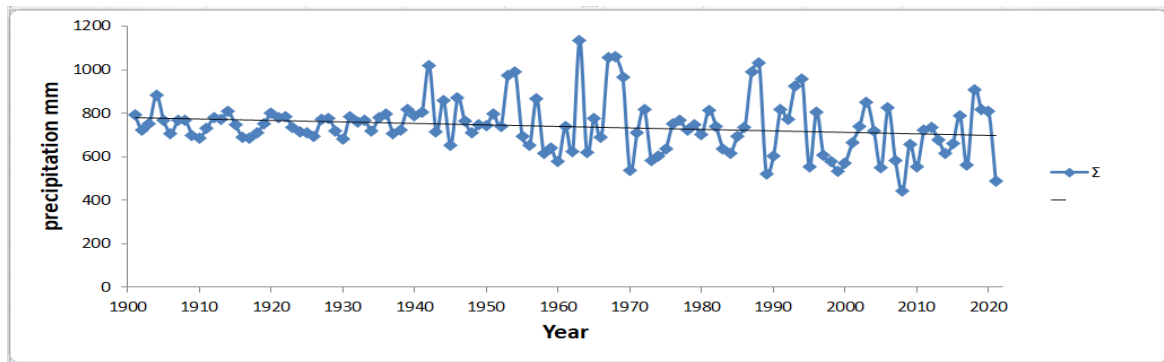


Figure 2. Rainfall for the years (1900-2022) AD for the Sarsink site.

From Figure (1) we find that the average rainfall for the Dohuk region in the year (1901) AD was about (580) mm annually, while we note in the year (2022) AD that the average rainfall was about (500) mm annually, the general trend of the rainfall line for this site is towards a gradual decline. Figure (1) shows that there is stability in rainfall around the average from the year (1901) AD until (1928) AD and its amount was approximately close to the average. From that period until (2020) AD, we notice an increase in the fluctuation in rainfall between different years, especially between the years (1960-1970) AD. Rainfall has great importance in its impact on biodiversity, the spread of trees, and their growth, as it affects the structure of the tree, its density, vitality, and total production. We see that rainfall has a great impact on the development processes that take place on the trees. O'Donnell et al. 2021 (So knowing its distribution and amount of vitality [13] shape of rainfall, its quantities, and the fluctuations that occur in it for the sites (Dohuk - Sarsink), data were taken for the years (1900-2022) AD, and Table (1) was prepared.

Table 1. Maximum and minimum rainfall and standard deviation for the sites (Dohuk - Sarsink) for the years (1900-2022) AD.

Region	Min	Max	Mean	S.D
Dohuk	0	282.1	46.3387	44.9838
Sarsink	0.1	330.8	61.68	53.8150

In general, the fluctuations in rainfall can be observed through the standard deviation (S.D) (44.9838), especially in recent years, and are attributed to the climate changes occurring in this location [14]. As for the Sarsink site, as in Figure (2), we note that the rainfall rate in the year (1901) AD was (800) mm, and the general trend line for rainfall is towards the decline of the data for this site, as the rainfall rate for the year (2022) AD reached about (680) mm annually. We also find that there is stability in rainfall around the average approximately since the year (1900-1940) AD, then find there is a fluctuation in rainfall and the downward direction since that period, and this is what became clear to us from the standard deviation of rainfall for this site, which was estimated at (53.8150). This indicates that both sites are affected by global climate changes, and this is what Standardized precipitation index (SPI) [15]. The distribution of rainfall and its density varies from one location to another and thus affects the growth rates of trees or plants growing in it. Knowing the amount of moisture present in the location and the extent to which it meets the requirements of the plant is of great importance to the administrator who works to develop the sites in a way that obtains sustainable resources for long periods. The lack of moisture affects these goals. This includes estimating the standard precipitation index (SPI), which depends on rainfall, as it is an important indicator that must be predicted to observe and monitor plant covers continuously, in addition to taking appropriate measures to overcome periods of moisture deficiency in a location. This is what was mentioned by [16]. Therefore, the initial data for the location (Dohuk-Sarsink) were taken in addition to other climatic factors, and we reached an estimate of the drought index that depends on rainfall, as in table (2):

Table 2. Standard drought index for the Dohuk and Sarsank regions for the years (1900-2022) AD.

Dohuk region.		Sarsink region.	
year	SPI	year	SPI
1900	-2.38823	1900	-2.38335
1910	-2.38782	1910	-2.38502
1920	-2.38735	1920	-2.38325
1930	-2.38953	1930	-2.38510
1940	-2.38702	1940	-2.38337
1950	-2.38744	1950	-2.38406
1960	-2.39091	1960	-2.38715
1970	-2.39348	1970	-2.38806
1980	-2.38684	1980	-2.38472
1990	-2.39245	1990	-2.38661
2000	-2.38938	2000	-2.38726
2010	-2.39155	2010	-2.38766
2020	-2.38646	2020	-2.38308

From Table (2) we notice that the range of this scale ranges between the highest and lowest (2.39522 _2.38230) for the Dohuk site and also ((-2.37946 _2.39068) for the Sarsink site. In this, we find that the trees that grow in such sites are drought tolerant and require higher humidity than that to grow well. This is because the value of this scale ranges from (-3 , 3) [17] (This means that there is high humidity. The value of this indicator is positive and reaches its maximum (3°). However, when this site is dry and the amount of rainfall is insufficient, it usually gives negative values. The higher its VALUE reaches approximately (-3), the more severe the drought and lack of humidity in the site. In other words, these trees The development in these sites is severely affected by water shortages and they grow abnormally and need different service operations that lead to reducing the pressure of low humidity through administrative and developmental procedures that work to raise the water stock in the soil [18]. This is what we noticed in the study

sites, which showed that the values for all years were negative and that the amount of these values is increasing. This is what we notice from the general trend line, as in Figures (3) and (4).

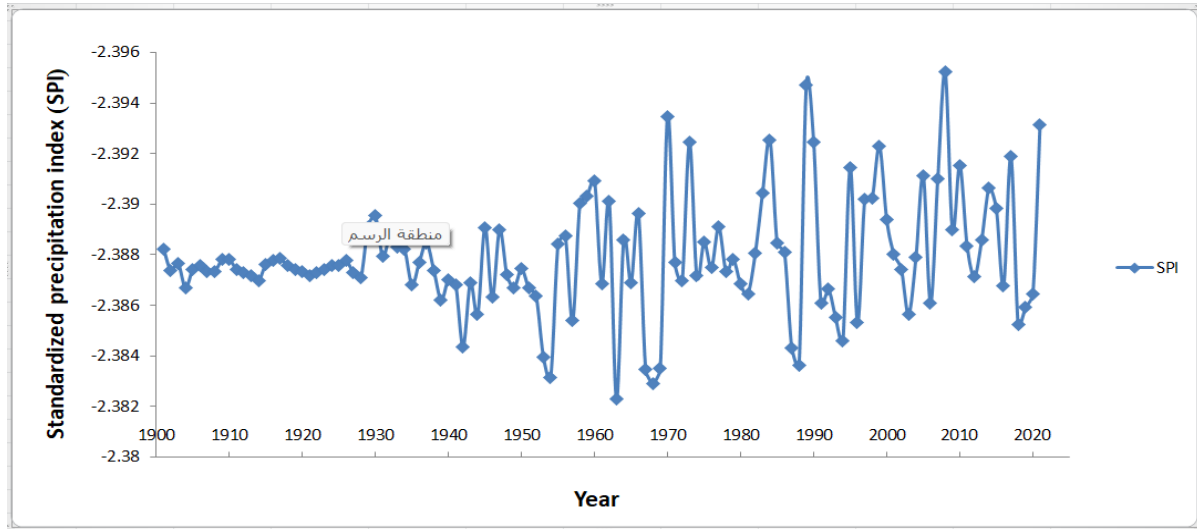


Figure 3. The relationship between the standard drought index and the years (1900-2022) AD for the Dohuk region.

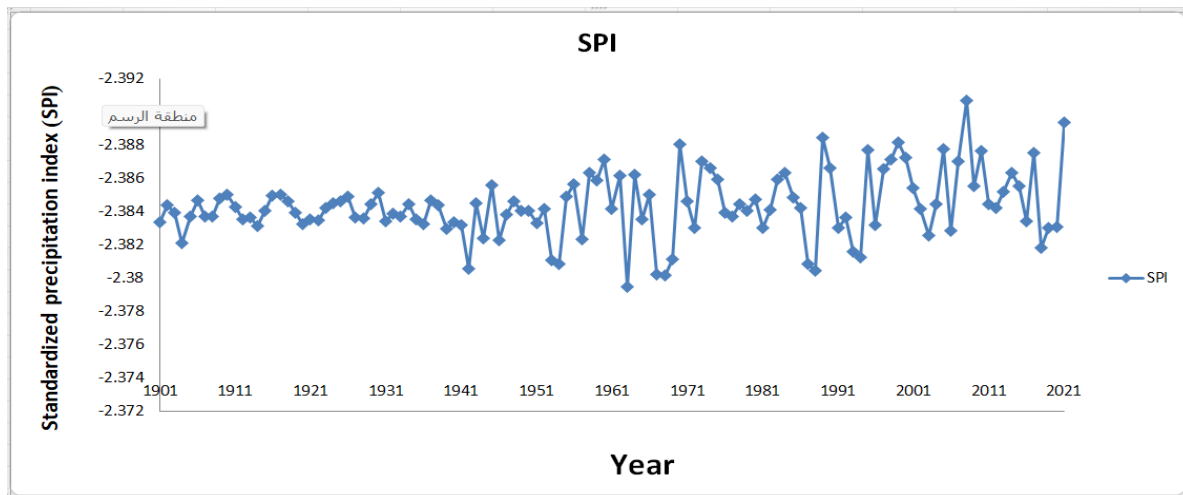


Figure 4. The relationship between the standard drought index and the years (1900-2022) AD for the Sarsink region.

Figures (3) and (4) show that there is a fluctuation and decrease in the amount of rainfall for both sites, and this is consistent with what we observe of environmental changes that accompany the low in rainfall, which is one of the main sources of moisture for tree growth [19]. We also note that the aridity index (SPI) was around (-2.387) and almost constant for the period from (1900) to (1931), but was a fluctuation in this index from (1931) to (1951), whether this was positive or negative at the general trend line. This indicates a decrease in the humidity percentage from (1931) to (1941), as the index read higher values than the axis of the general trend, while there were generally wet years in which rainfall increased from (1941) to (1951), where there was an increase in rainfall. From that period (1951) AD until (2020) AD, we notice a fluctuation in

rainfall around the general direction of the axis with a decrease or increase in this rainfall during these periods. There was an increase in humidity in the year (1963) AD and rainy years (1967-1968-1969) AD as well as in the years ((1987-1988 AD), while years passed in which rainfall decreased and the efficiency of rain decreased in the site in the year (1970) AD until (1984) AD and in the year (1989) AD as well as (2008) AD. We notice from Figure (4-13) that there is a wide fluctuation in the Dohuk site between the years and the difference in rainfall in it to a large extent, which affects the growth and development of the tree vegetation cover [20] for this site, also in Figure (4) gave to some extent the values of the efficiency of rainfall for the relationship between the years and the efficiency for a period from (1901) AD until (1940) AD, values that are somewhat close, which shows that the rainfall from this period is very close to each other, while we notice the emergence of fluctuations in it and differences in its proportions and its effect for the periods that exceeded that until (2020) AD, but the amount of this fluctuation, whether by increasing or decreasing rainfall to some extent, was less than the Dohuk site, this indicates that this site is more stable and suitable for the growth and development of trees, but in general, the values of the standard drought index range between(- 2.37946 _-2.39068) That is, there is a clear moisture deficit through the negative values that are less than zero and close to (-3). This indicates that the trees grow under the influence of high pressure due to low humidity. trees do not grow normally in a way that provides their water needs for growth and development [21]. This indicates that the site needs to exploit rainfall, when the plant is exposed to a decrease in humidity levels [22].

3.1. Precipitation Efficiency Index (PEI)

It is usually estimated based on the Standard Aridity Index (SPI) and the relationship prepared by [23] as follows:

$$PEI = 2 \times (SPI + 1) - 1$$

Where: PEI = Rainfall Efficiency Index. SPI = Standard Drought Index.

Through this index, we were able to calculate the values of the Rainfall Efficiency Index for the two locations (Dohuk - Sarsink (For this purpose and to know the fluctuations that occurred in this scale for different years and the changes that accompany them from environmental changes that affected the various climate elements, including rainfall, we prepared Figures (5) and (6).

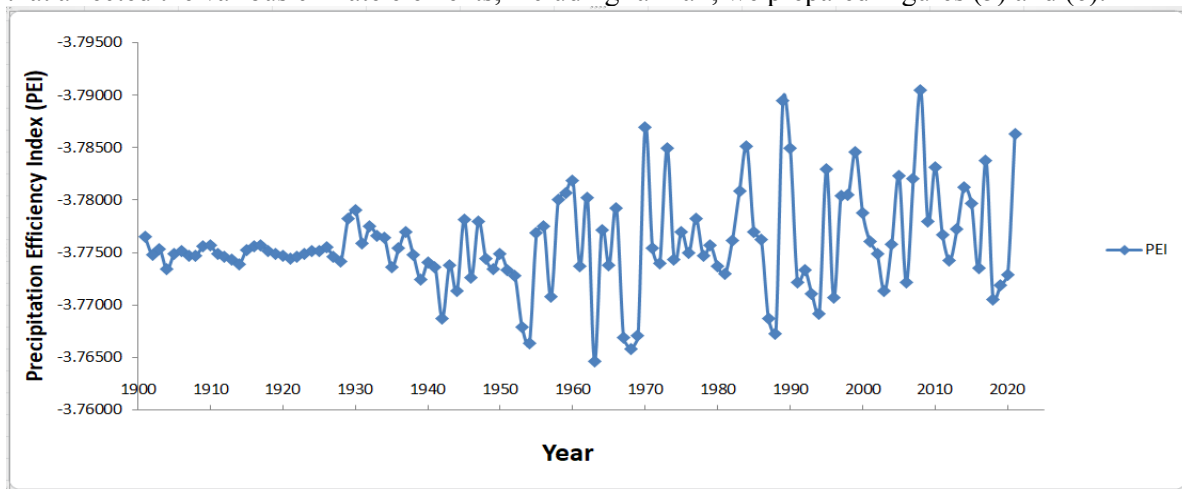


Figure 5. The relationship between the rain efficiency index and the years (1900-2022) AD for the Dohuk region.

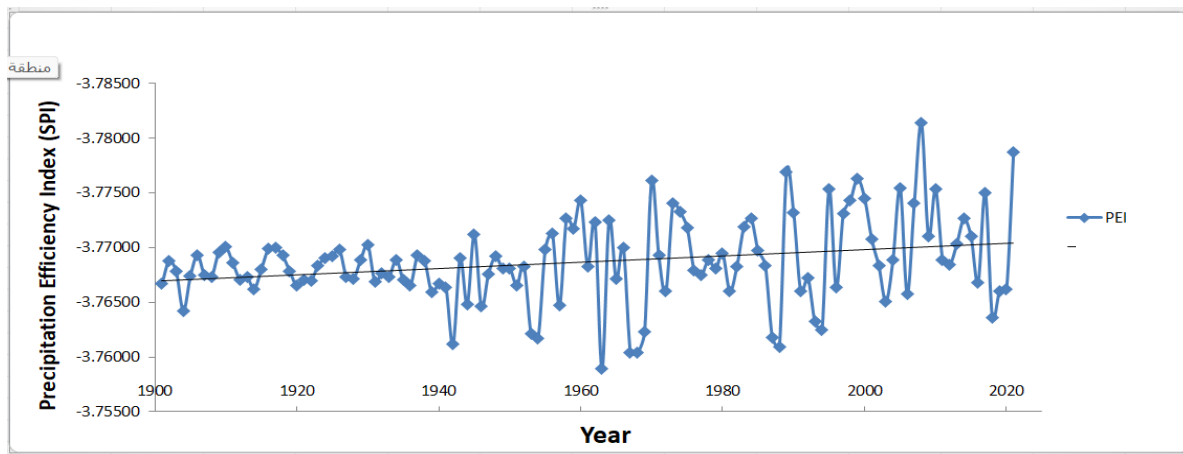


Figure 6. The relationship between the rainfall efficiency index and the years (1900-2022) AD for the Sarsink region.

We note from Figure (5) that the Rainfall Efficiency Index (PEI) is generally negative, as we mentioned previously in Table (4), but its fluctuation was somewhat limited at the beginning of the twentieth century until the year (1930) AD, there began to be wide deviations from the trend axis of the line drawn for the Rainfall Efficiency Index, as years showed a significant decrease in humidity, which is met in years of increase. In addition, the general trend axis line for this figure indicates an increase in the severity of the standard drought index, and this is consistent with what the Dohuk site is exposed to, as well as Sarsink, which we see in Figure (6), meaning that the climate changes that occurred have affected in two ways: increasing drought periods and decreasing humidity over the past three decades, while the percentage of rainfall has increased [24]. Usually, we note here that the amount of rainfall, its distribution, and its intensity has also varied greatly, and this is one of the effects of global warming, which has affected these sites, as these sites are considered to be sites Dry and semi-arid and classified within the Mediterranean basin [25].

Recommendations

We recommend using the stem analysis method to show the Rainfall Efficiency Index (PEI) to infer its effectiveness and impact on the amount of benefit growing in certain locations can gain to grow naturally and as an indicator of great importance for the vitality of trees and their natural growth.

References

- [1] Nebojsa Nakicenovic ;(2000).Special Report on Emissions Scenarios (SRES) – A Special Report of Working Group III of the Intergovernmental Panel on Climate Change July Edition: 1Publisher: Cambridge University PressISBN: 0521800811.
- [2] Lebourgeoisab. F ,Gomezc. N. Pinto, P. Mériana.; (2013). Mixed stands reduce Abies alba tree-ring sensitivity to summer drought in the Vosges mountains, western Europe, Journal ; Forest Ecology and Management,Volume 303, Pages 61-71.
- [3] Al-Khafagi, D.E. Qahtan.(2018).DISTEBUTION STUDY IN SOME MICROELEMENTS UNDER DIFFERENT CLIMATIC CONDITIONS IN SELECTED SOILS FROM NORTHERN IRAQ. Journal of Kirkuk University for Agricultural Sciences, 9,(3) 92.
- [4] Majeed. H. M.(2023). Urban Geomorphology of Duhok city. DIYALA.
- [5] Salila, E., Sharma, M. P., & Singh, R. (2020). Climate Change Impacts on Water Quality of Water Bodies-A Review. International Journal of Environment and Climate Change, 10(3), 26-37.

- [6] Serquet, G., Marty, C., Dulex, J. P., & Rebetez, M. (2011). Seasonal trends and temperature dependence of the snowfall/precipitation-day ratio in Switzerland. *Geophysical research letters*, 38(7).
- [7] Naresh, R., Tripathi, A., & Sharma, D. (2009). Modelling and analysis of the spread of AIDS epidemic with immigration of HIV infectives. *Mathematical and computer modelling*, 49(5-6), 880-892.
- [8] Edwards, D. C., & McKee, T. B. (1997). Characteristics of 20th century drought in the United States at multiple time scales.
- [9] Bhagat, A. D., Gorantiwar, S. D., & Malunekar, V. S. (2022). Assessment of Meteorological Drought using Standard Precipitation Index (SPI) in Western Maharashtra, India. *Journal of Agriculture Research and Technology*, 47, 152-156.
- [10] Abramowitz, M., & Stegun, I. A. (1964). National Bureau of Standards Applied Mathematics Series. Handbook of mathematical functions, 55.
- [11] Al-Rijabo, W. I., & Bleej, D. A. (2010). Variation of Rainfall with space and Time in Duhok Governorate. *Journal of Education and Science*, 23(1), 32-43.
- [12] Seleiman, M. F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., ... & Battaglia, M. L. (2021). Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants*, 10(2), 259.
- [13] Bista, R., Mohr, M., Saldaña, D., Angulo, G., & Chhetri, P. K. (2021). Tree-ring climate response of Jeffrey pine in the Cascade Creek Watershed, Northern California. *Journal of Forest Science*, 67(6), 285.
- [14] Udayashankara, T. H., Murthy, B. S., & Madhukar, M. (2016). Impact of climate change on rainfall pattern and reservoir level. *Journal of Water Resource Engineering and Management*, 3(1), 10-14.
- [15] Brien, R. J., LEBRIJA-TREJOS, E. D. W. I. N., Zuidema, P. A., & MARTÍNEZ-RAMOS, M. I. G. U. E. L. (2010). Climate-growth analysis for a Mexican dry forest tree shows strong impact of sea surface temperatures and predicts future growth declines. *Global Change Biology*, 16(7), 2001-2012.
- [16] Kasim, S. M., Youns, A. M., & Mahmood-Agha, O. M. (2021). Temporal and Spatial Analysis of Drought Using the Standard Precipitation Index for the Northwestern Region of Iraq. *Al-Rafidain Engineering Journal (AREJ)*, 26(1), 115-127.
- [17] Gao, Y., Gao, Z., Sun, W., & Hu, Y. (2016). Selective flotation of scheelite from calcite: A novel reagent scheme. *International Journal of Mineral Processing*, 154, 10-15.
- [18] Pachauri, R. K., & Reisinger, A. (2007). Climate change 2007: Synthesis report. Contribution of working groups I, II and III to the fourth assessment report of the Intergovernmental Panel on Climate Change. IPCC.
- [19] Guttman, N. B. (1998). Comparing the palmer drought index and the standardized precipitation index 1. *JAWRA Journal of the American Water Resources Association*, 34(1), 113-121.
- [20] Elbeltagi, A., Pande, C. B., Kumar, M., Tolche, A. D., Singh, S. K., Kumar, A., & Vishwakarma, D. K. (2023). Prediction of meteorological drought and standardized precipitation index based on the random forest (RF), random tree (RT), and Gaussian process regression (GPR) models. *Environmental Science and Pollution Research*, 30(15), 43183-43202.
- [21] Pasho, E., Camarero, J. J., de Luis, M., & Vicente-Serrano, S. M. (2011). Impacts of drought at different time scales on forest growth across a wide climatic gradient in north-eastern Spain. *Agricultural and Forest Meteorology*, 151(12), 1800-1811.
- [22] Vicente-Serrano, S. M., Beguería, S., & López-Moreno, J. I. (2010). A multiscalar drought index sensitive to global warming: the standardized precipitation evapotranspiration index. *Journal of climate*, 23(7), 1696-1718.
- [23] Hanadé Houmma, I., Gadal, S., El Mansouri, L., Garba, M., Gbetkom, P. G., Mamane Barkawi, M. B., & Hadria, R. (2023). A new multivariate agricultural drought composite index based on random forest algorithm and remote sensing data developed for Sahelian agrosystems. *Geomatics, Natural Hazards and Risk*, 14(1), 2223384.



- [24] Pendergrass, A. G., & Hartmann, D. L. (2014). Changes in the distribution of rain frequency and intensity in response to global warming. *Journal of Climate*, 27(22), 8372-8383.
- [25] Salhab, H. M., Ali, W., & Abboud, L. (2018). Influence of Precipitation and Temperature on Tree-Ring Width of Brutia Pine (*Pinus Brutia* Ten.) in Tartous-Syria. *ACADEMIA*: San Francisco, CA, USA.

Investigation of the Causal Agent of Wilt and Root Rot Disease *Fusarium spp.* on Pepper Plants in Certain Areas of Baghdad and Its Morphological and Molecular Identification

Baraa Abbas Ahmed Al-jubory¹ and Mena Waleed Hatem²

^{1,2}Department of plant protection, College of Agriculture Engineering Sciences, University of Baghdad, Baghdad, Iraq.

¹E-mail: baraa.abbas2204m@coagri.uobaghdad.edu.iq

²E-mail: mena.waleed@coagri.uobaghdad.edu.iq

Abstract. The study was conducted during the period 2022-2024 to identify the causal agent of pepper plant wilt in certain fields of Baghdad Governorate. Samples of infected plants were collected from several fields in Baghdad (Abu Ghraib, Yusufiyah, Radwaniyah, Arab Jbour, Sayafiya, and Samra). The pathogenicity of the isolates was tested on pepper seeds, and the isolate from Abu Ghraib (G1R3) showed the highest inhibition rate of pepper seed germination, reaching 100%. The most pathogenic isolate was identified both morphologically and molecularly, and the morphological and molecular diagnoses matched. Under the accession number PP446821, the sequences of the discovered isolate were deposited with the National Centre for Biotechnology Information (NCBI). The isolate's nucleotide sequence matched that of *F. solani* by 99%, according to the data.

Keywords: *Fusarium*, *Fusarium solani*, *Capsicum annuum*, Pepper.

1. Introduction

The pepper plant (*Capsicum annuum L.*) is one of the main vegetable crops and belongs to the Solanaceae family [1]. Sweet peppers contain essential nutrients such as carbohydrates, proteins, fats, and minerals, with potassium, calcium, phosphorus, and iron being the most important. They also provide vitamins (Vitamin A, Vitamin B, and Vitamin C) [2].

Pepper plants are susceptible to several soil-borne pathogens that cause economic losses, including pre-emergence and post-emergence damping-off, seedling mortality, and root rot [3, 4, 5]. The most significant of these pathogens include *Phytophthora capsici*, *Fusarium solani*, *F. oxysporum*, *Verticillium spp.*, *Rhizoctonia solani*, *Pythium aphanidermatum*, and *Macrophomina phaseolina* [6-10].

Many plant species are susceptible to wilt and root rot caused by *Fusarium spp.*, a major soil-borne fungal pathogen [11, 12]. Wilt in peppers is caused by certain members of the genus *Fusarium* [13, 14]. A kind of harmful fungus known as *Fusarium* lives in the soil as spores [15]. There are hundreds of species in the genus *Fusarium*, which is in the family Hypocreaceae. Many of these species live in soil, while others are connected with plants [16]. Worldwide, *Fusarium* causes enormous agricultural losses and is one of the most damaging plant infections from an

economic perspective [17, 18]. It infects nearly all economically important plants. *Fusarium* causes plant diseases by producing mycotoxins such as Trichothecenes [19]. *Fusarium* produces a variety of harmful secondary metabolites, such as fumonisins, zearalenone, and trichothecenes, which contaminate agricultural commodities and pose health risks to human consumption [20-23]. *Fusarium* causes diseases such as wilt, rot, and cankers in many plants [24, 25, 26, 27]. Since *Fusarium* is a soil-borne pathogenic fungus, it can survive in the soil for long periods by decomposing plant residues. The infected soil can then be spread to new areas through animals or farming tools, facilitating the transmission of the pathogen to new regions [28]. Given that seed rot and seedling damping-off are among the most damaging diseases affecting pepper seedlings, the aim of this study was to isolate and identify *Fusarium spp.*, the causal agent of seed rot and seedling damping-off in pepper, both morphologically and molecularly.

2. Materials and Methods

2.1. Sample Collection

Pepper plants exhibiting symptoms of wilt were collected from fields in several areas of Baghdad Governorate (Abu Ghraib, Yusufiyah, Radwaniyah, Arab Jbour, Sayafiya, and Samra). The infected plants were placed in polyethylene bags, labeled with the collection date and location, and transported to the Plant Diseases Laboratory, Plant Protection Department, College of Agricultural Engineering Sciences, for fungal isolation. The pathogen was then identified morphologically using a microscope with magnification power of (X10-X40) and molecularly using Polymerase Chain Reaction (PCR) techniques.

2.2. Fungal Isolation from Pepper Roots

The Plant Diseases Laboratory, which is part of the Plant Protection Department, received plants that were showing signs of disease. The bases of the roots and stems that displayed symptoms of decay and cankers were sampled. Before being sliced into small pieces (0.5-1 cm in length), these parts were rinsed under running water to remove any soil that stuck to them. Then, they were surface-sterilized with a 1% sodium hypochlorite solution (which contains free chlorine) for two minutes. After rinsing with distilled water that was sterile, the sections were dried with filter paper that was also sterile, and then moved to a clean area using forceps that had been sterilized. The components were set on 9 cm² Petri dishes that contained 15-20 mL of Potato Dextrose Agar (PDA) medium. This medium was made by combining 300 g of potatoes, 20 g of dextrose, and 15 g of HIMEDIA agar; it was treated with 200 mg L⁻¹ of the antibiotic tetracycline. The medium was made by combining 41 grammes of PDA with 1 litre of water and then subjecting it to a 15-minute autoclaving cycle at 121°C and 1.5 kg/cm² pressure. The plates were incubated at 25°C. After three days, fungal isolation was performed by transferring 0.5 cm discs from the fungal growth margin using a cork borer to new sterilized PDA Petri dishes. These plates were incubated for five days for morphological identification. The different fungal isolates were purified and examined under a compound microscope at magnifications of X10 and X40 to observe the fungal mycelium and its structures.

2.3. Morphological Identification

Sixteen isolates of *Fusarium spp.* were identified morphologically in the laboratory based on the shape and color of the fungal colonies, as well as the structures and forms of the conidiophores. Following established taxonomic guidelines, the identification was carried out at the Plant Diseases Laboratory, Plant Protection Department, College of Agricultural Engineering Sciences,

University of Baghdad, using a light microscope with magnifications ranging from X10 to X40. The classification was done using established taxonomic keys [29-34].

2.4. Pathogenicity Test of *Fusarium spp.* Isolates on Pepper Seeds

The most pathogenic isolate of *Fusarium spp.* was tested by inoculating the center of a Petri dish containing PDA medium with a 5-day-old fungal culture (0.5 cm in diameter). The plates were incubated at 25°C. After 3 days, pepper seeds were surface-sterilized, dried in the isolation chamber, and planted at a rate of 10 seeds per dish. The plates were incubated at 25°C. After 7 days, the germination percentage of the seeds was calculated using the following formula:

$$\text{seed germination \%} = \frac{\text{No. of germinated seeds}}{\text{Total number of seeds}} \times 100$$

2.5. Molecular Identification of *Fusarium spp.*

The most pathogenic isolate on pepper seeds was selected for molecular identification to confirm the morphological diagnosis. DNA extraction from the *Fusarium spp.* isolate was performed at the Molecular Diagnostics Laboratory of Jisr Al-Musayyib Company (Baghdad, Iraq) using the Master Mix extraction kit manufactured by Pioneer Company (Korea), following the manufacturer's recommended protocol.

The amplification of the target DNA region was carried out using the following pair of primers:

- ITS1 forward (F: 5'- TCCGTAGGTGAACCTGCGG- 3')
- ITS4 reverse (R: 5'- TCCTCCGCTTATTGATATGC- 3')

The targeted DNA region was amplified through Polymerase Chain Reaction (PCR) [35].

3. Results and Discussion

3.1. Isolation and Morphological Identification of *Fusarium spp.* Associated with Pepper Roots in the Laboratory

The results of the fungal isolation on PDA medium indicated that *Fusarium solani* was present in most of the samples collected from various areas of Baghdad Governorate. The fungal colonies exhibited varying colors, ranging from white cottony to reddish-brown (Figure 1), which suggests the presence of genetic variations among the isolates or that they belong to different strains of the same fungus [36, 37].



Figure 1. The appearance of *Fusarium solani* colony on PDA medium.

The results of the morphological identification showed that the fungus produces three types of asexual reproductive structures. These are:

- Microconidia: These form 2-3 days after being cultured on PDA medium, appearing on single, long to medium-length, branched conidiophores. The microconidia are either one or two-celled, oval, spherical, or gelatinous in shape.
- Macroconidia: After 4-7 days, large conidia (macroconidia) are formed on short, branched conidiophores. These are spindle-shaped or crescent-shaped, with curved ends and contain 3-6 transverse septa. Sometimes, the sub-basal cell is relatively wide or arched, while the apical cell is pointed or beak-like.
- Chlamydospores: These have smooth or rough walls and are produced singly, in pairs, or sometimes in chains [29-34,38,39].

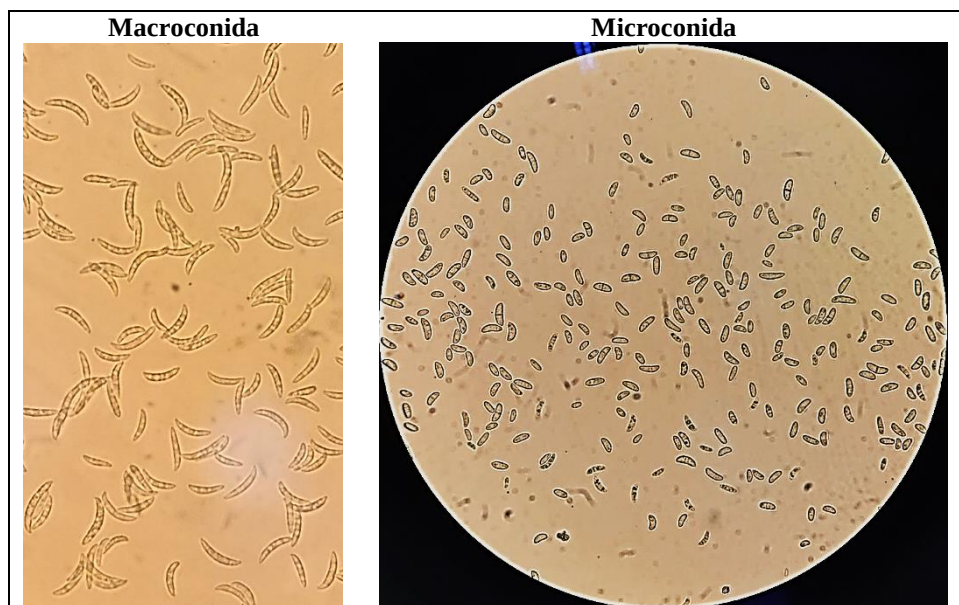


Figure 2. Macroconidia and Microconidia.

3.2. Pathogenicity Test of *Fusarium* spp. Isolates Using Pepper Seeds

Table 1 shows that the germination rate of pepper seeds was significantly reduced in the laboratory by all isolates of *Fusarium* spp. Among the tested isolates, the one from Abu Ghraib (G1R3) performed the best, with a germination rate of 0% compared to the control's 100%. Different individuals of the same species were isolated from different parts of the world, which could explain why their inhibition rates vary. Additionally, differences in the production of pectin- and cellulose-degrading enzymes, as well as variations in secondary metabolites, can lead to seed rot and prevent germination [39-41].

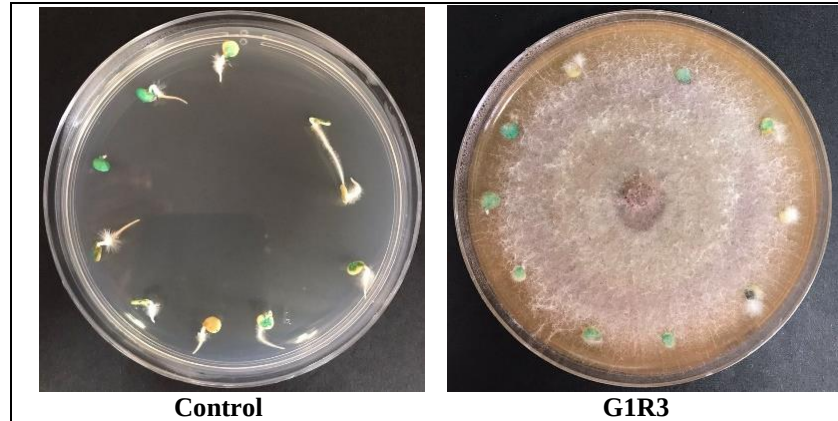


Figure 3. Fusarium spp. Isolates Using Pepper Seeds.

Table 1. The effect of pathogenic fungal isolates on the germination rate of pepper seeds in the laboratory on PDA medium.

ID	Location	Isolate code	Germination %
1	Control (Pepper seeds)	Control	100
2	Samra	MR6r1	10.0
3	Samra	MC2r1	13.3
4	Sayafiya	Z2R4r3	15.0
5	Radwaniyah	Y3R6r2	40.0
6	Radwaniyah	Y3R2r2	21.7
7	Sayafiya	Z2R6r1	20.0
8	Arab Jbour	Y2R3r4	8.3
9	Yusufiyah	Y1C1r1	15.0
10	Yusufiyah	Y1C2r1	13.3
11	Yusufiyah	Y1R3r1	13.3
12	Yusufiyah	Y1C1r2	16.7
13	Arab Jbour	Y2C1r2	23.3
14	Abu Ghraib	G1R3	0
15	Abu Ghraib	G1S4	16.7
L.S.D			16.11

*Each number in the table represents the average of 3 replicates.

3.3. Molecular Identification of *F. solani* Using Polymerase Chain Reaction (PCR) Technique

Prior to conducting PCR to amplify the ITS region, DNA was extracted from the fungal mycelium of the isolate with the highest pathogenicity. The primer pair used for this purpose was ITS1-ITS4. Under UV illumination, a distinct band was observed in the gel electrophoresis results (Figure 4). Gel electrophoresis revealed that the amplification result had a molecular weight of 525 bp, which was lower than the DNA ladder. A nucleotide sequence match with *F. solani* was found to be 99% in the results. The sequences of the known isolates were submitted to the National Centre for Biotechnology (NCBI) with the accession number PP446821.

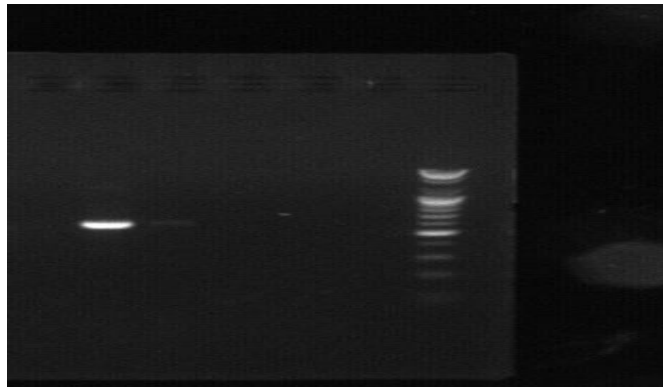


Figure 4. PCR product the band size 525 bp.

Conclusions

Fusarium spp. was found in all the regions from which pepper plant samples were collected, showing symptoms of wilt. *Fusarium solani* was identified as one of the major causes of root rot and wilt in pepper plants. All isolates had varying effects on seed germination in the laboratory, with the Abu Ghraib isolate (G1R3) showing the highest inhibition rate of 0%. The most pathogenic isolate was confirmed to be *Fusarium solani*.

References

- [1] Bosland PW, Votava EJ. 2000. Peppers: Vegetable and Spice Capsicums. Crop Production Science in Horticulture. Wallingford, CT: CAB International Publishing: 204.
- [2] Ismail F, Anjum MR, Mamon AN, Kazi TG. 2011. Trace metal contents of vegetables and fruits of Hyderabad retail market. Pakistan Journal of Nutrition. 10(4):365-372.
- [3] Abood NT, Abdul-Moohsin RG, Altaie AH. 2020. Isolation and diagnosis of *Fusarium solani* that causes root rot in soybean and evaluating the efficiency of bacteria *Bacillus subtilis* and *Azotobacter* spp. in controlling the disease. EurAsian Journal of BioSciences. 14:4617-4623.
- [4] Al-Aamel AN. 2023, The integrative efficacy of biological control agents *Trichoderma harzianum* and *Pseudomonas fluorescens* and chelated iron in controlling wilt disease in peppers. M.Sc. Thesis. College of Agricultural Engineering Sciences, University of Baghdad.
- [5] Manda RR, Addanki VA, Srivastava S. 2020. Bacterial wilt of solanaceous crops. International Journal of Chemical Studies. 8(6):1048-1057.
- [6] Al-Rubaie AR. 2022, Induction of resistance against damping-off and root rot diseases in pepper plants using some biological and chemical agents. M.Sc. Thesis. College of Agricultural Engineering Sciences, University of Baghdad.
- [7] Wilson KG, Sandoz EK, Kitchens J, Roberts M. 2010. The Valued Living Questionnaire: Defining and measuring valued action within a behavioral framework. The Psychological Record. 60:249-272.
- [8] El-Kazzaz, M.K., Ghoneim, K.E., Agha, M.K.M., Helmy, A., Behiry, S.I., Abdelkhalek, A., Saleem, M.H., Al-Askar, A.A., Arishi, A.A. and Elsharkawy, M.M. 2022. Suppression of pepper root rot and wilt diseases caused by *Rhizoctonia solani* and *Fusarium oxysporum*. *Life*, 12(4), p.587.
- [9] Khan, M.A., Riaz, H., Ramzan, M., Ullah, U.N., Ali, H. and Ishfaq, T. 2023. Morpho-Molecular Study and Biochemical Management of *Fusarium Solani* in Bell Pepper. *BioScientific Review*, 5(3), pp.42-54.
- [10] Engalycheva, I., Kozar, E., Frolova, S., Vetrova, S., Tikhonova, T., Dzhos, E., Engalychev, M., Chizhik, V., Martynov, V., Shingaliev, A. and Dudnikova, K. 2024. *Fusarium* Species Causing Pepper Wilt in Russia: Molecular Identification and Pathogenicity. *Microorganisms*, 12(2), p.343.

- [11] Abu-Taleb MA, El-Deeb K, Al-Otibi FO. 2011. Assessment of antifungal activity of *Rumex vesicarius* L. and *Ziziphus spina-christi* (L.) wild extracts against two phytopathogenic fungi. *African Journal of Microbiology Research*. 5(9):1001-1011.
- [12] Risan, M., 2012. DIAGNOSIS OF *Fusarium oxysporum* FUNGUS ISOLATIONS AND *Bacillus* BACTERIA THE OF EFFICIENCY THE OF EVALUATION OF INHIBITION THE IN PHOSPHATE POTASSIUM AND *subtili*. *Iraqi Journal of Science*, 53(3), pp.513-523.
- [13] Shaheen N, Khan UM, Azhar MT, Tan DK, Atif RM, Israr M, Yang SH, Chung G, Rana IA. 2021. Genetics and genomics of *Fusarium* wilt of chilies: A review. *Agronomy*. 11(11):2162.
- [14] Al-aamel, A.N.A. and Al-maliky, B.S.A., 2023. Control pepper *Fusarium* wilting by biocontrol agent *Trichoderma harzianum* and chelated iron Fe-EDDHA. *Baghdad Science Journal*, 20(6).
- [15] Ropalia R. 2017. Potency of bamboo root endophytes as biocontrol to *Fusarium oxysporum* pathogen causing yellowing disease on pepper plant. *AGROSAINSTEK: Jurnal Ilmu Dan Teknologi Pertanian*. 1(2):80-85.
- [16] Babadoost M. 2018. *Fusarium*: Historical and continued importance. In: *Fusarium—Plant Diseases, Pathogen Diversity, Genetic Diversity, Resistance and Molecular Markers*. pp.13-24.
- [17] Aoki T, O'Donnell K, Geiser DM. 2014. Systematics of key phytopathogenic *Fusarium* species: current status and future challenges. *Journal of General Plant Pathology*. 80:189-201.
- [18] Hatem, M.W., Shukri, H.M., Rasheed, K.A., Nawar, M.H., Hasan, S.M. and Adnan, R., 2020. The Effect of Magnetically Treated Water Against *Fusarium* Wilt Disease in Tomato Caused by the Fungus *Fusarium oxysporum* and Its Effect on Production Under Fertilized Farming Conditions. *Plant Archives*, 20(1), pp.533-6.
- [19] Asam S, Habler K, Rychlik M. 2017. *Fusarium* mycotoxins in food. In: *Chemical contaminants and residues in food*. Woodhead Publishing; p. 295-336.
- [20] Bhat R, Rai RV, Karim AA. 2010. Mycotoxins in food and feed: present status and future concerns. *Comprehensive Reviews in Food Science and Food Safety*. 9(1):57-81.
- [21] Azliza, I.N., Hafizi, R., Nurhazrati, M. and Salleh, B. 2014. Production of major mycotoxins by *Fusarium* species isolated from wild grasses in peninsular Malaysia. *Sains Malays*, 43, pp.89-94.
- [22] Toghueo, R.M.K., 2020. Bioprospecting endophytic fungi from *Fusarium* genus as sources of bioactive metabolites. *Mycology*, 11(1), pp.1-21.
- [23] Perincherry, L., Lalak-Kańczugowska, J. and Stępień, Ł. 2019. *Fusarium*-produced mycotoxins in plant-pathogen interactions. *Toxins*, 11(11), p.664.
- [24] Woloshuk CP, Shim WB. 2013. Aflatoxins, fumonisins, and trichothecenes: a convergence of knowledge. *FEMS Microbiology Reviews*. 37(1):94-109.
- [25] Hatem, M.W. and Nawar, M.H., 2024. Evaluation of the Efficiency of some Botanical Oils in Controlling wilt and Root Rot Disease in Pepper Plant Caused by *Fusarium solani*. *Jornal of Al-Muthanna for Agricultural Sciences*, 11(supplement 1).
- [26] Thilagam, R., Kalaivani, G. and Hemalatha, N. 2018. Isolation and identification of phytopathogenic fungi from infected plant parts. *International Journal of Current Pharmaceutical Research*, 10(1), pp.26-28.
- [27] Zakaria, L. 2023. *Fusarium* species associated with diseases of major tropical fruit crops. *Horticulturae*, 9(3), p.322.
- [28] Muimba-Kankolongo A. 2018. Food Crop Production by Smallholder Farmers in Southern Africa: Challenges and Opportunities for Improvement
- [29] Booth C. 1977. *Fusarium: Laboratory Guide to the Identification of the Major Species*. Commonwealth Mycological Institute, Kew, Surrey, England: 58.
- [30] Stalpers JA, Andersen TF. 1996. A synopsis of the taxonomy of teleomorphs connected with *Rhizoctonia* spp. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G, editors. *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology, and Disease Control*. p. 49-63.
- [31] Ndiaye M. 2007. Ecology and management of charcoal rot (*Macrophomina phaseolina*) on cowpea in the Sahel. PhD thesis, Wageningen University, The Netherlands. *British Journal of Nutrition*. 35(1):109-114.

- [32] Leslie JF, Summerell BA. 2006. *Fusarium* laboratory workshops—a recent history. *Mycotoxin Research*. 22(2):73-74.
- [33] Hafizi R, Salleh B, Latiffah Z. 2013. Morphological and molecular characterization of *Fusarium solani* and *F. oxysporum* associated with crown disease of oil palm. *Brazilian Journal of Microbiology*. 44:959-968.
- [34] Robles-Carrión A, Leiva-Mora M, Herrera-Isla L, Sánchez-Rodríguez A, Torres-Gutiérrez R. 2016. Morphological and molecular identification of *Fusarium* species associated with vascular wilt of babaco (*Vasconcellea heilbornii* var. *pentagona* Badillo). *Revista de Protección Vegetal*. 31(3):184-193.
- [35] Sidharthan VK, Aggarwal R, Surenthiran N, Shanmugam V. 2018. Selection and characterization of the virulent *Fusarium oxysporum* f. sp. *lycopersici* isolate inciting vascular wilt of tomato. *International Journal of Current Microbiology and Applied Sciences*. 7(2):1749-1756.
- [36] Abdullah BA. 2020. Evaluation of the efficiency of grafted watermelon on *Lagenaria siceraria* in controlling root rot and vascular wilt diseases caused by *Fusarium solani* and *Fusarium oxysporum*. M.Sc. Thesis. College of Agricultural Engineering Sciences, University of Baghdad; 98 p.
- [37] Heflish AA, Behiry SI, Al-Askar AA, Su Y, Abdelkhalek A, Gaber MK. 2023. *Rhaphiolepis indica* fruit extracts for control of *Fusarium solani* and *Rhizoctonia solani*, the causal agents of bean root rot. *Separations*. 10(7):369.
- [38] Asif R, Muzammil S, Yasmin R, Ahmad H, Ambreen A. 2023. Isolation and characterization of *Fusarium oxysporum* f. sp. *vasinfectum*, causative agent of cotton wilt disease in Punjab, Pakistan. *Pakistan Journal of Phytopathology*. 35(1):103-110.
- [39] Zakaria L. 2023. *Fusarium* species associated with disease of major tropical fruit crops. *Journal of Horticulture*. 9(3):322-325.
- [40] Zheng N, Zhang LP, Ge FY, Huang WK, Kong LA, Peng DL, Liu SM. 2018. Conidia of one *Fusarium solani* isolate from a soybean-production field enable to be virulent to soybean and make soybean seedlings wilted. *Journal of Integrative Agriculture*. 17(9):2042-2053.
- [41] Kadhim JH, Mohammed AE, Allwbawi SAAJ, Mohammed AA. 2019. The effect of $MgSO_4$ on the behavior of the pathogenic fungus, *Fusarium solani* and the rate of seedlings damping-off disease on sesame. *Biochemical and Cellular Archives*. 19(2):3047-3053.

The Use of Alcoholic and Aqueous Extracts of E Co-Friendly and Safe Buckthorn Leaves Combating the Hairy Grain Beetle Insect *Trogoderma granarium* (Coleoptera : Dermestidae)

Misem Qasim Jalab¹ and Majeed Hameed Nawar²

^{1,2}Department of Plant Protection, Agriculture Engineering Sciences College,
Baghdad University, Baghdad, Iraq.

¹E-mail: maisam.jabr2204m@coagri.uobaghdad.edu.iq

²E-mail: majeed.hameed@coagri.uobaghdad.edu.iq

Abstract. The insect of Al -Khalafa is one of the most important stores pests in destructive products and is a major problem in the State of Iraq because of its severe damage to the products by feeding it on the stored products, leaving behind flour caused by their nutrition, preferences and other damage, which causes the pollution of the stored products and the classic strategy strategies were Using gases and pesticides, but these methods are contaminated to the environment and not safe, so modern science has tended to use safe and Eco-friendly methods, including the use of extracts. The study included the use of extract of alcoholic cards, ethanol and water, and for 2%, 5%, and 10%to combat the exaggeration in all its phase, which are) eggs, larva. A third phase, a fifth phase larva, virgins, adult (. %On the water extract, as the killing rate at the treatment of the third phase reached 17.78, while the fifth phase reached the killing rate 18.33, while the role of the Virgin was given the alcoholic extract is ethanol and the water. For all the concentrations, the same rate of inhibiting the dawn of adults is 0.00, while the treatment of adults, where the alcoholic extract exceeds the concentration of 10%and 5 % And the water extract is 10% on the rest of the concentrations, as it gave the same killing rate 16.67.

Keywords. Khapra, Buckthorn, Concentration, Alcohol.

1. Introduction

Wheat one of the most important sources of food, as they constitute the most important strategies for countries to maintain the stability of their nations [1]. Which requires protecting these crops from harm, spoilage, and infection with insect pests during storage in order to preserve them. Therefore, the khapra insect, i.e. the hairy grain beetle *T. granarium*, which belongs to the family of leather beetles Dermestidae within the Coleoptera order, is one of the most important major insect pests with many families that It causes damage to stored products, ranging from 20 to 40% of the annual global crop productivity [2-9] Among these damages they cause during the storage period is a decrease in seed germination rates due to the larval stage of the Khabra beetle feeding on them. It also leads to the emergence of secondary insect pests and encourages the growth of fungi as a result of their feeding on them. Grains feed on the seeds and grains of all types of

crops, dried fruits, and animal foodstuffs, causing damage to the grains, loss of weight, a decrease in their quality, a change in the chemical composition, and the appearance of undesirable colors and odors in the grains, which causes economic and marketing losses [10-13]. In order to keep pace with modern developments, the search began for safe and alternative methods to chemical pesticides, as modern science has tended in recent years to find products that possess many biologically active substances that are highly effective in protecting stored grains and combating various insect pests, with rapid decomposition, and low toxicity to animals. It is produced naturally from some plant extracts [14,15]. Such as phenols, alkaloids, saponins, terpenoids, coumarins, tannins, and others. They suppress the insect's immunity (khapra), or change the feeding behavior in terms of reducing the insect's nutrition, or preventing it from feeding, or making it sterile, or affecting the insect's nutrition. The biology of the insect or repellent affects the physiology of the khapra insect or reduces its development, reproduction, and survival in some secondary compounds [16-21]. From the above, we prove the importance of researching the use of the *Ziziphus spina-christi* plant as a plant pesticide that contains effective substances such as (flavonoids) and other substances in combating the Khabra insect [22-25].

The study aimed to find a control: method that is safe for the environment and humans and conduct a study on it, which is the use of Buckthorn leaf extract to combat the Khabra insect, because it contains effective products that harm the Khabra insect and do not harm the environment or humans.

2. Material and Methods

2.1. Breeding of the Hairy Grain Beetle (khapra) T granarium

The *T. granarium* insect was obtained from the College of Agricultural Engineering Sciences / University of Baghdad in the year 2023, September, from infected wheat grains. After that, the *T. granarium* khapra insect was transferred to the laboratory and placed inside 50 ml plastic containers, and healthy, sterilized wheat grains were placed in it, where it was They were sterilized by exposing them to freezing at -20 degrees for a period of 14 days. After that, the plastic containers were covered with a light organza cloth over the nozzles of the plastic containers, and then closed with plastic strips so that they were tightly closed. Then they were placed in a binder type incubator and a measuring device was placed with it. Humidity and temperature Hygrometer at a temperature of (35 + 1) degrees Celsius and a humidity level of (36 + 1) in order to obtain all the stages of the Khabra insect, and then the plastic boxes are sieved between 3-7 days from the time they are placed inside the incubator in order to get rid of the flour resulting from feeding the insect And the molting skins. Their activity is increased by placing intact grains inside the boxes, after which the insect stages are isolated. When the practical test begins, the pupal stages are isolated using a brush and intact grains are placed with them in order to obtain newly emerged adults at the age of 12 hours to 24 hours, after which ten pairs are placed. Inside each petri dish with a frequency of 50 in order to obtain the eggs and the rest of the instars at their first age to conduct tests for the study's research [26,27].

2.2. Preparing the Extract of Cedar Leaves and Treating the Effects of the Khapra Insect With It

The Buckthorn leaves were obtained from Al-Muthanna Governorate in October 2023, where the Buckthorn leaves were obtained and washed well in order to get rid of the soil stuck to them. They were then sterilized with a 1% sodium hypochlorate solution, then washed with distilled water and dried using filter paper, then wrapped in newspapers. Then it was placed in the electric oven at a temperature of 50 degrees Celsius for a day with constant stirring, after which it was ground using an electric grinder to obtain a fine powder. It was then placed in plastic boxes with a

capacity of 60 ml and closed well, then covered with cellophane so that the powder does not absorb moisture and is preserved. It is used to prepare the alcoholic and aqueous extract [28]. The aqueous extract was prepared in the animal nutrition laboratory, Animal Production Department, College of Agricultural Engineering Sciences, University of Baghdad, where 25 grams of Buckthorn leaf powder was taken using a Sartorius digital scale, then placed in a 500 ml beaker, then distilled water was added to it, after which the beaker was shaken in order for the water to become homogeneous. The powder is then placed on the vortex device for 30 minutes at a temperature of 40 degrees Celsius, after which it is filtered using a piece of filtered cloth, which is placed on another beaker. Then the solution is poured onto the filtered cloth and left for a period of time to be completely filtered, then it is poured into glass dishes and is Place it in the oven for 24 hours at a temperature of 65 degrees Celsius, then scrape it off and place it in plastic bottles with a capacity of 60 ml. It is closed tightly and covered with cellphone in order to preserve it. After that, it is ready to make the concentrations, which are 2%, 5%, and 10%, where 2 grams of Buckthorn extract are weighed, then It is placed inside a beaker with a capacity of 100 ml and then completes 100 ml. As for the 5% concentration, it is done by weighing with a scale 5 grams of the extract and placed inside a beaker and then completing the volume of 100 ml. As for the 10% concentration, it is done by weighing with a scale 10 grams of the extract and placed inside a beaker and then completing the volume of 100 ml then It is ready to treat the stages of the insect, where 10 pairs of all stages of the Habra insect are isolated in each dish with a diameter of 9 ml and a depth of 1.5 ml, with three repetitions. Then the dishes containing the stages of the Habra insect are treated with the extract by spraying them and leaving a period of 10 to 15 minutes to dry, after which they are placed in the incubator. In addition to the control treatment with distilled water and three replicates for all stages of the treated insect for the purpose of comparison, in the same way, 10 pairs of pupae are isolated inside the dish with replicate 3 and treated in the same way as the adult with the extract. Then the rest of the stages of the khapra insect, larvae and eggs, are isolated in the same way as isolating the adult and pupae. As for preparing The alcoholic extract is made by weighing 100 grams of powder, then placing it in a 500 ml beaker, then adding 250 ml of distilled water and 250 ml of ethanol alcohol, then shaking it to homogenize it, then placing it in a refrigerator for a full day, then filtering it using filter paper, then placing it in the oven for a period. 24 hours at a temperature of 65 degrees Celsius, then it is scraped off and placed inside plastic containers with a capacity of 60 ml, closed and covered with cellphone in order to preserve it. After that, it is ready to make the concentrations, which are 2%, 5%, and 10%, in the same way as the previous treatment of the aqueous extract of the leaves *Ziziphus spina-christi*.

3. Results and Discussion

3.1. Effect of Alcoholic and Aqueous Extract of Buckthorn Leaves Ziziphus Spina-Christi on Egg Hatching Rate

Table (1) showed that all concentrations of gave rates of non-hatching of khapra beetle eggs, as the hatching rate at a concentration of 10% was 00.0, at a concentration of 5% it was 00.0, and at a concentration of 2% it was 00.0 on the third day.

The results of Table (1) demonstrated that the alcoholic ethanol extract and the aqueous extract at concentrations of 10%, 5%, and 2% all led to the inhibition of egg hatching. This is due to several reasons, including that the alcoholic and aqueous extracts contain secondary compounds, including phenols, which are toxic, which leads to the inhibition of the egg hatching process and also inhibits The development and growth of the fetus, as it penetrates into the egg, which leads to the eggs not hatching, or it affects the muscle tissue of the fetus, causing the fetus to be unable

to breathe, which leads to death. Or the compounds found in the alcoholic and aqueous extracts act as hormonal agents that hinder the growth of the fetus in the advanced stages of life, which leads to the appearance of deformities that appear in the larval growth stage, or prevents the complete or partial formation of the insect in the other stages of its life, or the compounds present in the alcoholic and aqueous extract cause a disruption in the endocrine glands that control the secretion of the glands of the spherical bodies that secrete the youth hormone. Through hormones, As he pointed out [29,30].

Table 1. Effect of alcoholic and aqueous extract of Buckthorn leaves *Ziziphus spina-christi* on egg hatching rate.

Extract	Concentration/ mm	Time/Day						Extract × Concentration
		3	5	7	9	11	15	
Control		50.00	30.00	20.00	0.00	0.00	0.00	16.66
Alcoholic/ethanol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Watery	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
lsd5% Extract		Time × extract						Extract
Alcoholic/ethanol		0.00	0.00	0.00	0.00	0.00	0.00	0.00
Watery		0.00	0.00	0.00	0.00	0.00	0.00	0.00
lsd5% Focus		Time × focus						Focus
0.02		0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.05		0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.10		0.00	0.00	0.00	0.00	0.00	0.00	0.00
lsd5% Time		0.00	0.00	0.00	0.00	0.00	0.00	
lsd5%								

3.2. Effect of Alcoholic and Aqueous Extract of Buckthorn Leaves (*Ziziphus Spina -Christi*) on the Killing Rate of Third Instar Larvae

Table (2) showed that the highest percentage of killing third-instar larvae was at a concentration of 10% alcohol on the third day, where it reached 36.67 compared to the rest of the treatments, where the killing percentage at a concentration of 5% was 33.33, and at a concentration of 2% it was 23.33. On the same day, the table also showed that there were highly significant differences at 10% concentration, reaching 17.78 compared to the 5% concentration, which reached 16.11 and the 2% concentration, which reached 12.22. It is noted from the table that there is a direct relationship between the concentration and the inhibition rate.

That is, as the concentration increases, the percentage of killing third-instar larvae increases for the concentrations of 2%, 5%, and 10%. There is also an inverse relationship between the number of days and the killing percentage, meaning that the third day at the 10% alcohol concentration is 36.67. The highest percentage of killing of third-instar larvae compared to the fifteenth day, where

It reached 0.00. There is a significant difference between the interventions, as well as in the extract, concentration, and time.

Table 2. Effect of alcoholic and aqueous extract of Buckthorn leaves (*Ziziphus spina-christi*) on the killing rate.

Extract	Concentration/ mm	Time/Day						× Extract Concentration
		3	5	7	9	11	15	
Alcoholic/ethanol	0.02	23.33	20.00	16.67	13.33	0.00	0.00	12.22
	0.05	33.33	26.67	20.00	16.67	0.00	0.00	16.11
	0.10	36.67	26.67	23.33	20.00	0.00	0.00	17.78
Watery	0.02	16.67	16.67	13.33	13.33	0.00	0.00	10.00
	0.05	30.00	20.00	20.00	16.67	0.00	0.00	14.45
	0.10	33.33	23.33	20.00	16.67	0.00	0.00	15.56
lsd5%				3.02*				1.23**
Extract				Time × extract				Extract
Alcoholic/ethanol		31.11	24.45	20.00	16.67	0.00	0.00	15.37
Watery		26.67	20.00	17.78	15.56	0.00	0.00	13.33
lsd5%				1.75**				0.71**
Focus				Time × focus				Focus
0.02		20.00	18.34	15.00	13.33	0.00	0.00	11.11
0.05		31.67	23.34	20.00	16.67	0.00	0.00	15.28
0.10		35.00	25.00	21.67	18.34	0.00	0.00	16.67
lsd5%				2.14*				0.87**
Time		28.89	22.22	18.89	16.11	0.00	0.00	
lsd5%				1.23				

ns There are no significant differences.

* There are significant differences at the probability level 5.0%.

3.3. Effect of Alcoholic and Aqueous Extract of Buckthorn Leaves (*Ziziphus Spina-Christi*) on the Killing Rate of Fifth Instar Larvae

Table (3) showed that there were no significant differences when interacting between extract and concentration. The table also showed that there is no significance between the ethanol alcoholic extract and the aqueous extract. The table showed that the 10% concentration had the highest killing rate compared to the other concentrations, as it reached 16.67, while the 5% concentration reached 15.28, while the 2% concentration reached 10.83. The table also showed that there was high significance between the concentrations. It is noted from the table that there is a direct relationship between the concentration and the inhibition rate. That is, as the concentration increases, the percentage of killing fifth-instar larvae increases for concentrations of 2%, 5%, and 10%. There is also an inverse relationship between the number of days and the killing percentage, meaning that the third day at the 10% alcohol concentration is 40.00, the highest percentage of killing of fifth-instar larvae compared to the fifteenth day, where It reached 0.00. There is a significant difference between the interventions, as well as at the extract, at the concentration, and at the time.

It was noted from the results of Table (2 and 3) that the alcohol-ethanol extract of Buckthorn leaves was superior to the aqueous extract in killing third- and fifth-instar larvae. This is due to the efficiency of ethanol alcohol in extracting the active substances of Buckthorn leaves that negatively affect the khapra insect, leading to greater killing rates. In larvae treated with ethanol alcoholic extract compared to the aqueous extract, this supports what was indicated by, The death of the larvae may be due to their sensitivity to toxic substances found in Buckthorn leaves, and

that these substances work inside the plant as defensive substances to protect the plant, or that these substances cause poisoning of the cells of the digestive tract, which are responsible for absorbing food. It was also noted that there is a direct relationship between the difference in the duration of the larval stage and the increase in the concentration of the alcoholic extract, ethanol or the aqueous extract. He also noticed a difference in the size and shape of the larvae, which leads to the shrinkage of the larvae treated with the alcoholic extract, ethanol or the aqueous extract, and this supports what was pointed out by. These small larvae gave small sized pupae and adults compared to the pupae and adults not treated with the alcoholic Buckthorn leaf extract, ethanol and aqueous extract. Or that the ethanol alcoholic extract and the aqueous extract target the nerve reception sites through an effect on the tissues of the nervous system, causing paralysis, shock, and then rapid death, and this supports what was explained [31-3]. The moulting process is also affected by the khapra insect larvae as a result of hormonal disturbances that affect the moulting, which has a negative impact on the larvae, leading to the death of the larvae.

Table 3. Effect of alcoholic and aqueous extract of Buckthorn leaves (*Ziziphus spina-christi*) on the killing rate of fifth instar larvae.

Extract	Concentration / mm	Time/dAy						× Extract Concentration
		3	5	7	9	11	15	
Alcoholic/ethanol	0.02	23.33	16.67	16.67	13.33	0.00	0.00	11.67
	0.05	36.67	23.33	20.00	16.67	0.00	0.00	16.11
	0.10	40.00	26.67	23.33	20.00	0.00	0.00	18.33
	0.02	16.67	16.67	13.33	13.33	0.00	0.00	10.00
Watery	0.05	26.67	20.00	20.00	20.00	0.00	0.00	14.45
	0.10	30.00	20.00	20.00	20.00	0.00	0.00	15.00
	Isd5%			2.46**				ns
Extract				Time × extract				Extract
Alcoholic/ethanol		33.33	22.22	20.00	16.67	0.00	0.00	15.37
Watery		24.45	18.89	17.78	17.78	0.00	0.00	13.15
Isd5%				1.42**				Ns
Focus				Time × focus				Focus
0.02		20.00	16.67	15.00	13.33	0.00	0.00	10.83
0.05		31.67	21.67	20.00	18.34	0.00	0.00	15.28
0.10		35.00	23.34	21.67	20.00	0.00	0.00	16.67
Isd5%				1.74**				0.71**
Time		28.89	20.56	18.89	17.22	0.00	0.00	
Isd5%				1.01**				

ns There are no significant differences.

* There are significant differences at the probability level 5.0% .

3.4. Effect of Alcoholic and Aqueous Extract of *Ziziphus Spina-Christi* Leaves on the Emergence Rate of *Khabra* Beetle Adults

Table (4) showed that all concentrations of gave the highest rates of non- emergence of *Khabra* beetle adults, as the emergence rate at a concentration of 10% was 00.0, at a concentration of 5% it was 00.0, and at a concentration of 2% it was 00.0.

Table (4) showed that both the alcoholic ethanol extract and the aqueous extract led to the inhibition of the emergence of adults, and this is due to the alcoholic and aqueous extracts containing active compounds that hinder the development of pupae, as they have the ability to enter the skin of pupae and kill adult pupae, especially at the early age of pupae, because It is transparent and thin, and the material penetrates through it, unlike the hardened skin of advanced ages. It has also been observed that the duration of the emergence of adults is prolonged due to

the compounds of Buckthorn leaf extract affecting the nervous system, which leads to sending nerve signals that affect the neurosecretory cells, which causes the endocrine glands to be stimulated to secrete the sebum hormone, which in turn leads to prolonging the catabolism stage and disrupting growth by inhibiting the processes. Various biological diseases, which affect the productivity of the insect, as well as the occurrence of deformities represented by shortening, wrinkling, or lengthening of the wings, or spreading the wings in an unnatural and permanent way, or shortening of the sheathed wings. Or the appearance of a distorted middle stage between the pupae and the adult, or it causes deformation of the khapra insect and the loss of the melanin pigment that is formed inside the cuticle wall through the process of hardening. Or that the khapra insect treated with one of its phase with the extracts delays laying eggs, does not lay eggs, lays few eggs, or lays eggs that do not hatch. This indicates that the alcoholic and aqueous extract of Buckthorn leaves works as a growth regulator or growth inhibitor compared to the control, As he pointed out [38-42].

Table 4. Effect of alcoholic and aqueous extract of *Ziziphus spina-christi* leaves on the emergence rate of Khabra beetle adults.

Extract	Concentration/ mm	Time/Day						Extract × Concentration
		3	5	7	9	11	15	
control		40.0	30.0	30.0	00.0	0.0	0.0	16.66
		0	0	0	0	0	0	
Alcoholic /ethanol	0.02	0.00	0.00	0.00	0.00	0.0	0.0	0.00
	0.05	0.00	0.00	0.00	0.00	0.0	0.0	0.00
	0.10	0.00	0.00	0.00	0.00	0.0	0.0	0.00
	0.02	0.00	0.00	0.00	0.00	0.0	0.0	0.00
	0.05	0.00	0.00	0.00	0.00	0.0	0.0	0.00
Watery	0.05	0.00	0.00	0.00	0.00	0.0	0.0	0.00
	0.10	0.00	0.00	0.00	0.00	0.0	0.0	0.00
Isd5% Extract		Time × extract						Extract
Alcoholic /ethanol		0.00	0.00	0.00	0.00	0.0	0.0	0.00
Watery		0.00	0.00	0.00	0.00	0.0	0.0	0.00
Isd5% Focus		Time × focus						Focus
0.02		0.00	0.00	0.00	0.00	0.0	0.0	0.00
0.05		0.00	0.00	0.00	0.00	0.0	0.0	0.00
0.10		0.00	0.00	0.00	0.00	0.0	0.0	0.00
Isd5% Time		0.00	0.00	0.00	0.00	0.0	0.0	
Isd5%						0	0	

3.5. Effect of Alcoholic and Aqueous Extract of Buckthorn Leaves (*Ziziphus Spina-Christi*) on the Killing Rate of Adults of the Khabra Beetle

Table (5) showed that the binary interaction between the extract and concentration gave the highest percentage of killing of adult khabara, which was at the concentration of 10% and 5% alcohol, as it reached 16.67 on the third day, compared to the concentration of 2% on the third day, where it reached 16.67. The table also showed that there are differences. High significance at a concentration of 10% alcohol, which reached 16.67 compared to a concentration of 2% and 5%, as there was no significance. As for a concentration of 2% and 5% aqueous, it is noted that there is a significant difference, as at a concentration of 2% it reached 12.22, and at a concentration of 5% it reached 15.00. And the table also showed that The alcoholic ethanol extract is better than the aqueous extract, with a high significant difference, as the alcoholic extract reached 16.48, while the aqueous extract reached 14.63. The table also showed that the best concentration is 10%, with a high significant difference compared to the remaining concentrations. It is noted from the table that there is a direct relationship between the concentration and the inhibition rate. That is, as the concentration increased, the rate of killing of adults increased for the concentrations of 2%, 5%, and 10%. There is also an inverse relationship between the number of days and the rate of killing, meaning that the third day at the 10% alcohol concentration is 80.00, the highest rate of killing of adults compared to the fifteenth day, when it reached 0.00. There is a significant difference between the interventions, as well as at the extract, at the concentration, and at the time. It was noted from the results of the table that the alcohol-ethanol extract of Buckthorn leaves was superior to the aqueous extract of Buckthorn leaves. This is due to the alcoholic extract containing compounds, including (phenols, alkaloids, terpenes, and others) that affect the khapra insect, as it leads to the death of the khapra insect through contact and penetration of the active compounds of the extract layer. Cuttlefish through the respiratory openings and flexible areas, and the killing of adult females by the khapra insect, or that the adult lays eggs or does not lay eggs, or lays few eggs, or lays eggs that do not hatch, As he pointed out [43-52].

Table 5. The effect of alcoholic and aqueous extract of Buckthorn leaves (*Ziziphus spina-christi*) on the killing rate of adults of the Khabra beetle.

Extract	Concentration / mm	Time/Day						Extract × Concentration
		3	5	7	9	11	15	
Alcoholic/ethanol	0.02	73.33	13.33	10.00	0.00	0.00	0.00	16.11
	0.05	80.00	10.00	10.00	0.00	0.00	0.00	16.67
	0.10	80.00	10.00	10.00	0.00	0.00	0.00	16.67
	0.02	60.00	6.67	6.67	0.00	0.00	0.00	12.22
Watery	0.05	70.00	10.00	10.00	0.00	0.00	0.00	15.00
	0.10	80.00	10.00	10.00	0.00	0.00	0.00	16.67
	lsd5%	2.19**						0.89**
Extract		Time × extract						Extract
Alcoholic/ethanol		77.78	11.11	10.00	0.00	0.00	0.00	16.48
Watery		70.00	8.89	8.89	0.00	0.00	0.00	14.63
lsd5%		1.26**						0.52**
Focus		Time × focus						Focus
0.02		66.67	10.00	8.34	0.00	0.00	0.00	14.17
0.05		75.00	10.00	10.00	0.00	0.00	0.00	15.83
0.10		80.00	10.00	10.00	0.00	0.00	0.00	16.67
lsd5%		1.55**						0.63**
Time		73.89	10.00	9.45	0.00	0.00	0.00	
lsd5%		0.89**						

ns There are no significant differences.

* There are significant differences at the probability level 5.0%.

Conclusion

Although there are many methods and chemicals in combating the Khabra insect, *Trogoderma granarium*, they are not safe, as there are plant extracts that have an effective effect in combating all the phase of the Khabra insect and are safe for the environment, including the extract of Buckthorn leaves, but it requires time, unlike chemical methods, as it was found that it increases The killing rate of the khapra insect by increasing the concentration of the extract.

References

- [1] Sonia D, Ana M, Graca B, Maria OC. Stored products insects in Portugal – New data and overview Journal of Stored Products Research;2024. Volume 105, February , 102230.
- [2] Mawlood NA, . Abdul-Rassoul MS..NOTES ON TROGODERMA SPECIES (COLEOPTERA,DERMESTIDAE) OF IRAQ Authors;2000. Vol. 9 No. 2.
- [3] Hill DS . Pests of storage foodstuffs and their control. Kluwer Academic Publishers, New York, NY; 2003. p. 1-453.
- [4] Arthur FH, Ondier GO, Siebenmorgen TJ.Impact of *Rhyzopertha dominica* (F.) on quality parameters of milled rice, Journal of Stored Products Research Volume 48 ; 2012. P. 137-142.
- [5] Asrar. Muhammad , Naila Ashraf SM , Hussain, Khuram Zia , Bilal Rasool .Toxicity and repellence of plant oils against *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Trogoderma granarium* (E.) ;2016, Vol. 38, No. 1: 55-63 ref. 39.
- [6] Suleiman RA, Rosentrater KA . Grain storage in developing countries. In: Rosentrater KA (ed) Storage of cereal grains and their products, 5th edn. Woodhead Publishing, Sawston;2022, pp: 113–133.
- [7] Banga Sheetal Km , Nachiket Kotwaliwale, Debabandya Mohapatra, Saroj Kumar Giri..Techniques for insect detection in stored food grains: An overview; 2018, Volume 94, December , : 167-176.
- [8] FAO, Wang D , Saleh NB, Byro A, Zepp R, Demessie ES, LuxtonTP, Ho KT, Burgess MF, White JC, Su Ch,Nano-enabled pesticides for sustainable agriculture and global food security, Cite this article. 2022 ;17: pages347–360 <https://www.fao.org/news/story/en/item/1187738/icode>.
- [9] Payam Azizi Farsani, Nader Sakenian Dehkordi, Rahim Ebrahimi, Alireza Nemati b, Maryam Taghizadeh Dehkordi ..Bioacoustics of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) ; 2024,Volume 27, Issue 1, March 2024, 102189.
- [10] Starratt AN ,and Bond EJ. Recovery of glutathione levels in suscep-tible and resistant strains of *Sitophilus granarius* (L.) (Coleoptera : Cur-culionidae) following methyl bromide treatment. Journal of stored products research;1990, 26 (1) : 39-41.
- [11] Rajendran S. Insect Pest Management in Stored Products Outlooks on Pest Management;2020, 31(1): 24-35.
- [12] Moore J A, Pubantz J .The New United Nations International Organization in the Twenty-First Century Edition3rd Edition First Published First Published Pub. LocationNew York ImprintRoutledg : Edition3rd Edition First Published2022: eBook Published30 November ;2022, Pub. LocationNew York ImprintRoutledge .Pages400 eBook ISBN9781003281535DOI<https://doi.org/10.4324/9781003281535>
- [13] Athanassiou CG, Phillips TW ,Wakil W. Biology and control of the khapra beetle, *Trogoderma granarium*, a major quarantine threat to global food security. Annu. Rev. Entomol;2019., 64 : 131–148.
- [14] Mawlood NA, Abdul- Rassoul MS, Notes on trogoderma species (coleoptera, dermestidae) of Iraq.2000; Vol. 9: No. 2.
- [15] Scheff SD , Arthur FH,† Domingue M J, Myers SW.. Combination Insecticide Treatments with Methoprene and Pyrethrin for Control of Khapra Beetle Larvae on Different Commodities, 2024;15(1), 77. <https://doi.org/10.3390/insects15010077>

- [16] Abedi Z, olizadeh A, Soufbaf M, Hassanpour M, Nodoushan AJ, Akhava HR. Effect of Different Fruit Developmental Stages and Biochemical Metabolites of Pomegranate (Punicaceae) on Life History Parameters of *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) Journal of Economic Entomology, ;2020,113 (2): 964–973.
- [17] Shishehbor PA, Hemmati S. Life Table Parameters and Digestive Enzyme Activity of *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae) on Selected Legume Cultivars Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz ;2022,61357-43311, Iran. 13(7) : 661.
- [18] Attia MA, Wahba TF, Shaarawy N, Moustafa FI, Guedes RN, Dewer Y. Stored grain pest prevalence and insecticide resistance in Egyptian populations of the red flour beetle *Tribolium castaneum* (Herbst) and the rice weevil *Sitophilus oryzae* (L.). J Stored Prod;2020, Res 87:101611.
- [19] Yadav SK, Srivastava C, Sabtharishi S. Phosphine resistance and antioxidant enzyme activity in *Trogoderma granarium* Everts. J Stored Prod ;2020,Res 87:101636.
- [20] Cui K, Zhang L, He L, Zhang Z, Zhang T, Mu W, Lin J, Liu F. Toxicological effects of the fungal volatile compound 1-octen-3-ol against the red flour beetle, *Tribolium castaneum* (Herbst). Ecotoxicol Environ Saf ;2021,208:111597. [Return to ref 2021 in article](#)
- [21] Amjad F, Hasan MU, Ahmad F, Sahi ST. Pyrethroid resistance and selection against *Trogoderma granarium* (Everts) in Punjab, Pakistan. Int J Trop Insect Sci;2022,42:191–202.
- [22] Kumar J, Ramlal A, Mallick D, Mishra V. An Overview of Some Biopesticides and Their Importance in Plant Protection for Commercial Acceptance. Plants, ;2021,10, 1185.
- [23] Gad HA, Al-Anany MS, Atta AA, Abdelgaleil SA. Potential of low application rate combinations of three chitin synthesis inhibitor insecticides with spinosad for the control of *Sitophilus oryzae* on stored wheat. J Stored Prod Res ;2022 ,95:101926
- [24] Shaibani.Elham AS, Al-Ameri.Dawlat, Ali A, Al-Hegami.Mohammed A. The Effect of Zizyphus spina-christi Extracts on hyperglycemia induced by hyperlipidemic diet in Albino rats; 2024, Vol. 2 No. 1
- [25] Dawood,Hend M, Eman Shawky, Menna-Allah Elsayed Zayed, Merna Elsayed Tayea, Doaa A, Ghareeb d, Reham S, Darwish, Metabolomics and chemometrics approaches unravel the metabolic diversity and in-vitro antidiabetic potential of two Zizyphus species. Volume ; 2024,212, June , 118288
- [26] Kavallieratos NG, Nika EP, Skourti A, Spinozzi E, Ferrati M, Petrelli R, Maggi F, Benelli G. *Carlina acaulis* essential oil: a candidate product for agrochemical industry due to its pesticidal capacity. Ind Crops Prod .2022 ;188:1155.
- [27] Wakil W, Kavallieratos NG, Ghazanfar MU, Usman M. Laboratory and field studies on the combined application of *Beauveria bassiana* and fipronil against four major stored-product coleopteran insect pests. Environ Sci Pollut Res.2022 ; 29:34912–34929.
- [28] Sahi, Shaima Najm. Study of the effect of ethyl alcohol and petroleum ether extract of the leaves and seeds of the *Sesbania sesban* L. plant on the different phase of the beetle *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae). Master Thesis. College of Agricultural Engineering Sciences. Baghdad University; 2019, 96 pages.
- [29] Sabet FA, Sabr SH. Evaluation the Efficacy of Ozone and High Temperature to Control Eggs and Pupae Stages Laboratory for Hairy Grain Beetle (khapra) *Trogoderma granarium* Everts Coleoptera Dermestidae: Iraqi Journal of Science. 2015, Volume 56, Issue 3B, Pages 2164-2169.
- [30] Abbas, Najwan Delph. 2016. Evaluation of the effectiveness of the alcoholic extract and volatile oils of the leaves of *Myrtus communis* L. and *Eucalyptus camaldulensis* Dehnh. On some biological aspects of the southern cowpea beetle *Callosobruchus maculatus* (Fab.). Master's thesis, College of Agriculture. Baghdad University; 2016, 110 pages.
- [31] Losic D, Korunic Z. Diatomaceous earth, a natural insecticide for stored grain protection: recent progress and perspectives. In: Losic D (ed) Diatom nanotechnology: progress and emerging applications. Royal Society of Chemistry, Croydon, pp; 2018. 219–247.

- [32] Brunner-Mendoza C, Reyes-Montes MD, Moonjely S, Bidochka MJ, Toriello C. A review on the genus *Metarhizium* as an entomopathogenic microbial biocontrol agent with emphasis on its use and utility in Mexico. *Biocontrol Sci Technol*.2019; 29:83–102
- [33] Trinh DN, Ha TK, Qiu D. Biocontrol potential of some entomopathogenic fungal strains against bean aphid *Megoura japonica* (Matsumura). *Agriculture* .2020; 10:114
- [34] Ramadan M M, Abdel-Hady A A, Awadalla H S, Guedes RN C, Hashem A S. Larval competition in the khapra beetle, *Trogoderma granarium* everts (Coleoptera: Dermestidae) ; *Journal of Stored Products Research*, 2024.Volume 106 , 102278
- [35] Abdullah L M. Effect of feeding on different cereals on some biological parameters of grain moth *Sitotroga cerealella* (Oliv.) (Lepidoptera: Gelechiidae). *The Iraqi J. Agric. Sci.*, 2008 ;39 (1):69-75
- [36] Batta YA, Kavallieratos NG. The use of entomopathogenic fungi for the control of stored-grain insects. *Int J Pest Manag*.2018; 64:77–87.
- [37] Wakil W, Kavallieratos NG, Nika EP, Ali A, Yaseen T, Asrar M . Two are better than one: the combinations of *Beauveria bassiana*, diatomaceous earth, and indoxacarb as effective wheat protectants. *Environ Sci Pollut Res* .2023a; 30:41864–41877.
- [38] Abdelaziz, N. F., H.A. Salem and E.A. Sammour. Insecticidal effect of certain ecofriendly compounds on some scale insects and mealy bugs and their side effects on antioxidant enzymes of mango nurslings *Arch Phytopathol. Plant Protect*. 2014; 47: 1-14
- [39] Sabr, Saadi Hussein Al-Jourani, Reda Saqb and Abdul-Jabbar, Tamader Marawan. The effect of the alcoholic extract of the Eucalyptus plant on the life of the greater waxworm, *Galleria mellonella* L.. *Iraqi Agricultural Sciences Journal* .2004 ; 35 (4): 89-94.
- [40] Uddin MA, Ara N . Temperature effect on the toxicity of six insecticides against red flour beetle, *Tribolium castaneum* (Herbst). *J Life Earth Sci* .2006 ; 1:49–52.
- [41] Wilches DM, Laird RA, Floate KD, Fields PG Control of *Trogoderma granarium* (Coleoptera: Dermestidae) Using High Temperatures, *Journal of Economic Entomology*, 2019 ; Volume 112 (2) :Pages 963–968, <https://doi.org/10.1093/jee/toy379>
- [42] Shakoori FR, Feroz A, Gondal A, Akram S, Riaz T. Impact of λ -cyhalothrin on carbohydrate metabolizing enzymes and macromolecules of a stored grain pest, *Trogoderma granarium*. *Pak J Zoo* . 2018 ; 150:1467–1474.
- [43] Jarjis, Salem Jamil and Al-Jubouri, Abdul Razzaq Younis. Sublethal effects of crude extracts of some medicinal plants on the bean beetle *Trogoderma granarium*, *Iraqi Agriculture Journal*, 2005; 10 (1): 83 – 93
- [44] Hamza A . Toxicity and combined action of some insecticides and clove oil against *Rhyzopertha dominica* in wheat grain. *J Plant Prot Res*. 2018;58:192–201.
- [45] Farsani PA, Dehkordi NS, Ebrahimi R, Nemati Ab, Dehkordi MT . Bioacoustics of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) .2024;Volume 27, Issue 1, 102189.
- [46] Ahmadi E, Khajehali J, Jonckheere W, Van Leeuwen T . Biochemical and insecticidal effects of plant essential oils on insecticide resistant and susceptible populations of *Musca domestica* L. point to a potential cross-resistance risk. *Pest Biochem Physiol*, 2022 ; 184 : 105115 <https://doi.org/10.1016/j.pestbp.2022.105115>
- [47] Akermi S, Smaoui S, Elhadek K, Fourati M, Louhichi N, Chaari M, Chakchouk Mtibaa A, Baanannou A, Masmoudi S, Mellouli L (2022) *Cupressus sempervirens* essential oil: exploring the antibacterial multitarget mechanisms, chemcomputational toxicity prediction, and safety assessment in zebrafish embryos. *Molec*, 2022; 27 : 2630. <https://doi.org/10.3390/molecules27092630>.
- [48] Almadhiy AA, Nenaah GE, Albogami BZ, Shower DM, Alasmari S . *Cupressus sempervirens* essential oil, nanoemulsion, and major terpenes as sustainable green pesticides against the Rice Weevil. *Sustainability* , 2023; 15:8021. <https://doi.org/10.3390/su15108021>
- [49] Guettal S, Tine S, Tine-Djebbar F, Soltani N . Evaluation of *Citrus limonum* (Sapindales: Rutaceae) L. essential oil as protectant against the granary weevil, *Sitophilus granarius* (L.)



- (Coleoptera: Curculionidae). Allel J, 2020; 51(1) : 79– 92. <https://doi.org/10.26651/allelo.j/2020-51-1-1292>
- [50] Guettal S, Tine S, Tine-Djebbar F, Soltani N . Effect of *Citrus limonum* essential oil against granary weevil, *Sitophilus granarius* and its chemical composition, biological activities and energy reserves. Int J Trop Insect Sci, 2020; 41:1531–1541. <https://doi.org/10.1007/s42690-020-00353-y>
- [51] Guettal S, Tine S, Kaouther H, Tine-Djebbar F, Soltani N . Combined effects of Azadirachtin and *Citrus limonum* essential oil against *Sitophilus granarius*: toxicity and Biological activities. Pest Res J , 2021; 33(1):78–86. <https://doi.org/10.5958/2249-524X.2021.00023.6>
- [52] Jameel MAR, Baker SZ, Nawar MH. Control of the Khapra Beetle *Trogoderma granarium* (Everts.) (Coleoptera : Dermastidae) using Nanoparticles of Neem Leaf Extract Published under licence by IOP Publishing Ltd IOP Conference Series: Earth and Environmental Science.2022; Volume 1225, The 3rd International Conference on Agricultural Sciences and Sustainable Environment (ICASE-2022) 15/11/2022 - 16/11/2022 Samawah, Iraq.

Effect of Crop Type and Soil on the Population Density of *Bacillus subtilius* and *Pseudomonas putida* in Some Soil Properties

Israa Kadhim Majed¹ and Jawad Abdul Kadhim Kamal²

^{1,2}College of Agriculture, University of Al-Qadisiyah, Al-Qadisiyah, Iraq.

¹E-mail: agr22.mas10@qu.edu.iq

²E-mail: jawad.alshabbany@qu.edu.iq

Abstract. The study aims to know the effect of crop type and soil on *Bacillus subtilius* and *Pseudomonas putida* bacteria. The experiment was conducted in autumn season of 2023 in Al- Diwaniyah Governorate - Afak District fields. The experiment was designed according to the Randomized Complete Block Design (R.C.B.D) with three replicates. The treatments were distributed randomly. Where one factor was used in the soil (silty, sandy clay, clay and sandy) and the seeds of okra, corn, Sorghum and cowpea were cultivated in four types of soils: silty, sandy clay, clay and sandy. The averages of the treatments were compared using the least significant difference (LSD) and at a probability level of 5%. The results showed that the treatments showed a significant increase in *Bacillus subtilius* and *Pseudomonas putida* bacteria and the properties of available and absorbed phosphorus compared to other soils. The highest value was for the sandy clay soil treatments with the other soil treatments in each of the okra, corn, Sorghum and cowpea crops (15.00, 16.33, 13.67, 14.67 $\times 10^6$ CFU $^{-1}$ g dry soil), (9.67, 13.33, 11.00, 12.00 $\times 10^6$ CFU $^{-1}$ g dry soil), (14.33, 67.14, 67.15, 33.15 $\times 10^6$ CFU $^{-1}$ g dry soil), (10.00, 00.12, 33.11, 67.12 $\times 10^6$ CFU $^{-1}$ g dry soil), respectively, as well as available and absorbed phosphorus (26.26, 68.31, 75.30, 87.31 mg P kg $^{-1}$ soil), (22.20, 77.27, 44.27, 61.28 mg P kg $^{-1}$ soil), (298.3, 165.3, 723.2, 893.2 mg P pot $^{-1}$), (714.2, 604.2, 302.2, 580.2 mg P pot $^{-1}$) respectively.

Keywords. *Bacillus subtilius*, *Pseudomonas putida*, Available phosphorus, Absorbed phosphorus.

1. Introduction

The rhizosphere is the area surrounding and affected by the roots, where biological activities increase [1]. In this area, the state of coexistence between microorganisms and roots and the mutual activities between them appear, which directly affect plant growth and crop production. *Bacillus subtilius* is one of the most important types of root zone bacteria, characterized by various biomechanics that stimulate plant growth by increasing its supply of nutrients and the process of preparing phosphorus for the plant, one of the most important activities through which organic acids are secreted by bacteria and works to dissolve insoluble phosphorus and release it from inorganic phosphate compounds in the soil, such as dicalcium and triphosphate, in addition to phosphate rock [2]. Mycorrhizal fungi are one of the most common soil fungi that directly affect the plant host through different mechanisms. For example, they directly affect the

absorption of phosphorus and mineral elements, increase the plant's resistance to water stress, and protect against pathogens. The importance of fungi has increased over time because they conflict with different plant species, which necessitates the identification and study of these fungi, including the genera, species, and isolates that live in agricultural soils [3]. The relationship between fungi and plants is a symbiotic relationship because it benefits both fungi and plants. The role of fungi in preparing the plant with major and minor elements also plays a role in protecting the plant from pathogens present in the soil and its role in increasing the plant's ability to withstand stress and drought conditions [4]. *Pseudomonas putida* is among the microorganisms that have received great attention in the field of biological resistance to *S. sulphate*, which is one of the bacteria that stimulate plant growth, and has shown an important ability to eliminate the activity of many plant pathogens [3]. The okra crop *Hibiscus esculentus* L. is also used in many industries, including sugarcane juice filtration as an adhesive for paper and canning stored fruits as an anti-transpiration material, as these extracts reduce moisture loss because they contain pectin and wax-like substances [4]. Sorghum (*Sorghum bicolor* L.) is one of the important grain crops and is used in the dye and wax industry [5]. Corn (*Zea mays* L.) belongs to the Poaceae family and is one of the most important crops of this family, as it comes after wheat and rice in terms of economic importance due to its multiple uses [6]. The cowpea crop (*Vigna unguiculata* L.) is widely grown for human consumption, and it is one of the most nutritious legumes grown in dry and semi-dry areas [7]. The study aimed to:

- Effect of soil type on the population density of *Aspergillus niger* fungus.
- Effect of soil type on soil fertility and chemical properties and crop growth.

2. Materials and Methods

A pot experiment was carried out for the autumn agricultural season 2023-2024 on one of the farms located in Afak district for four types of soil (silty, sandy clay, clay and sandy) to grow four types of crops (Okra, corn, Sorghum and cowpea).

2.1. Experimental Design

The experiment was designed according to the randomized complete block design (RCBD) (3×4). The experiment included one factor in the soil (silty, sandy clay, clay, sandy) and three replicates for each treatment. The treatments were distributed randomly to the experimental units, so that the number of units became 48 experimental units.

Table 1. Shows the physical and chemical properties before planting.

traits	silty	Sandy clay	clay	sandy	units
Ec 1:1	1.41	2.5	2.30	2.16	Ds.m ⁻¹
pH 1:1	7.60	7.8	7.77	7.4	
CEC	24.00	15.30	24.5	10.7	Centimoles charge kg ⁻¹ soil
available Nitrogen	28.00	20.12	32.50	24.04	mg kg ⁻¹ soil
textuer					
Sand	120.00	255	88	806.00	g kg ⁻¹ soil
Silt	830.0	325	244	142.2	
Clay	50.00	420	668	52.00	

2.2. Biological and Chemical Analyses

It was estimated by the method of dilution and counting in plates according to the method [8]. 10 g of soil was weighed and placed in a bottle containing 90 ml of distilled water. The mixture was

shaken well for 10 minutes, then transferred to another bottle containing 90 ml of distilled water. The dilution process continued to obtain the dilution -6 10, then 1 ml of it was transferred to Petri dishes containing the dilution medium Pikovskaya Medium for the growth of *Bacillus subtilis* bacteria. The numbers of *Pseudomonas Putida* bacteria were calculated by the method of dilution and counting in plates using the diagnostic medium *Pseudomonas* Agar Base prepared by the company (HMEDIA India). And placed in the incubator at a temperature of 28 C 0 then taken out to count the growing colonies.

2.3. Absorbed Phosphorus in Vegetative Growth of the Plant

2.0 g of powdered vegetative part of the plants were taken and digested with concentrated sulfuric acid and concentrated perchloric acid and the percentage of phosphorus in the plant was measured as shown in (Haynes 1980) and the absorbed phosphorus was calculated.

$$\text{Absorbed phosphorus (mgP pot}^{-1}\text{)} = \text{Dry weight of the vegetative part (g)} \times \text{Percentage of phosphorus in the vegetative part} \times 1000.$$

3. Results and Discussion

3.1. Effect of Crop Type and Soil on the Number of Bacteria *Bacillus subtilis* (10^6 CFU⁻¹ g dry soil) in the Flowering Stage

The results of the statistical analysis in Table (2) showed significant differences in the average numbers of *Bacillus subtilis* bacteria in the flowering stage between the types of silty, sandy clay, clay and sandy soils and the crops Okra, corn and Sorghum, where the sandy clay soil reached the highest value for the crop Okra, corn, Sorghum and cowpea the highest value (15.00, 16.33, 13.67, 14.67 $\times 10^6$ CFU⁻¹ g dry soil). This is due to phosphorus in the extension of the roots and the increase in the density of root hairs, which is a medium colonized by microorganisms. The relationship between enzyme activity and the level of phosphorus in the soil depends on the characteristics of the soil and the requirements of the plant and microorganisms. In addition, phosphorus is of great importance to the cells of microorganisms, as it enters into the formation of nucleic acids, phospholipids and energy compounds such as ATP [9,10] While the silty soils gave the values for Okra crops Corn, Sorghum and cowpea were 13.33), 67.15, 33.12, 33.11 $\times 10^6$ CFU⁻¹ g dry soil) respectively. Clay soil did not differ significantly from silty soil in cowpea crops.. Then came the clay soils which gave values (00. 11, 33.13, 00.10, 33.10) $\times (10^6$ CFU⁻¹ g dry soil) respectively. They did not differ significantly from cowpea crop. As for sandy soils, they reached the lowest average values (8.67, 67.9, 33.6, 67.6 $\times 10^6$ CFU⁻¹ g dry soil) respectively. This is due to the fact that soils with high moisture content have the ability to release phosphorus more than soils with low content. Also, available phosphorus was observed to increase significantly with increasing moisture content from (75 to 100)% of field capacity [11]. Also, increasing the moisture content of the soil helped increase the form of dissolved, available, organic and total phosphorus in sandy soil, which contributes to improving the physical and biological properties [12].

Table 2. Effect of crop type and soil on the number of bacteria *Bacillus subtilis* (CFU⁻¹ g dry soil) at the stage.

Soil Type	Okra	Corn	Sorghum	Cowpea
silty	13.33	15.67	12.33	11.33
Sandy clay	15.00	16.33	13.67	14.67
clay	11.00	13.33	10.00	10.33

Sandy	8.67	9.67	6.33	6.67
LSD 0.05	2.47	1.67	1.79	1.20

3.2. Effect of Crop Type and Soil on the Number of *Bacillus subtilis* Bacteria (10^6 CFU⁻¹ g dry soil) at the End of the Season Stage

The results of the statistical analysis in Table (3) showed that there were significant differences in the average numbers of *Bacillus subtilis* bacteria at the end of the season stage between the types of silty, sandy clay, clay and sandy soils and the crops Okra, corn and Sorghum, where the sandy clay soil of the crops Okra, corn, Sorghum and cowpea reached the highest value (9.67, 13.33, 11.00, 12.00×10^6 CFU⁻¹ g dry soil) and the reason for this is the ability of *Bacillus subtilis* species to provide the necessary phosphorus to build the root system and the activity of microorganisms in the root zone, which increases the absorption of nitrogen by the root [13]. The effectiveness of *Bacillus subtilis* bacteria in dissolving phosphorus and converting it to forms ready for absorption, which provides appropriate concentrations for root growth and development and enhancing the construction of its tissues, since phosphorus is included in the structures of the growing tips of the root [14].

Then came the silty soils, which gave values for Okra, corn, white and cowpea crops (8.33, 33.12, 33.9, 33.9×10^6 CFU⁻¹ g dry soil). This is due to their ability to dissolve phosphorus from insoluble sources, which is an essential element in building the root system and its branches [15]. As for the clay soil, it gave values (67.6, 67.9, 67.6, 67.7×10^6 CFU⁻¹ g dry soil) respectively.

As for sandy soil, the lowest average values were (33.4, 67.4, 00.3, 33.3×10^6 CFU⁻¹ g dry soil) respectively. From the results, we notice a decrease in the number of bacteria at the end of the season for all treatments. This decrease may be due to root cells, as root secretions have a significant effect in increasing the activity of microorganisms in the soil, especially bacteria, and their numbers decrease during the advanced stages of the plant's life, where the secretions are compounds that are not easily decomposed or food sources decrease [16].

Table 3. Effect of crop type and soil on the number of *Bacillus subtilis* bacteria (CFU/gm soil⁻¹ dry) at the end of the season stage.

Soil Type	Okra	Corn	Sorghum	Cowpea
silty	8.33	12.33	9.33	9.33
Sandy clay	9.67	13.33	11.00	12.00
clay	6.67	9.67	6.67	7.67
Sandy	4.33	4.67	3.00	3.33
LSD 0.05	1.85	1.33	1.60	1.60

3.3. Effect of Crop Type and Soil on the Number of *Bacteria Pseudomonas Putida* (10^6 CFU⁻¹ g dry soil) in the Flowering Stage

The results of the statistical analysis in Table (4) showed that there were significant differences in the average numbers of bacteria *Pseudomonas Putida* in the flowering stage between the types of silty, sandy clay, clay and sandy soils and the crops Okra, corn and Sorghum, where the sandy clay soil of the crops Okra, corn, white and cowpea reached the highest value (14.33, 67.14, 67.15, 33.15×10^6 CFU⁻¹ g dry soil) respectively. This increase may be due to the availability of energy sources and ready phosphorus, which are important in increasing the activity and reproduction of organisms added to the soil, which leads to the secretion and formation of some organic acids that increase the availability of some nutrients in the soil. The addition of biofertilizer also has an encouraging effect on plant growth through its production of effective stimulating substances such as vitamins, nicotinic acid, acetic acid (IAA) and gibberellins. These

substances contribute to seed germination and increased root and vegetative growth, which is positively reflected in increasing the dry weight of the plant [17,18,19]. The silty soil did not differ significantly from the sandy clay soil in corn crops, followed by the clay soil which gave (10.00, 33.11, 00.11, 00.11×10^6 CFU⁻¹ g dry soil) respectively, and the clay soil did not differ significantly from silty soil in corn crops. while the sandy soil reached the lowest average value (67.7, 00.7, 67.7, 33.7 $\times 10^6$ CFU⁻¹ g dry soil) respectively. The reason for this is that the relationship between the plant and the phosphorus-dissolving bacteria is a synergistic relationship in nature, as the bacteria provide the plant with dissolved phosphorus and in turn provide the bacteria with carbon compounds, mainly sugars, which are important for bacterial growth [20]. Studies also indicated the ability of *Pseudomonas* bacteria to dissolve insoluble phosphorus. Soluble in both basic and acidic soils, and the effect of these bacteria in dissolving phosphorus appears through the secretion of the phosphatase enzyme and the production of organic acids such as formic acid, acetic acid, citric acid, and lactic acid. *Pseudomonas* bacteria have another advantage in their high ability to colonize the rhizosphere of many crops [21]. [22] explained that adding phosphorus-dissolving bacteria, including *Pseudomonas*, led to a positive increase in the dry weight of the root system and the dry weight of the vegetative system of tomato plants. The results of the experiment [23] also showed that when squash plants were inoculated with *Pseudomonas* bacteria, it leads to a significant increase in the content of leaves of phosphorus, potassium, copper, zinc, and some hormones such as auxins, cytokines, and gibberellins compared to uninoculated treatments. The IAA secreted by *Pseudomonas Putida* bacteria had a major role in the growth and elongation of roots in the bean plant [24]. The activity of the phosphatase enzyme in three soils to determine the effect of temperature on the activity of the enzyme was found that the activity of the enzyme increases with increasing temperature from (10-60) degrees Celsius, but the activity of the enzyme decreases when the temperature increases from (60-80) degrees Celsius. [25] In a study conducted by [26] on the activity of the alkaline phosphatase enzyme in the Rhizosphere area of the bean plant when adding levels of molybdenum (12.6.0) g. kg⁻¹ seed and adding lime (3.2.0) tons. ha⁻¹ and inoculating with rhizobium, it gave the highest value for the activity of the alkaline phosphatase enzyme in the treatment added to it 12 g. kg⁻¹ seed molybdenum and 3 tons. ha⁻¹ lime compared to the control treatment.

Table 4. Effect of crop type and soil on the numbers of *Pseudomonas Putida* bacteria (CFU⁻¹ g dry soil) at the end of the season stage.

Soil Type	Okra	Corn	Sorghum	Cowpea
silty	12.00	13.00	13.67	12.67
Sandy clay	14.33	14.67	15.67	15.33
clay	10.00	11.33	11.00	11.00
Sandy	7.67	7.00	7.67	7.33
LSD 0.05	1.60	1.79	1.53	1.37

3.4. Effect of Crop Type and Soil on the Number of Bacteria *Pseudomonas Putida* (106 CFU⁻¹ g dry soil) at the End of the Season

The results of the statistical analysis in Table (5) showed significant differences in the average numbers of *Pseudomonas Putida* bacteria at the end of the season stage between the types of silty, sandy clay, clay and sandy soils and the crops Okra, corn and Sorghum, as the sandy clay soil of the crops Okra, corn, white and cowpea reached the highest value (10.00, 00.12, 33.11, 67.12 $\times 10^6$ CFU⁻¹ g dry soil) respectively. It was noted that at the end of the season stage, the number of bacteria decreased, and this reason is due to the competition for energy sources between the

microorganisms themselves, in addition to the competition for nutrients in the soil and between the growing plants and microorganisms, which leads to a reduction in their numbers at the end of the season stage, as well as a decrease in the activity of the plant roots and microorganisms at this stage, and this result is consistent with what was reached by [27]. Then came the silty soil which gave (67).8, 33.10, 67.9, 67.10×10^6 CFU⁻¹ g dry soil) respectively silty soil did not differ significantly from the sandy clay soil in okra, corn and cowpea crops, followed by the clay soil which gave (33.7, 67.8, 33.7, 67.8×10^6 CFU⁻¹ g dry soil) respectively. The clay soil did not differ significantly from silty soil in okra, corn and cowpea.. As for the sandy soil, it reached the lowest average value (67).3, 33.3, 67.2, 00.3×10^6 CFU⁻¹ g dry soil) respectively.

Table 5. Effect of crop type and soil on the number of *Pseudomonas Putida* bacteria (CFU/gm soil⁻¹ dry) at the end of the season stage.

Soil Type	Okra	Corn	Sorghum	Cowpea
silty	8.67	10.33	9.67	10.67
Sandy clay	10.00	12.00	11.33	12.67
clay	7.33	8.67	7.33	8.67
Sandy	3.67	3.33	2.67	3.00
LSD 0.05	1.60	2.21	1.45	2.23

3.5. Effect of Crop Type and Soil on Available Phosphorus Content in Soil (mg P kg⁻¹ soil) in Flowering Stage

The results of the statistical analysis in Table (6) showed significant differences in averages in the flowering stage between the types of silty, sandy clay, clay and sandy soils and the crops Okra, corn and Sorghum, where the sandy clay soil of the crops Okra, corn, white and cowpea reached the highest value (26.26, 68.31, 75.30, 87.31 mg P kg⁻¹ soil) respectively. This may be due to the fact that the biofertilizer has bacterial isolates active in dissolving insoluble phosphorus compounds and their ability to release phosphorus from phosphate fertilizers added to the soil through different mechanisms, the most important of which is the production of organic acids [28,29,30]. Followed by the silty soil which gave values of 24.41, 40.28, 76.28, 02.29 mg P kg⁻¹ soil) respectively. The competition of PSB with other bacteria and sometimes with plants to represent phosphorus in the soil results in the accumulation of larger amounts of phosphorus in organisms that release this stored phosphorus to the soil when exposed to stress conditions or after the death of these organisms so that plants and other organisms can benefit from the available phosphorus in the soil and meet their phosphorus requirements [31] while the clay soil gave values of 73.22, 71.24, 61.25, 67.25 mg P kg⁻¹ soil) respectively. As for the sandy soil, it reached the lowest average value of 72.19, 11.20, 64.21, 74.20 mg P kg⁻¹ soil) respectively. Effect of crop type and soil on available phosphorus content in soil (mg P kg⁻¹ soil) at the end of the season stage.

The results of the statistical analysis in Table (7) showed that there were significant differences in averages at the end of the season stage between the types of silty, sandy clay, clay and sandy soils and the crops Okra, corn and Sorghum, as the sandy clay soil for the crops Okra, corn, white and cowpea reached the highest value (22.20), 77. 27, 44.27, 61. 28 mg P kg⁻¹ soil) respectively. Despite the decrease in the percentage of phosphorus at the end of the season compared to the flowering stage due to the advanced age of the plant, this is due to the role of bacteria in dissolving and preparing phosphorus as well as their secretion of growth-stimulating substances that improve plant growth and the formation of a dense root system, which leads to an increase in the absorption capacity of nutrients, including phosphorus [32]. Followed by the silty soil, which gave values of 19.35, 88.24, 21.24, 76. 25 mg P kg⁻¹ soil) respectively. silty soil did not differ

significantly from the sandy clay soil in corn crops. This is because *Pseudomonas* bacteria work to increase the availability of phosphorus in the soil in addition to their work in producing growth regulators and developing the root system, which leads to increased absorption of the phosphorus element [33,34]. While the clay soil gave values (19). 18, 78.21, 05. 20, 93. 21, mg P kg⁻¹ soil) respectively. The clay soil did not differ significantly from silty soil in okra and corn crops.. This is due to the activity of living organisms, as *Bacillus subtilis* and *Pseudomonas putida* bacteria secrete growth regulators such as IAA and GA₃, which encourage the growth of the vegetative and root systems, making them capable of absorbing nutrients [35].As for sandy soil, the values for the averages were 75 (15, 49.16, 85.14, 35. 16 mg P kg⁻¹ soil) respectively.

Table 6. Effect of crop type and soil on the content of available phosphorus in the soil (mg P kg⁻¹ soil) at the flowering stage.

Soil Type	Okra	Corn	Sorghum	Cowpea
silty	24.41	28.40	28.76	29.02
Sandy clay	26.26	31.68	30.75	31.87
clay	22.73	24.71	25.61	25.67
Sandy	19.72	20.11	21.64	20.74
LSD 0.05	1.40	3.07	1.24	1.95

Table 7. Effect of crop type and soil on the content of available phosphorus in the soil (mg P kg⁻¹ soil) at the end of the season stage.

Soil Type	Okra	Corn	Sorghum	Cowpea
silty	19.35	24.88	24.21	25.76
Sandy clay	22.20	27.77	27.44	28.61
clay	18.19	21.78	20.05	21.93
Sandy	15.75	16.49	14.85	16.35
LSD 0.05	1.35	3.04	3.05	2.71

The effect of crop type and soil on phosphorus absorbed in the vegetative part at the flowering stage (kg P h⁻¹) The results of the statistical analysis in Table (8) showed that there were significant differences in averages at the flowering stage between the types of silty, sandy clay, clay and sandy soils and the crops Okra, corn and Sorghum, as the sandy clay soil for the crops Okra, corn, white and cowpea reached the highest value (298.3, 165.3, 723.2, 893.2 mg P pot⁻¹) respectively. It was followed by the silty soil, which gave values (2.966, 882.2, 568.2, 635). 2 mg P pot⁻¹) respectively. silty soil did not differ significantly from sandy clay soil in Sorghum crops.. This may be due to the ability of *Bacillus* bacteria to increase the availability of nutrients to the plant due to the growth regulators it secretes such as gibberellins and cytokinins [36]. In addition, the presence of *Bacillus* bacteria that dissolve phosphate in the root environment contributes to increasing the available phosphorus in the soil and thus encourages the absorption of phosphorus by the plant [37]. The dissolution of phosphorus is one of the important mechanisms in the soil geochemical cycle that enhances plant growth [38] by encouraging the branching of roots to root hairs, and thus increasing the density of roots, which leads to increasing the capacity of the roots to absorb phosphorus. The increase in the levels of added mineral fertilizer led to an increase in the availability of phosphorus that can be absorbed by the roots, which in turn is reflected in the concentration of phosphorus in the plant, as phosphorus strengthens and activates the root system of plants, thus increasing the absorption of phosphorus [39,40], while the soil gave The clay soil values were 563.2, 459.2, 227.2, 363.2 mg P pot⁻¹) respectively. As for the sandy soil, the lowest average value was 842.1, 727.1, 634.1, 915.1 mg P pot⁻¹) respectively.

Table 8. Effect of crop type and soil on the content of phosphorus absorbed in the vegetative part at the flowering stage (mg P pot^{-1}).

Soil Type	Okra	Corn	Sorghum	Cowpea
silty	2.966	2.882	2.568	2.635
Sandy clay	3.298	3.165	2.723	2.893
clay	2.563	2.459	2.227	2.363
Sandy	1.842	1.727	1.634	1.915
LSD 0.05	0.207	0.226	0.236	0.127

3.6. Effect of Crop Type and Soil on Phosphorus Absorbed in the Vegetative Part at the End of the Season Stage (mg P pot^{-1})

The results of the statistical analysis in Table (9) showed that there were significant differences in averages at the end of the season between the types of silty soils, sandy clay, clay and sandy soils, and the crops Okra, corn and Sorghum, where the sandy clay soil for the crops Okra, corn, white and cowpea reached the highest value 714.2, 604.2, 302.2, 580. 2 mg P pot^{-1}) respectively. The reason for the decrease in the average absorbed phosphorus at the end of the season compared to the absorbed at the flowering stage is due to its transfer from the vegetative part to the grains. This is due to the fact that phosphate-dissolving bacteria work in dissolving and preparing phosphorus through the processes of mineralizing organic phosphorus in the soil and dissolving insoluble phosphate compounds in the soil solution, which leads to an increase in its absorption in the plant. The effect of the added organisms appears through their efficiency in producing organic acids and secreting the phosphatase enzyme, which affects the increase in the availability of phosphorus in the soil and its absorption by the plant, as indicated by [41]. Then came the silty soil, which gave values of 2.348, 311.2, 070.2, 269.2 mg P pot^{-1}) respectively. While the clay soil gave values of 165.2, 042.2, 778.1, 020.2 mg P pot^{-1}) respectively. As for sandy soil, the lowest average value was 303.1, 281.1, 172.1, 486.1 mg P pot^{-1}) respectively.

Table 9. Effect of crop type and soil on the content of phosphorus absorbed in the vegetative part at the end of the season stage (mg P pot^{-1}).

Soil Type	Okra	Corn	Sorghum	Cowpea
silty	2.348	2.311	2.070	2.269
Sandy clay	2.714	2.604	2.302	2.580
clay	2.165	2.042	1.778	2.020
Sandy	1.303	1.281	1.172	1.486
LSD 0.05	0.181	0.139	0.190	0.160

References

- [1] Sylvia, D.M.; J.J.Fuhrmann; P.G.Hartel and D.A. Zuberer .2005. Mycorrhizal symbioses, in : Principles and Applications of soil. Microbiology, eds., 2td ed., Pearson, Prentice Hall, New Jersey, pp. 263-282.
- [2] R Hindersah, M R Setiawati, B N Fitriatin, P Suryatmana, P Asmiran, Chemical characteristics of organic-based liquid inoculant of Bacillus spp., IOP Conf. Series: Earth and Environmental Science 393 (2019) 012005.
- [3] Cherr, C.M.: J.M.S. Scholbere and R. Mesorley . 2006. Green manure approaches to crop production. Agronomy J. 98:302-319.
- [4] Salah Mahdi Laibi and Jawad Abdul Kadhim Kamal.2023. Effect of T. harzianum and G. mosseae Biological Inoculation and Phosphate Rocks on the Availability of NPK in the Rhizosphere of

- Barley Crop (*Hordium Vagari* L.). Fifth International Conference for Agricultural and Environment Sciences. 169.224.86.57.
- [5] Tabarak Rahim Shuppar and Jawad Abdl-Kadhim Kamal.2021. The Effect of Biological Inoculation of *Azotobacter chroococcum* and *Glomus mosseae* on Growth and Yield of *Zea mays* L. under Different Levels of Water Stress. 2nd Virtual International Scientific Agricultural Conference. 169.224.86.57.
- [6] Lottmann, J., H. Hener, J. Devries, A. Mahn, K. During, W. Wackernagel, K. Smalla and G.Berg.2000.Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community.FEMS Microbiol. Ecol.33(1).41 -49.
- [7] Sathish Kumar, D. Eswar Tony, A. Praveen Kumar, K. Ashok Kumar, D. Bramha Srinivasa Rao, Ramarao Nadendla. 2013. A review on: *Abelmoschus esculentus* (Okra). International research Journal of Pharmaceutical and Applied Science (IRJPAS). Int. Res. J. Pharm. App Sci., 2013; 3 (4): 129-132.
- [8] Rampho, E. T. 2005 . National be barium, Pretoria, South Africa. Rane, J. Lakkineni, K.C. Kumar, P .A And Abrol, Y .P.1995. Salicylic acid protects nitrate reductase activity of corn leaves. Plant physiol. biochem. 22 : 119-121.
- [9] Orhum, G.E., 2013. Maize for Life. Int .J. Food Sci. and Nut. Eng.,3(2)13-16.
- [10] Carvalho, M.; Muñoz-amatrián, M.; Castro, I.; Lino-neto, T Matos, M.; Egea-cortines, M.; Rosa, E.; Close, T.; Carnide of Iberian cowpeas compared to world-wide cowpea accessions using high V. 2017. Genetic diversity and structure of Peninsula density SNP markers, BMC Genom. 18. 891.
- [11] Black, C. A. 1965b. Methods of soil Analysis part (2). Chemical and Microbiological Properties. Am. Soc. Agron INC. Publisher, Madison Wisconsin, U.S.A
- [12] Behravan, H. R.; R. Khorassani : A. Fotovat : A. A. Moezei and M. Taghavi 2019. The Effect of Humic Acid and Phosphorus Fertilizer on Phosphatase Enzymes, Active Carbon and Available Phosphorus in Sugarcane Rhizosphere. Iran Water and Soil Research, Volume 50.
- [13] Touhami, D. : R. W. Dowell and L. M. Condron .2020. Role of Organic Anions and Phosphatase Enzymes in Phosphorus Acquisition in the Rhizospheres of Legumes and Grasses Grown in a Low Phosphorus Pasture Soil. Plants, 9, 1185; doi:10.3390/plants9091185.
- [14] Al-Ansari, Abdul Mahdi Saleh, Sabah Shafi Al-Hadi, and Rashad Adel Omran. 2012. The effect of soil moisture and depth on some physical properties of soil and growth of barley (*Hordeum vulgare* L.) Basra Journal of Agricultural Sciences 124-138: (1) 25.
- [15] Eissa, M. A.: M. Nafady: H. Raghe and K.Attia .2013. Effect of Soil Moisture and Forms of Phosphorus Fertilizers on Corn Production under Sandy Calcareous Soil. Word Applied Sci. J. 26 (4):540-547.
- [16] Karlidag, H., Esitken, A., Yildirim, E., Donmez, M.F., Turan, M., 2010. Effects of plant growth promoting bacteria on yield, growth, leaf water content, membrane permeability and ionic composition of strawberry under saline conditions. J. Plant Nutr. 3434-45.
- [17] Manaf, Dunya Faeq and Mohammed Abdul Rabiei 2015 The effect of adding partially acidified phosphate rock and compost on phosphorus availability and wheat growth. Al-Qadisiyah Journal of Agricultural Sciences. Issue /1, Volume /5.
- [18] Lim, J.H.& Kim, S.D. 2009. Synergistic plant growth promotion by the indigenous auxins-producing PGPR *Bacillus subtilis* AH18 and *Bacillus licheniformis* K11 Journal Of The Korean Society For Applied Biological Chemistry 52: 531-538.
- [19] Al-Hamadi, Iman Al-Sadiq Mansour 2014. Identifying the effect of different plant growth on the microbial community in both soil and rhizosphere (rice and sapphire). Assiut Journal of Agricultural Sciences, Volume 45, Issue 5.
- [20] Zahir, A.; Z.M. Arshad and F. Frankenberg. 2004. Plant growth promoting rhizobacteria: Advances in Agronomy 81:97-168.
- [21] El-Kholi, M.A. S. El-Ashry and A.M. Gomam . 2005. Biofertilization of maize crop and its impact on yield and grains nutrient content under low rates of mineral nutrient content under low rates of mineral fertilizers. J. Applied Science Res. 1 (2):117-121.

- [22] Yazdani, M.: M. K. Bahmanyar; H. Pirdashti: M.A . Esmale. 2009. Effect of phosphate solubilization microorganism (PSM) and plant growth promotion rhizobacteria (PGPR) on yield componenets of corn (*Zea mays* L.): World Academy of Science,Engineering and Technology.37:90-92.
- [23] Perez. E, Sulbaran, M.. Ball, M. M.. and Yarzabal. L. A. 2007. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biology and Biochemistry*, 39(11), 2905-2914.
- [24] Bano. N... and Musarrat, J. 2003. Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential potential biocontrol agent.*Current microbiology*, 46(5), 0324-0328.
- [25] Turan, M., Ataoglu, N., and Sahin, F. 2007. Effects of *Bacillus* FS-3 on growth of tomato (*Lycopersicon esculentum* nplants and availability o phosphorus in soil. *Plant Soil and Environment*. 53(2), 58-64 USDA 1954, Diagonosis and improvement of saline and alkaline soils Agric. Hand book, No. 60
- [26] Al-Khafaf, Alaa Abdul Ali, Samira Kazim Hamid, and Zainab Rahman Jassim. 2007. The effect of treatment with a preparation containing *Pseudomonas fluorescens* and *Bacillus cere* bacteria on the content of some nutrients and hormones in zucchini leaves. *Iraqi Journal of Agricultural Sciences*, 33(3): 37-42
- [27] Patten, C. L., and Glick, B. R 2002, Role of *Pseudomonas putide* indoleacetic acid in development of t the host plant root system. *Appl. Environ. Microbiol.*, 68(8), 3795-3801.
- [28] Al-Ansari , A. M .S ; M.A . Abdul Kareem and L.A. Omar . 1999 . Characteristics of enzymes in recently reclaimed land : II Alkaline and acid phosphatases activity . *Iraqi .Agric .Sci .* 30 (2): 563-572.
- [29] Joachim , H.J.R ; M.S , Bambara and P.A .Nadakidemi . 2010 . Rhizosphere phosphatase enzyme activities and secondary metabolites in plants as of affected by the supply of rhizobium , lime and molybdenum in *phaseolas vulgaris* L.Aus.J.Crop .Sci . 4(8) : 590-597.
- [30] Al-Khalil, Shireen Muzaffar Ali. 2011.. The effect of integration between mineral, organic and biological fertilization on the productivity of tomato crop (*Lycopersicon esculentum* Mill)) in plastic houses. Master's thesis. Department of Soil Sciences and Water Resources, College of Agriculture - University of Baghdad.
- [31] Al-Assafi, Idham Ali Abdul. 2002. Using microbial technology to increase the availability of phosphorus and other elements from phosphate rock. PhD thesis, College of Science, University of Anbar.
- [32] Al-Shater, Muhammad Saeed and Muhammad Manhal Al-Zaghbi and Mustafa Ahmed Al-Balkhi. 2007. Study of the joint effect of *Rhizobium* bacteria and phosate-dissolving microorganisms on the dissolution of phosphate rock and the productivity of soybean plants. *Basil Al-Assad Journal of Engineering Sciences* 23: 36-56.
- [33] Turan , M. ; N . Ataoglu and F. Sahin . 2007 . Effects of Bacllus S-3 on groeth of tomoato (*Lycopersicon esculentum* Mill) plants and availability of phosphorus in soil . *plant Soil Environ* .53 (2): 58-64 .
- [34] Walpolo, B.C. and M.H. yoon. 2012. prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soil. *African journal of Microbiology Research*.6(37). 6600-6605.
- [35] El-Komy, H. 2004. Coimmobilization of *Azospirillum lipoferum* and *Bacillus megaterium* for successful phosphorus and nitrogen nutrition of wheat plants. *Food technology and biotechnology*, 43(1), 19-27.
- [36] Ahemad, M., and Khan, M S. 2011. *Pseudomonas aeruginosa* strain PSI enhances growth parameters of greengram [*Vignaradiata* (L.) Wilczek] in insecticide-stressed soils. *Journal of pest science*, 84(1), 123-131.
- [37] Esitken, A Pirlak. L. Turan. M.. and Sahin, F. 2006. Effects of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry. *Scientia Horticulturae*, 110(4) 324-327.



- [38] Hao, X. H., Liu, S. L., Wu, J. S., Hu, R. G., Tong, C. L., and Su, Y. Y2008. Effect of long-term application of inorganic fertilizer and organic amendments on soil organic matter and microbial biomass in three subtropical paddy soils. *Nutrient Cycling in Agroecosystems*, 81(1), 17-24.
- [39] Bhardwaj,D. ; Ansari, M.W. ; Sahoo ,R. K. and Tuteja, N.2014. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial Cell Factories*, 13:66
- [40] Dahich, S.K.; Somani,L.L. and shilpkar, D. 2011. Effect of integrated use of fertilizer p, FYM and bio fertilizers on soil properties and productivity of soybean- wheat crop sequence. *Journal of Advances in Developmental Research*. 2(1):42-46.
- [41] Jungwook, Y.; Joseph, W. K. and Choong-Min, R. 2009. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science*.14.1.

An Economic and Econometric Study of the Gross Domestic Product in Iraq Through the Oil, Industrial and Agricultural Sectors

Dawood Fahad Abdullah

Department of Medical and Industrial Plants, College of Medicinal and Industrial Plants, University of Kirkuk, Iraq

E-mail: dawood.f.abdullah@uokirkuk.edu.iq

Abstract. This study aims to study the impact of the main production sectors, oil, industry and agriculture, on the Iraqi GDP during the specified for the period (2009 – 2019). It also seeks to determine the contribution rates of these sectors to the GDP, by using the method of least squares (OLS) in the analysis to achieve these objective. Through results the economic and econometric analysis, the domestic oil product variable (X1) had a positive and significant effect at a 5% level with a value of (0.320). Increasing domestic oil product leads to increased government revenues, which can be used to finance development and infrastructure projects. This can lead to increased demand for local services and products, thus enhancing the country's economic growth. The domestic industrial product variable (X2) showed a negative and insignificant effect at a 5% level with a value of (-0.180). The negative impact of domestic industrial product on the GDP, it is due to infrastructure problems, lack of investment, lack of raw materials, weak production efficiency in this sector, as well as lack of financial and technical support and failure to improve the business environment and promote innovation and technology. The domestic agricultural product variable (X3) on the other hand, had a positive and significant impact at a 5% level with a value of (0.280). The positive impact of domestic agricultural product on GDP indicated that improving and increasing agricultural production contributes to enhancing food security and increasing rural income, enhancing economic activity in rural areas, and contributing to improving living standards and creating new job opportunities, as agriculture has a direct impact on the local economy. The study recommended some recommendations, including: The necessity of implementing suitable agricultural and industrial policies that have a positive impact on these sectors, such as credit facilities, financial aids, and others. Attracting foreign investment in all three economic sectors, enhances local investment by creating new investment opportunities for local companies.

Keywords. Iraq, GDP, Agriculture, Oil, Industry.

1. Introduction

Economic growth, from a theoretical perspective, is one of the most important topics related to economic development. It is one of the key measures used to assess developmental performance. Economic growth refers to the relative change in gross domestic product, which reflects the volume of goods and services generated by a particular economy [1]. The oil, industry, and

agriculture sectors are crucial economic sectors in Iraq, requiring significant attention from the state. This is especially important because the Iraqi economy faces numerous challenges, including a decline in the gross domestic product growth rate, deterioration of the industrial and agricultural sectors, a diminishing role of the private sector, infrastructure collapse, high unemployment rates, and capital flight. Iraq's involvement in continuous wars in the past has hindered the achievement of the primary goal of utilizing oil revenues to generate growth in the non-oil sector, which is not directly linked to oil revenues. Consequently, the economy has become weaker and less resilient in the face of external shocks. The well-known volatility of the oil market, resulting from price fluctuations, makes planning in this sector a challenging task. Therefore, other sectors such as agriculture and industry must be given a role that mitigates the impact of shocks on the Iraqi economy, reducing their severity compared to the oil sector. Additionally, the oil sector has limited capacity to absorb unemployment due to its capital-intensive nature. Developing and adapting the agriculture, industry, and services sectors, which are characterized by labor-intensive activities, will help absorb unemployment.

Today, Iraq has high potential for diversification in productive sectors. If the vast agriculturally suitable lands are utilized, self-sufficiency in agricultural production, particularly for strategic crops such as wheat, barley, and rice, can be achieved. Iraq suffers from a shortage in agricultural production compared to its local needs, due to the low contribution of agriculture to the GDP. This problem is attributed to several factors, including limited water resources, soil quality, economic challenges, backward agricultural production methods, in addition to the social problems facing rural areas in the country [2]. Increasing agricultural growth or agricultural productivity is a major factor influencing economic growth, as it allows this growth to focus on developing other sectors. In addition, agricultural growth contributes to boosting exports, which helps stabilize the exchange rate in the country [3]. The industrial sector, being an effective driver of economic growth and capable of creating real platforms for development, has the potential to establish a contemporary oil and petrochemical industry. Furthermore, attention should be given to mining industries (iron and other minerals) and the development of extractive industries such as phosphate and sulfur extraction. Additionally, continuous expansion and development of existing branches in transformative industries such as food, textiles, construction materials, and others should be pursued [4].

1.1. Study Problem

The study problem is the deterioration of the industrial and agricultural sectors, as they suffer from low growth rates, despite the significant increase in oil sector revenues. Evaluating the Gross Domestic Product (GDP) of the oil, industrial, and agricultural sectors, identifying their contribution to the country's GDP, and uncovering the significant causes are crucial aspects.

1.2. Study Aim

This study aims to determine the extent of the impact of the main productive sectors, namely oil, industry and agriculture, on the gross domestic product of Iraq for the period (2009-2019). It also seeks to reveal the percentages of contribution of each of these sectors to the gross domestic product, by using the method of least squares (OLS) in the analysis to achieve these objectives.

1.3. Study Importance

The significance of the study is found the significance of the studied sectors, which are considered vital sectors contributing to the formation of the Iraqi Gross Domestic Product, serving as an economic indicator for the country's economic situation.

1.4. Study Hypothesis

The study assumes that the oil, agricultural and industrial sectors contribute to large varying degrees to the economic growth of the gross domestic product in Iraq during the period (2009-2019), as the oil sector plays the largest and most important role in increasing the value of the gross domestic product, while other the economic sectors, including the agricultural and industrial sectors, have a very little and weak contribution and need continuous support and funding.

2. Materials and Methods

The ordinary least squares method (OLS) is employed in the analysis. This method is widely used in statistical applications, and depends on the existence of a relationship between one or more variables [5].

The oldest use of (OLS) was linear regression, which corresponds to the problem of finding a line that best fits a set of data points. In the standard formulation, a set of N pairs of observations $\{Y_i, X_i\}$ is used to find a function relating the value of the dependent variable (Y) to the values of an independent variable (X). With one variable and a linear function, the prediction is given, by the following equation [6].

$$Y = a + bX$$

This equation involves two free parameters which specify the intercept (a) and the slope (b) of the regression line. The least square method defines the estimate of these parameters as the values which minimize the sum of the squares hence the name least squares between the measurements and the model (i.e) the predicted values as the following equation [7].

$$E = \sum (Y_i - \hat{Y}_i)^2 = \sum [Y_i - (a + bX_i)]^2$$

where E stands for error which is the quantity to be minimized, the estimation of the parameters is obtained using basic results from calculus and specifically uses the property that a quadratic expression reaches its minimum value when its derivatives vanish. Taking the derivative of E with respect to a and b and setting them to zero gives the following set of equations [8].

$$\frac{\partial E}{\partial a} = 2N a + 2b \sum X_i - 2 \sum Y_i = 0$$

&

$$\frac{\partial E}{\partial b} = 2b \sum X_i^2 + 2a \sum X_i - 2 \sum X_i Y_i = 0$$

Solving the normal equations gives the following least square estimates of a and b, as following equation [9].

$$a = M_Y - bM_X$$

with MY and MX denoting the means of X and Y, as following equation [10].

$$b = (\sum (Y_i - M_Y)(X_i - M_X)) / (\sum (X_i - M_X)^2)$$

The description of the model, which forms the initial basis for standard analysis, begins with defining the problem to be studied and the factors affecting it, which contributes to its clear interpretation. This approach draws on the logic of economic theory and previous empirical studies to determine, the relationship between the variables included in the model and the expected signs and magnitudes of the predestined parameters. These expectations are used as a theoretical test to evaluate the estimation results [11].

Regression analysis is a statistical technique used to understand the relationship between two or more variables that are causally related, and to make predictions or estimates based on this relationship. Through this method, a mathematical model is constructed to explain the relationship between the variables, and this model is called a regression model [12].

The linear regression equation is where Y is the dependent variable, X_n is the independent variable, b_1 is the unknown parameter of this variable, and e_i is the unobserved error term as follows.

$$Y = b_0 + b_1 X_1 + b_2 X_2 \dots b_n X_n + e_i$$

Description of the study variables for the oil, industrial, agricultural sectors and GDP for the period (2009-2019):

- Dependent variable: (Y) - Gross Domestic Product at constant prices (million dinars).
- Independent variables: Several economic factors were considered as independent variables, as follows:

X_1 - Oil production (million dinars).

X_2 - Industrial production (million dinars).

X_3 - Agricultural production (million dinars).

The mathematical form of the model used and the explanation of the representation of the study variables in the mathematical equations are as follows:-

$$Y = f(X_1, X_2, X_3)$$

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + e_i \text{ (linear model)}$$

$$\ln Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + e_i \text{ (semi-logarithmic)}$$

$$Y = b_0 + b_1 \ln X_1 + b_2 \ln X_2 + b_3 \ln X_3 + e_i \text{ (inverse logarithmic)}$$

$$\ln Y = b_0 + b_1 \ln X_1 + b_2 \ln X_2 + b_3 \ln X_3 + e_i \text{ (double logarithmic)}$$

as follows:-

Y : Dependent variable.

b_0 , the intercept coefficient, also known as the constant, is the coefficient that takes a particular value when all independent variables are equal to zero in a linear regression model.

$b_1 \dots b_3$, the regression coefficients, they indicate the amount of effect resulting from changing the independent variable by one unit on the dependent variable.

$X_1 \dots X_3$, Independent variables.

e_i : A random variable that represents random effects or factors that were not included.

An explanation of the variables mentioned, with an initial overview of the nature of the relationship between these variables and the dependent variable, based on the concepts of economic theory and prior research.

Firstly, the dependent variable:-

(Y) Gross Domestic Product at constant prices (million dinars).

(GDP) at constant prices refers to the value of a country's economic output measured in terms of constant prices over a specific period. Constant prices are used to eliminate the effects of inflation

and provide a more accurate representation of changes in the real output of an economy. It is a vital economic indicator that measures the economic situation of the country and is considered more accurate because it accounts for the effects of inflation. Inflation reduces the value of money.

Secondly, the independent variables:

(X1). Oil production (million dinars)

Domestic oil product represents the value of oil production during the study period (million dinars). This variable is included to measure its impact on the dependent variable (Gross Domestic Product at constant prices) in Iraq.

(X2). Industrial production (million dinars)

Domestic industrial product represents the value of industrial production during the study period (million dinars). This variable is included to measure its impact on the dependent variable (Gross Domestic Product at constant prices) in Iraq.

(X3). Agricultural production (million dinars)

Domestic agricultural product represents the value of agricultural production during the study period (million dinars). This variable is included to measure its impact on the dependent variable (Gross Domestic Product at constant prices) in Iraq.

The accuracy of the estimated model parameters will be evaluated through statistical and standard tests, as well as examining the extent to which the parameters and magnitudes of the estimated model agree with the concepts of economic theory.

2.1. Statistical Tests

2.1.1. t-Test

This test assesses the significance of regression coefficients by calculating the t-value and comparing it to the critical value of t. If the calculated t-value at the 5% level exceeds the critical value, the regression coefficient is statistically significant. If the calculated t-value is less than the critical value, the regression coefficient is not statistically significant. [13].

2.1.2. F-Test

This test is used to determine the significance of the relationship between the dependent variable and the independent variables, the significance of the overall model equation is measured by calculating the F value and comparing it to the critical F value. If the calculated F value at the 5% level is greater than the critical value, the relationship is significant. If the calculated F value is less than the critical value, the relationship is not statistically significant [14].

2.1.3. Coefficient of Determination (R^2 test)

This index measures the proportion of variance, and ranges from 0% to 100%. The closer the ratio is to 100%. The better the selection of variables affecting the relationship with the dependent variable. It also determines the proportion of variance in the dependent variable that can be explained by the independent variables [15].

2.2. Standard Tests

2.2.1. Durbin-Watson Test (D-W TEST)

This test is used to detect the phenomenon of autocorrelation between the values of successive random variables, based on calculating the residuals resulting from the regression method using ordinary least squares, with the aid of the (D-W) (d^*) statistic. The test involves comparing the

computed value of Durbin-Watson with two critical values: the upper limit (d_u) and the lower limit (d_L). The interpretation is as follows, [16].

If $d^* < d_L$, we reject the hypothesis of no autocorrelation, indicating the presence of positive autocorrelation.

If $d^* < 4 - d_L$, we reject the hypothesis of no autocorrelation, indicating the presence of negative autocorrelation.

If $d^* < 4 - d_u < d_u$, we accept the hypothesis of no autocorrelation, indicating the absence of autocorrelation.

If $4 - d_L < d^* < 4 - d_u$, the test is inconclusive regarding the critical region.

2.2.2. Klein Test

This test is used to determine whether there is a problem with linear association between the independent variables. This is done by comparing the square root of the coefficient of determination (R^2) to the simple correlation coefficient between any two independent variables. If the square root of the coefficient of determination is greater than or equal to the correlation coefficient between any two independent variables, this indicates that there is no problem with linear association between the independent variables. Conversely, if the square root of the coefficient of determination is less than or equal to the correlation coefficient between any two independent variables, this indicates that there is a problem with linear association between the independent variables [17].

3. Results and Discussion

The table below illustrates the values of the Gross Domestic Product (GDP) for the period (2009-2019) and the values of both the oil production, industrial production, and agricultural production, as well as the contribution of each sector to the overall GDP. The values presented in the table (1) indicate that the oil production has the highest contribution to the overall GDP compared to the industrial production and the agricultural production. The highest contribution of the oil production to the overall GDP was observed in the year 2016 with a value of (64.68%), while the lowest contribution was in the year 2013 with a value of (49.39%). The agricultural production ranked second, with its highest contribution observed in the year 2011 with a value of (4.53%) and its lowest contribution in the year 2017 with a value of (1.88%). The industrial production ranked third, with its highest contribution observed in the year 2009 with a value of (2.12%) and its lowest contribution in the year 2016 with a value of (0.86%).

The averages for both the Gross Domestic Product (GDP) and the local oil production amounted to 175 million dinars and 100 million dinars, respectively. The domestic oil production had an average contribution of approximately 57%, while the domestic industrial production amounted to around 2 million dinars with an average contribution of 1.4%. On the other hand, the average domestic agricultural production reached approximately 5.5 million dinars with an average contribution of 3.3%.

Due to the challenging circumstances and economic crises faced by Iraq, fluctuations and variations in the values of the domestic agricultural production, particularly the domestic industrial production, were observed during the period of 2009-2019. This implies that the agricultural and industrial sectors suffer from significant neglect. Additionally, it is evident that the Iraqi economy relies primarily on the oil sector.

Table 1. Gross Domestic Product at constant prices for a base year (2007=100), the gross product of the oil, industrial and agricultural sectors, and the relative importance of each sector for the period (2009-2019). (Million dinars).

Years	GDP at constant prices	Domestic Oil product	Domestic Industrial product	Domestic Agricultural product	Relative importance of domestic oil product %	Relative importance of domestic industrial product %	Relative importance of domestic agricultural product %
2009	124702848	67752773	2637793	4898773	54.33	2.12	3.93
2010	132687029	68401972	2805041	5560828	51.55	2.11	4.19
2011	142700217	74185745	2870486	6465656	51.99	2.01	4.53
2012	162587533	83805694	2930766	6019561	51.54	1.80	3.70
2013	174990175	86435889	2653458	7459174	49.39	1.52	4.26
2014	178951407	93811857	2064946	7309016	52.42	1.15	4.08
2015	183616252	108624648	1723532	4613211	59.16	0.94	2.51
2016	208932110	135142910	1787447	4598971	64.68	0.86	2.20
2017	205130067	129589085	1926417	3863223	63.17	0.94	1.88
2018	202776269	127885396	1957710	4358551	63.07	0.97	2.15
2019	211789775	132700224	2459863	6048361	62.66	1.16	2.86
Average	175351244	100757836	2347042	5563211	56.72	1.42	3.30

Source: Republic of Iraq - Ministry of Planning and Development Cooperation, Central Statistical Organization and Information Technology, Department of National Accounts, (2009-2019) [18].

The multiple linear regression function was analyzed using the ordinary least squares (OLS) method, which is a common method in statistical applications that relies on the existence of a relationship between two or more variables. The available regression models include several types, such as linear, semi-logarithmic, inverse logarithmic, and double logarithmic regression. The double logarithmic model was chosen as the best model based on its outstanding performance in statistical and diagnostic tests such as (D.W test, t test, F test, R² test) tests and its compatibility with economic logic;

As following equation;-

$$\ln Y = 11.340 + 0.320 \times \ln X_1 - 0.180 \times \ln X_2 + 0.280 \times \ln X_3$$

$$t = (2.90) \quad (2.90) \quad (-1.02) \quad (2.27)$$

^{2}

$$R = 0.79 \quad F = 7.88 \quad D.W = 1.82$$

Based on the above results, the coefficient of domestic oil product variable, X₁, exhibited a significant and positive effect at a 5% level with a value of (0.320), this is consistent with economic theory. Indicating that an increase in domestic oil product leads to an increase in the gross domestic product (GDP). Increased domestic oil product leads to increased government revenues, which can be used to fund development and infrastructure projects. When domestic oil product increases, it can lead to increased demand for local services and products, which boosts economic growth.

On the other hand, the coefficient of domestic industrial product variable, X₂, showed a negative and insignificant effect at a 5% level, with a value of (-0.180). This contradicts economic theory, as a decrease in industrial output would typically result in a decrease in GDP. The reasons for the negative impact of domestic industrial product on GDP are infrastructure problems, lack of investment, lack of raw materials and weak production efficiency in this sector, as well as failure to improve the business environment, lack of financial and technical support, and failure to promote innovation and technology.

Regarding the coefficient of domestic agricultural product variable, X3, it had a positive and significant effect at a 5% level with a value of (0.280), this aligns with economic theory, indicating that an increase in domestic agricultural product leads to an increase in GDP. The positive impact of domestic agricultural product indicates that improving domestic agricultural product can contribute to enhancing food security and increasing rural income. Agriculture is an important source of employment and has a direct impact on the local economy. Also, increasing domestic agricultural product enhances economic activity in rural areas, which contributes to improving living standards and creating new job opportunities.

Additionally, the t-test revealed that the independent variables are capable of explaining the variations in the dependent variable. The calculated t-values for variables X1 and X3 were greater than the tabulated t-value at a 5% level indicating that these estimated parameter are statistically significant. However, the variable X2 had a negative and insignificant coefficient at a 5% level. Furthermore, the model was found to be significant at a 5% level based on the F-test, as the calculated F-value exceeded the tabulated F-value. R^2 test that the aforementioned independent variables explain approximately 79% of the variations in the dependent variable, while the remaining 21% can be attributed to other variables not included in the model. Additionally, the Durbin-Watson test indicated the absence of autocorrelation among the random variable values. as indicated by the Klein test the problem of multicollinearity among the independent variables did not emerge.

To measure the response of the gross domestic product to changes in domestic oil, industrial, and agricultural product variables in Iraq, the elasticity of the relevant variables was calculated during the study period. The elasticity for domestic oil product was (0.320), indicating that a 10% increase in domestic oil product leads to a 3.2% increase in the gross domestic product. The elasticity for domestic industrial product was (-0.180), suggesting that a 10% decrease in domestic industrial production results in a -1.8% decrease in the overall GDP. As for the elasticity of domestic agricultural product, it was (0.280), meaning that a 10% increase in domestic agricultural production leads to a 2.8% increase in the overall GDP.

Conclusions

- The oil, industrial, and agricultural sectors are essential economic sectors in Iraq due to their importance and contribution to increasing the gross domestic product (GDP).
- Amid the difficult conditions and economic crises faced by Iraq, fluctuations and variations in the values of domestic agricultural product and particularly domestic industrial product were observed during the period of (2009-2019).
- It is also evident that the Iraqi economy primarily relies on the oil sector, while the agricultural and industrial sectors suffer from significant neglect.
- The obtained results were consistent with the hypothesis of the study.
- The domestic oil product variable (X1) had a positive and significant effect at a 5% level with a value of 0.320. Increasing domestic oil product leads to increased government revenues, which can be used to finance development and infrastructure projects. This can lead to increased demand for local services and products, thus enhancing the country's economic growth.
- The domestic industrial product variable (X2) showed a negative and insignificant effect at a 5% level with a value of (-0.180). The negative impact of domestic industrial product on the GDP, it is due to infrastructure problems, lack of investment, lack of raw materials, weak production efficiency in this sector, as well as lack of financial and

technical support and failure to improve the business environment and promote innovation and technology.

- The domestic agricultural product variable (X3) on the other hand, had a positive and significant impact at a 5% level with a value of 0.280. The positive impact of domestic agricultural product on GDP indicated that improving and increasing agricultural production contributes to enhancing food security and increasing rural income, enhancing economic activity in rural areas, and contributing to improving living standards and creating new job opportunities, as agriculture has a direct impact on the local economy.

Recommendations

- The need to focus on the industrial and agricultural sectors by encouraging investors through state support and expanding support provided for agricultural and industrial projects. the role of government support plays a crucial and important in increasing industrial and agricultural production.
- Diversification of the economic base by working towards increasing the contribution of non-oil sectors (agricultural and industrial sectors) to the overall GDP and enhancing the role of the private sector in development, job creation, and reducing reliance on oil revenues.
- The necessity of implementing suitable agricultural and industrial policies that have a positive impact on these sectors, such as credit facilities, financial aids, and others.
- Giving more attention to the industrial sector. Developing a clear strategy for the industrial sector to achieve a wide industrial base, industrial growth, and provide employment opportunities at various skill levels, as advanced industries create advanced trade.
- Attracting foreign investment in all three economic sectors (oil, industrial, agricultural), enhances local investment by creating new investment opportunities for local companies.
- Encouraging citizens to start their own projects and establish various development zones, providing them with tax and trade incentives to attract investment and support job creation.

References

- [1] Hussain, I. M, 2020, Effect of some monetary and financial factors on the level of economic growth in Jordan during the period (1987 – 2013). University of kirkuk jouranl for administrative and economic sciences. Vol (10) No (2).
- [2] Ghazal, K. N. & Khalil, R. A, 2019, Development the ratios of agriculture products participating in the total agriculture products in Iraq for (1990-2016) University of kirkuk Journal for Agricultural Sciences. Vol (10) No. (4).
- [3] Anwar, M. M., Farooq, i. S. & Khan, G, Y, 2015, Agriculture sector performance, an analysis through the role of agriculture Sector share in GDP - Journal of Agricultural Economics, Extension and Rural Development: ISSN-2360-798X, Vol, 3(3), pp 270-275, April, 2015. Copyright - Spring Journals.
- [4] Muzahar, M. S, 2011, Research on the Role of the Oil Sector in Economic Development in Iraq - p. 10.
- [5] Kazem, A. H. & M, B. SH, 2002, Advanced Econometrics, Theory and practice - National Library - Baghdad - Iraq - p, 16.
- [6] Abdi, H.& Valentin D. Edelman, B.E, 1999, Neural networks - Thousand Oaks - Sage.
- [7] Bates, D.M., and Watts D.G, 1988, Nonlinear regression analysis and its applications - New York - Wiley.



- [8] Greene, W.H., 2002, Econometric analysis. New York - Prentice Hall.
- [9] Nocedal, J. and Wright, S., 1999,. Numerical optimization, New York – Springer:
- [10] Plackett, R.L, 1972, The discovery of the method of least squares. Biometrika - 59, (239–251).
- [11] Chang, Alphas, 1988. Fundamental Methods Of Mathematic Economic - Third Edition MC GREW - Hill - Singapore.
- [12] Davies P, L, Gather U., 1993, The Identification of Multiple Outliers. Journal of Statistical Planning and Inference - (122, 65-78).
- [13] Salvatore, Ph.D, 1982, Theory and Problems of Statistics, and Econometrics - p, (147).
- [14] Ghazal, K. N, 2003, The Impact of Globalization on Agricultural Performance in Selected Developing Countries - PhD thesis - College of Administration and Economics - University of Mosul.
- [15] Koutsoyiannis, A, 1979, Modern microeconomics, Second edition. - ST-Martins Press - New York p (121).
- [16] Pindyck, R.S.R. & D. L, 1976, Econometrics Model and Economic Forecasts - MC Graw - Hill - Kogakusha Ltd -, Tokyo.
- [17] Gujarati, N, 2003, Basic Econometrics - 4nd ed, MC Graw Hill – Book - NEW YORK - p (350).
- [18] Republic of Iraq - Ministry of Planning and Development Cooperation, Central Statistical Organization and Information Technology, Department of National Accounts, (2009-2019).

Economic Analysis of the Impact of Some Economic Variables on Investment in the Iraqi Agricultural Sector for the Period (1990-2022)

Ahmed Ali Hussein Al-Jumaili¹ and Mohamed Omar Sharif²

^{1,2}Department of Economics and Agricultural Extension, Agricultural Economics, College of Agriculture, Tikrit University, Iraq.

¹E-mail: Ahmed.ali.hu542@st.tu.edu.iq

²E-mail: mohmeedomear@tu.edu.iq

Abstract. The study deals with the impact of agricultural investments on wheat production in

Iraq. It aims to provide a comprehensive analysis of the factors affecting the effectiveness of investment and propose strategic solutions to enhance productivity and sustainability in the wheat sector. A mixed-methods approach was used, combining quantitative data analysis using the ARDL methodology to estimate the factors affecting wheat production such as cultivated area, size of the workforce working in the agricultural sector, value of imports, and size of agricultural investment using time series data of 33 years from 1990 to 2022. However, many challenges, including inadequate infrastructure, the spread of pests and diseases, are the most important production problems, and the difficulty of transporting the product to markets is the most important marketing problem from the point of view of the sample members. In addition to limited access to finance, which hinders the full potential of these investments. The adoption of modern technologies and practices was identified as a critical factor in improving productivity. The study recommended encouraging a participatory approach among farmers in terms of ownership of the means of production, stimulating large-scale investment in wheat cultivation as one of the promising sectors, and developing the infrastructure that provides attraction for investments, especially in the field of technology transfer. Keywords: Agricultural investment, public investment, investment in wheat cultivation.

Keywords. Investment, Agricultural , Economic.

1. Introduction

The agricultural sector in Iraq is one of the important productive sectors in the national economy, and is one of the main tributaries for achieving real development that affects various economic, social and environmental aspects. Without a doubt, the current indicators that show the role of this sector in employing workers, especially agricultural workers, achieving self-sufficiency and food security, preserving natural resources such as water and agricultural soil, improving the standard of living and life of rural areas, and contributing to the gross domestic product are not consistent with the components it enjoys.

In this regard, it must be emphasized that the area of Iraqi lands suitable for agriculture constitutes about 16% of the total area, and is a basic pillar for maintaining the sustainability and development of the agricultural sector. In addition, the lands actually included in the circle of

agricultural investment are distributed between irrigated lands and rainfed lands that extend across various parts of Iraqi regions [1], and depend on various water resources, especially traditional water resources such as surface water, groundwater and rain.

1.1. Research Problem

The research problem lies in the lack of research that has addressed current agricultural investment in Iraq in terms of its components, development, factors affecting it, its effects, efficiency, and how to deal with it in a manner that is consistent with its components and the challenges it faces, noting that Iraq has worked and is still working hard to invest in agricultural infrastructure, human capital, scientific research, training, qualification, and other basic pillars to maintain the sustainability and development of the agricultural sector, not to mention the efforts made to activate the role of the private sector and secure the necessary financing and transform its monetary form into a service-based production form for the purpose of creating a new agricultural capital formation, expanding the existing one, and adding production capacities so that real agricultural development can be achieved that achieves food security, bridges the gap between production and consumption on the one hand, and between savings and investment on the other hand, and raises the level of growth in profitability and employment. This bright and promising image of the Iraqi agricultural sector, which reflects the ambitions placed on agricultural investment, does not find its balance on the ground today, as the manifestations of backwardness are multiple in the Iraqi agricultural sector, and the old Iraq, which was nothing but an agricultural country par excellence, no longer exists today for various reasons, as a clear weakness in agricultural investment is observed, which casts its shadow on the entire agricultural development process as it is the driving element of agricultural activity economically and socially.

Despite Iraq's great agricultural potential, especially in wheat production, the country faces many challenges that hinder the effectiveness of agricultural investments, specifically, assessing how factors such as adopting technology, financial investment and policy support affect production efficiency and wheat cultivation outputs in Iraq.

Thus, the research problem can be formulated in a set of questions, which are:

- How did agricultural investment develop in Iraq during the previous period?
- Which factors affect agricultural investment in Iraq?

1.2. Importance of the Research

The importance of the research lies in the importance of the agricultural sector in Iraq and the pivotal role of agricultural investment in its development to occupy the position that befits the existing agricultural capabilities and other motivating factors, and contribute effectively to the national economy and change its structure by advancing the wheel of agricultural activity. Following up on the development and efficiency of agricultural investment in Iraq and its impact on the development of the agricultural sector, describing its nature, and identifying the factors that accompanied and coincided with this investment process,

1.3. Research Objectives

General objective: Evaluating the effectiveness of agricultural investments in improving wheat production in Iraq and proposing strategic measures to enhance investment results. A set of objectives branch out from it, namely:

- Study the development of agricultural investment in Iraq for the period (1990-2022).
- Analyzing the efficiency of agricultural investment in Iraq for the period (1990-2022).

1.4. Research Methodology

The descriptive analytical approach was adopted to study "Agricultural investment in Iraq for the wheat crop". This approach aims to describe the reality of agricultural investment in the wheat sector in Iraq and analyze the factors that affect crop productivity and maximize the benefit from investments. This approach will be used to determine the relationship between the various factors affecting the agricultural process and its outputs.

2. The Concept of Investment

The main purpose of doing business in the past was to make a profit, but today and in our current era, institutions have completely different goals, and the owners of these institutions have different goals and it has become necessary to increase the value of the institution. The value of the institution is formed through its development and technical progress as well as the quantitative and qualitative increase in production.

The topic of investment has become one of the topics that occupies a basic and important position in the priorities of economic, financial, banking and other studies that are concerned with the developments taking place in advanced societies, and countries have given it great attention, as through it the productive capacity of societies is built and the attention of economic entities is attracted, as it is a way to achieve profits that represent an income for business owners, owners and shareholders, and it also captures the attention of people as it is their means of obtaining job opportunities that save them from unemployment and provide them with the conditions of their efficiency.

Investment activity is built on a complex content. On the one hand, it contains a series of activities that involve an effort represented in formulating, discussing and proving investment options within the project in terms of economic efficiency, choosing the project, making investment decisions, ensuring the financing budget for each project and preparing the technical and economic conditions to achieve investment objectives. On the other hand, investment activity cannot be separated from its effects, which are fixed assets that are operated, supporting some functions, increasing production and GDP, or other effects resulting from the management plan [2]. The term investment represents a flexible, broad and rapidly evolving concept that cannot be defined or limited to a rigid concept. As for international agreements, setting a specific definition may be a point of dispute between countries because the definition may agree with the economic policies of some countries and differ with the policies of other countries at the same time. This requires leaving the freedom to the parties to the agreement to add a specific concept to the term investment that agrees with their economic policy and gives countries greater flexibility in applying it [3]. It is not easy to reach a specific definition of the term investment in economic and legal jurisprudence. This is due to the different aspects from which this term is viewed. The term investment itself is an economic and legal term that is affected by the economic policy followed in different countries on the one hand and the international economic policy as a whole on the other hand, in addition to the rapid and significant development that occurs in the phenomenon of investment on an ongoing basis. There are many definitions of investment in global economic terms, and each of these definitions reflects the point of view of its owner according to his specialization. We will mention some of them below. Some have defined it as: "Allocating capital with the aim of obtaining new means of production and production machines or developing available means in order to increase production capacity" [4]. It has also been defined as: "The productive employment of capital by directing savings towards uses that lead to the production of goods and services with the aim of meeting the economic needs of society and increasing its welfare". One of these definitions presents investments as: "Owning an asset to achieve capital gains at the time of its sale or to obtain a fixed income or a combination of both". Investment is

also defined as: "Allocating funds or capital to an institution with the aim of achieving profit". Despite the multiplicity of definitions, the meaning is one, which is allocating cash or capital at the present time to obtain a gain in the future, whether it is a fixed income over time, or a capital gain obtained at the time of reselling assets. We can say, based on all the previous definitions, that investment is harnessing material and non-material resources and moral resources And intellectual, whether national, foreign or joint, to establish new development projects in various fields or support existing projects in order to achieve mutual interests for the parties to the investment process.

2.1. Characteristics of Investment

The basic principle that should govern private or public investment policies in modern systems should enable and support investment in human and social capital. This rule can be applied to relevant systems that ensure well-being and quality of life as well as in various parts of social and economic development [5]. The main characteristics of investment are as follows:

- Time: Spending occurs now and then profits come after a period of time, thus there is a time lag between the time of making the investment and the time of making the profit.
- Risks: This characteristic represents the sacrifice made at the time of investment and its profit and size are uncertain. In other words, if the expenditures have a reasonable degree of certainty, the effects assume only hopes.
- Efficiency: The investment is accepted and activated only when the effects (i.e. the absolute total amount of future revenues) are higher than the initial expenditures.
- The tangible material content of investment efforts: Investments represent a flow of values whose starting point is a structure of resources diverse in nature and volume (financial assets, part of income and savings, human resources, etc.) [6]. Investment in economic thought:

When talking about investment, it is necessary to talk about projects and the human activity from which these projects are derived. Projects are an outgrowth of the will of human work, which the Austrian school defines as intentional and deliberate work that seeks to achieve a specific goal. Humans work to produce, earn money, or meet human needs, and they create institutions as a means to achieve these goals. The Austrian school has formulated the concept of utility, which motivates people who self-determine the value of the goal they want to achieve and then choose the means that they believe will enable them to reach that goal [7].

Investment represents a type of sacrifice of money and other resources today in order to obtain more money (or wealth) in the future. In this context, there are several elements that deserve special mention. People spend time and money (or resources in general) and these resources are considered scarce and therefore valuable. Investments deal with the effective management of money today in the hope of obtaining more money (or returns) in the future. This leads us to the next element of investment, which is uncertainty about the future. Investing involves sacrifice, however, there is a fundamental difference between saving and investing, as saving does not involve risk (or perhaps very little) but investing involves risk, and investment assets generally carry different amounts of risk ranging from none to very high risk [8].

Investing has always been a plan that gives most people security from the uncertainty of the future, and investing in finance is a monetary asset that is purchased with the idea that the asset will provide income in the future or that it will rise and then be sold at a higher price, and if we want to make some investments, we must have savings that allow us to achieve these investments, and if we do not have savings, we can obtain these savings from others in return for repayment and interest and vice versa.

The traditional way of investing is to buy gold, deposits in banks, savings in post offices and buying land in real estate, but due to industrial development and the growth of societies, investment has changed to the next level of investing money in stocks, buying bonds, debt securities, mutual funds, cryptocurrency, etc. [9].

Today's earnings sacrificed for tomorrow's life are called investment and investment plays a crucial role in differentiating between developed, developing and underdeveloped countries. Investments will certainly serve as an indicator of the countries' GDP, inflation, wholesale price index, national income etc., which is why developing countries accept foreign direct investment in the country and these countries face a tough task of generating sufficient capital for their development efforts. Most of these countries find it difficult to come out of the vicious circle of poverty of low income, low savings, low investment, low employment etc. With high capital production ratio developing countries need very high rates of savings and investments to leap forward in their efforts to achieve high levels of economic growth. Household savings play a vital role in the contribution of investment and disposable income of households basically consists of income generated from labour and from the operation of individual enterprises, plus receipts of interest, dividends and social benefits minus current tax payments, interest and social contributions. As Rao pointed out, "Increasing savings, using increased savings to increase capital formation, and using increased capital formation to increase savings for further increase in capital formation constitute the underlying strategy behind economic growth"[10]. The topic of investment has attracted the attention of economists in various economic schools, and below we will talk about the development of the concept of investment in some economic schools:

2.1.1. First: Investment in Classical Economic Thought

The classical school played an important role in strengthening the beginnings of progress for capitalist society, which was trying to discover the laws and foundations that govern the course of capitalist production and the prospects for its growth. This was clearly demonstrated in the works of Adam Smith and David Ricardo, and we will discuss below the most important ideas of these two economists on the subject of investment. A- Adam Smith:

Adam Smith focused in his book "The Wealth of Nations" on the importance of the division of labor and the accumulation of capital as two basic elements that help in the growth of wealth. Smith believes that only workers who work within productive fields contribute to increasing production. He emphasized this point by defining productive labor accurately and setting two main conditions for considering work as productive labor, which are:

- This work should lead to the creation of a surplus and can contribute to future investments.
- This work should lead to the production of material goods.

Regarding the accumulation of capital, Smith stressed the necessity of it preceding the division of labor process, and focused on the importance of achieving a sufficient level of savings, which he considered the basic foundation for investment and thus increasing capital formation. In this context, he also stressed the important role played by capitalists in implementing new investments and thus increasing capital formation, due to their great ability to save their high profits compared to the income (wages) of workers. Thus, Smith believes that the division of labor is the basic foundation for achieving wealth and increasing capital accumulation, which is considered a primary necessity for this division process. [11]. B- David Ricardo: David Ricardo assumed through the theory of distribution (distribution of national income to the three elements of production: land, labor, and capital) that the entire share taken by the landowner from its production will be consumed in the form of rent, while the largest part of the profits will be saved and reinvested in order to maximize production and increase employment. Profits, according to

Ricardo, come from two sources: interest and the profit of the organizer. He determined the interest rate at the rate of profit that the organizer can achieve. As for what exceeds this rate of interest, it represents the profit of the organizer. As for the source of profit that depends on the interest rate, Ricardo believed that it is determined by the productivity of capital. Ricardo considered that capital Money is an advance payment to workers, while profits are represented by the increase in the value of the product of these advance payments, which means that the rate of profit and thus the expansion of production depends on wages, as they are linked in an inverse relationship, so profits increase when wages decrease and vice versa. In this context, Ricardo says: At all times and in all countries, profits depend on the amount of work required to provide workers with necessities, whether those who work on the land or workers who employ capital that is no less profitable [12].

2.1.2. Second: Investment in Keynesian Thought

The economist John Keynes was greatly interested in the concept of investment, and Keynes focused in particular on the determinants of investment volume, as he believed that the volume of investment is determined by comparing the expected returns (marginal adequacy of capital) with the expected revenues from the capital asset, and Keynes indicated that investment demand is based on two basic factors:

- The monetary interest rate.
- The marginal adequacy of capital.

Keynes considered investment to be a function of the interest rate, and savings to be a function of income, and equilibrium is achieved by equalizing savings with investments, and the interest rate determined by the size of investments depends primarily on the rate of return. If it is possible to obtain the interest rate at a certain level and provide a schedule of demand for investment at different levels of capital adequacy, then it is possible to determine the size of the investment that requires its employment. Many criticisms have been directed at Keynes's views, the most prominent of which was that he dealt with the factors determining the investment function within a capitalist economic framework dominated by capitalist relations, and that what is true in a capitalist economy may not be true in developing countries dominated by economic relations that are very far from capitalist relations.

2.1.3. Third: Investment in Marxist Thought

Marx emphasized that the process of capital accumulation takes place only through property owners, i.e. capitalists who enjoy surplus value. Marx relied on the theory of value and surplus value from the classical English school, in which the value of goods is determined by the number of working hours required to produce them. According to the theory of value, the worker sells his labor power and the capitalist buys it from him. Marx emphasizes that after the capitalist buys his labor power from the worker, he employs it for a number of hours greater than the hours he paid for it, and the difference between the two represents the surplus value, which is considered the basis for the formation of capital according to Marx. Marx believes that surplus value (and hence capital formation) arises from variable capital (wages) without fixed capital, and for this reason he defined the degree of exploitation of workers (the rate of surplus value) as the ratio of surplus value to variable capital (wages), while he defined the rate of profit as the ratio of surplus value to capital. Thus, the inverse relationship between profits and wages becomes clear. When profits rise, wages must fall, and in order for profits to rise, profits must increase by a greater percentage than wages, and this can only happen by reducing the percentage of workers' wages from income or employing them for a greater number of hours at the same wage levels. Researcher's opinion: Thus, we see that the issue of investment and its relationship to economic growth has attracted the

attention of economists of different backgrounds and economic schools, and each of them has his own opinions and they may agree or disagree on how to increase economic growth rates and how to exploit investments for this growth.

2.2. The Importance of Investment

Investment has always been a matter of great importance in the commercial world, and while some enjoy a good percentage of returns, others get huge returns from investments that involve higher risks, and on the other hand there are people who lose due to lack of experience and the absence of strategy and knowledge in the world of investment. A person must be wise in order to be able to understand the sensitivity that exists in the current world of investments[14].

The rate of economic growth depends on the ability to attract investments and cash flows, and investment basically controls the rate of economic growth and how this growth is also, and this depends on the ability to provide facilities and incentives that tangibly affect capital owners when they make investment decisions in a country, as well as the impact on the volume of investments required from internal and external sources, and we will mention below the main points that summarize the importance of investment in the economic life of countries:

- Investment is the main factor in companies achieving profits.
- Investment achieves quantitative and qualitative growth for companies.
- Investment is an important factor in economic growth because it leads to the optimal use of resources that economic entities compete for in the free market.
- Increasing investments allows companies to keep pace with competition, which translates into higher profits, while institutions that do not invest deteriorate over time and tend towards decline.
- Increasing investment expenditures also translates into innovation, which is very important at the present time and leads to better management of resources.
- Investment contributes to combating unemployment by increasing the number of workers, and combating poverty and ignorance due to its significant repercussions on the lives of individuals and their future.
- Investment helps support the infrastructure of countries, as investing in a project is often accompanied by the construction of a building, bridge, garden, or other.
- Investment contributes to increasing national wealth and increasing national income because it is considered an addition to the available resources or maximizing them.
- Investment contributes to employing the money of savers, as there are many savers of money, but they do not know how to operate it, and here lies the role of investment in employing these savings and providing returns to savers.

2.3. Investment Objectives

The objectives of investment vary depending on the party carrying out the investment process, as the objective of the investment process may be to achieve public benefit, as is the case with public projects undertaken by the state, such as building bridges and paving roads, etc., and the objective of the investment process may be to achieve profits, as is the case with projects undertaken by the private sector. It can be said in general that most of the research that studies the subject of investment focuses mainly on investments in the business sector, i.e. investment aimed at achieving profits that are accompanied by a certain level of risk. We can divide the most important objectives of the investment process and classify them as follows:

2.3.1. Economic Objectives

The most important of which are:

- Increasing service and commodity production that can be marketed effectively, thus achieving appropriate incomes for production factors as well as increasing national income.
- Maximizing profit because it is the objective that investment projects seek to achieve as a return on invested capital and the increase to increase its growth and development[15].
- Increasing the project's ability to optimally use the elements of production, especially energy and raw materials, by introducing modern technology methods into the work.
- Working on developing the national industry and locally produced intermediate goods to raise their added value and thus increase the economic return and return.
- Preserving the real value of assets: i.e. preserving the value of the original capital invested in the project, and to achieve this, one must resort to selection and comparison after studying the economic feasibility until choosing the appropriate investment opportunity or alternative from among the many proposed opportunities (the alternative that achieves the highest return and the lowest degree of risk).
- Achieving an appropriate return from the investment process: The main goal of any investor is to obtain an appropriate return that enables him to maintain the continuity of the project.
- Ensuring the availability of the necessary liquidity: i.e. securing an appropriate level of liquidity to cover the requirements of the production process in order to continue covering unforeseen cases and emergencies that the production process may face.
- Ensuring the continuity of income and working to increase it: This means that the investor aims permanently, through investing his money in investment projects, to obtain a continuous return and to develop and increase it continuously.

2.3.2. Social Objectives

The most important of which are:

- Achieving balanced social development in various regions of the country by using investment projects as tools to achieve development and progress.
- Working to combat unemployment and the hotbeds of social corruption that it leaves behind, and achieving justice in the distribution of wealth to the owners of production factors.
- Achieving social stability and reducing cases of tension and social anxiety by providing the community's needs for essential goods and services.
- Changing the behaviors of individuals and their regularity in organizations, projects and entities that make them an effective force in society that increases the security of the homeland.

2.3.3. Technological Objectives

The most important of which are:

- Working to develop technology and local production methods to become more capable of meeting the needs of the country and citizens.
- Developing technology and production methods that were imported from abroad to become suitable for local conditions.
- Choosing modern technological methods and patterns that suit the needs of international growth and development.

- Working to keep pace with the prevailing technological progress in the world in order to provide the optimal model that was taken and emulated by similar and competing projects.

3. Agricultural Investment

The world's population is constantly and steadily increasing, and with it the need for food production is increasing, which in turn should contribute to the flow of investments into the agricultural sector. The effectiveness of developing modern agriculture is largely due to the ability to attract investments in this sector [16].

The global food system is increasingly threatened by severe pressures resulting from a number of factors, including climate change, environmental degradation, high population growth, rising energy prices, increased demand for meat and dairy products, and competition for land. Most governments and companies in the world today understand the fact that there is a need for urgent and large-scale investment, and this investment must come from the public and private sectors, as each has a crucial role to play.

Investment is the main driver in increasing the national economy's capacity for production, renewal and development. Agricultural investment is one of the most important sources on which the economies of developing countries are based and the most important topics of their economic policies, especially countries that depend on agriculture, as it is characterized by diversity in terms of its branches, and the agricultural sector is one of the strategic sectors in attracting investments due to its human and natural resources that facilitate the process of implementing these projects, in addition to the amount of financial amounts allocated to support and develop investment in this sector [17]

3.1. The Concept of Agricultural Investment

Agricultural investment is one of the most important factors contributing to stimulating the economic activity of the state, and it is one of the basic requirements in all economies of the world due to its great importance in developing the agricultural economy through agricultural infrastructure, technology and agricultural scientific research [18]. A distinction must be made between public agricultural investment carried out by governments and their affiliated bodies and private agricultural investment carried out by individuals at the level of small holdings or investors on a large scale. Private agricultural investment in all its forms has the potential to have a significant positive impact on the economy, as the presence of 500 million small farms in developing countries supports nearly two billion people, or about a third of the world's population. On the other hand, public agricultural investment plays a major role, especially in providing services that support the poorest and least market-ready small food producers, with whom companies have little incentive to deal. The public sector also has a very important function, which is to set the right policies to regulate investment so that it does not cause any harm, and to set the appropriate incentives to encourage investment and increase its production. For this reason, the private sector cannot replace the public sector in agricultural investment. Although the nature of the relationship between public agricultural investment and private agricultural investment is unclear, many economic studies have long confirmed the existence of an inverse relationship between them by assuming that public investment has a crowding out effect on private investment due to its monopolization of the largest part of available local resources, in addition to the government support directed to this investment. Agricultural investment can be defined as: "Investing capital in agricultural projects (whether plant or animal) in order to obtain good profits and returns for companies or individuals or to secure countries'

needs for the necessary food materials to provide food security and achieve economic and social development.

It is also defined as: "Reconnecting economic resources and using the necessary means in order to achieve the maximum possible agricultural production.

It is also defined as: "The process of integrating the available production factors in the agricultural sector (land, labor, capital) and operating them in order to produce food materials sufficient to meet the requirements and needs of consumers, and to obtain the best possible results."

We can say based on the aforementioned definitions that agricultural investment is the human investment of science, money and modern technology in order to harness and harness the natural resources available around him in order to achieve the best satisfaction of his current and future needs.

3.2. The Importance of Agricultural Investment

Today, there is an urgent need for huge investments in agriculture to help fix the crumbling global food system. Private sector investments can play a vital role in achieving comprehensive economic growth, environmental sustainability and poverty reduction. In order to do so, these investments must be organized appropriately and adhere to some basic principles, such as focusing on local food markets, working with producer organizations, and respecting the rights of small producers, workers and communities.

There is a need to increase investment that is not limited to encouraging production in a way that "does no harm" but in a way that "leads to more good", and what must be achieved through positive agricultural investment is comprehensive economic growth, environmental sustainability and poverty reduction in the long term.

The importance of agricultural investment stems from its importance in agricultural development, and this importance can be summarized in the following points:

- Agricultural investment is the driving force for agricultural development, and increasing agricultural investment and raising its efficiency are essential factors for achieving higher rates of economic and social growth in various countries of the world.
- Agriculture is the main source of securing the food needs of individuals, and for this reason, agricultural investment leads to increasing the ability of countries to meet the growing demand for food.
- Agricultural investment plays the most prominent role in exploiting available resources, energies and inert capacities, thus leading to increased agricultural production in particular and an increase in the country's gross national product in general.
- Agricultural investments contribute to expanding the market for industrial products, as they lead to increased incomes of farmers and thus increased their purchasing power, especially for industrial products, which in turn leads to increased growth in the industrial sector.
- Investments in agriculture represent one of the determinants of economic growth in rural communities and have a positive impact on economic growth in rural areas. The relationship between the level of investments in agriculture and the value of agricultural production is the most important.
- Agricultural investments play an important role in improving the country's trade balance by providing markets for the disposal of agricultural products surplus to local needs, thus shifting from import to export.

- Investment in the agricultural sector is one of the sources of obtaining the foreign currency necessary to secure production requirements.

4. Results and Discussion

4.1. Estimating Wheat Productivity Using the ARDL (Autoregressive Distributed Lag)

Methodology

The ARDL (Autoregressive Distributed Lag) methodology is a method used to analyze the relationships between variables in time series, especially when there is a difference in the degree of stability $I(0)$ or $I(1)$. This methodology is commonly applied in economic studies to analyze the effects of shocks and policies. The following are the basic steps for applying the ARDL methodology

4.1.1. Testing the Time Series Stability

Before applying the ARDL model, the time series stability must be verified using unit root tests such as the Augmented Dickey-Fuller (ADF) test or the Phillips-Perron (PP) test. And make sure that the variables are not of the second degree of integration ($I(2)$) because the ARDL model is not suitable for dealing with variables of this degree.

4.1.2. Selecting the Time Orders of the Model (Lag Orders)

Determine the degrees of lag using the optimal time criteria for the model using criteria such as the Akaike Information Criterion (AIC) or the Schwartz Bayesian Criterion (SBC).

4.1.3. Determining the ARDL Model

Constructing the appropriate ARDL model using the selected time orders. The model takes the following form

$$Y_t = a + \sum_{i=1}^p [\gamma_i B_i + \sum_{j=0}^q [\delta_{xj} t - j]] + \varepsilon_t$$

Where y is the dependent variable, x is the independent variable, and ε is the random error
Bound Test:

Perform a bound test to check for a cointegration relationship between the variables. This is done by comparing the calculated Fisher statistic with the appropriate critical values.

Estimating the Long-Run Model: If the bound test shows a cointegration relationship, the long-run model that describes the relationship between the variables in the long run can be estimated.

Estimating the Short-Run Model and Error Correction Model (ECM):

An error correction model is estimated to determine the short-run dynamics and correct the inverse error from the long-run equilibrium.

4.2. Diagnostic Tests

Conduct diagnostic tests to verify the validity of the model, such as the autocorrelation test, the heteroscedasticity test, and the normal distribution test.

4.3. Result Analysis

Interpret the estimated results of the model and evaluate the short-term and long-term effects based on the coefficient estimates.

Variables involved in the estimation process:

A number of variables were relied upon to determine the factors affecting wheat production, namely:

Wheat production (Prod) / ton.

Cultivated area (ARIA) / thousand hectares

Size of the workforce working in the agricultural sector (WF) / thousand workers

Value of imports (Imp) / million dinars.

Volume of agricultural investment (VAI) / million dinars.

Time series data of 33 years were taken from 1990 to 2022.

Time series stationarity test:

Time series independence test was conducted using the extended Dickey-Fuller (ADF) test using EVIEWS program

in the three cases (presence of a categorical, presence of a general and categorical trend, absence of a general and categorical trend) where the null hypothesis of the test H_0 : states the presence of a unit root and thus the non-stationarity of the time series, and the results are shown in Table 1.

Table 1. Results of testing the stationarity of time series using the ADF test.

Variable	Stability level	With Constant		With Constant & Trend		Without Constant & Trend	
		t-Statistic	Prob.	t-Statistic	Prob.	t-Statistic	Prob.
Production	At the level	-2.23	0.20	-4.29	0.01	0.59	0.84
	at the first difference	-6.46	0.00	-6.32	0.00	-6.38	0.00
Aria	At the level	-4.90	0.00	-4.83	0.00	-0.66	0.42
	at the first difference	-5.24	0.00	-5.12	0.00	-5.31	0.00
Imports	At the level	-2.54	0.12	-2.49	0.33	-1.54	0.11
	at the first difference	-6.40	0.00	-5.42	0.00	-6.52	0.00
Workforce	At the level	-1.74	0.40	0.26	1.00	1.51	0.96
	at the first difference	-8.30	0.00	-8.96	0.00	-7.37	0.00
VAI	At the level	-1.02	0.74	-4.78	0.00	0.38	0.79
	at the first difference	-9.50	0.00	-9.33	0.00	-9.32	0.00

Source: The table was prepared based on the outputs of the Eviews program.

From the table shown above:

- For the production variable: It is clear that the series is stable at the level when there is a general trend and a secant, but at the first difference the series becomes stable in all cases (presence of a secant, presence of a general trend and a secant, absence of a general trend and a secant).
- Area variable: It is clear from the table above that the area variable is stable at the level when there is a secant and a general trend and in the presence of a secant, and at the first difference the series becomes stable in all cases (presence of a secant, presence of a general trend and a secant, absence of a general trend and a secant).
- Imports variable: It is clear from the table above that the imports variable is not stable at the level, but at the first difference the series becomes stable in all cases studied (presence of a secant, presence of a general trend and a secant, absence of a general trend and a secant).
- Labor force variable: It is clear from the table above that the labor force variable is not stable at the level, but at the first difference the series becomes stable in all cases studied

- (presence of a secant, presence of a general trend and a secant, absence of a general trend and a secant).
- Agricultural investment size variable: It is clear from the table above that the agricultural investment size variable is stable at the level when there is a secant and a general trend, and also at the first difference the series becomes stable in all cases (presence of a secant, presence of a general trend and a secant, absence of a general trend and a secant).

From the previous results, it is clear that there is stability in the series variables, as production and area were stable at the level, while for the imports and labor force variables, they are stable at the first differences, and therefore we reject the null hypothesis stating the existence of a unit root, and thus these results enable us to conduct the joint integration test according to the autoregressive distributed lags methodology (ARDL). 2- Determining the optimal lag periods:

The lag periods are chosen based on a number of criteria, which are:

- Corrected coefficient of determination criterion (adj-R-sq)
- Schwarz SC (Schwarz Information Criterion)
- AIC (Akaike Information Criterion)

These criteria refer to the Autoregressive Distributed Lag Methodology ARDL(p,q1,q2,...) where the variable p indicates the lag periods of the dependent variable, while the variable q1,q2,... indicates the lag periods of the independent variables. The results came as shown in the following figure:

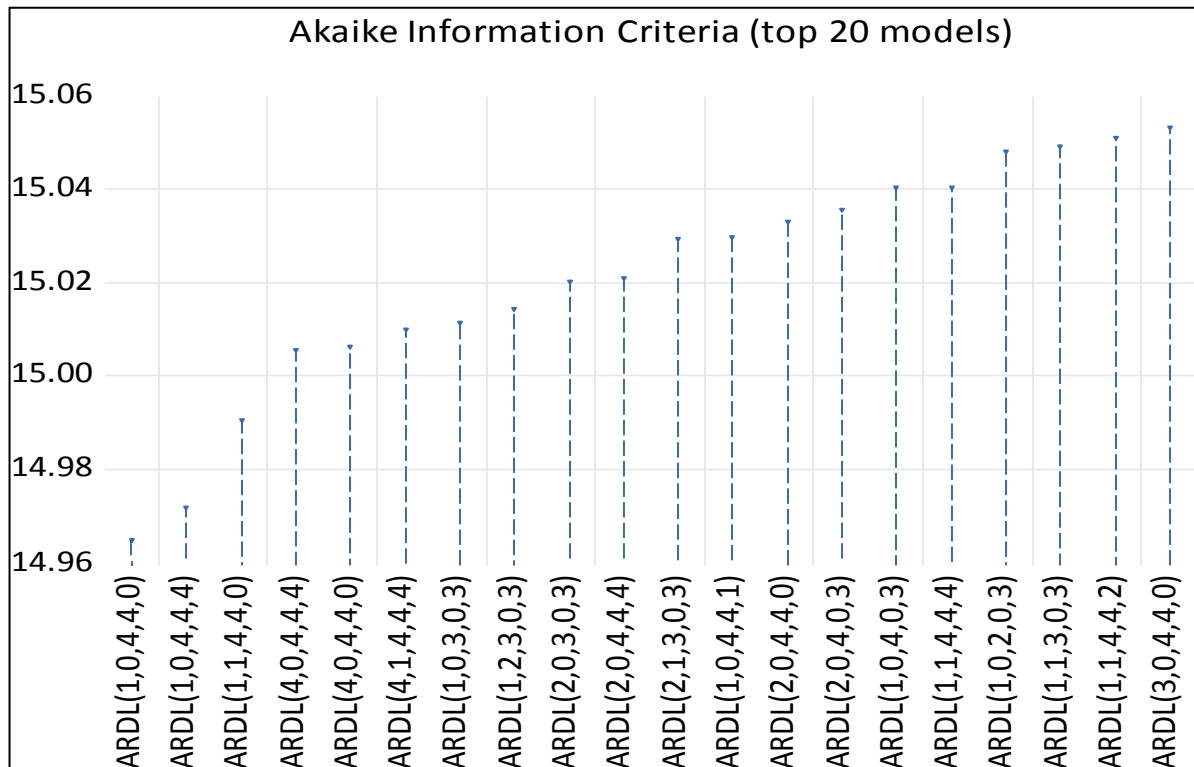


Figure 1. Estimation of optimal deceleration periods.

4.4. ARDL Bounds Test

This test is used to detect the existence of a long-term equilibrium relationship by comparing the calculated Fisher value of the coefficients of the independent explanatory variables that have been lagged with the Fisher tabular values and depends on the following two hypotheses:

H0: There is no long-term cointegration relationship between the variables.

H1: There is a long-term cointegration relationship between the variables.

Table 2. Cointegration test using the autoregressive distributed lag approach ARDL.

Statistical Test	Value	Number of independent variables	Decision
F-statistic	20.11	K=4	We reject the null hypothesis and accept the existence of a long-run cointegration relationship between the variables.
Tabular Values of Limits	I(0)Min	I(1)Maximum	
10%	2.2	3.09	
5%	2.56	3.49	
1%	3.29	4.37	

Source: The table was prepared based on the outputs of the Eviews program.

The previous table shows that the value of the F-statistic is greater than the tabulated values for the upper limit (I(1), so the null hypothesis will be rejected and the alternative hypothesis will be accepted, thus confirming the existence of a long-term equilibrium relationship between the dependent variable, which is production, and each of the independent variables, during the period studied. Table 3 shows the model estimation in the long run.

Table 3. Model estimation in the long-run function.

ARDL Long Run Form and Bounds Test				
Dependent Variable: D(PROD)				
Selected Model: ARDL(1, 0, 4, 4, 0)				
Case 2: Restricted Constant and No Trend				
Date: 06/30/24 Time: 05:45				
Sample: 1990 2022				
Included observations: 29				
Conditional Error Correction Regression				
Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	-753.9897	502.7788	-1.499645	0.1545
PROD(-1)*	-1.096090	0.111500	-9.830404	0.0000
ARIA	4.563626	0.705287	6.470596	0.0000
IMPORTS(-1)	-0.967368	0.466513	-2.073613	0.0558
VAI(-1)	0.461834	0.069086	6.684906	0.0000
WF	-0.553038	0.390192	-1.417350	0.1768
D(IMPORTS)	-0.601202	0.342222	-1.756759	0.0993
D(IMPORTS(-1))	0.274390	0.437692	0.626902	0.5401
D(IMPORTS(-2))	0.692785	0.385722	1.796076	0.0926
D(IMPORTS(-3))	0.869450	0.385780	2.253743	0.0396
D(VAI)	0.157055	0.049878	3.148762	0.0066
D(VAI(-1))	-0.207189	0.073303	-2.826496	0.0128
D(VAI(-2))	-0.195319	0.070189	-2.782759	0.0139
D(VAI(-3))	-0.096396	0.055895	-1.724595	0.1051
* p-value incompatible with t-Bounds distribution.				
Levels Equation				
Case 2: Restricted Constant and No Trend				
Variable	Coefficient	Std. Error	t-Statistic	Prob.
ARIA	4.163550	0.676551	6.154081	0.0000
IMPORTS	-0.882562	0.411927	-2.142522	0.0490
VAI	0.421347	0.053224	7.916507	0.0000

WF	-0.504556	0.359854	-1.402110	0.1812
C	-687.8904	470.4666	-1.462145	0.1643
EC = PROD - (4.1636*ARIA -0.8826*IMPORTS + 0.4213*VAI -0.5046*WF - 687.8904)				

Source: EVIWES software output.

4.5. Estimation of the Error Correction Model (ECM)

Table 4 shows the Error Correction Model (ECM) which shows the speed of transformation of factors from the short term to the long term and requires that the coefficient be negative and significant.

Table 4. Error Correction Model (ECM).

ARDL Error Correction Regression				
Dependent Variable: D(PROD)				
Selected Model: ARDL(1, 0, 4, 4, 0)				
Case 2: Restricted Constant and No Trend				
Date: 06/30/24 Time: 05:30				
Sample: 1990 2022				
Included observations: 29				
ECM Regression				
Case 2: Restricted Constant and No Trend				
Variable	Coefficient	Std. Error	t-Statistic	Prob.
D(IMPORTS)	-0.601202	0.241239	-2.492146	0.0249
D(IMPORTS(-1))	0.27439	0.259495	1.0574	0.3071
D(IMPORTS(-2))	0.692785	0.273209	2.535736	0.0228
D(IMPORTS(-3))	0.86945	0.260129	3.342383	0.0045
D(VAI)	0.157055	0.037475	4.190937	0.0008
D(VAI(-1))	-0.207189	0.045179	-4.585962	0.0004
D(VAI(-2))	-0.195319	0.046385	-4.210849	0.0008
D(VAI(-3))	-0.096396	0.034451	-2.798068	0.0135
CointEq(-1)*	-1.09609	0.086413	-12.68438	0
R-squared	0.926495	Mean dependent var		63.92041
Adjusted R-squared	0.897093	S.D. dependent var		995.7617
S.E. of regression	319.4324	Akaike info criterion		14.62009
Sum squared resid	2040741	Schwarz criterion		15.04443
Log likelihood	-202.9914	Hannan-Quinn criter.		14.75299
Durbin-Watson stat	1.924194			

Source: EVIWES software outputs.

It is clear from the above table for estimating the ECM error correction model that the value of CointEq(-1)* is equal to -1.09, meaning that the error correction condition is met since the value is negative and significant and the model has an error correction rate capable of converting the results from the short term to the long term at a speed equal to 1.9 per period. Therefore, the equation in the long term becomes

$$\text{PROD} = (4.1636 \times \text{ARIA} - 0.8826 \times \text{IMPORTS} + 0.4213 \times \text{VAI} - 0.5046 \times \text{WF} - 687.8904)$$

Accordingly, it is clear from the equation that the relationship between the relationship between production and area is a positive moral relationship, which is consistent with economic logic in terms of the long-term association of production with cultivated areas. Also, the relationship between production and imports is an inverse relationship, which is consistent with economic logic. The increase in imports may indicate the weakness of government measures that encourage

production and motivate investors to improve and increase their production. Also, the model indicated the existence of a direct moral relationship between production and the volume of agricultural investment, which is also consistent with economic logic. The more investment increases, the wheat crop is a strategic crop and is linked to achieving food security and is considered an attractive field if the investment components are available. As for the human workforce in the agricultural sector, it was found that the relationship is inverse between production and the volume of the human workforce working in the agricultural sector, but this relationship is not significant and can be The result is explained by the wheat's reliance on agricultural mechanization to a large extent in the work stages and the reliance on human resources has become relatively less with the availability of modern irrigation methods, plowing, harvesting, threshing, packaging, etc.

4.6. Diagnostic Tests for the Model

4.6.1. Residual Diagnostics (LM Test)

To detect the presence of a serial correlation problem between errors, where the test hypotheses take the following form:

H0: There is no serial correlation between the residuals.

H1: There is a serial correlation between the residuals.

The Breusch-Godfrey test was used and the results came out as follows:

Table 5. Serial correlation problem test.

Breusch-Godfrey Serial Correlation LM Test:			
Null hypothesis: No serial correlation at up to 2 lags			
F-statistic	0.086022	Prob. F(2,13)	0.9181
Obs*R-squared	0.378776	Prob. Chi-Square(2)	0.8275

Source: EVIWES software outputs.

The F coefficient value was 0.086, and the probability value of the test indicates that the treatment is not significant at the 0.05 level, and thus it is clear that there is no problem of serial correlation between the residuals

Testing the problem of heteroskedasticity of variance test where the ARCH test was used, whose null hypothesis states that there is no problem of difference in error variance, meaning that the error variance is homogeneous, as shown in Table 6.

Table 6. Shows the results of the ARCH test.

Heteroskedasticity Test: ARCH			
F-statistic	0.192108	Prob. F(1,26)	0.6648
Obs*R-squared	0.205368	Prob. Chi-Square(1)	0.6504

Source: EVIWES software outputs.

The results show that both the F, R2 values were insignificant at the 0.05 level, and therefore we accept the null hypothesis that there is no problem of error variance difference, meaning that the error variance is homogeneous.

Normal distribution test of residuals:

It represents the difference between the actual and estimated values and checks the extent to which the estimated values are close to the actual values. The results shown in Figure 2 show the results of the Jarque-Bera test, whose null hypothesis states that the data are normally distributed.

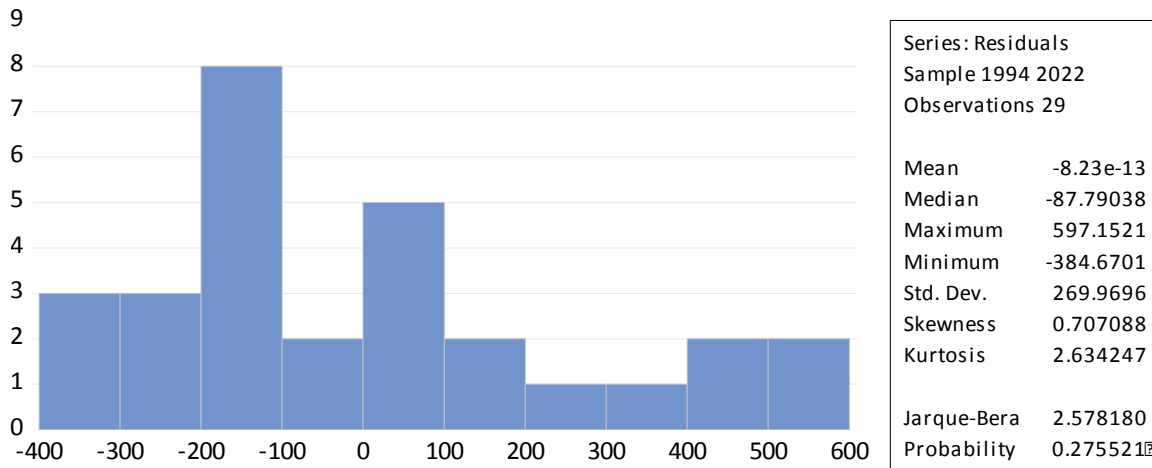


Figure 2. Results of the normal distribution test for the series residuals.

The Jarque-Bera test value was 2.578, where the probability value was 0.27, which is greater than the probability value of 0.05, and therefore we accept the null hypothesis that the residuals are normally distributed.

4.7. Analysis of the Results

The results concluded that the use of quantitative methods helps in understanding economic theories, as when accurate quantitative data is available and in light of the revolution of modern technologies, the use of modern quantitative methods helps in solving problems and making decisions, and it is clear from the estimation of the previous model that all the studied variables agree with the economic logic in interpreting the studied model, and the standard tests indicated that the model is free of standard errors, and therefore the model can be used in prediction.

Conclusions

- The results of the analysis of the factors affecting wheat production showed that the relationship between production and area is a positive significant relationship, which is consistent with economic logic in terms of the long-term association of production with cultivated areas.
- The existence of an inverse relationship between production and imports indicates the weakness of government measures that encourage production and motivate investors to improve and increase their production.
- The existence of a direct significant relationship between production and the volume of agricultural investment, which is also consistent with economic logic, as investment in wheat, which is considered a strategic crop, is linked to achieving food security and is considered an attractive field if investment requirements are available.
- The existence of an inverse relationship between production and the volume of human resources working in the agricultural sector, but this relationship is not significant and the result can be explained by the large dependence of wheat on agricultural mechanization in the work stages and the reliance on human resources has become relatively less with the availability of modern irrigation methods, plowing, harvesting, threshing, packaging, etc.

Recommendations

- Developing methods used in transporting crops and moving towards using large containers designated for transporting wheat.
- Developing infrastructure that provides attraction for investments, especially in the field of technology transfer and providing irrigation sources for farmers, in addition to improving roads and methods of transporting the crop.
- Providing an investment map that encourages investors to invest and provides them with all information about the possibility of investing in the studied area.
- Using kinetic models in studying the factors affecting wheat production and productivity, as these models have the ability to deal with large data, and provide mechanisms to understand the relationship between variables.

References

- [1] Hamid, Wissam Ali and Sakab, Anwar Mohsen (2020), Estimates of the spread and water requirements for agricultural executive agriculture, Al-Kut Journal of Economic and Administrative Sciences, Volume 12, Issue 38, p. 571.
- [2] Paaca, N.M. 2021. Investment and the Importance of Investment in the European Economy Comparative Analysis. "Ovidius" University Annals, Economic Sciences Series Volume XXI, Issue 2. P453-454.
- [3] Mahmoud, Ammar Shaker. 2020. The concept of investment in Iraqi law (comparative study). Journal of Economic, Administrative and Legal Sciences. Volume 4, Issue 1, pp. 185-193.
- [4] Taama, Basem Alwan. 2013. Iraqi Investment Law No. (13) of 2006 amended in Al-Mizan. A research published in the Journal of the Message of Law, University of Karbala, Issue 2. P. 8
- [5] Oprean, C and Stan, S. 2015. The Importance of Investing in Education for Sustainable Human Development the case of Romania. 2015. Economics 9 (174), P66.
- [6] Popa, F. 2020. Some aspects regarding the impact of foreign direct investment in economic development. Studies and Scientific Researches. Economics Edition, No 31. P70-75.
- [7] Fedorowicz, K and Łopatka, A. 2022. The importance of investment in the development of enterprises in Poland in 2009-2018. 26th International Conference on Knowledge-Based and Intelligent Information & Engineering Systems (KES 2022). P4171-4178.
- [8] Laopodis, N.T. 2020. Understanding Investments Theories and Strategies. Second edition. P4-5.
- [9] Jeyadevi, C. 2023. Type of Investment. Research Trends in Multidisciplinary Subjects-Volume-2. P46.
- [10] Sangeetha, D. 2015. Investment and its Role in Economic Development. International Journal of Science and Research (IJSR). Volume 4 Issue 9, P703-705.
- [11] Messaoui, Walid Qasoum. 2018. The Impact of Investment Promotion on Economic Growth in Algeria since 1993. PhD Thesis. Mohamed Khider University - Biskra - Faculty of Economics, Commercial Sciences and Management Sciences. P5-6.
- [12] Yang, xinye. 2020. Economic Theory Foundations for the Long-Term Investment. DePauw University. P2.
- [13] Hawish, Louay Taha Al-Mulla and Al-Jabouri, Hanan Muhammad Shukr. 2017. Conceptual Approaches and Definitions of Agricultural Investment in Economic Thought. Journal of the College of Basic Education. Volume 23. Issue 97. P633-646.
- [14] Pathan, H.S. 2017. Importance of Investing & the World of Investment. IJRAR- International Journal of Research and Analytical Reviews. Volume 4, Issue 2. P36-40.
- [15] Mokhtaria, Belmahidi and Bakhta, Busta. 2016. The role of local investment in economic development in Algeria for the period 2010-2015. Master's thesis. Ibn Khaldoun University - Tiaret - Faculty of Economics, Business and Management Sciences. Pp. 18-20.
- [16] Lysa, O., Oleksenko, R., Azhazha, M., Venger, O., and Sergiienko, T. 2022. State regulation of investment in agriculture. IOP Conf. Series: Earth and Environmental Science 949 (2022) 012009. P1-4.



- [17] Hania, Belbakouche and Ghada, Bayoud. 2020. The role of support and accompaniment bodies in financing agricultural investment in Algeria. A case study of the National Unemployment Insurance Fund - Mila Agency - Master's thesis. Abdelhafidh Boussouf University Center, Mila, Institute of Economics, Business and Management Sciences. Pp. 2-13.
- [18] Al-Aqabi, Duaa Ali Abdul Hussein. 2018. Requirements for agricultural investment in Iraq in light of its accession to the World Trade Organization. Journal of Economic and Administrative Sciences. Issue 106, Volume 24, pp. 272-294.

Okra *Abelmoschus esculentus* is a New Host of Cowpea Mild Mottle Virus *Carlavirus vignae* in Iraq

S. Owaid¹ and N. Al-Kuwaiti²

^{1,2}Plant Protection Department, College of Agricultural Engineering Sciences,
University of Baghdad, Baghdad, Iraq.

¹E-mail: saad.mohsen1104a@coagri.uobaghdad.edu.iq

²E-mail: n.alkuwaiti@coagri.uobaghdad.edu.iq

Abstract. Viral pathogens associated with symptomatic okra plants in a field in Baghdad, were investigated. Next generation sequencing confirmed the presence of cowpea mild mottle virus *Carlavirus vignae* sharing maximum 95% nucleotide identity to the reference sequences from the NCBI. Phylogenetic tree showed a high relatedness when grouped the CpMMV to the equivalent virus isolate from Babylon-Iraq, indicating a common origin. The report of cowpea mild mottle virus infecting okra in Baghdad-Iraq is the first, based on our knowledge.

Keywords. Carlaviruses, NGS, Phylogeny.

1. Introduction

Okra is a cash crop grown in Iraq both in protected and open fields [1,2]. It can be attacked by several diseases including those caused by viruses [3]. Cowpea mild mottle virus (CpMMV) *Carlavirus vignae* one of whitefly transmitted carlaviruses is belonging to the family *Betaflexiviridae* [4]. It has a monopartite genome comprises a positive sense single stranded ssRNA molecule, of about 8000 nucleotides, and encapsidated in a flexuous filamentous particle [5]. CpMMV impacts a wide range of crops including legumes, tomato and eggplant, worldwide [6]. Beside the whitefly *Bemisia tabaci*, it can be transmitted through seeds [7]. In 2013, CpMMV was reported for the first time on cowpea, in Iraq, using carlavirus specific primers followed by sequencing and commparision [8]. A complete genome sequence of the virus was announced, later on [9]. During September 2023, sever mosaic and leaf curl symptoms were observed on okra plants grown in a protected field near Baghdad (Figure 1). This disease was associated with a high whitefly infestation. This study aimed to investigate the viral pathogen in symptomatic okra plants based on next generation sequence (NGS).



Figure 1. Okra plant exhibiting sever mosaic symptoms on leaves.

2. Materials and Methods

About 0.5 cm leaf tissue of symptomatic okra sample was preserved in RNA later buffer (Merck, USA) and sent to DNA Link Inc. (South Korea) to perform total RNA extraction and sequencing. The kit TruSeq Stranded Total RNA combined with Ribo-Zero Plant, Illumina Novaseq6000 platform and bcl2fastq software (V 2020) were used for RNA sequence to generate 101 bp raw data. Geneious software was used for RNA sequence data assembly and analyses. Further sequence data were processed using the software packages MEGA 11 [10] and SDT v1.3 [11].

3. Results and Discussion

About 78,921 reads of viral contigs were obtained from the raw data of okra transcriptomes following trimming, assembly and mapping to reference sequences (Figure 2).

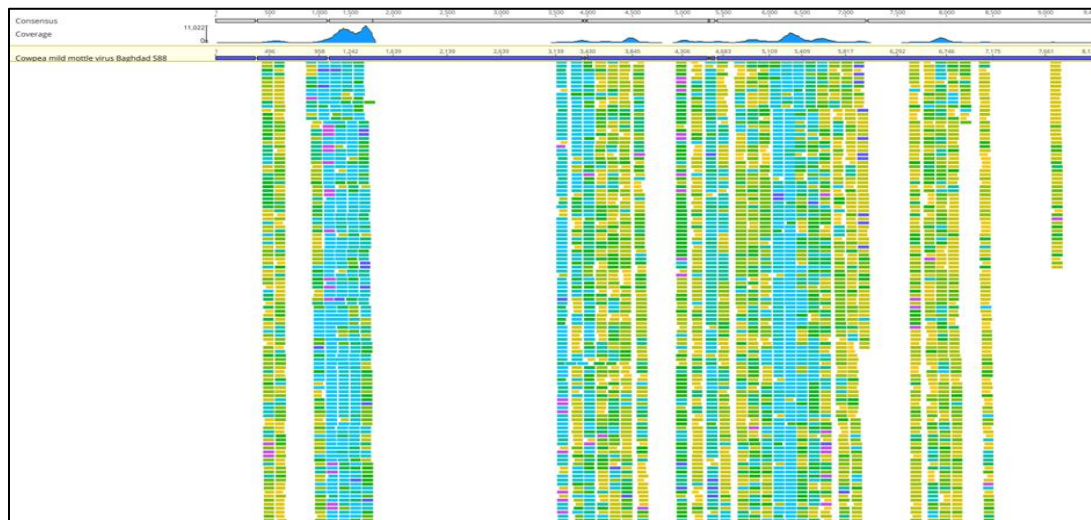


Figure 2. Sequence alignment of 151 nt contigs obtained (referred to as Baghdad S88) after raw data processing to the reference sequence of Cowpea mild mottle virus (CPMMV) *Carlavirus vignae*.

Amongst 5040 different virus sequences compared, viromic analysis confirmed the association of cowpea mild mottle virus *Carlavirus vignae* within the genome of symptomatic okra plant. CpMMV obtained shared maximum 95% nucleotide (nt) sequence identity with the relevant CpMMV isolates from NCBI (Figure 3).

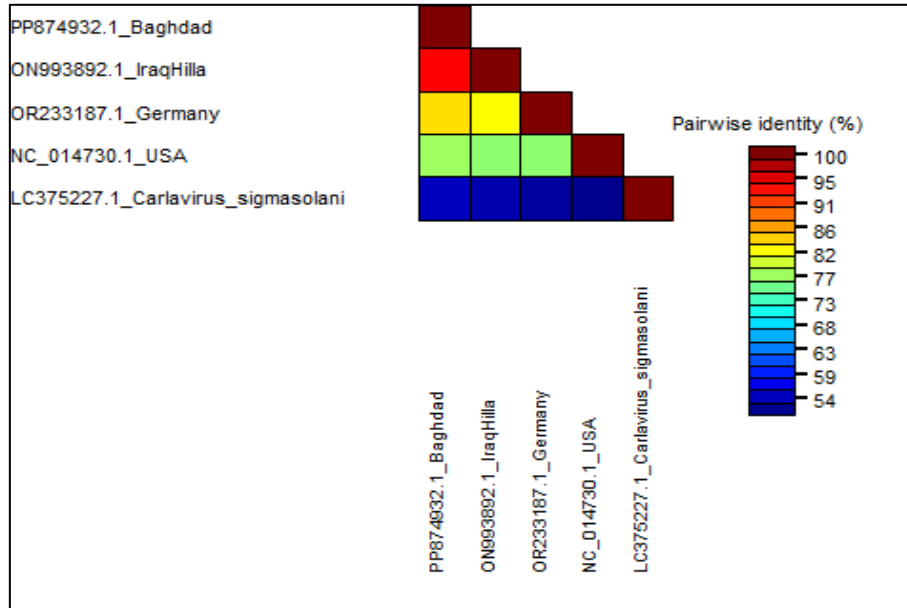


Figure 3. Nucleotide sequence identity percentages interpreted into colored matrix generated from cowpea mild mottle virus *Carlavirus vignae* identified (referred to as Baghdad) and equivalent NCBI nucleotide sequences. Potato virus S *Carlavirus sigmasolani* is an out-group comparison.

Based on Neighbor joining phylogeny, the sequence obtained (referred to as Baghdad) grouped to cowpea mild mosaic virus infecting cowpea form Babylon [9], suggesting a high relatedness (Figure 4).

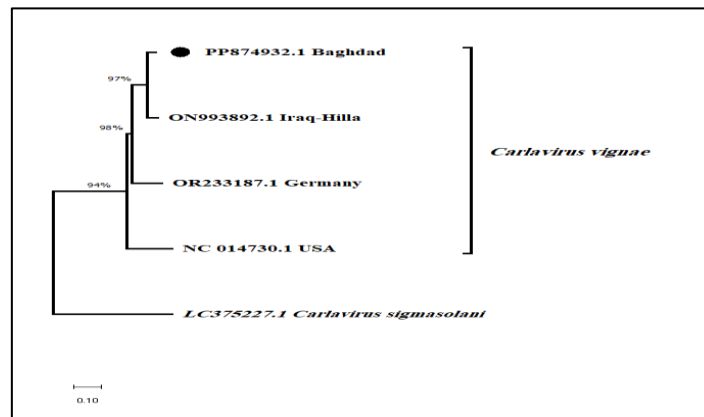


Figure 4. Neighbor-Joining phylogenetic trees constructed from cowpea mild mottle virus *Carlavirus vignae* identified (Marked with ●) and equivalent NCBI sequences. Potato virus S *Carlavirus sigmasolani* is an out-group comparison.

Proteomic analysis of the 1,788 nt sequence length of CpMV obtained revealed it included four open reading frames (ORFs) 3, 4, 5 and 6 encoding triple gene block protein 2, triple gene block protein 3, coat protein and nucleic acid-binding protein proteins, respectively (4, 9) (Figure 5).

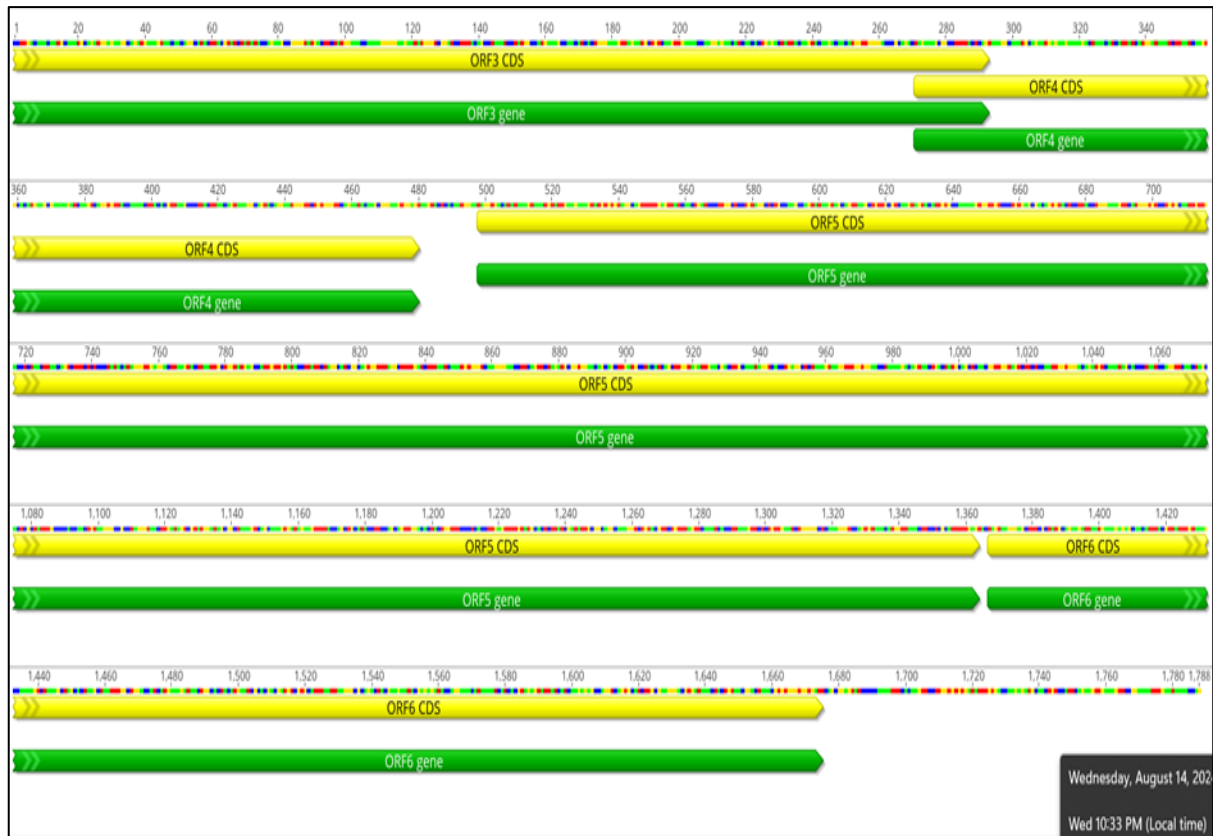


Figure 5. A diagram shows Proteomic analysis of Cowpea mild mottle virus (CPMMV) *Carlavirus vignae* Baghdad S88 isolate genome regions compared to the reference sequence.

Although CpMMV infection was reported on malvaceous hosts, okra was not among the virus host range (6,7). In this study, metagenomic analysis showed to be a powerful tool enabled to distinguished the viral pathogen associated with symptomatic okra plant in a single sample, provided sufficient data regarding the pathogen without prior information and it could differentiate CpMMV RNA from the host RNA [12].

Conclusion

Reporting the incidence of cowpea mild mottle virus on okra in Baghdad-Iraq is the first, based on our knowledge. This virus can be a threat to okra production in Iraq, especially when disseminating through whitefly and seeds. Besides, okra can be an alternative host to CpMMV, threatening legume crops, as well. Further metagenomic studies and precaution actions are required to manage *Carlavirus vignae* and its whitefly vector.

Acknowledgement

This work was sponsored by University of Baghdad-Iraq.

References

- [1] CSI (2023). Central Statistical organization, statistical group part3: Agricultural statistic. [Online] [Available from: <https://cosit.gov.iq/documents/AAS2023/3.pdf>]
- [2] Alwardi, H. J. M., Ali, S. H., and Al-Majeed, A. T. A. (2024). Measurement the Producing Efficiency and Marketing Efficiency of Okra Crop (Covered) in Baghdad Governorate for the Season 2022. *International Journal of Professional Business Review*, 9(1), e4230. <https://doi.org/10.26668/businessreview/2024.v9i1.423>
- [3] Adhab, M., Al-Kuwaiti, N. and Al-Ani, R. (2021). Biodiversity and occurrence of plant viruses over four decades: Case study for Iraq. 3rd International Sustainability and Resilience Conference: Climate Change. [online] USA: IEEE, pp.159–164. Available at: <https://doi.org/10.1109/IEEECONF53624.2021.9668128>.
- [4] ICTV, 2024. Virus Taxonomy: The Classification and Nomenclature of Viruses. The Online (10th) Report of the ICTV. https://talk.ictvonline.org/ictv-reports/ictv_online_report
- [5] Menzel, J.E., Schaefer, L.M., Burke, N.L., Mayhew, L.L., Brannick, M.T. and Thompson, J.K., 2010. Appearance-related teasing, body dissatisfaction, and disordered eating: A meta-analysis. *Body image*, 7(4), pp.261-270.
- [6] EPPO (2024) Carlavirus vignae. EPPO datasheets on pests recommended for regulation. <https://gd.eppo.int> (accessed 2024-08-21)
- [7] Brown, J. K. (2022). Cowpea mild mottle virus (angular mosaic of beans). CABI Compendium, CABI International, cabicompndium:15735. doi:10.1079/cabicompndium.15735
- [8] Al-Kuwaiti N 2013 Molecular characterization of viruses infecting potato and vegetables in Iraq. PhD thesis, University of Greenwich, UK.
- [9] Kareem A, Alisawi O, Al Fadhl F. (2023). Coding-Complete Sequence of Cowpea Mild Mottle Virus Isolated from Iraq. *Microbiol Resour Announc*. 12(3): e0122422. doi: 10.1128/mra.01224-22. Epub 2023 Feb 1. PMID: 36722964; PMCID: PMC10019285.
- [10] Tamura, K., Stecher, G. & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38, 3022–3027. <https://doi.org/10.1093/molbev/msab120>.
- [11] Muhire, B. M., Varsani, A. and Martin, D. P. (2014). SDT: A Virus Classification Tool Based on Pairwise Sequence Alignment and Identity Calculation. *PLoS ONE*, 9, e108277. doi: 10.1371/journal.pone.0108277
- [12] González-Pérez, E.; Chiquito-Almanza, E.; Villalobos-Reyes, S.; Canul-Ku, J.; Anaya-López, J.L. 2024. Diagnosis and Characterization of Plant Viruses Using HTS to Support Virus Management and Tomato Breeding. *Viruses*, 16, 888.

Estimating the Percentage of Vitamins, Gibberellin and Indole for Different Soil Textures in the Root Zone

Noor Abdul Hassan Tarhan¹ and Jawad Abdul Kadhim Kamal²

^{1,2}College of Agriculture, University of Al-Qadisiyah, Al-Qadisiyah, Iraq.

¹E-mail: Agr22.mas2@qu.edu.iq

¹E-mail: jawad.alshabbany@qu.edu.iq

Abstract. This experiment was conducted to estimate the percentage of vitamins and plant hormones in different soil textures. The experiment was conducted during the winter period of 2023 according to the complete randomized block design (RCBD). The experiment included one factor in the soil (loam, silty, clay, and sandy). The seeds of wheat, barley, broad bean, and radish were planted with three replicates for each treatment. The treatments were distributed randomly to the experimental units so that the number of units became 48 experimental units using the least significant difference (LSD) and at a probability level of 5%. The results showed that the treatments showed a significant increase in the percentage of vitamins. The treatment of the loam soil texture excelled on the other soil texture factors for wheat, barley, broad bean, and radish plants. It achieved a significant increase in the concentration of IAA in the flowering and end-of-season stages (9.767, 9.219, 9.862, 9.323 mg L⁻¹). (5.079, 5.104, 5.268, 4.961 mg L⁻¹), respectively, and achieved significant differences in the concentration of GA3 in the flowering and end-of-season stages (14.691, 14.429, 14.737, 14.259 mg L⁻¹), respectively, (10.316, 10.186, 10.663, 10.082 mg L⁻¹), respectively. It also achieved a significant increase in the concentration of each of vitamins B1, B3, B9 in the flowering and end-of-season stages (7.459, 7.355, 7.640, 7.465, µg g⁻¹), (6.396, 6.558, 6.396, 6.320 µg g⁻¹), respectively. (5.736, 5.481, 5.785, 5.358 µg g⁻¹), (5.169, 4.741, 5.226, 4.809 µg g⁻¹), respectively (6.736, 6.567, 7.004, 6.609 µg g⁻¹), (6.133, 5.800, 6.354, 5.928 µg g⁻¹), while the concentration of vitamin B12, the treatment of silt soil texture was superior to the treatments of other soil textures for wheat, barley, broad bean and radish plants in the flowering and end-of-season stages (3.813, 3.744, 3.832, 3.683 µg g⁻¹), (2.681, 2.580, 2.868, 2.477 µg g⁻¹).

Keywords. Soil, Vitamins B, Indole, Gibberellin.

1. Introduction

Soil texture is a volumetric distribution of soil particles, sand, silt and clay, that pass through a sieve to determine the proportions of sand, silt and clay aggregates. It is an important factor in classifying soils and knowing what the soil contains of water, air and various elements [1]. The root zone is the place where the roots and root hairs are aligned and it shows the state of coexistence between root microbes and roots and their mutual activity directly on plant growth. The basis of the relationship between living organisms and plants is the secretions secreted by plants from the roots in the soil surrounding the root zone [2]. The living organisms in the root zone that directly affect the plant host through different mechanisms, for example, it directly

affects the absorption of nutrients and minerals and increases the plant's resistance to water stress [3] The vitamins that plants secrete are not entirely manufactured by the plant but are also taken up by the root system from external sources and then released back into the soil [4] The relationship between the fungus and the plant is a symbiotic relationship because it benefits the fungus and the plant and the role of the fungus in preparing the plant with major and minor pressures. It also plays a role in protecting the plant from pathogens present in the soil and its role in increasing the ability of plants to withstand stress and drought conditions [5]. The study aims to

- Effect of soil texture on gibberellin and indole hormones.
- Effect of soil texture on vitamins B1, B3, B9, B12.

2. Materials and Methods

2.1. Experimental Location

The experiment was conducted during the winter period of 2023 in the Abu Ghraq Al-Awsat area of Babylon province, which is 15 km away from the center of Babylon province for four types of textures (clay, silty, loam, sandy) to grow four types of plants (wheat, barley, broad beans, and radish).

2.2. Experimental Design

The experiment was designed according to the complete randomized block design RCBD (3×4) and the experiment included one factor in the soil (loam, silty, clay, and sandy) with three replicates for each treatment and the treatments were distributed randomly to the experimental units so that the number of units became 48 experimental units.

2.3. Extraction Method Hormones (GA3, IAA), mg.L⁻¹

2.3.1. The Hormones Indoleacetic Acid and Gibberellin Were Extracted

3 g of the sample was taken and ground well using a ceramic mortar, then it was placed In a volumetric bottle, 20 ml of methanol was added to it and placed on a shaker for 4 hours, after which the sample was transferred to a centrifuge at a speed of 5000 rpm for 15 minutes, then the filtrate was transferred to a new tube and the pH was adjusted to 9 using 1 M KOH to preserve the hormone indoleacetic acid. To increase the polarity of the extract, 2 ml of ethyl flat and 5 ml of distilled water were added, the solution was shaken well and transferred to the centrifuge again at a speed of 5000 rpm for 15 minutes, the aqueous layer was collected and stored in the refrigerator until the analysis was conducted. 1. Analysis conditions: The examination was conducted in the laboratories of the Ministry of Science and Technology to detect the hormone indoleacetic acid and gibberellin using the high-performance liquid chromatography (HpLC) technique, SYKAM model, made in Germany, according to the conditions shown in the table below.

Table 1. SYKAM model.

No.	Name	Description
1	Carrier Phase	Acetonitrile: Distilled water (volume:volume) 26:74
2	Separation Column	C18_ODS(25cm*4.6mm)
3	Detector	280mm_UV
4	Flow Rate	mm/1min

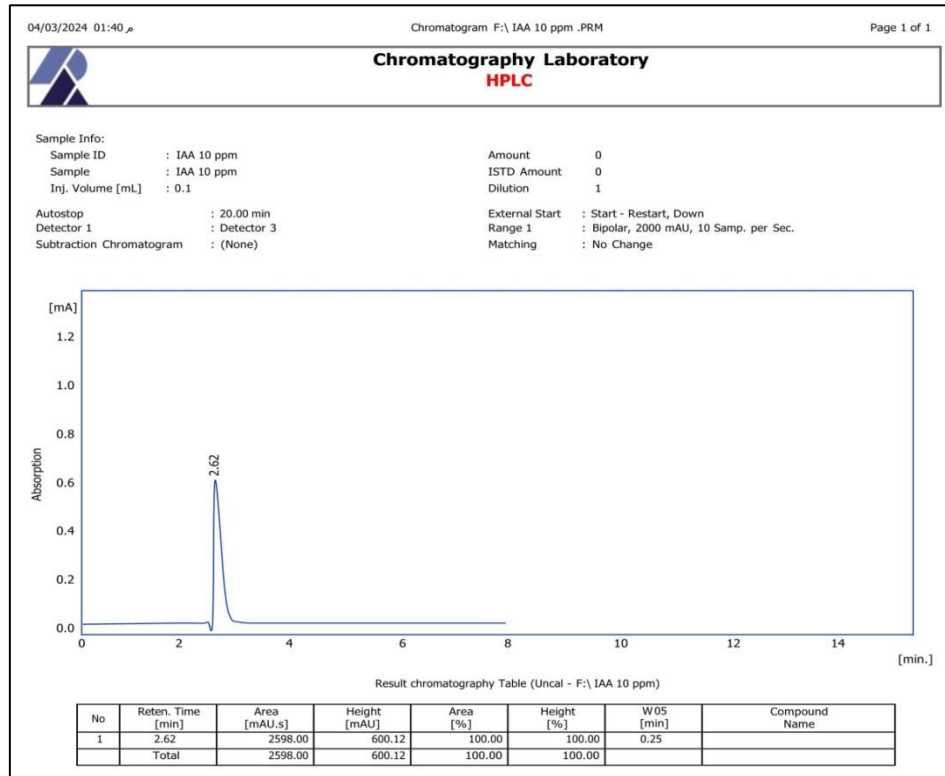


Figure 1. Standard chromatographic diagram of IAA hormone using HBLC device.

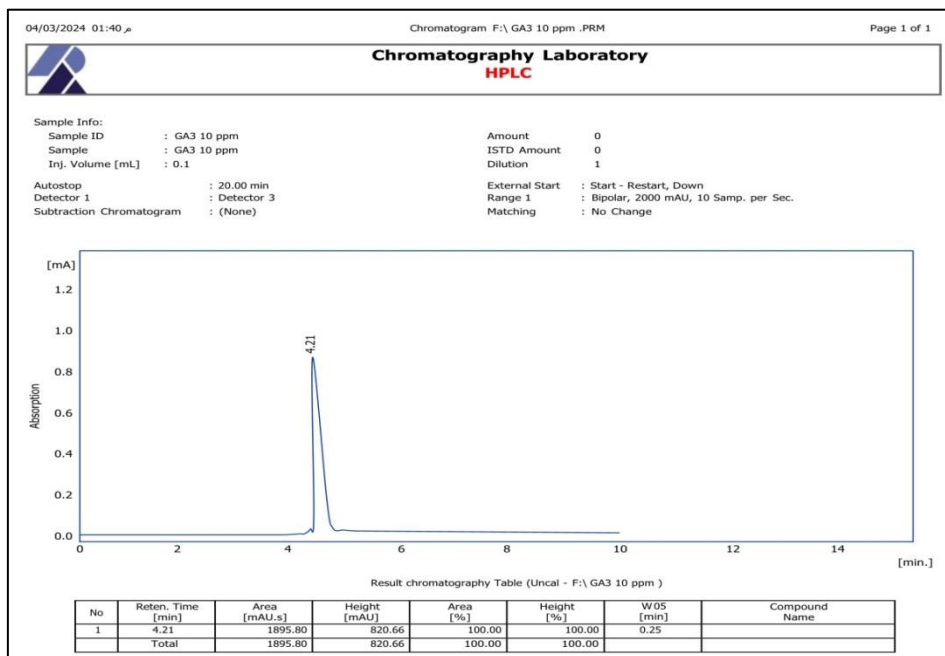


Figure 2. Standard chromatographic diagram of GA3 hormone using HBLC device.

2.4. Vitamins

3 g of the sample was taken and ground well using a ceramic mortar. Then it was placed in a volumetric flask and 20 ml of methanol was added to it. It was placed on a vibrating device for 4 hours. After that, the sample was transferred to a centrifuge at a speed of 5000 rpm for 15 minutes. Then the filtrate was transferred to a new tube and the PH was adjusted to 9 using 1 molar KoH. 100 microlysates of each standard compound were injected separately to determine the retention time of each standard compound. The results showed a peak with a retention time of 2.0, which is due to vitamin B3, another peak with a retention time of 2.5, which is due to B1, a third peak with a retention time of 3.8, which is due to B9, and a peak with a retention time of 4.5, which is due to vitamin B12, as in the attached figures (1 - 2 - 3 - 4). After that, 100 microlysates of each sample were injected into the HPLC device to detect the vitamin in soil samples. The results showed the presence of several values in one sample. After conducting the diagnostic process (comparing the retention time of the standard substance with the closest time that appeared in the sample), the results showed that the samples contained vitamins, as in the figures:

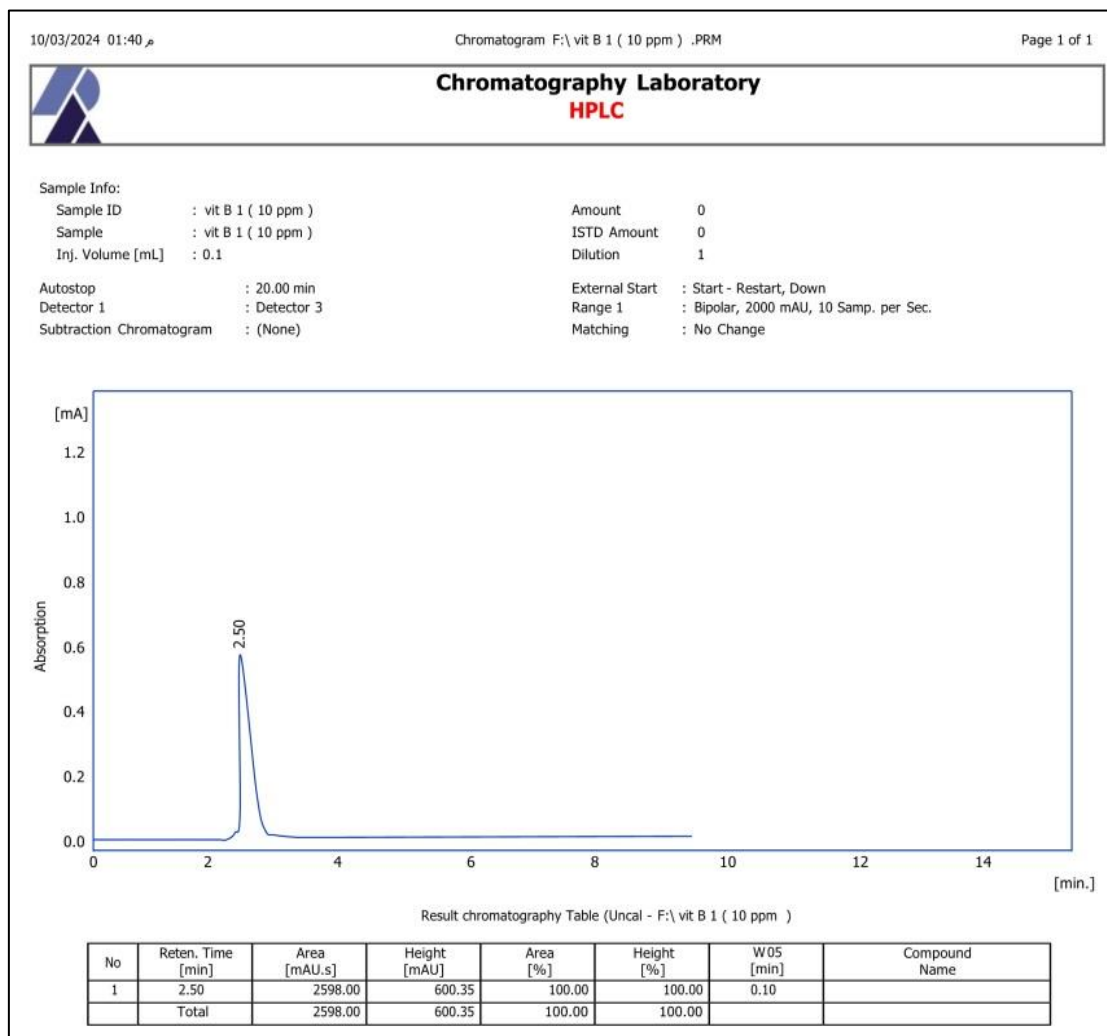


Figure 3. Standard chromatographic diagram of vitamin B1 using HBLC device.

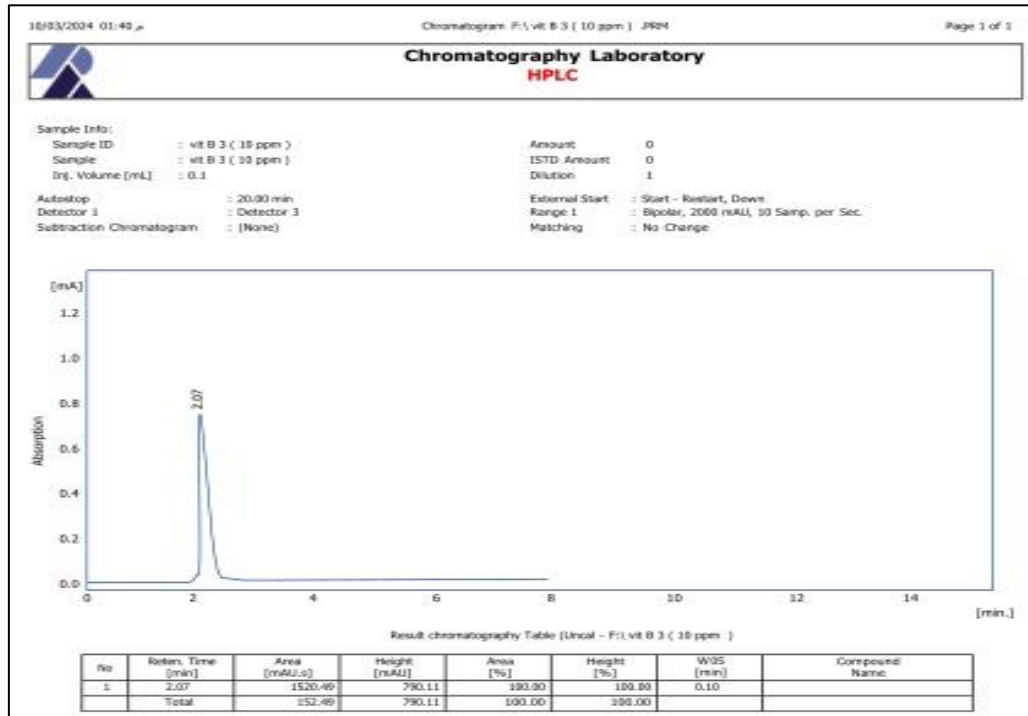


Figure 4. Standard chromatographic diagram of vitamin B3 using HBLC device.

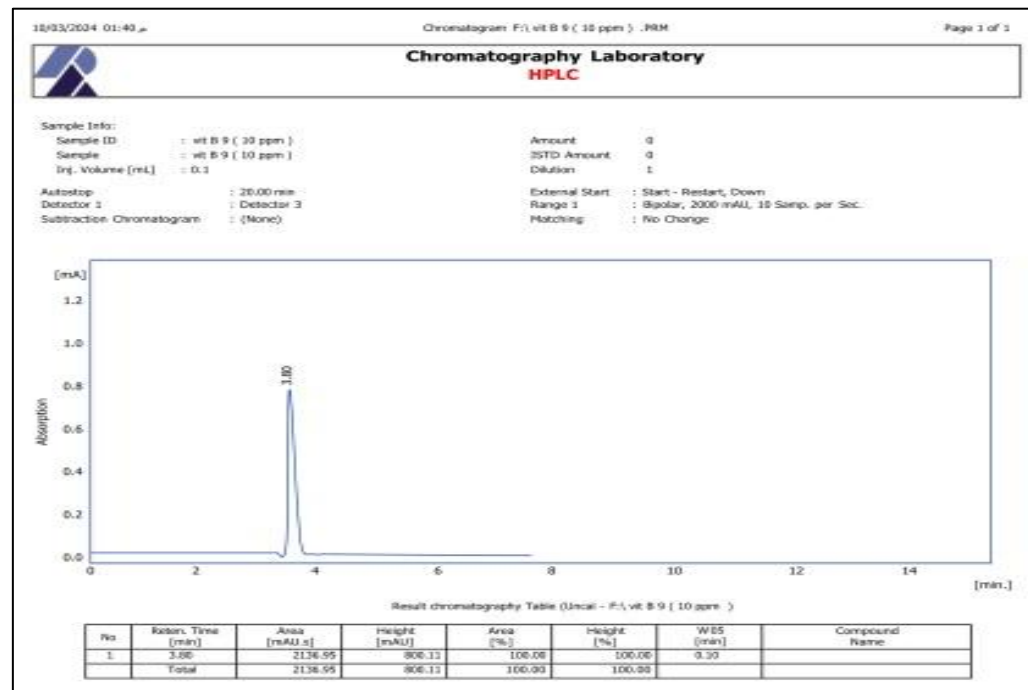


Figure 5. Standard chromatographic diagram of vitamin B9 using HBLC device.

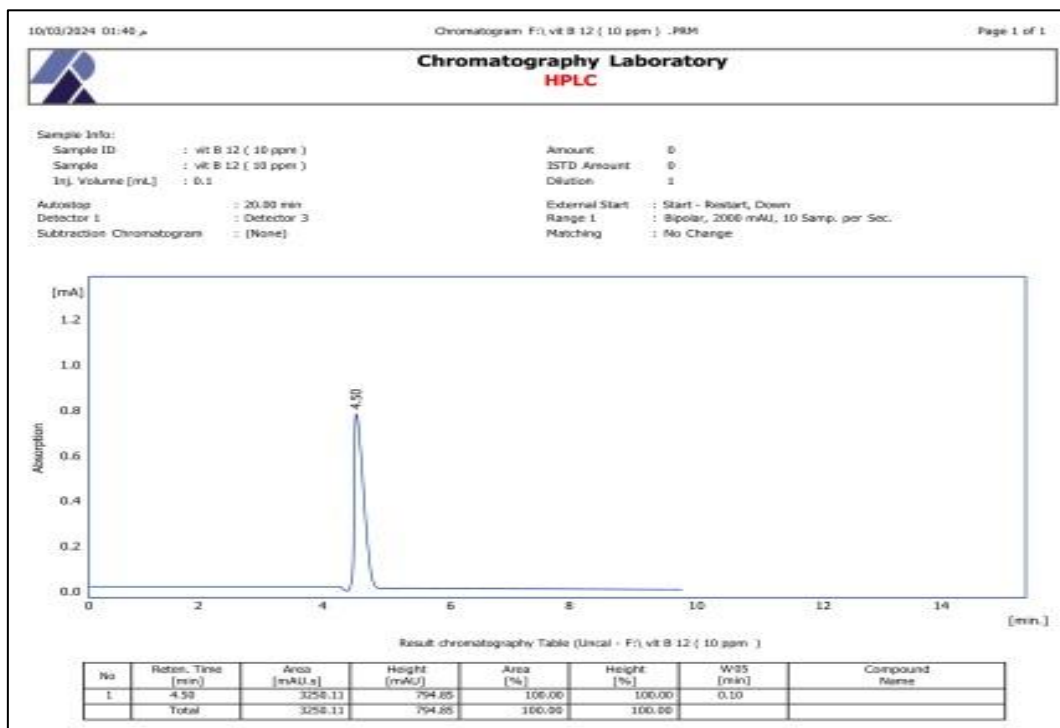


Figure 6. Standard chromatographic diagram of vitamin B12 using HBLC device.

2.5. Extraction Method

20 gm was taken from each sample and dried in an oven at 35C°, then 10 ml of (1) mol sodium hydroxide and 25 ml of regular solution (phosphate with poorer) were added and left in the dark for 24 hours, after which the sample was filtered using filter paper and 50 ml was taken from it and placed in a centrifuge at 3000 rpm. The filtrate was taken and stored in the refrigerator until analysis.

2.6. Analysis Conditions

The examination was carried out using the high-performance liquid chromatography (HPLC) technique, SECAM water-based model, to detect vitamins according to the conditions shown in the table below.

Table 2. Detect vitamins according SECAM water-based model.

No.	name	Description
1	Transport phase	Acetone ethyl: distilled water, formic acid 3 47 50
2	Separation column	C18:ODC (25cm × 4.6 mm)
3	Detector	215UV
4	Flow rate	1ml/min

The retention time was relied upon to diagnose the compounds and the concentration calculation equation was relied upon to calculate the concentrations of the compounds:

$$\text{Concentration of the substance} = (\text{Concentration of the standard substance per sample area} \times \text{Dilution}) / (\text{Area of the standard substance} \times \text{Weight of the sample}) \times 100$$

3. Results and Discussion

3.1. Effect of Soil Texture on IAA Content in Soil (mg L^{-1}) at Flowering Stage

The results of the statistical analysis in Table (3) showed significant differences in the averages of IAA numbers - flowering stage between the textures of clay, silty, loam and sandy soils and wheat, barley and broad bean plants, where the texture of the loam soil reached the highest average values ($9.767\_9.219\_9.862\_9.323 \text{ mg L}^{-1}$) respectively. This is attributed to effective role in producing the growth regulator IAA due to the different carbon and nitrogen sources contained in this fertilizer in developing microorganisms for the purpose of producing the growth regulator GA3 and IAA [6, 7], followed by the silty soil ($9.572\_8.971\_9.588\_8.916 \text{ mg L}^{-1}$) respectively. The texture of the silty soil did not differ significantly from the texture of the loam soil in wheat plants and Barley is due to the fact that the Azospirillum bacteria produce many strains of plant hormones in the rhizosphere and the main hormone produced is Indol-3-acetic acid (IAA). There are other hormones produced in small quantities but with significant effects, namely Indol (ILA), Indol-3-ethanol, Indol acid, Indol-3-butyric acid (IBA), lactic methanol, other indole compounds, a number of gibberellins, and cytokinins. [8]while the clay soil texture that gave the values ($8.979\_8.612\_9.31\_8.356 \text{ mg L}^{-1}$) respectively, or the sandy soil texture gave the lowest average values ($7.718\_7.748\_7.915\_7.698 \text{ mg L}^{-1}$) respectively

Table 3. Effect of soil texture on the IAA content in the soil (mg L^{-1}) at the flowering stage.

Soil Type	Wheat	Barley	broad bean	Radish
clay	8.979	8.612	9.031	8.356
silt	9.572	8.971	9.588	8.916
loam	9.767	9.219	9.862	9.323
sand	7.718	7.748	7.915	7.698
LSD0.05	0.211	0.288	0.258	0.290

3.2. Effect of Soil Texture on IAA Content in Soil (mg L^{-1}) at the End of the Season

The results of the statistical analysis in Table (4) showed significant differences in the averages of IAA numbers - end of the season between the textures of clay, silty, loam and sandy soils and wheat, barley and broad bean, where the texture of the loam soil reached the highest average values ($5.079\_5.104\_5.268\_4.961 \text{ mg L}^{-1}$) respectively. The reason for this is that plant hormones, unlike animal hormones, affect many and different physiological processes in the plant body, which is reflected in more than one aspect of its growth. Therefore, what we observe of the growth and development of the plant may be the result of effect of the secretion of different hormones. There are several types of plant hormones that differ from each other in their chemical composition and biological effect. They may be stimulating hormones: auxins, especially AIA, gibberellins and cytokinins, or inhibitory hormones such as ethylene and abscisic acid [9], followed by the silt soil. ($4.914\_4.823\_5.066\_4.617 \text{ mg L}^{-1}$) respectively. The reason for the increase in this is the ability of bacteria to produce and IAA from the rhizosphere surrounding the plant roots, as bacteria have a high ability to colonize the rhizosphere [10,11], while the clay soil texture that gave the values reached ($4.582\_4.355\_4.707\_4.100 \text{ mg L}^{-1}$) respectively, while the sandy soil texture gave the lowest average values ($3.807\_3.755\_3.965\_3.584 \text{ mg L}^{-1}$) respectively.

Table 4. Effect of soil texture on IAA content in soil (mg L^{-1}) at the end of the season.

Soil Type	Wheat	Barley	Broad bean	Radish
clay	4.582	4.355	4.707	4.100
silt	4.914	4.823	5.066	4.617

loam	5.079	5.104	5.268	4.961
sand	3.807	3.755	3.965	3.584
LSD0.05	0.150	0.236	0.167	0.220

3.3. Effect of Soil Texture on GA3 Content in Soil (mg L^{-1}) at Flowering Stage

The results of the statistical analysis in Table (5) showed significant differences in the average numbers of GA3 - flowering stage between the clay, silty, loam and sandy soil textures and wheat, barley and fava beans plants, where the loam soil reached the highest average values ($14.691\_14.429\_14.737\_14.259 \text{ mg L}^{-1}$) respectively, which is attributed to the secretion of growth regulators and amino acids by living organisms in the root zone [11]. Then came the silty soil texture ($14.114\_14.096\_14.300\_13.937 \text{ mg L}^{-1}$) respectively. The silty soil texture did not differ significantly from the loam soil texture in barley plants, which is attributed to the difference in the effect of the texture on the physical, chemical and biological properties of the soil, which affects the reproduction, growth and activity of microorganisms. And its secretions [12].

While the clay soil texture that gave the values ($13.516\_13.478\_13.639\_13.163 \text{ mg L}^{-1}$) respectively, the sandy soil texture gave the lowest average values ($12.560\_12.287\_12.882\_12.154 \text{ mg L}^{-1}$) respectively, and the reason is due to the intermediate state of the clay separator, which increases the secretion of growth hormones by living organisms, including GA3 [13].

Table 5. Effect of soil texture on GA3 content in soil (mg L^{-1}) in flowering stage.

Radish	Broad bean	Barley	Wheat	Soil Type
13.163	13.639	13.478	13.516	clay
13.937	14.300	14.096	14.114	silt
14.259	14.737	14.429	14.691	loam
12.154	12.882	12.287	12.560	sand
0.493	0.467	0.440	0.560	LSD0.05

3.4. Effect of Soil Texture on the Content of GA3 in the Soil (mg L^{-1}) at the End of the Season

The results of the statistical analysis in Table (6) showed significant differences in the averages of GA3 numbers - at the end of the season between the textures of clay, silty, loam and sandy soils and wheat, barley and fava beans plants, where the texture of the loam soil reached the highest average values ($10.316\_10.186\_10.663\_10.082 \text{ mg L}^{-1}$) and the reason is attributed to the secretions of microorganisms in the soil and hormones that encourage plant growth in the rhizosphere [14].

respectively, then followed by the silty soil ($9.775\_9.503\_10.117\_9.372 \text{ mg L}^{-1}$) respectively, which did not differ significantly between the texture of the silty soil and the texture of the loam soil in radish plants. Radish The difference in the effect of the texture on the various soil characteristics is due to the difference in the proportion of its joints and the intensity of its effect, which affects Secretions of nitrogen-fixing organisms [15]. The reason is due to the intermediate state of the clay separator, which increases the secretions of nitrogen-fixing microorganisms. [16] While the clay soil texture that gave the values ($9.205\_9.091\_9.340\_8.715 \text{ mg L}^{-1}$) respectively, the sandy soil texture gave the lowest average values ($7.344\_7.734\_8.094\_7.691 \text{ mg L}^{-1}$) respectively.

Table 6. Effect of soil texture on the content of GA3 in the soil (mg L^{-1}) at the end of the season stage.

Soil Type	Wheat	Barley	Broad bean	Radish
clay	9.205	9.091	9.340	8.715
silt	9.775	9.503	10.117	9.372
loam	10.316	10.186	10.663	10.082
sand	7.344	7.734	8.094	7.691
LSD0.05	0.406	0.375	0.385	0.471

3.5. Effect of Soil Texture on the Content of Vitamin B1 in the Soil ($\mu\text{g g}^{-1}$) in the Flowering Stage

The results of the statistical analysis in Table (7) showed significant differences in the average concentrations of vitamin B1 - flowering stage between the clay, silty, loam and sandy soil textures and wheat, barley and broad beans plants, where the loam soil texture reached the highest average values ($7.459_7.355_7.640_7.465 \mu\text{g g}^{-1}$), respectively. This is attributed to the release of water-soluble vitamins such as niacin, thiamine, riboflavin, pantothenic acid and biotin from plant roots [17], but they are also produced at levels that indicate the high infection rate of bacteria and fungi that produce vitamins in the root zone of the plant to the manufactured vitamins and the direct contribution of these compounds to plant growth [18]. Then came the silty soil texture ($7.088_6.963_7.145_6.877 \mu\text{g g}^{-1}$), respectively, while the clay soil texture reached The values were given ($6.698_6.382_6.804_6.142 \mu\text{g g}^{-1}$), respectively. The soil texture of clay soil did not differ from the texture of silty soil in wheat plants. As for sandy soils, they gave the lowest average values ($4.726_4.534_5.085_4.806 \mu\text{g g}^{-1}$), respectively.

Table 7. Effect of soil texture on the content of vitamin B1 in the soil ($\mu\text{g g}^{-1}$) at the flowering stage.

Soil Type	Wheat	Barley	Broad bean	Radish
clay	6.698	6.382	6.804	6.142
silt	7.088	6.963	7.145	6.877
loam	7.459	7.355	7.640	7.465
sand	4.726	4.534	5.085	4.806
LSD0.05	0.253	0.324	0.296	0.307

Effect of soil texture on the content of vitamin B1 in the soil ($\mu\text{g g}^{-1}$) at the end of the season

The results of the statistical analysis in Table (8) showed significant differences in the average concentration of vitamin B1 - at the end of the season between clay, silty, loam and sandy soils and wheat, barley and broad beans plants, where the loam soil reached the highest average values ($6.396_6.558_6.922_6.320 \mu\text{g g}^{-1}$), respectively, followed by the silty soil ($5.927_5.978_6.430_5.805 \mu\text{g g}^{-1}$), respectively, while the clay soils, which were given values ($5.644_5.452_5.966_5.039 \mu\text{g g}^{-1}$), respectively, and the reason is due to the spread of nitrogen-fixing and growth-promoting bacteria, as well as fungi that contribute to the secretion of vitamins to the roots in the rhizosphere of plants, and the availability of suitable conditions for soil texture, temperature, humidity and ventilation. Suitability [19]. As for the sandy soil texture, it gave the lowest average values ($3.940_3.725_3.861_3.506 \mu\text{g g}^{-1}$), respectively.

Table 8. Effect of soil texture on the content of vitamin B1 in the soil ($\mu\text{g g}^{-1}$) at the end of the season stage.

Soil Type	Wheat	Barley	Broad bean	Radish
clay	5.644	5.452	5.966	5.039
silt	5.927	5.978	6.430	5.805

loam	6.396	6.558	6.922	6.320
sand	3.940	3.725	3.861	3.506
LSD0.05	0.235	0.418	0.354	0.328

3.6. Effect of Soil Texture on the Content of Vitamin B12 in the Soil ($\mu\text{g g}^{-1}$) at the Flowering Stage

The results of the statistical analysis in Table (9) showed that there were significant differences in the averages of vitamin B3 concentration - at the end of the season between the textures of clay, silty, loam and sandy soils and wheat, barley and fava beans plants, where the texture of the soil and loam soils reached the highest average values (5.169_ 4.741_ 5.226_ 4.809 $\mu\text{g g}^{-1}$), respectively, followed by the texture of the silty soil (4.427_ 4.217_ 4.739_ 4.425 $\mu\text{g g}^{-1}$), respectively, while the textures of the clay soils, which were given values (4.189_ 4.066_ 4.403_ 3.978 $\mu\text{g g}^{-1}$), respectively, and the texture of the clay soil did not differ significantly from the texture of the silty soil from the barley plant, and the reason for the superiority of these textures over the sandy texture is due to The fineness of its particles and the increase in its surface area lead to the regularity of these joints, which is reflected in improving the structure and consequently the growth of nitrogen-fixing and colonizing bacteria to provide suitable conditions for growth and secretion of substances that stimulate plant growth, including vitamins [21]. As for the sandy soil texture, it gave the lowest values for the averages (3.586_ 3.359_ 3.634_ 3.292 $\mu\text{g g}^{-1}$), respectively.

Table 9. Effect of soil texture on the content of vitamin B12 in the soil ($\mu\text{g g}^{-1}$) at the flowering stage.

Soil Type	Wheat	Barley	Broad bean	Radish
clay	3.174	2.953	3.254	3.096
silt	3.813	3.744	3.832	3.683
loam	3.468	3.216	3.610	3.351
sand	2.564	2.446	2.695	2.250
LSD0.05	0.241	0.290	0.308	0.329

3.7. Effect of Soil Texture on the Content of Vitamin B12 in the Soil ($\mu\text{g g}^{-1}$) at the End of the Season

The results of the statistical analysis in Table (10) showed that there were significant differences in the average concentrations of vitamin B12 - at the end of the season between the textures of clay, silty, loam and sandy soils and wheat, barley and broad bean, where the texture of the silty soil reached the highest average values (2.681_ 2.580_ 2.868_ 2.477 $\mu\text{g g}^{-1}$), respectively, followed by the texture of the loam soil (2.338_ 2.286_ 2.410_ 2.206 $\mu\text{g g}^{-1}$), respectively, The reason is due to the effect of soil texture on the physical, chemical and biological properties of the soil, the proportion of its components, water retention and the high surface area that encourages bacteria and fungi to secrete enzymes alongside the roots in the soil rhizospheres [22]. while the texture of the clay soil reached the values (1.917_ 1.866_ 2.061_ 1.730 $\mu\text{g g}^{-1}$), respectively, while the texture of the sandy soil gave the lowest values For the averages (1.276_ 1.468_ 1.544_ 1.084 $\mu\text{g g}^{-1}$), respectively.

Table 10. Effect of soil texture on soil vitamin B12 content ($\mu\text{g g}^{-1}$) at the end of the season stage.

Soil Type	Wheat	Barley	Broad bean	Radish
clay	1.917	1.866	2.061	1.730
silt	2.681	2.580	2.868	2.477
loam	2.338	2.286	2.410	2.206

sand	1.276	1.468	1.544	1.084
LSD0.05	0.302	0.264	0.295	2.245

3.8. Effect of Soil Texture on the Content of Vitamin B3 in the Soil ($\mu\text{g g}^{-1}$) in the Flowering Stage

The results of the statistical analysis in Table (11) showed that there were significant differences in the average concentrations of vitamin B3 - flowering stage between the textures of clay, silty, loam and sandy soils and wheat, barley and broad bean, where the texture of the soil and loam soils reached the highest average values ($5.736_5.481_5.785_5.358 \mu\text{g g}^{-1}$), respectively, followed by the texture of the silty soil ($5.085_4.864_5.446_4.909 \mu\text{g g}^{-1}$), respectively, perhaps due to the fungus *Trichoderma* in providing the internal elements (2015, Lee), while the texture of the clay soils, which were given the values ($4.861_4.549_5.031_4.324 \mu\text{g g}^{-1}$), respectively. The reason is attributed to the fact that the soil texture is one of the main factors in determining the elements adsorbed on the surfaces of the separators, especially the soft ones, as well as determining the ability of these separators to adsorb, whether they are microscopic organisms that fix nitrogen or elements that contribute to increasing the activity of the secretions of microorganisms and roots of vitamins [23]. As for the sandy soil texture, it gave the lowest average values ($4.190_3.831_4.356_3.428$ respectively).

Table 11. Effect of soil texture on the content of vitamin B3 in the soil ($\mu\text{g g}^{-1}$) in the flowering stage.

Soil Type	Wheat	Barley	Broad bean	Radish
clay	4.861	4.549	5.031	4.324
silt	5.085	4.864	5.446	4.909
loam	5.736	5.481	5.785	5.358
sand	4.190	3.831	4.356	3.428
LSD0.05	0.168	0.282	0.306	0.223

3.9. Effect of Soil Texture on the Content of Vitamin B3 in the Soil ($\mu\text{g g}^{-1}$) at the End of the Season

The results of the statistical analysis in Table (12) showed that there were significant differences in the average concentrations of vitamin B3 - at the end of the season between the textures of clay, silty, loam and sandy soils and wheat, barley and broad bean, as the texture of the soil and loam soils reached the highest average values ($5.169_4.741_5.226_4.809 \mu\text{g g}^{-1}$), respectively, The reason is attributed to the increase in the population density of bacteria and fungi inhabiting soil textures, which differed in their levels of vitamin secretion, in addition to their colonization of plant roots [24]. followed by the texture of the silty soil ($4.427_4.217_4.739_4.425 \mu\text{g g}^{-1}$), respectively, while the texture of the clay soils, which gave the values ($4.189_4.066_4.403_3.978 \mu\text{g g}^{-1}$), respectively, and the texture of the clay soil did not differ from The texture of the silt soil was significantly higher than that of barley, while the texture of the sandy soil gave the lowest average values ($3.586_3.359_3.634_3.292 \mu\text{g g}^{-1}$), respectively.

Table 12. Effect of soil texture on the content of vitamin B3 in the soil ($\mu\text{g g}^{-1}$) at the end of the season.

Soil Type	Wheat	Barley	Broad bean	Radish
clay	4.189	4.066	4.403	3.978
silt	4.427	4.217	4.739	4.425
loam	5.169	4.741	5.226	4.809
sand	3.586	3.359	3.634	3.292
LSD0.05	0.265	0.232	0.278	0.208

3.10. Effect of Soil Texture on the Content of Vitamin B9 in the Soil ($\mu\text{g g}^{-1}$) in the Flowering Stage

The results of the statistical analysis in Table (13) showed that there were significant differences in the average concentrations of vitamin B9 - flowering stage between the textures of clay, silty, loam and sandy soils and wheat, barley and broad bean, where the texture of the soil and loam soils reached the highest average values ($6.736\_6.567\_7.004\_6.609 \mu\text{g g}^{-1}$), respectively, followed by the texture of the silty soil ($6.145\_5.828\_6.497\_5.962 \mu\text{g g}^{-1}$), respectively, while the texture of the clay soils reached the values ($5.744\_5.570\_6.045\_5.621 \mu\text{g g}^{-1}$), respectively, The reason is attributed to the superiority of fine soil textures because they have a high specific surface area that contributes to the reproduction and growth of microorganisms by retaining moisture for a longer period, in addition to maintaining their biological activity in the rhizosphere region in semi-arid conditions. As for the sandy soil texture, it gave the lowest average values ($1.276\_1.468\_1.544\_1.084 \mu\text{g g}^{-1}$), respectively. while the texture of the sandy soils The lowest values of the averages were given ($5.204\_4.989\_5.268\_4.844 \mu\text{g g}^{-1}$), 4.844 respectively.

Table 13. Effect of soil texture on the content of vitamin B9 in the soil ($\mu\text{g g}^{-1}$) at the flowering stage.

Soil Type	Wheat	Barley	Broad bean	Radish
clay	5.744	5.570	6.045	5.621
silt	6.145	5.828	6.497	5.962
loam	6.736	6.567	7.004	6.609
sand	5.204	4.989	5.268	4.844
LSD0.05	0.359	0.272	0.410	0.257

3.11. Effect of Soil Texture on the Content of Vitamin B9 in the Soil ($\mu\text{g g}^{-1}$) at the End of the Season

The results of the statistical analysis in Table (14) showed that there were significant differences in the average concentrations of vitamin B9 - at the end of the season between the textures of clay, silty, loam and sandy soils and wheat, barley and broad bean, where the texture of the soil and loam soils reached the highest average values ($6.133\_5.800\_6.354\_5.928 \mu\text{g g}^{-1}$), respectively, followed by the texture of the silty soil ($5.582\_5.240\_5.676\_5.381 \mu\text{g g}^{-1}$), respectively, while the texture of the clay soils, which gave the values ($5.172\_4.751\_5.338\_4.878 \mu\text{g g}^{-1}$), respectively, while the texture of the sandy soils gave the lowest values For the averages ($4.346\_4.127\_4.428\_3.917 \mu\text{g g}^{-1}$), respectively.

Table 14. Effect of soil texture on the content of vitamin B9 in the soil ($\mu\text{g g}^{-1}$) at the end of the season stage.

Soil Type	Wheat	Barley	broad bean	Radish
clay	5.172	4.751	5.338	4.878
silt	5.582	5.240	5.676	5.381
loam	6.133	5.800	6.354	5.928
sand	4.346	4.127	4.428	3.917
LSD0.05	0.328	0.198	0.277	0.347

References

- [1] Al-Mawsili, Mazhar Ahmad. 2011. Basics of General Soils for Horticulture, College of Agriculture - University of Mosul, Dar Dijlah Press 2012.
- [2] Pinton, R.: Z. Varavnini and p. Nannipieri. 2001. the rhizosphere as a site of biochemical interactions amogosoil Components, plants and microorganisms, in: the Rhizosphere Biochemistry and Organic Substances at the Soil - plant Interface, N.Y., pp. 1-17.

- [3] Salah Mahdi Laibi' and Jawad Abdul Kadhim Kamal. Effect of *T. harzianum* and *G. mosseae* Biological Inoculation and Phosphate Rocks on the Availability of NPK in the Rhizosphere of Barley Crop (*Hordium Valgari* L.). Fifth International Conference for Agricultural and Environment Sciences. IP address 169.224.86.57.
- [4] Azaizeh, H.A., Neumann, G. and Marschner, H. (1995) Effects of thiamine (Vitamin B-1) application on soil bulk and rhizosphere microorganisms and on its release from bean (*Phaseolus vulgaris* L) seedlings. See also: 158, 549-556.
- [5] Tabarak Rahim Shuppar and Jawad Abdl-Kadhim Kamal.2021. The Effect of Biological Inoculation of *Azotobacter chroococcum* and *Glomus mosseae* on Growth and Yield of *Zea mays* L. under Different Levels of Water Stress. Virtual International Scientific Agricultural Conference IOP. IP 169.224.86.57
- [6] Kosuge, T. ; Heskett, M. C. and Wilson, E. E. 1966. Microbial synthesis and degradation of Indole - 3- acetic acid the conversion of L-tryptophan to Indole-3 acetic acid by enzyme system from *Pseudomonas savastanoi* . J. Biological Chemistry, 241(16).3738-3744
- [7] Al-Husseini Ayad Kadhim Ali 2010 Inheritance and evolution of the profit horizons of some soils of northern Iraq. PhD thesis - College of Agriculture - University of Baghdad
- [8] Tien, T. m., Gaskins, M. H. and Hubbel, D. H. 1979. Plant growth substances produced. by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.) Appl. Environ. Microbiol., 37: 1016-1024.
- [9] Heller R, et Lance C., (2000). Physiologie vegetables. Part 2: Development 1 and 2 cycles, 6 months old, Dunod sciences. Paris.p: 64-134.
- [10] Kamble,K.D. and Galero, D.K. 2015. Indole acetic acid Production from *Pseudomonas* sp. isolated from rhizosphere of garden plants in Amaravati. Int. J.Adv.Pharmacy Bio. Chem.,4(1):23-31.
- [11] Bano, N., and Musarrat, J. 2003. Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. Current microbiology, 46(5), 0324-0328.
- [12] Zahir, Z. A., Asghar, H. N.. Akhtar. M. J., and Arshad, M. 2005. Interaction for Precursor (L-tryptophan)-inoculum (*Azotobacter*) improving yields and nitrogen uptake of maize. Journal of plant nutrition, 28(5), 805-817.
- [13] Forghani, A; H, A. Almodares and A, A. Ehsanpour. 2018. Potential objectives for gibberellic acid sweet sorghum (*Sorghum bicolor* L. Moench cv. Sofra).P,A. Vol:61. pp: 113-124.
- [14] Gartlet, K. L., 2011. Recommended soil testing procedures for the Northeastern United States, Recommended methods for measuring saolabe salts in soils. Chapter (10), cooperative Bullentin No. 493.
- [15] Zahir, Z. A., Asghar, H. N.. Akhtar. M. J., and Arshad, M. 2005. interaction for Precursor (L-tryptophan)-inoculum (*Azotobacter*) improving yields and nitrogen uptake of maize. Journal of plant nutrition, 28(5), 805-817.
- [16] Atta, Gomaa Mohamed. 2010. Soil texture, Alexandria University- Faculty of Agriculture-Agricultural Research Station, 2010.
- [17] Gartlet, K. L., 2011. Recommended soil testing procedures for the Northeastern United States, Recommended methods for measuring saolabe salts in soils. Chapter (10), cooperative Bullentin No. 493.
- [18] Rovira, A. D. and Harris, J. R. (1961) Plant root excretions in relation to the rhizosphere effect. Plant and Soil 14, 199-213.
- [19] Mozafar, A. (1994) Plant Vitamins. Agronomical, Physiological and Nutritional Aspects. Boca Raton, Florida, USA: CRL Press Benson, H. J. 2001. Microbial application laboratory in general microbiology, 8t edition. McGraw-Hill Companies.
- [20] Lee, B. H. (2015). Fundamentals of Food Biotechnology, 2nd Edn. Hoboken, NJ; Wiley Blackwell.
- [21] Salahin, N., Alam, M. D. K., Islam, M. D. M., Naher, L., & Majid, N. M. (2013) Effects of green manure crops and tillage practice on maize and rice yields and soil properties. Aust. J. Crop Sci, 7, 1901–1911.



- [22] Al-Amri, Abbas Ali 2013. A published lecture on the basics of soil science, educational website, College of Agriculture - University of Karbala.
- [23] Pearson, K. 2003. The basic of salinity and sodicity effects on soil physical properties. Water quality and irrigation management. Montana state University. Bozeman. Wate .
- [24] Sierra, S., Rodelas, B., Martínez-Toledo, M. V., Pozo, C., and González- López, J. (1999). Production of B-group vitamins by two Rhizobium strains in chemically defined media. J. Appl. Microbiol. 86, 851-858. doi: 10.1046/j.1365-2672.1999.00765.

Effect of Two Hybrids of Cherry Tomato (*Solanum Lycopersicum* L.) Grown in Greenhouse and Sprayed with Calcium and Iron on Yield and its Components

Azhar Ward Aboud Al-Maliki¹, Abdullah A. Abdullah² and Abbas Kadhim Obaid³

¹Basrah Agriculture Directorate, Basrah, Iraq.

^{2,3}Department of Horticulture and Landscape Architecture, College of Agriculture, University of Basrah, Basrah, Iraq.

¹E-mail: azharwardbwdalmalky@gmail.com

³E-mail: abbas.obaid@uobasrah.edu.iq

Abstract. The experiment was conducted during the winter season 2023/2024 in one of greenhouse at the Research Station of the College of Agriculture, University of Basrah. The study aims to know the effect of hybrids and spraying with calcium and iron on the quantitative and qualitative components of the yield of the Cherry tomato *Solanum Lycopersicum* L. plant. The experiment included 24 factorial treatments, which are two hybrids of Cherry tomato (POMODORO Principe Borghese V1) and (Principe EOR V2) and four concentrations of chelated calcium (zero, 1, 2 and 3 ml.L⁻¹), and three concentrations of chelated iron (zero, 100 and 200 mg.L⁻¹). The plants were sprayed three times, the first after 30 days of transplanting and with an interval of 14 days between each spray. The experiment was a factorial split-split Plot design according to the Randomized Complete Block Design (R.C.B.D.) with three sectors. The arithmetic means of the treatments were compared using the least significant difference test at the probability level (0.05). The results showed that the plants of the first hybrid V1 were significantly excelled on the second hybrid V2 in most of the quantitative yield traits in the number of fruits, fruit weight, and the yield of one plant, which amounted to 4.035 kg.plant⁻¹ and also excelled in the qualitative traits of the yield, which included TSS and lycopene, while there was no significant difference between the hybrids in other qualitative traits such as dry matter. Spraying with calcium had a significant effect in increasing the yield in quantity and quality, as the concentration of 3 ml.L⁻¹ was excelled in most of the yield traits, which are the number of fruits, the yield of one plant, and the increase in the concentration of total soluble solids (TSS), while the concentration of 2 ml.L⁻¹ was excelled in fruit weight and the lycopene trait, and the concentration of 1 ml.L⁻¹ was excelled in increasing the percentage of dry matter compared to other concentrations. Spraying with iron had a significant effect on improving traits of tomato plant yield in terms of quantity and quality, as the concentration of 200 mg.L⁻¹ achieved the highest values in the number of fruits, weight of one fruit, and yield of one plant, which reached 3.674 kg.plant⁻¹, and increased the concentration of total soluble solids (TSS) and dry matter in the fruits, while the concentration of 100 mg.L⁻¹ was significantly excelled in the lycopene characteristic. Most of the binary and triple interactions showed a significant effect for all the studied traits

Keywords. Quantitative and qualitative yield, Cherry tomato hybrids, Calcium, Iron, Foliar spray, Greenhouse.

1. Introduction

Cherry tomato (*Solanum Lycopersicum* L. (Var. *cerasiforme*) belonging to the Solanaceae family is one of the important summer vegetable crops characterized by its small fruits that are distinguished by important nutrients such as vitamin C, carotenoids, flavonoids and lycopene, which are essential for human health and protect it from cancer [1]. The speed of increasing productivity per unit area can be achieved by using a new hybrid type, using new seeds and using modern technologies [2]. Cherry tomato is a type of regular tomato and is small in size, and contains 94.5 g of water, 75.4 kcal of energy, 0.9 g of protein and 0.2 g of fat Carbohydrates 3.9g, calcium 10mg, potassium 237mg, vitamin A 833mg, vitamin C 12.7mg in 100g [3] (Cherry tomato is characterized by its high quality and high sweetness, which makes it more acceptable to the consumer, and it is characterized by its distinctive taste that many prefer over regular tomatoes [4]. Tomato is a vegetable crop with medium tolerance to salinity [5]. Many studies have indicated the variation of cultivars among themselves in terms of quantitative and qualitative yield traits, as [5] found when studying ten strains of Cherry tomato, where five pure strains were selected and an evaluation of the genetic structures was conducted, where (4451LA) excelled in the weight of the fruit, which reached (8.41 g) and the yield of one plant reached (2.72 g), while (LA4689) excelled in the number of fruits, which reached (512.33 fruits. Plant⁻¹). (4451LA) was excelled in the percentage of total soluble solids (TSS) reaching (5.11%). Wanous et al. (2023) found when studying four strains of Cherry tomato (of different origins) in Syria and planted under a protected environment, that the strain AS2 was excelled in the number of fruits, reaching (16.3) fruits. plant⁻¹, while the strain Sa recorded the highest fruit weight, reaching (49.62 g). [6] found when planting 11 samples of Cherry tomato under a protected environment, where (9) cultivars and hybrids were compared with the Cherry tomato (Marvarid) cultivar as a standard cultivar, where the cultivar (Black cherry) was excelled to the rest of the cultivars in the number of fruits, reaching (18 fruits. plant⁻¹), and the cultivar (Quality seed) was excelled on the rest of the cultivars in the average fruit weight, reaching (43 g). Foliar nutrition is one of the advanced agricultural methods that ensures the effective and faster delivery of nutrients to the plant compared to root nutrition, and saves on the amount of fertilizers and overcomes soil problems such as loss and sedimentation. Foliar spraying is (10-20) times more efficient than soil spraying [7]. Among the important elements for tomatoes are calcium and iron. [8] found that when tomato plants (Oula F1) were sprayed with liquid organic fertilizer containing calcium Ca at three concentrations (0, 1.25, 2.5%) ml.l⁻¹, the concentration (2.5 ml.l⁻¹) was excelled in production indicators, as the number of fruits reached (37.77 fruits. plant⁻¹) and the weight of the fruit reached (82.38 g. fruit⁻¹) compared to control treatment. [9] confirmed from his study on okra plant (btayrh) cultivar grown in greenhouses in Maysan Governorate that spraying with calcium at a concentration of 1 ml. L⁻¹ significantly excelled the number of pods per plant, the weight of one pod, and the yield of one plant compared to the control and the two concentrations (0.5, 1.5 ml. L⁻¹). [10] indicated that spraying tomato plants with calcium at concentrations of (0, 0.2, 0.3)% in the form of calcium chloride caused a concentration of 0.3% to significantly increase the number of fruits, while both concentrations of 0.2 and 0.3% showed a significant increase in the weight of one fruit compared to control treatment and [11] when spraying tomato plants with three concentrations of calcium (0, 0.3, 0.6%), as the concentrations (0.3, 0.6%) excelled in increasing the total number of fruits compared to control treatment, while spraying had no significant effect on increasing fruit weight and total soluble solids, [12] observed that when

calcium was added to tomato plants in the form of Ca actsoal containing 12% calcium oxide and 15% humic and folic acid at levels (0, 500, 1000, 3000, 5000) mg.kg⁻¹, the results showed that the level of 3000 mg.kg⁻¹ was significantly excelled to the level of 3000 mg.kg⁻¹ in fruit weight, fruit dry matter percentage, and total soluble solids percentage compared to the other levels and control treatment. [13], observed in a study conducted on two cultivars of tomatoes grown in a greenhouse and exposed to salt stress and sprayed with calcium on the leaves daily in the form of (CaSO₄.5H₂O) at three concentrations (5, 10, and 15 mm) in addition to control treatment, where the concentration was excelled (10 mm) on the rest of the concentrations, a significantly excelled, as the percentage of TSS reached (5.75%) compared to control. [14] explained that when tomato plants were sprayed with seven calcium treatments, foliar spraying at a concentration of (0.5% at 75 days) was excelled in most of the yield traits compared to the rest of the treatments, as the number of fruits per plant reached (31.66 fruits. plant⁻¹), and the yield of one plant reached (37.83 kg. plant⁻¹) compared to control. [15] found that when spraying tomato plants at the flowering stage with iron (Fe-EDTA (foliar spray at three concentrations (100, 150, 200 µM), the concentration (150 µM) excelled the rest of the concentrations and control treatment in fruit weight, reaching (3.891 g), while it was (2.802 g) in the comparison. [16] obtained when spraying tomato plants, Wajdan cultivar, with four levels of iron (0, 25, 50, 75) kg Fe, where the level of 75 kg Fe significantly excelled in increasing the percentage of dry matter in the fruits, the percentage of total soluble solids, the average fruit size, the number of fruits, the fruit weight, and the total yield compared to control treatment. [17] found that when Cherry tomato plants were sprayed with a group of micronutrients, including ferrous sulfate (FeSO₄) at an amount of (100) parts per million, foliar spray, this treatment excelled control treatment in a number of traits, including the number of fruits per plant, which reached (125.02), the average fruit weight reached (14.48 g), the yield per plant reached (1.81 kg), and the amount of TSS reached 7.23%. Based on the above and the lack of studies on the effect of hybrids and Foliar nutrition with calcium and iron on the quantitative and qualitative components of the cherry tomato plant in Basra province, this study was conducted.

2. Materials and Methods

The experiment was conducted in the winter season 2023-2024 and the transplanting method was used in planting tomatoes [18] in one of the unheated greenhouses affiliated with the Agricultural Research Station affiliated with the College of Agriculture, University of Basra, with dimensions of (42 x 8.5 m) and an area of 357 m². The soil of the greenhouse was analyzed before planting by taking samples randomly from different places and at a depth of (0-30 cm). Samples of irrigation water were also taken for the purpose of conducting some chemical analyses on it. Table (1) shows some of the physical and chemical properties of the soil of greenhouse and irrigation water as they were analyzed in the laboratories of the Marine Science Center/University of Basra. The soil of the greenhouse was prepared on 8/15/2023 by plowing it deeply twice using a rotary plow in a perpendicular manner, smoothing it, and adding decomposed animal manure to it (cow waste) at an average of 10 m³. dunum⁻¹, equivalent to 30 tons. ha⁻¹ [19] with the addition of superphosphate at an average of 1.5 kg. dunum⁻¹. It was leveled and sterilized by exposing it to direct sunlight. The land of the house was divided into six lines with a length of (36 m) and a width of (50 cm) and a distance of (128 cm) between the line Another one, 30 cm deep, and a space was left on each side of the house, and the lines were buried in the field soil at a height of (15) cm, and the house was sprayed with the fungicide ((Beltanol), and the drip irrigation system was extended in the middle of the lines and the lines were covered with the black cover Mulching Soil, and 10 kg of DAP was added with 10 kg of urea before planting, and the treatments were distributed randomly to the experimental units in each line and each experimental unit contains 10

plants, 5 plants on each side of the line and in opposite directions and with a planting distance of 30 cm between one plant and another. In this experiment, seeds of the Italian-originated Cherry tomato hybrid (V1 POMODORO Principe Borghese) were used, as this hybrid is characterized by a germination rate of 90% and a purity of 99%, and the second hybrid (V2 EOR Principe) is of Russian origin, and the germination rate of this hybrid is 90% and a purity of 99%, the weight of the seeds (gm) for each of them, the seeds were planted on 10/16/2023 in white cork dishes, each cork containing 198 (11×18) eyes, each type four corks, where the corks were filled with peat moss after moistening and softening the peat moss, at an average of one seed per eye and covering it with a layer of peat moss, and the seeds were watered after planting by light spraying with a special sprinkler, and the corks were placed inside a cooled shade and shaded with green mesh saran cloth to reduce sunlight, and all operations related to seedling production were carried out, as the seedlings were sprayed after the emergence of True leaves (3-4 true leaves) with high phosphorus compound fertilizer at an average of 1 g.L⁻¹, only two sprays with a two-day interval between each spray and then sprayed with balanced NPK fertilizer 20:20:20 at an average of 1 g.L⁻¹. The seedlings were also sprayed with a fungicide (Minatol) at an average of 1 cc.L⁻¹ on the vegetative group and soil. After that, the plants were hardened by lifting the corks from the soil and reducing the number of irrigations to reduce the shock of the seedlings.

Table 1. Some chemical and physical properties of the greenhouse soil and irrigation water.

Traits	Values	Method
Electrical conductivity (EC) dSm ⁻¹	4.14	
Physiodynamics (pH)	6.45	
Total nitrogen (mg. g ⁻¹)	0.567	
Phosphorus (mg. g ⁻¹)	0.014	
Potassium (mg.g ⁻¹)	0.02	Page <i>etal.</i> (1982)
Calcium (mg.g ⁻¹)	0.8	
Iron (mg.g ⁻¹)	0.345	
Organic matter %	3.3	
Soil separators(%)		
Sand (%)	65	
Silt(%)	25	
Clay(%)	10	Folk(1974)
soil texture	Sand silty	
irrigation water		
Electrical conductivity (E.C) dSm ⁻¹	2.58	
pH	8.24	

The greenhouse was prepared and then divided into six lines with a length of (36 m) and each line was divided into 12 experimental units with a length of (1.5 m) and every 24 experimental units are considered a sector where every three experimental units take one of the four concentrations of calcium and the three concentrations of iron are distributed on them. The planting process was conducted according to the transactions and at a distance of (30 cm) between one plant and another and on both sides of the line in an opposite manner. The number of plants in the experimental unit was 10 plants. The area of one experimental unit was (1.92 m²) and the plant density was (5.21 plants m²). The seedlings were transferred to the greenhouse 30 days after planting the seeds, where they were transferred to the lines on 11/24/2023 by making a hole with Mulching and in an opposite manner on both sides of the same line, and at a distance of 30 cm between one plant and another. The seedlings were then watered with a drip irrigation system. On 11/26/2023, a fungicide (Beltanol) was sprayed and insect traps were set up inside the house.

After that, the patching process was carried out and the failed holes were replanted. After that, on 12/21/2023, a high-phosphorus foliar fertilizer (20:50:20) was sprayed at an amount of (1 g. L⁻¹). After a week, it was sprayed Balanced fertilizer (20:20:20), at an average of (25) g/20 liters of water), and spraying is repeated twice. The plants were also sprayed with insecticides and fungicides periodically to combat insects and diseases. Among the insecticides used was the systemic insecticide (LOMATE) (contains 90% methomyl and 10% additives) (10g/20L/1L of water). In addition to preventing wilt disease, the plants were sprayed with the fungicide (Top Tapsen 70 WP) containing (70% thiophanate methyl and 30% additives) and in the amount of (1g/L/1) as a first preventive spray and was repeated periodically every two weeks. All service operations were also conducted, including weeding, weeding, and regular watering, in addition to carrying out the process of carcinogenesis, removing the side branches, and raising the plant on one stem only. In addition, the process of hanging the plants with ropes to allow them to grow vertically was carried out. The process of spraying chelated calcium was carried out a month after the transplanting process, with three sprays between each spray, two weeks apart. The experiment was carried out as a factorial experiment including two Cherry tomato hybrids and four concentrations of chelated calcium (0, 1, 2, 3) ml. L⁻¹ and three concentrations of iron (0. 100. 200) mg. L⁻¹ split twice (Split Split Plot design) according to a Randomized Complete Block Design (R.C.B.D) with three sectors where the cultivar is the main factor (Main Plot) and the calcium spray concentrations are the secondary factor (Sup Plot) and the iron spray concentrations are the sub-secondary factor (Sup Sup Plot) at an average of three sprays where all treatments were sprayed on the vegetative group in the early morning, the first spray after thirty days from transplanting and the second spray after 14 days from the first spray and the third spray after 14 days from the second spray so that the number of experimental units becomes (2×4×3×3=72 experimental units). Each two adjacent lines are considered a sector in which the two cultivars are randomly distributed on one of the lines. Then the results were analyzed using analysis of variance, and the least significant difference (L.S.D) test was chosen to compare the averages at a probability level of 0.05 [20]. There was a time difference of one day between spraying the plants with calcium and spraying the plants with iron. Harvesting began on 10/3/2024 and continued until 7/5/2024. The number of harvests reached (7) harvests during the experimental season using the appearance, texture and red color of the fruits. Measurements were taken for six plants from each experimental unit and for each replicate randomly. The following measurements were taken:

Number of fruits, fruit weight, yield per plant, percentage of dry matter in the fruits, TSS, fruit content of lycopene, acidity, vitamin C, as follows:

2.1. Total Number of Fruits (fruit.plant⁻¹)

The cumulative number of fruits for each experimental unit was calculated and divided by the number of plants for that unit.

2.2. Average Fruit Weight (g)

The weight of one fruit was calculated based on dividing the yield of the six plants in the experimental unit by the number of fruits in the same experimental unit.

2.3. Plant Yield (kg.plant⁻¹)

The total cumulative plant yield for the experimental unit was calculated divided by the number of plants and the reading was recorded as an average for the experimental unit.

2.4. Percentage of Dry Matter in Fruits (%)

Five fruits were taken from the six plants previously marked for each experimental unit and their weight was calculated, then dried at a temperature of 70°C until the weight was fixed in the oven for 48 hours, then the dry weight of the fruits was taken according to the equation:

$$\text{Dry matter (\%)} = \text{dry weight (g)} / \text{fresh weight (g)} \times 100$$

2.5. Total Soluble Solids (TSS) (%)

According to the method described by [21].

2.6. Lycopene in Fruits (mg.gm⁻¹ fresh weight)

According to the method described by [22].

3. Results and Discussion

Table 2 shows that the study factors and their interactions had a significant effect on the number of fruits and weight of one fruit in the plant, as the plants of the first hybrid V1 were significantly excelled compared to the second hybrid V2 with an increase rate of 3.69 and 63.82% for the two traits, respectively. The same table shows that spraying with calcium had a significant effect on both traits, as plants sprayed with a concentration of 3 ml.l⁻¹ were significantly excelled compared to other concentrations, with an increase rate of 20.76, 8.61, 18.74% for the first, second and third concentrations, respectively, in the trait of number of fruits, while spraying with calcium at a concentration of 2 ml.l⁻¹ was significantly excelled on the rest of the concentrations in the trait of fruit weight, with an increase rate of 22.51, 14.25, 9.39%, respectively. Spraying with iron had a significant effect on both plant traits, as plants sprayed with iron at a concentration of 200 mg.L⁻¹ significantly excelled the control and other concentrations, with an increase rate of 20.09, 7.46, 21.67, and 8.85% for both traits, respectively. As for the interactions, the results of the table showed that the interaction between hybrids and spraying with calcium had a significant effect on both traits, as the first hybrid V1 plants sprayed with calcium at a concentration of 3 ml.L⁻¹ gave the highest number of fruits, reaching 165.00 fruits.plant⁻¹, while the lowest number was 127.11 fruits.plant⁻¹ in the first hybrid V1 plants not sprayed with calcium. The first hybrid V1 plants sprayed with calcium at a concentration of 2 ml.L⁻¹ had a significantly excelled in fruit weight reaching 31.37 g, while the lowest weight was 14.85 g in the second hybrid V2 plants sprayed with calcium at a concentration of 1 ml.L⁻¹. The interaction between the hybrid and iron factors did not have a significant effect on the number of fruits, while the interaction had a significant effect on the fruit weight trait, as the first hybrid V1 plants sprayed with iron at a concentration of 200 mg.L⁻¹ gave the highest fruit weight reaching 29.17 g compared to the lowest weight which was 14.01 g in the second hybrid V2 plants not sprayed with iron. The interaction between calcium and iron had a significant effect on both traits, as plants sprayed with calcium at a concentration of 3 ml. L⁻¹ and iron at a concentration of 200 mg. L⁻¹ gave the highest number of fruits, reaching 171.83 fruits. plant⁻¹, while the lowest number was 111.67 fruits. plant⁻¹ in plants not sprayed with calcium and iron. Plants sprayed with calcium at a concentration of 2 ml. L⁻¹ and sprayed with iron at a concentration of 200 mg. L⁻¹ significantly excelled the rest of the treatments in the trait of fruit weight, reaching 30.06 g, while the lowest weight was 17.20 g in plants not sprayed with calcium and iron. The results of the table showed that the triple interaction had a significant effect on increasing the number of fruits and increasing the weight of the fruit in the plant, as the plants of the first hybrid V1 sprayed with calcium at a concentration of 3 ml.L⁻¹ and sprayed with iron at a concentration of 100 mg. L⁻¹ excelled and gave the highest number of fruits amounting to 174.67 fruits. plant⁻¹, while the lowest number of fruits was 107.33

fruits. plant⁻¹ in the plants of the second hybrid V2 not sprayed with calcium and iron. Meanwhile, the plants of the first hybrid V1 sprayed with calcium at a concentration of 2 ml. L⁻¹ and sprayed with iron at a concentration of 200 mg. L⁻¹ excelled and gave the highest weight of the fruit amounting to 38.02 g, while the lowest weight was 11.43 g in the plants of the second hybrid V2 not sprayed with calcium and iron. The difference between hybrids in both traits may be attributed to the genetic factors specific to the cultivar and its response to environmental factors inside the greenhouse, such as soil and climatic factors. This result is consistent with [23] and that the increase in fruit weight and number of fruits as a result of spraying with calcium may be attributed to its role in encouraging and activating some enzymes that lead to increased cell division, increasing their number and size, and forming cell walls by entering into their composition in the form of calcium pectate, giving them hardness, regulating their permeability, and making them more elastic, in addition to its role in preparing auxin IAA [24]. which encourages vegetative growth and increases the process of photosynthesis, thus increasing the accumulation of nutrients, leading to an increase in fruit weight. Calcium also works to strengthen the connection of the fruit to the mother plant, prevent its fall, regularize its shape, and thus increase the number of fruits in the plant. This result is consistent with what was obtained by [8,10,11] and The increase in the average fruit weight and number of fruits as a result of spraying with iron is due to its role in the representation of nucleic acids and plastids, which led to an increase in the chlorophyll content, which led to an increase in the efficiency of photosynthesis and thus an increase in growth rates by increasing the rate of cell division and elongation and thus increasing the weight of the fruit and its numbers in the plant [25]. This result is consistent with [16].

Table 2. Effect of hybrids, foliar spraying with calcium and iron, and their interactions on the number of fruits (fruit.plant⁻¹) and fruit weight (g).

Hybrids	Calcium ml.L ⁻¹	Total number of fruits (fruit/plant ⁻¹)			Fruit weight (g)		
		Iron (mg.L ⁻¹)			Iron (mg.L ⁻¹)		
		0	100	200	0	100	200
V1	0	116.00	128.00	137.33	127.11	22.97	22.39
	1	131.00	146.00	161.33	146.11	28.62	28.08
	2	130.00	134.33	150.33	138.22	28.74	27.36
	3	146.00	174.67	174.33	165.00	25.35	34.65
V2	0	107.33	139.33	155.33	134.00	11.43	16.34
	1	137.33	140.00	155.33	144.22	12.44	16.23
	2	126.00	130.00	126.00	127.33	15.34	17.96
	3	130.00	151.67	169.33	150.33	16.83	17.72
LSD 5%		8.82			6.48		
		Average effect of Hybrids			Average effect of Hybrids		
Interaction between Hybrids and Iron	V1	130.75	145.75	155.83	144.11	26.42	28.12
	V2	125.17	140.25	151.50	138.97	14.01	17.06
LSD 5%		NS			3.44		
		Average effect of Calcium			Average effect of Calcium		
Calcium-Iron	0	111.67	133.67	146.33	130.56	17.20	19.37

Hybrids	Calcium ml.L ⁻¹	Total number of fruits (fruit/plant ⁻¹)			Fruit weight (g)		
		Iron (mg.L ⁻¹)			Iron (mg.L ⁻¹)		
		0	100	200	0	100	200
Interaction	1	134.17	143.00	158.33	145.17	20.53	22.15
	2	128.00	132.17	138.17	132.78	22.04	22.66
	3	138.00	163.17	171.83	157.67	21.09	26.18
LSD 5%			6.62		5.16	1.40	
Average iron effect		127.96	143.00	153.67		20.21	22.59
LSD 5%			2.78			0.64	

The results of Table 3 show that the study factors and their interactions have a significant effect on the yield of a single plant, as the plants of the first hybrid V1 significantly excelled the plants of the second hybrid V2, with an increase rate of 71.77%, while the hybrid plants did not differ among themselves in the percentage of dry matter of the fruits.

The same table shows that spraying with calcium had a significant effect on both traits, as spraying with calcium at a concentration of 3 ml.L⁻¹ was significantly excelled compared to the rest of the concentrations, with an increase rate of 42.73, 16.33, 9.31% for the first, second and third concentrations, respectively, in the trait of plant yield, while spraying with calcium at a concentration of 1 ml.L⁻¹ was significantly excelled compared to the rest of the concentrations, with an increase rate of 6.18, 2.41% for the first and third concentrations, and did not differ significantly with the fourth concentration in the trait of dry matter in fruits. The results show that spraying with iron concentrations had a significant effect, as the concentration of 200 mg.L⁻¹ caused a significant increase compared to control and the other concentration, with an increase rate of 41.09, 11.43, 8.88, 3.28% for the two traits, respectively. As for the interactions, the results of the table showed that the interaction between hybrids and spraying with calcium had a significant effect on both traits, as the plants of the first hybrid V1 sprayed with calcium at a concentration of 3 ml.L⁻¹ gave the highest plant yield of 4.490 kg.plant⁻¹, while the lowest yield was 2.039 kg.plant⁻¹ in the plants of the second hybrid V2 not sprayed with calcium. The interaction between hybrids and spraying with calcium also showed a significant effect on the dry matter trait of the fruits, as the plants of the first hybrid V1 sprayed with calcium at a concentration of 1 ml.L⁻¹ excelled and gave the highest rate of dry matter of 6.72%, while the lowest value was 6.01% in the plants of the second hybrid V2 not sprayed with calcium. The interaction between hybrids and spraying with iron did not significantly affect the yield of a single plant, while it significantly affected the dry matter of the fruits, as the plants of the first hybrid V1 sprayed with iron at a concentration of 200 mg.L⁻¹ were significantly excelled, as the dry matter value reached 6.82% compared to the lowest value of 6.02% in the plants of the first hybrid V1 not sprayed with iron. The interaction between spraying with calcium and spraying with iron significantly affected the yield of the plant, as the plants sprayed with calcium at a concentration of 3 ml.L⁻¹ and sprayed with iron at a concentration of 100 mg.L⁻¹ gave the highest yield of the plant, reaching 4.369 kg.plant⁻¹, while the lowest yield was 1.942 kg.plant⁻¹ in the plants not sprayed with calcium and iron. While the interaction between spraying with calcium at a concentration of 1 ml.L⁻¹ and spraying with iron at a concentration of 200 mg.L⁻¹ gave the highest percentage of dry matter, reaching 6.95%, compared to the lowest value, which was 5.56%, in plants not sprayed with calcium and iron. The same table also shows that the triple interaction had a significant effect, as the plants of the first hybrid V1 sprayed with calcium at a concentration of 3 ml. L⁻¹ and sprayed with iron at a concentration of 100 mg. L⁻¹ were significantly excelled and gave the highest yield per plant, reaching 6.055 kg. plant⁻¹, while the

lowest yield was 1.223 kg. plant⁻¹ in the plants of the second hybrid V2 not sprayed with calcium and iron. The plants of the first hybrid V1 sprayed with calcium at a concentration of 1 ml. L⁻¹ and sprayed with iron at a concentration of 100 mg. L⁻¹ were significantly excelled and gave the highest dry matter of the fruits, reaching 7.16%, compared to the lowest value, which was 5.55% in the plants of the first hybrid V1 not sprayed with calcium and iron. The superiority of the first hybrid V1 in the trait of the single plant yield is attributed to the degree of suitability of this cultivar compared to the other cultivar for the prevailing conditions inside and outside the house of soil, humidity and temperature. This is consistent with [5]. The increase in the single plant yield and the percentage of dry matter in the fruits is due to the spraying with a high concentration of calcium 3 ml.L⁻¹ by giving the highest rates for these two traits. This had a positive impact on the vegetative growth of the plant, which led to an increase in the efficiency of the photosynthesis process, energy production and transfer of its food products from the leaves to the fruits, and an increase in the weight and numbers of the fruit (Table 2), and then an increase in the accumulation of dry matter in it. This is consistent with what was obtained by [12,14]. The increase in the plant yield of fruits when sprayed with a high concentration of iron 200 mg.L⁻¹ is attributed to its effective role in the representation of nucleic acids, plastids and enzymes that encourage increased cell divisions and cell elongation [26] in addition to being a cofactor for the growth of 140 enzymes that stimulate vital reactions and increase the effectiveness of vital processes in the plant [27] which contributes to increasing the yield. This result is consistent with what was obtained by [16,17].

Table 3. The effect of hybrids and foliar spraying with calcium and iron and the interactions between them on the yield of a single plant (kg. plant⁻¹) and dry matter in fruits (%).

Hybrids	Calcium ml.L ⁻¹	Yield per plant (kg.plant ⁻¹)			Dry matter in fruits (%)		
		Iron (mg.L ⁻¹)			Iron (mg.L ⁻¹)		
		0	100	200	0	100	200
V1	0	2.660	2.866	3.801	3.109	5.556	3.767
	1	3.506	4.227	4.770	4.168	5.917	6.167
	2	3.733	3.671	5.710	4.372	6.186	5.165
	3	3.700	6.055	3.716	4.490	6.436	2.069
V2	0	1.223	2.271	2.623	2.039	5.586	2.216
	1	1.708	2.269	2.468	2.148	5.586	4.067
	2	1.933	2.333	2.783	2.350	6.726	1.116
	3	2.371	2.683	3.517	2.857	6.546	1.186
LSD 5%		0.28			0.18		
		Average effect of Hybrids			Average effect of Hybrids		
Interaction between Hybrids and Iron	V1	3.400	4.205	4.499	4.035	6.026	5.668
	V2	1.809	2.389	2.848	2.349	6.116	2.226
LSD 5%		NS			0.17		
		Average effect of Calcium			Average effect of Calcium		
Calcium-Iron Interaction	0	1.942	2.568	3.212	2.574	5.566	2.296
	1	2.607	3.248	3.619	3.158	5.746	7.869
	2	2.833	3.002	4.247	3.361	6.456	3.162
	3	3.036	4.369	3.616	3.674	6.496	1.196

LSD 5%	0.21	0.14	0.15	0.09
Average iron effect	2.604	3.2973.674	6.066.396.60	
LSD 5%	0.10		0.07	

Table 4 shows that the study factors and their interactions had a significant effect on both the percentage of total soluble solids (TSS) and the concentration of lycopene in the fruits, as the plants of the first hybrid V1 significantly excelled the plants of the second hybrid V2, with an increase rate of 7.73 and 11.11% for both traits, respectively. Calcium spraying also had a significant effect, as spraying at a concentration of 3 ml.L⁻¹ significantly excelled the trait of total soluble solids compared to the rest of the concentrations, with an increase rate of 14.8, 2.93, and 6.79% for the first, second, and third concentrations, respectively, while spraying at a concentration of 2 ml.L⁻¹ significantly excelled in increasing the lycopene content of the fruits compared to the rest of the concentrations, with an increase rate of 29.46 and 8.20% for the first and second concentrations, respectively, but it did not differ significantly with the fourth concentration. The results show that spraying with iron concentrations had a significant effect, as the concentration of 200 mg.L⁻¹ caused a significant increase compared to control and the other concentration, with an increase rate of 5.86, 1.94%, respectively, in the percentage of total dissolved solids, while the concentration of 100 mg.L⁻¹ caused a significant increase compared to control and the other concentration, reaching 12.82, 13.63%, respectively, in the content of lycopene in the fruits.

The table shows that the interaction between hybrids and spraying with calcium had a significant effect on both traits, as the plants of the first hybrid V1 sprayed with calcium at a concentration of 3 ml.L⁻¹ excelled and gave the highest TSS and the highest values of lycopene, reaching 6.26%, 1.60 mg.g⁻¹, respectively, while the lowest value was 4.9%, 1.09 mg.g⁻¹ in the plants of the second hybrid V2 not sprayed with calcium for both traits, respectively. The interaction between hybrids and spraying with iron concentrations had no significant effect on the percentage of total dissolved solids, while the interaction between hybrids and spraying with iron concentrations had a significant effect on the lycopene trait, as the plants of the first hybrid V1 were significantly excelled, and those sprayed with iron at concentrations (100, 200 mg. L⁻¹) achieved the highest value of lycopene pigment, which reached 1.56 mg. g⁻¹ for both treatments, compared to the lowest value, which was 1.08 mg. g⁻¹ in plants not sprayed with iron. The interaction between spraying with calcium and spraying with iron also had a significant effect on both traits, as plants sprayed with calcium at a concentration of 3 ml. L⁻¹ and sprayed with iron at a concentration of 200 mg.L⁻¹ gave the highest TSS value, which reached 6.08%, compared to the lowest value, which was 4.43%, in plants not sprayed with calcium and iron. While the plants sprayed with calcium at a concentration of 3 ml.L⁻¹ and sprayed with iron at a concentration of 100 mg.L⁻¹ gave the highest value of lycopene, which reached 1.88 mg.g⁻¹, compared to the lowest value, which was 0.90 mg.g⁻¹, in plants not sprayed with calcium and iron. The same table also shows that the triple interaction had a significant effect on both traits, as the plants of the first hybrid V1, sprayed with calcium at a concentration of 1 ml.L⁻¹ and sprayed with iron at a concentration of 200 mg.L⁻¹, gave the highest TSS value, which reached 6.46%, compared to the lowest TSS value, which was 4.20%, in the plants of the second hybrid V2, not sprayed with calcium and iron. While the first hybrid V1 plants sprayed with calcium at a concentration of 3 ml.L⁻¹ and sprayed with iron at a concentration of 100 mg.L⁻¹ gave the highest value of lycopene pigment, reaching 2.57 mg.g⁻¹, compared to the lowest value, which was 0.84 mg.g⁻¹, in the first hybrid V1 plants not sprayed with calcium and iron. The excelled of the first hybrid V1 compared to the second hybrid is due to the difference between the genetic factors of the cultivar and their suitability to the prevailing environmental factors inside the greenhouse, such as soil and climatic

conditions. This result is consistent with [5]. The increase in the percentage of total soluble solids and the increase in the fruit content of lycopene pigment as a result of spraying with calcium is attributed to its important role in regulating the permeability of membranes and encouraging cell division, expansion and elongation [28] and its role in activating the photosynthesis process through its effect on light reactions, the manufacture and accumulation of carbohydrates and their transfer to different parts of the plant, accelerating the growth of fruits and improving their quality by accumulating pigments, including lycopene pigment, sugars, acids, vitamins, total dissolved solids and dry matter [12]. This result is consistent with [13]. The increase in the percentage of total soluble solids in the fruits and their content of red lycopene pigment when sprayed with iron is attributed to its effective role in raising the efficiency of the green group in manufacturing carbohydrates and increasing and accumulating them in the fruits, which increases them in the fruits. This result is consistent with [16,17].

Table 4. The effect of hybrids and foliar spraying with calcium and iron and their interactions on the concentration of total soluble solids in the fruits (%) and lycopene (mg. g⁻¹ fresh weight).

Hybrids	Calcium ml.L ⁻¹	TSS%			Lycopene (mg.g ⁻¹ fresh weight)		
		Iron (mg.L ⁻¹)		Interaction between Hybrids and Calcium	Iron (mg.L ⁻¹)		Interaction between Hybrids and Calcium
		0	100200		0	100200	
V1	0	4.66	5.466.23	5.45	0.841.271.36		1.15
	1	5.60	6.206.46	6.08	0.851.032.41		1.43
	2	6.16	5.365.30	5.61	1.611.371.27		1.41
	3	6.36	6.436.00	6.26	1.032.571.20		1.60
V2	0	4.20	5.265.36	4.94	0.971.420.87		1.09
	1	5.46	5.605.50	5.52	1.151.421.17		1.25
	2	5.70	5.705.33	5.57	1.631.731.06		1.47
	3	5.80	5.365.90	5.68	1.331.201.19		1.24
LSD 5%		0.36		0.27	0.12		0.06
		Average effect of Hybrids			Average effect of Hybrids		
Interaction between Hybrids and Iron	V1	5.70	5.866.00	5.85	1.081.561.56		1.40
	V2	5.29	5.485.52	5.43	1.271.441.07		1.26
LSD 5%		NS		0.27	0.05		0.03
		Average effect of Calcium			Average effect of Calcium		
Calcium-Iron Interaction	0	4.43	5.365.80	5.20	0.901.351.11		1.12
	1	5.53	5.905.98	5.80	1.001.221.79		1.34
	2	5.93	5.535.31	5.59	1.621.551.17		1.45
	3	5.95	5.906.08	5.97	1.181.881.19		1.42
LSD 5%		0.22		0.12	0.08		0.05
Average iron effect		5.46			1.171.501.32		
LSD 5%		0.12			0.04		

Conclusions

We conclude the following: the plants of the first hybrid V1 were significantly excelled on the second hybrid V2 in most of the quantitative yield traits, Spraying with calcium had a significant

effect in increasing the yield in quantity and quality, as the concentration of 3 ml.L⁻¹ was excelled in most of the yield traits

References

- [1] Wold,A.B;H.J. Rosen feld ; K.Holte; H. Baugerod; R.Blom boff and K.Haffiner(2004). Color of post harvest viped and vine ripened tomatoes (*Lycopersicon esculentum* Mill) as related to total autioxidant capacity and chemical composition . International journal of food science and Technology .39: 295-302.
- [2] Tomar B.S;G.S.Jat and Jogendra singh (2017) Adsances in hybrids seed production vegetables crops .university of Horticultue and forestry Nauni -173.230 Solan Himachal Pradesh .
- [3] Singh, B.;S. Kasera; K.Sudhir; S.Roy;S.Rana; and.D.Singh(2017). Gro yieldand quality of cherry tomato (*Lycopersicon esculentum* var *cerasiforme*) as influenced by foliar application of Zinc and Boron Journal of Pharmacognosy and Phytochemistry SP1: 911-914.
- [4] Hmiz, D. J. and Ihsan J. Ithbayyib.(2021). Effect of the Root Zone Temperature and Salt Stress on Plant Growth, Main Branches and some other Chemical Traits of Tomato Fruit *Solanum lycopersicum* L. cv. Memory. Basrah J. Agric. Sci., 34(1): 156-170.
- [5] Alwan, Othman Khalid and Mohammed Salman Mohammed (2023). Evaluation of genetic structures and the reverse effect of hybrid traits resulting from cross-breeding in cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*). Syrian Journal of Agricultural Research 10 (2): 87-96.
- [6] Egamberdiyev, S. Y. ; and A. S. Yunusov.(2023). Study on selection of high yield cherry tomatoes cultivars. IOP Conf. Series: Earth and Environmental Science 1142.
- [7] Zaman, Q. and A.W. Schumann,. (2006). Nutrient management zones for citrus based on variation in soil properties and tree performance. Precision Agri. 7: 45-6.
- [8] Majeed, H. H. and A. S. Al-Bayati.(2023). Role of Foliar Application of Organic Liquid Fertilizers Fortified with Phosphorus and Calcium in Tomato Yield and Fruit Quality. International Journal of Aquatic Science, Vol 14, Issue 01.
- [9] Alhamrani , K. S. L. ; Abdullah A. Abdullah and Diaa A. Taain .(2019). Effect of Planting Dates, Soil Mulching and Foliar Spraying Calcium on The Quantitative Yield Traits of Okra Plant Grown in plastic Greenhouse. Basrah J. Agric. Sci:171-182.
- [10] Ashraf, M.I.; S.Sajad ; B.Hussain ; M.Sajjad ;M.Adnan and M.Ismail.(2018).Foliar application effect of boron ,calcium and nitrogen on vegetative and reproductive attributes of tomato (*Solanum lycopersicum* L.).Journal of Agricultural Science and Food Research,9(1):1-3.
- [11] Rab, A. ; and I. Haq (2012). Foliar application of calcium chloride and borax influences plant growth, yield, and quality of tomato (*Lycopersicon esculentum* Mill.) fruit. Turk J Agric., 36(6):695-701 .
- [12] Ekinci, M.; A.Esringu; A.Dursun;E.Yildirim; M.Turan; M.R.Karaman and T.Arjumend.(2015).Growth,yield and calcium and boron uptake of tomato(*Lycopersicon esculentum* L.) and cucumber(*cucumis sativus*) as affected by calcium and boron humate application in green house conditions. Turksh journal of Agriculture and Forestry, 39:613-632.
- [13] Islam, M. ; K. J. ; Arpita S. ; T. A. U. ; M. Moynul Haque ; H. M. Ali; Manzer H. Siddiqu and Y. Murata .(2023). Exogenous Application of Calcium Ameliorates Salinity Stress Tolerance of Tomato (*Solanum lycopersicum* L.) and Enhances Fruit Quality. Antioxidants (12)558.
- [14] Chauhan, S. ; De. Y. ; S. K ; R. K. and A. Kumar. (2023). Effect of Calcium on the Growth and Yield of Tomato (*Solanum lycopersicum* L.). Biological Forum – An International Journal 15(10): 1162-1166.
- [15] Zhang, Y. ; Yi L. ; Jing H. ; X. H. ; Xiaojing L ; H. Z. ; L. B. ; Yu S and G. J.Ahammed. (2023). Interactive Effects of Iron and Photoperiods on Tomato Plant Growth and Fruit Quality. Journal of Plant Growth Regulation. 42:376–389.
- [16] Abdul Amir, Hamid Kazim, Sabah Latif Asi and Mohammed Tarfan Abu Al-Mikh (2018). The effect of spraying potassium and iron on some yield and quality indicators of tomato plants grown

- in greenhouse *Lycopersicon esculentum* Mill. Kufa Journal of Agricultural Sciences. 268-280:(1)10
- [17] Akanksha; A. K. and Navjot Singh Dhillon.(2022). Estimation of yield and quality traits of cherry tomato under the influence of micronutrients in protected condition. The Pharma Innovation Journal 2022; 11(3): 629-632.
- [18] Al-Rifai, Faisal Abdul Rahman, Issam Hussein Ali Al-Dughji and Jamil Hassan Haji (2006). Effect of planting method on vegetative and floral growth and yield of tomato cultivar (Super Marimmond) grown under plastic tunnels in desert lands in southern Iraq. Basra Journal of Agricultural Sciences. 19(2).
- [19] Matloub, Adnan Nasser, Ezz El-Din Sultan Mohammed and Karim Saleh Abdul (1989). Vegetable production. Part Two, Revised Edition, Higher Education Press, Mosul-Iraq: 337 pp.
- [20] Al-Rawi, Khashe' Mahmoud and Abdul Aziz Mohammed Khalaf Allah (1980). Design and analysis of agricultural experiments. Dar Al-Kutub for Printing and Publishing, Mosul/Iraq: 448 pp.
- [21] A.O.A.C. (1970). Association of Official Analytical Chemists .Official method of analysis, 12th. ed Washington D.C.
- [22] Al-Shammari, Aziz Mahdi Abdul (2005). Complete crosses and estimation of genetic parameters for some traits in tomato grown under plastic tunnels. PhD thesis. College of Agriculture. University of Baghdad. Iraq.
- [23] Wanous, Siham, Shaza Mihoub, Ali Dio B, Ataf Bal, Nada Yousef and Nasreen Mustafa (2023). Evaluation of some strains derived from cherry tomato ((*Solanum lycopersicum*(L) varcerasiforme(MILL) under protected cultivation conditions in the Syrian coast. Syrian Journal of Agricultural Research 10 (4): 77-86.
- [24] David, J.P.(2007).Hand book of Plant nutrition-philips.Morley wight Salads Ltd.Arreton.united King dom : P:121-144.
- [25] Amadi, Tariq Hassan (1991). Micronutrients in agriculture, Dar Al-Hikma for Printing and Publishing, Ministry of Higher Education and Scientific Research, University of Baghdad, Republic of Iraq.
- [26] Mengel, K. and A. Kirkby .(2001). Principles Plant nutrition,Kluwer Academic Publishers .
- [27] Taiz, L. and E. Zeiger.(2010).Plant Physiology.5th ed.Sin Aner Associates Ins.Publisher Sunderland.Massachus –Atis.USA.
- [28] Bothwell, J.H.F. and C.K.Y.Ng(2003). The evolution of Ca²⁺Signaling in Photosynthetic eukaryotes New Phytol.M., 166:21-38.

***Tetranychus urticae* Koch Mites Severity Response in Some Eggplant Varieties Treated with Salicylic Acid Concentrations and Their Relationship to Some Anatomical Characteristics of Plant Leaves Fieldally**

Yusra Jamal Talib

Marshes Research Centre, University of Thi-Qar, Thi-Qar, Iraq.

E-mail: yusra.iraq83@gmail.com

Abstract. A field study was conducted during the agricultural season 2023/2024 to show the effect of salicylic acid concentrations in the *Tetranychus urticae* (Koch) severity and its relationship with some anatomical characteristics of the eggplant varieties leaves in field. The results o showed that the eggplant varieties have varied in the *Tetranychus urticae* severity. In general, the Barcelona eggplant variety was the most sensitive plant variety, followed by the Thuraya variety, which was moderately sensitive, and the karimah variety was identified as the most resistant variety for a two-spotted spider mite. The 0.5 mM concentration of salicylic acid has high controlling on the *T. urticae* severity, reaching the lowest 30.79% compared with control treatment, which has the highest *T. urticae* 's severity, which amounted to 38.8%. The study results confirmed that eggplant varieties differed in the leaf tissue thickness, which effected in the two-spotted spider mite population density. The results recorded that a significant positive correlation between the upper epidermis thickness of the eggplant plants leaves with the two-spotted spider mite severity, a significant negative correlation between the spongy layer thickness of the eggplant plants leaves with the two-spotted spider mite severity.

Keywords. Salicylic acid, Two spotted spider mite, *Tetranychus urticae*, Anatomical characteristics, Eggplant Varieties.

1. Introduction

The eggplant is exposed to many pests that may cause economic losses of up to 70%, foremost of which is *Tetranychus urticae* Koch [1,2].

This mite species causes direct plant damage by absorbing plant sap from the leaves lower surface [3], which leads to reduced growth, flowering and crop yield [4], in addition to the indirect damage as a result of its spider webs weaving that leads to many damages to the plant such as blocking sunlight and being a protective cover for spider mite colonies from its natural enemies, rain and pesticides [5].

The plants have advanced defense systems against pest infestation such as the secondary compounds production, including Salicylic acid (SA) and Ethylene (ET) [6], which leads to the stimulation of the physical and chemical plant resistance mechanisms against pests, which is known as Acquired Systemic Resistance (ASR) [7]. This resistance type is activated after the plant is attacked by pests, therefore the other plant parts is acquired a resistance which is highly dependent on the phytohormone salicylic [8]. Many studies confirmed that exogenous application of these compounds on the shoot system may induce plant resistance against pests [9]. Salicylic acid is involved in many physiological processes regulation in plants that it is characterized by

being rapidly transmissible inside the plant from treated areas to other areas to create a defensive response [10].

[11] reported in their study in Brazil to show the salicylic acid efficacy in four tomato plant genotypes inducing resistance to two-spotted spider mite *T. urticae* and tomato leafminer *Tuta absoluta*, plants treatment by salicylic acid reduced the egg-laying of the both pests, the two-spotted spider mite traveled a shorter distance on the plant leaves that treated by acid, reduced the tomato leafminer infestation, and concluded that the treatment by salicylic acid induced plant resistance and was effective in reducing the two-spotted spider mite and tomato leafminer infestation and considering it efficient means for integrated pests management on the tomato plant. [12] indicated in his study in Brazil that the 50 mg/L concentration of salicylic acid that was among the acid concentrations used (0, 25, 50, 75 and 100 mg/L) on strawberries two varieties (Aromas and Sweet Charlie) to stimulate their resistance to *tetranychus urticae* infestation was the most efficient in reducing the spider mites individuals on the strawberries two varieties and the Sweet Charlie variety was the most reactive with acid coefficients in reducing the eggs number on the leaves for concentrations 25 and 50 and 75 mg/L compared with the Aromas variety.

[13] confirmed that spraying three concentrations of salicylic acid (0, 0.2 and 0.4 mmol) on the mustard plant *Brassica* spp. was effective in inducing plant resistance, a 0.4 mmol concentration caused that the highest reduction the aphids on the plants. Increased accumulation of salicylic phytohormone in tomato tissues leads to increased whitefly oviposition non-preference [14].

A positive effect of the salicylic acid on the anatomical characteristics leaves for some plant species has been recorded, when salicylic acid was sprayed on the Egyptian lupine plant *Lupinus termis* Forssk, it caused to increased the leaf dimensions and thickness of the [15]. [16] indicated to that when treating canola plant (*Brassica napus* L.) by 50, 100, and 200 mg/L salicylic acid concentrations led to an improvement the leaves anatomical characteristics. [17] noticed that after wheat seeds treated with salicylic acid for 12 hours, this led to changes in the leaves anatomical characteristics, including an increase in the thickness of mesophyll and epidermis layers.

In view of the importance of studying *Tetranychus urticae* and the inducing resistance of the eggplant *Solanum melongena* L. against the two-spotted spider mite using salicylic acid, this study aimed to find out the salicylic acid effect in the some eggplant varieties resistance to the pest in addition to its effect in some leaves anatomical characteristics and their relationship with the pest.

2. Material and Method

2.1. Study Location

A field experiment was conducted in a greenhouses in Al-Gharraf city of Thi-Qar Governorate during 2023-2024 season. The soil was prepared for cultivation after plowing, leveling, and dividing it into three lines (three replicates) with a length of 25, a width of 40 cm, and a height of 20 cm, with a distance of 100 cm between each line and other.

Organic fertilizer, triple superphosphate and NPK were added to the lines according to the approved fertilizer recommendation. Use a drip irrigation system, each line covered with mulches to maintain soil moisture and prevent to bush growth.

2.2. Eggplant Varieties Used in the Study

Karimah, Barcelona, and Thuraya eggplant varieties seeds were used.

2.3. Salicylic Acid Concentrations Used in the Study

The salicylic acid concentrations were seen to be 0 (distilled water alone), 0.5, 0.75, and 1 mM.

2.4. Planting Seeds

The seedlings were transferred to the greenhouse after the fourth true leaf appeared for seeds that it were planted in cork container during the last week of September 2023.

The planting lines were divided into experimental units with a length of 1.5 meter, with 12 experimental units per line (3 varieties*4 concentrations). A 30 centimeter distance was left between each experimental unit and another without planting, 8 seedlings were planted for each experimental unit on both sides of the line, a 30 centimeter distance were left between plant and another, the plants watered as needed, a continuous program fertilization of urea, NPK and complex fertilizer were followed.

The plants were treated with salicylic acid concentrations in the last week of October 2023.

2.5. Salicylic Acid Concentrations Effect in Two-Spotted Mite's Severity on Eggplant Varieties

The study began in the first week of November/2023, continued for six months, every two weeks until the first week of May/2024. The sample collection mechanism included the following: three plants were randomly selected from both each experimental unit and each replicate, from each plant, three leaves were taken (from three levels: the upper, middle and lower third of the plant) to be examined later by using a dissecting microscope (40X). The mite individuals' number (the motile stages only: larvae, nymphs, and adults) were calculated per leaf. The following scale were adopted to determine of the two-spotted spider mite's severity:

0: Individuals Number 0/ No infestation.

1: Individuals Number 1-10 / light infestation.

2: Individuals Number 11-20 / moderate infestation.

3: Individuals Number 21-30 / severe infestation.

4: Individuals Number 30 / very severe infestation.

The equation below was used to convert the severity degrees (determined by the above scale) into numerical values[18]:

$$\text{Injury severity (\%)} = [(\text{Leaves number from a grade of } 0 \times 0) + (\text{Leaves number from a grade of } 1 \times 1) + (\text{Leaves number from a grade of } 2 \times 2) + (\text{Leaves number from a grade of } 3 \times 3) + (\text{Leaves number from a grade of } 4 \times 4)] / \text{The total leaves number} \times \text{the highest value of scale}$$

2.6. Estimation of Leaves Anatomical Indicators

Plant leaves were taken from each experimental unit, treatment and replicate in 1/12/2023. [19] method were followed in making tissue sections and uploading them on slides, the working mechanism were included:

- Fixation: Parts of plant leaves were placed in Fixing Solution (FAA) (Formalin Acetic Acid), prepared by adding 5 ml formalin + 5 ml glacial acetic acid + 90 ml 70% ethyl alcohol for 24 hours.
- Washing: Parts of plant leaves were washed by ethyl alcohol (70%) to eliminate traces of the fixative solution.
- Dehydration: Plant leaves were placed in an ascending series of 70%, 80%, and 90% ethyl alcohol for 1 hour at each concentration, then in absolute ethyl alcohol (100%) overnight.
- Clearing: The samples were transferred to containers containing a mixture of absolute alcohol: Xylene at a ratio of 1:3, 1:1 and 3:1 for 30 minutes in each solution, then it was

- left in pure xylene for 30 minutes, then transferred it to a mixture consisting of xylene: paraffin wax (1:1) in oven of 60-65 °C for 4 hours.
- Infiltration: The samples were left in pure paraffin wax at oven of 60-65 °C overnight.
 - Embedding: Liquid paraffin at 60-65 °C was poured into cubic- plastic molds, the samples were placed in them after marked them, left for 24 hours until they are ready for cutting.
 - Trimming & Sectioning: The wax molds were trimmed using a sharp blade by microtom device (MSE type), then they were cut with 10-15 micrometers per slice.
 - Mounting: They were placed in a water bath with 50-60°C, then on the slides (after placing drops of Mayer's solution on the slide) (egg albumen: glycerin) (1:1), placed a cover on the slide, transferred the slides to an oven (50-60°C) for half an hour.
 - Staining: The paraffin wax were removed from the sections by transferring the slides to a Coplin jar (containing: absolute xylene) for an hour for three times, then passed through a descending series of 100, 95, 90, 80, 70, and 50% ethyl alcohol (from the highest to the lowest concentration) for 15 minutes for each.

The slides were transferred to a Coplin tester filled with Safranin dye (1g dye dissolved in 100ml of 70% ethyl alcohol) for 30-60 seconds. The slides were placed in 50% ethyl alcohol to remove excess dye, then were placed in Fast Green dye (1gm dye dissolved in 100 ml of ethyl alcohol) for 15-30 seconds, then were washed with absolute alcohol to remove excess dye.

The slides were passed with xylene (three times) for 5 minutes each time, then they left to dry.

The cover slide was gently placed on the slide after adding a drop of DPX (Distrene Plasticize Xylene) on the slide, they were transferred to a hot plate at 60°C for several hours until the slides were ready for microscopic examination.

2.7. Measuring the Layered Dimensions Plant Leaves

The following characteristics of the histological sections were measured: the thickness of the upper, lower epidermis, upper epidermis's cuticle, lower epidermis's cuticle, palisade parenchyma layer and Spongy cells layer (micrometer) using an AxioCamICc3 camera, attached to a ZEISS microscope.

2.8. Statistical Analysis

The experiments were designed using Randomized Complete Block Design (R.C.B.D.) with two factors (acid concentrations * eggplant varieties) and adopted the least significant design (L.S.D.) test to compare the averages at a probability level of 0.05 and used the statistical program SPSS (Statistical Package for the Social) Science version 21 in data analysis.

3. Result and Discussion

3.1. The Effect of Salicylic Acid Concentrations in the Two-Spotted Spider Mite Severity on Eggplant Varieties

The results (Table 1) indicated to the significant effect of each of eggplant varieties, salicylic acid concentrations and the overlap between them in the two-spotted spider mite severity on eggplant. The severity highest percentage was 35.08% on the Barcelona variety, while the severity lowest percentage was 32.9% on the karimah variety.

The control treatment had the severity highest percentage, reaching 38.8%, while the 0.5 mM concentration had the severity lowest percentage, reaching 30.79%.

As for the overlap between eggplant varieties and salicylic acid concentrations, the Barcelona variety had the severity highest percentage in the control treatment, as it amounted to 39.72%,

whereas the karimah variety with a 0.5 mM concentration had the severity lowest percentage reached 29.19%, with a significant difference among the treatments.

Table 1. Effect of Salicylic acid concentrations in Severity of *T. urticae* on leaves of eggplant.

Average (%)					
Conc. (mM)	0	0.5	0.75	1	Var.
Var.					
karimah	37.85	29.19	35.22	29.33	32.9
Barcelona	39.72	29.97	36.52	34.1	35.08
Thuraya	38.81	33.2	35.04	31	34.51
Conc.	38.8	30.79	35.6	31.48	
LSD(0.05)	Var.= 0.65 *	Conc.= 0.75 *	Var.*Conc.= 1.29 *		

The statistical analysis results shown in Table 1 confirmed that the eggplant varieties was varied in the two-spotted spider mite severity. In general, the results indicated that the Barcelona eggplant variety was the most sensitive plant variety, followed by the Thuraya variety, which was moderately sensitive, while the karimah variety was the most resistant variety to the two-spotted spider mite infestation.

The study results was similar to [20] study in Taiwan to estimate the resistance of 120 eggplant varieties scarlet (*Solanum aethiopicum* L.) and 5 eggplant varieties gboma (*Solanum macrocarpon* L.) to the two-spotted spider mite infestation, in which it confirmed that the resistance highest levels to the pest appeared in the VI050393 and VI050444 varieties (*Solanum macrocarpon* L. species), while the VI042749 and VI042753 varieties (*Solanum aethiopicum* L. species) were medium resistant, on the contrary the other varieties (*Solanum aethiopicum* L. species) showed high sensitivity to the two-spotted spider mite infestation, and the varieties resistant (*Solanum macrocarpon* L.) can be used directly or before breeding and improvement programs which aimed at breeding resistant lines of the most commonly used eggplant varieties. This confirms that the plant varieties vary in many important qualitative characteristics, including the pests resistance level such as insects and mites [21].

The results of the statistical analysis (Table 1) showed that the 0.5 mM concentration was the highest efficiency in the two-spotted spider mite controlling. Salicylic acid has been widely used in plant production for its important role in improving plant qualities, as well as its role in pests and diseases plant resistance, many studies have mentioned that the salicylic acid efficiency in inducing plant resistance to pest infestations such as insects and mites. [22]'s study on the strawberry plant (Sweet Charlie variety) to show the 5 concentrations efficiency of Salicylic acid (0, 25, 50, 75 and 100 mg/L) in the plant resistance against *T. urticae*, the two-spotted spider mite were less preference, fewer eggs laid and fewer live females on the acid-treated plant leaves compared with the control treatment.

The salicylic acid negative effect in population density, female fertility and the laying eggs process in insects may be due to it's effect in inducing plant tissues to release some compounds that has negatively affect in the insects activities, this is confirmed by [23] in their study when spraying tomato plants by salicylic acid, they found that it has a significant effect on the release, quantity and type of volatile substances such as Methyl Salicylate and Limonene- δ , which have an important role in reducing fertility, longevity and the whitefly preference for the plant compared with control plants.

3.2. The Effect of Salicylic Acid Concentrations in Some Anatomical Characteristics Leaves and Their Relationship with the Two-Spotted Spider Mite Severity on Eggplant Varieties

3.2.1. The Effect of Salicylic Acid Concentrations in the Upper Epidermis's Cuticle Thickness

The results (Table 2) confirmed that absence the statistical effect of the salicylic acid concentration, while had observed that a clear statistical significance of both the eggplant variety and the interaction between the eggplant variety and the salicylic acid concentration in the upper epidermis's cuticle of the eggplant varieties leaves.

The Thuraya variety leaves have the highest thickness of the upper epidermis's cuticle, which amounted to 5.08 μm , while The karimah variety leaves has the lowest thickness of upper epidermis's cuticle, which reached to 4.08 μm , with a significant difference among the varieties.

The highest and lowest thickness of the upper epidermis's cuticle, were 8.33 and 3 μm in the Thuraya and karimah leaves with 1 mM salicylic acid concentration respectively, in the interaction between eggplant variety and the salicylic acid concentration, with a significant difference among the treatments.

The results has recorded a negative non-significant correlation coefficient between the thickness of the upper epidermis's cuticle of eggplant leaves with the two-spotted spider mite severity.

3.2.2. The Effect of Salicylic Acid Concentrations in the Lower Epidermis's Cuticle Thickness

The results in Table 2 recorded that a significant difference effect in each eggplant variety, the salicylic acid concentration and the overlap between them in the lower epidermis's cuticle thickness.

the 1 mM concentration has the maximum lower epidermis's cuticle thickness of the eggplant varieties leaves, as it reached 5.56 μm , while the minimum lower epidermis's cuticle thickness was 3.44 μm in the eggplant leaves treated with 0.75 mM concentration, with a significant difference among the concentrations.

The Thuraya variety leaves have the maximum lower epidermis's cuticle thickness, which amounted 5 μm , compared to the karimah variety leaves which have the minimum lower epidermis's cuticle thickness, which reached to 3.58 μm , with a significant difference among the varieties.

As the interaction between the eggplant variety with the salicylic acid concentration, the maximum lower epidermis's cuticle thickness was 7 μm in the Thuraya variety leaves treated with 1 mM concentration, while the minimum lower epidermis's cuticle thickness was 2.67 μm in the karimah variety leaves treated with 0.5 mM concentration of salicylic acid with a significant difference among the treatments.

The results has recorded a negative non-significant correlation coefficient between the thickness of the lower epidermis's cuticle of eggplant leaves with the two-spotted spider mite severity.

3.2.3. The Effect of Salicylic Acid Concentrations in the Upper Epidermis Thickness

The results (Table 2) showed the significant effect of salicylic acid concentrations, as the 0.75mM concentration has a positive effect in increasing the upper epidermis thickness reached to 22.33 μm , while the 1 mM concentration has a negative effect in the upper epidermis thickness of the eggplant leaves, as it reached 17.11 μm , with a significant difference among the concentrations.

The Barcelona variety leaves were distinguished by having the highest upper epidermis thickness, which amounted to 20.5 μm , while the karimah variety leaves have the lowest upper epidermis thickness, which amounted to 17.17 μm , with a significant difference among the varieties.

The results showed that a significant effect of the overlap between the eggplant variety and the salicylic acid concentration in the upper epidermis thickness of eggplant leaves. The highest upper epidermis thickness reached 29 μm in the Barcelona variety leaves-treated with 0.75 mM concentration, whereas the lowest upper epidermis thickness was 15 μm in the karimah variety leaves-treated with 1 mM, with a significant difference among the treatments.

The results recorded a positive-significant correlation coefficient between the upper epidermis thickness of eggplant leaves with the two-spotted spider mite severity.

3.2.4. The Effect of Salicylic Acid Concentrations in the Lower Epidermis Thickness

The results (Table 2) showed the significant effect of the salicylic acid concentration, while there was not any significant effect for both the eggplant variety and the interaction between the eggplant variety with the salicylic acid concentration in the lower epidermis thickness of eggplant leaves.

the 0.75 mM concentration has the maximum lower epidermis thickness, as it reached 14 μm , while the minimum lower epidermis thickness was 10.44 μm in the eggplant varieties leaves treated with 1 mM concentration, with a significant difference among the salicylic acid's concentrations.

The results recorded a negative- non significant correlation coefficient between the lower epidermis thickness of eggplant leaves with the two-spotted spider mite severity.

3.2.5. The Effect of Salicylic Acid Concentrations in the Palisade Parenchyma Layer Thickness

The results of the statistical analysis shown in Table 2 confirmed that there is a statistically significant effect for each the salicylic acid concentration, the eggplant variety and the interaction between them in the palisade parenchyma layer thickness of eggplant leaves. The 1 mM concentration of salicylic acid has the highest palisade parenchyma layer thickness, as it reached 90 μm , while the lowest palisade parenchyma layer thickness was 80.56 μm in the control treatment, with a significant difference among the acid concentrations.

The Barcelona variety leaves has the highest palisade parenchyma layer thickness, as it reached 98.33 μm , while the lowest palisade parenchyma layer thickness was amounted 71.5 μm in the Thuraya variety leaves with a significant difference among the varieties.

As the results of the overlap between the eggplant variety and the salicylic acid concentration showed that the highest and lowest of the palisade parenchyma layer thickness of the eggplant leaves were 125 and 62.67 μm in both Barcelona and Thuraya varieties leaves respectively which treated with 0.75 mM, with a significant difference among the treatments.

The results recorded a negative-non significant correlation coefficient between the palisade parenchyma layer thickness of eggplant leaves and the two-spotted spider mite severity.

3.2.6. The Effect of Salicylic Acid Concentrations in the Spongy Cells Layer Thickness

The results in Table 2 showed that a statistically significant effect for each the salicylic acid concentration, the eggplant variety and the overlap between them in the spongy cells layer thickness of the eggplant varieties leaves. As the highest of the spongy cells layer thickness was 115.67 μm in the leaves treated with a 1 mM concentration, while the lowest of the spongy cells layer thickness was 87.78 μm in the leaves treated with a 0.75 mM concentration, with a significant difference among the acid concentrations.

The karimah variety leaves were distinguished by having the highest spongy cells layer thickness, which amounted to 108.33 μm , while the lowest spongy cells layer thickness was 90.83 μm in the Barcelona variety leaves, with a significant difference among the varieties.

As the overlap between the eggplant variety and the salicylic acid concentration, the highest spongy cells layer thickness was 150 μm in the karimah variety leaves-treated with 1 mM, while the lowest spongy cells layer thickness was 60 μm in the Thuraya variety leaves-treated with 0.75 mM, with a significant difference among the treatments.

The results recorded a negative-significant correlation coefficient between the spongy cells layer thickness of eggplant leaves and the two-spotted spider mite severity.

Table 2. Effect of salicylic acid concentrations in the anatomical characteristics of the eggplant varieties's leaves.

Characteristic	Conc. (mM) / Var.	Thickness (μm) (average)					LSD (P≤0.05)	r
		0	0.5	0.75	1	Var.		
Upper epidermis's Cuticle	karimah	3.33	6	4	3	4.08	Var.= 0.5*	- 0.31 ^{NS}
	Barcelona	4	5	5	4	4.5	Conc.=	
	Thuraya	5.67	3	3.33	8.33	5.08	0.57 ^{NS}	
	Conc.	4.33	4.67	4.11	5.11		Var.* Conc.= 0.99*	
Lower epidermis's Cuticle	karimah	4	2.67	3	4.67	3.58	Var.= 0.52*	- 0.17 ^{NS}
	Barcelona	3.67	4.33	4	5	4.25	Conc.= 0.6*	
	Thuraya	5	4.67	3.33	7	5	Var.* Conc.=	
	Conc.	4.22	3.89	3.44	5.56		1.03*	
Upper epidermis	karimah	17	19.33	17.33	15	17.17	Var.= 1.84*	0.35*
	Barcelona	17.33	17	29	18.67	20.5	Conc.=	
	Thuraya	21.67	20.33	20.67	17.67	20.08	2.13*	
	Conc.	18.67	18.89	22.33	17.11		Var.* Conc.= 3.68*	
Lower epidermis	karimah	14.67	9.33	9.33	12.33	11.42	Var.= 1.77 ^{NS}	- 0.03 ^{NS}
	Barcelona	7.33	11.33	17	9.33	11.25	Conc.=	
	Thuraya	10	12.67	15.67	9.67	12	2.04*	
	Conc.	10.67	11.11	14	10.44		Var.* Conc.= 3.53 ^{NS}	
Palisade parenchyma layer	karimah	90	90	67	100	86.67	Var.= 5.97*	- 0.18 ^{NS}
	Barcelona	86.67	91.67	125	90	98.33	Conc.=	
	Thuraya	65	78.33	62.67	80	71.5	6.89*	
	Conc.	80.56	86.67	84.78	90		Var.* Conc.= 11.94*	
Spongy cells layer	karimah	100	80	103.33	150	108.33	Var.= 7.26*	-0.41*
	Barcelona	70	100	100	93.33	90.83	Conc.=	
	Thuraya	96.67	113.33	60	103.67	93.42	8.39*	
	Conc.	88.89	97.78	87.78	115.67		Var.* Conc.= 14.53*	

r = indicates to the correlation coefficient between the characteristic and the severity of *T. urticae*.

The statistical analysis results shown in Table 2 confirmed that the eggplant varieties varied in the leaves tissues thickness. this variation may be effected in the two-spotted spider mite severity, therefor it is believed that there is a relationship between the leaves tissues thickness and the two-spotted spider mite.

The plant anatomical characteristics such as the leaves waxy layer and the cuticle layer thickness are Playing an important role in the plant's resistance against pests, it's representing the first line of defense against insects as a barrier that prevents the insects nutrition, as well as plant secondary compounds and these traits may be effect in growth, development, mating, feeding and

laying eggs insects [24; 25]. A relationship has been observed between the leaf tissues thickness and the population density of some species insects [26]. The leaves anatomical characteristics such as leaf plate thickness, are playing an important role in reducing the eggs number of some insects species such as whitefly [27].

The negative effect of some anatomical characteristics of leaves such as the epidermis thickness and the mesophyll in some insects species may be due to their impact in some pest life activities such as research and nutrition behavior and the laying eggs process [28].

[29] have confirmed that the leaf thickness affects in the Lepidoptera larvae nutrition, especially newly hatched larvae, and may be unable to reach the epidermis to feed.

The statistical analysis results, shown in Table 2, showed that the positive effect of salicylic acid concentrations in most of the anatomical characteristics of the eggplant leaves and it's effect in the two-spotted spider mite population density. These results were consistent with the several studies results that showed the salicylic acid importance in improving the leaves anatomical characteristics and its effect in the two-spotted spider mite population density, [22] confirmed in their study on the strawberry plant (Sweet Charlie variety) to efficacy demonstrate of the salicylic acid concentrations (25, 50, 75 and 100 mg/L) in resisting against *T. urticae*, that the laid eggs numbers and the females live numbers were negatively correlated with the anatomical characteristics of the leaves acid-treated, including leaf thickness, mesophyll thickness, palisade parenchyma cells, adaxial and abaxial, by using Scanning Electronic Microcopy (SEM).

The reason of the salicylic acid efficiency may be attributed to its role in increasing the size of plastids and grana plates (thylakoid-containing chlorophyll plates) [30], thereby increasing physiological processes such as cell's division and expansion [31].

Conclusions

This study concluded that the karimah eggplant variety was the most resistant, while the Barcelona eggplant variety was the most sensitive against the two-spotted spider mite infestation. The 0.5 mM concentration of salicylic acid was highly efficient in reducing the two-spotted spider mite severity.

Eggplant varieties varied in the leaves anatomical characteristics thickness, higher salicylic acid concentrations (0.75 and 1 mM) were the most efficient in affecting of the most of the anatomical traits studied.

The two-spotted spider mite severity was positively correlated with the upper epidermis thickness of eggplant leaves, negatively correlated with the spongy cells layer of eggplant leaves .

References

- [1] Hodge, S.; A. Bluon and C. N. Merfield. (2018). Pore size requirements for mesh crop covers to protect potatoes from aphids. *Journal of New Zealand Entomologist*, 41(2): 66-71. doi.org/10.1080/00779962.2018.1556914.
- [2] Kamaloddin, A. ; F. Yaghoub and B. Mohammad. (2020). Control of *Tetranychus urticae* by three predatory mites (Acari: Phytoseiidae) in a commercial greenhouse rose. *Journal of Crop Protection*, 9(1): 17-27.
- [3] Wang, L.; Y. Zhang; W. Xie; Q. Wu and S. Wang. (2016). Sublethal effects of spinetoram on the two-spotted spider mite, *Tetranychus urticae* (Acari:Tetranychidae). *Journal of Pesticide Biochemistry and Physiology*, 132:102-107. doi:10.1016/j.pestbp.2016.02.002.
- [4] Yu, X.; Y. Zhang; Y. Liu; Y. Li and Q. Wang. (2019). Synthesis and Acaricidal- and Insecticidal-Activity Evaluation of Novel Oxazolines Containing Sulfiliminyl Moieties and Their Derivatives [Research-article]. *Journal of Agricultural and Food Chemistry*, 67(15): 4224-4231. <https://doi.org/10.1021/acs.jafc.9b00657>.

- [5] Murase, A.; F. Kazuo and Y. Shuichi. (2017). Behavioural flexibility in spider mites: oviposition site shifts based on past and present stimuli from conspecifics and predators. *Journal of Royal Society Open Science (RSOS)*, 4(7): 1-10.
- [6] Smith, C. M. and W. P. Chuang. (2014). Plant resistance to aphid feeding: behavioral, physiological, genetic and molecular cues regulate aphid host selection and feeding. *Journal of Pest management science*, 70(4): 528- 540.
- [7] Ossowicki, A.; Tracanna. V. ; M. C. Petrus ; G. V. Weze ; J. H. Medema and P. Garbeva . (2020). Microbial and volatile profiling of soils suppressive of wheat. *Journal of Proceedings of the Royal Society B*, 287(1921): 1- 10.
- [8] Gao, Q. M.; S. Zhu; P. Kachroo and A. Kachroo. (2015). Signal regulators of systemic acquired resistance. *Journal of Frontiers in Plant Science*, 6: 228. doi: 10.3389/fpls.2015.00228.
- [9] Kumari, A. and S. K. Singh. (2022). Chapter 18 - Role of plant hormones in combating biotic stress in plants. In: Aftab, T. and A. Roychoudhury (eds.). *Plant Perspectives to Global Climate Changes*. Academic Press, Pp: 373-391. <https://doi.org/10.1016/B978-0-323-85665-2.00010-8>.
- [10] Çetinbaş-Genç, A. and F. Vardar. (2021). The Role of Salicylic Acid in Plant Reproductive Development. *Salicylic Acid-A Versatile Plant Growth Regulator*, p:35-45. Springer, Cham.
- [11] Pulga, P. S.; J. M. Henschel; J. T. V. Resende; A. R. Zeist; A. F. P. Moreira; A. Gabriel; M. B. Silva and L. S. A. Gonçalves. (2020). Salicylic acid treatments induce resistance to *Tuta absoluta* and *Tetranychus urticae* on tomato plants. *Journal of Horticultura Brasileira*, 38(4): 288-294. <http://dx.doi.org/10.1590/S0102-053620200308>.
- [12] Favaro, R.; J. Resende; A. Gabriel; A. Zeist; E. Cordeiro and J. J. Favaro. (2019). Salicylic acid: Resistance inducer to two-spotted spider mite in strawberry crop. *Journal of Horticultura Brasileira*, 37(1): 60-64. DOI: 10.1590/s0102-053620190109.
- [13] Mony, F. T. Z.; M. S. Ali; M. N. Islam; S. Choudhury and M. S. Hossain. (2017). Effect of salicylic acid and varieties on incidence of aphid and yield of mustard. *Journal of Multidisciplinary Engineering Science Studies*, 3(2): 1331-1340.
- [14] Ueda, H.; S. Kugimiya; J. Tabata; H. Kitamoto and I. Mitsuhara. (2019). Accumulation of salicylic acid in tomato plant under biological stress affects oviposition preference of *Bemisia tabaci*. *Journal of Plant Interactions*, 14(1): 73-78.
- [15] Gomaa, E. F.; R. M. Nassar and M. A. Madkour. (2015). Effect of foliar spray with salicylic acid on vegetative growth, stem and leaf anatomy, photosynthetic pigments and productivity of Egyptian Lupine plant (*Lupinus termis* forssk). *International Journal of Advanced Research*, 3(1): 803-813.
- [16] El-Hamahmy, M. A. M.; M. F. Mahmoud and T. Y. Bayoumi. (2016). The effect of applying exogenous salicylic acid on aphid infection and its influence on histo-physiological traits and thermal imaging of canola. *Journal of Cercetari Agronomice in Moldova*, 49(2): 67-85.
- [17] Agami, R. A. and G. F. Mohamed. (2013). Exogenous treatment with indole-3-acetic acid and salicylic acid alleviates cadmium toxicity in wheat seedlings. *Journal of Ecotoxicology and Environmental Safety*, 94: 164-171. DOI: 10.1016/j.ecoenv.2013.04.013.
- [18] Sháaban, A. and N. M. Al Mallah. (1993). *Pesticides*. Mousul University, p: 520.
- [19] Al-Khafagy, M. A.(2001). *Plant microtechnology*. Faculty of Agriculture, Mansoura University, p: 312.
- [20] Taher, D.; M. Rakha; S. Ramasamy and S. Solberg. (2019). Sources of Resistance for Two-spotted Spider Mite (*Tetranychus urticae*) in Scarlet (*Solanum aethiopicum* L.) and Gboma (*S. macrocarpon* L.) Eggplant Germplasms. *Journal of Hortscience*, 54(2): 240-245. Doi:org/10.21273/HORTSCI13669-18.
- [21] Vidavski, F.; H. Czosnek; S. Gazit; D. Levy and M. Lapidot. (2008). Pyramiding of genes conferring resistance to Tomato yellow leaf curl virus from different wild tomato species. *Journal of Plant Breeding*, 127(6): 625- 631.
- [22] de Resende, J. T. V.; R. Matos; D. M. Zeffa; L. V. Constantino; S. M. Alves; M. U. Ventura; N. C. V. Resende and K. Youssef. (2021). Relationship between salicylic acid and resistance to mite in strawberry. *Journal of Folia Horticulturae*, 33(1): 107–119. DOI: 10.2478/fhort-2021-000.

- [23] Shi, X.; G. Chen; L. Tian; Z. Peng; W. Xie; Q. Wu and Y. Zhang. (2016). The salicylic acid-mediated release of plant volatiles affects the host choice of *Bemisia tabaci*. *International Journal of Molecular Sciences*, 17(7): 1048. doi: 10.3390/ijms17071048.
- [24] Agrawal, A. A.; M. Fishbein; R. Jetter; J. P. Salminen; J. B. Goldstein and A. E. Freitag. (2009). Phylogenetic ecology of leaf surface traits in the milkweeds (*Asclepias* spp.): chemistry, ecophysiology and insect behavior. *Journal of New Phytologist*, 183(3): 848-867.
- [25] He, J.; F. Chen; S. Chen; G. Lv; Y. Deng and W. Fang. (2011). *Chrysanthemum* leaf epidermal surface morphology and antioxidant and defense enzyme activity in response to aphid infestation. *Journal of plant physiology*, 168(7): 687-693.
- [26] Peeters, P. J. (2002). Correlations between leaf structural traits and the densities of herbivorous insect guilds. *Biological Journal of the Linnean Society*, 77(1): 43-65.
- [27] Hasanuzzaman, A. T. M.; M. N. Islam; Y. Zhang; C. Y. Zhang and T. X. Liu. (2016). Leaf morphological characters can be a factor for intra-varietal preference of whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) among eggplant varieties. *Journal of Plos One*, 11(4): E0153880.
- [28] Lei, H.; J. C. Van Lenteren and W. F. Tjallingii. (1999). Analysis of resistance in tomato and sweet pepper against the greenhouse whitefly using electrically monitored and visually observed probing and feeding behaviour. *Journal of Entomologia Experimentalis Et Applicata*, 92(3): 299-309.
- [29] ShuSheng, L.; C. FeiZhou and M. P. Zalucki. (2002). Development and survival of the diamondback moth (Lepidoptera: Plutellidae) at constant and alternating temperatures. *Journal of Environmental Entomology*, 31(2): 221-231.
- [30] Nour, K. A. M.; N. T. S. Mansour and G. S. A. Eisa. (2012). Effect of some antioxidants on some physiological and anatomical characters of snap bean plants under sandy soil condition. *Journal of New York Science*, 5(5): 1-9.
- [31] Abd El-Mageed, T. A.; W. M. Semida; G. F. Mohamed and M. M. Rady. (2016). Combined effect of foliar-applied salicylic acid and deficit irrigation on physiological-anatomical responses, and yield of squash plants under saline soil. *South African Journal of Botany*, 106: 8-16. DOI:10.1016/j.sajb.2016.05.005.

Adopting Thermodynamic Criteria to Evaluate Potassium Available in Saline Soils Under Different Temperatures

Haneen H. A. Al-Jubory¹ and Qahtan D. Essa Al-Khafagi²

¹Center for Arid Farming and Conservation Agriculture Research, Mosul University, Mosul, Iraq.

²Soil Science and Water Resources Department, College of Agriculture and Forestry, Mosul University, Mosul, Iraq.

¹E-mail: haneen.husham@uomosul.edu.iq

²E-mail: qahtan_darwish@uomosul.edu.iq

Abstract. The Beckett method was used to study the thermodynamic parameters of the capacity and intensity relationships (Q/I) of the element potassium for the purpose of evaluating the behavior of this element in saline soils within the Nineveh Governorate in northern Iraq. The study was conducted on three sites that differed in salt content, and each site was divided into two depths (surface and subsurface depth). Thermodynamic parameters were studied at three temperatures (278°, 298°, 318° Kelvin). The results of the study indicated that the highest value of the ionic activity of potassium ARK was at a temperature of 298° Kelvin, reaching an average of $(8.63 \times 10^{-3}) \text{ mol.l}^{-0.5}$, while the lowest value of the ionic activity reached an average of $(3.20 \times 10^{-3}) \text{ mol.l}^{-0.5}$ at a temperature of 278° Kelvin. As for the labile potassium (ΔK) values, the highest value was recorded at a temperature of 318° Kelvin, as its value reached an average of $(-10.49) \text{ Cmol.kg}^{-1}$, while the lowest values were recorded at a temperature of 278° Kelvin and was an average of $(-6.09) \text{ Cmol.kg}^{-1}$. As for the potassium buffering capacity (PBC_K), the highest value was recorded at a temperature of 278° Kelvin, with a rate of $2241.33 (\text{Cmol.kg}^{-1}/\text{mol.l}^{-1})^{-0.5}$. When the temperature was increased to 298° Kelvin, the lowest rate was reached and its value was $1243.19. (\text{Cmol.kg}^{-1}/\text{mol.l}^{-1})^{-0.5}$. The values of the change in the free energy of potassium, ΔG_K , in the studied soils ranged from the highest rate, which reached $(-13.52 \times 10^{-3} \text{ kJ})$ at a temperature of 278° Kelvin, to the lowest rate, which reached $(-12.133 \times 10^{-3} \text{ kJ})$ at a temperature of 298° Kelvin. The course of the exchange process also indicated the presence of two clear parts with different degrees of inclination of the (Q/I) curves, as the straight part expresses the exchanged ion that is liberated from sites where the binding energy is low, while the curved part indicates the ion that is liberated from strongly held sites. The largest of these sites are called specialized sites.

Keywords. Ionic activity of K^+ , Buffering capacity of K^+ , Free energy of K^+ .

1. Introduction

Soil temperature has a role in the available and movement of K^+ in the soil system, in addition to its role in the process of its absorption by the plant. In general, the speed of any chemical reaction increases with increasing temperatures whenever the temperature increases by 10 degrees Celsius. From this standpoint, [1] see the necessity of studying K^+ reactions under the influence of different temperatures, especially in Iraqi environmental conditions, due to the large temperature fluctuations during the seasons of the year and during the day and night in a single day. In their study on the adsorption of potassium in calcareous soil from northern Iraq at three absolute temperatures (278°, 298°, 318° kelvin) using mixed displacement techniques, the two researchers concluded that there is a highly significant effect of temperature on the kinetics and thermodynamics of K^+ adsorption and release and the state of equilibrium between them. In this field, [2], showed that low temperature restricts plant growth and the rate of absorption of K^+ , as the flow of potassium towards the roots of the corn crop at a temperature of 15°C was half the

flow at a temperature of 29°C, and the growth of the roots at this temperature thermal growth was eight times greater than its growth at 15 degrees celsius. [3] explained that there is an effect of temperature on the equilibrium rate between dissolved and exchangeable potassium in the soil solution, especially in mixed and sandy soils, and the reason for this is due to the change in the free energy of the reaction, as the process of releasing K^+ is an endothermic reaction, while the process of potassium adsorption is an exothermic reaction. As for the High Potassium Institute [4], it showed that low soil temperatures reduce the absorption of potassium by plants, and that the ideal soil temperature for potassium absorption by crops such as corn, for example, is 29.5°C. The high temperature of agricultural soils has a significant impact on the ability of minerals to fix potassium, which affects its readiness for plants [5]. In this regard, both [6] indicated that the high temperature of calcareous soils leads to an increase in the amount of soluble K^+ and exchangeable K^+ , thus increasing the rate of K^+ release. On the other hand, the ability to release potassium from the soil may be limited due to the change in the structure of potassium-bearing soil minerals and the decrease in the diffusion of potassium ions in the interlayer spaces of the minerals. Estimating available K^+ using chemical extracts is a traditional and widespread method that cannot be completely relied upon in assessing the amount of available potassium for plants, and there is a need to use other unconventional methods to describe the potassium supply strength in the soil [7]. [8] explained the importance of using thermodynamic criteria to describe the state and available of potassium in Iraqi soils because they well expressed the state, behavior and dynamics of K^+ in the studied soils. [9] also pointed out that evaluating soil potassium by thermodynamic methods is more discreet and efficient than traditional methods and reflects the behavior of the soil towards the ongoing changes in K^+ . In the past few years, attempts have emerged to propose modern means and methods in which potassium available is expressed, most of which are based on the use of energy (thermodynamic) functions in the soil-water system, as the use of these measures succeeded in revealing the relationship between the amount of ion in the exchange complex and its concentration in the equilibrium solution [10]. There are many studies conducted in this field in order to determine the ability of soils to maintain an appropriate preparation of potassium for plants, and the information derived from these curves is important in giving a better understanding and visualization of the available of K^+ for plants from the soil solution [11].

The use of thermodynamic criteria in evaluating soil fertility to express the state of potassium and its behavior in the soil, which helps in revealing an appropriate management method for each soil [12-14]. The evaluation of potassium fertility continued for long periods and after it was confirmed recent studies have shown that there is a clear deficiency in the fertility assessment according to the traditional chemical standard, which led researchers to shift to new concepts that are more realistic and related to the potassium available of the plant. The researchers considered that the thermodynamic criteria for potassium available gave the best quantitative description for evaluating the available K^+ in the soil and understanding the fertility state of the soil in order to determine fertilizer needs for K^+ [15]. In this context, [16] pointed out the importance of using thermodynamic criteria, including the ionic activity of K^+ , the free energy of potassium, the quantity/intensity, and buffering capacity of potassium to evaluate the potassium available capacity of different soils in Nineveh governorate with similar content of exchangeable potassium, and with different soil characteristics, clear differences appear in the values. Thermodynamic standards.

2. Materials and Methods

2.1. Select of Study Sites

The study included three sites in the Rabia area within Nineveh Governorate - Iraq, for saline soils with different salinity levels. Soil samples were collected from the surface depth (0 - 25 cm) and the subsurface depth (25 - 50 cm) to conduct routine analyses. The soil samples were air-dried and ground using a wooden hammer, it was then sieved with a 2 mm sieve, and the quantity of the three separations - clay, silt, sand, total calcium carbonate (CaCO_3), organic matter (OM), exchange capacity of positive ions (CEC), pH, and electrical conductivity (EC) were estimated using the methods described [17]. as shown in table (1).

Table 1. Some of chemical and physical properties for the studied soils.

Depth	pH	EC dS.m^{-1}	CaCO_3 gm.kg^{-1}	O.M gm.kg^{-1}	CEC Cmol.kg^{-1}	Soil Separates(gm.kg^{-1})			Texture
						Sand	Silt	Clay	
First Location									
Surface	7.3	2.70	225	15.98	29.56	15.05	54.25	30.70	S C Loam
Sub Surface	7.4	5.10	220	18.36	32.17	9.70	44.50	45.80	Silty Clay
Second Location									
Surface	7.2	3.00	220	11.90	29.56	34.70	34.50	30.80	Clay Loam
Sub Surface	7.3	6.00	190	12.24	29.56	31.40	38.50	30.10	Clay Loam
Third Location									
Surface	7.4	7.80	195	15.98	26.95	16.50	31.00	52.50	Clay
Sub Surface	7.6	7.80	225	13.94	26.08	11.50	33.50	55.00	Clay

2.2. Thermodynamic Adsorption of Potassium

Transparent plastic cans of 100 ml with a tight seal were used to study this adsorption. The cans were prepared with the number of concentrations of potassium prepared from potassium chloride (KCl) added as follows (0, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2) mmol.L^{-1} , 2.5 grams of study soil were placed in each box, and these concentrations were added in the form of a volume and the volume was completed to 50 ml with a 0.01 M CaCl_2 solution. After that, the plastic boxes were shaken with a shaker for two hours and left to stabilize for 48 hours at three temperatures (278°, 298°, 318° Kelvin), then the suspension was filtered in the cans to obtain the equilibrium solution in which K^+ , Ca^{+2} , Mg^{+2} , pH, and EC were determined. The amount of potassium adsorbed on the surface of the solid phase, K - quantity, was calculated, as well as the intensity of potassium in the equilibrium solution, K - intensity, which is expressed as the relative effectiveness of potassium in the equilibrium solution according to the ratio law, according to what was mentioned by [18], and it was calculated from the following equation:

$$\text{AR}_K = (a K) / (a \text{Ca} + a \text{Mg})$$

AR_K : Activity relative potassium

The mathematical relationship was drawn between the amount of adsorbed potassium, expressed by ($\pm \Delta K$), which represents potassium in the solid soil phase (Q) quantity on the y-axis, and the intensity of potassium in the liquid soil phase (I) intensity on the x-axis, which is (AR_K), and through this relationship the following constants can be found for potassium:

- Mobile potassium: Labile – K: It was calculated from the extension of the linear relationship towards the y-axis - ΔK .
- Activity relative potassium AR_K : It represents the point where Q/I meets the x-axis.
- potential buffering capacity of K^+ (PBC_K): Calculate mathematically from the quotient of mobile potassium / Activity relative potassium.
- free energy (ΔG): It can be calculated from the equation proposed by [19] $-\Delta G = 2.303 \text{ RT log AR}_K$

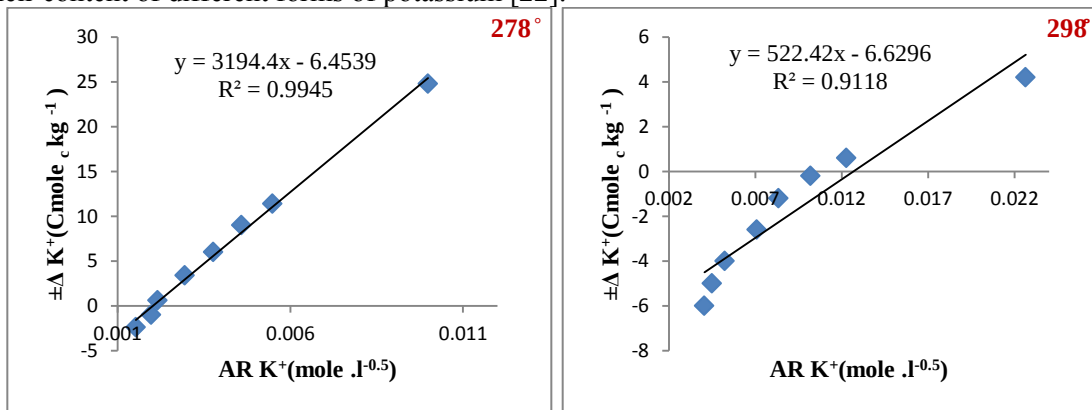
- The Quantity of potassium adsorbed on the exchange surface was calculated from the following relationship:

$$\text{Adsorbed Quantity} = (\text{Balanced } K^+ - \text{added } K^+) / (\text{Soil weight}) \times \text{Volume of solution}$$

3. Results and Discussion

3.1. Function of Potassium Intensity and Quantity (Q/I) at Different Temperatures

Figures (1, 2, 3, 4, 5 and 6) show the relationship between the factors Quantity and Intensity of the potassium ion in the study soil, which represents the amount of mobile potassium labile ion present in the solid phase of the soil (released or adsorbed) as a function of the potassium effectiveness ratio in the balanced soil solution, expressed as the intensity of the chemical potential. For the mobile potassium relative to the chemical potential of the calcium and magnesium ions moving towards the soil solution, [18] defined the relationship between the quantity (Q) and the intensity (I) of the ion as the relationship between the quantity of the mobile ion present in the solid phase of the soil and its intensity in the liquid phase of the soil. He used the concept of AR, which expresses the effectiveness of the ion over the square root of the total effectiveness ($Ca^{+2} + Mg^{+2}$) in the equilibrium solution. This concept has been applied by many researchers [20], [21], [22], [16]. The slope of the straight lines, represented by the slope of the angle of the straight line, represents the buffering capacity of the potassium effort, which means the difference in the buffering capacity of this effort in the study soils. Likewise, the intersection of the straight lines with the y-axis represents the mobile potassium. It is clear from these shapes that the values of the slope coefficient and the secant value for the study soils differ. This clearly reflects the effect of the type of added solution, the type of ion accompanying it, and the binary and ternary equilibrium systems on the movement of potassium between the two phases and its release. The following figures show the differences in the study soils in their slope values and the intercept values for the relationships of potassium intensity and quantity, due to the relationship of these values to the mineral composition of the soil and to the differences in soil textures and their content of different forms of potassium [22].



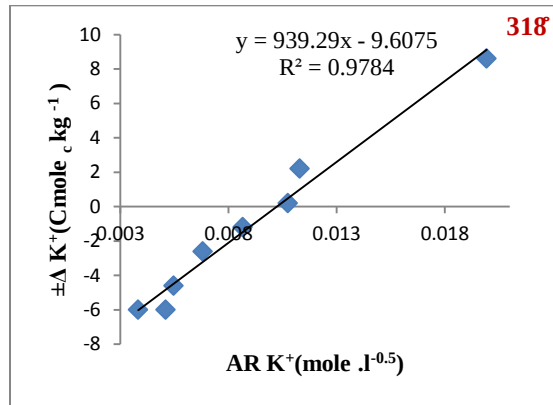


Figure 1. Quantity/Intensity curve (first location-surface) at three temperatures.

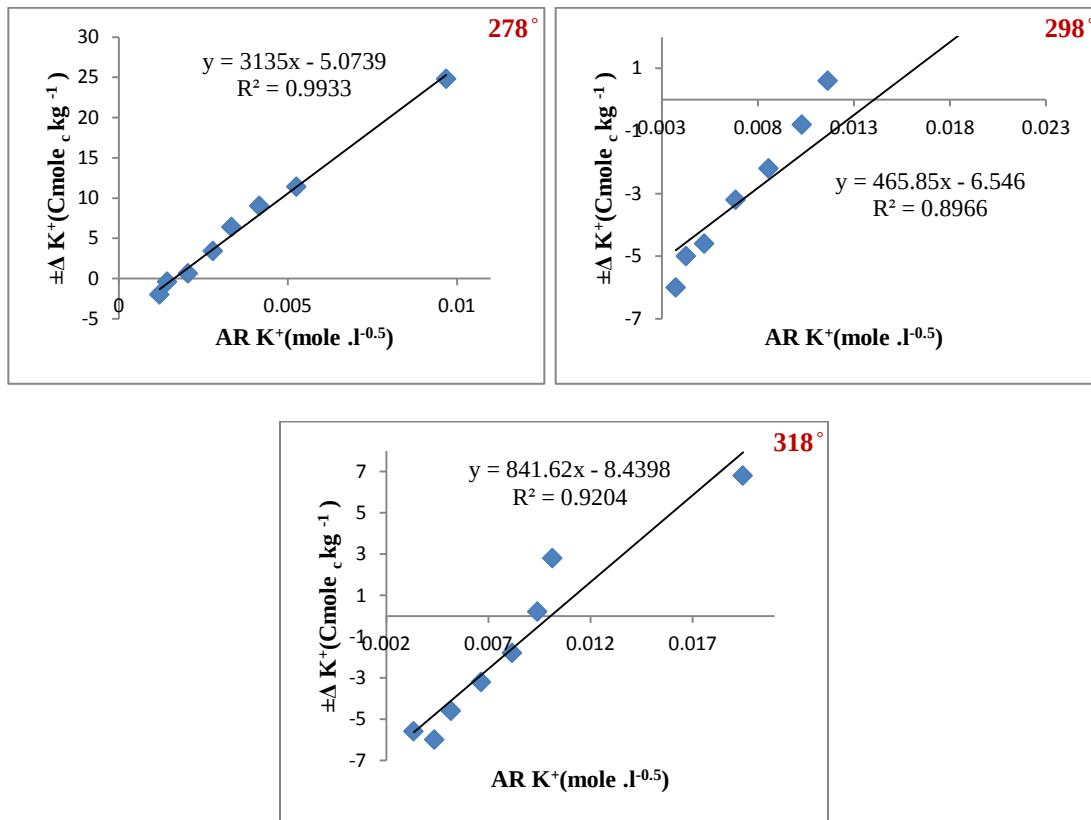


Figure 2. Quantity/Intensity curve (First location-Sub surface) at three temperatures.

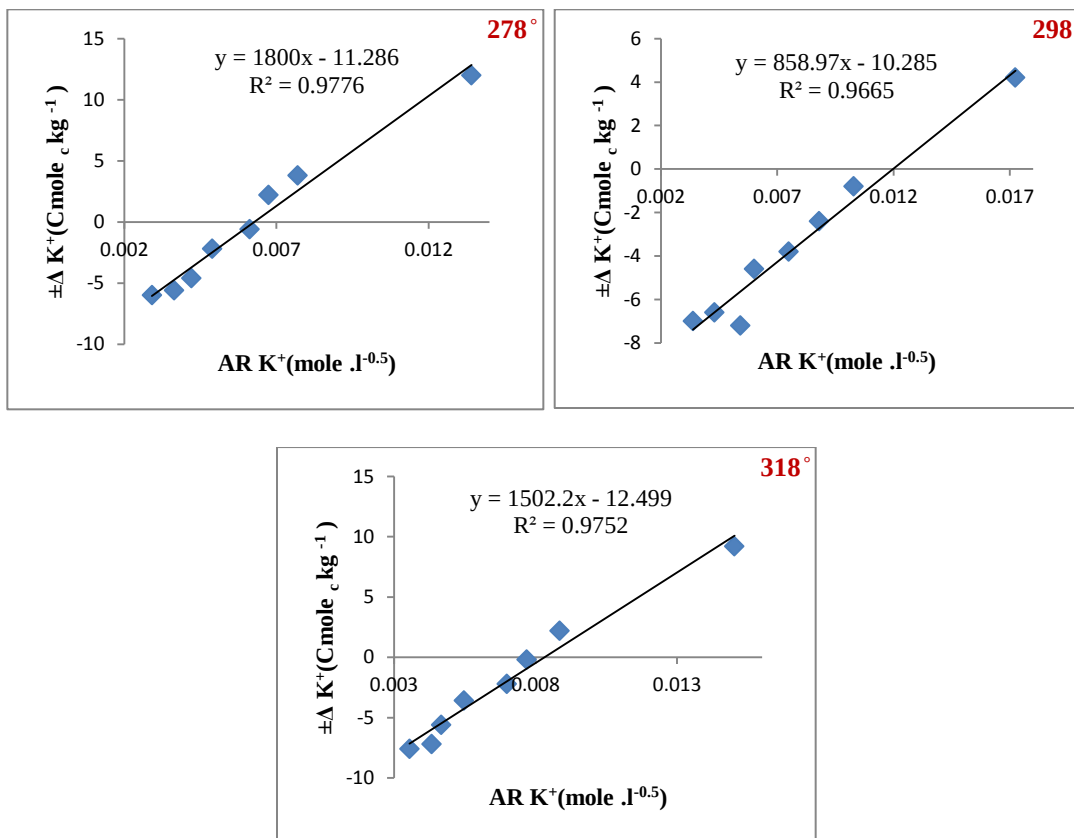
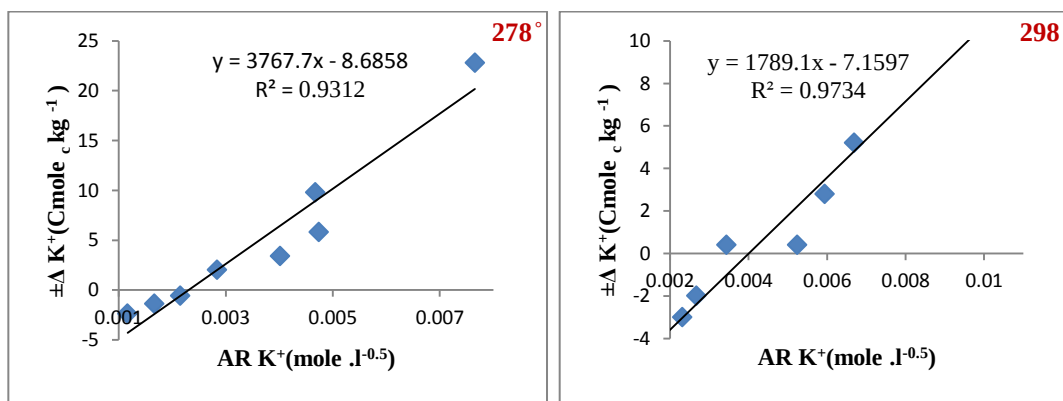


Figure 3. Quantity/Intensity curve (second location-surface) at three temperatures.



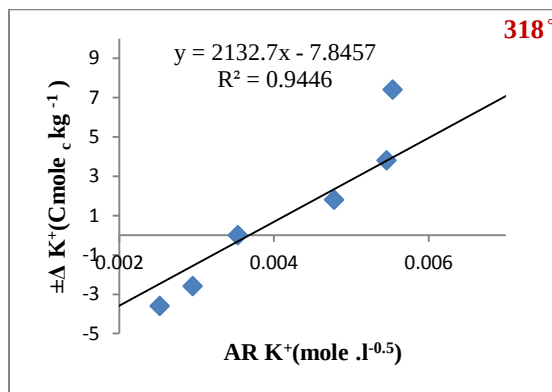


Figure 4. Quantity/Intensity curve (second location-sub surface) at three temperatures.

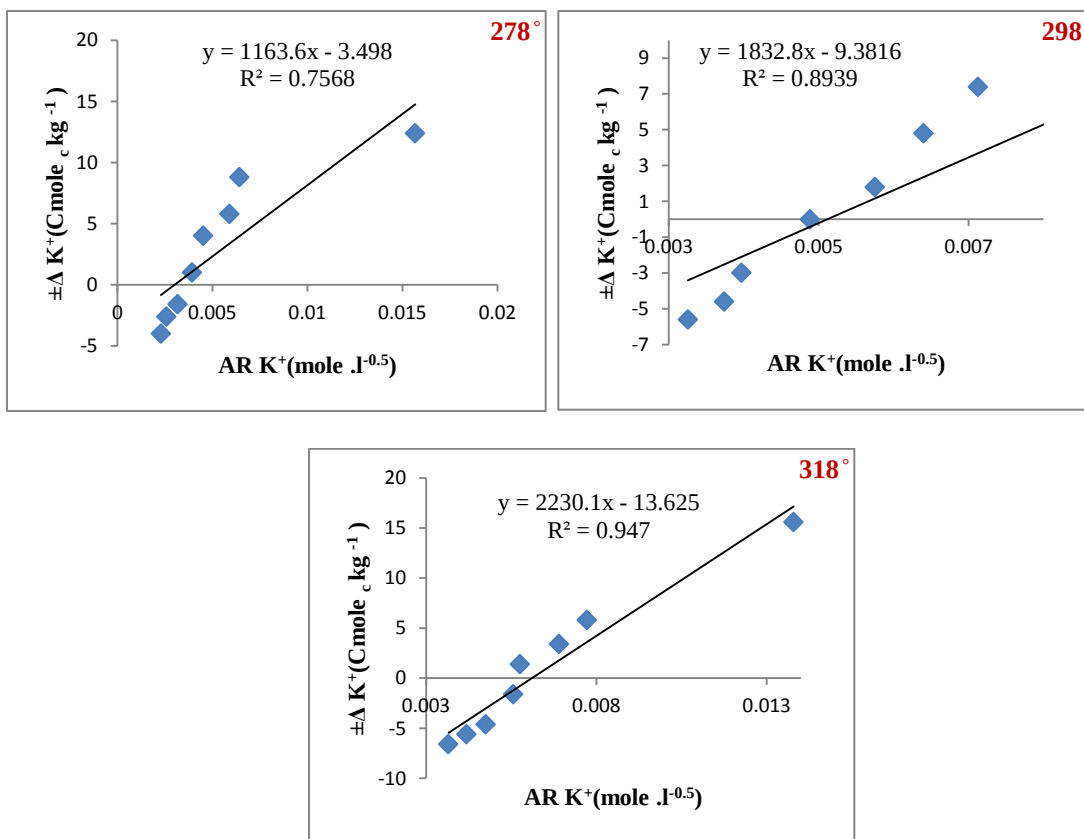


Figure 5. Quantity/Intensity curve (third location-surface) at three temperatures.

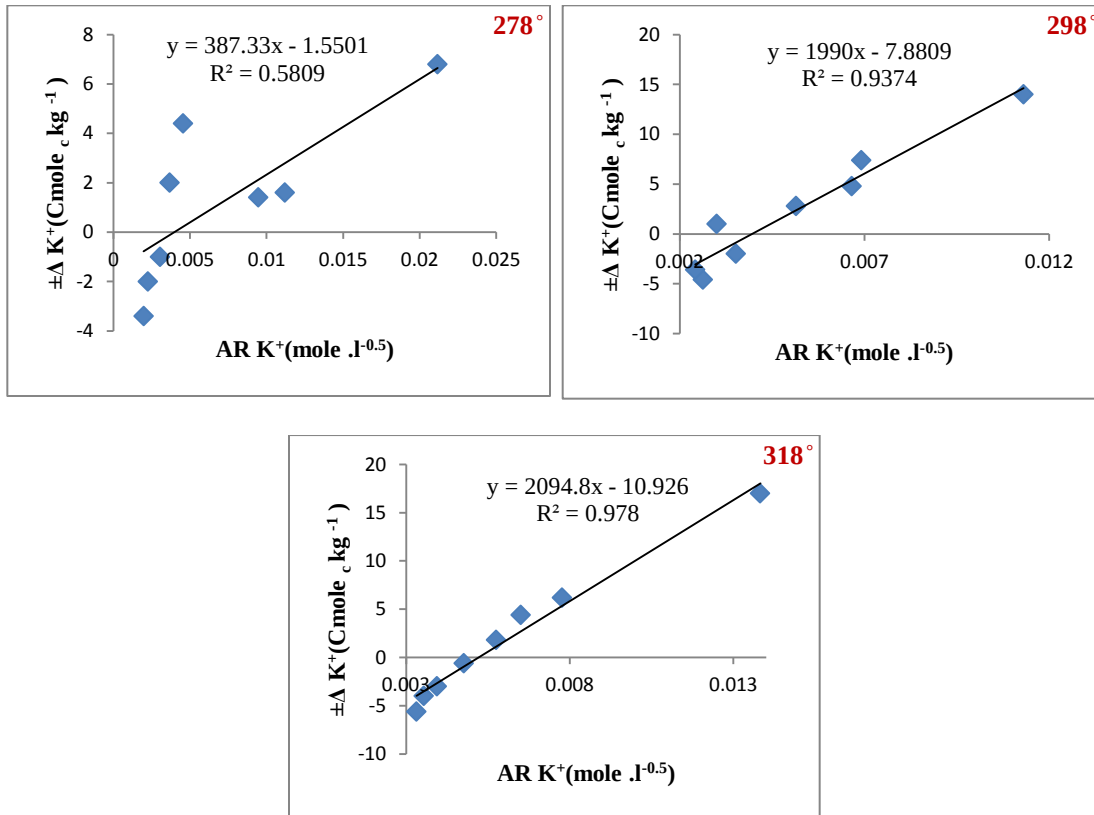


Figure 6. Quantity/Intensity curve (third location-sub surface) at three temperatures.

These shapes also illustrate the difference in the ability of buffering soils to prepare potassium and the extent of their need for potassium fertilizer, in addition to the shape of these straight lines and their behavior of the Q/I relationship, which is a distinctive characteristic of each soil and describes the behavior of potassium added to the soil, which is distributed between the solid and liquid phases of the soil [23].

3.2. Effect of Temperature on the Ionic Activity Ratios of Potassium at Equilibrium (AR^0_K)

Ionic activity has been used in research to evaluate the available of some nutrients, including potassium, and for the purpose of identifying locations where adsorption occurs on the surfaces of the solid phase of the soil, we have relied on the relative effectiveness values, and the relative effectiveness values of potassium (AR^0_K) represent the intensity of potassium in the solid phase of the soil when it does not occur loss or gain of potassium in the soil system is a measure of the instantaneous available content of the soil and has no relation to the soil's ability to release or prepare the ion over a long period of time [24]. The lowest value of ionic activity was recorded at the subsurface depth of the first site (1.61846×10^{-3}) $mol \cdot l^{-0.5}$, while the highest value of ionic activity rates at the surface depth of the second site was (6.270×10^{-3}) $mol \cdot l^{-0.5}$, at a temperature of 278° Kelvin at a rate of (3.20×10^{-3}). When the equilibrium temperature was increased to 298° K, it led to a high increase in the ionic activity values. We noticed that the lowest value was at the subsurface depth of the third site (3.96×10^{-3}) $mol \cdot l^{-0.5}$, and the highest value appeared at the surface depth of the site. The first amounted to (14.0517×10^{-3}) $mol \cdot l^{-0.5}$, with a rate of (8.63×10^{-3}) $mol \cdot l^{-0.5}$. As for the temperature of 318° K, we noticed that the lowest values were in the subsurface depth of the site fourth was (3.6787×10^{-3}) $mol \cdot l^{-0.5}$, and the highest values reached

(10.2284×10^{-3}) mol.l^{-0.5} at the surface depth of the first site, at a rate of (7.37×10^{-3}) mol.l^{-0.5} (table 2). These values indicate that adsorption occurs in locations between layers of minerals as well as at the edges, as indicated by [25] that values that are more than (1×10^{-2}) mol.l^{-0.5} indicate the dominance of adsorption over (Positions Planner), while values less than (1×10^{-2}) mol.l^{-0.5} indicate the dominance of adsorption (edge site). This confirms that the adsorption process is accompanied by stabilization processes that are slow and occur between layers of clay minerals and are the result of rapprochement between the ions and the surface soil exchange [26]. We notice in general that a rise in temperature above 278° K generally led to a clear increase in the (AR⁰_K) values. This effect of temperature was clear at the temperature of 298° K, and this confirms the assumption that the adsorption process with these soils does not include superficial adsorption on surfaces only soil particles, but this is accompanied by slow processes of fixing potassium between the layers of clay minerals. In this regard, [27] indicated that the interaction of potassium with soil components includes an initial adsorption reaction, and this reaction is completed within minutes of its beginning and after this is followed by a slower reaction, and increasing the reaction temperature will increase the rate of reaction speed that follows the surface adsorption process.

Table 2. Value of activity relative at equilibrium (AR⁰_K) for three temperatures.

Depth	AR ⁰ _K × (10 ⁻³) mole.l ^{-0.5} at temperature		
	278° Kelvin	298° Kelvin	318° Kelvin
First Location			
Surface	2.02037	12.6901	10.2284
Sub Surface	1.61846	14.0517	10.0280
Second Location			
Surface	6.2700	11.9736	8.3204
Sub Surface	2.3053	4.0018	3.6787
Third Location			
Surface	3.0061	5.1187	8.3204
Sub Surface	4.0020	3.9602	3.6787

We conclude from the above that increasing the temperature from 278° to 298° K led to an increase in the ionic effectiveness rate by 232.72%, while increasing the temperature from 278° to 318° represented an increase of 201.83%. The reason for the increase in ionic effectiveness values with increasing temperature to a certain extent it is to increase the speed of the chemical reaction until a state of dynamic equilibrium is reached between the soil phases. That is, at this state (state of equilibrium), the ionic activity values reach the highest values and begin to decrease when a state of dynamic imbalance occurs in the soil system (solid-liquid phase of the soil).

3.3. Effect of Temperature on Mobile Potassium (K-Labile)

The results in table (3) indicate the K-labile values of potassium, which was calculated from extending the linear relationship of quantity and intensity, the mobile potassium values ranged between (-11.286 and -1.550) Cmol.kg⁻¹ at the surface depth of the second site and the subsurface depth of the third site, respectively, at a temperature of 278° K, at a rate of (-6.09) Cmol.kg⁻¹. While when the temperature was increased to 298° K, the values ranged between (-10.285 and -6.546) Cmol.kg⁻¹ at the subsurface depth of the first site and the surface depth of the second site respectively, and as a general average it reached (-7.98) Cmol.kg⁻¹. As for the temperature of 318° K, the values of mobile potassium increased, as we note that the lowest values reached (-13.625) at the surface depth of the third site, and the highest values reached (-7.845) Cmol.kg⁻¹ at the subsurface depth of the second site, at a rate of (-10.49) Cmol.kg⁻¹, the variation in the values of

mobile potassium in the soil of the study is caused by the variation in the values of the potassium potential buffering capacity, and this is due to the difference in the various soil properties, these properties play an essential role in potassium adsorption reactions, and the reason for the decrease in the value of mobile potassium is due to the increased strength of binding to the exchange sites, which makes its release difficult [28]. Regarding the effect of temperature on the values of mobile potassium, as is clear, it had a major role in increasing the amounts of reactants (released or adsorbed), when this relationship is expressed statistically, we notice that increasing the temperature from 278° K to 298° K led to an increase in the values of mobile potassium by a percentage an amount of 11.88%, and an increase in temperature from 278° to 318° K led to an increase in the percentage of mobile potassium values amounting to 43.91%.

Table 3. Value of potassium labile (ΔK) for three temperatures.

Depth	Cmole.kg ⁻¹ at temperature ΔK		
	278° Kelvin	298° Kelvin	318° Kelvin
First Location			
Surface	-6.453	-6.629	-9.607
Sub Surface	-5.073	-6.546	-8.439
Second Location			
Surface	-11.286	-10.285	-12.499
Sub Surface	-8.685	-7.159	-7.845
Third Location			
Surface	-3.498	-9.381	-13.625
Sub Surface	-1.550	-7.880	-10.926

3.4. Effect of Temperature on Potassium Buffering Capacity (PBC)

The slope representing $\Delta Q/\Delta I$ in intensity and quantity relationships is a measure of potassium buffering capacity. The values of PBC are a criterion for the soil's ability to maintain potassium potential in the soil and are important for the soil's ability to compensate through the process of depletion from the solid or liquid phase of the soil. The ability of the soil to maintain the change that occurs to the ion $-\Delta I$ can be measured, which makes it an indicator of releasing. [29] explained that the value of the PBC gives an impression of the soil's ability to maintain continued ion preparation in the nutrient solution, and therefore it reflects positively on the ion preparation for crops and thermodynamic equilibrium depends on the process of balance between loss and preparation between the liquid and solid phases, and that high values of PBC they indicate the presence of sufficient storage in the soil when the element is deficient as a result of its depletion in the (water - soil - plant) system, while low values indicate the need for fertilization with the element for the purpose of compensating for the deficiency that occurs in the root zone and at close intervals. Soils that are characterized by a low of PBC relatively speaking, it requires larger amounts of fertilization due to a decrease in this capacity and to fill the deficiency, if any, and this is what is recommended [30]. Table (4) shows that the highest value of PBC was in the subsurface depth of the second site, 3767.7 (Cmol.kg⁻¹/mol.l⁻¹)^{-0.5}, while the lowest value was recorded in the subsurface depth of the third site, 387.33 (Cmol.kg⁻¹/mol.l⁻¹)^{-0.5} at a temperature of 278° K and a rate of 2241.33 (Cmol.kg⁻¹/mol.l⁻¹)^{-0.5}, When the temperature was increased to 298° K, the highest value was recorded at 1990.0 (Cmol.kg⁻¹/mol.l⁻¹)^{-0.5} at the subsurface depth of the third site, while the lowest value was recorded at the subsurface depth of the first site at 465.85 (Cmol.kg⁻¹/mol.l⁻¹)^{-0.5} the average amounted to 1243.19 (Cmol.kg⁻¹/mol.l⁻¹)^{-0.5}, while at a temperature of 318° K the highest value reached 2230.1 (Cmol.kg⁻¹/mol.l⁻¹)^{-0.5} at the surface depth of the third site, and the minimum value reached 841.62 (Cmol.kg⁻¹/mol.l⁻¹)^{-0.5} at the second depth

of the first site, at a rate of $1623.45 \text{ (Cmol.kg}^{-1}/\text{mol.l}^{-1})^{-0.5}$. This difference in the values of the PBC is due to the difference in soil density of surface charges present on the exchange surfaces, and is also due to the difference in the number of specialized adsorption sites in the soil and the extent of their ability to hold ions [31]. These results that we obtained indicate that increasing the equilibrium temperature led to a significant decrease in the buffering ability of the soil, When the temperature was increased from 278° to 298° K, the amount of decrease in PBC amounted to 154.67%, while the continued increase in the equilibrium temperature to 318° K led to a decrease in the amount of PBC by 97.36% compared to its value at a temperature of 278° K, and the reason for the decrease in the amount the buffering capacity with an increase in temperature may be due to the ion exchange reaching a state of equilibrium, and this does not allow the soil to regain its natural position in regulating the amounts added or removed from it. [29] explained that the value of PBC gives an impression of the soil's ability to maintain continuous ion preparation in the nutrient solution and therefore reflects positively on the ion preparation for crops and thermodynamic equilibrium depends on the process of balance between loss and preparation between the liquid and solid phases, and that high values of PBC indicate there is sufficient storage in the soil when the element is deficient as a result of its depletion in the system (water - soil - plant), while low values indicate the need for fertilization with the element for the purpose of compensating for the deficiency that occurs in the root zone.

Table 4. Value of potassium buffering capacity (PBC) for three temperatures.

Depth	$(\text{Cmole.kg}^{-1} / \text{mole.l}^{-1})^{-0.5}$ at temperature PBC		
	278° Kelvin	298° Kelvin	318° Kelvin
First Location			
Surface	3194.4	522.42	939.29
Sub Surface	3135.0	465.85	841.62
Second Location			
Surface	1800.0	858.97	1502.2
Sub Surface	3767.7	1789.1	2132.7
Third Location			
Surface	1163.6	1832.8	2230.1
Sub Surface	387.33	1990.0	2094.8

3.5. Effect of Temperature on the Substitution Free Energy (ΔG) of Potassium

The free energy ΔG is one of the thermodynamic functions used to describe the spontaneity of the reaction because it represents the result of two factors: the change in heat capacity ΔH and the factor of change in the randomness of the reaction ΔS [20], and the supply capacity of any soil can be found from the energy levels of the nutrients in the solution. Soil, as the level of this energy can represent the total differential of the supply forces of the element, which includes the nature of the ion and its salts and their quality for specific sites as well as the ions accompanying them, the free energy can be calculated from the following equation ($\Delta G = -RT \ln AR^0$) proposed by [19] based on the values of the ionic activity ratios AR^0 at equilibrium for potassium when no gain or loss of it occurs, and that a negative value for the amount of change in energy means that the reaction is spontaneous. It does not require heat, while a positive value means that the reaction is not spontaneous and requires heat. Through the results we obtained, it was shown that the values of the change in free energy ΔG for potassium in the study soil were all negative, which means that the reaction is spontaneous. Table (5) shows the results for free energy, from which it is noted that the values of the change in the free energy of potassium ΔGK in the soil ranged from $(-14.855 \times 10^{-3} \text{ KJ})$ in the subsurface depth of the first site to $(-11.724 \times 10^{-3} \text{ KJ})$ in the surface depth of the second site reached a rate of $(-13.52 \times 10^{-3} \text{ KJ})$ at a temperature of 278° K, while the

values ranged from $(-13.707 \times 10^{-3} \text{ KJ})$ at the subsurface depth of the third site to $(-10.568 \times 10^{-3} \text{ KJ})$ in the subsurface depth of the first site, and an average amounted to $(-12.133 \times 10^{-3} \text{ KJ})$ at a temperature of 298° K, as the temperature increased to 318° K, the values ranged from $(-14.821 \times 10^{-3} \text{ KJ})$ at the subsurface depth of the second site to $(-12.117 \times 10^{-3} \text{ KJ})$ at the surface depth of the first site, with an average of $(-13.19 \times 10^{-3} \text{ KJ})$. This difference in the free energy values of the study soils is directly related to the equilibrium conditions between the soil solution and its solid phase, as well as to the difference in exchange sites and its mineral composition [32]. Negative values of free energy indicate a high available of the potassium ion, and the higher the value with a negative sign, the greater the available of potassium quantitatively in the soil. Conversely, the greater the values of free energy with a positive sign mean a decrease in the available of potassium in those soils, which means the soil needs to add potassium [20]. We found that the temperature showed a variation between the increase and decrease in the free energy values of the process of dissolving reactions and the release of potassium during the depths within one site as well as between the sites. When the temperature rose from 278° to 298° K, the percentage of decrease in the average energy values reached the free rate was 16.26%, and when the temperature continued to rise to 318° K, the percentage of decrease reached 7.49% compared to the temperature of 278° K. This is due to the fact that the high equilibrium temperature works to provide the soil with thermal energy, and thus the value of the remaining free energy in the solution decreases. This variation in values is directly related to the change in the ratio between the activity of K^+ and the activity of Ca^{+2} and Mg^{+2} , which changes with changes in the equilibrium conditions of the soil solution with its solid phase. This is due to the effect of temperature on the speed of the adsorption reactions that occur after adding potassium to the soil. As [33] showed, the free energy values are related to the form in which the ion is present, when the ion is present in easily soluble compounds, the amount of dissolution and release is greater, and thus the free energy value is high compared to complex compounds that are difficult to dissolve.

Table 5. Value of free energy at equilibrium (ΔG) for three temperatures.

Depth	(Cal / mole ⁻¹) at temperature Free energy (10^{-3})		
	278° Kelvin	298° Kelvin	318° Kelvin
First Location			
Surface	-14.342	10.821-	12.117-
Sub Surface	-14.855	10.568-	12.170-
Second Location			
Surface	-11.724	10.965-	12.663-
Sub Surface	-14.037	13.681-	14.821-
Third Location			
Surface	-13.424	13.071-	13.480-
Sub Surface	-12.762	13.707-	13.898-

References

- [1] Al-Obaidi, M. A. J, and Al-Zubaidi A. H. (2005). The effect of temperature on the adsorption and release of potassium in soil. *Mesopotamia Agriculture Journal*. 33(4), 47-56
- [2] Asaad, S. Abdel Karim (2016). Evaluation study for some extraction method of available soil potassium and their relationship with absorbed potassium and some parameters of Barley crop growth (*Hordeum vulgare* L.). Master's thesis, College of Agriculture, University of Baghdad.
- [3] Al-Yasiri, M. N. Hussein (2012). The effect of infusions and levels of nitrogen and potassium fertilizer on the readiness and release of ammonium and potassium in the soil and on the growth and yield of wheat (*Triticum aestivum* L.). Master's thesis. College of Agriculture - University of Baghdad.

- [4] IPI, International Potash Institute. (2016). Technical manual on Potash Fertilizer. Addis Ababa, Ethiopia
- [5] Al-Shibini, J. M. (2007). Potassium in the ground and plants. First edition. Egyptian Library for Printing, Publishing and Distribution, p. 207.
- [6] Najafi-Ghiri, M., Boostani, H. R., Mirsoleimani, A., Mohaviye-Asadi, N., Beizavi, M., Shafiei, M., & Mirdoraghi, M. (2020). Potassium fixation and release in some calcareous soils under orange cultivation. *Eurasian Soil Science*, 53, 978-985.
- [7] Dobermann, A. P.; C. Sta. Cruz. and K. G. Cass man .(1995). Potassium balance and soil potassium supplying power in intensive rice ecosystems potassium in Asia , proceedings of the 24th Colloquium of the International Potash Institute held at Chaing Mai/Thailand P. 199-234.
- [8] Al-Zubaidi, A. H. and Al-Rubaie, S. M, (2002) The thermodynamics of potassium in Iraqi soils. *Jerash Journal of Research and Studies*. Volume Seven, Issue One, 23-32.
- [9] Abdel Rasoul, Q. Jamal (2007). Evaluating the effect of organic and mineral fertilization (N, K) on the condition, release and absorption of potassium and production of potato tubers (*Solanum tuberosum* L.). Doctoral thesis, College of Agriculture, University of Baghdad.
- [10] Majeed, D. A. (2020). The effect of potassium, organic matter, and deficit irrigation on potassium forms of maize (*Zea mays* L.). Master thesis, College of W Agriculture, University of Wasit- Iraq.
- [11] Shil, N. C., Alam, M. K., Saleque, M. A., Islam, R. M., & Jahiruddin, M. (2021). Quantity-to-intensity (Q/I) relationships can efficiently characterize intensively cultivated agricultural soils in Bangladesh for better potassium supplying capacity. *Spanish Journal of Agricultural Research*, 19(2).
- [12] Sulaiman, H. S., Abd Alrazaq, O., & Kaba, R. (2021). Study of the mineral composition of some soils of the Syrian island.
- [13] Isnaini, S., & Rivaie, A. A. (2023). Comparison of potassium quantity-intensity relationships in tropical paddy soil under tillage and no-tillage systems after fifteen growing seasons. *Plant, Soil & Environment*, 69(1).
- [14] Rani, K., Datta, A., Jat, H. S., Choudhary, M., Sharma, P. C., & Jat, M. L. (2023). Assessing the availability of potassium and its quantity-intensity relations under long term conservation agriculture based cereal systems in North-West India. *Soil and Tillage Research*, 228, 105644.
- [15] Mam-Rasul, G., & Sheikh-Abdullah, S. (2022). Potassium Mobility Potential of Forest Soil In Kurdistan Region, Iraq, As Estimated By Quantity-Intensity (Q/I) Relationships. *Journal of Applied Science and Technology Trends*, 3(01), 34-39.
- [16] Al-Qattan, R. A. (2023). Impact of Wetting, Drying and Soil Texture on Dynamic and Kinetic Equilibrium for Cations in Some Soils of Nineveh Governorate. Master thesis, College of Agriculture and Forestry, University of Mosul.
- [17] Carter, M.R. and E.G. Gregorich (2008). Soil sampling and methods of analysis Lewis Publishers. Canadian Society of Soil Science. CRC, Press. Inc. Canola.
- [18] Beckett, P. H. T. (1964). Studies on soil potassium: confirmation of the ratio law: I. measurement of potassium potential. II. The immediate Q/I relation of labile potassium in soil. *J. Soil Sci.* 15: 1 – 23.
- [19] Woodruff, C.M. (1955). Ionic equilibrium between clay and dilute salt solution.
- [20] Al-Obaidi, M. A. Jamal, Raida I. Abdullah, and Fateh A. Hassan (2010). The effect of wetting and drying cycles on the release of potassium added to some calcareous soils from northern Iraq. *Al-Rafidain Agriculture Journal*, Volume (38), Issue (1).
- [21] Ahmed, G., and Sheikh-Abduallah, S. (2020). Potassium mobility potential of forest soil in Kurdistan region, Iraq, as estimated by quantity-intensity (Q/I) relationships. *Journal of Geoinformatics and Environmental Research*, 1(01), 11-19.
- [22] Al-Sultan, N. M., and Al-Obaidi, M. A. J. (2022). Potassium Buffering Potential in Some Burne Soils of North Iraq. *Tikrit Journal for Agricultural Sciences*, 22(4), 81-95.
- [23] Wang, J.J.; D.L., Harvell and P.F., Bell (2004). Potassium buffering characteristics of three soils low in exchangeable potassium. *Soil Sci. Soc. Am. J.* 68: 654-661.

- [24] Wakeel, A., & Ishfaq, M. (2022). Potash use and dynamics in agriculture (pp. 7-17). Singapore: Springer
- [25] Hamed , M.H.Amin , Abu Zied (2017). Evaluation of potassium quantity_ intensity in some soils of EL_dakhlaosis, new valley , Egypt.Alexandria sci_ Exch.J .38, 112_119.
- [26] Biliyas , F, and barbayiannis , N (2019). Potassium availability : An approach using thermodynamic. Parameters derived from quantity_intensity relationships. Geoderma , 338, 353, _364.
- [27] Strauss, C.R. and G.W. Brummener (1989). Einfluss der Kristallinität Van Phosphata desorption. M: Mitteilungen der Deutschen Bodenkundliche Gesellschaft.
- [28] Elbana,T.A ; selim, H.M; akrami , N , newman , A; shaheen , S.M., and rinklebe , J.(2018). freundlich sorption parameters for cadmium, copper , nickel ,lead , and zinc for different soils; influence of kinetics . Geoderma , 324, 86,88.
- [29] Suttanukool,p;darunsontaya T ,and Jindaluang, W. (2019). A study on the quantity /Intensity relationships of potassium of sugarcane growing soils, Eastern Thailand . communications in soil science and plant analysis 50:2,153_163.
- [30] Subba Rao, A., and T.R. Srivastava .2001. Assessing potassium availability in Indian. In: N.S. Pasricha and S.K. Bansal (eds) "Potassium in India Agriculture". Potash Research Institute of India/International Potash Institutes, Basel, Switzerland, pp. 125-157.
- [31] Islam, A.; Karim, A. S., Solaiman, A. R. M., Islam, M. S., and Saleque, M. A. (2017). Eight-year long potassium fertilization effects on quantity/intensity relationship of soil potassium under double rice cropping. Soil and Tillage Research, 169, 99-117.
- [32] Mahmmdani, L. A. (2008). Study the effect of carbonate minerals and iron oxides removal on potassium adsorption- desorption from clay fraction in some soils of northern Iraq. Doctoral thesis, College of Agriculture, University of Mosul.
- [33] Al-Khafagi, Q. D. (2021). Physical and chemical behavior of iron and manganese in some forest and agricultural soils in northern Iraq. Ph.D thesis, College of Agriculture and Forestry, University of Mosul, Iraq.

Effect Type of Nutrient Medium and Cytokinin on the Production and Development of the Embryonic Callus of *Phoenix dactylifera* L., Albarhi date Palm Variety, in Vitro

Mohammad H. Tuaimah¹, Osama N. Jaafar² and Manal Z. Sabti³

^{1,2}Palm Research Centre, University of Basrah, Basrah, Iraq.

³College of Agriculture, University of Basrah, Basrah, Iraq.

¹E-mail: mhady4822@gmail.com

²E-mail: dr.osama74@gmail.com

³E-mail: manal.al_myhi@uobasrah.edu.iq

Abstract. The study was carried out in the tissue culture laboratory of the Palm Research Center, University of Basra for the year 2022-2023, as three types of food media were used, namely MS, WPM and B5, with two types of cytokines Zeatin and 2ip with four concentrations of each (0, 0.5, 1, 2) mg L⁻¹ in the production of fetal callus and its development into vegetative embryos for the Barhi variety outside the vivo. The results showed that the nutrient medium WPM and MS recorded the shortest period for the appearance of embryo callus and period of appearance of embryos and an increase in fresh and dry weight and amounted to 39.22, 40.32 days, 30.4, 31.18 days, 1.55, 1.58 g, 0.067 and 0.087 g, respectively, with a significant difference from the B5 medium in all studied traits. The results showed that the medium WPM and MS equipped with cytokinin 2ip and Zeatin at a concentration of (0 and 0.5) mg liter⁻¹ recorded the shortest period of appearance of embryo callus and amounted to 36 and 38.2 days 36.4 and 38.8 days respectively and a significant difference from the rest of the interventions and it was noted that the superiority of the medium MS equipped with 2ip at a concentration of 0.5 mg liter⁻¹ and the medium of WPM Zeatin at a concentration of 0.5 mg liter⁻¹ in the average fresh weight and amounted to 2.23 g and 2.03 g, While it was noted that the MS medium with 2ip at a concentration of 0.5 mg L⁻¹ gave the highest rate of dry weight and a significant difference from the rest of the interactions of another medium.

Keywords. Nutrient medium, WPM; Zeatin, Embryonic callus, Cytokinin.

1. Introduction

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous, diploid, monoecious, dioecious plant and is one of the most important fruit crops due to its nutritional and economic value [1]. It is grown in many countries of the arid regions of West Asia, North Africa, and the Middle East [2]. Plant tissue culture is one of the most important and recent scientific and applied specialties, and its importance has emerged as an alternative method to traditional methods in the multiplication and genetic improvement of plants of all kinds[3,4].

Researchers in most countries of the world have been able to harness this technology for the widespread propagation of plants, and plant tissue culture has proven its efficiency in terms of the number of plants that can be produced from one plant and the matching of the resulting plants to

their genetic origins [5]. Date palms are multiplied tissue-cultured either by organogenesis (Organogenesis) from the cultivation of the growing apex and axillary buds or by somatic embryogenesis (Somatic embryogenesis) by passing through the callus stage from which the green embryos are formed by planting plant tissues in sterile industrial nutrient media [6].

The induction of callus and the production of somatic embryos have been stimulated by many researchers for date palms, such as [7,8]. Nutrient media are one of the most important factors that determine the success of tissue culture, as they play an active role in growth, specialization, and division by providing the plant part with all the nutritional needs, provided that the balance in the components of those media is taken into account[9].

The nature of the nutrient medium and the concentrations of growth regulators added affect the success of plant tissue culture [10]. The type and concentration of cytokinin are one of the most important factors that affect the success of plant tissue culture, and the balance between internal plant hormones and added growth regulators is essential for the success of date palm tissue culture[11]. Auxins and cytokines are the most important components of the nutrient medium that affect the success of tissue culture [12]. Auxins play a fundamental role in the formation of callus and its development into green embryos and their germination, while cytokines are important factors in the induction of lateral and adventitious buds[13,14]. Kin. Zeatine, BA, and 2ip are among the most commonly used cytokines[15].

Based on the above, we decided to use three nutrient media, namely the Woody plant medium, the B5 medium, and the MS medium, for comparison in order to know the difference in the results that will be obtained compared to the MS nutrient medium, as it is the most commonly used nutrient medium in tissue culture, in terms of the period for inducing embryonic callus and the appearance of green embryos and its development into green embryos that later give seedlings ready for acclimatization.

2. Material and Methods

The study was carried out in the Plant Tissue Culture Laboratory of the Palm Research Center at the University of Basrah during the period 2022-2023.

2.1. \ Preparation and Sterilization of Culture Media

Three nutrient media were used in the cultivation, namely the MS medium [13] the Woody plant medium [16] which is denoted as WPM, and the B5 medium which is denoted as B5, and manufactured by the American company Caisson Lab. with vitamins as shown in Table (1). Nutrient medium salts and growth regulators were added after they were prepared as basic solutions, sucrose, and agar, as shown in Table (2). The pH of the nutrient medium was adjusted to 5.7 by adding drops of 0.1 N sodium hydroxide or hydrochloric acid solution. The concentrations of cytokines added were (0, 0.5, 1, 2) mg l⁻¹ for each of Zeatin and 2ip, each separately, with a constant concentration of the auxin Naphthalene Acetic Acid (NAA) in all research experiments in the nutrient medium at a concentration of 1 mg l⁻¹. The nutrient medium was heated to 90 °C using a heater equipped with a magnetic stirrer; then the nutrient medium was distributed in 500 ml jars and distributed at a rate of 50 ml/jar, closed with plastic lids, then placed in an autoclave and sterilized at a temperature of 121 °C and atmospheric pressure of 1.05 kg cm⁻² for 20 minutes. After that, it was taken out of the sterilization device, shaken, and left to cool and solidify until the time of cultivation.

2.2. Primary Callus Culture

The primary callus resulting from the cultivation of the floral shoots of the Barhi variety obtained from the Date Palm Research Center/Tissue Culture Laboratory was planted on MS, WPM, and

B5 nutrient media equipped with concentrations of 0, 0.5, 1, and 2 mg l⁻¹ for each of Zeatin and 2ip, with a weight of 50 mg callus per jar. The cultures were incubated in the growth chamber at a temperature of 27 ± 1 °C and a light intensity of 1000 lux for 16 hours of light and 8 hours of darkness. After the formation of the embryonic callus, it was transferred to another medium to stimulate the formation of embryos, which included the same components of the previous medium with the stability of growth regulators to show the effect of the medium in this regard. The replanting process was once every 4-5 weeks. The data and observations were recorded daily, represented by the duration required to start the formation of the embryonic callus, the appearance of the embryos and their number. After two months of the experiment, the following traits were measured:

- The duration required for the start of the formation of the embryonic, callus (days).
- Calculation of the fresh and dry weight of the embryonic callus (g).
- The duration required for the appearance of green embryos (days).



Figure 1. Cultivation of primary callus.

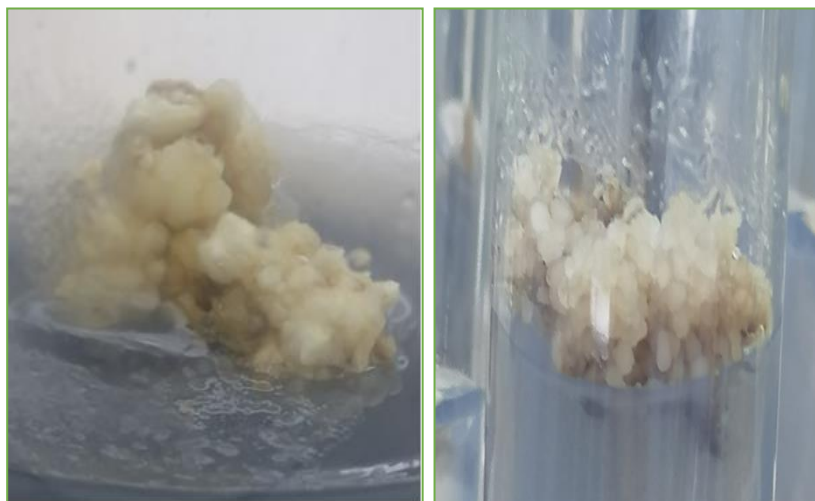


Figure 2. Effect of type of nutrient medium and cytokines at different concentrations On the start of the appearance of embryonic callus in date palms.

Table 1. Composition of nutrient media based on (mg l⁻¹).

Substance name	MS	WPM	B5
A- Macronutrients			
NH ₄ No ₃ Ammonium nitrate	1650	400	-
Calcium nitrate CaNo ³	-	556	-
Potassium nitrate KNo ₃	1900	-	2500
Ammonium sulfate So ₄ No ₃	-	-	134
KSo ₄ Potassium sulfate		990	
Magnesium sulfate MgSo ₄ .H ₂ O	370	180	122
Calcium chloride CaCl ₂	440	72,47	113,24
Potassium phosphate KHPo ₄	170	170	-
Sodium phosphate NaHPo ₄	-	-	150
Micronutrients B-			
Boric acid H ₃ BO ₃	6,20	6,20	3
Cobalt chloride CoCl ₂ .6H ₂ O	0,025	0,160	0,025
Manganese sulfate MnSo ₄	22,3	22,30	10
Copper sulfate CuSo ₄ .5H ₂ O	0,025	0,160	0,025
Sodium molybdate NaMoO ₄ .2H ₂ O	0,25	0,25	0,25
Potassium iodide KI	0.83	-	0.75
Zinc sulphate ZnSo ₄ .7H ₂ O	8,60	8,60	2
Aqueous ferrous sulfate FeSo ₄ .7H ₂ O	27,84	27,85	27,80
chelating substance Na ₂ EDTA	36,7	37,30	37,26
C- Vitamins and amino acids			
Thiamine HCl	0.5	1	10
Nicotinic acid	0.5	0.5	1
Pyridoxine	0.5	0.5	1
Claesin	2	2	-
My inositol	100	100	100

Table 2. Concentrations of substances added to the nutrient medium.

Substance	Quantitative mg l ⁻¹
Sucrose	30000
Sodium hydrogen orthophosphates	170
Adenine sulphates	40
Amino acid glutamine	100
Activated charcoal	1000
Agar	6000

2.3. Statistical Analysis

All experiments carried out a completely randomized design (CRD) with factorial experiments, and the significance of the means was tested according to the least significant difference (L.S.D) test with a probability level of 0.05. The statistical analysis program GenstatV.3 was used to analyze the results, according to what was stated [17].

3. Results and Discussion

3.1. Effect of the Type of Medium, Type of Cytokinin, and its Concentration on the Rate of the Period Required for the Appearance of Embryonic Callus (day)

Table (3) results indicate the effect of the type of nutrient medium and cytokinin, its concentration, and the interaction between them in the period required for the appearance of embryonic callus (day). The results showed that the WPM medium recorded the shortest period for the onset of fetal callus, followed by the MS median, amounting to 39.22 and 40.32 days, respectively, with a significant difference from the B5 mean, which recorded the longest period, reaching 54.67 days, while the effect of the growth model type was not recorded, giving any statistically significant differences, as the results showed that the effect of cytokinin concentration in the period required for the appearance of sexual callus, as it was noted that the concentration of 0.5 mg liter⁻¹ recorded the shortest period and amounted to 42.20 days in a row compared to the rest of the concentrations. The results of the interference between the type of medium and cytokinin showed that the WPM medium, equipped with cytokinin Zeatin 2ip, had recorded the shortest period required to start fetal callus, amounting to 38.85 and 39.6 days, respectively, followed by MS nutrient medium with cytokinin 2ip and olives, with a rate of 39.8 and 40.85 days, respectively, while the longest period for the appearance of embryonic callus was 56 days recorded for developing callus on the B5 nutrient medium with cytokinin 2ip. As for the effect of interaction between the type of medium and the concentration of cytokinin, the results showed that the WPM medium with a concentration of 0 and 0.5 mg liter⁻¹ with the lowest period of embryonic callus appearance was 36 and 37.1 days respectively compared to other interactions of the same medium and the medium B5 and MS except for the MS medium equipped with a concentration of 0.5 mg.L⁻¹ gave the shortest period of embryonic callus appearance, reaching 36.3 days. As for the effect of overlapping the type of growth regulator and its concentration, the results showed that there are no significant differences, as for the effect of triple interference for the type of medium, the type of cytokinin, and concentration, it was found through the results that the shortest period of appearance of embryonic callus was recorded at the medium WPM and MS with cytokinin 2ip and olives 0 and 0.5 mg liter⁻¹ and amounted to 36, 38.2 days, 40.2 and 36.4 days respectively, with a significant difference from the rest of the interactions for the same two mediums and the medium of B5. The plant parts grown outside the living body are lopsided in their nutrition depending on the organic compounds provided by the nutrient medium that lead to their growth and development [18]. In addition, low concentrations were more responsive in the induction of embryonic callus This may be because high concentrations lead to a decrease in the rate of cell division and development[19]. As the medium WPM is considered a suitable nutrient medium for woody plants and its content of major nutrients is ammonium nitrate, calcium nitrate and potassium sulfate in appropriate quantities compared to the medium MS and B5, which lead to the growth and development of plant tissue, especially that the nitrogen element, which enters the construction of amino and nucleic acids and proteins, which encourages the growth and development of the plant part cultivated [20] as well as it was found that high concentrations of growth regulators lead to the death of callus tissue as a result of the release of ethylene in large quantities, which leads to rapid cell divisions and the occurrence of a state of imbalance of growth and lack of development and thus the death of the formed callus tissue. It was also noted that the addition of growth regulators in high concentrations to the nutrient medium higher than the ideal concentration leads to an impact on the work of enzymes responsible for the construction of the cell wall and their decomposition, which affects the mechanical properties. Of cell wall and influence on cell division and embryonic callus formation [21] and these results were consistent with what he found [22,23] .

Table 3. Effect of the type of nutrient medium and cytokinin, its concentration, and the interaction between them in the period required for the appearance of embryonic callus (day).

Type Medium A	Type Cytokinin B	C concentration Cytokinin mg L ⁻¹			Average A	Average B	A*B
		0	1	2			
MS	2ip	40.2	37.4	45.2	40.32	45.13	39.8
	Z	40.2	43.2	43.8		44.35	40.85
	MS*C	40.2	40.3	44.5			
WPM	2ip	36	43.2	43.2	39.22		39.6
	Z	36	40.2	41			38.85
	WPM*C	36	41.7	42.1			
B5	2ip	58.2	56.8	55	54.67		56
	Z	58.2	50.2	52.6			53.35
	B5*C	58.2	53.5	53.8			
B*C	2ip	44.8	45.8	47.8			
	Z	44.8	44.53	45.8			
	Average C	44.8	45.17	46.8			
L.S.D.= 0.05	A=1.254	A*B=1.773					
	B=NS	A*C=2.508					
	C=1.448	B*C=NS					
	A*B*C=3.546						

3.2. Effect of the Type of Medium, Type of Cytokinin, and its Concentration on the Rate of the Fresh Weight of the Embryonic Callus (g)

The results of Table (4) indicate the effect of the type of nutrient medium and cytokinin, its concentration and the interaction between them on the average soft weight of embryonic callus (g). The results showed that the nutrient medium MS and WPM have given the highest rate of soft weight for fetal callus, as it reached 1.58 g and 1.55 g, respectively, with a significant difference from the medium B5, which recorded the lowest average soft weight of 1.256 g, as for the effect of the type of growth regulator, no significant differences were recorded, while the concentration of cytokinin recorded a significant effect, as the concentration exceeded 0.5 mg liter⁻¹ for all concentrations at a rate of 1.73 g, The results of the interaction between the type of medium and cytokinin showed that there are no significant differences in the average soft weight of embryonic callus that the medium MS provider 2IP, while the effect of the interaction between the type of nutrient medium and the concentration of cytokinin. The results showed that the nutrient medium MS and WPM with a concentration of 0.5 mg liter⁻¹ gave the highest rate of soft weight for fetal callus, as it amounted to 1.96 g and 1.95 g, respectively, with a significant difference from the rest of the interactions for the same two mediums and the medium of B5. The results of the effect of the interaction between the type of growth regulator and its concentration showed the superiority of the growth regulators Zayatin and 2ip at a concentration of 0.5 each by giving the highest rate of the soft weight of 1.723 and 1.796 g respectively and with a significant difference from the rest of the interactions The results of the triple intervention showed that the highest rate of the soft weight of embryonic callus has been observed in the MS nutrient medium with a concentration of 0.5 mg liter-1 of 2ip as it reached 2.23 g, followed by the WPM medium equipped with Zeatin at a concentration of 0.5 Amalgam L⁻¹ gave an average soft weight of 2.03 g with a significant difference from the rest of the interventions, while the lowest soft weight was recorded at the medium B5 equipped with both types of cytokinin for all concentrations. Plant parts grown outside the living body are discarded in their nutrition depending on the organic compounds provided by the nutrient medium that lead to their growth and development [24] The

reason for the superiority of the two mediums MS and WPM may be due to the difference in the composition of the components of the medium and their high content of macro and micronutrients compared to the medium B5, especially the element nitrogen, which is involved in the construction of amino and nucleic acids and proteins, which encourages the growth and development of the cultivated plant part [20] as well as the presence of growth regulators in low concentrations helps the embryonic callus to divide and grow, as auxin stimulates the formation of RNA as it leads to flexible energy that They are exploited by tissues for division and growth [24] and food media with a high content of growth regulators cause cell suppression, resulting in poor growth[25] and these results were in line with what he found [26].

Table 4. Effect of the type of medium, type of cytokinin, and its concentration on the rate of the fresh weight of the embryonic callus (g).

Type Medium	Type Cytokinin B	Cytokinin, Concentration mg L ⁻¹ C			Average A	Average B	A*B
		0	1	2			
MS	2ip	1.598	1.65	0.936	1.584	1.421	1.603
	Z	1.598	1.394	1.576		1.507	1.565
	MS*C	1.598	1.522	1.256			
WPM	2ip	1.488	1.586	0.936	1.552		1.471
	Z	1.488	1.554	1.458			1.633
	WPM*C	1.488	1.57	1.197			
B5	2ip	0.995	1.168	1.304	1.256		1.188
	Z	0.995	1.56	1.294			1.324
	B5*C	0.995	1.364	1.299			
B*C	2ip	1.36	1.468	1.059			
	Z	1.36	1.503	1.443			
	Average C	1.36	1.485	1.251			
L.S.D.= 0.05	A=0.089	A*B=0.127					
	B=NS	A*C=0.179					
	C=0.103	B*C=NS					
	A*B*C=0.253						

L.S.D.= 0.05

3.3. Effect of the Type of Medium, Type of Cytokinin and its Concentration on the Rate of the Dry Weight of the Embryonic Callus (g)

The results shown in Table (5) indicate that the results of Table (4) indicate the effect of the type of nutrient medium and cytokinin, its concentration, and the interaction between them on the average dry weight of embryonic callus (g). The results showed that the food medium MS and WPM have given the highest rate of dry weight for fetal callus, as it amounted to 0.087 g and 0.067 g, respectively, with a significant difference from the medium B5, which recorded the lowest dry weight rate of 0.034 g, as for the effect of the type of growth regulator, no significant differences were recorded, while the concentration of cytokinin recorded a significant impact on the rate of dry weight, as the concentration exceeded 0.5 mg liter⁻¹ for all concentrations at a rate of 0.10 g. The results of the interaction between the type of medium and cytokinin showed that the medium MS with growth regulator 2ip gave the highest rate of dry weight of 0.119 g compared to the same medium with olive and medium WPM and B5 equipped with both growth regulators, while the results of the effect of the interaction between the type of nutrient medium and the concentration of cytokinin The results showed that the MS nutrient medium with a concentration of 0.5 mg liter⁻¹ gave the highest rate of dry weight for fetal callus, as it amounted to 0.167 g And a significant difference from the rest of the interferences for the same medium, WPM medium, and B5 medium. The results of the effect of the interaction between the type of

growth regulator and its concentration showed the superiority of the growth regulator 2ip at a concentration of 0.5 mg liter⁻¹ by giving the highest rate of dry weight of 0.140 g and a significant difference from the remaining interactions for both growth regulators. The results of the triple intervention showed that the highest rate of dry weight of embryonic callus has been observed in the MS nutrient medium with a concentration of 0.5 mg liter⁻¹ of 2ip as it reached 0.289 g and a significant difference from the rest of the interventions. Plant parts grown outside the living body are discarded in their nutrition dependent on the organic compounds provided by the nutrient medium that lead to their growth and development [18]. Accordingly, the increase in the soft and dry weight of callus is a reflection of changes in the different contents of its cells depending on its growth in the quality of the food medium used and on the quality and concentration of growth regulators added, and generally accompanied by the process of cell division of callus an increase in the contents of the task to sustain division and growth such as proteins and amino acids with internal changes leading to division and growth and then specialization [29]. The reason for the superiority of the MS medium may be attributed to its high content of macro and micronutrients, especially nitrogen, which is involved in the construction of amino, and nucleic acids and proteins within the plant tissue, which encourages the growth and development of the cultivated plant part [18]. In addition the presence of growth regulators in low concentrations helps the embryonic callus to divide and grow, as auxin stimulates the formation of RNA as it leads to mRNA by providing energy that is exploited by tissues for division and growth[23] and the food media that contain a high content of growth regulators cause inhibition of cells, resulting in poor growth [26] and these results were in line with what he found [27].

Table 5. Effect of the type of medium, type of cytokinin and its concentration on the rate of the dry weight of the embryonic callus (g).

Type of Medium (A)	Type of cytokinin (B)	Concentration Cytokinin mg L ⁻¹ (C)			Averag e A	Averag e	A*B
		0	1	2			
MS	2ip	0.06	0.062	0.056	0.087	0.072	0.119
	Z	0.06	0.06	0.048		0.054	0.056
	MS*C	0.06	0.061	0.052			
WPM	2ip	0.06	0.06	0.056	0.067		0.066
	Z	0.060	0.066	0.064			0.069
	WPM*C	0.06	0.063	0.06			
B5	2ip	0.025	0.031	0.035	0.034		0.032
	Z	0.025	0.042	0.038			0.037
	B5*C	0.025	0.036	0.037			
B*C	2ip	0.048	0.051	0.049			
	Z	0.048	0.056	0.050			
Average C		0.048	0.054	0.049			
L.S.D.= 0.05	A=0.023	A*B=0.033					
	B=NS	A*C=0.046					
	C=0.027	B*C=0.038					
	A*B*C=0.066						

3.4. Effect of the Type of Medium, Type of Cytokinin, and its Concentration on the Period Required for the Appearance of Embryos (day)

The results of Table (6) indicate the effect of the type of nutrient medium and cytokinin, its concentration, and the interaction between them in the period required for required of embryos (day) The results showed that the medium WPM and MS recorded the shortest period for required of vegetative embryos amounted to 30.4 and 30.18 days respectively, with a significant difference from the food medium B5, which recorded the longest period of 41.75 days. As for the effect of the type of growth regulator, there were no statistically significant differences, while the effect of concentration of the growth regulator gave a significant effect, as the results showed that the concentrations of 0 and 0.5 mg liter⁻¹ had a significant superiority by giving the least period of emergence of vegetative embryos compared to other concentrations, as it reached 33.93 and 33.63 days respectively. As for the effect of the interaction between the type of medium and the growth regulator, no significant differences were recorded, while the results of the interaction between the type of food medium and the concentration of the growth regulator showed significant differences, as it was noted that the MS medium with a concentration of 0 and 0.5 mg liter⁻¹ had recorded the shortest period of embryo appearance, amounting to 28.8 and 30.4 days, respectively, followed by the WPM food medium with a concentration of 0.5 and 0 mg liter⁻¹ at a rate of 29 30.2 days respectively and a significant difference from the rest of the interventions for the same Middle and middle B5. As for the effect of bilateral interference between the type of growth regulator and its concentration, the results showed no significant differences, and the results of the triple overlap showed no statistically significant differences in the average period of emergence of vegetative embryos. The plant parts grown outside the living body are thrown in their nutrition depending on the organic compounds provided by the nutrient medium that lead to their growth and development, as well as the development of vegetative embryos occurs as a result of the reduction of cytokinin concentration due to the long period of implantation or as a result of its consumption and spread in the cells of the growing tissue or its absorption by activated charcoal [29,30]. After the exhaustion of the growth regulator in the nutrient medium, the development of spherical embryos occurs by stopping them about division as its pole begins to meristem division and growth accompanied by rupture of the solid shell of the embryo spherical and thus occur elongation of cotyledon and required of the cylindrical shape of the embryo [5] The reason for the superiority of the two mediums MS and WPM may be due to the difference in the composition of their components of macro and minor nutrients, for example, the presence of potassium nitrate and calcium nitrate compared to the medium B5 or their high content of elements, especially the element nitrogen, which is involved in the construction of amino and nucleic acids and proteins, which encourages the growth and development of the cultivated plant part [18] as well as these results were in line with what he found [27].

Table 6. Effect type of nutrient medium, cytokinin, its concentration and their interactions on the period required for the appearance of embryonic callus (day).

(A) type of medium	(B) Type of cytokinin	Cytokinin of concentration mg L ⁻¹			Average A	Average B	A*B
		(C)					
		0	1	2			
MS	2ip	28.8	32	34.4	31.18	43.63	31.45
	Z	28.8	33.4	31.2		34.25	30.9
	MS*C	28.8	32.7	32.8			
WPM	2ip	30.2	32.2	31.2	30.4		30.65
	Z	30.2	30.4	31			30.15
	WPM*C	30.2	31.3	32.8			
B5	2ip	42.8	41.6	41.2	41.75		41.8
	Z	42.8	42	40.6			41.7

	B5°C		42.8	41.8	40.9
B*C	2ip		33.93	35.27	35.6
	Z		33.93	35.72	34.27
	Average (C)		33.93	35.27	34.75
		A=0.657		A*B=NS	
		B=NS		A*C=NS	
L.S.D.= 0.05		C=0.759		B*C=1.073	
			A*B*C=NS		

Conclusion

The results demonstrated that the shortest embryo callus appearance period was recorded by the WPM and MS mediums supplemented with cytokinin 2ip and Zeatin at concentrations of 0 and 0.5 mg liter⁻¹, respectively, with durations of 36 and 38.2 days. The average fresh weight was 2.23 g for the MS medium and 2.03 g for the WPM medium, while the highest rate of dry weight was observed in the MS medium supplemented with 2ip at a concentration of 0.5 mg L⁻¹, distinguishing it significantly from the other interventions.

References

- [1] Vardareli, N., Doğaroğlu, T., Doğaç, E., Taşkın, V. and Göçmen Taşkın, B. (2019). Genetic characterization of tertiary relict endemic *Phoenix theophrasti* populations in Turkey and phylogenetic relations of the species with other palm species revealed by SSR markers. *Plant Syst. Evol.*, 305: 415–429.
- [2] Trigiano, R.N., and D.J.Gray. (2000). *Plant Tissue Culture Concepts and Laboratory Exercises*. CRC Press LLC, USA.p.44-60.
- [3] Al-Mayahi, Ahmed Wahid Madi (2010). Effect of NAA, 2ip, Inorganic Sulfate, PEG and adenine sulfate on the maturation of vegetative embryos of date palm Alkantar variety abundant outside the vivo. *Basra Journal of Date Palm Research*.9 (1)2010.
- [4] Al-Qatrani, M. K. J., Al Khalifa, A. A. S., & Obaid, N. A. (2021). Effect of Jasmonic acid on stimulating the growth and development of date palm callus (*Phoenix dactylifera* L.) cultivar Shukar in vitro under salt stress conditions. *IOP Conference Series. Earth and Environmental Science*, 923(1), 012017. <https://doi.org/10.1088/1755-1315/923/1/012017>
- [5] Al-Khayri, J. M. And Naik, P. M. (2017).Date Palm Micropropagation: Advances and Applications', *Ciência E Agrotecnologia*, 41(4), Pp. 347–358.
- [6] Khan, A. (2009) 'In vitro micropropagation of "Khalas" date palm (*phoenix dactylifera* l .), an important', in vitro, 17(1), pp. 15–27.
- [7] Al-Mir, O. N. J. (2020). The effect of cytokinin and auxin on the growth and rooting of vegetative branches of the two varieties of date palm, Halawi and Ashqar, ex vivo, *Basra Journal of Date Palm Research*, 19 (1) 2020.
- [8] Eke CR, Akomeah P, Asemota. O (2005). Somatic embryogenesis in date palm (*Phoenix dactylifera* L.) from apical meristem tissues from 'zebra and 'loko' landraces. *Afr J Biotechnol*. 4: 244-246.
- [9] El-Dawayati,M.,Baki,M.A.A And Abdelgalil,L.M.(2018).Effect Of Different Conservation Periods with Different Surface Concentrations On Conserving Somatic Embryo Clusters Of Date Palm (*Phoenix Dactylifera* L.)Under Minimal Growth Conditions.*Applied Science Reports* 21(1) 14-21.
- [10] Ibrahim, M. A., Waheed, A. M. and Al-Taha, H. (2013). Plantlet regeneration from root segments of Date palm tree (*Phoenix dactylifera* L. cv. Barhee) produced by in vitro culture. *A. A. B. Bioflux.*, 5(1): 45–50
- [11] Al-Asadi, A. Z., Abdul Wahid, A. H., & Al-Mayahi, A. M. (2019). The Effect of Thidiazuron on Callus and in vitro Shoots Development of Date Palm (*Phoenix dactylifera* L.) cv. Barhee. *Basrah Journal of Agricultural Sciences*, 32, 258–265. <https://doi.org/10.37077/25200860.2019.170>

- [12] Bhatia, S. And Bera, T. (2015). Somatic Embryogenesis and Organogenesis, Modern Applications of Plant Biotechnology in Pharmaceutical Sciences. Elsevier Inc. Doi: 10.1016/B978-0-12-802221-4.00006-6.
- [13] Al-Rawi, K. M., and Khalaf Allah, M. A. (2000) Design and analysis of agricultural experiments. Ministry of Higher Education and Scientific Research, Dar Al-Kutub Foundation for Printing and Publishing, University of Mosul. 488 p.
- [14] Corchete, M.P., J.M.Sanchez.C.Cacho, M.Moran, and J.F.Tarrag (1990). Ardionlide content in cultures derived from root and leaf callus of digitalis therapists L.J.Plant Physiol.137.196-200Lloyd, G.; and McCown, B. (1981). Commercially feasible micropropagation of mountain laurel, *Lamia Latifolia*, by use of shoot tip culture. Proc. Inter Plant Propagation Soc, 30: 421-427.
- [15] Al-Rawi, K. M., and Khalaf Allah, M. A. (2000) Design and analysis of agricultural experiments. Ministry of Higher Education and Scientific Research, Dar Al-Kutub Foundation for Printing and Publishing, University of Mosul. 488 p.
- [16] Krueger, R. R. (2021). Date Palm (*Phoenix dactylifera* L.) Biology and Utilization. In *The Date Palm Genome*, Springer: New York.
- [17] Trigiano, R.N. and D .j. Gray.(2005). Plant Development and Biotechnology.CRC PRESS LLC.
- [18] Hopkins, W.G., and N.P.A.Huner. (2004). Introduction to Plant Physiology. The University of Western Ontario .PP.67-70.
- [19] Taiz, L and E, Zeiger. (2006). Plant Physiology. Sinauer Associates, Inc Publishers. Sunderland. USA .p. 290-300.
- [20] Sharif, Hussein Jassim and Al-Mayahi, Ahmed Madi Waheed and Mohsen, L. Q. (2016). Micropropagation of the agricultural date palm *Pholifera* L. Persian cultivar ex vivo. Dhi Qar University Journal of Agricultural Research 5(1):472-484.
- [21] Mohsen, K. A. (2004) Studies on improving the formation of somatic embryos and their germination of date palm *Phoenix dactylifera* L. Barhi cultivar ex vivo, Master's thesis - College of Agriculture - University of Basra - Iraq 78. s.
- [22] Krueger, R.J., and D.P.Carew. (1978). *Catharanthus roseus* tissue culture: The effects of precursors on growth and alkaloids production.Liodia 41:327-331.
- [23] Mohsen, K. A., Abbas, M., Batoul, H., Faleh, A. (2014) The effect of yellow corn extract and naphthalene acetic acid on the development of embryonic callus and the formation of vegetative embryos and their germination of date palm Barhi cultivar ex vivo, Basra Research Journal (Al-Alamiyat), Issue 40, Part B.4: 15-23.
- [24] Mohsen, K. A. (2007). Date palm *Phoenix dactylifera* L. Sharifi variety of various extravivo apical parts. Basra Journal of Date Palm Research.6(1):64-80.
- [25] Hamad, Muhammad Shehab and Jassim, Noura Jabr (2011). The effect of components of the nutrient medium and plant part on callus induction of belladonna plants ex vivo. Iraqi Agricultural Sciences Journal.42(3):59-70.
- [26] Wiesman, Z.; Riov, J. and Epstein, E. (1989). Characterization and rooting ability of indol-3-butyric acid conjugates formed during rooting of mung bean cuttings.Plant Physiol. 91: 1080-1084.
- [27] Saad, A. & Elshahed,A.(2012).plant tissue culture media.In: Leva, A. and Rinaldi, L.M.R.,(eds)recent advanced in plant in Vitro Culture, chap. 2, Intech, Winchester, pp 29-40.
- [28] Khalil, A. I. (2002) Using some alternatives to plant growth regulators in propagating the date palm *Phoenix dactylifera* L. ex vivo. Master's thesis, Department of Horticulture and Palm Trees, College of Agriculture, University of Basra, Iraq. Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture Physiol. Plaut.15:473-497.
- [29] Lloyd, G.; and McCown, B. (1981). Commercially feasible micropropagation of mountain laurel, *Lamia Latifolia*, by use of shoot tip culture. Proc. Inter Plant Propagation Soc, 30: 421-427.
- [30] Sen, M. K., Nasrin, S., Rahman, S. and Jamal, A. H. (2014). In vitro callus induction and plantlet regeneration of *Achyranthes aspera* L., a high-value medicinal plant. Asian Pacific Journal of Tropical Biomedicine, 4(1): 40-46

Effect of Using Different Levels of Active Biochar on Increasing Soil Fertility and Radish Crop Growth

Nisreen S. Al-Shewaili¹, Lina A. Hameed², Shirin S. Al-Shewaili³ and Ahmed A. Al-Salhi⁴

¹Department of Horticulture and Landscape Architecture, Continuing Education Center, Al-Qasim Green University, Babylon, Iraq.

^{2,3}Department of Horticulture and Landscape Architecture, College of Agriculture and Marshes, University of Thi-Qar, Thi-Qar, Iraq.

⁴ Department of Animal Production, College of Agriculture and Marshes, University of Thi-Qar, Thi-Qar, Iraq.

¹E-mail: Nisreen@uoqaasim.edu.iq

²E-mail: Linaalihameed93@gmail.com

⁴E-mail: ahmed.a.k.alsalhi@gmail.com

Abstract. This study was conducted in the Department of Horticulture and Landscape Engineering, College of Agriculture and Marshlands, University of Thi Qar, during the period from 1/10/2024 to 4/11/2024; for study the effect of adding different levels of activated biochar extracted from home water purification stations, on soil fertility and the vegetative group of radish plants, as the pH rate and the activity of *Azotobacter* and *Bacillus* bacteria in the soil were studied, in addition to studying the number of leaves, plant height and leaf area, after adding biochar to the soil at four different levels (5, 10, 15, and 20) % per kg of soil for treatments T₂, T₃, T₄, T₅, respectively, compared to the control treatment T₁, which was free of the addition. The results indicated a significant improvement ($P \leq 0.05$) in some soil properties represented in pH and in the content of *Azotobacter* and *Bacillus* bacteria in the soil, in favor of the treatments that included the addition of activated biochar at levels (5 and 10)% to the soil, compared to the control treatment. The results also indicated a significant superiority ($P \leq 0.05$) in the characteristics of the vegetative group of the studied radish plant, in favor of the third treatment, T₃, compared to some other treatments, T₁, T₄, T₅, while there were no significant differences between treatments T₂ and T₃ on the one hand, and treatments T₂ and T₁, T₄, T₅, on the other hand.

Keywords. Radish plant, Activated charcoal, Bio fertilizer, *Azotobacter*, *Bacillus*.

1. Introduction

Some agricultural soils in Iraq suffer from problems related to low fertility levels, which negatively affects crop productivity and quality, as low fertility is often attributed to the deterioration of organic content and low levels of essential nutrients as a result of repeated agricultural depletion and harsh environmental conditions [1]. In light of these challenges, the search for effective and sustainable ways to improve soil fertility has become a major priority for researchers and farmers. The use of activated biochar in agriculture may represent a promising

solution to increase soil fertility, which is known for its unique properties such as containing fine pores that help improve aeration and soil water retention, and it also contributes to enhancing microbial activity within the soil, which increases the ability of plants to absorb nutrients [2]. Several studies have indicated that adding biochar can contribute effectively to improving soil properties and increasing the productivity of agricultural crops, including radish, which is one of the fast-growing vegetables and is characterized by its susceptibility to changes in soil fertility [3]. A previous study conducted by [4] showed that adding biochar to soil planted with radish plants, and the results confirmed that adding biochar enhanced leaf growth and increased leaf size due to improved soil properties and increased availability of nutrients, which contributed to enhancing plant growth in general. The results of another study conducted by [5] showed that adding biochar led to a significant increase in the length and width of radish leaves, and the study confirmed that biochar contributed to improving the response of radish to nitrogen, which led to enhancing the overall growth of the plant.

A study conducted by [6] concluded that adding biochar to soil effectively contributes to adjusting the pH and increasing soil fertility, especially in highly acidic soils. This effect is due to the basic properties of biochar and its content of alkaline carbonates and oxides that neutralize acids in the soil, which contributes to improving crop productivity in general. Other studies have been conducted to investigate the effect of biochar on soil fertility and its ability to improve crop growth. A study by [7] showed that adding biochar to soil increased soil carbon content and improved soil moisture retention capacity. A new study by [8] confirmed that soil supplemented with biochar showed improvements in microbial activity and nutrient distribution, leading to an increase in radish crop yield by up to 20% compared to untreated soil. Finally, a study by [9] showed that biochar enhances soil water retention capacity and improves growth in radish plants. Charcoal was found to have positive effects on soil fertility. In continuation of previous studies and the importance of biochar as a means of improving soil quality and enhancing crop growth, especially radish, by improving soil properties and supporting the availability of essential nutrients for plants, which requires a deep understanding and recent results; to confirm and support previous results, we decided to present this study; to demonstrate the effect of using the best level of biochar extracted from water purification plants; to ensure obtaining the highest level of soil fertility at the lowest costs, and in preparation for its use instead of some manufactured chemical fertilizers that have a negative effect on plants and human consumers.

2. Materials and Methods

A set of different scientific and practical procedures were carried out, allocated for this study, starting from soil preparation and ending with statistical analysis, according to the following paragraphs:

- Soil preparation: The mixed soil was brought from agricultural nurseries in the city of Nasiriyah.
- Biochar preparation: Water purification filters (for home water purification stations) were purchased, which contain activated biochar in the form of a fine powder, as it was extracted and collected in a special container, and Figure 1 shows the biochar material used in the study.
- Mixing biochar with soil: The activated biochar was mixed with soil at four graded levels (5, 10, 15, and 20) % per kg of soil, for treatments T₂, T₃, T₄, T₅ respectively, in addition to the control treatment T₁, which was free of addition .
- Distribution of treatments and replicates: The replicates were distributed randomly, after being packed in small anvils; to ensure that all experimental units were exposed to the

- same field conditions for growing the crop and according to the recommendations of the company supplying the radish seeds, It was distributed as 5 treatments, each treatment had 5 replicates, and each replicate had 5 experimental units.
- Seed distribution: Seeds were distributed equally on all anvils, 5 seeds per anvil. To monitor the rate of plant height, leaf areas and numbers.
 - Watering the plants: The radish plants were watered by regular surface spraying, on a daily basis, taking care not to dry out the soil, according to [10] indicated in the book Principles of Field Crop Production in the second edition.
 - Vegetative measurements: Plant height, leaf area and number were measured according to [10], and Figure 2 shows the details of the vegetative radish plant in the second week of the experiment.
 - Chemical tests: pH of the soil was measured using a pH meter, according to [11].
 - Microbial tests: Microscopic tests for microbial counting were prepared using the pouring plate method according to what was indicated by [12]; to estimate the number of (*Azotobacter*) bacteria to infer soil fertility; due to its ability to fix nitrogen in aerated soil, as well as (*Bacillus subtilis*) bacteria in the soil, for its effective role in improving soil health and combating some pathogens [13,14].
 - Statistical analysis: The data were analyzed statistically using a completely randomized design (CRD), and the significant differences between the means were compared using the [15] multiple-nominal test at a significance level of 0.05, and the [16] program was used in the statistical analysis.



Figure 1. Biochar material used in the experiment.

3. Results and Discussion

3.1. Effect of Using Different Levels of Biochar on Bacterial Activity and Soil pH

It is clear from Table 1. Which represents the effect of using different levels of biochar on the activity of *Azotobacter* and *Bacillus* bacteria and soil pH, as it is clear that there was a significant improvement ($P \leq 0.05$) in the concentration of hydrogen ions (pH) in favor of the treatments that included adding activated biochar at levels (5 and 10)% to the soil compared to the control treatment, while the treatment T₅, which included adding (20)% of activated biochar to the soil, recorded a poor level in the pH rate, compared to the other experimental treatments. The treatment T₃ recorded a significant superiority ($P \leq 0.05$) in the content of *Azotobacter* and

Bacillus bacteria in the soil, compared to the control treatment, while there were no significant differences between all other biochar treatments when estimating the number of *Bacillus* bacteria, and it also recorded a significant superiority ($P \leq 0.05$) over the treatment T_5 when estimating the number of *Azotobacter* bacteria.

Table 1. Effect of using different levels of biochar on bacterial activity and soil pH (Mean $\pm$ standard error).

Treatment	pH	<i>Azotobacter</i> (cfu/ g)/Log	<i>Bacillus subtilis</i> (cfu/ g)/Log
T_1	6.52 $\pm$ 0.20 ^c	3.96 $\pm$ 0.33 ^b	4.89 $\pm$ 0.95 ^b
T_2	7.50 $\pm$ 0.23 ^b	4.67 $\pm$ 0.27 ^{ab}	5.92 $\pm$ 0.56 ^{ab}
T_3	7.65 $\pm$ 0.27 ^b	7.08 $\pm$ 0.35 ^a	8.33 $\pm$ 0.50 ^a
T_4	8.18 $\pm$ 0.21 ^b	4.93 $\pm$ 0.50 ^{ab}	7.28 $\pm$ 1.15 ^{ab}
T_5	9.12 $\pm$ 0.17 ^a	4.50 $\pm$ 1.55 ^b	6.35 $\pm$ 1.26 ^{ab}
Sec.	*	*	*

T_1 : Control treatment (without addition).

T_2, T_3, T_4, T_5 : adding (5, 10, 15, and 20) % of biochar per kg of soil, for each Treatments respectively.

*Different letters indicate significant differences at the 0.05 probability level.

The improvement of the properties of the studied soil when adding activated biochar at a level of 10% occurred due to a number of combined factors, most notably adjusting the pH level; because it contains an alkaline composition that can balance acidic soil, which creates a suitable environment for plants and beneficial bacteria, in addition to its effective role in improving the soil structure by providing additional pores for the soil, which increases its ability to retain water and air, and enhances the growth of beneficial microorganisms that prefer well-ventilated environments. It also contributes to increasing organic carbon: because it is rich in carbon, it acts as a long-term food source for beneficial bacteria such as *Azotobacter* and *Bacillus*, which helps in increasing their biological activity and spread in the soil, and also works to store nutrients, which reduces their loss through filtration and ensures their availability to living organisms and plants [17]. These results were consistent with [6] observed that adding biochar to soil effectively contributes to adjusting the pH and increasing soil fertility, and were also consistent with what [8] found in soil supplied with biochar, which witnessed improvements in microbial activity and nutrient distribution, leading to an increase in radish crop productivity by up to 20% compared to untreated soil.

3.2. The Effect of Using Different Levels of Biochar on Some Characteristics of the Vegetative Group of Radish Plants

It is noted through Table 2. which indicates the effect of using different levels of biochar on some characteristics of the vegetative group of radish plants, represented in the number of leaves, plant height and leaf area, as it is noted that there is a significant superiority ($P \leq 0.05$) in the characteristics of the vegetative group of the radish plant studied, in favor of the treatment T_3 , compared to some other treatments T_1, T_4, T_5 , while there are no significant differences between treatments T_2 and T_3 on the one hand, and treatments T_2 and T_1, T_4, T_5 , on the other hand.

Table 2. The effect of using different levels of biochar on some characteristics of the vegetative group of radish plants (Mean $\pm$ standard error).

Treatment	Number of Leaves (Leaf)	Plant height (cm)	Plant leaf area (cm ²)
T_1	9.00 $\pm$ 1.22 ^b	21.25 $\pm$ 3.11 ^b	60.12 $\pm$ 5.59 ^b
T_2	10.50 $\pm$ 2.02 ^{ab}	24.00 $\pm$ 1.82 ^{ab}	68.25 $\pm$ 1.65 ^{ab}
T_3	14.25 $\pm$ 0.85 ^a	27.50 $\pm$ 1.04 ^a	81.25 $\pm$ 3.14 ^a

T ₄	8.75±1.25 ^b	20.00±4.08 ^b	63.25±4.75 ^b
T ₅	7.50±0.28 ^b	19.75±2.65 ^b	60.00±9.02 ^b
Sec.	*	*	*

T₁: Control treatment (without addition).

T₂, T₃, T₄, T₅: adding (5, 10, 15, and 20) % of biochar per kg of soil, for each Treatments respectively.

*Different letters indicate significant differences at the 0.05 probability level.

N.S indicates that there are no significant differences between the averages of the coefficients.

The addition of 10% activated biochar to the soil contributed to improving its qualitative properties such as improving the soil structure and increasing its ability to retain water, which helped the radish plant to obtain the necessary moisture better, and thus positively reflected on the growth of leaves and the height of the plant, in addition to the effective role it provides in fixing nutrients in the soil, and making them more sustainably available to the roots of the radish plant, which contributed to improving its vegetative growth and increasing the area and number of leaves, in addition to increasing microbial activity that works to improve soil fertility, which creates an ideal environment for radish roots to obtain nutrients efficiently, and thus increases the strength of its growth, and finally, biochar contributed to improving cation exchange, which provides additional strength in absorbing essential nutrients such as potassium, calcium, and magnesium, which contribute directly to increasing the size of the plant and the number of its leaves [18]. The results of this study agreed with the findings of [4], which indicated that adding biochar to the soil planted with radish plants enhances the growth of leaves and increases their size due to improved Soil properties and increased availability of nutrients, which contributed to enhancing plant growth in general. With [5] concluded, that adding biochar led to a significant increase in the length and width of radish leaves significantly. With [9] concluded that biochar enhances the soil's water retention capacity and improves the growth of the vegetative group of radish plants.

Conclusion

We conclude from this study that the use of activated biochar, present in water treatment plants, at a rate of 10% leads to a significant improvement ($P \leq 0.05$) in some soil properties, represented in the pH rate, and the activation of the bacterial content of the numbers of *Azotobacter* and *Bacillus* bacteria in the soil; this will be reflected, in the end, positively in improving the characteristics of the vegetative group of the radish plant, and it is not recommended to increase this percentage to 15% or 20% in the soil; as it gave negative results, compared to adding a percentage of 10%, not to mention the economic cost resulting from the unjustified increase in the added material and its subsequent impact on the economic feasibility of the crop.

References

- [1] Smith, J., Liu, Y., & Perez, R. (2023). Soil fertility challenges in arid regions and potential solutions. *Journal of Soil Science*, 58(3), 251-262.
- [2] Jones, K., Matthews, A., & Shah, T. (2022). Biochar as a soil amendment: Benefits and applications. *Environmental Agriculture Journal*, 45(2), 172-189.
- [3] Lee, S., & Kim, D. (2021). Effect of biochar on radish growth and nutrient uptake. *Plant Science Journal*, 29(5), 398-410.
- [4] Pratiwi, L., et al. (2016). Effects of biochar application on rice soil properties and plant growth, including radish growth indicators. *Soil Science and Plant Nutrition*, 62(2), 130-145.
- [5] Poudel, D., et al. (2018). Influence of biochar on leaf dimensions and nitrogen uptake in radish. *Environmental Research in Agriculture*, 23(5), 207-215.

- [6] Masud, M. M., Zhang, Y., & Zhao, H. (2020). Biochar's dual impact on soil acidity management and crop yield enhancement: A meta-analysis. Research Square. <https://doi.org/10.21203/rs.3.rs-28795/v1>
- [7] Brown, P., Li, J., & Wang, H. (2023). Reducing greenhouse gas emissions through biochar soil amendments. *Journal of Sustainable Agriculture*, 62(4), 317-330.
- [8] Gonzalez, M., Brown, P., & Ahmed, Z. (2023). Improving soil fertility with biochar: Case studies and practical applications. *Agricultural Innovations*, 13(1), 45-59.
- [9] Van Zyl, J., et al. (2024). The impact of biochar on soil fertility and radish growth. *Journal of Agricultural Studies*, 15(4), 102-115.
- [10] Martin, J. H., Leonard, W. H., & Stamp, D. L. (1976). *Principles of field crop production* (3rd ed.). Macmillan Publishing Co.
- [11] Olsen, S. R., & Sommers, L. E. (2023). Soil pH and Soil Acidity. In *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties* (pp. 227-248). Soil Science Society of America.
- [12] Da Silva, N.; Taniwaki, M. H.; Junqueira, V. C. A.; de Arruda Silveira, N. F.; Okazaki, M. M. and Gomes, R. A. R. (2019). *Microbiological examination methods of food and water: A laboratory manual*. 2nd edn., CRC Press. Taylor and Francis Group, London, UK.526p.
- [13] Lehmann, J., & Joseph, S. (2021). *Biochar for Environmental Management: Science, Technology, and Implementation*. Routledge.
- [14] Zhang, A., Bian, R., Hussain, Q., & Li, L. (2020). Biochar and Soil Biota. In Lehmann, J. & Joseph, S. (Eds.), *Biochar for Environmental Management: Science, Technology, and Implementation* (pp. 519-544). Routledge.
- [15] Duncan, D. B. (1955). Multiple ranges test and Multiple F – test. *Biometrics*. 11: 1 – 42.
- [16] SPSS: Statistical Package for the Social Sciences (2018). SPSS users guide. Statistics, Version 25. IBM SPSS Statistics, SPSS Institute, Inc, Chicago, IL, USA.
- [17] Lehmann, J., & Joseph, S. (2009). *Biochar for Environmental Management: Science and Technology*. London: Earthscan.
- [18] Kammann, C., Ratering, S., Eckhard, C., & Müller, C. (2011). Biochar and hydrochar effects on greenhouse gas (carbon dioxide, nitrous oxide, and methane) fluxes from soils. *Journal of Environmental Quality*, 40(6), 1797-1805.

Efficiency of the RAPD in Determining the Genetic Dimension of Genotypes of Cotton

M. H. Amoon¹, Zeyad A. Abdulhamed² and Abdulsamad H. Noaman³

¹⁻³Department of Field Crops, College of Agriculture, University of Anbar, Anbar, Iraq.

²E-mail: ag.zeyad.abdul-hamed@uoanbar.edu.iq

³E-mail: ag.abdulsamad.hashim@uoanbar.edu.iq

Abstract. The use of Random Polymorphic Multiplication (RAPD) technology to analyze genetic differences between ten varieties of cotton. This technique relies on polymerase chain reaction (PCR) to determine the genetic polymorphism of the taxa in question using well-known sequence primers. Through this study, DNA was extracted from the leaves of developing plants, and 12 known initiators were used to verify the presence of genetic multiplicity. The results showed that two of the initiators produced no results, while the other ten produced good and outstanding results. RAPD has shown clear differences in the number of duplicate DNA bands and their molecular weights, suggesting genetic variation between varieties. This type of analysis can be an effective tool in classifying cotton varieties and is useful in breeding studies and the selection of superior or disease-resistant varieties. The results of the study indicate that the RAPD technique showed a distinct genetic variation between the cotton varieties studied, where a total number of 258 packages were detected, of which 213 were differentiated packages and 45 were similar packages. These results indicate that there is clear genetic diversity between the different varieties. The highest number of packets in the initiator (-07 OPN) produced 31 packets, which is the highest number of packets, while the initiator (-17 OPQ) produced 10 packets, which is the lowest number of packets. The distinctive efficiency of the initiators was remarkable, as it gave the highest efficiency in detecting genetic variations to the initiator (-11 OPA), as its efficiency reached 13.17, while the lowest efficiency was for the initiator (-20 OPQ) with 2.71 efficiency. The highest genetic distance was between the descending taxon 87 and the descending class 310, where it reached 0.9677, indicating a large genetic variation between them. The smallest genetic distance was between the Assyrian variety and the diacritic variety, at 0.1002, which indicates a great genetic similarity between these two species. The study also showed that RAPD technology was able to detect unique DNA packets that distinguished between the studied varieties. This distinction can be very useful in using these packages as a genetic fingerprint to help preserve plant breeders' rights. The study also showed the efficiency of the RAPD technique in determining the degree of proximity and genetic distance between varieties, which contributes to revealing genetic diversity among cotton varieties cultivated in Iraq. RAPD can therefore be considered a very effective tool in the analysis of genetic diversity among plant varieties. This technique makes it possible to accurately distinguish varieties, which enhances the protection of breeders' rights and helps in variety improvement programs. This study can be a strong basis for future studies in the field of improving cotton cultivation in Iraq and the use of polymerase chain reaction (PCR) techniques to enhance agricultural efficiency.

Keywords. Genetic dimension, Initiator, RAPD, Divergent packages, DNA.

1. Introduction

Cotton belongs to the marshmallow family (Malvaceae), the genus *Gossypium*. There are currently twenty species in the world, four of which are grown on a commercial scale. Two species originated in the ancient world, herbaceum and arborium, while the other two species, hirsutum and barbadense, originated in the New World. In addition, most cotton varieties belong to the species, although most commercial cotton varieties in the world belong to the navigating species hirsutum [1-3].

Cotton fibers are fibers that have a distinguished global position among other fabric fibers despite the competition of synthetic fibers, as well as other uses of the products of this crop. In the Arab world, this crop occupies a distinctive position in the agricultural sector, especially in these countries, namely Egypt, Sudan and Syria.

The main goal of cotton breeding is to combine high productivity with good staple specifications. The breeding of the ideal genetic genotype, whether using traditional breeding methods or using the technique of genes marked in cotton, is a main goal of plant breeders. Recently, the demand for a special quality of fiber has become dependent on the final manufacturing requirements, which has led to the creation of a scheduling in the prices of the crop from year to year, according to the desired rank, length, softness and durability [4-7].

This research aims to produce superior hybrids with some qualitative characteristics and to study some other genetic features by cross-fertilization between genetic genotypes of cotton of different origins and genetic base, as well as to determine the electoral evidence for the qualitative outcome [8,9].

In recent years, remarkable developments have emerged in the use of molecular techniques in determining genetic spacing in plants. The advantage of random multiplication with multiple forms of the amino acid chain is one of the common and modern indicators that excels in the smooth use and speed of laboratory analyzes and the possibility of applying them to many genetic clans in addition to the possibility of using random and general prefixes. In this technique, it is allowed to cover multiple areas of the genomes of studied individuals [10,11].

Many researchers have used various types of DNA indicators to study genetic variation and molecular description of the cotton plant, but in Iraq there are few studies in which DNA indicators have been used to study the genetic diversity of the cotton plant, which is why we have used molecular indicators in the RAPD method to analyze genetic differences between the genetic genotypes of the cotton plant.

2. Materials and Methods

2.1. Extraction of DNA from Leaves

Aldna was extracted by a method .Permingeat *et al*,1998Took 0.5-1g of fresh leaves per sample, washed with distilled water and cut into small pieces, ground in a ceramic mortar using liquid nitrogen and transferred the powder to glass tubes and added 4ml of extraction solution) - 100 mM TrisHCl pH 8, 20 mM EDTA, 1.4 M NaCl, 2% (, incubated tubes in CTAB, 0.5 M glucose) 60 °C vibrating water bath for 60-90 minutes. I left the tubes to gain room temperature and added 4ml of chloroform solution: isoamyl alcohol (1:24per tube with continuous stirring for 15minutes, then transferred to the centrifuge and start the mixture at a speed of 8,000 cycles/minute at a temperature of 4°C for (10minutes) [8,9].

The upper aqueous layer was taken to new pipes and a similar volume of cooled isopropanol alcohol was added to it and blended with a quiet stirring until a white lump representing the strands of the .DNA The strands of DNA were pulled by a curly-end glass rod ,placed in another tube containing 200microliters of sterile distilled water and moved from time to

time until the DNA was completely dissolved. Aldna quality was tested based on the method of electrophoresis using acarose gel at a concentration of 1%. The DNA concentration in the samples was also measured using a Nanodrop device . Korea). Its purity, which ranged from 1.80-2.00 ,was calculated. The concentration of DNA samples was standardized to 25ng/μL using sterile distilled water [12-17].

3. Results and Discussion

3.1. Preparation of RAPD-PCR Interactions

Random DNA amplification reactions (RAPD-PCR) were performed according to Willams ACCU Power PCR [1822], (premix Kit supplied by Bioneer of Korea and according to the attached instructions. The tubes contain the basic components of a polymerase chain reaction which include a single unit of an enzyme, Taq DNA polymerase μM250 from a mixture of nitrogenous bases, dNTPs1,5 and KCL mM30, Tris-Hcl (pH9) mM10.MgCl2 mM added picomole 10from the initiator and 25nanograms of DNA and then the size of the reaction with sterile distilled water to μL20per tube. The tubes were well mixed and transferred to the Thermocycler device to complete the multiplicative reaction after it was programmed according to the program: one cycle for two minutes at a temperature of 94C for the initial monster of the DNA tape followed by 40 doubling cycles that include a one-minute cycle at a degree of 92 C for the monster of the mold and one minute at a degree of 36C to connect the prefixes to the mold and one minute at a temperature of 72C for elongation with a last cycle for 7minutes and at a degree of 72C for the final elongation. The outputs of the amplification process on the acarose gel were carried out at a concentration of 1.2% with the volumetric evidence consisting of DNA to supply the section with the enzyme Hind III and Eco RI for a period of 90minutes by (5V/ cm). The gel was examined after being dyed with ethyl bromide dye for 45-30 minutes under ultraviolet light (UV-Light Gel Documentation System) and photographed using [23-26].

3.2. Recording the Results of RAPD and Statistical Analysis

To record the results of random multiplication of the RAPD technique, the images of the electrical separation patterns of each prefix were examined and the bands of each prefix were recorded in a table so that they were treated as a binary trait representing the presence of the bundle (Band B (1) and the absence of the bundle (BandB) (0). From this table, the total number of RAPD bundles shown by the prefixes in the genetic genotypes of the studied cotton were calculated, the polymorphic bundles were identified, the monomorphic bundles were matched, and the degree of variation in the bundle products was calculated. RAPD The coefficient of genetic distance as well as the coefficient of similarity between the studied samples were calculated using the coefficient of (Nei and Li, Nei's 72), [26-29]. The unweight pair group was then analyzed using the method for the arithmetic average (UPGMA.) conducted the statistical analysis using the Numerical Taxonomy and Multivariate Analysis program [30-33].

Table 1. Sequence of prefixes used in the study.

No.	Primers	Sequence (3-5)	GC
1	OP-T19	GTCCGTATGG	60
2	OP-T20	GACCAATGCC	60
3	OP-V02	AGTCACTCCC	60
4	OP-V09	TGTACCCGTC	60
5	OP-V19	GGGTGTGCAG	70
6	OP-M20	AGGTCTTGGG	60

7	OP-M05	GGGAACGTGT	60
8	OP-R12	ACAGGTGCGT	60
9	OP-M14	AGGGTCGTTC	60
10	OP-P04	GTGTCTCAGG	60

Table 2. Products of the prefixes of the total and differential beams and their molecular sizes.

Primers	Molecular weight (Chem.) BP	Resultant Packages	Divergent Packages	% of disparate packets	Percentage of Distinguishing Ability %	Efficiency ratio of initiators
OP-T19	500-1500	18	12	66.67	8.70	10.23
OP-T20	400-1000	13	8	61.54	5.80	7.38
OP-V02	400-2000	7	5	71.42	3.68	11.36
OP-V09	400-1500	20	15	75.00	87 10	11.36
OP-V19	300-1100	17	14	82.35	10.15	9.66
OP-M20	200-1200	20	11	55.00	7.97	11.36
OP-M05	200-1500	21	18	85.71	13.04	11.93
OP-R12	200-1000	18	18	100.00	13.04	10.23
OP-M14	400-1300	19	17	89.47	12.32	10.80
OP-P04	200-1500	23	20	86.95	14.49	13.07
Total		176	138			
Average		17.6	13.8	78.41		

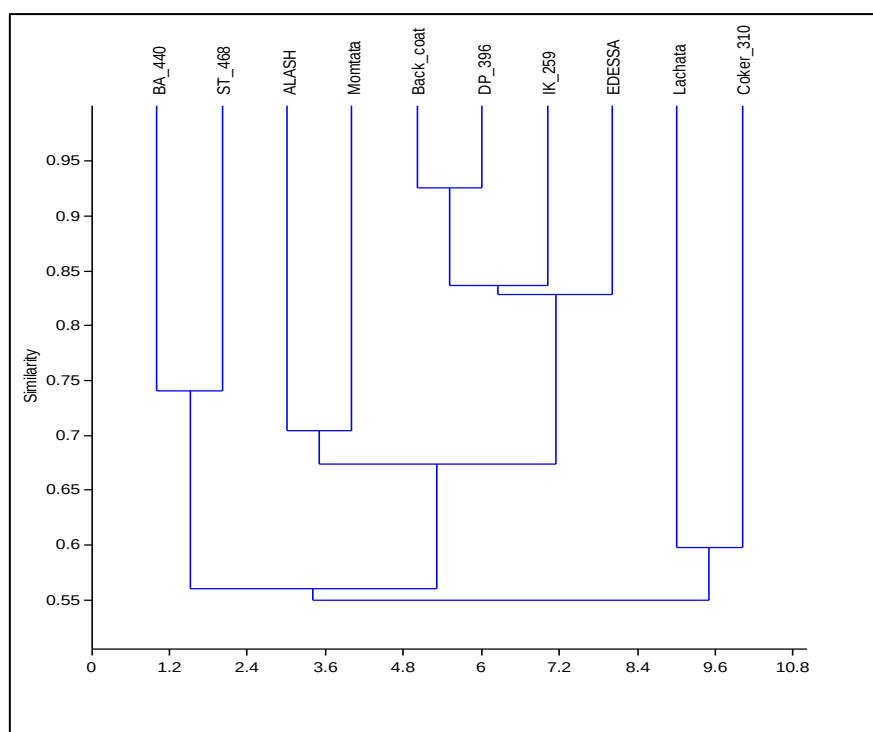


Figure 1. Dendrogram tree of ten cotton genotypes based on results from 176 clusters.

3.3. Similarity and Distance Indices

The results of the table show that there is a correlation as well as genetic differences between different variants. In this table, each number shows the amount of relationship between the specific variants between the genetic genotypes of the cotton plant that were introduced in the experiment [34-3], which showed us the genetic fingerprint used in the RAPD technique, through which the fingerprint used between the genetic genotypes can be detected, and this is by knowing the ten prefixes that have the ability to show genetic variations without the need to test many prefixes. This shows that the highest genetic spacing ratio is 0.949 between the class Coker_310 and the class ST_468, and the lowest genetic spacing ratio was 0.255 between the class Coker_310 and the class Lachata [37-40].

Table 3. Shows the values of the genetic dimensions of the varieties and their crosses for cotton using the RAPD technique.

	Coker_310	ELASH	Momtata	Lachata	BA_440	ST_468	Back_coat	DP_396	IK_259	EDESSA
Coker_310	1.000									
ELASH	0.791	1.000								
Momtata	0.901	0.569	1.000							
Lachata	0.255	0.899	0.833	1.000						
BA_440	0.927	0.306	0.793	0.884	1.000					
ST_468	0.949	0.791	0.792	0.939	0.410	1.000				
Back_coat	0.618	0.561	0.603	0.726	0.714	0.720	1.000			
DP_396	0.561	0.494	0.695	0.665	0.719	0.801	0.368	1.000		
IK_259	0.730	0.501	0.714	0.517	0.584	0.742	0.329	0.381	1.000	
EDESSA	0.709	0.670	0.714	0.548	0.826	0.841	0.495	0.278	0.317	1.000

Conclusion

This study showed the efficiency of the use of RAPD molecular technology in showing the difference between the cotton varieties studied and in measuring the degree of genetic spacing and convergence among them, which led to the detection of genetic diversity between cotton varieties grown in Iraq that can be worked on and developed for future use.

References

- [1] Al Nuaimi, A T, Al Assaf M A, and Saleh N S 2013 Response of cotton varieties to different plant densities - Mosul Agricultural Technical College - Cotton Research Unit - Diyala Journal of Agricultural Sciences, 5 (2): 144 145-
- [2] Abdul-Hamed, Z A, Abood, N M, & Noaman, A.H.2021. Heterosis in sunflower using cytoplasmic male sterility. IOP Conference Series: Earth and Environmental Science, 779, 012127.
- [3] Abdulhamed, Z A, Hwaidi, M I, & Alqaisi, M R M. 2023. Determination of maize genotypes performance under water deficit using ISSR molecular index. Plant Science Today, 10(1), 30–37.
- [4] Abdul-Hamed, Z A, Sarhan, I A, & Abbas, S A. 2017. Combining ability, heterosis and gene action using (line×tester) analysis in corn. The Iraqi Journal of Agricultural Science, 48(1), 294-301.
- [5] Sohair, E E D, Abdall, A A, Amany, A M, Hossain, M F and Houda, R A 2014 Foliar application of boron and zinc effects on growth yield and fiber properties of some Egyptian Cotton Cultivars. University Egypt. College of Agriculture. Journal of Agriculture, 7(13):1274-1282.
- [6] Abd, HS, Abdulhamed, Z A, & Ghadir, M A. 2021. Estimation of Genetic Parameter by Using Full Diallel Cross in Maize under Different Irrigation Interval. IOP Conference Series: Earth and Environmental Science, 904, 012054. doi: 10.1088/1755-1315/904/1/012054.

- [7] Mutlag, N A, Fayyad, S A, AbdulHamed, Z A, & Ibraheem, M M. 2018. Estimation of hybrid vigour, Combining ability and gene action using (Line x Tester) Analysis in Maize. The Iraqi Journal of Agricultural Science, 49(5), 740.
- [8] Hamad, H S, & Abdulhamed, Z A. 2023 ..Estimation of Combining Ability of Growth and Yield and Its Components of Maize under Salicylic Acid Concentrations. Journal of Bionature, 8(1).
- [9] Abd, MS, Abdulhamed, Z A, & Ghadir, M A. 2021. Response of Maize Hybrids and Inbred to Yield and its Components Under Irrigation Interval. IOP Conference Series: Earth and Environmental Science, 904, 012003. doi: 10.1088/1755-1315/904/1/012003.
- [10] Ayman, A Y 2014 The effect of fertilization with phosphorus and boron on the growth and yield of blossom cotton and its components (Lashata). College of Agriculture. Tikrit University. Tikrit University. Journal of Agricultural Sciences.(Special Issue) :242-247
- [11] Abdulhamed, Z A, Abas, S A, Noaman, A H, & Abood, N M. 2021. Genetic Performance of Inbred and Hybrids of Maize Under Irrigation Interval. IOP Conference Series: Earth and Environmental Science, 904, 012001. doi: 10.1088/1755-1315/904/1/012001.
- [12] Hamad, H S, & Abdulhamed, Z A. 2022. Role of Salicylic acid in stay green, growth and yield of two purposes maize hybrid. Bionatura, 7(4), 33. DOI: 10.21931/RB/2022.07.04.33.
- [13] More, VR, Khargkharate, VK, Yelvikar, NV and Matre, Y B, 2018 Effect of Boron and Zinc on Growth and Yield of Bt. Cotton under Rainfed Condition, Int. J. Pure App. Biosci. 6(4): 566-570 doi: <http://dx.doi.org/10.18782/2320-7051.6885>
- [14] Abdulhamed, Z A, Abas, S A, Noaman, A H, & Abood, N M. 2021. Review on the Development of Drought Tolerant Maize Genotypes in Iraq. IOP Conference Series: Earth and Environmental Science, 904, 012010. doi: 10.1088/1755-1315/904/1/012010.
- [15] Abdulhamed, Z A, Abood, N M, & Noaman, A H.2024. Recurrent selection for general and specific combining ability in maize. Iraqi Journal of Agricultural Sciences, 55(Special Issue), 99-110.
- [16] Fadel, A A, Abdulhamed, Z A, & Yousif, S A. 2022. RAPD Technique to Determine the Genetic Divergence of Barley Genotypes. IOP Conference Series: Earth and Environmental Science, 1060, 012123. doi:10.1088/1755-1315/1060/1/012123.
- [17] Abdulhamed. Z. A., A.O. Alfalahi. and N.M. Abood. 2020. Riboflavin and cultivars affecting genetic parameters in maize (*Zea mays* L.). AIP Conference proceedings. 2290.020020. <https://doi.org/10.1063/5.0027367>.
- [18] Al-Naqeeb, M and Askar F Y 2017 Comparison of flowering and fruiting behavior of some cotton varieties and its relationship to planting distances. Al-Furat Journal of Agricultural Sciences – 9(4), 1220 – 1236.
- [19] Amoon, M H, & Abdul Hamed, Z A. 2020. Determination genetic diversity of inbred lines and hybrids of maize using ISSR technic. Iraqi Journal of Agricultural Science, 51(1), 269-277.
- [20] Abdulhamed, Z A, Naoman, A H, Mohammed, Y A, & Abood, N M. 2019 .The genetic parameters of several oats cultivars under the influence of the biofertilizer (humiforte) spraying. Journal of Physics: Conference Series, 1294.
- [21] Abdulhamed, Z A, Abood, N M, & Noaman, A H. 2021 . Genetic Path Analysis and Correlation Studies of Yield and Its Components of Some Bread Wheat Varieties. IOP Conference Series: Earth and Environmental Science, 761, 012066.
- [22] Ibrahim, M M, & Abdulhamed, Z A.2024. Molecular genetic divergence of several sunflower genotypes using RAPD technology. IOP Conference Series: Earth and Environmental Science, 1060012123.
- [23] Al-Obaidi, O N J M 2022 Estimating the stability and path coefficient of cotton genotypes grown at plant densities in conservation agriculture. Doctoral thesis, Department of Field Crops, College of Agriculture, Tikrit University.
- [24] Ibrahim, M Muayad, & Abdulhamed, Z A.2023. Efficiency of Selection in Inducing Genetic-Molecular Variations in Sunflower. IOP Conference Series: Earth and Environmental Science, 1158, 062032. doi:10.1088/1755-1315/1158/6/062032.

- [25] Abdulhamed, Z A, & Al-Baurki, F R. 2023. Morphological and Molecular Characterization Using ISSR/PCR Markers for Half Diallel Crossing for Five Genotypes of Flax. AIP Conference Proceedings, 2977, 040040-1–040040-13.
- [26] Fadil , N and Al- Farttoosi H 2022 Effect of Nano - zinc and Nano – boron spraying on Cotton growth and yield . University Iraq . . college of Agriculture . Journal of Agriculture , 4 (41) . DOI. 10.21931/RB/2022.07.04.41.
- [27] Rawia, M. M.; Z. A. Abdulhamed & , M. A. M Alogaidi. 2022. Effect of planting dates on growth characteristics and yield of six bread wheat cultivars. Iraqi Journal of Desert Studies, 12(1), 69-78.
- [28] Fadel, A A, Abdulhamed, Z A, & Yousif, S.A. 2022. Study correlation and path coefficient analysis for barley under seed rates. Anbar Journal of Agricultural Sciences.20 (2) 436-449.
- [29] Saleh B.H., Abdulkareem ,B.M., Shahatha S. Sh., Mousa O. M., Abdulhamed, Z A., Abood, N M. and Alkaisy. A.U.M. 2024. The Growth and Yield of Oat Cultivars Influenced by Kinetin Application . IOP Conf. Series: Earth and Environmental Science 1371 (2024) 052041. doi:10.1088/1755-1315/1371/5/052041.
- [30] Shaker, S A, , Y E, MansyA E I, Darwesh, and S S M Badr, 2020 Evaluation and stability of some Egyptian cotton varieties under normal and late sowing conditions. Menoufia Journal of Plant Production, 5(2): 91-105.
- [31] Abas. S. A., Abdulhamed, Z A, & Abood, N M, 2024. Response of Four Barley Varieties to the Effect of Seed Rates. IoP Conference Series: Earth and Environmental Science . 1371 (2024) 052019. doi:10.1088/1755-1315/1371/5/052019
- [32] Wahid. S.A., Noaman, A H., Abdulhamed, Z A., Hamad. H.S., and Abdulkareem ,B.M., 2024. Genetic Parameters Using Line × Tester Multiplication for Growth, Yield and Quality of Maize. IOP Conf. Series: Earth and Environmental Science 1371 (2024) 052079. doi:10.1088/1755-1315/1371/5/052079.
- [33] Ibrahim, M M, & Abdulhamed, Z A 2024. The Selection by Honey Comb Design and its Effect on Disk Area and Yield Under Three Plant Densities in Sunflower. IOP Conference Series: Earth and Environmental Science, 1371 (2024) 052001 . 10.1088/1755-1315/1371/5/052001.
- [34] Abdulkareem ,B.M., Aoad . S.R., Salih , M.A.A., Abdulhamed, Z A., and Abood, N M. 2024. Response of Several Maize Cultivars to Potassium Fertilizer Levels. 1371 (2024) 052030. doi:10.1088/1755-1315/1371/5/052030
- [35] Oudah. A.M., Abdulhamed, Z A., and Hammadi . H.J. 2024. Efficiency of the RAPD Molecular Indicator to Determine the Genetic Polymorphism of Flax Genotypes. IOP Conf. Series: Earth and Environmental Science 1371 (2024) 052049. doi:10.1088/1755-1315/1371/5/052049.
- [36] Hamad SH, Abdulkareem BM, Abdulhamed ZA, Abood NM. Genotypic and phenotypic variance, correlation, and path coefficient analysis in maize. Anbar Journal of Agricultural Sciences. 2024; Available from: https://ajas.uoanbar.edu.iq/article_184519.html.
- [37] Hamad SH, Abdulhamed ZA, and Abdulkareem BM, 2024. Estimation the Heterosis Some Traits of Wheat Using Half Diallel. . IOP Conf. Series: Earth and Environmental Science.
- [38] Abas. S. A., Abdulhamed, Z A, & Abood, N M, 2025. Impact of climate change on the growth and yield, of sunflower cultivars under different plant densities.. Iraqi Journal of Agricultural Science, 56 (Special Issue) ,
- [39] Abdulhamed, Z A , Abdulkareem ,B.M, and Noaman, A H. 2023 .Role of Molecular Marker RAPD in Determining the Genetic Divergence between Hybrids and Inbreed Lines of Maize Using Full Diallel Cross Bionatura, 8(1), . DOI: 10.21931/RB/CSS/2023.08.03.68.
- [40] Ghasabeh, S R; Barzali M, Delkhosh B, and, Angaji S J 2021 Investigating the efficiency of different herbicides in double-cropped cotton (*Gossypium hirsutum* L.) after canola (*Brassica napus* L.) in Golestan province. Annals of the Romanian Society for Cell Biology: 21139-21145.

The Role of Thyme Oil on the Enzymatic Activity and Histological Changes in Common Carp (*Cyprinus carpio*) Exposed to Cadmium Chloride

Ali Abd Allateef Al-Ali¹, Anaheed A. Mohammed² and Sajad AbdulGani Abdullah³

¹Department of Biology, College of Education for Pure Science, University of Basrah, Basrah, Iraq.

^{2,3}Department of Biology, College of Education-Qurna, University of Basrah, Basra, Iraq.

¹E-mail: ali.abdalhassan@uobasrah.edu.iq

²E-mail: anahedodaa380@ gmail.com

³E-mail: sajad.aballah@uobasrah.edu.iq

Abstract. The present study was concerned with evaluating the health of common carp fish in their aquatic environment by relying on a number of environmental conditions. The present experiment included four groups: the control group, the group treated with thyme oil, the group treated with cadmium chloride and the group treated with both substances. The histopathological changes in the gills and liver of the treated fish were studied at periods (7, 15 and 30 days). The level of antioxidant activity was studied in the liver, gills and muscles at successive intervals for 30 days. The results of microscopic examination of the tissues of the organs of the fish treated with cadmium chloride showed the presence of a number of histopathological changes during the experimental periods. These changes increased with the length of exposure to cadmium, especially in the last two periods. These changes varied from slight reversible changes in the first period of the experiment to non-reversible changes. These changes reached the point of cell necrosis and death in the last periods of the experiment. A decrease in the severity of these histological pathological changes was observed in the group treated with thyme oil with cadmium, especially during the 30-day period of the experiment. In addition, no significant pathological changes appeared in the group of fish treated with thyme oil alone. This reflects the protective role of thyme in maintaining the integrity and health of fish tissues. The results of the activity of the enzyme superoxide dismutase (SOD1) in some organs (gills, liver and muscles) of common carp (*C. carpio*) showed the role of thyme oil in raising the level of activity of these antioxidant enzymes during periods (7, 15 and 30 days) of the experiment.

Keywords. Cadmium chloride, Thyme oil, Histopathological, Antioxidants enzyme (Superoxide dismutase 1).

1. Introduction

Fish wealth is a fundamental pillar in strengthening the economies of countries. Scientists and researchers pay great attention to it due to its vital role as a major source of human food.

Researchers are working hard to preserve this wealth and increase its production. The increase in production is proportional to the large population growth and the increase in demand for food, especially protein [1]. Carp (*C. carpio*) is a common fish in local aquatic environments. It can also be considered among the important species of the Cyprinidae family, which has great economic value in Iraqi inland waters [2]. Fish face many problems in their aquatic environments, which affect their growth and prosperity and sometimes threaten their ability to survive. These problems include unfavorable environmental conditions, increasing human activities, and heavy metal pollutants such as cadmium. This element is considered non-essential and has high toxicity even at low concentrations [3,4]. Cadmium accumulation leads to multiple health problems due to its negative effects on the immune system. It acts as a toxic agent by regulating apoptosis and stimulating the production of reactive oxygen species and oxidative stress [5]. To overcome these difficulties, fish farms seek to improve the quality of feed provided to fish by including nutritional elements and nutrients that enhance their ability to resist diseases and pollutants. Among these solutions, the addition of antioxidants to fish feed to enhance their immune system stands out. Many natural plant products have antioxidant properties, including thyme (*Thymus vulgaris*), which belongs to the Lamiaceae family and the *Thymus* genus. Thyme is an important medicinal and economic plant because it contains a group of essential phytonutrients, vitamins and minerals. Thyme leaves are a rich source of potassium, iron, magnesium and calcium [6]. Antioxidants work to eliminate free radicals by donating their electrons to free radicals to stabilize them. It is worth noting that antioxidants do not turn into free radicals when they lose electrons, thanks to their ability to stabilize in more than one form [7]. Several antioxidant enzymes, such as peroxidase, superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), contribute to the removal of free radicals [8]. SOD1 is considered one of the most powerful antioxidants and the first enzyme to detoxify the cell, as it represents the first line of defence against reactive oxygen species (ROS) (Ighodaro and Akinloye, 2018). SOD plays a crucial role in catalyzing the decomposition of superoxide ions (O_2^-) into less dangerous hydrogen peroxide (H_2O_2) and molecular oxygen [9]. Increased enzyme activity indicates high levels of superoxide ions, as fish are exposed to high levels of free radicals due to exposure to pollutants in the aquatic environment. The following equation illustrates the enzyme's stimulation of the decomposition process [10]. The study aimed to determine the effectiveness of thyme oil in reducing histopathological changes in some fish organs due to exposure to cadmium chloride and to evaluate the level of SOD1 enzyme activity.

2. Materials and Methods

2.1. Experimental Fish

The current study used common carp (*C. carpio*) with an average weight of (1.54 ± 39.37) g. The fish were transferred to the laboratory of the Department of Life Sciences, College of Education, Qurna, University of Basrah, operation with laboratories in The Ministry of Science and Technology Ethical approval No. ISO 17025 where they were reared under controlled laboratory conditions. The lighting cycle was set to be 12 hours of light and 12 hours of darkness. The temperature was (0.50 ± 24) °C, salinity (0.2 ± 1.076) g/L, dissolved oxygen (0.31 ± 8.42) mg/L, and pH (0.24 ± 7.9) . The fish were fed with diets at a rate of 5% of their live weight twice daily. The ponds were cleaned daily to remove waste and withdraw uneaten food. Two-thirds of the water in the ponds was withdrawn using the siphon process, and the deficit was compensated with clean water.

2.2. Fish Exposure

The fish were randomly divided into four groups, each group consisting of 16 fish, as follows: The first group: represents the control group, where the fish were raised in chlorine-free water and fed on commercial feed with protein content. The second group: included fish treated with thyme oil added to the feed at a ratio of (1 ml of thyme oil to 3 g of feed), supplied by the Indian company SHEER essence at a concentration of 100%. The third group: included fish treated with a concentration of (56.2) mg/L of cadmium chloride added to the water (Al Ali et al., unpublished research), supplied by the Indian company Thomas Baker. The fourth group: included fish treated with a concentration of (56.2) mg/L of cadmium chloride in water, with thyme oil added to the feed at a ratio of (1 ml of thyme oil to 3 g of feed). Fish were conditioned for 21 days on a diet containing thyme oil before being exposed to cadmium chloride. Fish were fed daily on a diet containing (37% protein, 8.84% fat, and 42.64% carbohydrates), at 5% of fish weight. Fish were dissected at (7, 15, and 30) days after treatment, with three replicates for each period, to study the level of the enzyme Superoxide Dismutase 1 (SOD1) and histopathological changes in the gills and liver.

2.3. Study of Histopathological Changes

Histopathological sections were prepared according to [11], method for gill, liver and kidney tissues. The samples were fixed with Bouin's fixative for 24 hours. Then, they were washed with 50% ethyl alcohol several times. After that, they were passed through an ascending series of alcohols starting from 70% alcohol, passing through 80%, 90% and 100% concentrations for two hours each. Then, the samples were cooled using xylene as a preliminary step for the penetration of paraffin wax. Then, the samples were embedded in molten paraffin wax at a temperature of 56°C using metal moulds. Then the molds were cut using a rotary microtome with a thickness of 5-7 ml. They were mounted on glass slides and stained with hematoxylin and eosin. The histopathological sections were examined and photographed using a Ziess microscope equipped with a Lieca digital camera.

2.4. Antioxidants

The experiment was conducted on some organs of common carp (liver, gills and muscles) for the purpose of studying the activity of the SOD1 enzyme during periods of (7, 15 and 30) days. An Iraqi company conducted the work.

2.5. Protein Extraction

Two organs of fish, gills and white muscles, were taken after dissecting the fish during successive periods (7, 15 and 30) days of the experiment. Antioxidants were studied depending on the special kit for each enzyme. Before that, the protein was extracted using an extraction buffer. It was carried out in steps that can be summarized as follows: 100 mg of each of the gill and muscle tissues were taken and mashed well. After adding 150 microliters of the extraction buffer to the mashed tissue, 10 microliters of protein inhibitor enzyme (2mM) were added instantly to the buffer solution. After performing the vortex process, the samples were left for half an hour at room temperature. Then, the samples were placed in a centrifuge at high speed (20000 revolutions for five minutes). Then, 100 microliters of supernatant were taken and added to the holes of the SOD1 plate. Estimation of SOD1 enzyme activity in liver, gill and muscle tissue of common carp fish by ELISA technique [12], using Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

The work was done according to the instructions mentioned in the kit manufactured by the Korean company Elabscience with the serial number Cat.No.:E-EL-H1113 Superoxide dismutase (SOD1) kit.

2.6. Statistical Analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) and using the SPSS v19 program. The mean values with standard error and significant differences were taken at the probability level ($P \leq 0.05$).

3. Results

Histopathological changes in some fish organs (gills and liver)

3.1. Gill Tissue

3.1.1. Control Group

(1) Microscopic imaging showed that the gills of the fish had normal structures, almost free of histological pathological changes. They consist of a number of gill arches, each arch consisting of a primary filament and secondary lamellae. The gill plate consists of epithelial tissue containing two layers of epithelial cells and a blood capillary (blood sinuses) Figure (1). (2) The group treated with thyme oil: The results showed healthy gill tissue with a slight change in the shape of some secondary gill plates during the experimental period (7, 15 and 30) days. As shown in Figures (2, 3 and 4). (3) The group was treated with a concentration of 56.2 mg / L of cadmium chloride. The results showed some histological pathological changes in the gills of the fish. During the 7 days, a change in the shapes of the secondary gill plates and their congestion was observed. In addition, hyperplasia reached the middle of the length of the secondary gill plates [5]. Swelling appeared in some epithelial cells of those plates, Figure (6). For the first time, necrosis was recorded in the epithelial tissue layer lining the secondary gill plates, Figure (7). Separation of the epithelial tissue layer was also observed, as shown in Figure (8). The histological pathological changes continued during the 15 days. They were more severe than during the 7 days. It was represented by severe hyperplasia in the epithelial tissue reaching the free ends along the secondary gill plates and a change in the shapes of the secondary plates with bleeding in some areas of the gill thread. Necrosis was observed in the secondary gill plates (Figures 9 and 10). As for the 30 days, the changes were more severe than in the previous periods. Severe hyperplasia was observed reaching the free ends of the gill plates, and the appearance of edema in the terminal ends of the secondary gill plates and adhesion of the secondary gill plates to each other (Figure (11)). 4. The group was treated with cadmium chloride at a concentration of (56.2 mg/L) with the addition of thyme oil. The results showed a decrease in the histopathological changes in the 7-day period, which were represented by slight hyperplasia and a change in the shape of the secondary gill plates with bleeding between the primary gill plates and congestion in the terminal ends of the capillary blood vessels of the secondary gill plates, as shown in Figure (12). However, in the 15 days, a decrease in the severity of the histopathological changes was observed. Some of these changes occupied smaller areas on the gill filaments, changing the shapes of fewer secondary gill plates. Capillary congestion with moderate hyperplasia was observed in other cases Figure (13). While in the 30 days, a decrease in the histopathological effects in the gills of the fish was observed. It was represented by slight hyperplasia, as the epithelial cells migrated from the base of the gill plates towards the terminal ends of the gill plates Figure (14).

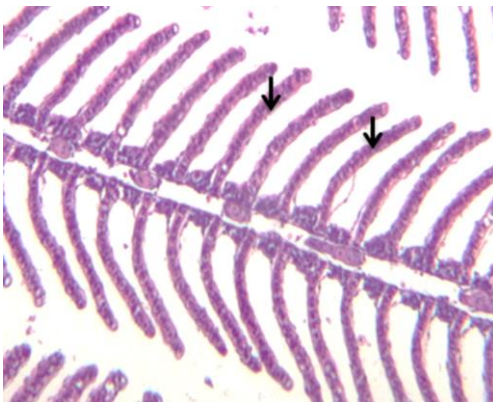


Figure 1. Section of the gill tissue of the control group fish, secondary gill plates (arrows), E&H, X 40.

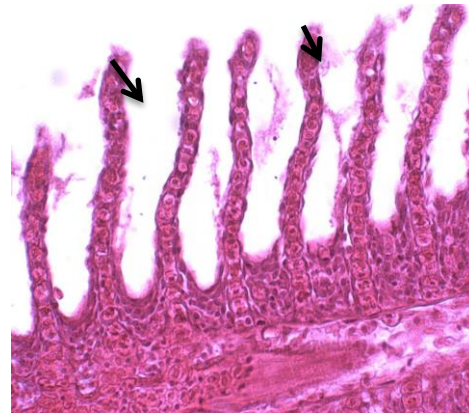


Figure 2. A section of the gill tissue of fish from the group treated with thyme oil only after 7 days of treatment. A slight change in the shapes of the gill plates (arrows) is observed, E&H, X 100.

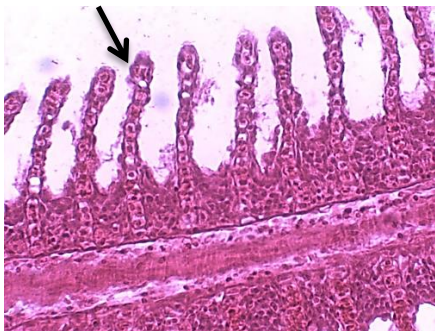


Figure 3. Section of the gill tissue of fish in the group treated with thyme oil only after 15 days of treatment, a slight change in the shapes of some secondary gill plates (arrows), E&H, 100X.

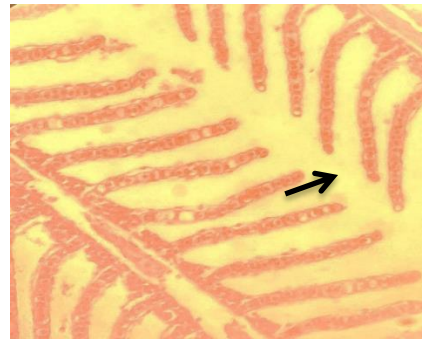


Figure 4. Section of the gill tissue of fish in the group treated with thyme oil after 30 days of treatment, a slight change in the shapes of a few secondary plates (arrows), E&H, X 40.

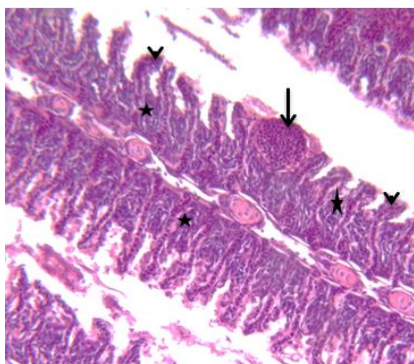


Figure 5. Section of the gill tissue of fish in the group treated with a concentration of 56.2 mg/L of cadmium chloride after 7 days of treatment, hyperplasia (stars), congestion of blood vessels (arrows), change in the shape of the secondary gill plates (arrowhead), E&H,

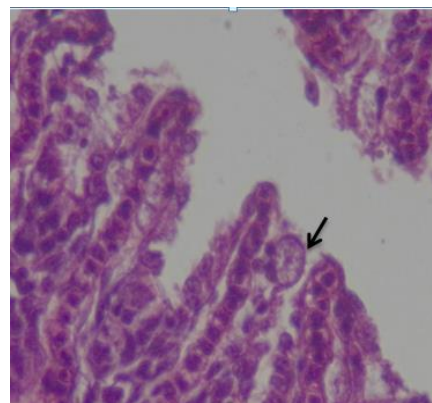


Figure 6. Section of the gill tissue of fish from the group treated with a concentration of 56.2 mg/L of cadmium chloride after 7 days of treatment, swelling (arrow), E&H, 100X.

40X.

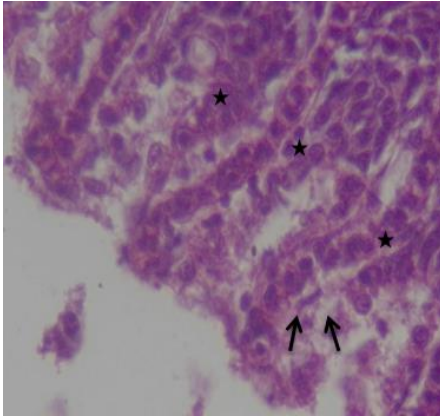


Figure 7. Section of the gill tissue of fish in the group treated with a concentration of 56.2 mg/L of cadmium chloride after 7 days of treatment, necrosis (arrows), hyperplasia (stars) E&H, X.

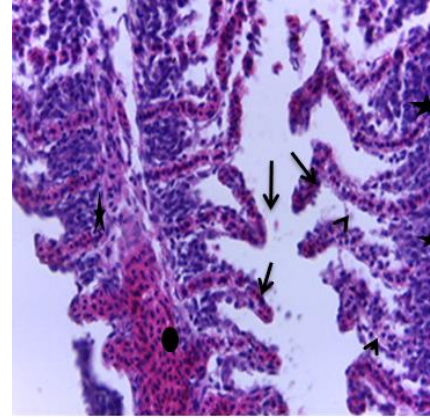


Figure 8. Section of the gill tissue of fish in the group treated with a concentration of 56.2 mg/L of cadmium chloride after 7 days of treatment, change in the shapes of the secondary gill plates (arrows), hyperplasia (star), congestion (circle), detachment of the epithelial tissue layer (arrowhead) E&H, X 40.

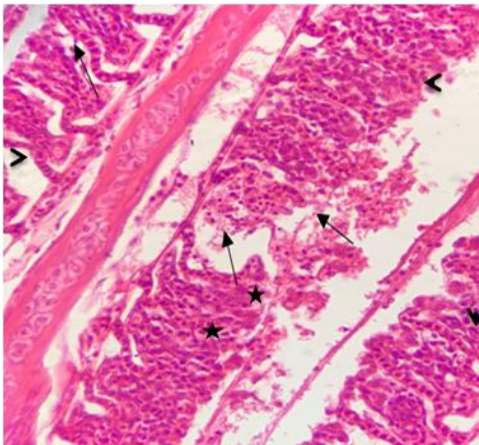


Figure 9. Section of the gill tissue of fish treated with 56.2 mg/L of cadmium chloride 15 days after treatment, necrosis of the secondary gill plates (arrows), hyperplasia (stars), change in the shape of the gill plates (arrowheads), E&H, X 40.

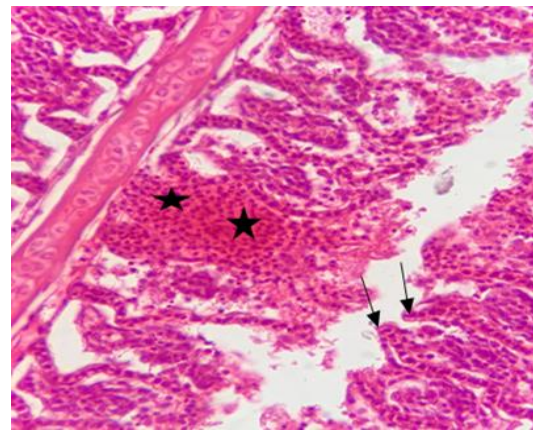


Figure 10. A section of the gill tissue of fish in the group treated with a concentration of 56.2 mg/L of cadmium chloride after 15 days of treatment. There was bleeding in some areas of the gill filament (stars), and the shapes of the secondary gill plates (arrows) changed. 40X, E&H.

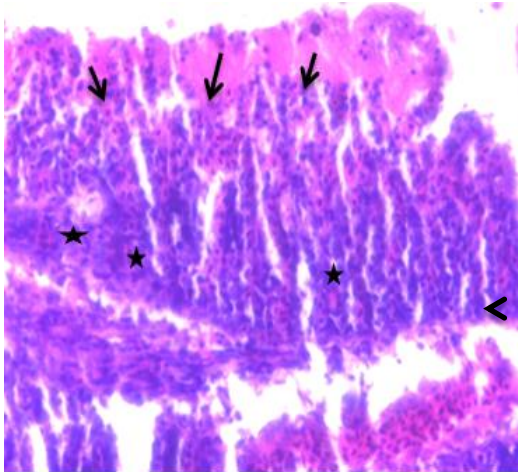


Figure 11. Section of the gill tissue of fish in the group treated with a concentration of 56.2 mg/L of cadmium chloride after 30 days of treatment, presence of edema in the secondary gill ends (arrows), severe hyperplasia (star), adhesion (arrowhead), E&H, 40X.

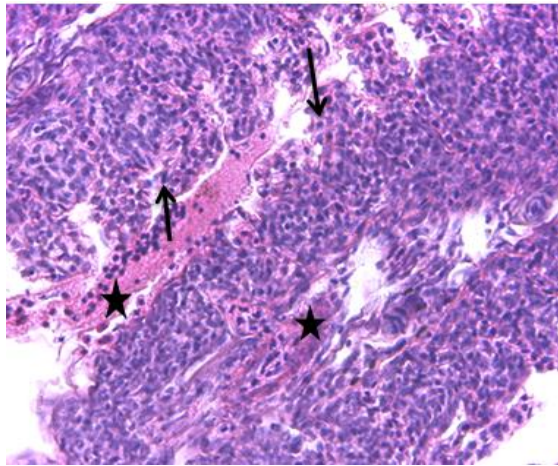


Figure 12. Section of the gill tissue of fish in the group treated with a concentration of 56.2 mg/L of cadmium chloride with thyme oil after 7 days of treatment, congestion and change in the shape of the secondary gill plates (arrow), bleeding (star) E&H, 100X.

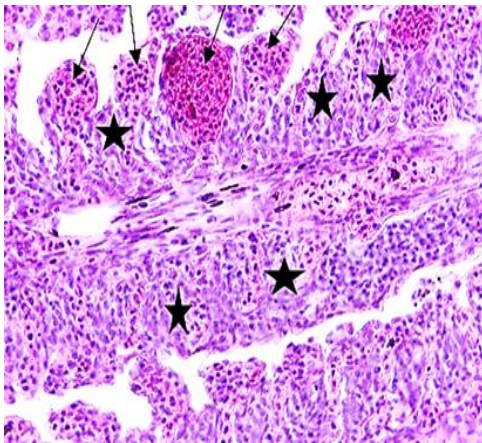


Figure 13. Section of the gill tissue of fish from the group treated with a concentration of 56.2 mg/L of cadmium chloride with thyme oil after 15 days of treatment, hyperplasia in the secondary gill plates (stars), congestion (arrows), E&H, X 40.

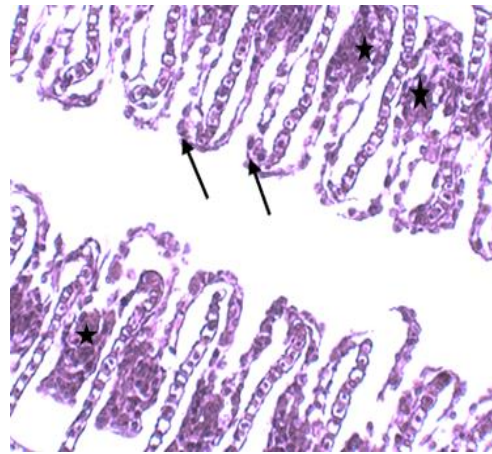


Figure 14. Section of the gill tissue of fish from the group treated with a concentration of 56.2 mg/L of cadmium chloride with thyme oil after 30 days of treatment, migration of epithelial cells from the base of the gill plates towards the terminal ends of the secondary plates (arrows), hyperplasia (stars), E&H, X40.

3.2. Liver Tissue

(1) The control group consisted of hepatocytes arranged around the central vein in a regular radial manner. Between the hepatocytes were liver sinusoids (sinuses) Figure (15). (2) The group treated with thyme oil showed no pathological tissue changes in the liver tissue for all periods. The tissue was observed to be intact with the presence of melanomacrophages, as shown in Figures (16, 17, and 18). (3) The group was treated with cadmium chloride at a concentration of

56.2 mg/L. The results showed several diverse pathological tissue changes during the 7 days. These changes were represented by the appearance of areas devoid of hepatocytes due to their necrosis and decomposition, in addition to the occurrence of bleeding in many areas of the liver and the accumulation of large masses of melanomacrophages that appear dark in color Figure (19). The diversity of pathological tissue changes increased and were more severe than in the previous period. In the 15 days, liver cells were observed to degenerate and necrotic, and areas devoid of tissue were observed, as shown in Figure (20). In the 30 days, the pathological changes were more severe and aggravated. They were represented by necrosis and atrophy of liver cells with the accumulation of melanomacrophages. Areas devoid of liver tissue were observed, with bleeding. Megakaryocytes appeared, and their numbers increased. This is the beginning of the process of phagocytosis, as shown in Figures (21, 22, and 23). (4) The group was treated with cadmium chloride at a concentration of 56.2 mg/L. Thyme oil was added. The results showed a decrease in the pathological changes in the tissue compared to the group treated with cadmium chloride alone. In the 7 days, there was a slight infiltration of blood cells, regeneration of liver cells, and the presence of melanomacrophages with a few necrotic cells were observed, as shown in Figure (24). In the 15 days, the tissue appeared healthy with the presence of melanomacrophage cell masses, as shown in Figure (25). Thyme oil had a protective role and was more effective with increasing the period, in the 30 days. It was noted that the pathological effects decreased, and the numbers of melanomacrophages decreased, with the presence of liver cell renewal in some areas of the liver that were suffering from cell necrosis. Some of these areas still contain spaces or voids devoid of liver cells, i.e. the presence of necrotic cells, Figure (26).

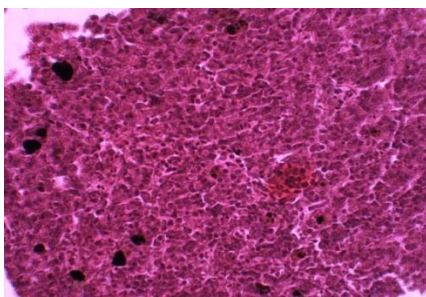


Figure 15. Section of liver tissue showing the treatment of control hepatocytes (arrows), E & H, 40 X.

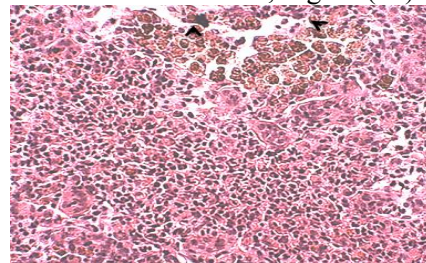


Figure 16. Section of the liver tissue of fish in the group treated with thyme oil after 7 days of treatment, showing the normal liver tissue, a collection of melanomacrophage cell masses (arrowhead), E & H, 40 X.

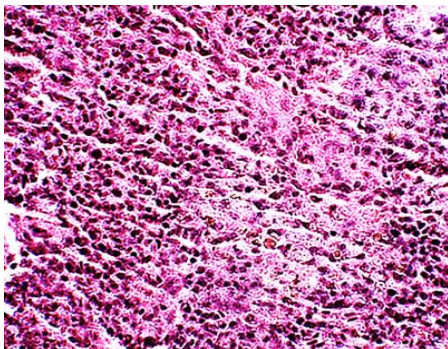


Figure 17. Section of the liver tissue of fish in the group treated with thyme oil after 15 days, the integrity of the liver tissue (the star), E & H, 40 X.

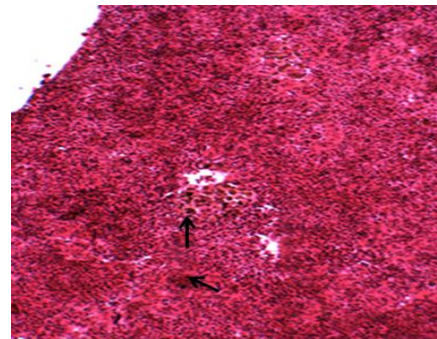


Figure 18. Section of the liver tissue of fish in the group treated with thyme oil only 30 days after treatment. The liver tissue appears normal, melanomacrophage cells (arrows) E & H, X10.

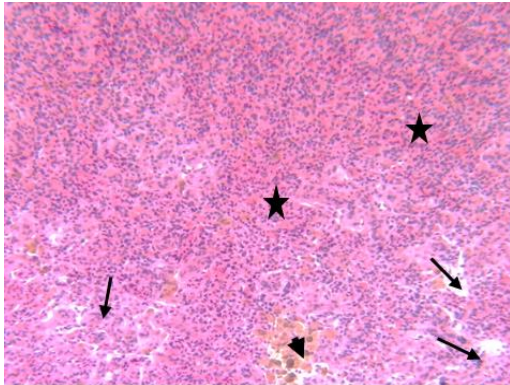


Figure 19. Section of liver tissue of fish treated with 56.2 mg/L of cadmium chloride 7 days after treatment. It shows hemorrhage in most areas of the liver (star), necrosis of hepatocytes (arrows), and aggregation of melanomacrophage cells (arrowhead), E & H, 40 X.

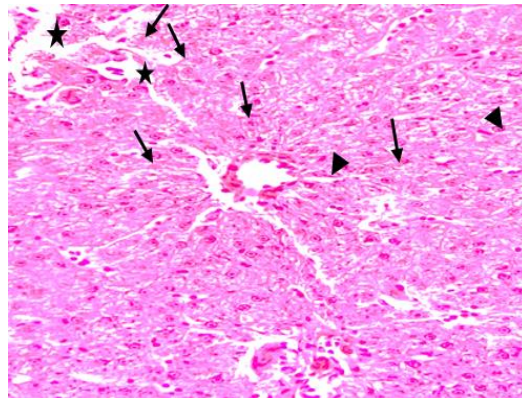


Figure 20. Section of liver tissue of fish treated with 56.2 mg/L of cadmium chloride 15 days after treatment, hepatic cell necrosis (arrows), hepatic cell degeneration (arrowheads), the appearance of tissue-free areas (stars), E & H, 40 X.

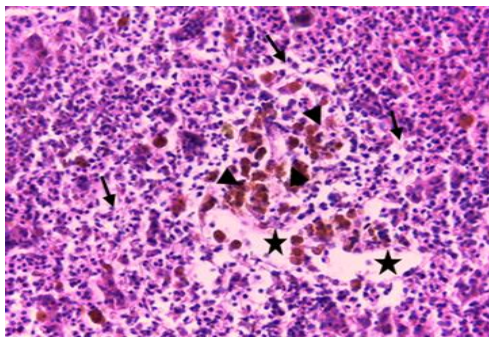


Figure 21. Section of liver tissue of fish treated with 56.2 mg/L of cadmium chloride 30 days after treatment, necrosis (arrows), appearance of areas devoid of tissue (stars), aggregation of melanomacrophage cells (arrowhead), E & H, X40.

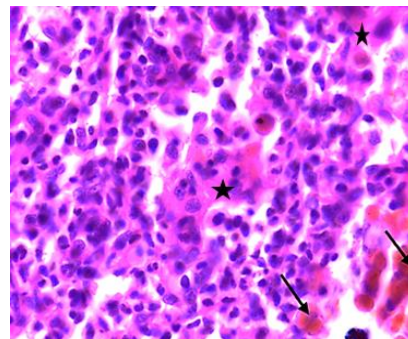


Figure 22. Section of liver tissue of fish treated with 56.2 mg/L of cadmium chloride 30 days after treatment, hemorrhage (inj) and appearance of megakaryocytes inflammatory cells (arrows), E & H, X100.

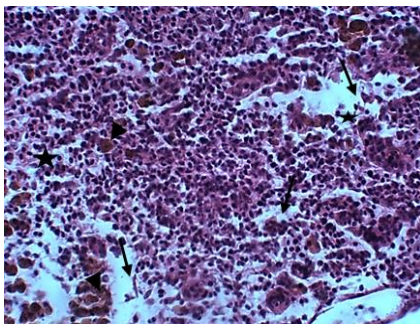


Figure 23. Section of liver tissue of fish treated with 56.2 mg/L of cadmium chloride 30 days after treatment, necrosis (arrows), cell atrophy (stars), melanomacrophage cell aggregation (arrowhead), E & H, X40.

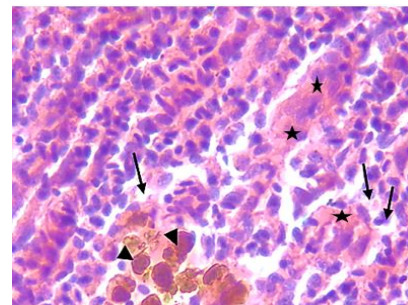


Figure 24. Section of liver tissue of fish in the group treated with a concentration of 56.2 mg/L of cadmium chloride with thyme oil after 7 days of treatment, showing clumps of melanomacrophage cells (arrowhead), necrosis in some hepatocytes (arrows), infiltration of blood cells (stars), E & H, 40 X.

3.3. Antioxidants

Estimation of protein expression of superoxide dismutase1 (SOD) in the organs of common carp *C. carpio* under the influence of study factors. The present study showed the activity of superoxide dismutase1 (SOD 1) in some organs of common carp *C. carpio* during three periods (7, 15 and 30 days) of the experiment for the following treatments (thyme oil, cadmium chloride and a combined treatment between thyme oil and cadmium chloride). The results showed a significant difference in the enzyme activity in the liver, gills, and muscles between the three treatment groups compared to the control group.

3.3.1. Comparison of SOD 1 Enzyme Activity in Fish Liver Between Periods in One Treatment

The results of the statistical analysis of the liver of carp fish treated with the study materials showed an increase in the activity of the enzyme (SOD 1) above the normal level under the influence of the two treatments of cadmium alone and thyme oil during the experimental period. It was observed in the treatment with cadmium chloride alone an increase in the activity of the enzyme (SOD 1) in 7 days. Then, it begins to gradually decrease with the length of the exposure period with a significant difference at the probability level of $P \leq 0.05$ Figure (25 A). As for the treatment with thyme oil, the highest activity of the enzyme was shown during 7 days with a significant difference of $P \leq 0.05$ from the rest of the periods. Then it decreased in 15 days to return to rise in 30 days Figure (25 B). The treatment with cadmium chloride and thyme oil together showed a significant difference in the activity of the enzyme. If its level was lower than the normal level and was close between the periods of 7 and 15 days, it increased during 30 days with a significant difference. Figure (25 C).

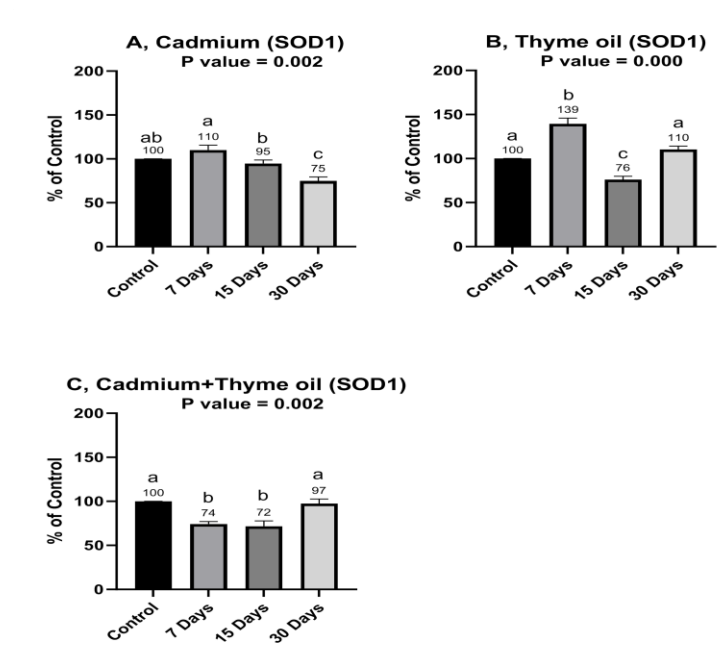


Figure 25. The activity of the enzyme superoxide dismutase 1 (SOD 1) in picograms/ml in the liver of common carp *C. carpio* during the periods (7, 15 and 30) days of the experiment for the treatments, A the group treated with cadmium chloride, B the group treated with thyme oil, C the group treated with cadmium chloride with thyme oil.

3.3.2. Comparison of the Activity of the Enzyme SOD 1 in the Liver of Fish Between the Treatments in One Period

When comparing the activity of the enzyme SOD 1 during one period in the liver of fish treated with the study materials, it was noted that the level of enzyme activity was close significantly in all periods with the normal level at a probability of ($P \leq 0.05$). However, there were some significant differences in the treatment with cadmium chloride during the 7-day period, which was higher than the rest of the treatments (Figure A 26). During the 15 days, significant differences were observed between the treatments, and the highest activity of the enzyme (SOD1) was in the treatment with thyme oil (Figure B 26). While after 30 days of the experiment, a significant convergence in the level of enzyme activity was observed between the treatment with thyme oil and the treatment with cadmium and between the control group, except for the combined treatment (cadmium chloride and thyme oil) with a significant difference at $P \leq 0.05$ (Figure (26 C).

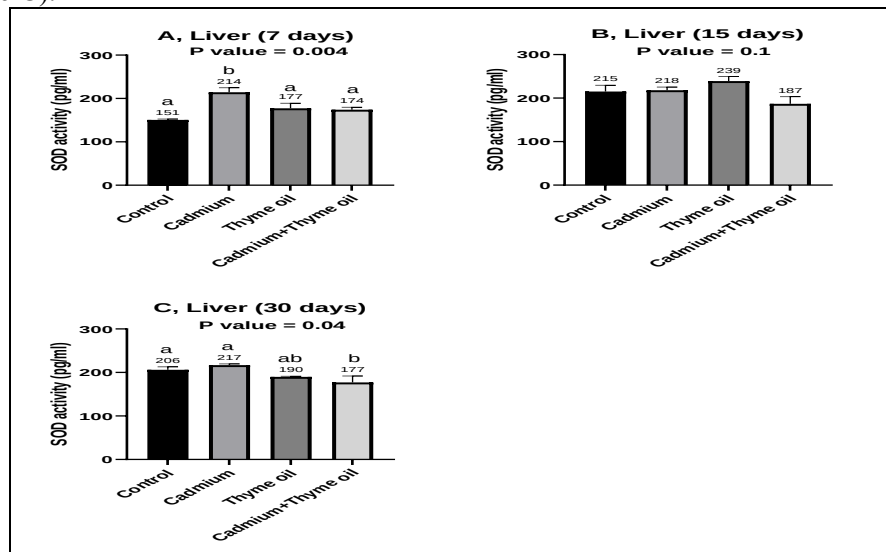


Figure 26. Shows the activity of the enzyme superoxide dismutase1 (SOD 1) in picograms/ml in the liver of common carp *C. carpio* for the three treatments, 7 days, B 15-day period, C 30-day period.

3.3.3. Comparison of SOD 1 Enzyme Activity in Fish Gills Between Periods in One Treatment

The results of the enzyme estimation in the gills of fish treated with the study materials showed SOD1 enzyme activity for each treatment during the experimental periods. It was noted that the treatment with cadmium chloride increased the enzyme activity above the normal level in the 7 days. Then, it began to gradually decrease with the length of the experimental period with a significant difference at $P \leq 0.05$ Figure (27 A). As for the treatment with thyme oil, the enzyme activity increased during the 7 days. Then, it decreased with a significant difference at the probability level of $P \leq 0.05$ during the 15 days. As for the 30 days, the enzyme activity level was significantly close to the normal level of the enzyme Figure (27 B). It was noted in the combined treatment (cadmium chloride and thyme oil) a significant closeness in the enzyme activity during the 7 and 15-day periods of the experiment. A significant convergence in the enzyme level in the 30 days with the normal level, Figure (27 C).

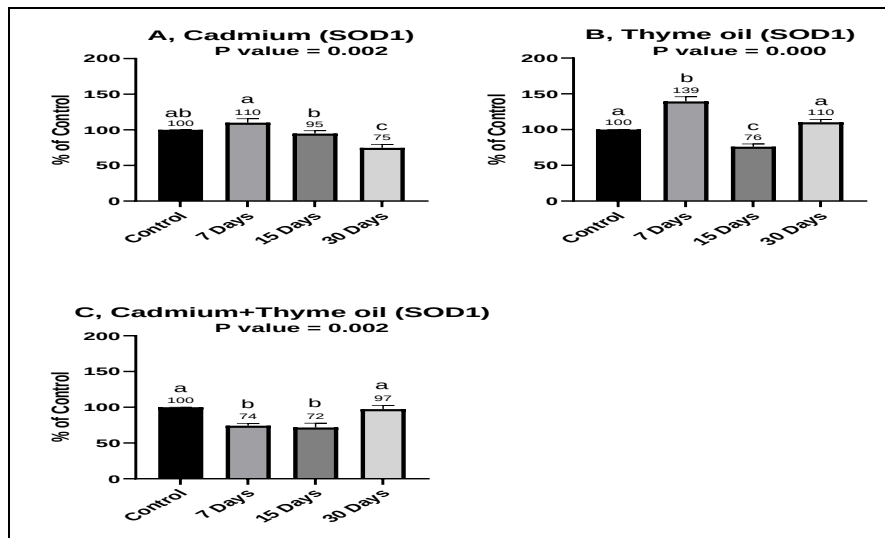


Figure 27. shows the activity of the enzyme superoxide dismutase I (SOD 1) in picograms/ml in the gills of common carp *C. carpio* during periods (7, 15 and 30) days of the experiment for treatments A, the group treated with cadmium chloride, B, the group treated with thyme oil, C, the group treated with cadmium chloride and thyme oil.

3.3.4. Comparison of the Activity of the Enzyme SOD 1 in the Gills of Fish Between Treatments in One Period

The results showed a difference in the activity of the enzyme SOD1 from the normal level. In the 7 days, a significant convergence of the enzyme activity was observed in the treatment with cadmium chloride with the control group. Also, the increase in the enzyme activity in the treatment with thyme oil decreased in the combined treatment with cadmium chloride and thyme oil with a significant difference at the probability level $P \leq 0.05$, Figure (A 28). After 15 days of the experiment, the enzyme activity level decreased below the normal level for all treatments. A significant convergence was observed between the two treatments with thyme oil and the combined treatment (cadmium and thyme oil) at a probability level of $P \leq 0.05$, Figure (28 B). During the 30 days, no significant differences were observed at $P \leq 0.05$ between the groups treated with thyme oil and the combined treatment (cadmium chloride and thyme oil) and the control group, except for the treatment with cadmium chloride alone, Figure (28 C).

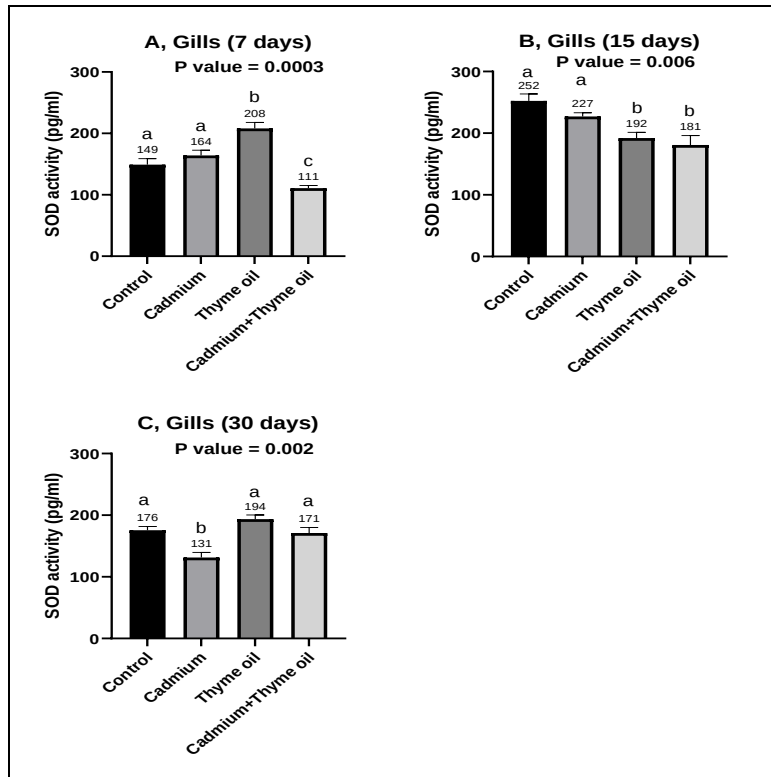


Figure 28. Shows the activity of the enzyme superoxide dismutase 1 (SOD 1) in picograms/ml in the gills of common carp *C. carpio* for the three treatments, 7 days, B 15-day period, and C 30-day period.

3.3.5. Comparison of SOD 1 Enzyme Activity in Fish Muscles Between Periods in One Treatment

The results of the statistical analysis in fish muscles for groups within one period showed that the group treated with cadmium chloride showed a gradual increase in enzyme activity with the length of exposure period with a significant difference at $P \leq 0.05$ Figure (29 A). As for the group treated with thyme oil only, significant differences were observed at the probability level of $P \leq 0.05$ between periods. The enzyme activity increased during the 7 days. Then, it began to gradually decrease during the 15 and 30-day periods of the experiment Figure (29 B). While in the group treated with cadmium chloride and thyme oil together, a convergence of SOD1 enzyme activity was observed during the 7 and 15-day periods and then decreased during the 30 days with a significant difference at the level of $P \leq 0.05$ Figure (29 C).

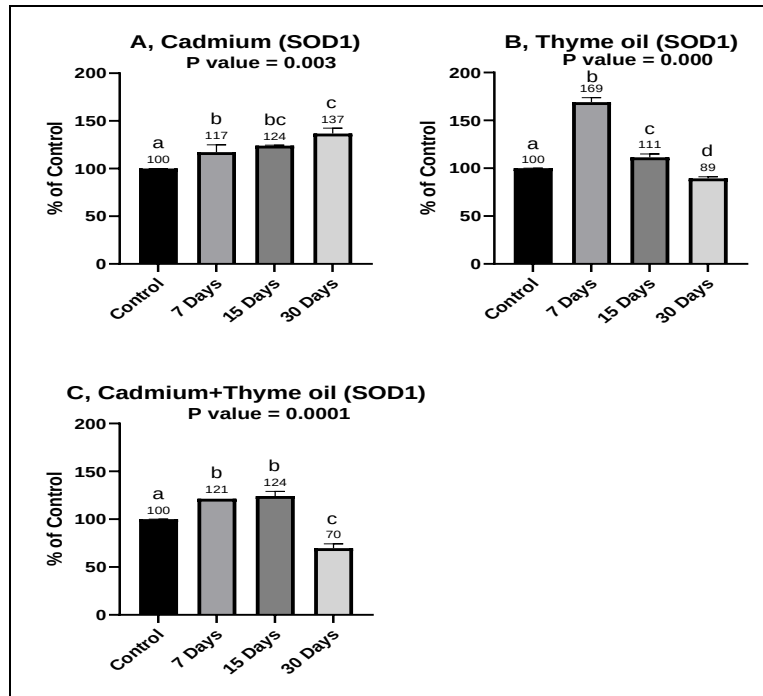


Figure 29. Shows the activity of superoxide dismutase 1 (SOD 1) enzyme in picograms/ml in the muscles of common carp *C. carpio* during periods (7, 15 and 30) days of the experiment for treatments A, the group treated with cadmium chloride, B the group treated with thyme oil, C the group treated with cadmium chloride and thyme oil.

3.3.6. Comparison of SOD 1 Enzyme Activity in Fish Muscles Between Treatments in One Period

The results of the study of the enzymatic activity of the SOD1 enzyme in fish muscles showed an increase in enzyme activity in all treatments for one period except for the 30 days when treated with the combined group. The enzyme activity was less than the normal level in the 7 days. The highest activity was in the thyme oil treatment. There was a significant difference between treatments at the probability level $P \leq 0.05$ Figure (30 A). However, after 15 days of the experiment, a significant convergence was observed between the treatment with cadmium chloride and the combined treatment Figure (30 B). After 30 days of the experiment, a difference in enzyme activity was observed. There were significant differences between treatments at $P \leq 0.05$, as the highest activity was in the treatment with cadmium chloride. The lowest activity was in the combined treatment of cadmium chloride and thyme oil, Figure (30 C).

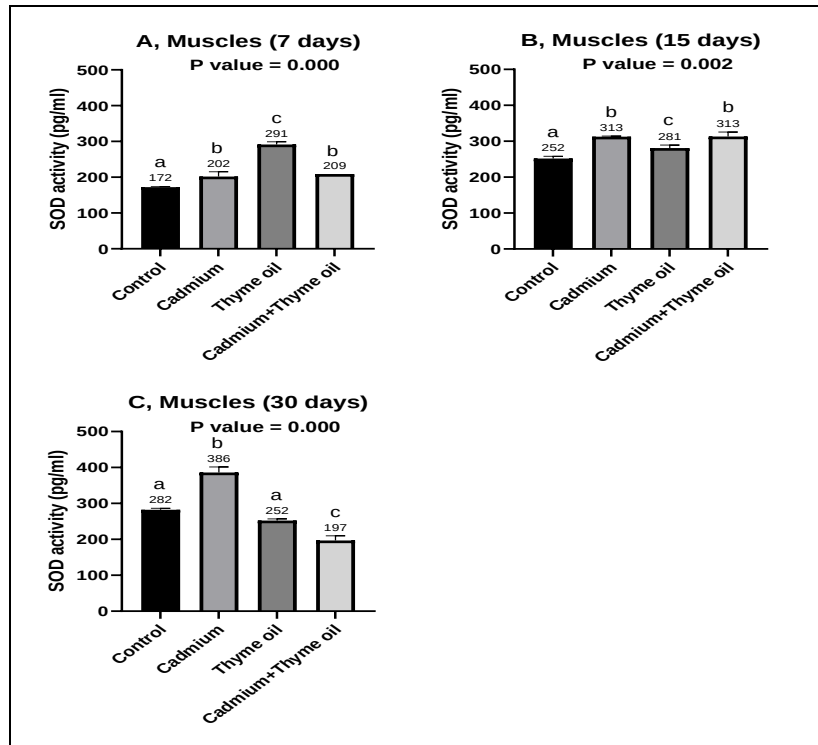


Figure 30. Shows the activity of the enzyme superoxide dismutase 1 (SOD 1) in picograms/ml in the muscles of common carp fish *C. carpio* for the three treatments, 7 days, B 15-day period, C 30-day period.

3.3.7. Comparison of the Activity of the Enzyme SOD 1 Between the Fish Organs in One Period and One Treatment

When comparing the activity level of the enzyme SOD in the organs of common carp fish (liver, gills and muscles) in one period of the experiment, a difference in the activity of the enzyme was found between the organs during one period. In Figure (31 A, B and C), the highest activity of the enzyme (SOD1) was in the treatment with cadmium chloride for 7 days in the liver. During the periods of 15 and 30 days of the experiment $P \leq 0.05$, the activity of the enzyme in the muscles increased significantly at the probability level ($P \leq 0.05$). As for the treatment with cadmium chloride and thyme oil together, an increase in the enzyme was observed during the periods of 7 and 15 in the muscles, while it increased in the last period of 30 days in the gills with a significant difference at ($P \leq 0.05$) as in Figure (32 A, B and C). The results showed that the increase in the enzyme activity in the treatment with thyme oil varied. It increased in the muscles during 7 days and 15 days. A significant convergence in the level of enzyme activity was observed in both the liver and muscles at the probability level ($P \leq 0.05$). After 30 days of the experiment, the enzyme activity level in the gills increased compared to the rest of the organs, while it was significantly closed in the liver and muscles (Figure 33 A, B, and C).

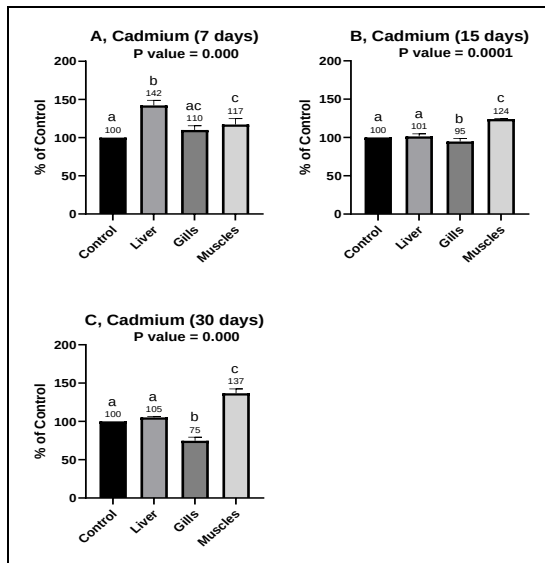


Figure 31. Shows the activity of the enzyme superoxide dismutase1 (SOD 1) in picograms/ml in the organs of common carp *C. carpio* (liver, gills, and muscles) for the group treated with cadmium chloride during the experimental periods, 7 days, B 15-day period, C 30-day period.

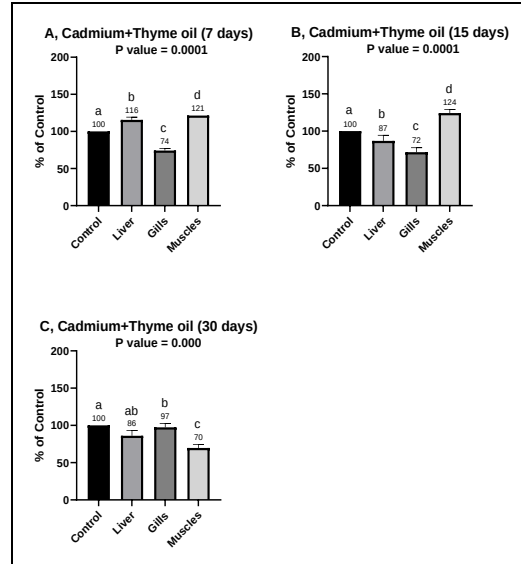


Figure 32. Shows the activity of the enzyme superoxide dismutase1 (SOD 1) in picograms/ml in the organs of common carp *C. carpio* (liver, gills, and muscles) for the group treated with cadmium chloride with thyme oil during the experimental periods, 7 days, B 15-day period, C 30-day period.

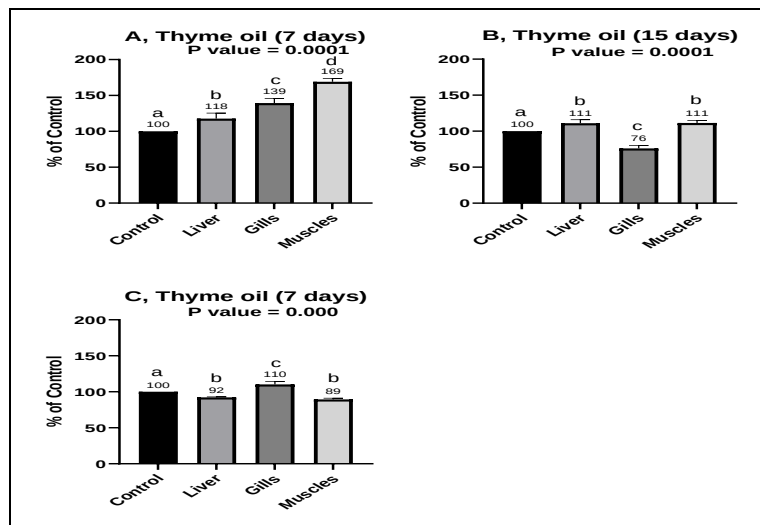


Figure 33. Shows the activity of the enzyme superoxide dismutase 1 (SOD 1) in picograms/ml in the organs of common carp *C. carpio* (liver, gills and muscles) for the group treated with thyme oil during the experimental periods, 7 days, B 15-day period, C 30-day period.

4. Discussion

4.1. Histopathological Changes

The results of the histological study showed the presence of some histopathological changes in the gills, liver and kidneys of fish treated with an effective dose of cadmium chloride at a concentration of 56.2 mg/L. These are considered natural reactions against many pollutants in the aquatic environment and are defensive means that thus work to obstruct the entry of pollutants [13]. The results showed a change in the shape and adhesion of the secondary gill plates in the early periods of exposure, followed by a separation in the epithelial layer, which is consistent with what was mentioned by [14]. The adhesion of the gill plates is due to the increase in mucus secretion from the mucous cells. It may be a protective reaction due to pollutants that stimulate the epithelial cells to increase the secretion of mucus. It is known that the composition of mucus is a glycoprotein [15]. Pollutants may change the composition of this glycoprotein, which leads to adhesions between the secondary gill plates [16]. The distance between the blood and blood vessels increases when the epithelial tissue layer of the secondary gill plates and pollutants in the external environment separate in order to reduce the effect of pollutants in the fish environment [17]. The occurrence of separation may be a result of the effect of cadmium on adhesion proteins and adhesion bonds between the membrane Basal and epithelial tissues, which leads to damage to these proteins and separation of the epithelial tissue, which leads to a malfunction in the function of the epithelial tissue such as absorption, secretion and exchange [18]. One of the forms of inflammatory response in fish tissues is vascular congestion as a result of the toxic effect of pollutants in the aquatic environment. This indicates its effect on blood flow and the factors controlling it, or it may be due to toxic pollutants that increase blood viscosity. This leads to slow blood flow and the appearance of congested blood vessels. It is known that endothelial cells lining the capillaries release factors that control blood flow [19]. Increased blood flow usually occurs towards a specific area, especially the area exposed to damage, and not others. This leads to congestion of the blood vessels and, thus, their expansion [20]. The toxic effect of cadmium on fish cells increases with continued oxygen deficiency, in addition to its toxic effect on cell membranes. It affects the lateral connections between the cells lining the blood vessels. This leads to the breakdown of these bonds and thus increases their permeability to leak red blood cells out of the blood vessel, leading to bleeding [21]. Vacuolar degeneration occurs due to the imbalance in osmotic pressure. This is reflected in the cells and leads to the accumulation of water inside the cells. When exposure to toxic substances continues, this leads to necrosis due to the weakness in blood delivery to the cells [22]. The occurrence of degeneration may be due to the inhibition of cellular protein synthesis, which leads to a decrease in adenosine triphosphate (ATP) as a result of the increase in the glycolysis process. This leads to a decrease in cellular pH [23]. It may be due to the accumulation of polysaccharides, water or fatty substances in the cell, which may appear in the form of vacuoles filling the cell cytoplasm, which arises as a result of a defect in the vital functions of the cell, such as metabolism, for example. This may lead to cell atrophy and, consequently, death [24]. It is noted that the cells go through gradual stages. It begins first with the degeneration of the cell cytoplasm. Then changes at the level of the nucleus. It includes the loss of its clear nucleus. A gradual decay of the hematoxylin stain in the nucleus accompanies this. These changes end with the decomposition of the cytoplasm and the nucleus, and nothing remains of the cells except their outer borders, which decompose, leaving spaces containing remnants of the decomposed cells. This is a natural reaction to the toxic effect of cadmium. The swelling that occurs in some liver cells is a result of the toxins of pollutants entering the fish body, which affect the permeability of the cell membranes by causing some changes in the composition of those membranes. This swelling is due to the association of those pollutants with

the proteins of the cell membranes, causing a change in the function of those membranes. This leads to a loss of the permeability property of the cell membranes, causing a malfunction and change in their function [24]. Hyperplasia was observed in the secondary gill plates and the cells spread at different distances in the space between the secondary gill plates. Hyperplasia reaches the free end of the gill plate with increasing exposure time to the pollutant. This is due to the reduction in the amount of oxygen exchange with the capillaries. This leads to hypoxemia, which is a lack of oxygen [25]. Hyperplasia is one of the tissue reactions when exposed to pathogens. It may lead to cancer with continued exposure of fish to these pathogens [26]. At the same time, the cause of gill congestion is due to a partial local increase in blood pressure above the normal level or as a result of a disturbance in the distribution of blood within the systemic circulation in the fish's body due to the presence of pollutants in the aquatic environment [19]. Increased blood flow usually occurs towards a specific area, especially the one exposed to damage, rather than others. This leads to congestion of the blood vessels and then their expansion [20]. The gills are the most sensitive organ when any change occurs in the environment, as the severity of the histological pathological effect in the gills depends on the concentration of cadmium and the duration of exposure [27]. In liver tissue, a number of histopathological changes were observed. Their severity increases with increasing concentration of pollutants and increasing the duration of exposure. The toxic effect of cadmium on fish cells increases under conditions of oxygen deficiency, in addition to its toxic effect on the cell membrane. It affects blood vessels and increases their permeability, causing red blood cells to leak out of the blood vessel by passing through the cells lining the blood vessel, leading to bleeding [21]. Vacuolar degeneration occurs due to the imbalance in osmotic pressure. This is reflected in the cells and leads to the accumulation of water inside the cells and the occurrence of. When exposure to toxic substances continues, this leads to necrosis due to the weakness in blood delivery to the cells [22]. It appears that the cells go through gradual stages, starting with the degeneration of the cell cytoplasm. Then, changes occur at the nucleus level, including the loss of its clear nuclei. A gradual decay of the hematoxylin stain in the nucleus accompanies this. These changes end with the dissolution of the nucleus and cytoplasm. Only the outer borders of the cells appear, which disintegrate, leaving spaces containing remnants of the disintegrated cells. This is a natural reaction to the toxic effect. Melanomacrophage cells are often observed to accumulate in areas of necrotic cells in the tissue. Melanomacrophage cells are often observed to accumulate in areas where cell necrosis appears. Their presence is a defensive condition against cadmium toxicity. These cells increase their oxygen consumption with the help of NADPH oxidase enzymes and generate different types of reactive oxygen species, such as hydrogen peroxide, hydroxyl radicals, and superoxide ions [28]. The results of the histopathological study in the groups treated with thyme oil and the groups treated with cadmium with the addition of thyme oil to the fish feed showed the presence of histopathological changes in the organs with a decrease in cadmium accumulation. This means that low concentrations of it may be harmful to tissues, or perhaps the reason for the appearance of these changes is due to the pollution of the environment in which the experimental fish were raised. This caused the accumulation of cadmium in the tissues. Thyme oil works to reduce the accumulation of cadmium over time. The antioxidant activity of thyme oil is due to its content of phenolic compounds and flavonoids that can eliminate free radicals, extinguish reactive oxygen, or decompose peroxides [29]. Thyme oil contains volatile oils and phenolic substances, the most important of which are thymol and carvacrol, which have medical benefits [30]. Our study is consistent with many previous studies [31-36] that explain the effective role of thyme oil due to its containing many active compounds.

4.2. Antioxidants

Enzymatic and non-enzymatic antioxidants are a complex defence network surrounding the body. These antioxidants fight free radicals as well as resist their harmful effects on biomolecules and body tissues [9]. The antioxidant enzyme (SOD1) is one of the sensitive indicators that has been widely adopted to assess oxidative stress [37]. Therefore, the activity of the SOD1 enzyme was determined in our current study in some organs of common carp fish. To know the effect of oxidative stress resulting from exposure to cadmium, which generates free radicals. Therefore, the cell enters a state of oxidative stress [38]. Cells exposed to oxidative stress usually show different reactions. Antioxidants are generated and seek to reach a state of balance between the levels of antioxidants and the level of free radicals [39]. It is known that the production of free radicals stimulates the generation of metallothionein proteins that participate in the balance of metal ions and detoxification in cells. Therefore, cellular interference may play a role in removing excess metal ions from the extracellular environment by stimulating them to produce antioxidant generation pathways [40]. The results of the study showed that the level of SOD1 enzyme activity was more evident in the muscles in the cadmium-treated group during the 15th and 30th days of the experiment. Cadmium causes oxidative stress to cells. This leads to increased production of free radicals that can cause damage to cells and living tissues. To get rid of these free radicals, the SOD1 enzyme plays a major role in dismantling and converting superoxide to H₂O₂. Thus, the catalase enzyme works to get rid of the toxic H₂O₂ in cells by breaking it down into oxygen molecules and water [41]. In the group treated with thyme oil, an increase in the activity of the SOD1 enzyme in the muscles was observed during the 7th and 15th days of the experiment. This is due to the presence of active groups that provide support for the activity of the SOD1 enzyme through its antioxidant role and work to eliminate free radicals and reduce oxidative stress [42]. This indicates that the muscles produced a large amount of antioxidant enzymes to protect the body in the middle and late stages of cadmium stress through self-regulation of the body [43]. The decrease in the level of the SOD1 enzyme in the studied organs is due to the metabolism and physiology of the cell and its dealing with cadmium. The entry of cadmium into the cell stimulates the production of metallothionein proteins, which in turn works to raise the level of antioxidants [44]. It is known that the formation of the metallothionein-cadmium complex does not remain inside the cell and is transferred to other areas in the body. The cell regulates its distribution by the action of metallothionein proteins. This transfer may be one of the reasons that led to the reduction in antioxidant levels in the cell [45]. The results of the current study are consistent with [46] is the increase in the level of enzyme activity in the liver of fish (*Leuciscus cephalus*) when exposed to heavy elements. The results showed the role of thyme oil in reducing the levels of free radicals and trying to restore the cell to the balance between antioxidants and free radicals. A (significant) increase was observed when treating fish with cadmium chloride with thyme oil compared to treatment with cadmium chloride alone. This is due to the fact that thyme oil contains active compounds that act as antioxidants [47]. On the other hand, the presence of free radicals is a catalyst for activating cellular signals specific to the production of antioxidants [48]. The results of the current study are consistent with previous studies that highlighted the role of thyme oil in supporting antioxidant responses within the organism [49,50,51,52,47]. They all confirm the ability of thyme oil to enhance the production of antioxidants. It is worth noting that the study conducted by [53,54] demonstrated the ability of thyme oil to reduce free radical levels. This enhances the body's natural defence capabilities against oxidative stress. This puts thyme oil in the spotlight for researchers and consumers alike as a valuable addition to a health-promoting diet. It is worth noting that the active compounds found in thyme oil play a pivotal role, such as thymol, thanks to its effective properties that work to raise antioxidant levels and eliminate free radicals, as shown by previous studies conducted by

[55,56,57], confirm the effectiveness of other active compounds found in thyme oil, such as) 1,2-Benzenediol, O-4-methoxybenzoyl), which acts as an antioxidant. This shows the active compounds found in thyme oil that contribute significantly to its antioxidant capabilities. Adding thyme oil to the diet, either directly or through nutritional supplements, can enhance the effectiveness of the body's natural defences against oxidative stress and contribute to maintaining biological balance. Among the plants that have been used as antioxidants after being added to fish feed such as the white mustard plant *Brassica hirta*, which grows in the Mediterranean. Mustard oil was used as a nutritional supplement to evaluate the effects of growth, immune responses, digestive enzyme activities and antioxidants in rainbow fish *Oncorhynchus mykiss*. It was observed that the activity of SOD, GPX and CAT enzymes in the liver of fish improved, and the level of free radicals decreased [58].

Conclusion

Based on the results of the current study, studies of histopathological changes can be adopted as biomarkers of aquatic environment pollution, which vary according to the organ and tissue type. Therefore, gills are the most sensitive to any environmental pollutant due to their direct contact with the environment. It was found that the responses of antioxidants (SOD1) depend on the concentrations of pollutants and the extent of exposure to them. Therefore, they can be considered sensitive biomarkers for biological monitoring of the aquatic environment polluted with cadmium. The results showed that thyme oil has an important role in reducing the toxicity of cadmium chloride in fish.

References

- [1] Lusková, V., Lusk, S., Halačka, K., & Vetešník, L. (2010). *Carassius auratus gibelio*—The most successful invasive fish in waters of the Czech Republic. *Russian journal of biological invasions*, 1(3), 176-180. <https://link.springer.com/article/10.1134/S2075111710030069#citeas>.
- [2] Kühlwein, H., Merrifield, D. L., Rawling, M. D., Foey, A. D., & Davies, S. J. (2014). Effects of dietary β -(1, 3)(1, 6)-D-glucan supplementation on growth performance, intestinal morphology and haemato-immunological profile of mirror carp (*C yprinus carpio* L.). *Journal of animal physiology and animal nutrition*, 98(2), 279-289.
- [3] Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The effects of cadmium toxicity. *International journal of environmental research and public health*, 17(11), 3782. <https://doi.org/10.3390/ijerph17113782>.
- [4] Zhong, C., & Langrish, T. (2020). A comparison of different physical stomach models and an analysis of shear stresses and strains in these system. *Food Research International*, 135, 109296. <https://doi.org/10.1016/j.foodres.2020.109296>.
- [5] Wang, Z., Sun, Y., Yao, W., Ba, Q., & Wang, H. (2021). Effects of cadmium exposure on the immune system and immunoregulation. *Frontiers in Immunology*, 12, 695484. <https://doi.org/10.3389/fimmu.2021.695484>.
- [6] Dauqan, E. M., & Abdullah, A. (2017). Medicinal and functional values of thyme (*Thymus vulgaris* L.) herb. *Journal of applied biology and biotechnology*, 5(2), 017-022. <http://creativecommons.org/licenses/by-nc-sa/3.0/>.
- [7] Tirzitis, G., & Bartosz, G. (2010). Determination of antiradical and antioxidant activity: basic principles and new insights. *Acta biochimica polonica*, 57(2). <https://bibliotekanauki.pl/articles/1040394.pdf>.
- [8] Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford university press, USA.
- [9] Ighodaro, O. M. & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire

- antioxidant defence grid. *Alexandria Journal of Medicine*, 54 (4): 287-293. doi: 10.1016/j.ajme.2017.09.001
- [10] Liu, R., Lai, T., Xu, Y. & Tian, S. (2013). Changes in physiology and quality of Laiyang pear in long time storage. *Scientia horticulturae*, 150: 31-36. <https://doi.org/10.1016/j.scienta.2012.10.017>.
- [11] Humason, G.L. (1972). Animal tissue techniques. Freeman, W.H. (3th ed.). *San Francisco press*. UAS.PP.64. <https://www.cabidigitallibrary.org/action/doSearch?do=Animal+tissue+techniques>.
- [12] Yin, X.L.; Mu,L.; Fu ,S.; Wu, L.; Wu, H.; Bian.X.; Wei,X. and Ye.W. (2019). Expression and characterization of Nile tilapia (*Oreochromis niloticus*) secretory and membrane-bound IgM in response to bacterial infection. *J. Aquac.* 508:214–222.
- [13] Ortiz, J.B.; Gonzalez,R; Canales, M.L. and Sarasqute, C. (2003). Histopathological changes induced by lindane in various organs of fish. *Sci. MAR.*, 67(1): 53-6 . <https://doi.org/10.3989/scimar.2003.67n153>.
- [14] Ahmeda, M.D.K.;Parvina, E. ;Islamb,M.D.M. ;Akterc ,M.S. ;Khand, S. and Al-Mamune, M.D.H.(2014). Lead- and cadmium-induced histopathological changes in gill, kidney and liver tissue of freshwater climbing perch *Anabas testudineus* (Bloch, 1792). *Chemistry and Ecology*, 2014 Vol. 30, No. 6, 532–540, <http://dx.doi.org/10.1080/02757540.2014.889123>.
- [15] Ferguson, H. W. (1989). Gill and pseudobranchs In: *Sysremic pathology of fish. Iowa State Univercity press/ames*, 11-40.
- [16] Sharma, R.R.; Pandey, A.K. and Shukla, G.R.(2001). Histopathological alterations in fish tissues induced by toxicity. *Aquacult.*, 2(1): 31-43.
- [17] Hinton, D.E. and Lauren, D.J. (1990). Integrative histopathological effects of environmental stressors on fishes . *J. Am. Fish Soc. Symp.*, 8: 51-66. <https://cir.nii.ac.jp/all?q=American%20Fisheries%20Society%20Symposium>
- [18] Yaung,B.;Woodford,P.H. and Dowd,G .O. (2014).wheaten furetinal *histologyand colour atlas*,6th ed.,452p.
- [19] Weissman, G.(1992). Inflammation :historical perspective. In Gallin, J.I *et al.* Inflammation: Basic principles and clinical correlates, 2nd ed. New York. Ravenpress, pp. 5-13
- [20] Atamanalp, M.; Sisman, T.; Geyikoglu, F. and Topal, A. (2008). The histopathological effects of copper sulphate on rainbow trout liver (*Oncorhynchus mykiss*). *J. Fish. Aquat. Sci.*, (In press). <http://www.academicjournals.net/2/c4p.php?id=2&theme=2&jid=jfas>
- [21] Slauson, D. O., & Cooper, B. J. (2002). Mechanisms of disease: A textbook of comparative general pathology (No. Ed. 3). Mosby Inc. <https://www.cabidigitallibrary.org/doi/full/10.5555/20033208813>.
- [22] Kumar VM, Cotran MD, Robbins MD. Basic pathology.7th editionSauners Philadelphia. (2003);p.4. <http://www.vetmedmosul.org/ijvs>.
- [23] Al-Ali, A. A., Al-Sultan, E. Y., & AL-Sultan, F. A. (2011). Histopathological effects of toxic alga *Nostoc muscurum* on juvenile grass carp fish (*Ctenopharyngodon idella* Val. 1844). *J Marsh Bull*, 6, 32-61.
- [24] Stevens,A.;Lowe,J.;Scott,I.andDamjanov,I.(2009).Corepathology. 3thed. Mosby ,*Elsvier*, P 632.
- [25] Stryer,D.S. and Rubin,E.(2012).Cell injury .Ch1,p35.
- [26] AL- Ali. A. Abdel Hassan (2009). Histopathological changes in black molly *Poecilia sphenops* and its embryos to a sublethal concentration cadmium. *Basra Research Journal*, 35(6), pp. 102-127.
- [27] Ribeiro, C. O., Fanta, E., Turcatti, N. M., Cardoso, R. J., & Carvalho, C. S. (1996). Lethal effects of inorganic mercury on cells and tissues of *Trichomycterus brasiliensis* (Pisces; Siluroidei). *Biocell: official journal of the Sociedades Latinoamericanas de Microscopia Electronica...* et al, 20(3), 171-178. https://pubmed.ncbi.nlm.nih.gov/?term=Cardoso+RJ&cauthor_id=9091098.
- [28] Roberts, R.J.; Agius, C.; Saliba, C.; Bossier, P. and Sung , Y.Y.(2010). Heat shock proteins (chaperones) in fish and shellfish and their potential role in relation to fish health: a review. *J. Fish Dis.*, 33: 789–801. <https://doi.org/10.1111/j.1365-2761.2010.01183.x>.

- [29] Wisam, S. U., Nahla, T. K., & Tariq, N. M. (2017). Antioxidant activities of thyme extracts. *Pakistan Journal of Nutrition*, 17(1), 46-50.
- [30] Khalil, S. R., Abd Elhakim, Y., Abd El-fattah, A. H., Farag, M. R., Abd El-Hameed, N. E., & Abd Elhakeem, E. M. (2020). Dual immunological and oxidative responses in *Oreochromis niloticus* fish exposed to lambda cyhalothrin and concurrently fed with Thyme powder (*Thymus vulgaris* L.): Stress and immune encoding gene expression. *Fish & shellfish immunology*, 100, 208-218. <https://doi.org/10.1016/j.fsi.2020.03.009>.
- [31] Nagoor Meeran, M. F., & Stanely Mainzen Prince, P. (2012). Protective effects of thymol on altered plasma lipid peroxidation and nonenzymic antioxidants in isoproterenol-induced myocardial infarcted rats. *Journal of Biochemical and Molecular Toxicology*, 26(9), 368-373.
- [32] Ulaiwi, H. K., Ghazi, A. M., & Auda, D. A. (2010). Assessment of protective effect of thymus on cadmium chloride that induced nephropathy and neuropathy in rats. *Kufa Journal for Veterinary Medical Sciences*, 1(1), 174-187.
- [33] Nagoor Meeran, M. F., Javed, H., Al Tae, H., Azimullah, S., & Ojha, S. K. (2017). Pharmacological properties and molecular mechanisms of thymol: prospects for its therapeutic potential and pharmaceutical development. *Frontiers in pharmacology*, 8, 260734.
- [34] Ahmed, O. M., Galaly, S. R., Mostafa, M. A. M., Eed, E. M., Ali, T. M., Fahmy, A. M., & Zaky, M. Y. (2022). Thyme oil and thymol counter doxorubicin-induced hepatotoxicity via modulation of inflammation, apoptosis, and oxidative stress. *Oxidative Medicine and Cellular Longevity*, 2022.
- [35] Ahmed, O. M., Galaly, S. R., Raslan, M., & Mostafa, M. A. M. (2020). Thyme oil and thymol abrogate doxorubicin-induced nephrotoxicity and cardiotoxicity in Wistar rats via repression of oxidative stress and enhancement of antioxidant defense mechanisms. *Biocell*, 44(1), 41.
- [36] Stojanović, N. M., Mitić, K. V., Nešić, M., Stanković, M., Petrović, V., Baralić, M., ... & Radulović, N. (2024). Oregano (*Origanum vulgare*) Essential Oil and Its Constituents Prevent Rat Kidney Tissue Injury and Inflammation Induced by a High Dose of L-Arginine. *International Journal of Molecular Sciences*, 25(2), 941.
- [37] Elabd, H., Mahboub, H. H., Salem, S. M., Abdelwahab, A. M., Alwutayd, K. M., Shaalan, M., ... & Youssuf, H. (2023). Nano-Curcumin/Chitosan Modulates Growth, Biochemical, Immune, and Antioxidative Profiles, and the Expression of Related Genes in *Nile tilapia*, *Oreochromis niloticus*. *Fishes*, 8(7), 333. <https://doi.org/10.3390/fishes8070333>
- [38] Pruski, A. M., & Dixon, D. R. (2002). Effects of cadmium on nuclear integrity and DNA repair efficiency in the gill cells of *Mytilus edulis* L. *Aquatic toxicology*, 57(3), 127-137. [https://doi.org/10.1016/S0166-445X\(01\)00192-8](https://doi.org/10.1016/S0166-445X(01)00192-8).
- [39] Jones, D. P. (2024). Redox Organization of living System. *J. Free Radical Biology and Medicine*, 217(1), 179-189. <https://www.nature.com/articles/s41580-024-00730-2#change-history>.
- [40] Finkel, T. (2011). Signal transduction by reactive oxygen species. *Journal of Cell Biology*, 194(1), 7-15. <https://doi.org/10.1083/jcb.201102095>.
- [41] Rosa, A. C., Corsi, D., Cavi, N., Bruni, N., & Dosio, F. (2021). Superoxide dismutase administration: A review of proposed human uses. *Molecules*, 26(7), 1844. <https://doi.org/10.3390/molecules26071844>.
- [42] Hoseini, S. M., & Yousefi, M. (2019). Beneficial effects of thyme (*Thymus vulgaris*) extract on oxytetracycline-induced stress response, immunosuppression, oxidative stress and enzymatic changes in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture nutrition*, 25(2), 298-309. <https://doi.org/10.1111/anu.12853>.
- [43] Kim, B. M., Rhee, J. S., Park, G. S., Lee, J., Lee, Y. M., & Lee, J. S. (2011). Cu/Zn- and Mn-superoxide dismutase (SOD) from the copepod *Tigriopus japonicus*: molecular cloning and expression in response to environmental pollutants. *Chemosphere*, 84(10), 1467-1475. <https://doi.org/10.1016/j.chemosphere.2011.04.043>.
- [44] Feng, Y., Huang, X., Duan, Y., Fan, W., Duan, J., Wang, K., ... & Yang, S. (2018). The Effect of Vitamin E and Metallothionein on the Antioxidant Capacities of Cadmium-Damaged Liver in

- Grass Carp *Ctenopharyngodon idellus*. *BioMed research international*, 2018(1), 7935396. <https://doi.org/10.1155/2018/7935396>.
- [45] Atif, F., Kaur, M., Yousuf, S., & Raisuddin, S. (2006). In vitro free radical scavenging activity of hepatic metallothionein induced in an Indian freshwater fish, *Channa punctata* Bloch. *Chemico-biological interactions*, 162(2), 172-180. <https://doi.org/10.1016/j.cbi.2006.06.006>.
- [46] Hermenean, A., Damache, G., Albu, P., Ardelean, A., Ardelean, G., Ardelean, D. P., ... & Dinischiotu, A. (2015). Histopathological alterations and oxidative stress in liver and kidney of *Leuciscus cephalus* following exposure to heavy metals in the Tur River, North Western Romania. *Ecotoxicology and environmental safety*, 119, 198-205.
- [47] Ghafarifarsani, H., Hoseinifar, S. H., Javahery, S., & Van Doan, H. (2022). Effects of dietary vitamin C, thyme essential oil, and quercetin on the immunological and antioxidant status of common carp (*Cyprinus carpio*). *Aquaculture*, 553, 738053. <https://doi.org/10.1016/j.aquaculture.2022.738053>.
- [48] Kalogeris, T., Bao, Y., & Korthuis, R. J. (2014). Mitochondrial reactive oxygen species: a double edged sword in ischemia/reperfusion vs preconditioning. *Redox biology*, 2, 702-714.
- [49] Honcharenko, V., Tkachenko, H., Nachychko, V., Prokopiv, A., & Osadowski, Z. (2018). Total Antioxidant Capacity in the Muscle Tissue of Rainbow Trout (*Oncorhynchus mykiss* Walbaum) Under in Vitro Incubation with Leaf Extracts of Some Thymus (Lamiaceae) Representatives. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, (2). <http://sandbox.agrobiodiversity.uniag.sk/scientificpapers/article/view/155>
- [50] Mirghaed, A. T., Hoseini, S. M., Hoseinifar, S. H., & Van Doan, H. (2020). Effects of dietary thyme (*Zataria multiflora*) extract on antioxidant and immunological responses and immune-related gene expression of rainbow trout (*Oncorhynchus mykiss*) juveniles. *Fish & Shellfish Immunology*, 106, 502-509. <https://doi.org/10.1016/j.fsi.2020.08.002>.
- [51] Yousefi, M., Ghafarifarsani, H., Hoseini, S. M., Hoseinifar, S. H., Abtahi, B., Vatikov, Y. A., ... & Van Doan, H. (2022). Effects of dietary thyme essential oil and prebiotic administration on rainbow trout (*Oncorhynchus mykiss*) welfare and performance. *Fish & Shellfish Immunology*, 120, 737-744. <https://doi.org/10.1016/j.fsi.2021.12.023>.
- [52] Jasim, S. A., Davoodi, R., Yasin, G., Shichiyakh, R. A., Alkadir, O. K. A., Taifi, A., ... & Abarghouei, S. (2023). Potential protective effects of thyme essential oil on growth, hematology, immune responses, and antioxidant status of exposed to malathion. *Annals of Animal Science*, 23(2), 481-493. <https://doi.org/10.2478/aoas-2022-0064>.
- [53] Aldosary, S. K., El-Rahman, S. N. A., Al-Jameel, S. S., & Alromihi, N. M. (2021). Antioxidant and antimicrobial activities of *Thymus vulgaris* essential oil contained and synthesis thymus (*Vulgaris*) silver nanoparticles. *Brazilian Journal of Biology*, 83, e244675. <https://doi.org/10.1590/1519-6984.244675>.
- [54] Lawrence, L., Chika, A., & Abel, A. (2023). Extraction and Characterization of the Essential Oil from the Leaves of *Ocimum basilicum* and Evaluation of its Antioxidant Properties. *Journal of Biochemistry International*, 10(1), 92-104. <https://doi.org/10.56557/job/2023/v10i18682>.
- [55] Alagawany, M., Farag, M. R., Abdelnour, S. A., & Elnesr, S. S. (2021). A review on the beneficial effect of thymol on health and production of fish. *Reviews in Aquaculture*, 13(1), 632-641.
- [56] Khalil, S. R., Zheng, C., Abou-Zeid, S. M., Farag, M. R., Elsabbagh, H. S., Siddique, M. S., ... & Elkhadrawey, B. A. (2023). Modulatory effect of thymol on the immune response and susceptibility to *Aeromonas hydrophila* infection in *Nile tilapia* fish exposed to zinc oxide nanoparticles. *Aquatic Toxicology*, 259, 106523. <https://doi.org/10.1016/j.aquatox.2023.106523>.
- [57] El-Guendouz, S., Aazza, S., Anahi Dandlen, S., Majdoub, N., Lyoussi, B., Raposo, S., ... & Graça Miguel, M. (2019). Antioxidant activity of thyme waste extract in O/W emulsions. *Antioxidants*, 8(8), 243. <https://doi.org/10.3390/antiox8080243>
- [58] Salem, M. O. A., Taştan, Y., Bilen, S., Terzi, E., & Sönmez, A. Y. (2022). Effects of white mustard (*Sinapis alba*) oil on growth performance, immune response, blood parameters, digestive and antioxidant enzyme activities in rainbow trout (*Oncorhynchus mykiss*). *Fish & shellfish immunology*, 131, 283-299. <https://doi.org/10.1016/j.fsi.2022.10.006>

Water Quality Index Estimation Al-Hawija Irrigation Project, Kirkuk, Iraq

Omar Mutlag Ali¹ and Awaz Bahrooz Mohammed²

^{1,2} Biology Department, College of Sciences, University of Kirkuk, Kirkuk, Iraq.

¹E-mail: scbm22093@uokirkuk.edu.iq

²E-mail: dr.awaz.eco@uokirkuk.edu.iq

Abstract. The current study aimed to Estimated Water Quality Index Al-Hawija Irrigation Project, Kirkuk, Iraq. Water samples were collected at different months, from September of the year (2023), until February 2024, using containers Poly Athelin is 2.0 liters tight, sample water was transferred into the containers repeatedly. The results showed that the potential salinity value of the Hawija irrigation project water ranged between (-0.650 to -1.306 mmEq. L⁻¹), where the highest potential salinity value was in the fourth site if it reached -0.650 in March, while the lowest potential salinity value was in the fourth site as well if it reached -1.306 in February. The sodium adsorption value of the Hawija irrigation project water ranged between (0.516 to 0.621 mmEq. L⁻¹), where the highest sodium adsorption value was at the fourth site, reaching 0.621 in May, while the lowest value of SAR was at the third site, reaching 0.516 in February. The value of residual sodium carbonate for the water of the Hawija irrigation project ranged between (2.885 to 3.16 mmEq. L⁻¹), where the highest value of residual sodium carbonate was in the first site if it reached 3.16 in March, while the lowest value of residual sodium carbonate was in the fourth site as well if it reached 2.885 in February. the results of the nitrate pollution index values in the water of the Al-Hawija irrigation project was indicated that the water is safe in terms of the nitrate pollution index and does not pose any danger. The results of the model indicate that all study samples are of the (medium quality) category for drinking and civil uses. This relative deterioration is mainly due to the high concentrations of total dissolved salts, nitrate ions, and fecal coliform bacteria, which negatively affected the values of the sub-index (Qi). Finally, the current study was found that the raw Euphrates River water was very good for watering livestock and poultry and for irrigation purposes, based on the results of the physical and chemical properties that were examined. The reason for the deterioration of water quality for drinking and domestic use is mainly due to bacterial contamination.

Keywords. Wastewater, Physical properties, Chemical properties, TDS.

1. Introduction

The Water Quality Index (WQI) is defined as a tool that describes the overall quality of water by combining complex and technical information about water quality into a single numerical value with inferential meaning [1]. The Water Quality Index (WQI) is an accurate and easy-to-understand method for assessing water quality for various purposes [2]. The Water Quality Index (WQI) is one of the most important tools used to assess water quality in general because it provides the final form in a single value. This concept was initially developed by the scientist

Horten in 1965. Since then, many other transformations have occurred in defining the Water Quality Index as proposed by different scientists and researchers. The index aims to facilitate the effective management of multiple water sources, whether surface or groundwater, and determines their suitability for various civil, agricultural and industrial uses [3]. Water Quality Index (WQI) is an important tool for determining the quality of drinking water in urban, rural and industrial areas. It is defined as an index that reflects the combined effect of different water quality properties that are considered to calculate the water quality index [4]. The WQI has shown a valuable and unique classification to depict the overall condition of water quality in a single term which helps in selecting the appropriate treatment technology to meet the problems concerned. However, the WQI depicts the combined effect of different water quality parameters and conveys water quality information to the public and legislative decision makers for the suitability of drinking water sources and other uses [5]. The water quality index has been shown to be an important criterion in reconciling water supply and demand by providing an adequate supply of fresh water to meet biological needs that are a vital aspect of integrated environmental management and sustainable development [6]. The water quality index reflects the interconnected effects of the various studied characteristics in determining water quality by providing a single value instead of large amounts of data and representing it in a simple way for experts in the field to understand as well as for non-specialists to understand [7]. So, the current study aimed to estimated Water Quality Index Al-Hawija Irrigation Project, Kirkuk, Iraq.

2. Materials and Methods

2.1. Sampling Methods

Al-Hawija irrigation project (fig: 1) is located in Al-Hawija district, Kirkuk Governorate, located (70) km southwest of Kirkuk city, on the left side of the Lower Zab River, bordered on the west by Al-Abbasi district and on the east by Riyadh district, and is located between latitudes (35°,38.55) and (35°,51.30) north, and longitudes (43°,56.30) and (43°,38.30) east. Containers have been cleaned with diluted nitric acid, and Poly Athelin is 2.0 liters tight. Once organic debris and pollutants have been removed, give them a thorough wash with premium distilled water.

The containers were then twice pushed full of sample water after that, and it remained filled with distilled water until the sample arrived. Glass 300 ml containers were utilized before sampling was done in each field sample. Each site was sample both before and after processing, with the nozzle facing toward the stream of water, while the container was held from below and filled with water to a depth of over 30 cm. After that, the cover was taken off to let water fill the container, and it was shut right after.

Lastly, instructions were given to the bottles on the date of the sample take. Additionally, until they were sent to the lab for sampling, all extra data and the containers were stored in fiber boxes with ice. In the refrigerator, the samples were kept at a specific temperature. Sulfate, phosphate, nitrate, TDS, EC, salinity, pH, and temperature samples were subjected to analysis at 4.0 0C. As soon as the laboratory reopened, samples totaling 150 milliliters were collected for the heavy element examination. The samples were filtered, and then they were acidified with a few drops of strong nitric acid (HNO₃). The three functions of drinking, irrigation, and livestock have the selected parameters grouped based on their relative importance. The selective parameters had been listed with their standard values in Table (1).

Table 1. WHO standard values for selective parameters and for three purposes.

Drinking purpose			Irrigation purpose			Livestock purpose		
Parameter	unit	Limits	Parameter	Unit	limits	Parameter	unit	Limits
pH	-	6.5-8.5	EC	µs/cm		TDS	mg/l	10000
CL	mg/l	250	SAR	(meq/l) ^{0.5}	2700	SO ₄	mg/l	1000
Na	mg/l	200	TDS	mg/l	15	pH	-	6.5-8.5
SO ₄	mg/l	200	Na	mg/l	1750	NO ₃	mg/l	440
TDS	mg/l	1000	CL	mg/l	200	EC	µs/cm	12500
Ca	mg/l	200	pH		250			
Mg	mg/l	50						
NO ₃	mg/l	10						
Turbidity	NTU	≤5						
EC	µs/cm	2000						

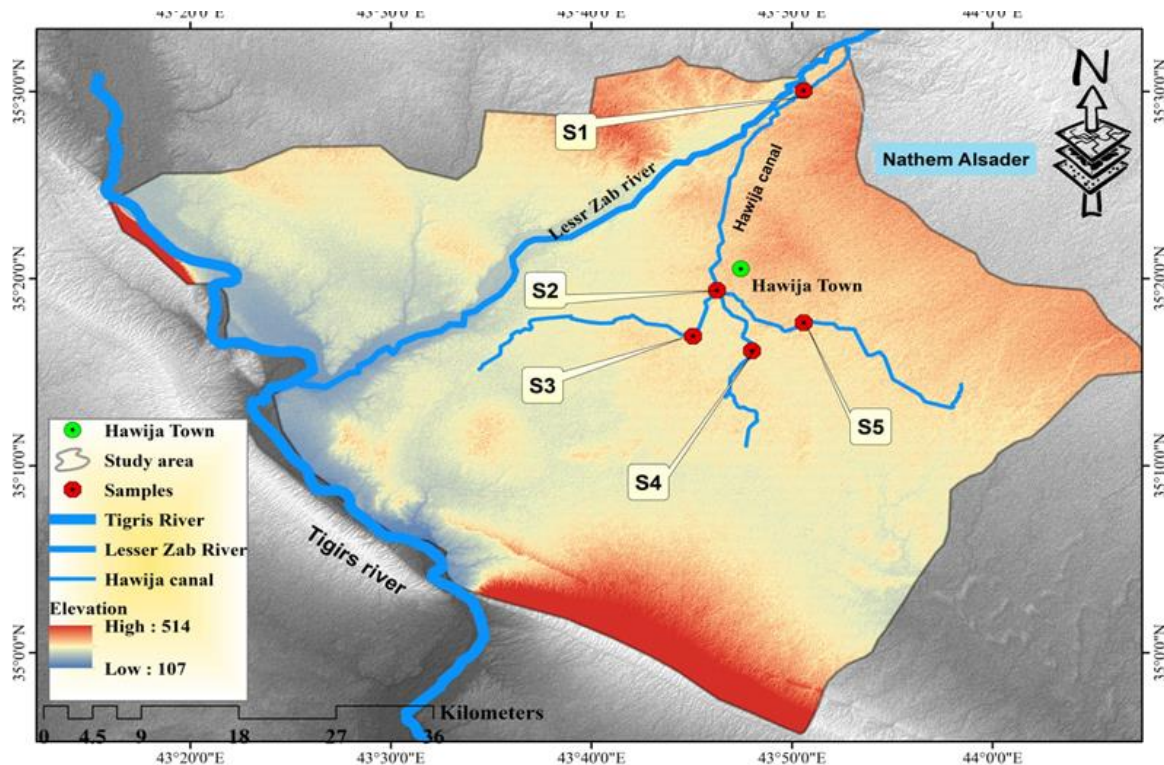


Figure 1. Detailed map of Al-Hawija irrigation project (GIS program).

2.2. Physical Tests of Water

- Turbidity: Turbidity was analyzed by using (Hach 2010) [8], and reported in Nephelometric Turbidity Units (NTU).
- Electric Conductivity- (EC): Specific Electrical Connectivity Using HANNA Model (EC-meter) Device H.199301 Microcosmenise/CM Unit.
- Total Dissolved Solid (T.D.S): Total dissolved solids in milligrams per liter (mg/L) using the HANNA model type device H.199301 standard (2000.0-0.0).
- Chemical tests of water: The pH of the samples was measured using a pH meter made by HANNA (Microprocessor HI 9321). Calcium (Ca²⁺), and Magnesium (Mg²⁺) were

measured by (EDTA titrimetric method), Sodium (Na⁺) and Potassium (K⁺) cations were determined by Flame Emission Photometer method, the analysis of the parameters for chloride (Cl⁻) according to [9]. The (Argentometric method) was used to determine Chloride (Cl⁻) [2].

3. Results and Discussion

3.1. Physical Factors

Table (2) shows the average of some physical and chemical properties of the study samples according to the collection site, and there were no significant differences in the properties between the study sites.

Table 2. Shows the average of some physical and chemical properties of the study samples according to the site.

Site /	1	2	3	4	5
Parameters					
temperature	19.17±6.99	19.33±7.61	19.17±7.76	18.67±7.45	18.5±8.09
Turbidity	40.88±35.2	34.75±11.8	38.0±21.8	32.17±14.9	30.67±13.1
E.C	429.83±42.3	435±28.2	438.5±24.8	434.83±22.8	436.5±23.5
TDS	275±24.7	276.83±20.2	276.7±18.9	276±20.3	275.83±18.6
pH	8.13±0.15	7.98±0.53	8.2±0.46	8.25±0.35	8.28±0.38
DO	11.6±0.19	11.6±0.29	11.3±0.25	11.5±0.12	11.5±0.29
BOD	1.03±1.2	0.62±0.25	1.02±0.51	1.0±0.76	0.73±0.16
Calcium	53.17±2.14	53.33±1.97	46.5±2.66	46.17±1.6	47.0±1.09
Magnesium	16.5±1.05	16.0±0.89	19.67±2.94	19.83±3.12	19.33±1.03
TH	199.8±2.04	203.7±4.5	199.3±1.03	196.5±4.5	196.0±2.19
Sodium	12.83±0.41	13.08±0.2	12.58±0.49	12.33±0.52	12.67±0.75
Chloride	18.17±1.17	18.67±0.52	17.67±0.82	18.33±0.52	18.17±0.75
Sulfates	47.5±2.26	47.33±0.82	45±0.89	45.83±0.98	45.17±1.83
Phosphate	0.164±0.25	0.015±0.03	0.018±0.02	0.016±0.02	0.011±0.01
Nitrite	0.008±0.007	0.004±0.003	0.006±0.005	0.005±0.003	0.006±0.004
Nitrates	4.47±6.39	4.37±6.69	4.28±6.01	4.27±6.27	1.93±0.45

Since they are the most obvious indicators of water quality, turbidity and total suspended solids (TSS) could be used to determine the quality of water with ease. Runoff, discharges, soil erosion, disturbed bottom sediments, and algae blooms are some of the sources of suspended solids particles [10]. Numerous factors can influence the turbidity and total suspended solids (TSS) of river water. These variables can be attributed to human activity, such as construction engineering and pollutant discharge, or to natural forces, such as excessive rainfall, mudslides, and landslides [11]. When the river approaches the city in the fall and winter, its turbidity and TSS levels are at their lowest; however, the rainy seasons of October and December cause the values to rise. During the rainy season, surface water, particularly in rivers, may have a high turbidity level [12]. The current study's results were also higher than those of Abdullah et al. [13], who discovered that the Euphrates River in southern Iraq had a mean value of 20.9 ± 6.26 and the lowest rate value of turbidity (12.27 NTU) in January and the highest (30.40 NTU) in October. The researcher explained why these results were different. The absence of rain, the large decline in river levels, and the increased development of fungi are the causes of the change in turbidity value and its rise in the summer. The present study's electrical conductivity results were not as high as those of Mohammed [14], who reported that, for the number of water treatment plants in Baghdad, the electrical conductivity values of raw water during the study period ranged between

(604-946) μ S/cm. By comparing the TDS results in the current study with Table (1), which shows the ideal limits for TDS, it is noted that the study stations are suitable for drinking purposes.

Table (5) shows pH values during the study period, the results show that the pH values in the raw water (river water) tended to be acidity with average (5.8 ± 1.24) and the reason for this was the type of soil in the study area, which is one of the main sources in changes of pH values [5], this result disagree with results of Al-Jeboory [15], who referred that the surface water in Iraq is alkalinity water. Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) ions are critical parameters for assessing water quality. Ca^{2+} and Mg^{2+} are directly related to hardness. The average calcium and magnesium levels in the samples were found to be (49.5 ± 2.73) and (18.8 ± 1.94), respectively. These levels fell short of the 100 mg/L and 30 mg/L WHO guidelines for calcium and magnesium, respectively [16]. In the study area, calcium concentration is higher than that of magnesium. This is due to the fact that limestone makes up the majority of the examined region's geological formation [17]. Furthermore, Bui et al. [18] report that these springs exhibit greater magnesium contents due to the abundance of carbonate rocks in the catchment area. High magnesium concentrations can irritate the gastrointestinal tract and promote water hardness [19]. A high chloride content may indicate the presence of sewage or industrial waste contamination, as well as the infiltration of salt water or seawater into an aquifer or freshwater body of water, even if the average chloride concentration of 18.5 ± 0.55 was less than the Iraqi limit of 250 mg/l [20]. Elevated levels of chloride are hazardous to most trees and plants and corrode metal buildings and pipelines. Reducing the number of chlorides in river water will make it easier to select the most effective experimental water treatment methods [21].

3.2. Irrigation Index's

3.2.1. Potential Salinity (P.S)

One of the important determinants of irrigation water resulting from the presence of chloride and sulfate ions, if their high concentration causes soil salinization, in addition to their negative effects on irrigated plants, which leads to a decrease in their productivity, as they cause burning (scorching) of plant leaves [22]. The potential salinity value of the Hawija irrigation project water, shown in Table (3) ranged between (-0.650 to $-1.306 \text{ mmEq. L}^{-1}$), where the highest potential salinity value was in the fourth site if it reached -0.650 in March, while the lowest potential salinity value was in the fourth site as well if it reached -1.306 in February.

Table 3. Levels, average and standard deviation of potential salinity for the study area (mmEq. L^{-1}).

Sites / Months	1	2	3	4	5	Mean	$\pm$ Sd
December	-1.094	-1.014	-1.099	-1.025	-1.029	-1.052	0.041
January	-1.046	-0.954	-1.032	-1.075	-0.996	-1.021	0.047
February	-1.218	-1.168	-1.181	-1.306	-1.060	-1.187	0.089
March	-0.788	-1.036	-1.049	-1.160	-1.078	-1.022	0.140
April	-1.100	-0.916	-0.949	-0.650	-0.879	-0.899	0.162
May	-0.936	-0.868	-0.914	-0.731	-0.813	-0.852	0.083

One of the important irrigation indicators that determine the quality of water is that when water is used for irrigation for successive years, low-soluble salts are deposited in the water and accumulate on the soil, which leads to damage to the soil [23]. Referring to Table (4) to compare the results with the global classifications of irrigation water, we find that all the values studied in

terms of potential salinity are suitable for irrigation due to the low concentrations of chloride and sulfate ions in the studied water [24].

3.2.2. Sodium Adsorption

The sodium adsorption value of the Hawija irrigation project water, shown in Table (5), ranged between (0.516 to 0.621 mmEq. L⁻¹), where the highest sodium adsorption value was at the fourth site, reaching 0.621 in May, while the lowest value of SAR was at the third site, reaching 0.516 in February. Sodium adsorption is considered an important indicator for determining the suitability of water for irrigation, as it is responsible for indicating the relationship between sodium, calcium and magnesium, as it affects soil properties and plant growth together in the event of a decrease in sodium versus an increase in calcium and magnesium. An increase in (SAR) values above the permissible limits leads to increased soil alkalinity and difficulty in reclaiming and cultivating it [25]. The results shown in Table (5) indicated that the SAR values of the studied samples ranged between (0.516) milliequivalent/liter in the water of the third site and (0.621) milliequivalent/liter in the water of the fifth site. In general, all the studied water samples, in terms of (SAR) values, were under the excellent category for irrigation according to the standards used in the study [26].

Table 4. Results of levels, average and standard deviation of the sodium adsorption ratio SAR for the studied water.

Sites / Months	1	2	3	4	5	mean	±Sd
December	0.567	0.567	0.537	0.521	0.527	0.544	0.022
January	0.563	0.597	0.517	0.514	0.527	0.544	0.036
February	0.538	0.545	0.516	0.498	0.519	0.523	0.019
March	0.572	0.563	0.563	0.559	0.580	0.567	0.009
April	0.554	0.577	0.595	0.575	0.585	0.577	0.015
May	0.540	0.580	0.616	0.611	0.621	0.594	0.034

3.2.3. Residual Sodium Carbonate (RSC)

The value of residual sodium carbonate for the water of the Hawija irrigation project, shown in Table (6), ranged between (2.885 to 3.16 mmEq. L⁻¹), where the highest value of residual sodium carbonate was in the first site if it reached 3.16 in March, while the lowest value of residual sodium carbonate was in the fourth site as well if it reached 2.885 in February. Bicarbonate is important in determining the quality of irrigation water. When its concentrations rise above the concentrations of calcium and magnesium combined, carbonates combine with calcium and magnesium and precipitate to form a solid layer on the soil surface [25, 27]. The results shown in Table 5 showed that all RSC values were positive, indicating the presence of sodium carbonate deposition on the soil due to the low concentration of carbonates and bicarbonates in the studied water samples compared to the concentrations of calcium and magnesium [28]. Therefore, the water is suitable and safe for use for irrigation in terms of RSC values and according to the international standards adopted in the study.

Table 5. Levels, average and standard deviation of bicarbonate ions for the study area (mmEq.L⁻¹).

Sites / Months	1	2	3	4	5	Mean	±Sd
December	2.89	2.97	2.885	2.984	2.934	2.933	0.045
January	2.97	2.98	2.902	2.984	2.967	2.961	0.033
February	2.98	2.98	2.967	2.885	2.967	2.956	0.040
March	3.16	2.98	2.967	2.885	2.967	2.992	0.101
April	2.98	3.00	2.967	2.984	2.984	2.983	0.012
May	2.93	3.03	2.984	2.967	2.967	2.976	0.036

3.3. Health Risks

3.4. Calculating the Health Risks of Nitrates HHR by Calculating the Daily Intake of Nitrates

Table (6) shows the extent of the chronic daily intake and the risk quotient of nitrate ions in the water of the Al-Hawija irrigation project.

Table 6. Chronic daily intake and risk quotient of nitrate ions in the studied waters.

Sites / Cohort			1	2	3	4	5
Children	Birth	CDI	0.531691	0.499911	0.509790	0.507886	0.230078
		HQ	0.332307	0.312445	0.318619	0.317429	0.143799
	3 month	CDI	0.611918	0.575342	0.586712	0.584521	0.264795
		HQ	0.382449	0.359589	0.366695	0.365325	0.165497
	6-2month	CDI	0.490879	0.461538	0.470659	0.468901	0.212417
		HQ	0.306799	0.288461	0.294162	0.293063	0.132761
	6-11 year	CDI	0.210390	0.197815	0.201724	0.200971	0.091042
		HQ	0.131494	0.123634	0.126078	0.125607	0.056901
	11-16year	CDI	0.146702	0.137933	0.140659	0.140133	0.063482
		HQ	0.091689	0.086208	0.087912	0.087583	0.039676
21-Old	16-18year	CDI	0.119901	0.112734	0.114962	0.114533	0.051885
		HQ	0.074938	0.070459	0.071851	0.071583	0.032428
	18-21year	CDI	0.191725	0.180265	0.183828	0.183141	0.082965
		HQ	0.119828	0.112666	0.114892	0.114463	0.051853
	F.P1 st tri	CDI	0.194014	0.182418	0.186023	0.185328	0.083956
		HQ	0.121259	0.114011	0.116264	0.115830	0.052472
	Male	CDI	0.162487	0.152775	0.155794	0.155212	0.070313
		HQ	0.101554	0.095484	0.097371	0.097008	0.043946
	Female	CDI	0.172206	0.161913	0.165113	0.164496	0.074519
		HQ	0.107629	0.101196	0.103195	0.102810	0.046574

3.4.1. Nitrate Pollution Index

Table (7) shows the results of the nitrate pollution index values in the water of the Al-Hawija irrigation project. It was found that the water is safe in terms of the nitrate pollution index and does not pose any danger.

Table 7. Results of nitrate pollution index values and water quality.

Site	NPI value	Water type
1	0.553	Clean level

2	0.580	Clean level
3	0.572	Clean level
4	0.573	Clean level
5	0.807	Clean level

3.5. Evaluation of Water Quality for Drinking

After applying the logarithmic model to evaluate water for drinking and civil use, the results shown in Tables (8,9) indicate that the water quality index values ranged between (413.26 to 428.70); the highest value in the studied water samples was for site 4 and the lowest value was in Site 1. When comparing the results in Table (9) for water quality classification, we note that all surface water in the study area was unfit for drinking.

Table 8. Standard limits, weight and relative weight of the studied drinking water standards.

Parameters	Standard limits	Attribute weight	Relative weight
Turb.	5.0	3	0.090909091
Ph	6.5-8.5	3	0.090909091
Ec25	1000	3	0.090909091
Do	5.0	4	0.121212121
T.Alk.	150	2	0.060606061
T. Hard.	500	2	0.060606061
Na	200	2	0.060606061
SO4	400	3	0.090909091
Cl	250	1	0.030303030
PO4	0.5	1	0.030303030
NO3	50	4	0.121212121
TPC.	10	5	0.151515152
Σ		33	1.000000000

Table 9. Results of the quality index values Qi, sub-index Sli, values and status of the quality of the water studied for drinking and domestic use.

Sites	Parameters	1	2	3	4	5
Turb.	Sli	74.327	63.273	77.273	59.636	55.745
	Qi	817.60	696.00	850.00	656.00	613.20
pH	Sli	8.6950	8.5348	8.7701	8.8235	8.8556
	Qi	95.647	93.882	96.471	97.059	97.411
Ec ₂₅	Sli	3.9090	3.9545	3.9909	3.9545	3.9727
	Qi	43.000	43.500	43.900	43.500	43.700
Do	Sli	6.5300	6.6020	6.6820	6.5662	7.2409
	Qi	53.879	54.466	55.127	54.172	59.737
T.Alk.	Sli	6.0280	6.0404	5.9394	5.9475	5.9798
	Qi	99.467	99.667	98.000	98.133	98.667
T. Hard.	Sli	2.4240	2.4727	2.4121	2.3879	2.3758
	Qi	40.000	40.800	39.800	39.400	39.200
Na	Sli	0.3880	0.3964	0.3818	0.3736	0.3848
	Qi	6.4000	6.5400	6.3000	6.1650	6.3500
SO4	Sli	1.0800	1.0757	1.0227	1.0409	1.0273
	Qi	11.875	11.833	11.250	11.450	11.300
Cl	Sli	0.1970	0.2040	0.2070	0.2034	0.2081
	Qi	6.4960	6.7320	6.8320	6.7120	6.8680
PO4	Sli	0.9940	0.0927	0.1073	0.0958	0.0648
	Qi	32.800	3.0600	3.5400	3.1600	2.1400
NO3	Sli	1.0840	1.0182	1.0424	1.0424	1.0182

Sites Parameters		1	2	3	4	5
TPC.	Qi	8.9400	8.4000	8.6000	8.6000	8.4000
	Sli	307.60	321.52	310.61	338.64	337.12
	Qi	2030.0	2122.0	2050.0	2235.0	2225.0
WQI	Value	413.26	415.17	418.43	428.70	423.99
	Status	Unfit	Unfit	Unfit	Unfit	Unfit

The NSFQI model is one of the most widely used global models due to its high accuracy in weighing each of the characteristics, which is reflected in the accuracy of the water quality assessment results [29,30]. The results of the model shown in (Table 8) indicate that all study samples are of the (medium quality) category for drinking and civil uses. This relative deterioration is mainly due to the high concentrations of total dissolved salts, nitrate ions, and fecal coliform bacteria, which negatively affected the values of the sub-index (Qi). The reason for the deterioration of water quality for drinking and domestic use is mainly due to bacterial contamination, as it is noted that the Sli values fluctuated between 20230 and 2235 resulting from the high Qi values that ranged between (307.6 to 338.6) followed by water turbidity, so water must be treated before use (turbidity precipitation and chlorine sterilization).

3.6. Evaluation of Water Quality for Watering Livestock and Poultry

The Weighted Subindex Model (WSM) developed by [31], was applied for watering livestock and poultry on eleven criteria

Table 10. Quality rating, subindex, water quality index, and water condition and type for watering animals.

Sites Prosperities		1	2	3	4	5
T.°C	Sli	4.44448	4.46763	4.35189	4.32874	4.35189
	Qi	80.0000	80.4267	78.3333	77.9166	78.3333
DO	Sli	4.48992	4.53883	4.59388	4.593881	4.97808
	Qi	53.8793	54.4662	55.1268	55.1268	59.7372
PH	Sli	9.99999	9.87653	10.1234	10.1852	10.2222
	Qi	90.0000	88.8889	91.1111	91.6667	92.0000
EC	Sli	2.23957	2.26562	2.28645	2.26562	2.27603
	Qi	26.8750	27.1875	27.4375	27.1875	27.3125
T.Alk.	Sli	1.01085	1.01288	0.99594	0.99729	1.00272
	Qi	18.1951	18.2317	17.9268	17.9512	18.0487
Ca ⁺²	Sli	0.13611	0.13556	0.13945	0.13667	0.13333
	Qi	4.90000	4.88000	5.02000	4.92000	4.80000
Mg ⁺²	Sli	0.26861	0.27542	0.27320	0.22222	0.22917
	Qi	9.67000	9.91500	9.83500	8.00000	8.25000
Na ⁺	Sli	0.35555	0.36333	0.35000	0.34167	0.35278
	Qi	4.26667	4.36000	4.20000	4.10000	4.23333
K ⁺	Sli	0.22639	0.22500	0.22222	0.22500	0.22639
	Qi	8.15000	8.10000	8.00000	8.10000	8.15000
Cl ⁻	Sli	0.30000	0.31111	0.31667	0.31111	0.31852
	Qi	5.40000	5.60000	5.70000	5.60000	5.73333
SO ₄ ⁼	Sli	0.79166	0.78833	0.75000	0.76333	0.75333
	Qi	9.50000	9.46000	9.00000	9.16000	9.04000
NO ₃ ⁻	Sli	0.47349	0.44192	0.45244	0.45244	0.44192
	Qi	3.40909	3.18180	3.25760	3.25758	3.18182

Sites Prosperities		1	2	3	4	5
PO ₄	Sli	0.01428	0.02077	0.02336	0.01947	0.21288
	Qi	0.51402	0.74770	0.84110	0.70093	7.66355
TPC	Sli	5.63889	5.88889	5.69445	6.22223	6.19445
	Qi	40.6000	42.4000	41.0000	44.8000	44.6000
WQI	Values	30.3898	30.6118	30.5734	31.0648	31.6937
	Status	Excellent	Excellent	Excellent	Excellent	Excellent
	Class	I	I	I	I	I

As for the rest of the studied characteristics, they were within the permissible limits for watering animals, despite their relative rise in some periods. For example, the temperature of the valley water rises to approach the upper permissible limits in some periods, reaching 28 degrees Celsius in the summer, and livestock and poultry need to drink relatively cold water, less than 18 degrees Celsius, to cool their body temperature and practice their activity in eating fodder. Therefore, the results of the current study confirm that the water of the Hawija irrigation project is suitable and excellent for watering livestock and poultry. The results of the current study agreed with the study of [20], in terms of the suitability of water for irrigation, as the researchers found that the raw Euphrates River water was very good for watering livestock and poultry and for irrigation purposes, based on the results of the physical and chemical properties that were examined.

Conclusions

The current study referred that the reason for the deterioration of water quality for drinking and domestic use is mainly due to bacterial contamination, as it is noted that the Sli values fluctuated between 20230 and 2235 resulting from the high Qi values, followed by water turbidity, so water must be treated before use (turbidity precipitation and chlorine sterilization).

References

- [1] Lukhabu D., Paul M., Noble K. Adapted Water Quality Indices: Limitations and Potential for Water Quality Monitoring in Africa. *Water*. 2023; 15:1736
- [2] Ewaid S. H., Salwan A., Nadhir A., Riyadh M. S. Development and Evaluation of a Water Quality Index for the Iraqi Rivers. *Hydrology* 2020, 7(3), 67.
- [3] Akinro A., Falowo O. Spatial assessment of groundwater quality and hydrochemical indices in Ore, Southwestern, Nigeria. *Journal of Engineering Environmental Science*, 2020; 4: 1-5.
- [4] Chaurasia AK, Pandey HK, Tiwari SK, Prakash R, Pandey P, Ram A (2018) Groundwater quality assessment using water quality index (WQI) in parts of Varanasi district, Uttar Pradesh, India. *J Geol Soc India* 92:76–82
- [5] Tyagi, Shweta, et al. "Water Quality Assessment in Terms of Water Quality Index." *American Journal of Water Resources* 1.3 (2013): 34-38.
- [6] Poonam T., Tanushree B., Sukalyan C. Water quality indices- important tools for water quality assessment: a review. *International Journal of Advances in Chemistry (IJAC)* 2013; 1(1): 1-8.
- [7] Al-Mashhdany M. H.S., Jasium A.A., Abdul Aziz Y.T. valuation of water for drinking and domestic purposes using (WQI). Case study : groundwater of Abu-Wagnah village, Tal-Afar district, Iraq. *Plant Archives*, 2020; 20(2): 3924-3928.
- [8] Abd Noor S. A. Study of The Standards Quality of A Wastewater Before and After Treatment In Kufa Treatment Plant. *JOURNAL OF KUFA FOR CHEMICAL SCIENCES*, 2013(8): 7-21.
- [9] Abdullah S. A., Abdullah A. H. J., Ankush M. A. (2019). Assessment of water quality in the Euphrates river, southern Iraq. *Iraqi Journal of Agricultural Sciences*, 50(1):221-228.
- [10] Majd Y., Csaba B., Szabó J., György D., Szilard S., Sándor N., István B., Alexandra S., János N., Imre S., Eva A., Istvan G. (2021). Changes in Algal Plankton Composition and Physico-Chemical Variables in a Shallow Oxbow Lake. *Water journal*. 13(17): 2339.

- [11] liu, W., Wang, S., Yang, R., Ma, Y., Shen, M., You, Y., Hai, K., Baqa, M.F., 2019. Remote sensing retrieval of turbidity in alpine rivers based on high spatial resolution satellites. *Rem. Sens.* 11, 1– 31.
- [12] Ali M. A. (2022). Turbidity and Total Suspended Solids as Physical Parameters for the Evaluating the Water Quality of Tigris River in Mosul City. *Journal of Engineering Science and Technology Review*, 15 (3): 153 – 157.
- [13] Abdullah S. A., Abdullah A. H. J., Ankush M. A. (2019). Assessment of water quality in the Euphrates river, southern Iraq. *Iraqi Journal of Agricultural Sciences*, 50(1):221-228.
- [14] Mohammed Z. B. (2015). Evaluation The Quality of Raw and Treated Water for Number of Water Treatment Plants in Baghdad, Using a Water Quality Index. *Engineering and Technology Journal*, 33(6): 429-1441.
- [15] Al-Jeboory, Muhsin Hamad Edham (2005). "A Study of Bacterial Indicators of Biological Pollution and Some Related Physical and Chemical Factors on River Tigris and Lower River Al-Zab in Al - Hawija and Tikrit Districts", M.Sc. Thesis, University of Tikrit, Iraq.
- [16] WHO. 2004. Guideline for Drinking Water Quality, 3rd Edition World Health Organization (WHO), Geneva.
- [17] Yasin S A., Alwan Q. A., Lanja O. T., Rezan S. A.(2023). Spring Water Quality Assessment Using Water Quality Index in Shawre Valley- Sulaymaniyah-Iraqi Kurdistan Region. *Zanco Journal of Pure and Applied Sciences*, 35(2): 118-130
- [18] Bui, Y., Lodhi, M. S., & Regional, N. E. 2020. Assessment of spring water quality using water quality index method: study from upper Subansiri district, Arunachal Pradesh, India. *International Journal of Science, Environment and Technology*, 9, 898-908.
- [19] Ibeneme, S.I., Ukiwe, L.N., Essien, A.G., Nwagbara, J.O., Nweze, C.A., Chinemelu, E.S. and Ivonye, C.A. 2013. Assessment of the Chemical Characteristics of a Spring Water Source at IfeOwutu, Ezinihite-Mbaise, Southeastern Nigeria. *American Journal of Engineering Research (AJER)*, 2 (10), 282-290.
- [20] Yasir M., Ali J., Laith B. (2023). Assessment of Water Quality of Tigris River and branches in Alkut City using Water Quality Index. *Wasit Journal of Engineering Sciences*, 10(3): 203-217.
- [21] Shukla, M.; Arya, S. (2018). Determination of chloride ion(Cl-) concentration in ganga river water by Mohr method at Kanpur, India. *Green Chem. Technol. Lett.*, 4: 6–8.
- [22] Rahimabadi, P.D., Masoudi, R., Alamdarloo, E.H., Khosravi, H., and Azarnivand, H. (2022). Assessment of groundwater quality and its suitability for irrigation using hydrogeochemical properties. *Environmental Resources Research*, 10(2), 221-236. Volume 10, Issue 2, December 2022, Pages 221-236.
- [23] Shil, S., Singh, U. K., and Mehta, P. (2019). Water quality assessment of a tropical river using water quality index , multivariate statistical techniques and GIS. *Applied water science*, 9, 1-21.
- [24] Al-Saffawi A Y T 2018 Application of the CCME WQI to Evaluate Water Quality for Drinking Purpose: A Case Study for Groundwater Quality of Al-Mahalibiyah Sub District, Nineveh Province/ Iraq. *Al-Rafidain Science J.*, Vol 27, Issue 5 / Botany / Special Issue of the Third Scientific Conference of Life Sciences, pp. 193-202.
- [25] Tegegne K. M., Solomon M. D., Mekonnen T. L., Ayelign M. K. Prevalence of Depression and Its Associated Factors among Prisoners in North Wollo Zone, Northeastern Ethiopia: A Cross-Sectional Study. *Depress Res Treat.* 2023; 2023: 261-2690.
- [26] Kasa T., Abeanezer L. B., Geleta T. N., Zenebe A., Daniel R. Investigation of Wabe River water fitness for agricultural and industrial purposes. 2022; 8(12): e11865.
- [27] Humadi, Adnan jassam (2021) Estimation of the water quality of selected wells from eastern wasit governorate for different human uses *Journal of Education and Scientific Studies Chemistry JESCS* Vol.17, No. 5, May 2021, ISSN 2413 – 4759
- [28] Gazzi, M. F., and Beg, A. A. F. (2022). Assessment of Groundwater Quality for Irrigation Purposes Using the Irrigation Water Quality Index (IWQI)/Al Rashidiya area-A Case Study. *Journal of College of Education*, 3(1813-0380).



- [29] APHA (1999) Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington DC.
- [30] Hamdan, A., Dawood, A. and Naeem, D. (2018). Assessment study of water quality index (WQI) for Shatt Al-arab River and its branches, Iraq. MATEC Web of Conferences 162, 05005.
- [31] Sharifinia, M., Ramezanpour, Z., Imanpour, J., Mahmoudifard, A. and Rahmani, T. (2019). Water quality assessment of the Zarivar lake using physico-chemical parameters and NSF-WQI indicator, Kurdistan province-Iran. Int. J. of Advanced Biol. and Biomedical Res. 7(1): 87-97.

Polycyclic Aromatic Hydrocarbons (PAHs) in Meat Cooked by Charcoal Grilled

Sameer Saleh Hazeem¹ and Awaz B. Mohammed²

^{1,2} Department of Biology, College of Science, Kirkuk University, Kirkuk, Iraq.

¹E-mail: scbhm013@uokirkuk.edu.iq

²E-mail: dr.awaz.eco@uokirkuk.edu.iq

Abstract. This study investigated the presence of polycyclic aromatic hydrocarbons (PAHs) in grilled chicken samples. The results, presented in Tables 1 and 2, revealed elevated levels of various PAHs, including benzo[a]pyrene, benzo[a]fluoranthene, benzo[a]pyrene (BaP), chrysene (Chr), naphthalene (Nap), fluoranthene (Flu), and pyrene (Phe). Chrysene, phenanthrene, pyrene, and benzo[a]pyrene was identified as the most prevalent PAHs in the grilled chicken samples. Despite the elevated levels of PAHs, a non-carcinogenic risk assessment based on the Hazard Index (HI) for BaP indicated that the grilled meat samples did not pose a significant carcinogenic risk. The HI values were below the threshold of concern, suggesting a negligible risk of cancer. This assessment was limited to BaP due to the lack of a reference dose for total PAHs. The results suggest that grilled meat can contain significant levels of PAHs, which pose a health risk. The type of meat and cooking method can influence PAH formation. Consumers should be aware of the potential risks associated with consuming grilled meat and consider alternative cooking methods or limit their consumption. Further research is needed to explore strategies for reducing PAH formation in grilled meat.

Keywords: Benzo[a]pyrene (BaP), PAHs, Charcoal grilled, Meat samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed around the world, owing primarily to long-term anthropogenic pollution sources [1]. Petroleum hydrocarbon contamination, forest fires and volcanic eruptions can contribute to PAHs emissions [2]. Additionally, it is possible to combine dripping fat from grilled meat with coals, which produces smoke containing PAHs [3]. Polycyclic aromatic hydrocarbons are organic pollutants that consist of two or more fused aromatic rings of carbon and hydrogen atoms. They are typically colorless, white, or pale-yellow solid substances [4,5]. There are allegedly 200 distinct PAHs chemicals compound in this group [6]. They have the ability to contaminate meat by directly pyrolyzing food components and by depositing smoke from incomplete combustion of organic materials [7]. Thus, smoked or barbecued meats frequently have significant levels of PAHs [8].

One polycyclic aromatic hydrocarbon (PAH) that is utilized as a biomarker of PAHs is benzo[a]pyrene (BaP). Burning wood, other organic resources, and fossil fuels is the main method used to generate it. Exposure to other PAHs is typically linked to human exposure to BaP [9]. It has been demonstrated that food, drinking water, and the air are the common sources of human exposure to BaP [10,11]. BaP is a Group I carcinogen, particularly its metabolite r-7, t-8-dihydrodiol-t-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE-I), forms mutagenic and carcinogenic adducts with DNA such as cancer of the lung, skin, bladder, breast, kidney, prostate, larynx, hematopoietic system, brain, and colon [9]. Epigenetic alterations are one of the mechanisms responsible for the negative consequences of BaP. BaP's principal modes of action include: (1) the formation of persistent and depurinated DNA adducts; (2) recurrent redox cycling that generates reactive oxygen species; (3) activation of the aryl hydrocarbon receptor (AhR); (4) immunosuppression; and (5) various epigenetic alterations [12,13].

2. Materials and Methods

2.1. Sample Collection

Samples of 60 charcoal-grilled meat (chicken, fish, and kebab) were obtained from Al-Hawija restaurants operation with laboratories in The Ministry of Science and Technology Ethical approval No. ISO 17025 starting from January/ 2022 to the end of March/ 2023. The grilled meat was then minced in an electric blender until it reached a dough-like consistency and stored in a freezer until further analysis. QuEChERS cleanup is a rapid, efficient, and accurate method for the determination of polycyclic aromatic hydrocarbons (PAHs) in meat samples. This method involves a quick and efficient extraction process, making it suitable for the determination of polycyclic aromatic hydrocarbons (PAHs) in meat samples. After extraction, the extracts were analyzed using a gas chromatography-mass spectrometry (GC-MS) system

2.2. Extraction and Purification of Grilled Meat Samples

Two grams of smoked meat sample were added to a 50 mL centrifuge tube and mixed with d-PAHs (surrogate compounds for polycyclic aromatic hydrocarbons). The mixture was thoroughly vortexed and left for 30 minutes at room temperature. Water (5 mL) was added and mixed, followed by 5 mL of ACN (acetonitrile). The tube was vigorously vortexed for one minute. Sodium chloride (0.5 g) and magnesium sulfate (3.0 g) were added to the tube, and the tube was immediately shaken for one minute after the addition of salts. The contents were centrifuged for 10 minutes at 3400 rpm (temperature = 20°C). The upper extract was transferred to a 15 mL tube containing a sorbent (Z-Sep) and 500 mg of magnesium sulfate, vortexed for one minute, and centrifuged for 10 minutes at 3400 rpm (temperature = 20°C). Finally, the extract was transferred to appropriate tubes and dried using a heating block (45°C) until the volume reached approximately 100 microliters.

2.3. HPLC Analysis for PAHs in Meat Samples

2.3.1. Chromatographic System and Conditions

10 microliters of the extracted sample were precisely injected into the gas chromatograph-mass spectrometer (GC-MS) using a specialized syringe. Once injected as a liquid, the sample vaporizes and enters a 30-meter long capillary column. Inside the column, the components of the sample are separated based on their volatility. After separation, the components pass through a detector, which converts the chemical information into electrical signals. These signals are then processed by a computer connected to the GC-MS and displayed as peaks on a chromatogram. The total analysis time for each sample is approximately 20-25 minutes, and the column temperature is maintained at 320°C.

2.3.2. Evaluation of Health Hazards Associated with Grilled Meat Consumption

To assess the health risks associated with total polycyclic aromatic hydrocarbons (tPAHs) and benzo[a]pyrene (BaP) in the studied meat samples, the following components were calculated:

- Carcinogenic risk (CR) assessment: The daily dietary exposure level (ED) to tPAHs and BaP was calculated using the following equation [14]:

$$E_D = BEC \times IRI$$

- BEC is the sum of total PAHs and BaP concentrations in grilled meat, based on toxic equivalents (TEFs). The TEFs for PAHs and BaP_{eq} are 1.211 and 1 µg/kg, respectively [15]. The BEC value is calculated using the following equation:

$$\text{BEC} = \text{Concentration} \times \text{TEFs}$$

- The meat consumption rate (IR_i) for different groups (boys, adult males, girls, and adult females) was 15.9, 21.8, 13.9, and 17.6 g/day, respectively [16]. The carcinogenic risk (CR) from daily grilled meat consumption due to dietary exposure to PAHs was calculated for each group using the equation provided by [17].

$$\text{CR} = (\text{E}_D \times \text{E}_F \times \text{E}_D \times \text{CS}_F \times \text{C}_F) / (\text{B}_W \times \text{A}_T)$$

- To determine the cancer risk levels from the final results, CR values below 1.00E-5 indicate that the risks were at acceptable levels, while CR values above 1.00E-5 suggest unacceptable adverse effects [18].

3. Result and Discussion

3.1. PAHs in Indoor Chicken Samples

Table 1 showed elevated levels of benzo[a]pyrene, benzo[a] fluoranthene, benzo[a] pyrene (BaP), chrysene (Chr), naphthalene (Nap), fluoranthene (Flu), and pyrene (Phe) in charcoal-grilled chicken samples. The values were 61.38, 11.530, 10.771, 8.166, and 5.513 µg/kg, respectively, compared to other PAH compounds.

To better understand the health risks associated with human exposure to PAHs through contaminated food, the European Food Safety Authority EFSA [19] classified PAHs into three categories based on their carcinogenic, mutagenic, and toxic activities: PAH₂, including Chr; PAH₄, including BaA, BaP, BbF, and Chr; and PAH₈, comprising BaA, BaP, Chr, BkF, BbF, IcdP, DahA, and BghiP [6]. Given their impact on human health, maximum levels for BaP and PAH₄ in food have been established to control human exposure to these compounds in many countries, including Turkey, according to the Turkish Food Codex [20] and the European Union Directive [21]. The maximum limit for BaP is set at 5 µg/kg and the total PAH₄ at 30 µg/kg. In recent years, the importance of PAHs has increased due to the numerous sources of exposure. Humans are primarily exposed to PAH-type particles through diet [22].

Polycyclic Aromatic Hydrocarbons (PAHs) formed during the smoking, grilling, or broiling of meat can damage DNA and increase the risk of cancer [23]. While there is no direct evidence that meat consumption directly triggers this mechanism, there is no definitive information about the safest cooking method for meat. Additionally, cooking methods such as grilling (direct contact with fire), roasting, and broiling reveal higher levels of carcinogenic compounds like PAHs in red meat [24].

Table 1. PAH concentrations (mg/l) in chicken samples cooked indoors.

PHAs / Places	acenaphthylene	phenanthrene	chrysene	naththalene	fluorene	Benzo{ghi}perylene	Benzo{a}fluoranthene	Benzo{a}pyrene	pyrene	total PAHs
S 1	0	0	0	0	0	0	0	10.2	0	10.2
S 2	0	0	0	0	0	0	53.72	7.64	2.7	64.06
S 3	13.59	0	14.8	0	0	0	0	16.12	8	52.51
S 4	0	0	0	0	0	0	0	8.78	0	8.78
S 5	0.71	0	0	0	0	0	0	4.66	0	5.37
S 6	0	0	0	28.97	0	0	0	4.17	0	33.14
S 7	0	0	0	0	0	0	0	13.49	0	13.49
S 8	0	23.37	0	0	0	0	0	11.88	0	35.25
S 9	0	0	0	0	0	0	0	8.11	0	8.11
S 10	0	0	0	0	0	0	0	11.02	4.82	15.84
Min	0	0	0	0	0	0	0	4.17	0	5.37
MAX	13.59	23.37	14.8	28.97	0	0	53.72	16.12	8	64.06
Average	1.43	2.337	1.48	2.897	0	0	5.372	9.607	1.552	24.675

In a study conducted by Aydın and Şahan [25], grilled beef, turkey, lamb, and chicken were evaluated for PAH4 (BaP, BaA, BbF, and Chr) concentrations. Significant differences were observed among meat types and PAH compounds, with chicken exhibiting the highest PAH4 levels (3.30 µg/kg), followed by turkey (3.14 µg/kg), lamb (1.74 µg/kg), and beef (1.10 µg/kg). These variations were attributed to differences in meat chemical composition. Furthermore, Sahin *et al.* [6] evaluated PAH4 concentrations in various meat products and found the lowest levels in meatballs (1.95 µg/kg) and the highest in grilled fish (3.30 µg/kg).

3.2. PAHs in Outdoor Chicken Samples

The current study results, presented in Table 2 for grilled chicken samples, showed that the highest levels of PAHs were found for chrysene (Chr), phenanthrene (Phe), pyrene, and benzo[a]pyrene (BaP), with concentrations of 14.738, 8.389, 5.178, and 4.943 µg/kg, respectively, compared to other compounds. Duedahl-Olesen *et al.* [8] found Σ16PAH concentrations of 10.2 and 6.3 µg/kg in grilled beef steak and chicken breast, respectively, in Denmark, Alomirah *et al.* [26] reported Σ16PAHs levels ranging from 48.2 to 342.0 µg/kg in grilled chicken meat, with an average of 222 µg/kg. PAHs are formed through the pyrolysis or incomplete combustion of organic matter during high-temperature meat cooking [27]. Chung *et al.* [28] found that grilled beef, chicken, and pork contained PAHs, with charcoal grilling resulting in the highest PAH levels. Grilled chicken samples cooked over charcoal exhibited the highest Σ PAHs levels at 9.46 µg/kg. The formation of PAHs in charcoal-grilled meats depends on the fat content of the meat, cooking temperature, and duration.

Table 2. Quantification of PAHs (mg/l) in outdoor chicken samples.

PHAs / Places	acenaphthylene	phenanthrene	chrysene	naththalene	fluorene	Benzo{ghi} perylene	Benzo{a} fluoranthene	Benzo{a} pyrene	pyrene	total PAHs
S 1	0	0	0	0	0	0	0	5	0	5
S 2	0	0	0	0	0	0	0	0.97	0	0.97
S 3	0	0	0	0	0	0	0	0.41	0	0.41
S 4	0	0	0	0	0	0	0	0.83	0	0.83
S 5	4.75	0	0	0	0	0	14.22	1.29	0	20.26
S 6	0	24.46	0	0	0	0	0	3.69	1.26	29.41
S 7	0	0	0	1.03	0	0	0	1.11	0	2.14
S 8	0	0	0	0	0	0	0	1.52	0	1.52
S 9	3.12	0	0	0	0	0	0	0.55	0	3.67
S 10	0	0	0	0	0	0	0	5.35	7.47	12.82
Min	0	0	0	0	0	0	0	0.41	0	0.41
MAX	4.75	24.46	0	1.03	0	0	14.22	5.35	7.47	29.41
Average	0.787	2.446	0	0.103	0	0	1.422	2.072	0.873	7.703

The results presented in Tables 3 demonstrate that the aromatic hydrocarbons found in grilled chicken, fish, and kebab are not carcinogenic, regardless of whether they were cooked indoors or outdoors. This non-carcinogenic risk assessment was based on the Hazard Index (HI) for the risk of polycyclic aromatic hydrocarbons (PAHs) from the consumption of grilled meat. The HI was calculated for BaP only due to the lack of a reference dose (RfD) for total PAHs. The RfD for BaP is 1.7 µg/kg. The HI was calculated using the following equation [16, 29].

$$HI = (C_i \times IR \times ED \times CF) / (BW \times AT \times RfD)$$

In most environmental studies, if the HI value is less than 1, individuals exposed to these compounds are not considered at risk for carcinogenic effects. However, if the HI value exceeds 1, there may be potential health risks, including cancer [16,30,31].

Meat and meat products are well-known to be nutritionally important [32]. Changes in consumer habits, economic conditions, and the diversity of processed foods have led to increased consumption of meat and meat products [33]. Some studies have examined the relationship between different types of meat, cooking methods, and the chemical composition of meat to determine PAHs [34]. In addition to the formation of PAHs, there was no data on dietary exposure to PAHs and the assessment of potential health risks in Turkey. The primary objective of this study was to investigate the types and quantities of PAHs in grilled chicken samples that were heat-treated by grilling. By considering the published literature based on international authorities, the dietary exposure and potential public health risks of PAHs were examined and evaluated in common grilled meat samples.

Table 3. Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in Grilled Chicken Samples.

Indoor sites	CR BaP				CR PAHs				Hi BaP			
	child male	child female	adult male	adult female	child male	child female	adult male	adult female	child male	child female	adult male	adult female
S1	4.49E-07	3.92E-07	1.54E-06	1.24E-06	4.49E-06	3.92E-06	1.54E-05	1.24E-05	3.62E-07	3.16E-07	1.24E-06	1E-06
S2	3.36E-07	2.94E-07	1.15E-06	9.3E-07	2.82E-05	2.46E-05	9.65E-05	7.79E-05	2.71E-07	2.37E-07	9.28E-07	7.49E-07
S3	7.09E-07	6.2E-07	2.43E-06	1.96E-06	2.31E-05	2.02E-05	7.91E-05	6.39E-05	5.71E-07	5E-07	1.96E-06	1.58E-06
S4	3.86E-07	3.38E-07	1.32E-06	1.07E-06	3.86E-06	3.38E-06	1.32E-05	1.07E-05	3.11E-07	2.72E-07	1.07E-06	8.61E-07
S5	2.05E-07	1.79E-07	7.02E-07	5.67E-07	2.36E-06	2.07E-06	8.09E-06	6.53E-06	1.65E-07	1.44E-07	5.66E-07	4.57E-07
S6	1.83E-07	1.6E-07	6.28E-07	5.07E-07	1.46E-05	1.27E-05	4.99E-05	4.03E-05	1.48E-07	1.29E-07	5.06E-07	4.09E-07
S7	5.93E-07	5.19E-07	2.03E-06	1.64E-06	5.93E-06	5.19E-06	2.03E-05	1.64E-05	4.78E-07	4.18E-07	1.64E-06	1.32E-06
S8	5.23E-07	4.57E-07	1.79E-06	1.45E-06	1.55E-05	1.36E-05	5.31E-05	4.29E-05	4.21E-07	3.68E-07	1.44E-06	1.16E-06
S9	3.57E-07	3.12E-07	1.22E-06	9.87E-07	3.57E-06	3.12E-06	1.22E-05	9.87E-06	2.87E-07	2.51E-07	9.85E-07	7.95E-07
S10	4.85E-07	4.24E-07	1.66E-06	1.34E-06	6.97E-06	6.09E-06	2.39E-05	1.93E-05	3.91E-07	3.42E-07	1.34E-06	1.08E-06
Outdoor sites	0	0	0	0	0	0	0	0	0	0	0	0
S1	2.2E-07	1.92E-07	7.54E-07	6.08E-07	2.2E-06	1.92E-06	7.54E-06	6.08E-06	1.77E-07	1.55E-07	6.07E-07	4.9E-07
S2	4.27E-08	3.73E-08	1.46E-07	1.18E-07	4.27E-07	3.73E-07	1.46E-06	1.18E-06	3.44E-08	3.01E-08	1.18E-07	9.51E-08
S3	1.8E-08	1.57E-08	6.18E-08	4.99E-08	1.8E-07	1.58E-07	6.18E-07	4.99E-07	1.45E-08	1.27E-08	4.98E-08	4.02E-08
S4	3.65E-08	3.19E-08	1.25E-07	1.01E-07	3.65E-07	3.19E-07	1.25E-06	1.01E-06	2.94E-08	2.57E-08	1.01E-07	8.14E-08
S5	5.67E-08	4.96E-08	1.94E-07	1.57E-07	8.91E-06	7.79E-06	3.05E-05	2.47E-05	4.57E-08	4E-08	1.57E-07	1.26E-07
S6	1.62E-07	1.42E-07	5.56E-07	4.49E-07	1.29E-05	1.13E-05	4.43E-05	3.58E-05	1.31E-07	1.14E-07	4.48E-07	3.62E-07
S7	4.88E-08	4.27E-08	1.67E-07	1.35E-07	9.41E-07	8.23E-07	3.23E-06	2.6E-06	3.93E-08	3.44E-08	1.35E-07	1.09E-07
S8	6.69E-08	5.85E-08	2.29E-07	1.85E-07	6.69E-07	5.85E-07	2.29E-06	1.85E-06	5.39E-08	4.71E-08	1.85E-07	1.49E-07
S9	2.42E-08	2.12E-08	8.29E-08	6.69E-08	1.61E-06	1.41E-06	5.53E-06	4.47E-06	1.95E-08	1.7E-08	6.68E-08	5.39E-08
S10	2.35E-07	2.06E-07	8.06E-07	6.51E-07	5.64E-06	4.93E-06	1.93E-05	1.56E-05	1.9E-07	1.66E-07	6.5E-07	5.25E-07

Conclusion

The results revealed elevated levels of various PAHs, including benzo[a]pyrene, benzo[a]fluoranthene, benzo[a]pyrene (BaP), chrysene (Chr), naphthalene (Nap), fluoranthene (Flu), and pyrene (Phe). Chrysene, phenanthrene, pyrene, and benzo[a]pyrene was identified as the most prevalent PAHs in the grilled chicken samples. Despite the elevated levels of PAHs, a non-carcinogenic risk assessment based on the Hazard Index (HI) for BaP indicated that the grilled meat samples did not pose a significant carcinogenic risk. The HI values were below the threshold of concern, suggesting a negligible risk of cancer. This assessment was limited to BaP due to the lack of a reference dose for total PAHs. The results suggest that grilled meat can contain significant levels of PAHs, which pose a health risk. consumption. Further research is needed to explore strategies for reducing PAH formation in grilled meat.

References

- [1] Patel, A. B., Shaikh, S., Jain, K. R., Desai, C., and Madamwar, D. (2020). Polycyclic aromatic hydrocarbons: sources, toxicity, and remediation approaches. *Frontiers in Microbiology*, 11, 562813.
- [2] Imam, A., Suman, S. K., Kanaujia, P. K., and Ray, A. (2022). Biological machinery for polycyclic aromatic hydrocarbons degradation: A review. *Bioresource Technology*, 343, 126121.
- [3] Babaoğlu, A. S. (2023). Assessing the formation of polycyclic aromatic hydrocarbons in grilled beef steak and beef patty with different charcoals by the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method with gas chromatography–mass spectrometry. *Food Science of Animal Resources*, 43(5), 826.
- [4] Abdel-Shafy, H. I., and Mansour, M. S. (2016). A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. *Egyptian journal of petroleum*, 25(1), 107-123.

- [5] Suman, S., Sinha, A., and Tarafdar, A. (2016). Polycyclic aromatic hydrocarbons (PAHs) concentration levels, pattern, source identification and soil toxicity assessment in urban traffic soil of Dhanbad, India. *Science of the Total Environment*, 545, 353-360.
- [6] Sahin, S., Ulusoy, H. I., Alemdar, S., Erdogan, S., and Agaoglu, S. (2020). The presence of polycyclic aromatic hydrocarbons (PAHs) in grilled beef, chicken and fish by considering dietary exposure and risk assessment. *Food Science of Animal Resources*, 40(5), 675.
- [7] Hamidi, E. N., Hajeb, P., Selamat, J., and Razis, A. F. A. (2016). Polycyclic aromatic hydrocarbons (PAHs) and their bioaccessibility in meat: A tool for assessing human cancer risk. *Asian Pacific Journal of Cancer Prevention*, 17(1), 15-23.
- [8] Duedahl-Olesen, L., and Ionas, A. C. (2022). Formation and mitigation of PAHs in barbecued meat—a review. *Critical Reviews in Food Science and Nutrition*, 62(13), 3553-3568.
- [9] Bukowska, B., and Sicińska, P. (2021). Influence of benzo (a) pyrene on different epigenetic processes. *International Journal of Molecular Sciences*, 22(24), 13453.
- [10] Bahroz, Awaz. 2017. "Isolation of Some Benzo(a)Pyrene- Degrading Bacteria from Contaminated Area in Kirkuk City, Iraq."
- [11] Amadou, A., Praud, D., Coudon, T., Deygas, F., Grassot, L., Faure, E., and Fervers, B. (2021). Risk of breast cancer associated with long-term exposure to benzo [a] pyrene (BaP) air pollution: Evidence from the French E3N cohort study. *Environment international*, 149, 106399.
- [12] Bahroz, Awaz, Maysoon Al, – Tae, and Fikrat Hassan. 2009. "The Study Of Some PAHs Compounds In Euphrates River Sediment From Al-Hindiya Barrageto Al-Kifil City – Iraq."
- [13] Birkett, N., Al-Zoughool, M., Bird, M., Baan, R. A., Zielinski, J., and Krewski, D. (2019). Overview of biological mechanisms of human carcinogens. *Journal of Toxicology and Environmental Health, Part B*, 22(7-8), 288-359.
- [14] Xia, Z., Duan, X., Qiu, W., Liu, D., Wang, B., Tao, S., Hu, X., (2010). Health risk assessment on dietary exposure to polycyclic aromatic hydrocarbons (PAHs) in Taiyuan, China. *Sci. Total Environ.* 408, 5331–5337.
- [15] USEPA, (1993). Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons. Environmental Protection Agency, Research Triangle Park, NC, EPA-600/R-93/089.
- [16] Li, Z., Ma, Z., vander Kuijp, T.J., Yuan, Z., Huang, L., (2014). A review of soil heavy metal pollution from mines in China: pollution and health risk assessment. *Sci. Total Environ.* 468, 843–853.
- [17] Chiang, K.C., Chio, C.P., Chiang, Y.H., Liao, C.M., 2009. Assessing hazardous risks of human exposure to temple air borne polycyclic aromatic hydrocarbons. *J. Hazard. Mater.* 166(2), 676–685.
- [18] Wu, B., Zhang, Y., Zhang, X.X., Cheng, S.P. (2011). Health risk assessment of polycyclic aromatic hydrocarbons in the source water and drinking water of China: quantitative analysis based on published monitoring data. *Sci. Total Environ.* 410, 112–118.
- [19] EFSA. "Scientific Opinion of the Panel on Contaminants in the Food Chain on a Request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food." *EFSA J.* 724 (2008): 1–114.
- [20] Turkish Food Codex [TFC] Food contaminants regulation (No. 28157. Date: 29.11.2011) Ankara, Turkey: Official Gazette; 2011.

- [21] European Union [EU] Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. L215/4-L215/8. Off J Eur Union. 2011;215:4–8.
- [22] Bansal, Vasudha, and Ki-Hyun Kim. 2015. “Review of PAH Contamination in Food Products and Their Health Hazards.” *Environment international* 84: 26–38.
- [23] Lee, Joon-Goo et al. 2016. “Effects of Grilling Procedures on Levels of Polycyclic Aromatic Hydrocarbons in Grilled Meats.” *Food Chemistry* 199: 632–38.
- [24] IARC, International Agency for Research on Cancer (2010) Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monogr Eval Carcinog Risks Hum 92:1–853
- [25] Aydın, Ömer Şerif, and Yasemin Şahan. 2018. “Bazı et Türlerinde Polisiklik Aromatik Hidrokarbon Oluşumuna Farklı Pişirme Yöntemlerinin Etkisi.” *Akademik Gıda* 16(4): 387–94.
- [26] Alomirah, H., Al-Zenki, S., Al-Hooti, S., Zaghoul, S., Sawaya, W., Ahmed, N., and Kannan, K. (2011). Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. *Food control*, 22(12), 2028-2035.
- [27] Jiang, Dafeng et al. 2018. “Occurrence, Dietary Exposure, and Health Risk Estimation of Polycyclic Aromatic Hydrocarbons in Grilled and Fried Meats in Shandong of China.” *Food Science and Nutrition* 6(8): 2431–39.
- [28] Chung, S.Y. et al. 2011. “Effects of Grilling and Roasting on the Levels of Polycyclic Aromatic Hydrocarbons in Beef and Pork.” *Food Chemistry* 129(4): 1420–26.
- [29] Cao, S., Duan, X., Zhao, X., Ma, J., Dong, T., Huang, N., Wei, F., (2014). Health risks from the exposure of children to As, Se, Pb and other heavy metals near the largest coking plant in China. *Sci. Total Environ.* 472, 1001–1009.
- [30] Pongpiachan, S., Tipmanee, D., Deelaman, W., Muprasit, J., Feldens, P., Schwarzer, K., (2013). Risk assessment of the presence of polycyclic aromatic hydrocarbons (PAHs) in coastal areas of Thailand affected by the 2004 Tsunami. *Mar. Pollut. Bull.* 76,370–378.
- [31] Zhao, L., Xu, Y., Hou, H., Shangguan, Y., Li, F. (2014). Source identification and health risk assessment of metals in urban soils around the Tanggu chemical industrial district, Tianjin, China. *Sci. Total Environ.* 468,654–662.
- [32] Arslan, Ali. 2013. Et Muayenesi ve et Ürünleri Teknolojisi. Medipres.
- [33] Singh, V., Negi, R., Jacob, M., Gayathri, A., Rokade, A., Sarma, H., and Qureshi, Q. (2023). Polycyclic Aromatic Hydrocarbons (PAHs) in aquatic ecosystem exposed to the 2020 Baghjan oil spill in upper Assam, India: Short-term toxicity and ecological risk assessment. *Plos one*, 18(11), e0293601.
- [34] Fatma Aygün, S, and Filiz Kabadayi. 2005. “Determination of Benzo [a] Pyrene in Charcoal Grilled Meat Samples by HPLC with Fluorescence Detection.” *International Journal of Food Sciences and Nutrition* 56(8): 581–85.

Effect of Seed Priming and Foliar Spray with Seaweed Extract on Some Growth Characteristics of Two Sunflower *Helianthus annuus* L. Cultivar

Hawraa Ali Abbas¹ and Saudi A. H.²

^{1,2}Field Crops Department, College of Agriculture Engineering Sciences,
University of Baghdad, Baghdad, Iraq.

¹E-mail: hawraa.ali2106p@coagri.uobaghdad.edu.iq

Abstract. A field experiment was carried out during the spring periods of 2022 and 2023 in the experimental fields of the College of Agricultural Engineering Sciences - University of Baghdad using a design RCBD according to factorial experiments with three sectors and three factors including tow cultivars of sunflower (Ishaqi 1 and Ishaqi 2), and the second factor included seed priming with seaweed extract at concentrations (0 and 3 and 4 gm L⁻¹), in addition to the third factor, spraying plants with seaweed extract at the above concentrations. The results showed the Ishaqi 2 cultivar was significantly superior in all studied traits and for both seasons, as it gave the highest average for plant height, leaf area and chlorophyll index which in the first season (198.1 cm, 0.733 m², and 42.60 Spad), while in the second season (261.86 cm, 0.781 m², and 43.04 Spad). The seed priming with seaweed extract at concentrations 4 gm L⁻¹ was superior in the chlorophyll index and biological yield (43.68 Spad and 12.356 ton ha⁻¹) in the first season, while in the second season it reached 43.65S Spad and 12.839 tons ha⁻¹. In most of the studied traits, the spraying treatment with a concentration of 4 gm L⁻¹ performed better than the vegetative group. This was evident in the highest average plant height, number of leaves, leaf area, chlorophyll index, and biological yield that reached 207.1 cm, 30.07 leaves, 0.779 m², 43.60 Spad, and 12.442 tons ha⁻¹ in the first season, and 265.16 cm, 32.35 leaves, 0.824 m², 43.85 Spad, and 12.812 ton ha⁻¹ in the second season. The effect of the binary and triple interactions of the study factors in their effect, but the interaction between the Ishaqi 2 variety, priming at a concentration of 3 gm L⁻¹, and spraying at a concentration of 4 gm L⁻¹ was the most distinctive in most of the traits studied. To achieve the best increase in sunflower yield, we can recommend adopting the cultivation of the Ishaqi 2 variety, revitalizing its seeds with a concentration of 3 gm L⁻¹, or spraying the shoots with a concentration of 4 gm L⁻¹ of seaweed extract.

Keywords. *Helianthus annuus* L., Plant extracts, Genotypes, Seed activity.

1. Introduction

The process of seed formation includes a series of developmental stages that begin with fertilization and the accumulation of nutrients and end with the drying and dormancy of the seed. Each of these stages includes variation in the morphological and physiological development of the seed, which can determine the underlying vitality of the seed and its ability to perform. Also, the activity of the seed and the uniformity of its germination and emergence are the most

important. The cornerstones of effective field preparation, which correlates to increased seed yields and active plant growth [1,2]. He promises sunflower plant (*Helianthus annuus* L.) is one of the most important oil crop plants [3], as its seeds contain 25-48% oil and 20-27% protein, a high percentage of polyunsaturated fatty acids and a low level of cholesterol [4]. As you promise sunflower seeds are among the most common seeds of crops in general and of oil crops in particular, whose vitality can be degraded, their strength reduced, and their qualitative characteristics damaged for several reasons, including unsuitable environmental conditions during seed growth and emergence while they are on the mother plant, mechanical damage during harvest, high oil content in the seeds, and preparation factors. Storing unsuitable seeds after harvest [5, 6]. High, rapid and homogeneous germination leads to good field establishment, which can be obtained by following agricultural applications that improve the performance of the seed during the germination stages, including seed priming technology (seed priming before planting, which is one of the low-cost procedures that shortens the period between seed planting and seedling emergence and reduces effort. It is also characterized by its high efficiency in revitalizing seeds and improving their performance, thus reducing the time required for germination and uniformity of seedling emergence [7]. The weakness of the seeds of many crops It may be due to the date of the seeds on the mother plant. It is possible that the seeds during their formation stages while they are on the mother plant are exposed to many factors that directly or indirectly affect the quality of the seeds produced, and one of these reasons may be a lack of nutrients in the stage of rapid seed filling [8].

It has been found that spraying shoots with extracts from marine algae can accelerate their growth. They play a role in the majority of crucial physiological processes in any crop and, when applied at low concentrations, promote plant growth. Low prices, non-polluting, and non-toxic are some of its defining characteristics [9,10]. A variety of nutrients and hormones included in marine algae extract help plants deal with biotic and abiotic challenges and speed up their metabolic reactions [11]. Many researchers have also pointed out that marine algae contain some carbon-reactive pigments that enable it to produce dry matter with the help of sunlight and nutrients present in the water [12]. This increases the production of dry matter in the plant and its transfer to estuaries, which is reflected in an increase in yield. The use of marine algae extract is not only limited to revitalizing seeds, but it is widely used in foliar nutrition of plants, the results of many studies have shown its direct effect in improving plant growth and raising its productivity, as when added to the soil, nutrients are subject to loss through washing, sedimentation, volatilization, or fixation. Therefore, foliar fertilization is preferred to stimulate rapid growth by meeting the plant's needs and bypassing soil problems [13]. Therefore, this study came with a goal knowing the role of marine algae extracts in revitalizing seeds or spraying green shoots on growth characteristics for two varieties of sunflower crop and the extent of variation and response of those varieties to the study factors.

2. Materials and Methods

A field experiment was carried out during the spring periods of 2022 and 2023 in the experimental fields of the College of Agricultural Engineering Sciences - University of Baghdad using a design RCBD according to factorial experiments with three sectors and three factors including tow cultivars of sunflower (Ishaqi 1 and Ishaqi 2), and the second factor included seed priming with seaweed extract at concentrations (0 and 3 and 4 mg L⁻¹), in addition to the third factor, spraying plants with seaweed extract at the above concentrations The plants were sprayed during three stages of plant growth: the first is the four-true-leaf stage, the second is the beginning of the emergence of flower buds, and the third is at the beginning of flowering. The experimental unit had a 2 × 3 m area, with five lines spanning 75 cm between them and 20 cm

between the holes. Both the first and second applications of 360 kg ha⁻¹ of urea fertilizer (46% N) were spaced at similar intervals, with the first applied after field emergence had finished and the second applied at the start of flowering. In one batch, before planting, 220 kg ha⁻¹ of triple superphosphate fertilizer (46% P₂O₅) and 200 kg ha⁻¹ of potassium sulfate fertilizer (41% K) were applied. The following traits were extracted from ten randomly selected guarded plants from each experimental unit:

2.1. Plant Height (cm)

While the plants were fully bloomed, a measuring tape attached to a wooden ruler was used to record the height of ten randomly selected plants from the median lines of each experimental unit. The measurement began at the soil surface and continued all the way to the end of the floral disc.

2.2. Number of Leaves (leaf plant⁻¹)

At the end of the blooming stage, we determined the number of effective leaves per plant by counting and dividing by 10 the leaves of 10 randomly selected guarded plants from the median lines of each experimental unit.

2.3. Leaf Area (cm²)

The formula that was used to get the plant's leaf area was: sum of squares of the sixth leaf's breadth × 4.31.

2.4. Stem Diameter (mm)

It was measured from the middle area of the stem exactly using the Vernier device.

2.5. Chlorophyll Index in Leaves (SPAD)

The chlorophyll index was estimated for both seasons by taking readings for the ten plants from each experimental unit at the stage of complete flowering using the (502 Chlorophyll Meter (model SPAD) which gives a direct reading of the total chlorophyll index in the leaf.

2.6. Biological Yield (Mg ha⁻¹)

Ten whole plants (stems + leaves + discs) were randomly taken from each experimental unit and cut after separating the discs and leaves from the stems. The stems were placed in potato bags and the discs and leaves in perforated paper bags, each separately. After air drying until the weight was constant, they were all weighed and the average biological yield of each plant was extracted. The weight was then converted to Mg and multiplied by the plant density to extract the biological yield per unit area.

2.7. Statistical Analysis

Statistical analysis was performed on the data using Genstat, a statistical analysis tool (version 20.1). The means were compared using the L.S.D. test at a probability level of P<0.05 [14].

3. Results and Discussion

3.1. Plant Height (cm)

The statistical analysis results show that the plants of the Ishaqi2 cultivar were significantly superior in both growing seasons, giving the highest average plant height of 198.1 and 261.86 cm, compared to the Ishaqi1 cultivar, which gave the lowest average of the trait, reaching 179.4 and 231.80 cm (Table 1). The difference between the two cultivars in the trait of plant height is due to

the genetic variation between the two cultivars, which is reflected in the anatomical and morphological traits of the cultivar, including plant height. [15,16] confirmed the dominance of genetic variation on this trait when environmental conditions are similar. This result confirms what was indicated by [17], who confirmed the existence of differences between sunflower cultivars in the trait of plant height. The 3 g L⁻¹ stimulation treatment outperformed by giving the highest average of the trait 193.1 and 264.13 cm for both seasons in succession, without any significant difference with the 4 g L⁻¹ seed stimulation treatment in the first season only, while the comparison treatment gave the lowest average of the trait for both seasons in succession 184.1 and 225.05 cm. The effect of seaweed extract stimulation on field emergence features and seedling strength may explain the observed increase in plant height. This, in turn, led to perfect field establishment and robust seedlings that fared better throughout their growth and development. Spraying with seaweed extract at a dosage of 3 g L⁻¹ caused a considerable rise in plant height, which had previously been at its lowest point of 169.2 and 235.63 cm in the comparison treatment. Plant height was found to increase when seeds were primed with seaweed extract. This had a positive effect on field emergence characteristics and seedling strength, leading to ideal field establishment and strong seedlings that grew and developed better. The highest average height for the trait was achieved in both growing seasons when the highest concentration of seaweed extract was sprayed (207.1 and 265.16 cm, respectively). Seaweed extract's nutrient content is responsible for plants growing taller; this is because the extract promotes plant growth in several ways, including by opening up cell membranes, boosting enzymatic reactions, and enhancing cell division, elongation, and expansion [18]. Ishaqi2 × seed priming at a concentration of 3 g L⁻¹ of seaweed extract had the highest interaction value of 205.2 cm for the first season and 278.61 cm for the second season, respectively, when it came to the effect of interactions between the study factors. However, there was no significant difference between this interaction and the interaction between the same variety whose seeds were activated at a concentration of 4 g L⁻¹ in the first season. The first season saw the lowest trait value of 178.9, 180.9, and 178.4 cm for the variety Ishaqi1 × all seed priming concentrations, while the second season saw the lowest trait value of 204.36 cm for the interaction that resulted from activating the Ishaqi1 seeds with distilled water. The binary interaction (SIF) between the Ishaqi2 cultivar and the vegetative group sprayed with 4 g L⁻¹ had the highest plant height values of 212.3 and 279.40 cm, respectively, while the interaction between the Ishaqi1 cultivar and the distilled water spraying treatment had the lowest plant height values of 153.6 and 217.37 cm, respectively. The binary interaction between the priming and spraying treatments with seaweed extract A×F (seed priming with a concentration of 3 g L⁻¹ × spraying with a concentration of 4 g L⁻¹) achieved the highest value of plant height for this interaction, which reached 218.5 cm in the first season, and the highest value of the trait reached 298.39 cm in the second season of the interaction (seed priming with a concentration of 3 g L⁻¹ × spraying with a concentration of 4 g L⁻¹), while the comparison treatment for both study factors showed the lowest value of this trait for both seasons, which reached 159.3 and 212.94 cm. As for the triple interaction between the study factors (S×A×F), the seeds of the cultivar Ishaqi 2 activated with seaweed extract at a concentration of 3 g L⁻¹, and whose plants were sprayed with a concentration of 4 g L⁻¹, gave the highest value of 223.0 and 316.29 cm, while the interaction (Ishaqi 1 × all seed priming concentrations × 0 g L⁻¹) compared showed the lowest interaction value of 153.3, 149.3 and 158.0 cm in the first season, respectively, while in the second season, the interaction (Ishaqi 1 × both comparison treatments for seed priming and spraying the vegetative group) recorded the lowest interaction value of 187.60 cm.

Table 1. Effect of seed priming (A) and foliar spray (F) with seaweed extract on plant plant height (cm).

Cultivars (S)	Season 2022					Season 2023				
	A (g L ⁻¹)	F(g L ⁻¹)			S×A	F			S×A	
		0	3	4		0	3	4		
Ishaqi 1	0	153.3	185.0	198.3	178.9	187.60	202.27	223.20	204.36	
	3	149.3	179.3	214.0	180.9	226.07	242.40	280.50	249.66	
	4	158.0	184.3	193.0	178.4	238.43	236.70	249.03	241.39	
Ishaqi 2	0	165.3	196.3	206.0	189.2	238.28	249.44	249.53	245.75	
	3	187.3	205.3	223.0	205.2	254.69	264.84	316.29	278.61	
	4	202.0	190.0	208.0	200.0	268.73	242.55	272.38	261.22	
Mean F		169.2	190.1	207.1		235.63	239.7	265.16		
LSD (0.05)		F = 5.28			7.46	F = 3.42			4.83	
		S × A × F = 12.93				S × A × F = 8.37				
S		V×K			Mean S	V×K			Mean S	
		0	3	4		0	3	4		
Ishaqi 1		153.6	182.9	201.8	179.4	217.37	227.12	250.91	231.80	
Ishaqi 2		184.9	197.2	212.3	198.1	253.90	252.28	279.40	261.86	
LSD (0.05)		7.46			4.31	4.83			2.79	
F		F×A			Mean F	F×A			Mean F	
		0	3	4		0	3	4		
0		159.3	190.7	202.2	184.1	212.94	225.85	236.37	225.05	
3		168.3	192.3	218.5	193.1	240.38	253.62	298.39	264.13	
4		180.0	187.2	200.5	189.2	253.58	239.63	260.71	251.31	
LSD (0.05)		7.46			5.28	5.92			3.42	

3.2. Number of Leaves (leaf plant⁻¹)

The plants of the cultivar Ishaqi2 achieved the highest average number of leaves in both seasons, reaching 27.77 and 31.13 leaf plant⁻¹, compared to the cultivar Ishaqi1, which gave the lowest average for the trait, reaching 25.53 and 28.07 leaf plant⁻¹ (Table 2). This is due to the genetic variation between the two cultivars, which is related to vegetative growth, as these genetic compositions differ in plant height (Table 1), number of nodes on the stem, and number of leaves. The results demonstrate that, for both seasons, the trait averaged 28.69 and 31.85 leaf plant⁻¹ in the seed priming treatment at a concentration of 3 g L⁻¹. In comparison, the trait averaged 23.86 and 25.84 leaf plant⁻¹ in the seed priming with distilled water treatment. One possible explanation for the observed increase in plant leaf number after seed activation with seaweed extract is that the extract has a beneficial effect on cell division and elongation, leading to an increase in leaf number. On average, plants sprayed with 4 g L⁻¹ of seaweed extract had 30.07 and 32.35 leaves per plant, respectively, in the first and second growth seasons, while plants in the comparison treatment had the fewest leaves, at 23.84 and 27.22, respectively. The amino acids and nutrients found in seaweed extract enhance photosynthetic efficiency and control the dispersion of the end product, which may explain why spray treatments with seaweed extract are so beneficial. Regarding the impact of the interplay between the variables under investigation, the binary interaction involving seed priming with seaweed extract (SĀ) and the varieties in the second spring season yielded the most significant results. Specifically, the interaction involving the Ishaqi variety and doses of 2 × 3 and 4 g L⁻¹ of seaweed extract, respectively, resulted in an interaction value of 33.31 and 33.34 leaves plant⁻¹, respectively, with no discernible difference between the two interactions. The trait's lowest value, reaching 24.93 leaves plant⁻¹, was produced

by the interaction Ishaqi 1 $\times$ comparison treatment. The interaction between the binary variables S $\times$ F and the spraying of a concentration of 4 g L⁻¹ of seaweed extract on the vegetative group yielded the highest value of 31.57 and 33.73 leaf plant⁻¹ for the two seasons, respectively, while the interaction between Ishaqi 1 and distilled water resulted in the lowest number of seeds at 22.12 and 24.93 leaf plant⁻¹ for the two seasons, respectively. The two-way interaction between seed priming and spraying with seaweed extract A $\times$ F (seed priming with seaweed extract at a concentration of 3 g L⁻¹ $\times$ spraying with seaweed extract at a concentration of 4 g L⁻¹) achieved the highest value for the number of leaves for this interaction, reaching 33.60 and 35.76 leaves plant⁻¹ for both seasons, respectively, which did not differ significantly from the interaction (seed priming with a concentration of 4 g L⁻¹ $\times$ spraying with a concentration of 4 g L⁻¹), while the comparison treatment for both study factors showed the lowest value for this trait for both seasons, reaching 21.42 and 23.56 leaf plant⁻¹. The triple interaction (Ishaqi 2 $\times$ 3 g L⁻¹ $\times$ 4 g L⁻¹) showed the highest value of 35.53 and 37.80 leaf plant⁻¹ for the two seasons respectively, while the interaction (Ishaqi 1 $\times$ 0 g L⁻¹ $\times$ 0 g L⁻¹) gave the lowest interaction value of 20.10 and 21.99 leaf plant⁻¹ for the two seasons respectively.

Table 2. Effect of seed priming (A) and foliar spray (F) with seaweed extract on number of leaves (leaf plant⁻¹).

Season 2022						Season 2023			
Cultivars (S)	A (g L ⁻¹)	F(g L ⁻¹)			S×A	F			S×A
		0	3	4		0	3	4	
Ishaqi 1	0	20.10	23.50	26.00	23.20	21.99	25.62	27.18	24.93
	3	23.43	27.07	31.67	27.39	28.62	28.80	33.72	30.38
	4	22.83	27.10	28.03	25.99	24.18	30.51	31.98	28.89
Ishaqi 2	0	22.73	24.27	26.53	24.51	25.13	27.20	27.93	26.76
	3	26.33	28.10	35.53	29.99	30.40	31.73	37.80	33.31
	4	27.63	26.20	32.63	28.82	32.97	31.57	35.47	33.34
Mean F		23.84	26.04	30.07		27.22	29.24	32.35	
LSD (0.05)		F = 0.69			NS	F=0.75			1.07
S × A × F = 1.71						S × A × F = 1.85			
S		V×K			Mean S	V×K			Mean S
		0	3	4		0	3	4	
Ishaqi 1		22.12	25.89	28.57	25.53	24.93	28.31	30.96	28.07
Ishaqi 2		25.57	26.19	31.57	27.77	29.50	30.17	33.73	31.13
LSD (0.05)		0.98			0.57	1.07			0.62
F		F×A			Mean F	F×A			Mean F
		0	3	4		0	3	4	
0		21.42	23.88	26.27	23.86	23.56	26.41	27.56	25.84
3		24.88	27.58	33.60	28.69	29.51	30.27	35.76	31.85
4		25.23	26.65	30.33	27.41	28.58	31.04	33.72	31.11
LSD (0.05)		1.21			0.69	1.31			0.75

3.3. Leaf Area (m²)

Table 3 shows that in both growing seasons, plants of the Ishaqi 2 cultivar had the largest average leaf area at 0.733 and 0.781 m², while plants of the Ishaqi 1 cultivar had the smallest at 0.635 and 0.689 m². This is because of differences in vegetative growth-related genetic variation between the two cultivars, which manifest in different plant traits like leaf area and plant height (Tables 1 and 2). Cultivar Ishaqi 2 performed better in these areas, and its superiority was borne out in the results. Based on the results, the seed priming treatment with a concentration of 3 g L⁻¹ produced

the best average trait values of 0.762 and 0.850 m² for the two seasons, respectively, while the comparison treatment produced the lowest average traits of 0.583 and 0.631 m² for the same periods. Priming seeds with seaweed extract is better than other methods because it provides the optimum expansion of the cell wall required for growth and division, which is a crucial step in the cell division and expansion process. [19], in addition to the effect of priming in improving the characteristics of field emergence and increasing the strength of the seedling, which was positively reflected in providing an ideal field establishment and strong seedlings that grew and developed better. The treatment of spraying plants with the highest concentration of seaweed extract 4 g L⁻¹ also gave the highest average leaf area of 0.779 and 0.824 m², and the comparison treatment gave the lowest average for the trait of 0.592 and 0.641 m² in both planting seasons. The reason for the increase in leaf area as a result of spraying the green group with seaweed extract is due to the positive effect of natural growth stimulants for plants rich in phenolic compounds, antioxidants and nutrients found in seaweed extract that help enhance plant growth and increase fresh biomass [20]. The binary interaction between varieties and seed priming with seaweed extract (S×A) in the second spring season, especially the interaction resulting from the variety Ishaqi 2 × 3 g L⁻¹ of seaweed extract, gave the highest interaction value of 0.851 m², while the interaction Ishaqi1 × control treatment gave the lowest value of the trait of 0.570 m². The binary interaction (S×F) of the cultivar Ishaqi 2× spraying the vegetative group with a concentration of 4 g L⁻¹ of seaweed extract in the first season and the interaction Ishaqi 2× spraying the vegetative group with a concentration of 3 g L⁻¹ of seaweed extract in the second season gave the highest value of 0.818 and 0.851 m² for the two interactions, respectively, while the lowest value for the trait was for the interaction Ishaqi 1× treatment of spraying with distilled water, which amounted to 0.562 and 0.570 m² for the two seasons, respectively. The double interaction between priming and spraying treatments with seaweed extract A×F (seed priming with seaweed extract at a concentration of 3 g L⁻¹ × spraying with seaweed extract at a concentration of 4 g L⁻¹) achieved the highest value of leaf area, which amounted to 0.882 and 0.916 m² for both seasons, respectively, while the comparison treatment for both study factors showed the lowest value for this trait for both seasons, which amounted to 0.515 and 0.543 m². The triple interaction (Ishaqi 2 × 3 g L⁻¹ × 4 g L⁻¹) showed the highest value, which amounted to 0.913 and 0.960 m² for both seasons, respectively, while the interaction (Ishaqi1 × 0 g L⁻¹ × 0 g L⁻¹) gave the lowest value of interaction, which amounted to 0.500 and 0.509 m² for both seasons, respectively (Table 3).

Table 3. Effect of seed priming (A) and foliar spray (F) with seaweed extract on leaf area (m²).

Cultivars (S)	A (g L ⁻¹)	Season 2022				Season 2023			
		F(g L ⁻¹)			S×A	F			S×A
		0	3	4		0	3	4	
Ishaqi 1	0	0.500	0.513	0.593	0.535	0.509	0.566	0.633	0.570
	3	0.580	0.693	0.850	0.708	0.654	0.747	0.873	0.758
	4	0.607	0.603	0.780	0.663	0.694	0.719	0.807	0.740
Ishaqi 2	0	0.530	0.700	0.660	0.630	0.576	0.738	0.764	0.693
	3	0.730	0.803	0.913	0.816	0.760	0.836	0.960	0.851
	4	0.607	0.777	0.880	0.754	0.655	0.835	0.905	0.798
Mean F		0.592	0.682	0.779		0.641	0.740	0.824	
LSD (0.05)		F = 0.027			NS	F = 0.017			0.024
		S × A × F = 0.067				S × A × F = 0.042			
		V×K				V×K			
S		0	3	4	Mean S	0	3	4	Mean S

Ishaqi 1	0.562	0.603	0.741	0.635	0.570	0.758	0.740	0.689
Ishaqi 2	0.622	0.760	0.818	0.733	0.693	0.851	0.798	0.781
LSD (0.05)		0.038		0.022		0.024		0.014
F		F×A				F×A		
	0	3	4	Mean F	0	3	4	Mean F
0	0.515	0.607	0.627	0.583	0.543	0.652	0.700	0.631
3	0.655	0.748	0.882	0.762	0.707	0.791	0.916	0.850
4	0.607	0.690	0.830	0.709	0.674	0.777	0.856	0.769
LSD (0.05)		0.047		0.027		0.029		0.017

3.4. Stem Diameter (mm)

In both seasons, the plants of the Ishaqi 2 cultivar had the largest average stem diameter, measuring 20.81 and 23.03 mm, whereas the plants of the Ishaqi 1 cultivar had the smallest average, measuring 19.09 and 20.84 mm, respectively (Table 4). Due to genetic variation, which is reflected in the anatomical and morphological traits of the cultivar, including stem diameter, there is a difference between the two sunflower cultivars in terms of stem diameter. This finding is supported by previous research that found differences in stem diameter between sunflower cultivars [15,16,21], which further supports the idea that genetic variation dominates this trait under similar environmental conditions. The priming treatment with the highest concentration (3 and 4 g L⁻¹) was superior by giving the highest average of the trait (20.83 and 20.81 mm), without any significant difference between them in the first season. In the second season, the seed priming with seaweed extract at a concentration of 3 g L⁻¹ was superior and gave an average of the trait of 23.72, while the comparison treatment (seed priming with distilled water) gave the lowest average of the trait of 18.20 and 19.21 mm for the two seasons respectively. The reason for the superiority of the seed priming with seaweed extract is due to the role of the contents of the seaweed extract of nutrients and growth stimulants in improving seed activity and accelerating physiological activities inside the seed, which contributed to accelerating germination and field emergence, which was positively reflected in providing ideal field establishment and strong seedlings that grew and developed better. The stem diameter increased as a result of increasing the concentrations of spraying with seaweed extract. After it was at its lowest average of 17.74 and 20.31 mm in the comparison treatment, it increased significantly when spraying with seaweed extract at a concentration of 3 g L⁻¹, then reached the highest average for the trait of 21.57 and 23.49 mm when spraying with the highest concentration of seaweed extract in both growing seasons. The nutrients found in seaweed extract are responsible for the spray's superiority in increasing stem diameter. Seaweed extract positively impacts plant growth by increasing cell membrane permeability, stimulating enzymatic reactions, improving cell division, cell elongation, and expansion, and so on [18]. As for the effect of interaction between the study factors, the binary interaction between the varieties and the seed priming with seaweed extract (S×A), especially the interaction Ishaqi2 × 3 g L⁻¹, gave the highest interaction value of 22.07 cm for the first season and 25.65 mm for the second season without any significant difference with the interaction between the same variety and the higher concentration of seaweed extract in the first season. While the interaction Ishaqi1×comparison treatment gave the lowest value for the trait of 18.06 and 18.95 mm for the two seasons respectively. The binary interaction (S×F) for the variety Ishaqi2×spraying the vegetative group with a concentration of 4 g L⁻¹ showed the highest value of 22.76 and 25.04 mm, while the lowest value for the disc diameter was for the interaction (Ishaqi 1 × spraying treatment with distilled water), which reached 16.74 and 19.63 mm for the two seasons respectively. The double interaction between the priming and spraying treatments with seaweed extract A×F (seed priming at a concentration of 3 g L⁻¹ × spraying at a

concentration of 4 g L⁻¹) achieved the highest value of stem diameter for this interaction, which amounted to 26.14 mm, while the comparison treatment showed the lowest value for this trait, which amounted to 17.45 mm for the second season only. As for the triple interaction between the study factors (S×A×F), it recorded the highest value, which amounted to 28.73 cm for the interaction (Ishaqi2 × 3 g L⁻¹ × 4 g L⁻¹), while the interaction (Ishaqi1 × 0 g L⁻¹ × 0 g L⁻¹) showed the lowest value of the interaction, which amounted to 16.82 mm for the second spring season.

Table 4. Effect of seed priming (A) and foliar spray (F) with seaweed extract on stem diameter (mm).

Cultivars (S)	A (g L ⁻¹)	Season 2022				Season 2023			
		F(g L ⁻¹)			S×A	F			S×A
		0	3	4		0	3	4	
Ishaqi 1	0	15.20	19.37	19.60	18.06	16.82	19.79	20.24	18.95
	3	17.13	20.60	21.07	19.60	20.79	21.01	23.56	21.78
	4	17.90	20.40	20.50	19.60	21.28	22.06	22.02	21.79
Ishaqi 2	0	16.97	18.40	19.67	18.34	18.09	18.96	21.39	19.48
	3	19.00	22.30	24.90	22.07	20.69	27.54	28.73	25.65
	4	20.30	22.07	23.70	22.02	24.18	22.72	25.01	23.97
Mean F		17.75	20.52	21.57		20.31	22.01	23.49	
LSD (0.05)		F = 0.650			0.919	F = 0.422			0.597
		S × A × F = NS				S × A × F = 1.034			
S		V×K			Mean S	V×K			Mean S
		0	3	4		0	3	4	
Ishaqi 1		16.74	20.12	20.39	19.09	19.63	20.95	21.94	20.84
Ishaqi 2		18.76	20.92	22.76	20.81	20.95	23.07	25.04	23.03
LSD (0.05)		0.919			0.531	0.597			0.344
		F×A			Mean F	F×A			Mean F
F		0	3	4		0	3	4	
0		16.08	18.88	19.63	18.20	17.45	19.38	20.81	19.21
3		18.07	21.45	22.98	20.83	20.74	24.27	26.14	23.72
4		19.10	21.23	22.10	20.81	22.73	22.39	23.52	22.88
LSD (0.05)		NS			0.650	0.731			0.422

3.5. Chlorophyll Index (SPAD)

Table 5 shows that when comparing the two growth seasons, the plants of cultivar Ishaqi2 had a considerably higher average chlorophyll index (42.60 and 43.04, respectively), while those of cultivar Ishaqi1 had the lowest average (41.67 and 42.52, respectively). Table 3 shows that among the vegetative development indicators, leaf area is the most important, and that cultivar Ishaqi2 has a higher chlorophyll index as a result. The growth in dry matter buildup and chlorophyll production were both mirrored by this upsurge. Priming the seeds at the highest concentration (4 g L⁻¹) resulted in the highest average trait value (43.68 SPAD in the first season and 43.72 SPAD in the second season), while the comparison treatment yielded the lowest average trait value (39.44 and 40.98 SPAD, respectively) for the two seasons. Seaweed extract had a beneficial influence on vegetative development markers, which showed up as an increase in leaf chlorophyll content, leading to higher chlorophyll concentrations.

These results are consistent with what [22] reached, who obtained a significant increase in the vitality of seeds and the growth of their seedlings activated by seaweed extract, and this was reflected in the improvement of subsequent characteristics, including the total chlorophyll content or chlorophyll index in the leaves. An increase in chlorophyll index occurred as a result of

increasing the concentrations of spraying with seaweed extract. After it was at its lowest average of 40.76 and 41.77 SPAD in the comparison treatment, it increased significantly when spraying with seaweed extract at a concentration of 3 g L⁻¹, then it reached the highest average for the trait of 43.60 and 43.85 SPAD when spraying with the highest concentration of 4 g L⁻¹ of seaweed extract in both growing seasons. The reason for the superiority of the spray treatments may be attributed to the extract containing amino acids that work effectively to increase the efficiency of the photosynthesis process. It may also be due to the fact that growth regulators and nutrients included in the extract work to regulate the distribution of the photosynthesis product. The hormones present in the extract also work to delay leaf aging, increase the chlorophyll content of the leaves, and increase the number of green leaves remaining on the plant [23].

This result is consistent with what was indicated by [24]. The binary interaction (S×F) of the cultivar Ishaqi2× spraying the vegetative group with a concentration of 4 g L⁻¹ showed the highest value of 44.21 and 44.40 SPAD, while the lowest value of the chlorophyll index was for the interaction (Ishaqi1× spraying with water only), which reached 39.72 and 41.12 SPAD for the two seasons respectively. The binary interaction between the seed priming and spraying with seaweed extract (seed priming with a concentration of 3 g L⁻¹× spraying with a concentration of 4 g L⁻¹ of seaweed extract) achieved the highest value of seed yield for this interaction, which reached 44.57 and 45.47 SPAD for both seasons respectively, while the comparison treatment for both study factors showed the lowest value for this trait for both seasons, which reached 36.73 and 40.24 SPAD. As for the triple interaction between the study factors (S×A×F), in which the interaction (Ishaqi2×3 g L⁻¹×4 g L⁻¹) gave the highest interaction value of 45.87 and 46.01 SPAD, without any significant difference with the interaction (Ishaqi2 × seed priming and spraying at a concentration of 4 g L⁻¹ of seaweed extract) in the second season, while the interaction (Ishaqi1×0 g L⁻¹×0 g L⁻¹) gave the lowest interaction value of 35.52 and 39.83 SPAD for the two seasons respectively.

Table 5. Effect of seed priming (A) and foliar spray (F) with seaweed extract on chlorophyll index (SPAD).

		Season 2022				Season 2023			
Cultivars (S)	A (g L ⁻¹)	F(g L ⁻¹)			S×A	F			S×A
		0	3	4		0	3	4	
Ishaqi 1	0	35.52	39.61	41.40	38.84	39.83	40.84	41.12	40.59
	3	42.72	43.34	43.26	43.11	42.28	43.60	44.94	43.61
	4	40.92	43.91	44.31	43.05	41.23	44.97	43.89	43.37
Ishaqi 2	0	37.94	40.00	42.16	40.03	40.65	41.17	42.29	41.37
	3	43.65	40.83	45.87	43.45	43.72	41.77	46.01	43.83
	4	43.80	44.52	44.61	44.31	42.91	43.99	44.89	43.93
Mean F		40.76	42.03	43.6		41.77	42.72	43.85	
LSD (0.05)		F = 0.66			NS	F = 0.36			NS
		S × A × F = 0.62				S × A × F = 0.89			
S		V×K			Mean S	V×K			Mean S
		0	3	4		0	3	4	
Ishaqi 1		39.72	42.29	42.99	41.67	41.12	43.14	43.31	42.52
Ishaqi 2		41.80	41.78	44.21	42.60	42.43	42.31	44.40	43.04
LSD (0.05)		0.93			0.54	0.51			0.29
F		F×A			Mean F	F×A			Mean F
		0	3	4		0	3	4	
0		36.73	39.80	41.78	39.44	40.24	41.01	41.70	40.98
3		43.18	42.08	44.57	43.28	43.00	42.69	45.47	43.72

Cultivars (S)	Season 2022				Season 2023			
	A (g L ⁻¹)	F(g L ⁻¹)			S×A	F		
		0	3	4		0	3	4
4		42.36	44.22	44.46	43.68	42.07	44.48	44.39
LSD (0.05)			1.14		0.66		0.63	

3.6. Biological Yield (ton ha⁻¹)

The plants of the cultivar Ishaqi2 recorded the highest average biological yield of 12.379 and 13.080 ton ha⁻¹ in both growing seasons, while the cultivar Ishaqi1 recorded the lowest average of the trait of 11.943 and 12.001 ton ha⁻¹ (Table 6). The superiority of the cultivar Ishaqi2 in seed yield is due to its superiority in the traits of vegetative growth and seed yield, which together led to the superiority of the cultivar Ishaqi2 in biological yield. The seed priming with the highest concentration (4 g L⁻¹) gave the highest average of the trait, which reached 12.356 and 12.839 ton ha⁻¹, while the comparison treatment recorded the lowest average of biological yield of 11.646 and 12.061 ton ha⁻¹ for the two seasons, respectively. The reason for the superiority of the seed priming with seaweed extract is due to the role of the contents of seaweed extract of nutrients and growth stimulants in improving seed activity and accelerating physiological activities inside the seed, which contributed to accelerating germination and field emergence and increasing seedling strength, which was positively reflected in providing ideal field establishment and strong seedlings that grew and developed better. These results confirm what [25] reached, who confirmed the important role of seaweed extract in increasing the germination and field emergence rate and providing strong and active seedlings and reducing the time required for seed germination. The spraying treatment with seaweed extract at the highest concentration of 4 g L⁻¹ gave the highest average for the trait, reaching 12.442 and 12.812 ton ha⁻¹, and it was at the lowest average for the biological yield, 11.967 and 12.225 ton ha⁻¹, for the control treatment in both planting seasons, respectively. The reason for the increase in the biological yield due to the effect of adding seaweed extract, whether by spraying it on the vegetative group, is the superiority of these treatments in the indicators of vegetative growth (which include plant height, number of leaves and leaf area), as well as the yield that was directly reflected in the increase in the biological yield of these treatments. This result confirms what was reached by [26], who obtained a significant increase in dry matter as a result of fertilization with seaweed extract. In terms of the impact of the interplay between the variables under investigation, the interaction between the varieties and priming with seaweed extract (SĀ) had the greatest value of interaction, reaching 12.832 and 13.504 tons ha⁻¹ for the first and second seasons, respectively, when the interaction was (Ishaqi 2 × seed priming with seaweed extract at a concentration of 3 g L⁻¹). In contrast, the trait value was lowest for the Ishaqi1 × control treatment interaction, which reached 11.460 and 11.648 tons ha⁻¹ for the two seasons, respectively. The interaction between the cultivar Ishaqi2 and the vegetative group sprayed with 4 g L⁻¹ had the highest binary interaction (SĪF) value, reaching 12.739 and 13.456 tons ha⁻¹ for the two seasons respectively. In contrast, the interaction between Ishaqi1 and distilled water spraying had the lowest biological yield value, at 11.850 and 11.862 tons ha⁻¹ for the two seasons. The binary interaction between the seed priming and spraying with seaweed extract (seed priming at a concentration of 3 g L⁻¹ × spraying with a concentration of 4 g L⁻¹ of seaweed extract) recorded the highest value of the biological yield for this interaction, reaching 12.778 and 13.095 ton ha⁻¹ for both seasons, respectively, while the comparison treatment for both study factors showed the lowest value for this trait for both seasons, reaching 11.473 and 11.690 ton ha⁻¹. As for the triple interaction between the study factors (S×A×F), in which the interaction (Ishaqi2×3g L⁻¹×4g L⁻¹) gave the

highest interaction value of 13.133 and 13.697 ton ha⁻¹, the interaction (Ishaqi1×0g L⁻¹×0g L⁻¹) gave the lowest interaction value of 11.333 and 11.503 ton ha⁻¹ for the two seasons respectively.

Table 6. Effect of seed priming (A) and foliar spray (F) with seaweed extract on biological yield (ton ha⁻¹).

Cultivars (S)	A (g L ⁻¹)	Season 2022				Season 2023			
		F(g L ⁻¹)			S×A	F			S×A
		0	3	4		0	3	4	
Ishaqi 1	0	11.333	11.473	11.573	11.460	11.503	11.687	11.753	11.648
	3	11.813	12.150	12.423	12.129	11.860	12.190	12.493	12.181
	4	12.043	11.880	12.437	12.240	12.223	12.040	12.260	12.174
Ishaqi 2	0	11.613	11.677	12.207	11.832	11.877	12.463	13.083	12.474
	3	12.483	12.880	13.133	12.832	12.447	13.643	13.697	13.262
	4	12.153	12.387	12.877	12.472	13.440	13.487	13.587	13.504
Mean F		11.967	12.074	12.442		12.225	12.585	12.812	
LSD (0.05)		F = 0.092			0.131	F = 0.116			0.163
		S × A × F = 0.226				S × A × F = 0.283			
S		V×K			Mean S	V×K			Mean S
		0	3	4		0	3	4	
Ishaqi 1		11.850	11.834	12.144	11.943	11.862	11.972	12.169	12,001
Ishaqi 2		12.083	12.314	12.739	12.379	12.588	13.198	13.456	13080
LSD (0.05)		0.131			0.075				0.094
F		F×A			Mean F	F×A			Mean F
		0	3	4		0	3	4	
0		11.473	11.575	11.890	11.646	11.690	12.075	12.418	12.061
3		12.148	12.515	12.778	12.481	12.153	12.917	13.095	12.722
4		12.278	12.133	12.657	12.356	12.832	12.763	12.923	12.839
LSD (0.05)		0.160			0.092	0.200			0.116

Conclusions

It is concluded from this study that the two varieties of sunflower differed in growth indicators, which is certainly due to the genetic makeup of each of them, which was reflected in the growth of their plants, and that the best increase in vegetative growth characteristics was achieved by the plants of the variety Ishaqi2 compared to the variety Ishaqi1. Also, seed priming the sunflower seeds with seaweed extract, especially at a concentration of 3 g L⁻¹, or spraying the vegetative system at a concentration of 4 g L⁻¹, achieved a clear improvement in vegetative growth characteristics. Therefore, it is preferable to plant the variety Ishaqi2 and seed priming or foliar spray with seaweed extract to obtain a good increase in vegetative growth characteristics, which must be reflected in increased production.

References

- [1] Saudi, A.H., M.F.H. Al-Hassan and J.W. Mahmoud. 2016. The effect of planting at different seed rates on the qualitative characteristics and seed vitality of four varieties of bread wheat (*Triticum aestivum* L.). Iraqi Journal of Agricultural Sciences, 47(2):452-460/2016.
- [2] Elshahookie, M.M. and S.H. Cheyed. 2011. Predicting percent of plants in the field giving cultivar mean grain yield and up (+ $\bar{X}$) by vigorous seedlings emerged from sand (SE/96h). Iraqi Journal of Agricultural Sciences, 42(5).
- [3] Ahmed, M., H.M. Noaman and H. Zahran. 2022. Combining ability estimation for yield and its components of sunflower inbred lines. Egyptian Journal of Chemistry, 65(3), pp.19-28.

- [4] Nasralla, A.Y., I.H. Al-Hilfy, H.M. Al-Abodi, O.A. Mohammed, and M. Mhmood. 2014. Effect of spraying some plant extractions and anti-oxidant on growth and yield of sunflower. *Iraqi Journal of Agricultural Sciences*, 45(7-special issue).
- [5] Cheyed, S.H. 2013. Effect of capitulum diameter and seed position on seed quality of sunflower. *The Iraqi Journal of Agricultural Sciences*, 44(3): 322-330.
- [6] Saudi, A.H. 2012. Evaluating the storability of sunflower seeds (*Helianthus annuus* L.). Treated with gibberellic acid and stored for different storage periods. *Dhi Qar Journal of Agricultural Research* 1(1)-2012.
- [7] Singh, S.K., R. Thakur, M.K. Singh, C.S. Singh and S.K. Pal, 2015. Effect of fertilizer level and seaweed sap on productivity and profitability of rice (*Oryza sativa*). *Indian Journal of Agronomy*, 60(3), pp.420-425.
- [8] Elsahookie, M.M. and S.H. Cheyed. 2023. Seed Growth Relationships. Ministry of Higher Education and Scientific Research. p. 174.
- [9] Saudi, A. H. 2017. The effect of priming parameters under salt stress on the vitality and strength of soybean seeds. *Anbar Journal of Agricultural Sciences* 15.(1).
- [10] Mustafa, A.S. and S.H. Cheyed. 2018. Effect of seed priming of organic, biochemical and mineral fertilizers on seed vigor of sorghum. *Int. J. Agric. Stat. Sci.*, 14(1), pp.239-244.
- [11] Pramanick, B., K. Brahmachari, S. Kar and B.S. Mahapatra. 2020. Can foliar application of seaweed sap improve the quality of rice grown under rice–potato–greengram crop sequence with better efficiency of the system,?. *Journal of Applied Phycology*, 32, pp.3377-3386.
- [12] Al-Kriti, S.A.K. 2021. Standardization of chemical fertilizers with seaweed extract and humic acid and its effect on the growth, yield and quality of seeds of some rice varieties. Ph.D. Thesis. College of Agriculture. University of Baghdad. A. S.
- [13] Al-Alusi, Y.A.M. 2009. The effect of ground and foliar fertilization with elements N, P, K growth and yield of bread wheat. *Iraqi Journal of Agricultural Sciences*, 40(1): 82-88.
- [14] Kadhim, F. A. and Abed, N. Y., 2017. Applications of Statistics and Analysis of Agricultural Experiments (Practical part) Dar Al-Doctor for Administrative and Economic Sciences. AL Mutanabi Street, Baghdad, Iraq, p.200.
- [15] Ulaiwee, A.M., M.M. Elsahookie, and L.I. Mohammed. 2015. Performance of selected sunflower in a saline sodic soil. *Iraqi Journal of Agricultural Sciences*, 46(5)
- [16] Hassan, W.A. 2016. Estimation of some genetic parameters of growth and yield characters of sunflower under three plant densities. *Iraqi Journal of Agricultural Sciences*, 47(4)
- [17] Sarheed, B.R., M.H.I.A. Ani and M. Abd Almnam Hasn. 2015. Effect of manganese foliar application on growth and yield of three sunflower (*Helianthus annuus* L.) varieties. *Anbar Journal of Agricultural Sciences*, 13.(2).
- [18] Attia, H.A, and S.H. Kazem 2017. Response of some vegetative growth traits to two sunflower genotypes and the number of humic acid sprays. *Karbala Journal of Agricultural Sciences*. 4(1):14-26.
- [19] Saudi, A.H., I.H.H. Al-Hilfy, A.S.M. Al-Rawi, H.M.K. Al-Abodi A.M. Mahdi and Kh. A. Salman. 2024. Effect of Seed Stimulation Treatments with Hydrogen Peroxide and Sowing Dates on Growth. Traits of Sorghum Cultivars. 5th International Conference of Modern Technologies in Agricultural Sciences. IOP Conf. Series: Earth and Environmental Science, 1371(2024) 052021. doi:10.1088/1755-1315/1371/5/052021.
- [20] Tanga, M., F. B. Lewu, A.O. Oyedeji and O. Oyedeji. 2020. Yield and morphological characteristics of burdock (*Arctium lappa* L.) in response to mineral fertilizer application. *Asian J Agric & Biol*. 2020;8(4):511-518.
- [21] Sarheed, B.R., M.H.I.A. Ani and M.H. Abd Almnam. 2015. Effect of manganese foliar application on growth and yield of three sunflower (*Helianthus annuus* L.) Varieties. *Anbar Journal of Agricultural Sciences*, 13.(2)
- [22] Gürsoy, M. 2022. Role of biostimulant priming applications on germination, growth and chlorophyll content of sunflower (*Helianthus annuus* L.) cultivars under salinity stress. *Selcuk Journal of Agriculture and Food Sciences*, 36(1), pp.75-81.



- [23] Al-Jubouri, K.M.S. 2002. The use of plant growth regulators in the adaptation of sunflower plants *Helianthus annuus* L. To tolerate drought and determine its water needs, PhD thesis, College of Agriculture, University of Baghdad.
- [24] Abdel Wahed, M.S., A.H. Saudi and S.A. Naji 2019. The effect of foliar spraying with seaweed extract on the yield and quality of four varieties of beans. (*Vicia fab* L.). *Al-Muthanna Journal of Agricultural Sciences*, 7 (4) 2019.
- [25] Zhang, X. and E.H. Ervin. 2004. Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. *Crop Science*, 44(5), pp.1737-1745.
- [26] Kadhim, J.J. and J.H. Hamza. 2021, November. Study of seed soaking and foliar application of ascorbic acid, citric acid and humic acid on growth, yield and active components in maize. In *IOP Conference Series: Earth and Environmental Science* (910(1), p. 012076). IOP Publishing.

Effect of Seaweed Extract and Bio-Health on the Growth of Statis Plant (*Limonium sinuatum*)

Kawthar Hadi Abboud

Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Najaf, Iraq.

E-mail: com.kutr@atu.edu.iq

Abstract. The experiment was conducted in lathouse the Department of Plant Production Al-Furat AL.Awsat Technical University, AL- Musaib Technical College for autumn season (2023) and the spring season 2024 to study the effect of seaweed extract and bio-health extract on the flowering growth of the statice plant a good uniformity *Limonium Sinuatum* as a factorial experiment according to Completely Randomized Design (CRD) It consists of two factors, the first factor is seaweed extract and three levels: A1 (control treatment), A1 (spraying at a concentration of 1.5 g liters⁻¹), A2 (spraying at a concentration of 2.5 g liters⁻¹) and the second factor bio-health extract with four levels: B1 (without adding) B2 (adding 3 g.L⁻¹), B3 (add 6 g L⁻¹), B4 (add 9 g L⁻¹) The results of this study can be summarized as follows: The results showed that spraying with seaweed extract has a significant effect on most of the studied traits, Where A3 spraying treatment achieved 2.5 g L⁻¹ By giving it the highest values for regular traits, the number of leaves reached 53 plant leaves⁻¹ The plant height is 45.58 cm, chlorophyll is 52.17 SPAD unit, the stem diameter is 6.06 mm, and the number of inflorescences is 37.17 Plant inflorescences⁻¹ While the A2 treatment gave 3 g. L⁻¹ The highest rates in Number of branches 10.93 plant branches⁻¹ The stalk flower length 35.35 cm. the treatment B3 (6 g .L⁻¹) significantly excelled in all the studied traits and represented by the number of leaves leaf.plant⁻¹ and plant height (cm) and the number of branches (branch. plant⁻¹) and the stalk flower length (cm) and the intensity of chlorophyll dye SPAD unit) and the stem diameter (mm) and the number of inflorescences (inflorescences.plant⁻¹). The Bi-interaction between the seaweed extract and the biological extract led to a significant increase in the studied traits, as the A3B2 treatment excelled in the number of leaves, plant height, chlorophyll dye intensity, stem diameter and number of inflorescences, while A2B2 treatment excelled in the number of branches and the stalk flower length.

Keywords. Seaweed extract, Bio-health, *Limonium sinuatum*, Inflorescences.

1. Introduction

Roses are a language traded by all humans in the world that does not need a translator, and when the language of speech is disrupted, the world of flowers speaks beautifully feeling all the colors of life, bright colors that reflect deep optimism and joy in life. The Statice plant belonging to the family *Plumbaginaceae* and the genus *Limonium*, whose scientific name is *Limonium Sinuatum*, is a herbaceous plant around winter or perennial according to the varieties and the environment in which it grows, native to the Mediterranean basin, southern Spain, North Africa, the Canary

Islands and even in Palestine [1], usually grows in sandy lands and tolerates salinity so it is suitable for cultivation on the shores of the sea, and tolerates cultivation in dry conditions [2]. Seaweed extract, including moss (*Ascophyllum Nodsum*), is one of the organic biostimulants commonly used in recent years, as these extracts contain many important compounds such as vitamins, plant hormones and some organic compounds that contribute to increasing and improving plant growth[3] as well as containing Betaine. It is a source of nitrogen in its low concentrations, a regulator of osmosis in its high concentrations, as well as improving the physical and biochemical properties of the soil [4].

The modern trend includes gradually dispensing completely or partially with chemical fertilizers and relying on biological sources that supply plants with their nutritional needs, as some biological sources secrete growth-promoting substances such as hormones and convert elements that are not ready for absorption into elements that are easy to absorb by the plant and also increase the readiness of some microelements, which reduces the use of chemical fertilizers by 25 % of it leads to lower production costs, as well as environmental conservation and improvement of soil properties [5]. Since the stasis plant is considered one of the filler harvest flowers in its fresh and dry states and the lack of studies about it in Iraq this study was found for the following aims: Study of the response of the stasis plant to the treatment with levels of seaweed extract and its effect on vegetative and flowering growth. Study the effect of levels of bio-health extract on the growth and flowering of stasis plant. Study the interaction effect of study factors on the growth and flowering of stasis.

2. Materials and Methods

The experiment conducted in lathouse the Department of Plant Production Al-Furat AL.Awsat Technical University, AL- Musaib Technical College for the autumn season of 2023 and the spring season of 2024 to study the effect of seaweed extracts and bio-health on the growth and flowering of stasis *limonium sinuatum* (good uniformity) with pink color

The seeds of the stasis plant were imported by the American company Johnny's Selected Seed and were planted in the greenhouse of the Agricultural House Company in Babylon province, AL-Mahawil district, on 11/21/ 2023 . Cork dishes divided into 128 parts were used in which the medium of mosquitoes was used for cultivation and the seed dishes were covered with a piece of restlessness and then the seeds were sprinkled with water The seeds began to germinate after about 7 Days of planting

The experiment included a study of two factors, the first factor is spraying marine algae extract, which is at three levels and is symbolized by the symbol (A):

A1 : spraying with water (control treatment) and A2 : spraying at a concentration of 1.5 g. L⁻¹ and A3: Spray at a concentration of 2.5 g. L⁻¹ Use Flowering seaweed extract obtained from Eden Modern Agriculture. Spanish S.L Figure (2) and its components are shown in Table No. (1)

Table 1. Ingredients of seaweed extract.

Nutrients	%
Seaweed extract from (<i>Ascophyllum Nodsum</i>)	26.30
Free amino acide	8.70
Phosphorus (P2O5)	10.00
Zinc (zn chelated with EDTA)	1.71
Boron (B)	0.85
Molybdenum (Mo)	0.27
Vitamius (B group)	0.30
Bio Saccharides	5.50

The spray solutions were prepared by dissolving the calculated weights of seaweed extract (Flowering Setting) in distilled water to obtain the concentrations required in the study with the addition of some drops of liquid soap to reduce surface tension and increase the absorption process, the process of spraying plants with seaweed extract began two weeks after planting seedlings in pots. Then repeated the spraying process every 10 days until the date of the beginning of flowering plants, spraying operations were conducted by a hand sprinkler in the early morning until the plant is completely wet, taking into account that the process of watering the plants a day before the spraying process.

The second factor (Bio-health, which has four levels and is symbolized by the symbol (B): B1: without addition (control treatment) and B2: adding 3 g. L⁻¹ and

B₃ : Add 6 g. L⁻¹ and B4 : add 9 g. L⁻¹

The biological preparation WSG Bio-health was added German origin from the company and after 21 days of planting seedlings in pots and at a rate of one addition every 21 days and was added after weighing the amount to be dissolved with a ml of distilled water with the addition of sugar to activate microorganisms in the preparation, taking into account that the soil of the pots at the addition is semi-wet and takes into account stirring before adding and in a simple way to ensure that the biological preparation reaches the root area of plants.

After the completion of the formation of four real leaves on the seedlings transferred to the pots and were distributed randomly at the study site and by 5 plants per experimental unit and three replicate, and took into account the removal of bushes from the pots whenever needed.

2.1. Studied Traits

2.1.1. Plant Height (cm)

The height of the plant was measured from the soil surface to the highest flower inflorescence in the plant using a metric tape measure for three plants and then the averages were calculated for each treatment.

2.1.2. Number of Leaves (leaf.plant⁻¹)

I calculated the number of leaves and three plants and then extracted the averages for each treatment.

2.1.3. The Intensity of Chlorophyll Pigment in the Leaves (SPAD Unit)

(Chlorophyllcontent meter SPAD-2252) and for three plants and then calculated the averages.

2.1.4. Stalk Flower Length (cm)

Using a metric tape and for three plants per experimental unit and then according to the rate for each treatment.

The length of the stalk flower was measured from the potted soil surface to the bottom of the base of the flower inflorescence.

2.1.5. Stalk Flower Diameter (mm)

At a height of 3 cm from the pot soil surface, the stalk flower Diameter and three plants were measured using the micro Vernier and the rate was calculated for each experimental unit for each treatment.

2.1.6. Number of Inflorescences (Stalk Flower Inflorescence⁻¹)

I calculated the number of blooming flower inflorescences and three plants of the experimental unit and then according to the rate and for each treatment.

2.1.7. Number of Stalk Flowers (plant stem⁻¹)

I calculated the number of stalk flowers formed and for three plants and then calculated the rate for each treatment.

3. Results and Discussion

3.1. Number of Leaves (leaf. plant⁻¹)

The results of Table (2) indicate that there are significant differences between the levels of study factors in influencing leaves number, the level of A3 was exceeded and gave the highest average leaves number amounted to 53.00 leaf.plant⁻¹ compared to control treatment, which gave the lowest average for this trait amounted to 37.71 leaf.plant⁻¹, as for the B factor, the level of B2 was significantly excelled and gave the highest average for this trait amounted to 50.06 Leaf.plant⁻¹ compared to control treatment, which gave the lowest average of 40.56 leaf.plant⁻¹, as for the effect of interaction between the two factors, the combination of interaction A3B2 significantly excelled and gave the highest average of 65.00 leaf.plant⁻¹ compared to control treatment, which gave the lowest average of 33.67 leaf.plant⁻¹.

Table 2. Effect of seaweed extract and. Bio-Health on the Number of Leaves of Statis Plant (Leaf.Plant⁻¹).

Bio-health / Seaweed extract	B1	B2	B3	B4	Average
A1	33.67	41.83	37.67	37.67	37.71
A2	38.33	43.33	36.67	40.50	39.71
A3	49.67	65.00	52.00	45.33	53.00
Average	40.56	50.06	42.11	41.17	
LSD	A	B	OFF		
	1.252	1.446	2.504		

3.2. Plant Height (cm)

The results of Table (3) indicate that there are significant differences between the levels of study factors in the impact on the plant height trait, the level of A3 has excelled and gave the highest average plant height of 45.68 cm compared to control treatment, which gave the lowest average for this trait amounted to 37.83 cm, as for the B factor, the level of B2 was significantly excelled and gave the highest average for this trait of 45.56 Compared to control treatment, which gave the lowest average of 37.22 cm, while the effect of interaction between the two factors, the combination of interaction A3B2 significantly excelled and gave the highest average of 50.00 cm compared to control treatment, which gave the lowest average of 32.00 cm.

Table 3. Effect of seaweed Extract and Bio-health on Statis Plant Height (cm).

Bio-health / Seaweed extract	B1	B2	B3	B4	Average
A1	32.00	42.33	40.67	36.33	37.83
A2	36.00	44.33	37.00	39.33	39.17
A3	43.67	50.00	45.00	43.67	45.68

Average	37.22	45.56	40.89	39.78
	A	B	OFF	
LSD	3.148	3.676	6.368	

3.3. Number of Stalk Flowers (*branch.plant⁻¹*)

The results of Table (4) indicate that there are significant differences between the levels of study factors in influencing the number of stem branches in the plant, the level of A2 was excelled and gave the highest average number of stalk flowers amounted to 10.92 plant branches⁻¹ Compared to control treatment, which gave the lowest average for this trait amounted to 8.13 plant branch⁻¹ As for the B factor, the level of B2 was Significantly excelled and gave the highest average for this trait amounted to 11.00 plant branches⁻¹, compared to control treatment that gave the lowest average of 8.33 plant branches⁻¹, As for the effect of interaction between the two factors, the combination of interaction A2B2 significantly excelled and gave the highest average of 11.67 plant branches⁻¹ Compared to control treatment that gave the lowest average of 6.33 plant branches⁻¹.

Table 4. Effect of seaweed extract and bio-health on the number of stem branches of statis (plant branch⁻¹).

Bio-health / Seaweed extract	B1	B2	B3	B4	Average
A1	6.33	10.67	7.17	8.33	
A2	11.67	11.67	11.67	8.67	
A3	7.00	10.67	8.33	8.00	8.50
Average	8.33	11.00	9.06	8.33	
	A	B	OFF		
LSD	1.218	1.407	2.436		

3.4. Stalk Flower Length (*cm*)

The results of Table (5) indicate that there are significant differences between the levels of the study factors in the impact on the characteristic of the length of the stalk flower, the level A2 excelled and gave the highest average length of the stalk flower amounted to 35.25 cm compared to control treatment, which gave the lowest average for this trait amounted to 28.71 cm, as for the B factor, the level of B2 was significantly excelled and gave the highest average for this trait amounted to 35.11 CM compared to control treatment, which gave the lowest average of 29.11 cm, while the effect of interaction between the two factors, the A2B2 interaction combination significantly excelled and gave the highest average of 39.33 cm compared to control treatment, which gave the lowest average of 25.50 cm.

Table 5. Effect of seaweed extract and Bio-health on Statis stalk flower length (cm).

Bio-health / Seaweed extract	B1	B2	B3	B4	Average
A1	25.50	30.33	31.50	27.50	28.71
A2	33.00	39.33	35.67	33.00	35.25
A3	28.83	35.67	32.00	33.50	32.50
Average	29.11	35.11	33.06	31.33	
	A	B	OFF		
LSD	1.670	1.929	3.341		

3.5. Intensity Chlorophyll Dye SPAD Unit

The results of Table (6) indicate that there are significant differences between the levels of study factors in the impact on the severity of chlorophyll SPAD unit, the level of A3 was excelled and gave the highest average of chlorophyll of 52.17 unit SPAD compared to control treatment, which gave the lowest average for this trait amounted to 48.00 SPAD unit, as for the B factor, the level of B2 was significantly excelled and gave the highest average for this trait amounted to 95.78 SPAD unit compared to control treatment, which gave the lowest average of 44.22 SPAD unit, while the effect of interaction between the two factors was significantly excelled to the A3B2 interaction combination and gave the highest average of 62.67 SPAD unit compared to control treatment that gave the lowest average of 41.33 SPAD unit.

Table 6. Effect of seaweed extract and Bio-Health on the Intensity of Chlorophyll of Statis Plant (Spad Unit).

Bio-health / Seaweed extract	B1	B2	B3	B4	Average
A1	41.33	59.00	44.33	47.33	48.00
A2	42.67	57.67	45.67	51.67	49.42
A3	48.67	62.67	47.33	50.00	52.17
Average	44.22	59.78	45.78	49.67	
LSD	A	B	OFF		
	1.383	1.597	2.766		

3.6. Diameter Stalk Flower (mm)

The results of Table (7) indicate that there are significant differences between the levels of the study factors in the impact on trait of the leg Diameter (mm), the level of A3 was excelled and gave the highest average leg diameter of 6.06 mm compared to control treatment, which gave the lowest average for this trait of 4.72 mm, as for the B factor, the level B2 was significantly excelled and gave the highest average for this trait of 6.22 mm compared to control treatment, which gave the lowest average of 4.34 mm, while the effect of interaction between the two factors was significantly excelled to the A3B2 interaction combination and gave the highest average of 6.97 mm compared to control treatment that gave the lowest average of 3.50 mm.

Table 7. Effect of seaweed extract and bio-health on statis stem diameter (mm).

Bio-health / Seaweed extract	B1	B2	B3	B4	Average
A1	3.50	5.47	4.77	5.13	4.72
A2	4.29	6.23	3.71	4.07	5.07
A3	5.22	6.97	6.20	5.87	6.06
Average	4.34	6.22	4.89	5.02	
LSD	A	B	OFF		
	0.196	0.227	0.393		

3.7. Number of Inflorescences (plant inflorescence⁻¹)

The results of Table (8) indicate that there are significant differences between the levels of study factors in influencing the number of inflorescences of the plant, the level of A3 has excelled and gave the highest average plant height of 37.17 inflorescences compared to control treatment, which gave the lowest average for this trait amounted to 29.75 inflorescence, as for the B factor, the level of B2 was significantly excelled and gave the highest average for this trait amounted to 39.33 Inflorescence plant⁻¹ compared to control treatment that gave the lowest average of 31.67

inflorescences plant⁻¹, as for the effect of interaction between the two factors, the combination of interaction A3B2 significantly excelled and gave the highest average of 46.33 floral inflorescences plant⁻¹ compared to control treatment that gave the lowest average of 26.00 inflorescences plant⁻¹.

Table 8. Effect of seaweed Extract and Bio-Health on the Number of Flower Inflorescences of Statis Plant (Plant Inflorescence⁻¹).

Bio-health / Seaweed extract	B1	B2	B3	B4	Average
A1	26.00	33.00	31.33	28.67	29.75
A2	34.67	38.67	34.00	31.33	34.67
A3	34.33	46.33	35.33	32.67	37.17
Average	31.67	39.33	33.56	31.89	
LSD	A	B	OFF		
	1.240	1.432	2.480		

4. Discussion

The results of tables (2-8) show that the seaweed extract has a significant impact on trait of vegetative and floral growth, represented in (the number of leaves, plant height, number of stem branches, stalk flower length, chlorophyll pigment intensity, stem Diameter, number of flower inflorescences) The results showed excelled of the level treatment A2 and A3 by giving them the highest values for vegetative and floral growth traits, and this may be attributed to the importance of marine algae extracts containing phosphorus, zinc, boron, amino acids, and molybdenum. And a group of B vitamins, in addition to containing various growth regulators such as gibberellins oxins and cytokinins important in the process of division and elongation of cells and containing manitol and alginic acid, which helps plants to absorb nutrients as well as containing the necessary iron in the photosynthesis that improves plant growth, if this contributes to increasing the metabolic activities of the plant, where phosphorus enters the construction of vital compounds, including the construction and composition Energy compounds necessary for photosynthesis and in many metabolic pathways, which led to this increase and improvement of vegetative growth traits, as well as the elements involved in the composition of seaweed extracts such as phosphorus and zinc have an important role in the process of photosynthesis and the transfer of electrons, which reflected this increase chlorophyll and activate various metabolic processes and then increase the plant content of the necessary elements that directly affected the improvement and increase vegetative growth [6] and does the increase in vegetative growth led to the formation of flower buds, which increased the number of flower inflorescences on the plant Table (7) In addition to improving trait of flower growth Stalk flower length and stalk flower diameter Table (4 and 6) This may be due to the content of the extract on organic compounds with a vital effect on plant growth and development in addition to its content of vitamins (vitamin B group, macroelements and phosphorus) and some micro (Zn, B, Mc), amino acids and may have a role in the control and organization of biochemical and physiological activities and the construction of carbon skeleton compounds and the resulting formation of primary and secondary metabolic compounds as the element phosphorus has an important role in the production of energy compounds ATP (*Adenosin triphosphate*) important in the vital processes of plants and nucleic acids DNA and RNA necessary in cell division [7].

The addition of the biological preparation also led to a clear increase in the traits of vegetative and syphilis growth containing *Tricoderma* fungi, *Bacillus* bacteria and humic acids, the results of tables (2-8) showed an increase in vegetative and floral growth represented in (the number of

leaves, plant height, number of stem branches, stalk flower length, chlorophyll pigment intensity, stem Diameter, number of flower inflorescences) and the reason may be attributed to the role of biofertilizers in the biological preparation in improving the root growth of the plant from During the encouragement of the formation of root hairs and an increase in the surface of the root system, which leads to an increase in the absorption of water and nutrients, and this is reflected in the improvement of vegetative growth [8] as well as the improvement of physical and chemical traits as a result of the secretion of plant hormones in the growth medium such as Auxins and Gibberellin, which work on cell division and increase their numbers [9] and the reason may be due to the fact that the biological preparation contains *the fungusTircoderma*, which played an important role in the analysis of organic matter and the formation of mineral organic complexes with microelements (Zn, Mn, Fe), which increased their readiness due to the reduction of the degree of soil neutrality (PH) as this fungus works to produce stimuli and regulators for growth and the formation of mechanisms to defend the plant, which greatly facilitates the increase in the readiness of nutrients in the root area and then easy absorption by the plant [10].As for *the Bacillus bacteria*, they have several mechanisms through which they contribute to improving plant growth, as they have the ability to dissolve phosphorus fixed in the soil, which facilitates its absorption by the plant by secreting many amino acids such as oxalic and citric [11] And secrete more plant hormones such as oxins, cytokinins, gibberellins and acetic acid indole, which work to increase cell division and expansion, which leads to an increase in the number of leaves and plant height The number of stem branches, the length of the stalk flower and the diameter of the stalk flower (1, 2, 3, 4, 6) also has an important and positive role in the process of biological fixation of nitrogen, as it converts nitrogen from the inactive form (N₂) to the active form (ammonium) with the help of the enzyme Nitrogenase and that the element N is an important element and enters directly into the formation of the chlorophyll molecule [12].Therefore, the intensity of chlorophyll pigment in the leaves increases Table (5), increasing the height of stability and the number of leaves Tables (1, (2) and increasing the plant's ability to produce nutrients and increase their accumulation in flowering and push the plant to increase the formation of flower buds Table (7) Or may be attributed to the role of *Bacillus* in providing phosphorus, which enters most of the biological processes and pushes towards the formation of flower buds and blooming [13]. The increase in syphilis leg Diameter is due to *Bacillus* bacteria by fixing nitrogen as well as their role in the secretion of plant hormones such as auxins and gibberellins that increase cell division and dilation [14].

Conclusions

- The seaweed extracts had a significant effect on all the traits studied and the concentration was 2.5 g per liter⁻¹ achieved the highest rates in that.
- The use of Bio-heath achieved the best results in all the studied traits and the concentration of 6 g L⁻¹ is the best in achieving the highest equations, while increasing the concentration to 9 g L⁻¹ was not significant in improving the studied traits.
- The interaction between seaweed extract and biopreparation achieved the best rates in the traits studied above.

References

- [1] Buira, A.; Aedo, C.; Medina, L.(2017). Spatial patterns of the Iberian and Balearic endemic vascular flora.
- [2] González-Orenga, S., Grigore, M. N., Boscaiu, M. & Vicente, O.(2021). Constitutive and induced salt tolerance mechanisms and potential uses of *Limonium* Mill. species. *Agronomy* 11, 413.

- [3] Zamani ,S,S.Khorasaninejad and B.Kashefi . (2013).The importance role of seaweeds of some characters of plant .Int. J. Agr. and crop Sci,5. (16).
- [4] Kageyama, H.; R. Waditee-sirisath; Y. Tanaka and T. Takabe. (2017). 'Osmoprotectant and sunscreen molecules form Halophilic Alge and Cyanobacteri',Algal Green Chemistry, <http://dx.doi.org/10.1016/B978-0-444-63784-0.00001-1>.
- [5] Reddy, G.C., Goyal, R.K., Puranik, S., Waghmar, V., Vikram, K.V. and Sruthy, K.S., (2020). Biofertilizers toward sustainable agricultural Development. In Plant Microbe Symbiosis (115-128).
- [6] Spagnuolo, Damiano and Domenico, Prisa. (2021). Evaluation of Growth Parameters on *Carpobrotus edulis*, *Kalanchoe daigremontiana* and *Kalanchoe tubiflora* in Relation to Different Seaweed Liquid Fertilizer (SLF) as a Biostimulant. International Journal of Current Microbiology and Applied Sciences. 10. 1-10. 10.20546/ijcmas.. 1010.xx.
- [7] Elasco-Ramirez AP, Hernandez-Herrera RM Garcia-Contreras FM. Maldonado-Villegas MM.(2020). Effect of liquid seaweed extract on potted growth of *Eustoma grandiflorum*(Raf) Shinn. Tropical and Subtropical . Agroecosystems;23.
- [8] Bhat, M. A.; R. Rasool and Ramzan, S. 2019. Plant Growth Promoting Rhizobacteria (PGPR) for Sustainable and Eco-Friendly Agriculture. Acta. Sci. Agri., 3(1): 23–25..
- [9] Wu, Z., Huang, Y., Li, Y., Dong, J., Liu, X. and Li, C., (2019). Bio-control of *Rhizoctonia solani* via induction of the defense mechanism and antimicrobial compounds produced by *Bacillus subtilis* SL-44 on pepper (*Capsicum annuum* L.). Frontiers in Microbiology, 10, p.2676.
- [10] Sharma, B., S. Rashi and Saha. A. (2012). In-vitro solubilization of tricalcium phosphate and production of IAA by phosphate solubilization bacteria isolated from Tea rhizosphere of Darjeelin Himalaya. P. Sci. Feed 2 (6): 96-99.
- [11] Dawwan, G. E.; A. Elbeltagy; H. M.Emara; I. H. Abbas and M. Hassan. M.M., (2013). Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. Annals of Agricultural Sciences, 58(2): 195-201.
- [12] Diab, saeed . 2012. The use of phosphate rock and superphosphate and the addition of fungal and bacterial fertilizers in the growth and yield of potatoes. Thesis Doctbehind him. Department of Horticulture, College of Horticulture, University of Baghdad,
- [13] Radhakrishnan, R., Hashem, A., and AbdAllah, E. F. (2017). *Bacillus*: a biological tool for crop improvement through bio-molecular changes in adverse environments. Frontiers in physiology, 8, 667.
- [14] Hayat, R.; S. Ali; U. Amara; R. Khalid and Ahmed, I. 2010. Soil beneficial bacteria and their role in plant growth promotion : a review . Ann Microbiol. Springer – Verlag and the University of Milan. Page 1-20.

HACCP Application in Next-Generation Sequencing in Assuring the Safety of Mosul Factories

Baqer T. Al-Emam¹ and Ayman Albanna²

^{1,2} College of Environmental Sciences, Environmental Science Department,
University of Mosul, Mosul, Iraq.

¹E-mail: baqer.22evp10@student.uomosul.edu.iq

²E-mail: aymanalbanna@uomosul.edu.iq

Abstract. Next-Generation Sequencing coupled with HACCP about the assessment of factory safety in Mosul provide the overall information about microbial presence in the above-mentioned industrial setup, ensuring air and waste water safety with quality control. The main findings of the study are about identified microbial hazards and strategies to reduce them in an attempt to enhance the safety of products and compliance with regulatory requirements from ice production, snack manufacturing, tahini processing, and flour grinding. This study systemically identified the microbial landscapes of both the wastewater and atmospheric environments in these factories, pinpointing sources of contamination and suggesting improvements to their current safety protocols. In this context, the research revealed the following contaminants: in ice factory water sources, there were *E. coli* and *L. monocytogenes*; while in the air, organisms frequently identified included *P. aeruginosa* and *V. cholerae*. This points to variable routes of contamination within similar setups. Furthermore, the presence of *Salmonella* and *Staphylococcus aureus* in water and air was found in snack food producing facilities. Most importantly, the results imply stringent measures of contamination avoidance. Surveillance on tahini and flour milling plants identified hazards against *Vibrio cholerae* and *Bacillus cereus*, which are critical intervention points for HACCP applications. The detailed pathogen mapping provided by NGS will now facilitate targeting critical control points management in the improvement of sanitation and safety across production lines. The study, therefore, provides support for adopting a harmonized approach to the implementation of HACCP frameworks with due regard to the complex interactions between airborne and aqueous microbial communities. Improving the way microbial hazards are overseen, enhancing safety performance, and improving regulatory adherence will be enhanced. The research can further enhance safety and operational efficiency in the industrial landscape of Mosul by providing accurate microbial mapping and proactive control initiatives.

Keywords. HACCP, NGS, Mosul Factories, Food safety.

1. Introduction

Together with different factories operating within the dynamic industrial setting of Mosul and forming a core link in the local and broader regional economy chain, it simply underlines the importance of safe and high-quality air and wastewater systems. The Hazard Analysis and Critical Control Points framework has long been recognized as a robust tool for managing risks in

food and beverage production environments [1, 2]. Coupled with technological development, however, the incorporation of NGS can create a truly revolutionary opportunity for boosting the safety protocols of those factories in terms of air quality monitoring [3,4].

Using these features high throughput, scalability, and speed it generates the genetic snapshot of microbial communities in air systems with exhaustive detail [5,6]. This technology far outclasses conventional methods of microbiology for quick identification of pathogenic and non-pathogenic organisms necessary for health compliances and operational safety alike [7]. It is with NGS that this hidden microbial dynamics can be revealed and traditional culturing techniques include assessment of air quality at Mosul factories, acting as a foundation for production safety and efficiency [8].

Subsequently, air filtration and ventilation practices in manufacturing processes grow in terms of complexity, underscoring the need for monitoring techniques such as NGS in industrial applications [9]. Conventionally, air quality assessment methods used to monitor manufacturing processes do not detect low-abundance pathogens or allow real-time assessment for microbial contamination as factories in Mosul try to attain local and international safety standards[10]. One of the things is that NGS not only fills this gap but also provides data-driven insight that complements HACCP's preventive approach in enhancing critical control and risk assessment capabilities.

Application of NGS in conjunction with HACCP principles fits squarely into the context of recovery and development at Mosul. Such initiatives in the post-conflict recovery of the region include strengthening economic stability through industrial growth, where ensuring environmental safety and public health is one of the priorities [11]. The integration of NGS in air safety management is therefore very much in line with the goals, providing a way of effectively tracing and rapidly responding to potential microbial hazards in the air used along the food and beverage industry, pharmaceuticals, and in chemical manufacturing [12].

This integration thus supports not only rigorous hygiene standards but also sustainability in terms of ensuring ventilation and filtration processes do not have any detrimental effects on the quality and safety of products. This broad-spectrum ability of NGS to detect biological markers can further facilitate the development of more targeted cleaning and maintenance protocols for better productivity with reduced downtime [13].

Overall, the integration of Next-Generation Sequencing technology into HACCP frameworks shows huge potential for leaps in safety management of both air and wastewater in factories located in Mosul. This paper allows for the exploration of practical applications, benefits, and challenges in implementing NGS within HACCP systems that will help address microbial risk assessment in industrial environments where the quality of air is important for safety and compliance. This innovative model shall hence be able to return Mosul factories to levels of safety, efficiency, and environmental responsibility that will secure the regional economy and public health.

2. Materials and Methods

2.1. Sample Collection

The current research attempted to evaluate the quality of microorganisms present in the air and wastewater of various Mosul factories through the adoption of integrated Next-Generation Sequencing and Hazard Analysis and Critical Control Points. In this study, there are 52 samples: 26 wastewater samples and 26 air samples from different types of factories.

2.1.1. Wastewater Samples

These were collected from four different factory types, which reflect the diversified industrial activities:

- Ice Factories: 10 samples.
- Crisp Factories: 6 samples.
- Tahini (Sesame Oil) Factories: 3 samples.
- Flour Grinding Factories: 7 samples.

2.1.2. Air Samples

Concurrently, the air samples were collected on open plates containing nutrient agar set within the working areas of the same factories to collect the communities of airborne microorganisms.

2.2. Processing and Transport of Samples

- Sterile Collection: All air and wastewater samples were collected using sterile techniques so that any kind of external contamination may be avoided [14].
- Controlled Transport: Samples were immediately sent to the laboratory under controlled conditions so that integrity may be maintained and microbial growth from external sources may be avoided [15].

2.3. DNA Extraction and Sequencing Preparation

- DNA Isolation: Microbial DNA was isolated from both air and wastewater samples using a high-efficiency DNA extraction kit for environmental samples [16].
- Library Preparation: Extracted DNA was used for library preparation for targeted amplicon sequencing [17]. This amplifying specific regions: 16S rRNA for Bacteria Targeting the V4 region, widely used for bacterial community analysis.

2.4. Next-Generation Sequencing and Bioinformatics Analysis

- Sequencing: Prepared libraries were then sequenced on an Illumina HiSeq platform. This platform was used for the capability of delivering high-throughput sequencing data with accuracy in its results [18].
- Data Analysis: Sequencing data was processed using state-of-the-art bioinformatics pipelines. First, quality control was assured to guarantee the integrity of the data, then the mapping of reads against reference microbial databases for identification and quantification of microbial species [19].
- Operational Taxonomic Unit Classification: The reads were further classified into operational taxonomic units that gave more information on microbial diversity and the likely pathogenic presence within the factory environments [20].

2.5. Integration of HACCP and Risk Assessment

- Data Integration: Targeted NGS generated detailed microbial profiles that were integrated into each factory's HACCP framework in the effort to identify and better manage microbial risks.
- Enhanced Safety Controls: Analyzed microbial data identifies the critical control points, allowing for targeted intervention developments to reduce microbial risks and enhance overall safety and quality controls within the factories.
- This methodology ensures that the microbial safety assessment is holistic by using the latest molecular tools to strengthen conventionally developed industry safety

management systems. In line with the latest developments in the area of microbial research, the use of targeted amplicon sequencing for both bacterial and fungal communities provides robust tools to ensure environmental safety in Mosul factories.

3. Results and Discussion

Next-generation sequencing techniques, coupled with hazard analysis and critical control points, provided the minute details of the microbial populations in aerosol and wastewater samples in the different types of industries in Mosul (table1). In this research, various factories producing ice, crisps, tahini, and flour were considered. Several major findings have come out from this study that may help in revisiting the safety rules of the factories or compliance. Pathogenic and spoilage organisms have been isolated from all samples of air and wastewaters collected from different factory environments, thus pointing to the need for an effective microbial control strategy in the manufacturing environment[21]. However, the differential growth pattern of microorganisms even within comparable segments of production lines would indicate differences either in standards of hygiene or effectiveness of control measures adopted [22]. In particular, the presence of organisms such as *Escherichia coli*, *Listeria*, and *Salmonella* draws attention to the fact that monitoring and intervention need to be done in order to avoid possible health risks related to these pathogens [23, 24].

Table 1. The microbiological results from the wastewater and air samples at various types of factories in Mosul, which proved the existence of major bacterial pathogens.

Mosul factories	Wastewater samples	Air samples
Ice Factories		
Ice Factory 1:	<i>Escherichia coli</i> , <i>Listeria monocytogenes</i>	<i>Pseudomonas aeruginosa</i> , <i>Vibrio cholerae</i>
Ice Factory 2:	<i>Salmonella enterica</i> , <i>Enterococcus faecalis</i>	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i>
Ice Factory 3:	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i>	<i>Salmonella enterica</i> , <i>Enterococcus faecalis</i>
Ice Factory 4:	<i>Vibrio cholerae</i> , <i>Bacillus cereus</i>	<i>Clostridium perfringens</i> , <i>Listeria monocytogenes</i>
Ice Factory 5:	<i>Clostridium perfringens</i> , <i>Campylobacter jejuni</i>	<i>Campylobacter jejuni</i> , <i>Bacillus cereus</i>
Ice Factory 6:	<i>Enterococcus faecalis</i> , <i>Salmonella enterica</i>	<i>Vibrio cholerae</i> , <i>Escherichia coli</i>
Ice Factory 7:	<i>Escherichia coli</i> , <i>Vibrio cholerae</i>	<i>Pseudomonas aeruginosa</i> , <i>Enterococcus faecalis</i>
Ice Factory 8:	<i>Listeria monocytogenes</i> , <i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i> , <i>Salmonella enterica</i>
Ice Factory 9:	<i>Staphylococcus aureus</i> , <i>Bacillus cereus</i>	<i>Listeria monocytogenes</i> , <i>Clostridium perfringens</i>
Ice Factory 10:	<i>Clostridium perfringens</i> , <i>Campylobacter jejuni</i>	<i>Campylobacter jejuni</i> , <i>Bacillus cereus</i>
Crisp Factories		
Crisp Factory 1:	<i>Escherichia coli</i> , <i>Salmonella enterica</i>	<i>Listeria monocytogenes</i> , <i>Clostridium perfringens</i>
Crisp Factory 2:	<i>Listeria monocytogenes</i> , <i>Vibrio cholerae</i>	<i>Escherichia coli</i> , <i>Vibrio cholerae</i>
Crisp Factory 3:	<i>Staphylococcus aureus</i> , <i>Enterococcus faecalis</i>	<i>Enterococcus faecalis</i> , <i>Pseudomonas aeruginosa</i>
Crisp Factory 4:	<i>Pseudomonas aeruginosa</i> , <i>Bacillus cereus</i>	<i>Staphylococcus aureus</i> , <i>Salmonella enterica</i>
Crisp Factory 5:	<i>Clostridium perfringens</i> , <i>Campylobacter</i>	<i>Campylobacter jejuni</i> , <i>Bacillus cereus</i>

Mosul factories	Wastewater samples	Air samples
	jejuni	
Crisp Factory 6:	Enterococcus faecalis, Escherichia coli	Escherichia coli, Listeria monocytogenes
Tahini Factories		
Tahini Factory 1:	Salmonella enterica, Staphylococcus aureus	Staphylococcus aureus, Campylobacter jejuni
Tahini Factory 2:	Listeria monocytogenes, Pseudomonas aeruginosa	Salmonella enterica, Vibrio cholerae
Tahini Factory 3:	Vibrio cholerae, Bacillus cereus	Pseudomonas aeruginosa, Bacillus cereus
Flour Grinding Factories		
Flour Grinding Factory 1:	Clostridium perfringens, Campylobacter jejuni	Clostridium perfringens, Escherichia coli
Flour Grinding Factory 2:	Enterococcus faecalis, Escherichia coli	Enterococcus faecalis, Listeria monocytogenes
Flour Grinding Factory 3:	Salmonella enterica, Staphylococcus aureus	Salmonella enterica, Staphylococcus aureus
Flour Grinding Factory 4:	Listeria monocytogenes, Vibrio cholerae	Pseudomonas aeruginosa, Vibrio cholerae
Flour Grinding Factory 5:	Pseudomonas aeruginosa, Bacillus cereus	Bacillus cereus, Escherichia coli
Flour Grinding Factory 6:	Clostridium perfringens, Campylobacter jejuni	Enterococcus faecalis, Campylobacter jejuni
Flour Grinding Factory 7:	Enterococcus faecalis, Escherichia coli	Clostridium perfringens, Salmonella enterica

The overlap of some microbes isolated from both air and wastewater samples in some factories might show the potential cross-contamination routes needing attention for improved environmental and process controls. Findings like this underline the importance of integrated approaches in HACCP plans, considering all possible sources and vectors of microbial contamination [25, 26].

3.1. Implications on Factory Safety and Implementation of HACCP

Such findings of the present study can provide valuable insights that could be helpful in the optimization of HACCP programs dealing with microbial hazards in an industrial setting. In addition, the monitoring of the System as a whole on a regular basis by NGS would further provide data to track control measure effectiveness and adjust these dynamically, if necessary. This proactive attitude will help in saving not only the safety and quality of the products but also health regulation compliance, hence public health from possible harm, while at the same time preserving the integrity of operational factories.[27].

3.2. Phylogenetic Analysis of Bacterial Isolates

The NGS results from the samples shed considerable light on the composition of bacterial communities existing within Mosul factories' industrial settings. On this note, construction of a phylogenetic tree was done to explain genetic relationships among the different bacterial strains isolated from the air and wastewater samples, all this being a follow-up to the 16S rRNA gene sequencing. We hereafter discuss the results depicted in the phylogenetic tree based on 16S rRNA sequences of identified bacterial species [28].

3.3. Phylogenic Tree Analysis

This history tree represents few of the most important bacteria from both the point of food safety and the industrial hygiene issue:

Escherichia coli (strain U 5/41) and *Salmonella enterica* (subsp. *enterica* serovar Typhimurium strain LT2) are sister groups with each other. They are very significant microorganisms in terms of causing food poisoning.

Vibrio cholerae (strain RC782) and *Pseudomonas aeruginosa* (strain DSM 50071) are in different clades, which could be indicating different niches in the environment and pathogenic potentials. In this case, *Vibrio cholerae* is a very big public health concern because it has been associated with cholera outbreaks. *Campylobacter jejuni* (strain NCTC 11351) and *Clostridium perfringens* (ATCC 13124) are in the same clade, suggesting some common genetic features.

A further relationship that exists with the *Listeria monocytogenes* and *Enterococcus faecalis* strains, underpinning the Maillard reaction once more. Both strains depict their importance in food safety, characterized by their persistence and resistance mechanisms in food processing environments.

Staphylococcus aureus (ATCC 12600) and *Bacillus cereus* (ATCC 14579) are strains whose importance is seen in the vast areas they cover in food spoilage and food poisoning events.

The bootstrap values for major nodes are indicated on the tree to statistically show the clade support, among which several nodes have 100% high support, underscoring their reliability as phylogenetic relationships (figure1).

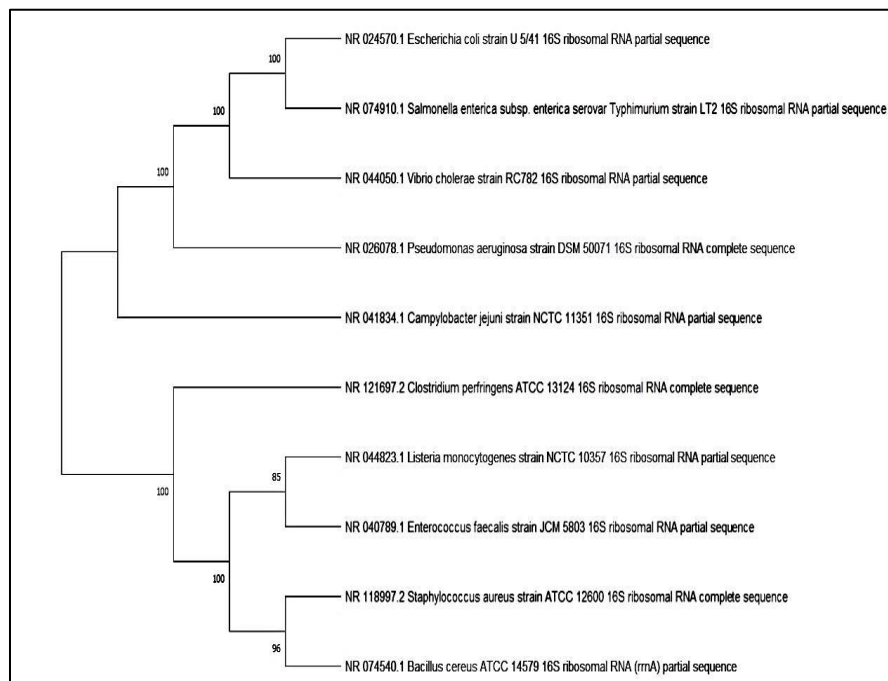


Figure 1. Phylogenetic tree of the genealogical relationships of bacterial strains identified in Mosul factories, based on 16S rRNA sequencing. All bootstrap values are indicated, showing statistical support of each clade. Key pathogenic species of relevance for industrial safety and health are highlighted, underlining their evolutionary and ecological relationships.

3.4. Implications for Factory Safety and HACCP Integration

The phylogenetic analysis provides a molecular fingerprinting framework that helps in traceability, sources of contamination, and its microbial dynamics within factory settings [29]. This information is very important in:

- Hazard Identification and Risk Assessment: Knowing specific relations among bacterial types that may create health risks would be useful for the identification of critical control points.
- Surveillance and Control: Using NGS to track regularly can be useful to track changes in the effectiveness of sanitation and hygiene practices with adjustment accordingly, based on phylogenetic insight.
- Regulatory Compliance: Microbial standards with regard to safety in line with the local and international guidelines.

Such phylogenetic insights can be integrated into HACCP systems operating in factories to better manage the risks associated with microbes and promote improved safety and business outcomes [23, 30]. This would safeguard public health, help factories operate within the ambit of environmental and health laws, and thus ensure a safe industrial environment for Mosul [31].

The application of NGS in this study has been opportunistic in deepening the identification and quantification of microbial contaminants that can become health risks in industrial settings [10]. Results from this study show that even as most factories were within control in terms of microbial contaminants, others showed blatancy in irregularities that may prove hazardous to health if allowed to go unchecked, thus calling for intervention[32].

3.5. Advantages of Integrating NGS Data into HACCP Systems Include

- Enhanced Detection: Next-generation sequencing provides a more sensitive and wide-ranging method for the detection of a wide array of pathogens that traditional methods might fail to detect.
- Data-driven interventions: With regard to the exact data of the nature and concentration of microbial contaminants, factories in an intervention phase shall have more efficient and targeted control measures.
- Regulatory Compliance: Detailed microbial profiles help to ensure that a facility meets health and safety standards, reducing the risk of regulatory noncompliance and the fiscal penalties brought about as a consequence.

Conclusion

Application of NGS in microbial assessment within the Mosul factories serves as a strong tool for enhancing food safety and operational efficiency. In that way, managers of the factories will be in a position to identify a location with potential risks at some point in production. According to HACCP, it is possible to allocate resources more efficiently at critical points along the process to enhance general safety and quality. Such a proactive approach in the management of safety will safeguard not only public health but also the industry's reputation and economic viability in the long term.

References

- [1] Uzoigwe, D.O., D.J.I.J.o.L.T.i.E. Kongolo, Management, and A. Science, Integration of Hazard Analysis and Critical Control Points HACCP with Maintenance Practices: Enhancing Food Safety in the Food and Beverage Industry; A Review. 2024. 13(4): p. 88-101.
- [2] Błaszczuk, I., The management of food safety in beverage industry, in Safety Issues in Beverage Production. 2020, Elsevier. p. 1-38.

- [3] Crippa, C., Artisanal food productions of animal origin: exploring food safety in the age of Whole Genome Sequencing. 2023.
- [4] Cook, N., M. D'Agostino, and K.C. Thompson, Molecular microbial diagnostic methods: pathways to implementation for the food and water industries. 2015: Academic Press.
- [5] Satam, H., et al., Next-generation sequencing technology: current trends and advancements. 2023. 12(7): p. 997.
- [6] McCombie, W.R., J.D. McPherson, and E.R.J.C.S.H.p.i.m. Mardis, Next-generation sequencing technologies. 2019. 9(11): p. a036798.
- [7] Lüth, S., Molecular surveillance of *Listeria monocytogenes* in Germany to control transmission along food supply chains and to prevent human listeriosis cases. 2021.
- [8] Boers, S.A., et al., Understanding and overcoming the pitfalls and biases of next-generation sequencing (NGS) methods for use in the routine clinical microbiological diagnostic laboratory. 2019. 38: p. 1059-1070.
- [9] of Engineering, N.A., et al., Acquiring and Applying Emerging Tools and Methods, in Transforming EPA Science to Meet Today's and Tomorrow's Challenges. 2023, National Academies Press (US).
- [10] Chawla, H., et al., A comprehensive review of microbial contamination in the indoor environment: sources, sampling, health risks, and mitigation strategies. 2023. 11: p. 1285393.
- [11] Zhou, J., P. Brereton, and K.J.F.C. Campbell, Progress towards achieving intelligent food assurance systems. 2024: p. 110548.
- [12] Maljkovic Berry, I., et al., Next generation sequencing and bioinformatics methodologies for infectious disease research and public health: approaches, applications, and considerations for development of laboratory capacity. 2020. 221(Supplement_3): p. S292-S307.
- [13] Cason, C., et al., Next-generation sequencing and PCR technologies in monitoring the hospital microbiome and its drug resistance. 2022. 13: p. 969863.
- [14] Walker, R.J., et al., An investigation of the microbial contamination of dental unit air and water lines. 2004. 54(6): p. 438-444.
- [15] Cutter, C.N.J.C.r.i.f.s. and nutrition, Microbial control by packaging: a review. 2002. 42(2): p. 151-161.
- [16] Olson, N.D. and J.B.J.B.r.n. Morrow, DNA extract characterization process for microbial detection methods development and validation. 2012. 5: p. 1-14.
- [17] Tay, T.K.Y., et al., Comparison of an amplicon-based large panel next generation sequencing (NGS) assay with conventional testing methods for MET and HER2 amplification in lung and breast cancers. 2024. 56(3): p. 325-333.
- [18] Barbadikar, K.M., et al., Assembly, Annotation and Visualization of NGS Data. 2024: p. 63-93.
- [19] Huang, L., et al., Bioinformatics-based analysis of programmed cell death pathway and key prognostic genes in gastric cancer: implications for the development of therapeutics. 2024. 26(1): p. e3590.
- [20] ESTIKOMAH, S.A., et al., Bacterial diversity in cheese wastewater using Next-Generation Sequencing (NGS). 2024. 25(2).
- [21] Vermelho, A.B., et al., Microbial Preservation and Contamination Control in the Baking Industry. 2024. 10(5): p. 231.
- [22] Loveniers, P.-J., F. Devlieghere, and I.J.I.J.o.F.M. Samplers, Towards tailored guidelines for microbial air quality in the food industry. 2024: p. 110779.
- [23] Mather, A.E., et al., Foodborne bacterial pathogens: genome-based approaches for enduring and emerging threats in a complex and changing world. 2024: p. 1-13.
- [24] Hazards, E.P.o.B., et al., Persistence of microbiological hazards in food and feed production and processing environments. 2024. 22(1): p. e8521.
- [25] Su, K., et al., The abundance and pathogenicity of microbes in automobile air conditioning filters across the typical cities of China and Europe. 2024. 472: p. 134459.
- [26] Santos, M.I., et al., Microbial Challenges in Fresh-cut Produce: A Comprehensive One Health Approach. 2024: p. 101.



- [27] Benson, C., et al., The impact of interventions on health, safety and environment in the process industry. 2024. 10(1).
- [28] Priyadarsini, S., et al., Sequencing And Phylogenetic Analysis of Microbiota Isolated from Different Types of Street Food Samples by Using 16S rRNA Sequencing and Insilico Analysis.
- [29] Bhadra, L., et al., Processed Food Microbiology and Safety: Risks, Trends and Future Perspectives, in Industrial Microbiology and Biotechnology: An Insight into Current Trends. 2024, Springer. p. 175-227.
- [30] Qian, J., et al., One Health: A holistic approach for food safety in livestock. 2022. 1: p. 100015.
- [31] Rahman, N.A.A., A. Hassan, and M.F. Mohammad, Halal logistics and supply chain management in Southeast Asia. 2020: Routledge.
- [32] Lai, H., et al., Microbiological Safety Assessment of Haccp-Certified Kitchens in Hotels and Restaurants: A Study in Eastern China.

Effect of DCI in Pumpkin Seed Alcoholic Extract on Type 2 Diabetic Rats Fed a Fatty Diet with Histological Study of Pancreas

Noor Abdulameer Kadhim Alriyahiu^{1,2} and Laith Fareed Hasan Al-Obaidi³

^{1,3}Department of Food Science, College of Agriculture, University of Kufa, Najaf, Iraq.

²Al-Farqadein University College, Basra, Iraq.

¹E-mail: noora.alriyahiu@student.uokufa.edu.iq

²E-mail: laithf.alobaidi@uokufa.edu.iq

Abstract. The study aimed to know the effect of pumpkin seed alcoholic extract containing a high percentage of DCI and DMSO on rats with type 2 diabetes and pancreatic tissues. The animals were given pumpkin seed alcoholic extract at a concentration of 400 mg/kg and metformin at a concentration of 250 mg/kg. DCI was diagnosed by HPLC of the alcoholic extract. The biological parameters (Glucose, Insulin, IR, Lipid profile) were measured after sacrificing the rats and drawing blood directly from the heart and removing the pancreas and placing it in a 10% formalin solution for histological study. The results showed that the alcoholic extract contained DCI at a rate of 2.136 mg/100 g, as well as the superiority of the alcoholic extract of pumpkin seeds at a concentration of 400 mg/kg in treating rats with type 2 diabetes. The results of the histological study indicated the ability of DCI to improve pancreatic functions and protect its tissue from complications of type 2 diabetes, and the ability of the alcoholic extract of pumpkin seeds to restore the tissue to normal, as well as reduce the level of fat.

Keywords. DCI, HPLC, Pumpkin seed, Pancreas, DMSO, Lipid profile, IR.

1. Introduction

Public and private health sector workers have begun to pay attention to the importance of functional foods in disease prevention [1]. Pumpkin seeds are characterized by blood sugar-lowering and anti-diabetic activities [2], including antioxidant activities, antimicrobial and blood sugar-lowering effects [3]. Antioxidant chemicals (pumpkin seeds) can help lower blood sugar levels in animals with impaired glucose metabolism [4]. Pumpkin seeds are also a good source of magnesium, one of the seven essential minerals. Magnesium is an important mineral that plays many roles in the body, including regulating blood sugar levels [5]. A study found that feeding diabetic rats 15 grams of pumpkin seed powder resulted in lower blood glucose, cholesterol, triglycerides, and LDL (low-density lipoprotein) levels [6]. Pumpkin seeds contain dihydrochlorothiazide (DCI), which is known for its blood sugar-lowering properties [7]. Recent studies have shown that DCI improves glucose metabolism in diabetic rats, as DCI treatment reduces insulin resistance, and DCI may be useful for improving insulin sensitivity of pancreatic alpha cells in the treatment of diabetes [8] [9]. DCI has been shown to stimulate insulin secretion

in beta cells and enhance glucose-stimulated insulin secretion in the islets of Langerhans [10]. DCI is originally an intermediate component of insulin action within cells, acting as a putative mediator to accelerate the dephosphorylation of glycogen synthase and pyruvate dehydrogenase, enzymes that determine the rate of glucose disposal. Administration of DCI to diabetic rats, Rhesus monkeys, and now to humans has accelerated glucose disposal and insulin sensitivity, with DCI restoring insulin sensitivity and glucose disposal at least partially [11]. Type 2 diabetes, also known as non-insulin-dependent diabetes mellitus (NIDDM), is a condition in which cells are unable to use insulin effectively, either with or without the complete absence of insulin. This particular classification was previously referred to as “adult-onset diabetes”[12]. This type occurs in approximately 90–95% of people with diabetes.[13] Insulin resistance in pancreatic alpha cells is associated with hypersecretion of glucagon in type 2 diabetes[8]. Many drugs have been used over the years to induce diabetes in experimental animals for study or to test therapeutic means prepared for it. The most commonly used chemical drugs are (Alloxan and streptozotocin (Alloxan is the most common [14]. It is one of the most important chemical compounds used to induce experimental diabetes mellitus in laboratory animals. Alloxan is a derivative of urea that causes damage to beta cells β -cells in the pancreatic islets [15]. Dimethyl sulfoxide (DMSO) is a clear, odorless liquid, and a means of penetration for various drugs [16], where the dose of DMSO plays an important role in causing negative reactions, as DMSO is safe to use in small doses [17]. Hence the idea of this research came and aimed to study the effect of the alcoholic extract of pumpkin seeds on some biochemical parameters and pancreatic tissue.

2. Materials and Methods

2.1. Alcoholic Extraction Method

The alcoholic extract of pumpkin seeds was prepared according to the method [18].

2.2. DCI Estimation In the Alcoholic Extract of Pumpkin Seeds

DCI was estimated in the alcoholic extract of pumpkin seeds by HPLC according to the method [19].

2.3. Experimental Animals

24 mature male Norwegian laboratory rats were provided at the age of 10 weeks and weighing 200-220 g. The experiment was conducted in the animal house of the College of Science, University of Kufa, Ethical approval No. 27086 in 7-12-2022.

2.4. Animal Groups

The 24 animals were divided into 4 groups, 6 animals per group, with similar weights as shown:

- The first group: The negative control group without treatment, with food and water given normally.
- The second group: The positive control group was injected with alloxan at a concentration of 90 mg/kg as a single dose and remained untreated, with food and water given normally.
- The third group: injected with alloxan at a concentration of 90 mg/kg and treated with pumpkin seed alcoholic extract at a concentration of 400 mg/kg + DMSO solvent diluted with distilled water at a rate of 0.1%.
- The fourth group: injected with alloxan at a concentration of 90 mg/kg and given DMSO solvent diluted with distilled water at a rate of 0.1%.

The duration of the experiment was 60 days divided into 30 days of feeding on Fatty feed The fatty feed was calculated by Professor Dr. Ali Mahmoud Al-Kassar, College of Veterinary Medicine, University of Kufa (Table 1).

Table 1. Feed components.

Components	Corn	Soybeans	Premix	Salt food	Limestone	Dicalcium phosphate	Fat	Total
percentage	55	35	2.5	0.3	0.7	1.5	5	100

Then, alloxan was injected and the animals were given the alcoholic extract of pumpkin seeds and DMSO for 30 days by Stomach tube. At the end of the experiment, the rats were anesthetized before sacrifice using an anesthetic mixture of 0.5 ml ketamine and 0.1 ml xylazine [20]. Blood samples were collected from the hearts of the rats and placed in a gel tube and then the centrifuge and the resulting serum was divided into Eppendorf tubes and the pancreas was placed in (10%) formalin for histological study.

2.5. Induction of Diabetes

It was done according to the method [21], as alloxan was prepared immediately before injection, where the dose was dissolved according to weight in 1 ml of physiological solution (Normal Saline 0.9%), and the rats were given a sugar solution with a concentration of 5% according to the method [22].

2.6. Blood Plasma Tests

Fasting plasma glucose Kit from (Spinreact (Spain)) was used using the Trinder method [23], Insulin Kit ELISA from (Biolabo (Spain)) according to the method [24], HOMA-IR index of insulin resistance using the formula:

$$\text{HOMA-IR} = [\text{Glucose (mg/dL)} \times \text{Insulin (}\mu\text{IU/ml)}] \div (405)$$

Estimation of total cholesterol (TC) level was based on the enzymatic method mentioned previously [25], estimation of triglycerides (TG) level was based on the enzymatic method mentioned previously [26], estimation of high-density lipoprotein (HDL-C) level was based on the enzymatic method

LDL-C concentration (mg/dl) = Total cholesterol - [(HDL-C) + (VLDL- C)], estimation of very low-density lipoprotein (VLDL-C) level

$$\text{VLDL concentration (mg/dl)} = (\text{Triglycerides} / 5)$$

2.7. Histological Study

Histological sections of the pancreas were prepared. The organs were sacrificed and treated according to [27].

2.8. Statistical Analysis

The statistical program Gentat V.12.10 was used in data analysis to study the effect of different treatments on the studied characteristics according to the completely randomized design (CRD), and the significant differences between the means were compared using Duncan's multiple range test DMRT.

3. Results and Discussion

3.1. *D-chiro inositol-1*

The percentage of DCI in the alcoholic extract was measured by HPLC, where the percentage in the alcoholic extract reached 2.136 mg/100 g.

3.2. *Biochemical Tests*

3.2.1. *Biochemical Tests for Rats After the Fatty Diet*

Table (2) shows the results of the biochemical tests for experimental rats after 30 days of feeding with a high-calorie fatty diet. The results indicated an increase in blood sugar for the High Fat Diet group, which reached 103.3 (mg/dl) compared to the control group 88 (mg/dl). This result is less than what was stated in [28,29]. As for insulin, the results indicated an increase in insulin for the High Fat Diet group, which reached 41.218 (unit/liter) compared to the control group 6.01 (unit/liter). This result is higher than what was stated in [30]. Insulin secretion increases as a result of the weakness of the glucose metabolism process, where High fat diet causes metabolic disorders [31]. The results of the table indicated an increase in insulin resistance for the High Fat Diet group, which reached 10.412 compared to the control group, which reached 0.39 (unit/liter). This result is higher than what was reached by [29], which was 6.11 (unit/liter). The results of the table showed an increase in VLDL, HDL, LDL, TC, and TG fats for the High Fat Diet group, reaching (195.44, 27.37, 36.1, 180.56, 258.93) (mg/dL) compared to the control group (66.7, 91.9, 26.7, 185.3, 133.5) (mg/dL) respectively. This result is higher than what was reported in [28], except for LDL, which was lower than what was reported. High-fat diets often cause an increase in triglycerides (TG) and cholesterol (TC) due to high calories and fat accumulation in adipose tissues, as high-fat diets lead to a decrease in high-density lipoprotein (HDL) levels [32,33]. Very low-density lipoprotein (VLDL) levels increase due to increased fat formation in the liver, as excess fatty acids are transported to the liver and converted into triglycerides, and the liver secretes high amounts of VLDL to transport triglycerides into the bloodstream. [34,35].

Table 2. Effect of fatty feed on the vital functions of rats.

No.	Tests	High Fat Diet	Control
1	Glucose	103.3	88
2	Insulin	41.218	6.01
3	IR-HOMA	10.412	0.39
4	TG	180.56	133.5
5	TC	258.93	185.3
6	HDL	27.37	66.7
7	LDL	195.44	91.9
8	VLDL	36.1	26.7

The numbers represent the average of three replicates in which blood was drawn directly from the heart.

3.2.2. *Biochemical Tests for Rats After Diabetes*

Table (3) shows the results of the biochemical tests for experimental rats after 30 days of developing type 2 diabetes. The results indicated an increase in blood sugar for the positive control group, which reached 463.7 (mg/dl) compared to the negative control group, which reached 95.7 (mg/dl). This result is higher than what was reported in [36,37]. Alloxan is considered a cytotoxic cytotoxic, as alloxan causes selective destruction of pancreatic beta cells, thus reducing insulin secretion in the cells and then increasing blood sugar levels [38]. The results of the table showed a significant decrease in blood sugar for the T1 group, which reached 268.7

(mg/dl) compared to the positive control group, which reached 463.7 (mg/dl). This result is higher than what was reached by [6], as experiments showed that pumpkin seeds work to reduce blood sugar [7]. Pumpkin seeds contain D-chiro inositol (DCI), which has an effect on lowering and regulating blood sugar. DCI also enhances glucose metabolism [9,39]. The results of the table indicated a non-significant decrease in blood sugar for the T2 group, which amounted to 448.7 (mg/dl) compared to the positive control group, which amounted to 463.7 (mg/dl), as DMSO did not affect blood sugar as stated in [40]. As for insulin, the results indicated an increase in insulin for the positive control group, which amounted to 8.69 (unit/liter) compared to the negative control group, which amounted to 2.45 (unit/liter). This result is higher than what was reached by [36], [41]. The reason for the increase in insulin is due to weakness in insulin production in beta cells, so the body compensates for this deficiency by producing insulin in a greater way, and as a result of the cells not responding due to insulin resistance [42,43]. The results of the table showed a significant decrease in insulin for the T1 group, which amounted to 5.88 (unit/liter) compared to the positive control group, which amounted to 8.69 (unit/liter), as pumpkin seeds work to renew beta cells and restore Insulin secretion [7], as DCI improves the insulin signaling pathway by restoring the phosphorylation of insulin receptor (IR), insulin receptor substrate 1 (IRS-1), and Akt in pancreatic alpha cells [8]. Studies have indicated the ability of DCI to improve insulin sensitivity [44]. The results of the table indicated a non-significant decrease in insulin for the T2 group, amounting to 8.67 (unit/liter) compared to the positive control group, 8.69 (unit/liter), as DMSO at a concentration of 0.1% did not affect the insulin level, while it can improve insulin secretion at high concentrations (1.25-2.5%) [45]. The results of the table indicated a significant increase in insulin resistance for the positive control group, which reached 10.67% compared to the negative control group, which reached 0.59%. This result is higher than what was reached by [36]. The toxic effects of alloxan cause metabolic changes, including increased lipid oxidation and inflammation, and lead to a weakening of the response to insulin and an increase in blood sugar, which contributes to the occurrence of insulin resistance [41,46]. The results of the table showed a significant decrease in insulin resistance for the T1 group, which amounted to 3.9% compared to the positive control group of 10.67%. Pumpkin seeds significantly reduce insulin resistance and enhance the regeneration of pancreatic beta cells, which contributes to improving blood glucose and insulin resistance [7], as pumpkin seeds contain the compound (DCI), which is a fatty acid that stimulates insulin resistance [8]. The results of the table indicated a significant decrease in insulin resistance for the T2 group, which amounted to 9.68% compared to the positive control group of 10.67%, as the effect of DMSO is simple on insulin resistance because DMSO at low concentrations does not have a direct effect [47]. The results showed a significant increase in triglycerides in the positive control group, reaching 230.3 (mg/dL) compared to the negative control group, 133.6 (mg/dL). This result is higher than what was reached by [6,36]. The increase in triglycerides in rats with alloxan-induced diabetes is due to high blood sugar resulting from the destruction of pancreatic beta cells. This condition leads to increased fat decomposition and the release of fatty acids into the blood. Then the liver converts fatty acids into triglycerides. Also, as a result of high blood sugar, the action of the lipoprotein lipase (LPL) enzyme is disabled, which leads to an exacerbation of blood fats [48], [49]. The results of the table showed a significant decrease in triglycerides for the T1 group, which reached 178.6 (mg/dl) compared to the positive control group, which reached 280.3 (mg/dl). This result is higher than what was reached by [6]. Pumpkin seeds reduce triglycerides and also reduce the accumulation of fat in the liver [50]. Pumpkin seeds contain selenium [51], as selenium has lipid-lowering properties [52]. The results of the table indicated a non-significant decrease in triglycerides for the T2 group, which reached 278 (mg/dl) compared to the positive control group, which reached 280.3 (mg/dl). DMSO did not have a clear effect on lipids [53], perhaps because the concentration was very low.

The results showed a significant increase in cholesterol for the positive control group, which reached 340 (mg/dl) compared to the negative control group, which reached 185.4 (mg/dl). The results of the table showed a significant decrease in cholesterol for the group (T1, T2), which reached (327,214.2) (mg/dl) compared to the positive control group, which reached 340 (mg/dl). This result is higher than what was reached by [6,54]. The reason for the increase is mainly attributed to the increase in fats and the disturbance of the metabolic process, as the lack of insulin due to the damage caused by alloxan in destroying beta cells leads to an increase in fatty acids from adipose tissue, which the liver converts into cholesterol [49]. Insulin resistance also leads to a greater increase in cholesterol levels [55], as no clear effect of DMSO on cholesterol was observed [53]. This is due to the decrease in The concentration used in the study.

The results showed a significant decrease in HDL for the positive control group, which reached 23.75 (mg/dL) compared to the negative control group, which reached 66.7 (mg/dL), and this result is less than what was reached [6,36]. The results of the table showed an increase in HDL for the group (T1, T2), which reached (27.15, 57.46) (mg/dL) compared to the positive control group, which reached 23.75 (mg/dL), and this result is less than what was reached [6]. The oil extract of pumpkin seeds works to increase the level of high-density lipoprotein cholesterol HDL [56]. DMSO has an effect on reducing the activity of the hepatic cholesterol enzyme 7α -hydroxylase ($CH-7\alpha$), which is the enzyme that determines the rate of bile acid synthesis, significantly in rats, as this leads to a decrease in bile acid synthesis and an increase in blood cholesterol levels, which is consistent with the significant increase in high-density lipoprotein HDL [57]. The results showed a significant increase in LDL for the positive control group, reaching 260.2 (mg/dL) compared to the negative control group, 91.9 (mg/dL), and this result is higher than what was reached [6,36]. The results of the table showed an increase in LDL for the group (T1, T2), reaching (245.1, 121) (mg/dL) compared to the positive control group, 260.2 (mg/dL), and this result is higher than what was reached [6], as the decrease in the LDL level increased with the increase in the concentration of pumpkin seed extract, as it was found that pumpkin seeds reduce the level of low-density lipoprotein LDL [56]. The decrease in the LDL level in rats given DMSO is primarily due to the inhibition of bile acid synthesis. DMSO significantly reduces the activity of hepatic cholesterol 7α -hydroxylase, which is the enzyme that determines the rate of bile acid production, leading to a decrease in bile acid levels and an increase in blood cholesterol levels. This change in cholesterol metabolism leads to To lower LDL levels, as the body adapts to changes in bile acid synthesis and cholesterol balance [57], [58]. The results showed a significant increase in VLDL for the positive control group, reaching 56.05 (mg/dL) compared to the negative control group 26.71 (mg/dL), and this result is higher than what was reported in [59,60]. The results of the table showed a significant decrease in VLDL for the T1 group, reaching 35.72 (mg/dL) compared to the positive control group 56.05 (mg/dL), and this result is higher than what was reported in [60], as the level of decrease increased with increasing the concentration of the alcoholic extract of pumpkin seeds. Pumpkin seeds have an effect on reducing the level of very low-density lipoprotein, as studies have indicated the effect of pumpkin seeds on reducing fats and cholesterol through sugar levels and reducing insulin resistance [9,28]. The results of the table indicated a non-significant decrease in VLDL for the T2 group, which amounted to 55.60 (mg/dl), compared to the positive control group, which amounted to 56.05 (mg/dl), as DMSO did not have a clear effect on VLDL [53], which may be because the concentration was very low.

Table 3. The effect of diabetes on the vital functions of rats.

Tests	G-	G+	T1	T2
Glucose	95.7a	463.7d	268.7c	497.8d
Insulin	2.45a	8.69d	5.88b	8.67d
IR-HOMA	0.59a	10.67e	3.9b	9.68d
TC	185.4a	340e	214.2b	327d
TG	133.6a	280.3d	178.6b	278d
HDL	66.7e	23.75a	57.46d	27.15b
LDL	91.9a	260.2e	121b	245.1d
VLDL	26.71a	56.05d	35.72b	55.60d

- Values are expressed as the arithmetic mean of six replicates
- The presence of different lowercase letters between groups indicates the presence of significant differences at the probability level ($P < 0.05$) according to Duncan's test
- C- represents the negative control treatment, C+ represents the positive control treatment, T1 represents the treatment treated with pumpkin seed alcoholic extract, and T2 represents the DMSO treatment.

3.2.3. Histological Study of the Pancreas

The results of the histological study of the pancreas sections in the negative control group G- showed the presence of normal islets of Langerhans, and normal pancreatic tissues as shown in Figure (1).

The results of the histological structure of the pancreas sections in the positive control group G+ and the DMSO group (T5) rats infected with alloxan-induced diabetes showed the appearance of many pathological changes, including: dissolution of pancreatic tissue and loss of some cells with atrophy of secretory cells, bleeding within the tissues, bleeding and dilation of some blood vessels. These results indicate a complex pattern of tissue damage, including cell lysis, vascular changes, and inflammatory response as shown in Figure (2)(4), this result is consistent with [14], [46], where alloxan destroys beta cells, leading to a significant decrease in the islets of Langerhans [61], DMSO did not affect the result as mentioned [53].

While the results of group 1T of diabetic rats treated with alloxan and alcoholic extract of pumpkin seeds at a concentration of 400 mg/kg indicated the appearance of normal islets of Langerhans and no side effects as shown in Figure (3), this result is consistent with [62], pumpkin seeds reduce diabetes complications and improve insulin levels [60], where DCI enhances sugar absorption and renews cells, which helps improve pancreatic functions in rats with type 2 diabetes [63].

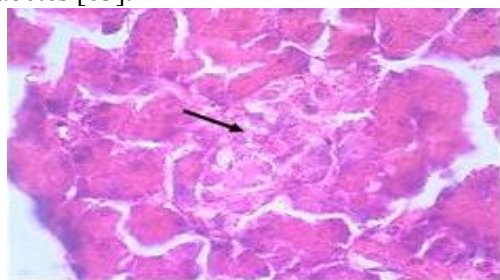


Figure 1. Shows a section of the pancreatic tissue of a rat from the G-negative control group and beta cells in the islets of Langerhans (black arrow), the tissue was stained with hematoxylin and eosin, magnification power (40X).

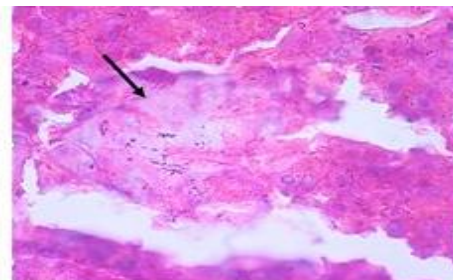


Figure 2. Shows a section of the pancreatic tissue of a rat from the G+ positive control group, with complete death and degeneration of beta cells from the islets of Langerhans (black arrow). The tissue was stained with hematoxylin and eosin, magnification (40X).

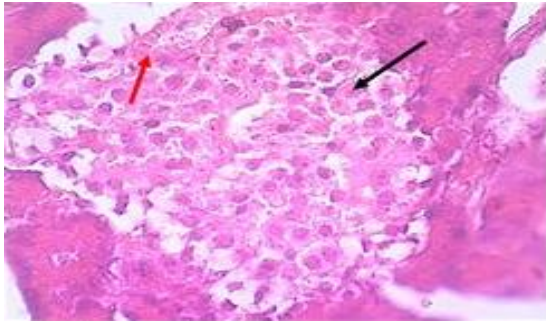


Figure 3. Shows a section of the pancreatic tissue of a rat from the T1 group, the alcoholic extract of pumpkin seeds at a concentration of 400 mg/kg, the normal structure of the pancreatic tissue and beta cells in the islets of Langerhans with slight degeneration of the beta cells (black arrow) and the presence of slight congestion in the pancreatic islets (red arrow), the tissue was stained with hematoxylin and eosin, magnification power (40X).

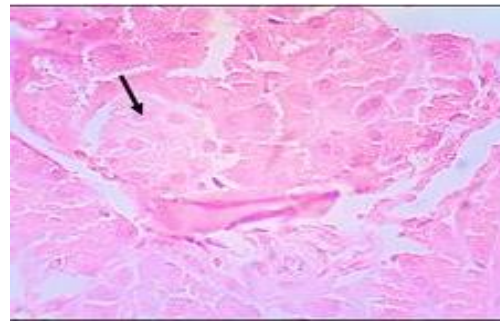


Figure 4. Shows a section of the pancreatic tissue of a rat from the DMSO T2 group, showing the abnormal structure of the liver tissue, where the hepatic cells appear damaged, with fatty liver degeneration and disappearance of the hepatic sinusoids (black arrow). The tissue was stained with hematoxylin and eosin, magnification power (40X).

Conclusions

This study revealed that the alcoholic extract of pumpkin seeds contains active substances that have the ability to alleviate the complications of type 2 diabetes, the most important of which are DCI.

And that feeding fatty feed for a month raised the level of cholesterol and fats, which led to insulin resistance, which is an important indicator of the possibility of developing type 2 diabetes. The ability of the alcoholic extract of pumpkin seeds at a concentration of 400 mg/kg to reduce the level of fats and improve the functions of liver tissue.

References

- [1] C. Yang, B. Wang, J. Wang, S. Xia, and Y. Wu, "Effect of pyrogalllic acid (1,2,3-benzenetriol) polyphenol-protein covalent conjugation reaction degree on structure and antioxidant properties of pumpkin (*Cucurbita* sp.) seed protein isolate," *Lwt*, vol. 109, no. January, pp. 443–449, 2019, doi: 10.1016/j.lwt.2019.04.034.
- [2] G. G. Adams *et al.*, "The hypoglycaemic effect of pumpkins as anti-diabetic and functional medicines," *Food Res. Int.*, vol. 44, no. 4, pp. 862–867, 2011, doi: 10.1016/j.foodres.2011.03.016.
- [3] Z. Jiang and Q. Du, "Glucose-lowering activity of novel tetrasaccharide glyceroglycolipids from the fruits of *Cucurbita moschata*," *Bioorganic Med. Chem. Lett.*, vol. 21, no. 3, pp. 1001–1003, 2011, doi: 10.1016/j.bmcl.2010.12.030.
- [4] S. Kainat *et al.*, "Sustainable novel extraction of bioactive compounds from fruits and vegetables waste for functional foods: a review," *Int. J. Food Prop.*, vol. 25, no. 1, pp. 2457–2476, 2022, doi: 10.1080/10942912.2022.2144884.
- [5] J. J. DiNicolantonio, J. H. O'Keefe, and W. Wilson, "Subclinical magnesium deficiency: A principal driver of cardiovascular disease and a public health crisis," *Open Hear.*, vol. 5, no. 1, 2018, doi: 10.1136/openhrt-2017-000668.
- [6] A. Hussain *et al.*, "In Vitro Role of Pumpkin Parts as Pharma-Foods: Antihyperglycemic and Antihyperlipidemic Activities of Pumpkin Peel, Flesh, and Seed Powders, in Alloxan-Induced Diabetic Rats," *Int. J. Food Sci.*, vol. 2022, 2022, doi: 10.1155/2022/4804408.
- [7] Z. Tasya, R. Amiruddin, A. Syam, and Y. Thamrin, "Pumpkin Seed Intervention to Control Diabetes Mellitus: A Systematic Review," *Open Access Maced. J. Med. Sci.*, vol. 10, no. F, pp. 535–540, 2022, doi: 10.3889/oamjms.2022.9850.

- [8] A. Filippello *et al.*, "Direct effects of d-chiro-inositol on insulin signaling and glucagon secretion of pancreatic alpha cells," *Biomolecules*, vol. 10, no. 10, pp. 1–15, 2020, doi: 10.3390/biom10101404.
- [9] C. Fan, W. Liang, M. Wei, X. Gou, S. Han, and J. Bai, "Effects of D-Chiro-Inositol on Glucose Metabolism in db/db Mice and the Associated Underlying Mechanisms," *Front. Pharmacol.*, vol. 11, no. March, pp. 1–13, 2020, doi: 10.3389/fphar.2020.00354.
- [10] R. Lazarenko, J. Geisler, D. Bayliss, J. Larner, and C. Li, "D-chiro-inositol glycan stimulates insulin secretion in pancreatic β cells," *Mol. Cell. Endocrinol.*, vol. 387, no. 1–2, pp. 1–7, 2014, doi: 10.1016/j.mce.2014.02.004.
- [11] J. Larner, "D-chiro-inositol-its functional role in insulin action and its deficit in insulin resistance," *Int. J. Exp. Diabetes Res.*, vol. 3, no. 1, pp. 47–60, 2002, doi: 10.1080/15604280212528.
- [12] S. R. Patil, A. B. Chavan, A. M. Patel, P. D. Chavan, and J. V. Bhopale, "A Review on Diabetes Mellitus its Types, Pathophysiology, Epidemiology and its Global Burden," *J. Res. Appl. Sci. Biotechnol.*, vol. 2, no. 4, pp. 73–79, 2023, doi: 10.55544/jrasb.2.4.9.
- [13] D. Care and S. S. Suppl, "6 . Glycemic Targets : Standards of Medical Care in Diabetes — 2022," vol. 45, no. January, pp. 83–96, 2022.
- [14] Y. F. Radjabovich, S. D. Shukhratovich, K. K. Khujayazovna, M. B. Madirimovich, and M. N. Odilbekovich, "IMPROVEMENT OF AN EXPERIMENTAL MODEL FOR THE DEVELOPMENT OF DIABETES MELLITUS IN LABORATORY," no. January, pp. 8–14, 2024.
- [15] G. Sudhakaran *et al.*, "Deacetylated nimbin analog N2 fortifies alloxan-induced pancreatic β -cell damage in insulin-resistant zebrafish larvae by upregulating phosphoenolpyruvate carboxykinase (PEPCK) and insulin levels," *Toxicol. Appl. Pharmacol.*, vol. 454, p. 116229, Nov. 2022, doi: 10.1016/j.taap.2022.116229.
- [16] M. Karim, R. S. Boikess, R. A. Schwartz, and P. J. Cohen, "Dimethyl sulfoxide (DMSO): a solvent that may solve selected cutaneous clinical challenges," *Arch. Dermatol. Res.*, vol. 315, no. 6, pp. 1465–1472, Dec. 2022, doi: 10.1007/s00403-022-02494-1.
- [17] M. Hilscher, D. Zetner, J. Rosenberg, and B. K. Madsen, "Adverse reactions of dimethyl sulfoxide in humans : a," *F1000Research*, pp. 1–21, 2019.
- [18] P. N. Muchirah, R. Waihenya, S. Muya, L. Abubakar, H. Ozwara, and A. Makokha, "Characterization and anti-oxidant activity of Cucurbita maxima Duchesne pulp and seed extracts," *J. Phytopharm.*, vol. 7, no. 2, pp. 134–140, 2018, doi: 10.31254/phyto.2018.7206.
- [19] V. Y. Ixtaina *et al.*, "Characterization of chia seed oils obtained by pressing and solvent extraction," *J. Food Compos. Anal.*, vol. 24, no. 2, pp. 166–174, 2011, doi: 10.1016/j.jfca.2010.08.006.
- [20] H. Şahin Kavakli, C. Koca, and Ö. Alici, "Antioxidant effects of curcumin in spinal cord injury in rats," *Ulus. Travma ve Acil Cerrahi Derg.*, vol. 17, no. 1, pp. 14–18, 2011, doi: 10.5505/tjtes.2011.31391.
- [21] O. M. Ighodaro, A. M. Adeosun, and O. A. Akinloye, "Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies," *Med.*, vol. 53, no. 6, pp. 365–374, 2017, doi: 10.1016/j.medici.2018.02.001.
- [22] C. Luo, D. Luo, L. Chen, H. Zhou, R. Zhou, and J. Wei, "Methods to Reduce the Hypoglycemic Mortality of Alloxan in Diabetic Rabbit Model," *Biosci. Med.*, vol. 12, pp. 242–255, 2024, doi: 10.4236/jbm.2024.125019.
- [23] R. Infirmary, "x 200.," vol. 6, pp. 24–27, 1969.
- [24] P. Phospho-irs-, E. Kit, and E. W. Buffer, "PathScan ® Phospho-IRS-2 (panTyr) Sandwich ELISA Kit," *cell Signal.*, vol. 2, pp. 24–27, 2024.
- [25] V. Sharma and S. M. Mobin, "peroxidase mimic CuO:graphene nanosphere composite as coloriCytocompatiblemetric dual sensor for hydrogen peroxide and cholesterol with its logic gate implementation," *Sensors Actuators, B Chem.*, vol. 240, pp. 338–348, 2017, doi: 10.1016/j.snb.2016.08.169.
- [26] A. S. Hamed and A. H. Farhan, "Evaluation of lipid profile with different ABO blood groups among Iraqi healthy people," *Al-Nisour J. Med. Sci.*, vol. 4, no. 2, pp. 186–193, 2022.
- [27] J. D. Bancroft and M. Gamble, "Theory and Practice of Histological Techniques, Sixth Edition," *Theory Pract. Histol. Tech. Sixth Ed.*, pp. 1–725, Jan. 2007, doi: 10.1097/nen.0b013e31817e2933.

- [28] A. Elsenousy, A. Farid, and K. Fararh, "Effect of pumpkin seed oil on lipid metabolism in experimental hyperlipidemic rats," *Benha Vet. Med. J.*, vol. 36, no. 1, pp. 302–309, 2019, doi: 10.21608/bvmj.2019.114449.
- [29] A. Ghahremanloo, R. Hajipour, M. Hemmati, M. Moossavi, and Z. Mohaqiq, "The beneficial effects of pumpkin extract on atherogenic lipid, insulin resistance and oxidative stress status in high-fat diet-induced obese rats," *J. Complement. Integr. Med.*, vol. 15, no. 2, pp. 1–7, 2018, doi: 10.1515/jcim-2017-0051.
- [30] X. xuan Guo, Y. Wang, K. Wang, B. ping Ji, and F. Zhou, "Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection," *J. Zhejiang Univ. Sci. B*, vol. 19, no. 7, pp. 559–569, 2018, doi: 10.1631/jzus.B1700254.
- [31] B. P. Melo *et al.*, "Thirty days of combined consumption of a high-fat diet and fructose-rich beverages promotes insulin resistance and modulates inflammatory response and histomorphometry parameters of liver, pancreas, and adipose tissue in Wistar rats," *Nutrition*, vol. 91–92, p. 111403, Nov. 2021, doi: 10.1016/j.nut.2021.111403.
- [32] Y. J. Jia, J. Liu, Y. L. Guo, R. X. Xu, J. Sun, and J. J. Li, "Dyslipidemia in rat fed with high-fat diet is not associated with PCSK9-LDL-receptor pathway but ageing," *J. Geriatr. Cardiol.*, vol. 10, no. 4, pp. 361–368, 2013, doi: 10.3969/j.issn.1671-5411.2013.04.007.
- [33] S. Lasker *et al.*, "High-fat diet-induced metabolic syndrome and oxidative stress in obese rats are ameliorated by yogurt supplementation," *Sci. Rep.*, vol. 9, no. 1, pp. 1–15, 2019, doi: 10.1038/s41598-019-56538-0.
- [34] Y. S. Sidorova, N. A. Petrov, Y. M. Markova, A. I. Kolobanov, and S. N. Zorin, "The Influence of a High-Cholesterol Diet and Forced Training on Lipid Metabolism and Intestinal Microbiota in Male Wistar Rats," *Int. J. Mol. Sci.*, vol. 25, no. 10, 2024, doi: 10.3390/ijms25105383.
- [35] A. Udomkasemsab and P. Prangthip, "High fat diet for induced dyslipidemia and cardiac pathological alterations in Wistar rats compared to Sprague Dawley rats," *Clin. e Investig. en Arterioscler.*, vol. 31, no. 2, pp. 56–62, 2019, doi: 10.1016/j.arteri.2018.09.004.
- [36] I. Ibrahim, Z. Abdel-razek Saleh, F. Ali, and E. Abdel-Hakeem, "Pathophysiological mechanisms of type 2 diabetes mellitus and the effects of metformin treatment in adult male albino rats.," *Minia J. Med. Res.*, vol. 0, no. 0, pp. 0–0, 2023, doi: 10.21608/mjmr.2023.184444.1271.
- [37] M. B. R. Al-Dbagh and S. M. Jwad, "Evaluation of efficiency of shrimp extract (Astaxanthin) histologically and immunologically in the treatment of induced diabetes mellitus with alloxan in albino male rats," *Int. J. Pharm. Res.*, vol. 12, no. 3, pp. 1932–1943, 2020, doi: 10.31838/ijpr/2020.12.03.268.
- [38] M. Naim *et al.*, "Comparative Study of Antidiabetic Activity of Hexane-Extract of Lemon Peel (Limon citrus) and Glimepiride in Alloxan-Induced Diabetic Rats," *Bangladesh Pharm. J.*, vol. 15, no. 2, pp. 131–134, 2012, doi: 10.3329/bpj.v15i2.12577.
- [39] S. Jane Monica *et al.*, "Chemical composition of pumpkin (*Cucurbita maxima*) seeds and its supplemental effect on Indian women with metabolic syndrome," *Arab. J. Chem.*, vol. 15, no. 8, 2022, doi: 10.1016/j.arabjc.2022.103985.
- [40] G. J. Lin *et al.*, "Dimethyl sulfoxide inhibits spontaneous diabetes and autoimmune recurrence in non-obese diabetic mice by inducing differentiation of regulatory T cells," *Toxicol. Appl. Pharmacol.*, vol. 282, no. 2, pp. 207–214, 2015, doi: 10.1016/j.taap.2014.11.012.
- [41] N. A. Shah and M. R. Khan, "Antidiabetic effect of *Sida cordata* in alloxan induced diabetic rats," *Biomed Res. Int.*, vol. 2014, 2014, doi: 10.1155/2014/671294.
- [42] M. G. Rad, M. Sharifi, R. Meamar, and N. Soltani, "The role of pancreas to improve hyperglycemia in STZ-induced diabetic rats by thiamine disulfide," *Nutr. Diabetes*, vol. 12, no. 1, pp. 1–8, 2022, doi: 10.1038/s41387-022-00211-5.
- [43] N. A. Qinna and A. A. Badwan, "Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats," *Drug Des. Devel. Ther.*, vol. 9, pp. 2515–2525, 2015, doi: 10.2147/DDDT.S79885.

- [44] K. I. Cheang *et al.*, "Insulin-stimulated release of d-chiro-inositol-containing inositolphosphoglycan mediator correlates with insulin sensitivity in women with polycystic ovary syndrome," *Metabolism*, vol. 57, no. 10, pp. 1390–1397, Oct. 2008, doi: 10.1016/j.metabol.2008.05.008.
- [45] D. R. Bijukumar, C. McGeehan, and M. T. Mathew, "Dimethyl sulfoxide acutely enhances regulated insulin secretion in the MIN6-K8 mouse insulinoma cell line," *Physiol. Behav.*, vol. 176, no. 1, pp. 139–148, 2022, doi: 10.1007/s00418-021-01984-z.Dimethyl.
- [46] R. M. Ibrahim, H. M. Abdelhafez, S. A. E. M. EL-Shamy, F. A. Eid, and A. Mashaal, "Arabic gum ameliorates systemic modulation in Alloxan monohydrate-induced diabetic rats," *Sci. Rep.*, vol. 13, no. 1, pp. 1–11, 2023, doi: 10.1038/s41598-023-31897-x.
- [47] M. Berenguer *et al.*, "Dimethyl sulfoxide enhances GLUT4 translocation through a reduction in GLUT4 endocytosis in insulin-stimulated 3T3-L1 adipocytes," *Biochimie*, vol. 93, no. 4, pp. 697–709, Apr. 2011, doi: 10.1016/j.biochi.2010.12.013.
- [48] P. Yin, Y. Wang, L. Yang, J. Sui, and Y. Liu, "Hypoglycemic Effects in Alloxan-Induced Diabetic Rats of the Phenolic Extract from Mongolian Oak Cups Enriched in Ellagic Acid, Kaempferol and Their Derivatives," *Molecules*, vol. 23, no. 5, 2018, doi: 10.3390/molecules23051046.
- [49] M. Banda, J. Nyirenda, K. Muzandu, G. Sijumbila, and S. Mudenda, "Antihyperglycemic and Antihyperlipidemic Effects of Aqueous Extracts of *Lannea edulis* in Alloxan-Induced Diabetic Rats," *Front. Pharmacol.*, vol. 9, no. September, pp. 1–8, 2018, doi: 10.3389/fphar.2018.01099.
- [50] L. M. de Farias *et al.*, "Hypotriglyceridemic and hepatoprotective effect of pumpkin (*Cucurbita moschata*) seed flour in an experimental model of dyslipidemia," *South African J. Bot.*, vol. 151, pp. 484–492, Dec. 2022, doi: 10.1016/j.sajb.2022.05.008.
- [51] I. Kreft, V. Stibilj, and Z. Trkov, "Iodine and selenium contents in pumpkin (*Cucurbita pepo* L.) oil and oil-cake," *Eur. Food Res. Technol.*, vol. 215, no. 4, pp. 279–281, 2002, doi: 10.1007/s00217-002-0563-5.
- [52] A. A. A. Tarmizi *et al.*, "The Ameliorative Effects of Selenium Nanoparticles (SeNPs) on Diabetic Rat Model: A Narrative Review," *Sains Malaysiana*, vol. 52, no. 7, pp. 2037–2053, 2023, doi: 10.17576/jsm-2023-5207-12.
- [53] P. Deol, J. Yang, C. Morisseau, B. D. Hammock, and F. M. Sladek, "Dimethyl sulfoxide decreases levels of oxylipin diols in mouse liver," *Front. Pharmacol.*, vol. 10, no. MAY, pp. 1–7, 2019, doi: 10.3389/fphar.2019.00580.
- [54] S. Dubey, C. Yadav, A. Bajpayee, and M. P. Singh, "Effect of pleurotus fossulatus aqueous extract on biochemical properties of liver and kidney in streptozotocin-induced diabetic rat," *Diabetes, Metab. Syndr. Obes.*, vol. 13, pp. 3035–3046, 2020, doi: 10.2147/DMSO.S265798.
- [55] A. Sarsenbayeva *et al.*, "Impaired hmg-coa reductase activity caused by genetic variants or statin exposure: Impact on human adipose tissue, β -cells and metabolome," *Metabolites*, vol. 11, no. 9, pp. 1–22, 2021, doi: 10.3390/metabo11090574.
- [56] B. K. Ramadan, S. A. Mohammad, E. S. Mahmoud, and E. A. Ouda, "Role of Pumpkin Seed Oil on Some Cardiovascular and Renal Aspects in Adult Male Albino Rats," *Al-Azhar Med. J.*, vol. 45, no. 4, pp. 931–956, 2016, doi: 10.12816/0034755.
- [57] A. S. Hassan, "The Effect of Dimethyl Sulfoxide on Cholesterol and Bile Acid Metabolism in Rats," *Exp. Biol. Med.*, vol. 186, no. 2, pp. 205–210, Nov. 1987, doi: 10.3181/00379727-186-42604.
- [58] T. B. Estridge *et al.*, *Identification of 4-aminopyrazolopyrimidine metabolite that may contribute to the hypolipidemic effects of LY2584702 in long evans diet-induced obese rats*, vol. 362, no. 1. 2017. doi: 10.1124/jpet.117.240242.
- [59] A. Ghadge, A. Harsulkar, M. Karandikar, V. Pandit, and A. Kuvalekar, "Comparative anti-inflammatory and lipidnormalizing effects of metformin and omega-3 fatty acids through modulation of transcription factors in diabetic rats," *Genes Nutr.*, vol. 11, no. 1, pp. 1–12, 2016, doi: 10.1186/s12263-016-0518-4.
- [60] ester abd-elnoor, "Hypoglycemic and Hypolipidemic Effects of Pumpkin Seeds Powder and Oil on Alloxan-Induced Diabetic in Rats," *Egypt. J. Food Sci.*, vol. 0, no. 0, pp. 0–0, 2019, doi: 10.21608/ejfs.2019.19348.1027.



- [61] M. Elmeligy, A. Farid, and K. Fararh, "Antioxidant and hepatoprotective effect of pumpkin seed oil in CCl₄-intoxicated rats," *Benha Vet. Med. J.*, vol. 36, no. 2, pp. 77–89, 2019, doi: 10.21608/bvmj.2019.13448.1030.
- [62] M. Makni, H. Fetoui, N. K. Gargouri, E. M. Garoui, and N. Zeghal, "Antidiabetic effect of flax and pumpkin seed mixture powder: Effect on hyperlipidemia and antioxidant status in alloxan diabetic rats," *J. Diabetes Complications*, vol. 25, no. 5, pp. 339–345, 2011, doi: 10.1016/j.jdiacomp.2010.09.001.
- [63] Y. Gao, M. Zhang, T. Wang, T. Wu, R. Ai, and Z. Zhang, "Hypoglycemic effect of D-chiro-inositol in type 2 diabetes mellitus rats through the PI3K/Akt signaling pathway," *Mol. Cell. Endocrinol.*, vol. 433, pp. 26–34, Sep. 2016, doi: 10.1016/j.mce.2016.05.013.

Effect of Nitrogen Fertilizer Fractionation and Adding Bio-Organic Fertilizer on the Content of Maize Leaves of Macronutrients and Chlorophyll Index

Zeyad Mudhafar Hashim Al-Ibraheem¹ and Mohammad Tarkhan Abo Almeekh²

^{1,2}Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Babylon, Iraq.

¹E-mail: ziadibnsina@gmail.com

²E-mail: Com.muh8@atu.edu.iq

Abstract. The experiment was conducted in one of the private fields in the Alexandria / Babylon region to study the effect of dividing nitrogen fertilizer and adding organic biofertilizer on the content of corn, Furat cultivars, of major elements. The experiment included: the first factor: urea fertilizer (N), and the second factor: organic and biofertilizer (B). Urea fertilizer was added to the soil in fifteen parts (after 15 days of planting, after 30 days of planting, after 45 days of planting, after 60 days of planting, after 15+ 30 days of planting, after 15+ 45 days of planting, after 15+ 60 days of planting, after 30+ 45 days of planting, after 30+ 60 days of planting, after 45+ 60 days of planting, after 15+ 30+ 45 days of planting, after 15+ 30+ 45 days of planting, after 15+ 30+ 60 ...45+ 60 days of planting, after 30+45+60 days after planting, after 15+30+45+60 days after planting) and symbolized by (N1, N2, N3, N4, N5, N6, N7, N8, N9, N10, N11, N12, N13, N14, N15) and according to the fertilizer recommendation (120 kg N ha^{-1}). Also, bio-fertilizers were added to the soil at two levels ($0\text{-}1000 \text{ kg ha}^{-1}$) and symbolized by (B1, B2) and added mixed with the soil before planting. A factorial experiment was conducted according to. Randomized Complete Block Design (RCBD). Three replicates were used and the treatment averages were compared using the Least Significant Difference (LSD) test at a probability level of 0.05 (Al-Rawi and Khalaf Allah, 1980). The results were as follows: The biofertilizer (B2) (1000 kg.ha^{-1}) treatment was significantly excelled and gave the highest rate of nitrogen, phosphorus and potassium in the leaves, reaching 3.61, 0.67, 3.50% respectively, and recorded the highest content of chlorophyll intensity in the leaves, 57.07 Spad. The N15 treatment was also significantly excelled and recorded the highest rate of nitrogen, phosphorus and potassium in the leaves, reaching 4.45, 0.75, 4.32%, and recorded the highest content of chlorophyll intensity in the leaves, 58.40 Spad. The interaction treatment (N15B2) was also significantly excelled. It gave the highest rate of nitrogen, phosphorus and potassium in the leaves, reaching 4.96, 0.76, 4.54%, and recorded the highest content of chlorophyll intensity in the leaves, 68.17 Spad.

Keywords. Biofertilizer, Maize leaves, Iraqi soils.

1. Introduction

Iraqi soils generally suffer from a high degree of soil reaction, a high content of calcium carbonate and a low percentage of organic matter. This leads to a decrease in the availability of nutrients originally present in the soil and a lack of efficiency in using added mineral fertilizers, especially nitrogenous fertilizers, as they are exposed to problems (sedimentation and adsorption) and their transformation into low-soluble compounds that are not available for absorption by the plant [1]. Urea fertilizer is the most nitrogen-supplying fertilizer in the soil and the most widely used in the world due to its high nitrogen content (46%) and low production costs [2]. The hydrolysis of the amide molecule in urea fertilizer by the action of the urease enzyme secreted by soil microorganisms affects the amount of nitrogen lost from the fertilizer through nitrate washing, ammonia volatility, or other biological processes, which reduces the efficiency of nitrogen use in the soil [3]. Due to the great expansion in the use of this fertilizer and to contribute to developing the efficiency of nitrogen use from fertilizer, several methods have been developed to reduce nitrogen loss and increase the efficiency of plant use of nitrogen. These methods include diversifying the methods of addition, finding the appropriate timing for addition, and using slow-release fertilizers [4]. Biofertilizers are a complementary source of chemical fertilizers that include organic fertilizers containing beneficial microorganisms, which when added can increase crop production by 15-50% [5]. Biofertilizers can be an effective way to reduce sources of chemical fertilizers and improve soil fertility. This fertilizer improves plant growth by increasing nitrogen availability through fixing atmospheric nitrogen and dissolving insoluble mineral phosphorus through the production of organic acids and enzymes and organic phosphorus mineral and promoting root growth [6]. The use of biofertilizers in agriculture and their use by farmers is still below the required level despite their importance and the results that encourage their use. Iraq is like most third world countries, where the use of these fertilizers is still below the required level. Corn (*Zea mays*) is one of the most important field crops that play a role in the food industry, as its grains are characterized by their high content of starch and oil, in addition to salts, minerals and vitamins, especially vitamin A, which is estimated to be twenty times higher than what it contains in wheat grains. These vitamins are essential in the manufacture of livestock feed and providing essential amino acids for animal growth[7,8]. In Iraq, the production of corn for the two seasons (spring and autumn) was estimated at (374.4) thousand tons for the cultivated area (325.9) thousand dunums, and the average yield per dunum for the two seasons (spring and autumn) for the cultivated area was estimated at (1148.8) kg dunum-[9] The study aims to:

- Study the effect of nitrogen fertilizer fragmentation on some soil properties and the growth and yield of corn.
- Know the effect of organic biofertilizer on some soil properties and the growth and yield of corn.
- Study of the joint combinations between organic biofertilization and nitrogen fertilizer in some soil properties and growth and yield of corn.

2. Materials and Methods

The experiment was conducted in one of the private fields in the Alexandria / Babylon area to study the effect of dividing nitrogen fertilizer and adding organic biofertilizer on the plant content of major elements. The experiment included the following factors: The first factor: urea fertilizer (N), and the second factor: organic and biological fertilizer (B). Urea fertilizer was added to the soil fifteen parts after (15, 30, 45, 60) and their interactions from the day of planting and according to the fertilizer recommendation (120 kg N ha⁻¹). (Ali, 2012) According to Table (1).

Also, biofertilizers were added to the soil at two levels (0-1000 kg h⁻¹) and are symbolized by (B0, B1) and are added mixed with the soil before planting. According to Table (1).

Table 1. Description of treatments of the first factor and the amount of fertilizers used in the experiment.

No.	Treatments	Amount of fertilizer added kg ha ⁻¹	Symbol treatments
1	After 15 days of planting	120	N1
2	After 30 days of planting	120	N2
3	After 45 days of planting	120	N3
4	After 60 days of planting	120	N4
5	After 15+ 30 days of planting	60+60	N5
6	After 15+ 45 days of planting	60+60	N6
7	After 15+ 60 days of planting	60+60	N7
8	After 30+ 45 days of planting	60+60	N8
9	After 30+ 60 days of planting	60+60	N9
10	After 45+ 60 days of planting	60+60	N10
11	After 15+ 30+ 45 days of planting	40+40+40	N11
12	After 15+ 30+ 60 days of planting	40+40+40	N12
13	After 15+ 45+ 60 days of planting	40+40+40	N13
14	After 30+ 45+ 60 days of planting	40+40+40	N14
15	After 15+ 30+ 45+ 60 days of planting	30+30+30+30	N15

The experiment was conducted on 7/27/2024 in a mixed soil. A soil sample was taken before planting, brought from the surface horizon 0-30 cm to determine some chemical and physical properties as in (Table 2). Plowing, smoothing and soil leveling operations were carried out. After that, the experimental soil was divided into three sectors (replicates) and each sector was divided into 30 experimental units. The dimensions of the experimental unit were (2*3) m, and the distance between the sectors was left with a width of 1 m, and a distance of 1 m was left between the experimental units. Corn seeds of the Furat variety were planted manually at a rate of 3 seeds per hole. After a week of germination, the number of plants was reduced to one plant per hole and the weeds were removed by hand weeding whenever necessary. The corn stem borer (*Sesamia cretica*) was controlled using granular diazinon pesticide at a concentration of 10% (5 kg ha⁻¹) in three batches. The first control was after (20) days of germination and the second batch after 10 days of the first control. The third control was after (25) days of the second control at a rate of three grains in the heart of the plant. A factorial experiment was conducted according to Randomized Complete Block Design (RCBD). Three replicates were used and the averages of the treatments were compared according to the least significant difference (LSD) test at a probability level of 0.05 [10].

Table 2. Analysis of some chemical, physical and biological properties of the studied soil.

Traits	Values	Units
PH	7.6	---
Ece	3.4	dSm ⁻¹
Organic matter	9.8	g kg ⁻¹
CEC	21.2	Centamol charge kg-1 soil
Positive ions	Ca ⁺²	12.8
	Mg ⁺²	7.7
	K ⁺¹	0.8
	Na ⁺¹	8.0
Negative ions	CO ₃ ⁻	NilL
	HCO ₃ ⁻	2.6

Cl ⁻	21.5	
SO ₄ ⁻	5.2	
Available Nitrogen	23.5	
Available Phosphorus	8.8	
Available Potassium	183.5	mgkg ⁻¹
Bulk Density	1.4	
Sand	340	
Silt	405	
Clay	255	gkg ⁻¹
Bulk Density	1.43	gm\cm ³
texture	loam	

2.1. Chemical and Physical Analysis of Soil Before Planting

2.1.1. Estimation of Soil Particle Size Distribution

The sizes of soil particles will be estimated using the hydrometer method according to [11].

2.1.2. Electrical Conductivity EC

The electrical conductivity in the 1:1 soil extract will be measured using the EC-meter according to [11].

2.1.3. Soil Reaction pH

The soil reaction pH will be measured in the 1:1 soil extract using the pH-meter according to [11].

2.1.4. Cation Exchange Capacity CEC

It will be estimated using ammonium acetate solution (1M) according to [11].

2.1.5. Percentage of Organic Matter in the Soil

The organic matter in the soil will be estimated by titrating the oxidized organic carbon with ammonium ferrous sulfate according to the Walkley and Black method mentioned by [11].

2.1.6. Soluble Calcium

The soluble calcium will be estimated in the 1:1 soil extract by titration with ferrous sulfate (Na₂EDTA) using Ammonium Purpurate reagent according to (Richards, 1954).

2.1.7. Soluble Magnesium

Calcium and magnesium will be estimated using the (EBT) reagent and titration with ferrous sulfate, subtracting calcium from the total calcium and magnesium according to [12].

2.1.8. Soluble Potassium

It will be estimated in the 1:1 soil extract using the FlamePhotomere device according to [12].

2.1.9. Sulphates

Sulphates will be estimated by turbidity method using barium chloride, then measured by Spectrophotometer according to [11].

2.1.10. Carbonates and Bicarbonates

They will be estimated in the 1:1 soil extract using the titration method with dilute sulfuric acid concentration 0.01N according to [12].

2.1.11. Available Nitrogen

Available nitrogen extracted with KCl (2N) solution will be estimated using Microkjeldahl device, ammonium was reduced using MgO and nitrate ion using Devarda alloy according to [11].

2.1.12. Available Phosphorus

Available phosphorus extracted with sodium bicarbonate solution (0.5M) will be estimated according to the Olsen method, measured using a (Spectro photometer) at a wavelength of 882 nm according to [11].

2.1.13. Available Potassium

Available potassium extracted with calcium chloride (0.5M) will be estimated, measured using a (Flame Photometer) according to the method [11].

2.2. Studied Traits

2.2.1. Estimation of Nitrogen, Phosphorus and Potassium Concentration in the Plant

Fully mature fresh leaves were taken from each plant and for each replicate and washed well with running water, then washed with distilled water to remove dust and dirt stuck to them, and placed in perforated paper bags and then dried in an electric oven (OVEN) at a temperature of 70 degrees Celsius for 72 hours until the weight was fixed, after which the samples were ground using an electric mill and 0.2 g of sample powder (dry leaves) was taken and digested by adding (3 ml of sulfuric acid) and (1 ml of perchloric acid) using a digestion device equipped by the German company Siemens at a temperature of (240 °C) for two hours, and after completing the digestion process, distilled water was added for cooling and the solution was filtered on filter papers and the filtrate was completed to 50 ml, [14] and stored in plastic containers with the name written on them The treatment was continued until the remaining elements were estimated. These elements were estimated in the laboratory of the Horticulture Department - College of Agriculture - Al-Qasim Green University as follows:

- Estimation of Nitrogen (%): Nitrogen was estimated using Microkjedhal device according to the method of [14].
- Estimation of Phosphorus (%): Phosphorus was estimated by soft digestion method using ammonium molybdate and ascorbic acid in Spectrophotometer (John, 1970).
- Estimation of Potassium (%): Potassium was estimated using Flame-Photometer according to the method followed by [15].
- 2-Leaf chlorophyll content (SPAD): Ten plants from the middle row will be measured randomly for each experimental unit for the leaf below the ear and the average of the readings will be taken using Chlorophyll meter.

3. Results and Discussion

3.1. Percentage of Nitrogen in Leaves

The results of Table (3) showed that adding biofertilizer had a significant effect in increasing the percentage of nitrogen in the leaves, as the biofertilizer treatment (B2) (1000 kg.ha-1) was

significantly excelled and gave the highest rate of nitrogen percentage in the leaves, reaching 3.61%, while the biofertilizer treatment (B1) recorded the lowest percentage of nitrogen in the leaves, reaching 3.09%. The results also showed that the treatments of adding Urea had a significant effect on the percentage of nitrogen in the leaves, as the N15 treatment was significantly excelled and recorded the highest rate of nitrogen percentage in the leaves, reaching 4.45%, followed by the N14 and N13 treatments, which recorded a rate of nitrogen percentage in the leaves of (4.35 and 3.29%), respectively, while the control treatment N1 (after 15 days of Agriculture) The lowest rate of nitrogen percentage in the leaves reached 2.47%.

The results of Table (3) also showed that the dual interaction between biofertilization and urea addition treatments had a significant effect on the percentage of nitrogen in the leaves, as the interaction treatment (N15B2) was significantly excelled and recorded the highest rate of nitrogen percentage in the leaves, reaching 4.96%, while the control treatment (N0B0) recorded the lowest rate of nitrogen in the leaves, reaching 2.30%.

Table 3. The effect of biofertilization, urea addition treatments and the interaction between them on the percentage of nitrogen in the leaves (%).

Biofertilizer (B) kg.ha ⁻¹	Nitrogen fertilizer kg N ha ⁻¹															Aver .
	N1	N2	N3	N4	N5	N6	N7	N8	N9	10	N1 1	N1 2	N1 3	N1 4	N1 5	
B1	2.3 0	2.5 5	2.6 7	2.7 7	2.8 2	2.8 5	2.9 0	2.9 4	3.0 5	3.2 5	3.3 6	3.4 3	3.7 4	3.8 3	3.9 4	3.09
B2	2.6 3	2.6 6	2.7 8	2.8 5	2.9 0	2.9 6	3.2 0	3.3 5	3.5 3	4.0 6	4.1 3	4.5 9	4.7 5	4.8 6	4.9 6	3.61
average	2.4 7	2.6 1	2.7 3	2.8 1	2.8 6	2.9 1	3.0 5	3.1 5	3.2 9	3.6 5	3.7 5	4.0 1	4.2 5	4.3 5	4.4 5	
	LSD					B=0.064					N=0.17					BN=0.25

The results of Table (4) showed that adding biofertilizer had a significant effect on increasing the percentage of phosphorus in the leaves, as the biofertilizer treatment (B2) (1000 kg.h⁻¹) was significantly excelled and gave the highest rate of phosphorus in the leaves, reaching 0.67%, while the biofertilizer treatment (B1) recorded the lowest percentage of phosphorus in the leaves, reaching 0.48%. The results also showed that the treatments of adding Urea had a significant effect on the percentage of phosphorus in the leaves, as the N15 treatment (after 15+30+45+60 days of planting) was significantly excelled and recorded the highest rate of phosphorus in the leaves, reaching 0.75%, followed by the N14 and N13 treatments, which recorded a rate of phosphorus in the leaves of (0.67 and 0.65%), respectively, while the control treatment N1 (after 15 days of planting) had the lowest rate of phosphorus percentage in the leaves, reaching 0.26%. The results of Table (4) also showed that the double interaction between biofertilization and urea addition treatments had a significant effect on the percentage of phosphorus in the leaves, as the interaction treatment (N15B1) was significantly excelled and recorded the highest rate of phosphorus percentage in the leaves, reaching 0.76%, while the control treatment (N0B0) recorded the lowest rate of phosphorus in the leaves, reaching 0.21%.

Table 4. The effect of biofertilization, urea addition treatments, and their interaction on the percentage of phosphorus in the leaves (%).

Biofertilizer (B) kg.ha ⁻¹	Nitrogen fertilizer kg N ha ⁻¹															Aver.
	N1	N2	N3	N4	N5	N6	N7	N8	N9	10	N11	N12	N13	N14	N15	
B1	0.21	0.25	0.31	0.32	0.35	0.38	0.40	0.43	0.48	0.51	0.67	0.67	0.69	0.72	0.76	0.48
B2	0.32	0.33	0.35	2.85	0.41	0.45	0.52	0.55	0.56	0.56	0.57	0.60	0.62	0.62	0.74	0.67
average	0.26	0.29	0.33	1.58	0.38	0.41	0.46	0.49	0.52	0.53	0.62	0.64	0.65	0.67	0.75	
	LSD					B=0.003					N=0.008					BN=0.012

The results of Table (5) showed that adding biofertilizer had a significant effect in increasing the percentage of potassium in the leaves, as the biofertilizer treatment (B2) (1000 kg.h⁻¹) was significantly excelled and gave the highest rate of potassium in the leaves, reaching 3.50%, while the biofertilizer treatment (B1) recorded the lowest percentage of potassium in the leaves, reaching 3.15%. The results also showed that the treatments of adding Urea had a significant effect on the percentage of potassium in the leaves, as the N15 treatment was significantly excelled and recorded the highest rate of potassium in the leaves, reaching 4.32%, followed by the N14 and N13 treatments, which recorded a rate of potassium in the leaves of (4.14 and 4.09%), respectively, while the control treatment N1 (after 15 days of planting) recorded the lowest rate of potassium in the leaves, reaching 2.27%

The results of Table (5) also showed that the double interaction between biofertilization and urea addition treatments had a significant effect on the percentage of potassium in the leaves, as the interaction treatment (N15B2) was significantly excelled and recorded the highest rate of potassium percentage in the leaves, reaching 4.54%, while the control treatment (N0B0) recorded the lowest rate of potassium in the leaves, reaching 2.01%.

Table 5. Effect of biofertilization, urea addition treatments and their interaction on the percentage of potassium in the leaves (%).

Biofertilizer (B) kg.ha ⁻¹	Nitrogen fertilizer kg N ha ⁻¹															Aver.
	N1	N2	N3	N4	N5	N6	N7	N8	N9	10	N11	N12	N13	N14	N15	
B1	2.01	2.15	2.30	2.45	2.67	2.99	2.12	3.39	3.54	3.61	3.87	3.94	4.01	4.06	4.11	3.15
B2	2.53	2.71	3.01	2.85	3.11	3.12	3.29	3.46	3.74	3.80	3.92	4.06	4.17	4.22	4.54	3.50
average	2.27	2.43	2.66	2.65	2.89	3.06	2.71	3.43	3.64	3.71	3.89	4.00	4.09	4.14	4.32	
	LSD							B=0.14				N=0.39			BN=0.55	

3.2. Chlorophyll Pigment Intensity in Leaves

The results of Table (6) showed that adding biofertilizer had a significant effect in increasing the intensity of chlorophyll pigment in leaves, as the biofertilizer treatment (B2) (1000 kg.h⁻¹) was significantly excelled and gave the highest rate of chlorophyll pigment intensity in leaves, reaching 57.07 Spad, while the biofertilizer treatment (B1) recorded the lowest rate of chlorophyll pigment intensity in leaves, reaching 44.88 Spad. The results also showed that the treatments of adding eucalyptus had a significant effect on the intensity of chlorophyll pigment in leaves, as the N15 treatment was significantly excelled and recorded the highest rate of chlorophyll pigment intensity in leaves, reaching 58.40 Spad, followed by the N14 and N13 treatments, which recorded the rate of chlorophyll pigment intensity in leaves, reaching (57.47 and 56.77 Spad), respectively, while the control treatment recorded N1 (after 15 days of planting) The lowest rate of chlorophyll pigment intensity in the leaves was 47.58 Spad

The results of Table (6) also showed that the dual interaction between biofertilization and urea addition treatments had a significant effect on the intensity of chlorophyll pigment in the leaves, as the interaction treatment (N15B2) was significantly excelled and recorded the highest rate of chlorophyll pigment intensity in the leaves, reaching 68.17 Spad, while the control treatment (N0B0) recorded the lowest rate of chlorophyll pigment intensity in the leaves, reaching 40.27 Spad.

Table 6. Effect of biofertilization, urea addition treatments and their interaction on the intensity of chlorophyll pigment in the leaves (%).

Biofertilizer (B) kg.ha ⁻¹	Nitrogen fertilizer kg N ha ⁻¹															Ave r.
	N1	N2	N3	N4	N5	N6	N7	N8	N9	10	N11	N12	N13	N14	N15	
B1	40.2	42.3	42.1	42.8	43.1	43.8	44.5	44.9	45.3	45.8	46.6	47.2	47.5	48.0	48.6	44.8
	7	7	3	7	0	7	3	0	0	7	0	0	7	3	3	8
B2	54.9	55.2	56.6	2.85	58.0	58.5	59.0	59.7	60.6	61.5	63.1	64.7	65.9	66.9	68.1	57.0
	0	3	7		3	3	3	3	3	0	0	3	7	0	7	7
average	47.5	48.8	49.4	22.8	50.5	51.2	51.7	52.3	52.9	53.6	54.8	55.9	56.7	57.4	58.4	
	8	0	0	6	7	0	8	2	7	8	5	7	7	7	0	
	LSD					B=0.11					N=0.30					BN=0.42

The increase in the content of leaves of nitrogen, phosphorus, potassium and chlorophyll (Tables 3-7) may be attributed to the role of biofertilizers containing some beneficial microorganisms, which is one of the methods that helps increase the soil content of organic matter and improves its fertility, thus increasing the cation exchange capacity. In addition, the continuous reproduction of these microorganisms is considered a source of organic carbon, which increases the percentage of organic matter in the soil, thus increasing plant growth and the aura content of chlorophyll. All of this led to an increase in the cation exchange capacity, in addition to the role of microorganisms in increasing plant growth, which led to the withdrawal of a quantity of salts present in the soil, which led to a reduction in electrical conductivity, in addition to the role of microorganisms in the secretion of organic and inorganic acids that lead to a reduction in the values of the degree of soil reaction. All of this was reflected in the increase in porosity and cation exchange capacity and the decrease in apparent density, electrical conductivity and the degree of soil reaction as a result of the increase in organic matter. These results are consistent with [16,17].

The results also showed that adding organic biofertilizer led to a significant increase in the above-mentioned characteristics. This is due to the fact that this fertilizer is a new organic source added to the soil, which is originally poor in its organic matter content, in addition to the fact that this fertilizer contains many beneficial microorganisms that play an important role in increasing decomposition and releasing nutrients to the soil, which led to the formation of a dense microbial biomass that played a role in increasing the organic content of the soil and thus increasing the absorbed elements and their accumulation in the leaves [18,19].

or the reason for this is due to the role of microorganisms in preparing energy sources and analyzing organic matter, which helps improve soil properties and reduce the loss of nitrogen, phosphorus and potassium, as well as their role in increasing the availability of phosphorus and potassium in the plant [16,20]. Or it may be attributed to its role in producing organic acids that dissolve phosphorus and producing calcium chelating compounds and releasing phosphorus, in addition to its role in the biological fixation of atmospheric nitrogen as well as its secretion of organic compounds (humic and fulvic acid) that work to reduce the degree of soil reaction, which contributed to increasing nitrogen, phosphorus and potassium in the soil, in addition to its role in increasing soil aggregates and stability as a result of its secretion of some polysaccharides and binding materials, which led to improving its structure and working to chelate positive ions responsible for fixing phosphorus, and all of this is reflected in the absorption of nitrogen, phosphorus and potassium. These results are consistent with [21,22]. Thus, increasing the percentage of nitrogen, phosphorus, potassium and chlorophyll in the leaves. The increase in the percentage of elements (NPK) in the leaves when adding urea is attributed to the direct addition of urea, which led to an increase in the plant's absorption of nitrogen and an increase in its content in the leaves and by increasing the absorption of these nutrients through the roots with the increase in the level of fertilizer added to the soil and its concentration in the vegetative group

increases, which leads to improving vegetative growth, increasing the efficiency of the photosynthesis process and increasing the effectiveness of the formation of carbohydrate and protein materials that plants need, and then transferring these materials from their production sites to areas of need and storage within the plant. Increasing the content of the leaves of the nitrogen element in the leaves leads to an imbalance in the nutritional balance of the elements within the plant, which requires the absorption of larger quantities of phosphorus and potassium to reach a state of nutritional balance within the plant [23] (Torkashvand et al., 2022) and thus increasing the percentage of phosphorus and potassium in the leaves.

Conclusions

We can conclude from the experiment that urea fractionation has a significant effect on the element content in the leaves. The N15 (After 15+ 30+ 45+ 60 days of planting) treatment was excelled.

References

- [1] Al-Naimi, Saadallah Najm Abdullah. 2011. Principles of Plant Nutrition (Translated). Ministry of Higher Education and Scientific Research. University of Mosul.
- [2] Selim, E.M.; Z.M. Elsirafy and A.A. Taha (2010).Effect of irrigation methods and N. application on the utilization of nitrogen by sugar beet grown under arid condition. Australian J. Basic and Applied Sci.. 4(7): 2114-2124
- [3] Parfitt, R.L.; Ll.A. Schipper; W.T. Baisder and A.H. Elliott (2006). Nitrogen inputs and out puts for New Zealand in 2001 at national and regional scales. Biogeochemistry. 80. 71-88.
- [4] Aziz, Eman and S.M. El-Ashry (2009).Efficiency of slow release urea fertilizer on herb yield and essential oil Production of lemon balm (*Melissa officinalis* L.) plant.American-Eurasian J. Agric. And Environ Sci., 5(2): 141-147.
- [5] Panhwar QA, Radziah O, Zaharah AR, Sariah M, MohdRazi I.2012. Isolation and characterization of phosphorus solubilizing bacteria from aerobic rice. Afr J Biotechnol. 2012; 11(11): 2711-2719.
- [6] Panhwar QA, Shamshuddin J, Naher UA, Radziah O, MohdRazi I.2014. Biochemical and molecular characterization of potential phosphate-solubilizing bacteria in acid sulfate soils and their beneficial effects on rice production. PLOS ONE , 9(10): e97241.
- [7] Huthily, K.H., K.A. Al-Dogagy and M.A. Kalaf. 2020. Effect of Nitrogen Fertilization and Foliar Application of Zinc in Growth and Yield of Maize (*Zea mays* L.). Int. J. Agricult. Stat. Sci., 16(Supplement 1), 1375-1380.
- [8] Abdullah, D. F. 2023, December. Evaluation of the Agricultural Reality of the Industrial Corn Crop in the Iraqi Province of Kirkuk. In IOP Conference Series: Earth and Environmental Science (Vol. 1262, No. 10, p. 102012). IOP Publishing.
- [9] Directorate of Agricultural Statistics. (2017). Production of Cotton, Maize and Potatoes. Central Statistical Organization, Ministry of Planning, Iraq. <http://www.cosit.gov.iq/ar/agri-stat/veg-prod>.
- [10] Al-Rawi, Khashe' Mahmoud and Abdul Aziz Khalaf Allah. 2000. Design and Analysis of Agricultural Experiments. Dar Al-Kutub for Printing and Publishing. University of Mosul. Iraq. P. 480.
- [11] Page, A.L., Miller, R.H. and Keeney, D.R. (1982) Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy. In Soil Science Society of America, Vol. 1159.
- [12] Richards, L.A. 1954. Diagnosis and improvement of Saline and Alkaline soils. USDA. Hand book 60. USDA., Washington , DC.
- [13] Cresser, M.S ., and .J.W.Parsons.1979.Sulphuric,perchloric acid and digestion of plant material for magnesium. Analytical Chemical .Acta.109:431-436.
- [14] Black , C. A. 1965. Methods of soil analysis . Physical and Micrological part 1 . Am. Soc. of Agron. Madison .Wisconsin
- [15] Hesse, P. R., & Hesse, P. R. (1971). A textbook of soil chemical analysis.



- [16] Itelima, J. U., Bang, W. J., Onyimba, I. A., Sila, M. D., & Egbere, O. J. 2018. Bio-fertilizers as key player in enhancing soil fertility and crop productivity: A review.
- [17] Otlewska, A., Migliore, M., Dybka-Stępień, K., Manfredini, A., Struszczyk-Świta, K., Napoli, R., ... & Pinzari, F. (2020). When salt meddles between plant, soil, and microorganisms. *Frontiers in plant science*, 11, 553087.
- [18] Havlin, J. L., J.D. Beaton, S. L. Tisdale, and W. L. Nelson. 2014. *Soil Fertility & Fertilizers: 8th Ed. An Introduction to Nutrient Management*. Upper Saddle River, New Jersey. Indian Reprint. P:516.
- [19] Ramos, F. T., Dores, E. F. D. C., Weber, O. L. D. S., Beber, D. C., Campelo Jr, J. H., & Maia, J. C. D. S. 2018. Soil organic matter doubles the cation exchange capacity of tropical soil under no-till farming in Brazil. *Journal of the Science of Food and Agriculture*, 98(9), 3595-3602.
- [20] Santos, K. F. D. N., Moure, V. R., Hauer, V., Santos, A. R. S., Donatti, L., Galvão, C. W., ... & Steffens, M. B. R. 2017. Wheat colonization by an *Azospirillum brasilense* ammonium-excreting strain reveals upregulation of nitrogenase and superior plant growth promotion. *Plant and Soil*, 415, 245-255.
- [21] Mortinho, E. S., Jalal, A., da Silva Oliveira, C. E., Fernandes, G. C., Pereira, N. C. M., Rosa, P. A. L., ... & Teixeira Filho, M. C. M. 2022. Co-Inoculations with Plant Growth-Promoting Bacteria in the Common Bean to Increase Efficiency of NPK Fertilization. *Agronomy*, 12(6), 1325.
- [22] Odoh, C. K., Sam, K., Zabbey, N., Eze, C. N., Nwankwegu, A. S., Laku, C., & Dumpe, B. B. 2020. Microbial consortium as biofertilizers for crops growing under the extreme habitats. *Plant microbiomes for sustainable agriculture*, 381-424.
- [23] Farahmandi, S. R., Samavat, S., Mostafavi, M., Torkashvand, A. M., & Kalatejari, S. (2022). Changes in physiological properties and vase life of gerbera (*Gerbera jamesonii*) under co-application of foliar applied L-glutamic acid, nitrogen and potassium.

Effect of the Type and Concentration of Salt Ions on the Growth of Bacillus Bacteria and Some Soil Traits

Hassan Hadi Jihad Al-Masoudi¹ and Alaa Edan Hasan²

^{1,2}College of Agriculture, University of Kufa, Najaf, Iraq.

¹E-mail: almasaody.h@gmail.com

²E-mail: alaa.albdairi@uokufa.edu.iq

Abstract. This study was conducted at the research station and laboratories affiliated to the College of Agriculture, University of Kufa, in the year 2023-2024 to know the effect of different salinity levels at concentrations of (75, 50, 25, 0) mM of calcium, sodium, potassium and magnesium chloride salts on the effectiveness and activity of *Bacillus* bacteria in sandy soil mixture. The experiment included adding the four salts (CaCl₂, KCl, NaCl, MgCl₂) at four different concentrations (75, 50, 25, 0) mM to small pots of 1 kg of soil and two types of bacteria (*B. subtilis*, *B. cereus*) were added to determine the extent of bacteria resistance to saline conditions. The readiness (NPK) was measured as well as the number of bacteria and the degree of interaction and the electrical conductivity of the soil was measured. The results indicated that adding salts to the soil had a significant effect. The results showed that KCl salt was significantly superior and gave the highest rate of total bacterial numbers before and after adding *B. subtilis*, which amounted to 52.08 and 59.58 × 10⁶ CFU g⁻¹ dry soil, respectively. As for adding *B. cereus* bacteria, MgCl₂ salt was significantly superior and gave the highest rate of bacterial numbers, which amounted to 44.50 × 10⁶ CFU g⁻¹ dry soil, while CaCl₂ salt gave the lowest total bacteria count before addition, reaching 21.00 × 10⁶ CFU g⁻¹ dry soil, while NaCl salt gave the lowest total bacteria count by adding *B. subtilis* and *B. cereus* bacteria, reaching 21.50 and 21.08 × 10⁶ CFU g⁻¹ dry soil, respectively. The results of the same table showed the effect of salts concentration on the total bacterial count. The concentration of 25 mM recorded the total bacterial count before addition and upon addition of *B. subtilis* bacteria at 52.92 and 47.50 × 10⁶ CFU/g⁻¹ dry soil, respectively, while the bacterial count recorded the highest bacterial count rate upon addition of *B. subtilis* bacteria at a concentration of 50 mM, reaching 39.58 × 10⁶ CFU. gm⁻¹ dry soil. The concentration of 50 mM recorded the highest rate of soil electrical conductivity before and after adding *B. subtilis*, which amounted to 2.55 and 2.64 dS m⁻¹, respectively. The results of the same table also showed the effect of Salts concentrations on soil electrical conductivity after adding *B. cereus*. The concentration of 75 mM recorded the highest rate of soil electrical conductivity after adding *B. cereus*, which amounted to 3.08 dS m⁻¹, while the rate of electrical conductivity decreased at the concentration of 0 mM and recorded 2.00. The concentration of 75 mM gave the highest rate of soil reaction degree before addition and after adding *B. cereus*, which amounted to 7.22 and 7.28, while the concentration of 0 and 25 mM recorded the highest rate of soil reaction degree after adding *B. subtilis*, which amounted to 7.22. The concentration of 50 mM also recorded the highest rate of soil nitrogen before addition, which amounted to 12.71 mg kg⁻¹ Soil. While the concentration of 75 mM recorded the highest rate of soil nitrogen with the addition of *B. subtilis* and *B. cereus* bacteria, which amounted to 12.74 and 10.89 mg kg⁻¹ soil, respectively, the results of the same table indicated the effect of salts concentrations on the ready soil phosphorus without addition, the concentration of 75 mM gave the highest rate of soil phosphorus, which amounted to

4.21 mg kg⁻¹ soil, while the concentration of 0 mM recorded the highest rate of soil phosphorus with the addition of *B.subtilis* bacteria, which amounted to 8.19 mg kg⁻¹ soil, while the concentration of 50 mM recorded the highest rate of soil phosphorus with the addition of *B.cereus* bacteria, which amounted to 11.95 mg kg⁻¹ soil, while the soil phosphorus rate decreased at the concentration of 50 and 25 mM and recorded 2.47 mg kg⁻¹ soil, respectively. The results also showed that the interaction between salts and concentrations had a significant effect on soil potassium. The interaction between KCl and concentration 75mM was excelled and recorded the highest rate of soil potassium, reaching 127.19 mg kg⁻¹ soil, while the interaction between NaCl and concentration 75mM was superior and recorded the highest rate of soil potassium by adding *B. subtilis* and *B. cereus* bacteria, reaching 169.63 and 141.45 mg kg⁻¹ soil, respectively.

Keywords. *B.subtilis*, *B.cereus*, Salt ions, NaCl, KCl.

1. Introduction

Microorganisms play an important role in soil fertility and supplying plants with nutrients. Groups of microorganisms that live in the soil and contribute to activating a series of biological reactions through which the availability of some important nutrients for absorption by the plant is fixed or increased are called biofertilizers [1]. Bacterial biofertilizers consist of bacterial cells that dissolve nutrients such as phosphorus and potassium from minerals and rocks containing these elements. *Bacillus* bacteria are one of the most important genera of root zone bacteria that are characterized by various biomechanics to stimulate plant growth by increasing its supply of nutrients and the process of preparing phosphorus for the plant is one of the most important activities studied by researchers, which is done through the secretion of organic acids by bacteria and works to dissolve insoluble phosphorus and release it from inorganic phosphate compounds in the soil such as calcium triphosphate, diphosphate in addition to phosphate rock. Among the bacterial species are *B. subtilis*, *B. cereus* [2] in addition to producing polysaccharides and Siderophores that prevent the movement of toxic ions and help maintain ionic balance and enhance water movement in plant tissues [3]. Studies have also shown that they affect the regulation of plant hormone biosynthesis pathways, modify ethylene levels in plants, and affect the emission of volatile organic compounds. compounds(VOCs) [4].

Positive ions have an important effect on the growth of soil organisms and depend on several factors and biological and chemical interactions. Positive ions play a vital role in the metabolic processes and energy transfer within plants, which directly affects their growth and development. Positive ions can also affect the composition and activity of microbes in the soil. Some soil organisms need these ions as essential nutrients, while they negatively affect the growth of some other species [5]. In general, it can be said that positive ions play a vital role in the growth of soil organisms and their interactions, and this effect depends on the ecological balance, soil composition, and the types of living organisms present in it[6].

Wheat (*Triticum astivum* .L) is one of the most important strategic crops belonging to the Poaceae family and is used as a basic food and an important vital source of plant protein. It is easy to process into different types of food products consumed by billions of people and ranks first in the world in terms of cultivated area. Iraq is one of the original habitats for the emergence of wheat crops, but the conditions and factors of erosion, salinization, and desertification due to the long period of rain withholding, weak vegetation cover, and the harmony of agricultural services with traditional ones play a major role in the decline in productivity and poor quality, as the percentage of cultivated area globally in 2020 amounted to about 215.4 million hectares [7].

The amount of wheat produced in Iraq for the year 2021 amounted to about 4,234,000 tons, a decrease of 32.1% compared to the year 2020, which recorded a production of 6,238,000 tons [8]. In view of the above, the study aims to:

- Study the effect of positive ions calcium, magnesium, sodium and potassium in the soil and in the nutrient medium on the growth of Bacillus bacteria.
- Know the minimum inhibitory concentration (MIC) for positive ions and determine the ion that most affects the growth of Bacillus bacteria.
- Study the duration of bacteria survival in the nutrient medium and soil under the influence of positive salt ions.

2. Materials and Methods

2.1. Experimental Location

This experiment was conducted in the research station affiliated with the College of Agriculture at the University of Kufa on 11/15/2023. Positive salts of chlorides (sodium, potassium, calcium, magnesium) were added at four concentrations (75, 50, 25, 0) mM of each salt to a sandy mixture soil (Table 2) in small pots with a capacity of 1 kg. After that, bacteria (Bacillus) were injected with its two genera (B. Subtilis, B. Cereus) each separately at an average of 5 ml per pot. The pot was incubated at field temperature for three months during which the degree of reaction (pH) and the degree of salinity (EC) were measured periodically every month. At the end of the experiment, the following measurements were taken: N, P, K, pH, EC, and total bacterial numbers after purification and isolation from the soil.

Table 1. Some physical and chemical properties of field experiment soil.

Traits	Units	Values
Soil particle size distribution	g.kg ⁻¹	Sand 661.4 silt 306.0 Clay 32.6
Texture		Sandy loam
Bulk density	Mg.m ⁻¹	1.2
Electrical conductivity (EC)	DS.m ⁻¹	1.9
pH	-	7.70
Organic matter (O.M)	g.kg ⁻¹	1.21
Chloride Cl-1 mmol.L-1	Chloride Cl-1 mmol.L-1	4.6
	Sulphate SO ₄ =	5.8
	Carbonate CO ₃ =	0.0
Dissolved ions	Bicarbonate HCO ₃ -	2.2
	Potassium K ⁺	0.3
	Sodium Na ⁺	6.4
	Magnesium Mg ⁺⁺	2.7
Available Nitrogen	mg kg ⁻¹	3.9
Available Phosphorus	mg kg ⁻¹	5.6
Available Potassium	mg kg ⁻¹	8.4
Total Bacteria	ML *10 ⁻⁶	10.3
Ion Exchange Capacity	Centi mole kg ⁻¹	77.00

2.2. Studied Traits

2.2.1. Number of Soil Bacteria

The total number of bacteria in the soil was estimated by dilution and plate counting using Nutrient Agar medium according to [9].

2.2.2. PH

The electrical conductivity of soil salts in the saturated paste extract was measured using a pH-Meter as mentioned in [9].

2.2.3. Electrical Conductivity EC

The electrical conductivity of soil salts in the saturated paste extract was measured using an EC-Meter as mentioned in [9].

2.2.4. Available Nitrogen

Available nitrogen was extracted using potassium chloride KCL and nitrogen was estimated using a Kjeldahl apparatus according to the method mentioned in [10].

2.2.5. Available Phosphorus

Soil phosphorus was extracted using sodium bicarbonate (NaHCO₃) and the color of the extract was developed using ammonium molybdate and ascorbic acid solution. Phosphorus was measured using a spectrophotometer at a wavelength of 882 nm according to the method mentioned in [10].

2.2.6. Available Potassium

Available soil potassium was extracted using 0.5 M calcium chloride and measured using a flame photometer as mentioned in [10].

3. Results and Discussion

3.1. Total Bacterial Numbers CFU $\times 10^6$

The salts added to the soil have a significant effect on the total bacterial numbers. The results showed (Table 2) that KCl salt was significantly excelled and gave the highest rate of total bacterial numbers before and after adding *B. subtilis*, reaching 52.08 and 59.58×10^6 CFU g⁻¹ dry soil, respectively. As for the bacterial numbers after adding *B. cereus* bacteria, MgCl₂ salt was significantly excelled and gave the highest rate of bacterial numbers, reaching 44.50×10^6 CFU g⁻¹ dry soil, while CaCl₂ salt gave the lowest total bacterial numbers before adding, reaching 21.00×10^6 CFU g⁻¹ dry soil, while NaCl salt gave The lowest number of bacteria with the addition of *B. subtilis* and *B. cereus* bacteria reached 21.50 and 21.08×10^6 CFU g⁻¹ dry soil.

The results of the same table showed the effect of salt concentration on the total bacterial count, as the concentration of 25 Mm recorded the total bacterial count before addition and upon adding *B. subtilis* bacteria 52.92 and 47.50×10^6 CFU g⁻¹ dry soil respectively, while the bacterial count recorded the highest rate of bacterial count with the addition of *B. subtilis* bacteria at a concentration of 50 mM, which reached 39.58×10^6 CFU. gm⁻¹ dry soil, while the number of bacteria decreased by adding *B. subtilis* and *B. cereus* bacteria at a concentration of (75) mM and recorded 32.50 and 28.17×10^6 CFU gm⁻¹ dry soil, respectively.

The interaction between salts and concentrations had a significant effect on the total bacterial counts. The interaction treatment between KCl with concentration 0mM was excelled and

recorded the highest rate of total bacterial counts before addition, reaching 60.00×10^6 CFU g⁻¹ dry soil, while the number of bacteria increased by adding *B. subtilis* bacteria. The interaction treatment between CaCl₂ and concentration 25mM gave the highest rate of bacterial counts, reaching 69.00×10^6 CFU g⁻¹ dry soil. As for the bacterial counts, by adding *B. cereus* bacteria, the interaction treatment between KCl and concentration 25mM gave the highest rate of bacterial counts, reaching 46.00, while the total bacterial counts in the soil decreased when CaCl₂ and concentration 75mM gave a rate of total bacterial counts of 12.00×10^6 CFU g⁻¹ dry soil, while NaCl treatment and concentration 75mM recorded the lowest number of *B. subtilis* and *B. cereus* bacteria, which amounted to 17.00 and 19.00×10^6 CFU g⁻¹ dry soil. The decrease in the number of bacteria is due to the fact that increasing the salt concentration leads to a change in the osmotic pressure, which affects the permeability of the cell wall and also works to change the concentration of the lipopolysaccharide layer of bacteria in response to the effect of salt [11]. The reason for this is attributed to the addition of biofertilizer containing bacteria, as well as *Bacillus* bacteria tolerating harsh environmental conditions because they carry internal spores Endospore.

Table 2. Effect of the type and concentration of positive salt ions and *Bacillus* bacteria on the total number of bacteria in the soil cfu $\times 10^6$

Salts	Concentrations mM				Average Salts
	0	25	50	75	
MgCl ₂	33.00	67.00	42.00	35.00	44.25
NaCl	35.00	54.00	49.00	45.00	45.75
KCl	60.00	55.67	53.67	39.00	52.08
CaCl ₂	20.00	35.00	17.00	12.00	21.00
Average concentrations	37.00	52.92	40.42	32.75	
<i>B. subtilis</i>					
MgCl ₂	39.00	35.00	33.00	27.00	33.50
NaCl	25.00	23.00	21.00	17.00	21.50
KCl	68.33	63.00	58.00	49.00	59.58
CaCl ₂	41.67	69.00	49.00	37.00	49.17
Average concentrations	43.50	47.50	40.25	32.50	
<i>B. cereus</i>					
MgCl ₂	44.00	36.00	65.00	33.00	44.50
NaCl	24.00	21.33	20.00	19.00	21.08
KCl	44.33	46.00	43.00	28.00	40.33
CaCl ₂	22.00	32.00	30.33	32.67	29.25
Average concentrations	33.58	33.83	39.58	28.17	
LSD 0.05					
		No bacteria	Salts	concentrations	interaction
		<i>B. subtilis</i>	1.735	1.735	3.47
		<i>B. cereus</i>	2.023	N.S	N.S
			1.588	1.588	3.176

3.2. Electrical Conductivity of Soil (DS.m⁻¹)

The results in Table (3) showed that NaCl salt was significantly excelled and gave the highest rate of electrical conductivity of soil, reaching 3.263 DS.m⁻¹, while 2MgCl salt was significantly excelled and gave the highest rate of electrical conductivity of soil after adding *B. subtilis*, reaching 2.83 DS.m⁻¹, and NaCl salt was significantly excelled and gave the highest rate of electrical conductivity after adding *B. cereus*, reaching 3.17 DS.m⁻¹, while KCl salt gave the lowest rate of electrical conductivity of soil, reaching 1.5 DS.m⁻¹, while NaCl salt gave the lowest rate of electrical conductivity of soil after adding *B. subtilis*, reaching 2.02, while MgCl₂ salt gave the lowest rate of electrical conductivity of soil after adding *B. cereus*, reaching 1.95 DS.m⁻¹.

The results indicated the effect of salt concentrations on the electrical conductivity of the soil. The concentration of 50 mM recorded the highest rate of electrical conductivity of the soil before and after adding *B. subtilis*, which amounted to 2.55 and 2.64 DS.m⁻¹, respectively. The results of the same table also showed the effect of salt concentrations on the electrical conductivity of the soil after adding *B. cereus*. The concentration of 75 mM recorded the highest rate of electrical conductivity of the soil after adding *B. cereus*, which amounted to 3.08 DS.m⁻¹, while the electrical conductivity rate decreased at the concentration of 0 mM and recorded 2.00, while the electrical conductivity rate of the soil decreased after adding *B. subtilis* at the concentration of 0 mM and recorded 1.87 dS m⁻¹. The electrical conductivity rate of the soil decreased after adding *B. cereus* at the concentration of 25 mM and recorded 2.13 dS m⁻¹. The interaction between salts The concentrations had a significant effect on the electrical conductivity of the soil, as the interaction factor between NaCl with a concentration of 50 mM was excelled and recorded the highest rate of electrical conductivity of the soil, which amounted to 4.17 dS m⁻¹, and the interaction factor between NaCl with a concentration of 75 mM was excelled and recorded the highest rate of electrical conductivity of the soil after adding *B. subtilis*, which amounted to 2.70 dS m⁻¹. The results showed that 2MgCl was significantly excelled and gave the highest rate of electrical conductivity of the soil after adding *B. subtilis*, which amounted to 2.83, while the electrical conductivity of the soil decreased when the MgCl₂ treatment and the concentration of 75 mM gave the lowest rate of 1.30 dS m⁻¹, while the electrical conductivity of the soil decreased after adding *B. subtilis* when the NaCl treatment and the concentration of 25 mM gave the lowest rate of 1.57 dS m⁻¹. This is due to the accumulation of salts with the increase in salinity of irrigation water, as a result of the difference in the ionic composition of the irrigation water used, which led to a change in the ionic composition of the soil solution as a result of the increase in ion concentrations due to irrigation with saline water and then increasing their concentration in the soil solution, which was reflected in the increase in the electrical conductivity of the soil, and this result is consistent with what was obtained by [12,13] who stated that the increase in salinity of irrigation water led to an increase in the electrical conductivity of the soil.

Table 3. Effect of the type and concentration of positive salt ions and *Bacillus* bacteria on the electrical conductivity of the soil (dS m⁻¹).

Salts	Concentrations mM				Average Salts
	0	25	50	75	
MgCl ₂	2.83	1.73	2.33	1.30	2.05
NaCl	2.17	3.67	4.17	3.03	3.26
KCl	1.40	1.77	1.40	1.50	1.52
CaCl ₂	1.60	1.60	2.30	2.20	1.93
Average concentrations	2.00	2.19	2.55	2.01	
<i>B. subtilis</i>					
MgCl ₂	2.13	2.27	3.53	3.37	2.83
NaCl	1.63	1.57	2.17	2.70	2.02
KCl	2.30	2.37	1.93	1.63	2.06
CaCl ₂	1.40	2.10	2.93	1.73	2.04
Average concentrations	1.87	2.08	2.64	2.36	
<i>B. cereus</i>					
MgCl ₂	2.33	1.70	1.67	2.10	1.95
NaCl	3.50	1.63	2.60	4.93	3.17
KCl	2.07	3.00	2.97	2.37	2.60
CaCl ₂	1.87	2.20	1.60	2.90	2.14
Average concentrations	2.44	2.13	2.21	3.08	

		Salts	concentrations	interaction
LSD 0.05	No bacteria	0.438	0.438	N.S
	<i>B. subtilis</i>	0.549	0.549	1.098
	<i>B. cereus</i>	0.49	0.49	0.98

3.3. pH

The positive ions added to the soil had a significant effect on the pH after three months of incubation. The results showed (Table 4) that KCl was significantly excelled and gave the highest rate of pH, reaching 7.42. 2CaCl₂ was significantly excelled and gave the highest rate of pH after adding *B. subtilis* and *B. cereus*, reaching 7.24 and 7.42, while NaCl salt gave the lowest rate of pH without adding and after adding *B. subtilis*, reaching 6.55 and 6.87.

The results showed the effect of salt concentrations on the pH. The concentration of 75 mM gave the highest rate of pH before addition and after adding *B. cereus*, reaching 7.22 and 7.28, while the concentration of 0 and 25 mM recorded the highest rate of pH after adding *B. subtilis*, reaching 7.22, while the rate of pH decreased At concentration 25 mM and recorded 6.94 while the pH rate decreased after adding *B. subtilis* at concentration 50 mM and recorded 7.03 The interaction factor between KCl with concentration 75 mM was excelled and recorded the highest pH rate of 7.48, while the interaction factor between CaCl₂ with concentration 25 mM was excelled and recorded the highest pH rate after adding *B. subtilis* of 7.57, while the pH rate decreased when NaCl treatment and concentration 50 mM and gave the lowest rate of 6.31 while the pH rate decreased after adding *B. subtilis* at NaCl treatment and concentration 25 mM and gave the lowest rate of 6.58.

These results are consistent with what was reached by [14, 13] as they showed a decrease in the pH rate towards neutrality with the increase in positive soil salts, as the organisms Microorganisms reduce the degree of pH by producing organic acids and work to chelate and sequester the divalent ions responsible for the adsorption and deposition of phosphorus in calcareous and basic soils [15].

Table 4. Effect of the type and concentration of positive salt ions and Bacillus bacteria on the degree of pH.

Salts	Concentrations mM				Average Salts
	0	25	50	75	
MgCl ₂	7.18	7.12	7.05	7.22	7.14
NaCl	6.55	6.63	6.31	6.71	6.55
KCl	7.48	7.33	7.39	7.48	7.42
CaCl ₂	7.34	7.45	7.00	7.48	7.32
Average concentrations	7.14	7.14	6.94	7.22	
<i>B. subtilis</i>					
MgCl ₂	7.16	7.27	7.19	7.08	7.17
NaCl	6.87	6.58	6.61	7.43	6.87
KCl	7.37	7.57	7.07	6.94	7.24
CaCl ₂	7.50	7.44	7.26	7.10	7.33
Average concentrations	7.22	7.22	7.03	7.14	
<i>B. cereus</i>					
MgCl ₂	7.17	7.13	7.16	7.18	7.16
NaCl	7.38	7.01	6.52	7.05	6.99
KCl	7.23	7.44	7.41	7.41	7.37
CaCl ₂	7.55	7.28	7.37	7.48	7.42
Average concentrations	7.33	7.21	7.12	7.28	
LSD 0.05		Salts	concentrations	interaction	

No bacteria	0.152	N.S	0.303
<i>B. subtilis</i>	0.175	0.175	0.350
<i>B. cereus</i>	0.080	0.080	0.160

3.4. Available Soil Nitrogen

The results of Table (5) showed that the positive ions added to the soil had a significant effect on soil nitrogen. The results showed that NaCl salt was significantly excelled and gave the highest rate of soil nitrogen, reaching 12.31 mg kg⁻¹ soil, while KCl salts were significantly excelled and gave the highest rate of available soil nitrogen by adding *B. subtilis* bacteria, reaching 11.71 mg kg⁻¹ soil, while MgCl₂ salt was significantly excelled and gave the highest rate of soil nitrogen by adding *B. cereus* bacteria, reaching 11.73 mg kg⁻¹ soil. While CaCl₂ salt gave the lowest rate of soil nitrogen before addition and after adding *B. subtilis* bacteria, reaching 8.89 and 10.77 mg kg⁻¹ soil, respectively. The results showed that KCl salt gave the lowest rate of soil nitrogen By adding *B.cereus* bacteria, it reached 8.96 mg kg⁻¹ soil. The concentration of 50 mM also recorded the highest rate of soil nitrogen before addition, reaching 12.71 mg kg⁻¹ soil, while the concentration of 75 mM recorded the highest rate of soil nitrogen by adding *B.subtilis* and *B.cereus* bacteria, reaching 12.74 and 10.89 mg kg⁻¹ soil, respectively. While the soil nitrogen rate decreased before and after adding *B.subtilis* bacteria at a concentration of 25 mM and recorded 10.08 and 9.48 mg kg⁻¹ soil, respectively, while the soil nitrogen rate decreased by adding *B.cereus* bacteria at a concentration of 0 mM and recorded 9.63 mg kg⁻¹ soil. The results also showed that the interaction between salts and concentrations had a significant effect on soil nitrogen, as the interaction coefficient between NaCl and concentration 75 mM was excelled and recorded The highest rate of soil nitrogen before and after adding *B.subtilis* and *B.cereus* bacteria was 13.80, 13.67 and 17.50 mg kg⁻¹ soil, respectively. While soil nitrogen decreased before adding when treated with CaCl₂ and concentration 75 mM and gave the lowest rate of 8.53 mg kg⁻¹ soil. As for soil nitrogen, by adding *B. subtilis* and *B. cereus* bacteria, when treated with NaCl and concentration 25 mM, it gave the lowest rate of 9.10 and 5.63 mg kg⁻¹ soil. This shows the effect of positive salts in increasing nitrogen availability, as the higher the level of positive salts, the lower the nitrogen availability. The reason for this increase in nitrogen is due to the ability of *Bacillus* bacteria to fix phosphorus and nitrogen, in addition to the growth regulators secreted by these bacteria. It is known that these materials improve plant growth and form a dense root system, which leads to increased absorption of nutrients, including nitrogen [7,10]. *Bacillus* bacteria secrete organic acids that work to reduce pH and thus increase nitrogen availability [16].

Table 5. Effect of the type and concentration of positive salt ions and *Bacillus* bacteria on available soil nitrogen (mg kg⁻¹).

Salts	Concentrations mM				Average Salts
	0	25	50	75	
MgCl ₂	10.43	11.20	13.53	9.70	11.22
NaCl	12.17	8.57	16.70	11.80	12.31
KCl	9.80	11.21	12.20	13.67	11.72
CaCl ₂	9.30	9.33	8.40	8.53	8.89
Average concentrations	10.43	10.08	12.71	10.93	
<i>B. subtilis</i>					
MgCl ₂	14.60	7.70	11.50	11.20	11.25
NaCl	10.10	9.10	11.53	13.80	11.13
KCl	11.20	9.73	13.33	12.57	11.71
CaCl ₂	8.50	11.40	9.77	13.40	10.77
Average concentrations	11.10	9.48	11.53	12.74	
<i>B. cereus</i>					

MgCl ₂	11.73	13.53	11.53	10.10	11.73
NaCl	5.63	9.80	10.57	17.50	10.88
KCl	9.93	9.67	8.57	7.67	8.96
CaCl ₂	11.20	9.50	9.20	8.30	9.55
Average concentrations	9.63	10.63	9.97	10.89	
		Salts	concentrations	interaction	
LSD 0.05	No bacteria	0.195	0.195	0.390	
	<i>B. subtilis</i>	0.217	0.217	0.433	
	<i>B. cereus</i>	0.127	0.127	0.253	

3.5. Available Soil Phosphorus

The results of Table (6) showed that the positive ions added to the soil had a significant effect on soil phosphorus. The results showed that KCl salt was significantly excelled and gave the highest rate of available soil phosphorus without adding, reaching 4.82 mg kg⁻¹ soil, while MgCl₂ salt was significantly excelled and gave the highest rate of soil phosphorus with the addition of *B. subtilis* bacteria, reaching 10.78 mg kg⁻¹ soil, while NaCl salt was significantly excelled and gave the highest rate of available soil phosphorus with the addition of *B. cereus* bacteria, reaching 23.44 mg kg⁻¹ soil. While CaCl₂ salt gave the lowest rate of soil phosphorus, reaching 1.55 mg kg⁻¹ soil, while KCl salt gave the lowest rate of soil phosphorus with the addition of *B. subtilis* bacteria, reaching 1.91 mg kg⁻¹ soil. While MgCl₂ salt The lowest rate of soil phosphorus with the addition of *B. cereus* bacteria was 2.43 mg kg⁻¹ soil. The results of the same table indicated the effect of salt concentrations on the available soil phosphorus without adding. The concentration of 75 mM gave the highest rate of soil phosphorus, reaching 4.21 mg kg⁻¹ soil, while the concentration of 0 mM recorded the highest rate of soil phosphorus with the addition of *B. subtilis* bacteria, reaching 8.19 mg kg⁻¹ soil, while the concentration of 50 mM recorded the highest rate of soil phosphorus with the addition of *B. cereus* bacteria, reaching 11.95 mg kg⁻¹ soil, while the soil phosphorus rate decreased at the concentration of 50 and 25 mM and recorded 2.47 mg kg⁻¹ soil, respectively. While the soil phosphorus rate decreased by adding *B. subtilis* bacteria at the concentration of 75 mM and recorded 2.28 mg kg⁻¹ soil, while the soil phosphorus rate decreased by adding *B. cereus* bacteria at the concentration of 0 mM and recorded 2.79 mg 1 kg of soil. The results also showed that the interaction between salts and concentrations had a significant effect on soil phosphorus. The interaction treatment between NaCl with a concentration of 75 mM was excelled and recorded the highest rate of soil phosphorus, which amounted to 7.65 mg kg⁻¹ soil, while the interaction treatment between 2CaCl with a concentration of 25 mM was excelled and recorded the highest rate of soil phosphorus by adding *B. subtilis* bacteria, which amounted to 15.78 mg kg⁻¹ soil, while the interaction treatment between NaCl with a concentration of 50 mM was excelled and recorded the highest rate of soil phosphorus by adding *B. cereus* bacteria, which amounted to 40.36 mg kg⁻¹ soil, while soil phosphorus decreased when treated with CaCl₂ and a concentration of 75 mM and gave the lowest rate of 0.49 mg kg⁻¹ soil. While soil phosphorus decreased when adding *B. subtilis* bacteria when treated with KCl and a concentration of 25 mM and gave the lowest rate of 0.66 mg kg⁻¹ soil. While soil phosphorus decreased by adding *B. cereus* bacteria when treated with CaCl₂ and concentration 50 mM and gave the lowest rate of 0.27 mg kg⁻¹ soil. The reason for the increase in phosphorus is due to the ability of *Bacillus* bacteria to increase the availability of phosphorus by dissolving compounds containing phosphorus in the unavailable form through its production of some organic acids such as (citric, oxalic, formic and malic or secreting enzymes such as phosphatase and phytase that increase the availability of phosphorus for the plant [17].

Table 6. Effect of type and concentration of positive salt ions and Bacillus bacteria on available soil phosphorus mg kg⁻¹.

Salts	Concentrations mM				Average Salts
	0	25	50	75	
MgCl ₂	3.20	0.45	3.96	3.73	2.84
NaCl	2.86	3.68	4.55	7.65	4.68
KCl	5.84	3.98	4.48	4.97	4.82
CaCl ₂	1.97	1.76	1.97	0.49	1.55
Average concentrations	3.47	2.47	3.74	4.21	
<i>B. subtilis</i>					
MgCl ₂	26.77	1.63	13.20	1.51	10.78
NaCl	4.96	0.96	1.94	2.81	2.67
KCl	0.76	0.66	2.87	3.34	1.91
CaCl ₂	0.25	15.78	2.98	1.47	5.12
Average concentrations	8.19	4.76	5.25	2.28	
<i>B. cereus</i>					
MgCl ₂	1.04	4.55	1.46	2.66	2.43
NaCl	3.06	10.10	40.36	40.23	23.44
KCl	5.92	5.66	5.55	2.59	4.93
CaCl ₂	1.13	0.27	0.44	1.20	0.76
Average concentrations	2.79	5.14	11.95	11.67	
LSD 0.05					
		No bacteria	Salts concentrations interaction		
		<i>B. subtilis</i>	0.071	0.071	0.143
		<i>B. cereus</i>	0.050	0.050	0.100
			0.065	0.065	0.130

3.6. Available Soil Potassium

NaCl salt was significantly excelled and gave the highest rate of soil potassium before addition and addition of *B.cereus* bacteria, which amounted to 103.98 and 121.94 mg kg⁻¹ soil, respectively (Table 7), while MgCl₂ salt was significantly excelled and gave the highest rate of soil potassium with addition of *B.subtilis* bacteria, which amounted to 139.90 mg kg⁻¹ soil, while CaCl₂ salt gave the lowest rate of soil potassium before addition and after addition of *B.cereus* bacteria, which amounted to 68.69 and 62.56 mg kg⁻¹ soil, respectively, while KCl salt gave the lowest rate of soil potassium with addition of *B.subtilis* bacteria, which amounted to 101.88 mg kg⁻¹ soil.

The results of the same table also showed the effect of salt concentrations on soil potassium. The concentration of 50 mM recorded the highest rate of soil potassium, reaching 80.16 mg kg⁻¹ soil, while the concentration of 75 mM gave the highest rate of soil potassium with the addition of *B. subtilis* and *B. cereus* bacteria, reaching 141.50 and 98.09 mg kg⁻¹ soil, respectively. The soil potassium rate decreased at the concentration of 25 mM and recorded 80.16 mg kg⁻¹ soil, while the soil potassium rate decreased with the addition of *B. subtilis* and *B. cereus* bacteria at the concentration of 0 mM and recorded 110.55 and 82.76 mg kg⁻¹ soil. The results also showed that the interaction between salts and concentrations had a significant effect on soil potassium. The interaction between KCl with a concentration of 75mM was excelled and recorded the highest rate of soil potassium, reaching 127.19 mg kg⁻¹ soil, while the interaction between NaCl with a concentration of 75mM was excelled and recorded the highest rate of soil potassium by adding *B. subtilis* and *B. cereus* bacteria, reaching 169.63 and 141.45 mg kg⁻¹ soil, respectively. While soil potassium decreased when treated with CaCl₂ and concentration 75 mM and gave the lowest rate of 65.61 mg kg⁻¹ soil, while soil potassium decreased by adding *B. subtilis* and *B. cereus* bacteria

when treated with CaCl_2 and concentration 25 mM and gave the lowest rate of 73.32 and 58.17 mg kg^{-1} soil. The reason is attributed to the effect of salinity on plant growth and absorption of nutrients, as the higher the soil salinity, the lower the amount of elements absorbed by the plant, which leads to plant weakness and deterioration of its growth, as the relationship is inverse between plant growth and high salinity in the soil due to the imbalance of ionic balance and the increase in the concentration of salts in the soil solution and the increase in osmotic pressure leads to an imbalance in absorption by the plant [18]. Also, the decrease in the percentage of potassium with increasing salinity levels is due to the increase in sodium absorption, which competes with potassium for absorption sites in the root.

Table 7. Effect of type and concentration of positive salt ions and *Bacillus* bacteria on soil potassium mg kg^{-1} .

Salts	Concentrations mM				Average Salts
	0	25	50	75	
MgCl_2	90.95	93.53	98.56	70.73	88.44
NaCl	98.58	80.06	130.45	106.83	103.98
KCl	80.07	78.63	95.27	127.19	95.29
CaCl_2	74.16	68.43	66.54	65.61	68.69
Average concentrations	85.94	80.16	97.70	92.59	
<i>B. subtilis</i>					
MgCl_2	183.53	87.72	140.62	147.75	139.90
NaCl	101.92	99.96	149.04	169.63	130.14
KCl	83.42	122.14	95.55	106.41	101.88
CaCl_2	73.32	152.52	72.67	142.23	110.18
Average concentrations	110.55	115.58	114.47	141.50	
<i>B. cereus</i>					
MgCl_2	120.41	93.50	92.92	71.57	94.60
NaCl	80.05	127.20	138.98	141.53	121.94
KCl	66.55	89.40	85.33	120.45	90.43
CaCl_2	64.04	58.17	69.20	58.83	62.56
Average concentrations	82.76	92.07	96.61	98.09	
Salts concentrations interaction					
LSD 0.05	No bacteria	0.105	0.105	0.210	
	<i>B. subtilis</i>	0.1158	0.1158	0.2316	
	<i>B. cereus</i>	0.183	0.183	0.367	

Conclusions

- The most harmful salts to bacteria are sodium chloride and magnesium chloride, and adding biofertilizers led to reducing the effectiveness and harm of these salts.
- Biofertilization led to reducing the negative effect of added salts and reducing the electrical conductivity value and the degree of pH.

References

- [1] Hari, M., & Perumal, K. (2010). Booklet on Bio-fertilizer (phosphabacteria). Shri AMM Murugappa Chettiar Research Center, Taramani, Chennai, 600, 113.
- [2] Cheng, J., Zhuang, W., Li, N. N., Tang, C. L., & Ying, H. J. (2017). Efficient biosynthesis of d-ribose using a novel co-feeding strategy in *Bacillus subtilis* without acid formation. *Letters in Applied Microbiology*, 64(1), 73-78.
- [3] Radhakrishnan, R., Hashem, A., & Abd_Allah, E. F. (2017). *Bacillus*: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in physiology*, 8, 667.

- [4] Shafi, J., Tian, H., & Ji, M. (2017). *Bacillus* species as versatile weapons for plant pathogens: a review. *Biotechnology & Biotechnological Equipment*, 31(3), 446-459.
- [5] Zhang H., Kim M. S., Sun Y., Dowd S. E., Shi H., Paré P. W. (2008) Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. *Molecular Plant Microbe Interaction*, 21, 737– 744.
- [6] Hussain, A. A., Kamran, K., Hina, M., Ishaq, M., Naz, M. Y., Bashir, S., ... & Quazi, M. M. (2023). Effect of electrokinetic treatment time on energy consumption and salt ions removal from clayey soils. *Materials Research Express*, 10(5), 055505.
- [7] Erenstein, O., Jaleta, M., Mottaleb, K. A., Sonder, K., Donovan, J., & Braun, H. J. (2022). Global trends in wheat production, consumption and trade. In *Wheat improvement: food security in a changing climate* (pp. 47-66). Cham: Springer International Publishing.
- [8] Central Statistical Organization. 2022. Wheat and barley production.
- [9] Black , C.A. .1965. *Methods of soil analysis . Part1 . Physical and mineralogical properties. Part2. Chemical and microbiological properties.* Am. Soc. Agron. , Inc. Madison , Wisconsin U.S.A
- [10] Page, A.L., Miller, R.H. and Keeney, D.R. (1982) *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties.* American Society of Agronomy. In *Soil Science Society of America*, Vol. 1159.
- [11] Al-Taie, Rand Abdul Hadi Ghazal. 2008. The role of phosphate-dissolving bacteria in the phosphate dissolution balance of phosphate from phosphate rock in calcareous soil. *Al-Rafidain Journal of Agriculture*. 36(4): 88-101.
- [12] Hess, T. M., and G. Molatakgosi.2009. Irrigation management practices of cabbage farmers in Botswana using saline groundwater. *Agricultural water Management*. 96 (2): 226-232.
- [13] Al-Zaidi, Hatem Salloum Saleh .. 2011 The combined effect of irrigation water quality, organic and phosphate fertilization on the growth and yield of cauliflower (*Brassica oleracea* var. botrytis). Master's thesis. Department of Soil Sciences and Water Resources. College of Agriculture. University of Baghdad. Iraq.
- [14] Takase, M., L.K. Sam-Amoah and J.D. Owusu Sekyere, 2011. The effects of four sources of irrigation water on soil chemical and physical properties. *Asian Journal of Plant Science*. 10 (1): 92-96.
- [15] Rabieyan, Z. ; M. Yarnia and H. Kazemi-Arbat. 2011. Effect of Biofertilizer on Yield and Yield Components of (*Cicer arietinum* /*icer arietinum* Id Biofertilizer Effect on Growth• Protein and Carbohydrate Content in *Stevia Rebaudiana* Var Berton.L.) under Different Irrigation Levels. *Australian Journal of Basic Applied Sciences*. 5(12): 3139-3145.
- [16] Panda, H. (2007). *Biofertilizers & Organic Farming*. D. Hota (Ed.). Gene-Tech Books.
- [17] Keston Oliver Willard Njira.2013. Microbial Contributions in Alleviating Decline in Soil Fertility. *British Microbiology Research Journal* 3(4): 724-742.
- [18] Al-Dulaimi, Ala Muwaffaq Sabri.. 2014 Evaluation of the efficiency of some *Bacillus* spp. isolates in phosphate solubilization under different salinity levels and growth and yield of barley. Master's thesis. College of Agriculture. University of Baghdad.

Effect of Different Levels of Cadmium and Bacterial Inoculum on Barley Growth, Soil Availability and Cadmium Content

Zahraa Juda Sadq¹ and Alaa Edan Hasan²

^{1,2}Faculty of Agriculture, University of Kufa, Najaf, Iraq.

¹E-mail: zahraaj.alarji@student.uokufa.edu.iq

Abstract. The experiment was conducted in the Department of Soil and Water Sciences and the Research Station at the College of Agriculture / University of Kufa in the year 2023-2024 to study the effect of different concentrations of cadmium and inoculation with bacteria on the growth of barley plants and some soil traits. One type of heavy elements (Cd) was used at four concentrations (0, 25, 50, 75) mM with two types of bacteria (*Bacillus cereus*, *Bacillus subtilis*). A portion of barley seeds (local variety) was soaked in a broth medium containing *Bacillus cereus* bacteria for an hour, and the other portion of barley seeds was soaked in the other type of bacteria *Bacillus subtilis* for an hour. After that, barley seeds were planted in pots on 11/8/2023 for a period of five months to find out the effect of different concentrations of cadmium on bacterial growth and barley growth and to study the effect of some soil characteristics at different cadmium concentrations. Heavy elements had a significant effect on the number of bacteria in the soil, as the cadmium treatment outperformed the lead treatment in reducing the number of bacteria of both types in the soil, as the control factor gave the highest average of 46.58×10^{-6} CFU ml⁻¹, while *Bacillus subtilis* gave the lowest average of 43.33×10^{-6} CFU ml⁻¹. Bacterial inoculation, especially inoculation with *Bacillus cereus* bacteria, also had a significant effect in improving soil properties, including available nitrogen and available phosphorus in the soil, as their rates reached 7.59 and 2.29 dS m⁻¹ and 10.7 mg kg⁻¹ and 14.55 mg kg⁻¹, respectively, and bacterial inoculation also reduced the residuals of heavy elements in the soil. The bacterial inoculation, especially *B. cereus*, also had an effect on reducing cadmium absorption in the vegetative part and roots, reaching 0.294 and 7.389 mg kg⁻¹, respectively. Cadmium levels had a significant effect on plant height and dry weight of the vegetative and root system, as the *Bacillus subtilis* treatment gave the highest average, reaching 28.46 cm, and the lowest average in the control treatment, reaching 23.58 cm. As for the dry weight of the vegetative system, it increased when inoculated with *B. subtilis* bacteria, reaching 63.06 g plant⁻¹.

Keywords. Environmental pollution, Soil, Cadmium and bacterial inoculation.

1. Introduction

One of the most important problems in the world in recent years is environmental pollution, as heavy elements come at the forefront of these problems, and these elements are the most toxic because they are not biodegradable, and they remain in the soil for a long time without any chemical change, after which their toxicity is not eliminated and remains in the soil and accumulates environmentally, and therefore it is likely to enter the food chain, and the higher their concentration in the plant exceeds the permissible limit, the more serious their impact on the

health of everyone who consumes the plant[1], due to cadmium and lead pollution, toxic symptoms that are harmful to the plant and its growth, as it reduces production and also affects the ionic balance and inhibits the work of enzymes and the absorption of essential elements and disrupts metabolic processes and photosynthesis in plants, while in humans it causes diseases such as kidney failure, high blood pressure and lung damage, and cadmium and lead are considered carcinogenic to humans [2]. There is a possibility of removing heavy elements biologically in the soil by using local isolates of *Bacillus* bacteria in contaminated soil, as the results of studies showed that the isolates proved their ability to remove heavy elements from the soil [3]. Barley *Hordeum vulgare* is a crop of the Gramineae family and is an annual winter crop, and one of the crops most adapted to difficult environmental conditions due to its tolerance to cold, salinity and drought, as indicated by [4]. Barley ranks fourth after wheat, rice and corn in terms of production and cultivated area, as the global area is 54 million hectares and is cultivated in most Iraqi governorates, as the cultivated area for the year 2018 for the barley crop reached 6101 thousand dunums. Barley production was estimated at 190 thousand tons, as the yield per dunum was 317.1 kg .dunum⁻¹, and Al-Qadisiyah Governorate ranked first. The first in terms of production with a percentage of 33.9% estimated at 65 thousand tons of the total production [5], and due to the importance of this topic, the study aimed to. Study the effect of cadmium and lead concentrations on bacterial activities, growth and yield of barley plants.

2. Materials and Methods

2.1. Experiment Location

The experiment was conducted in the laboratories of the Department of Soil and Water Sciences at the College of Agriculture / University of Kufa, at the research station / University of Kufa using a sandy mixture soil.

Table 1. Some chemical and physical properties of the study soil before cultivated.

Traits	Values	Units
Soil pH	7.70	
Electrical conductivity (EC)	1.9	DS.m ⁻¹
Organic matter (OM)	1.21	g.kg ⁻¹
Nitrogen available	5.6	
Phosphorus available	0.08	mg.kg ⁻¹
Potassium available	10.3	
Soluble ions	147.6	
Sodium	156.3	
Calcium	65.4	
Magnesium	162.7	Mmol.mL ⁻¹
Chloride	0.0	
Carbonates	134.2	
Bicarbonates	560.0	
Sulphates	0.0012	mg.L ⁻¹
Cadmium Cd	0.0002	mg.L ⁻¹
Lead Pb		Mg.g ⁻¹
Bulk density	1.43	
Soil separators	clay	
	silt	
	sand	
total bacteria	200 x 10 ⁶	CFU g ⁻¹ dry soil

The study included the effect of different concentrations of cadmium and bacterial inoculation on barley growth and some soil characteristics. One type of heavy metals (cd) was used at four concentrations (0, 25, 50, 75) mM with two types of bacteria (*Bacillus cereus*, *Bacillus subtilis*). A portion of barley seeds (local variety) was soaked in a broth medium containing *Bacillus cereus* bacteria for an hour, and the other portion of barley seeds was soaked in the other type of bacteria *Bacillus subtilis* for an hour. After that, barley seeds were planted in pots on 11/8/2023 for five months to know the effect of different concentrations of cadmium on bacterial growth and barley growth and to study the effect of some soil characteristics on different cadmium concentrations. Plastic pots were used to grow barley plants and measurements were taken at the end of the season on 20/4/2024.

2.2. Total Bacterial Counts (CFU g⁻¹ dry soil)

It was estimated by dilutions and plate counting according to Black, 1965. Field soil samples were taken at the end of the experiment and a series of dilutions of the soil suspension were prepared from 1-10 to 6-10 and Nutrient Agar was used as a medium, poured into sterile Petri dishes and 1 ml of the 6-10 dilution was added, then the dishes were placed in the incubator at 28°C for 48 hours. The live bacterial cells were calculated from the product of the number of colonies multiplied by the reciprocal of the dilution.

2.3. Plant Height (cm plant⁻¹)

The height of all plants was measured from the soil surface to the base of the upper leaf[6]

2.4. Dry Weight of the Vegetative Group (g plant⁻¹)

The dry weight of the vegetative group was measured after drying it in an electric oven at a temperature of 65°C until the weight stabilized, then it was weighed on a scale to determine the dry weight of the plant.

2.5. Dry Weight of the Root Group (g plant⁻¹)

It was measured at the end of the experiment by taking the root group of the plant after separating the vegetative group from the soil surface level for each experimental unit, then placing it in an electric oven at a temperature of 65°C for the purpose of drying until the weight stabilized, thus representing the dry weight of the root group.

2.6. Available Nitrogen

Available nitrogen was extracted using potassium chloride KCl and nitrogen was determined using Kjeldahl apparatus according to the method mentioned [7].

2.7. Available Phosphorus

Soil phosphorus was extracted using sodium bicarbonate (NaHCO₃) and the color of the extract was developed using ammonium molybdate and ascorbic acid solution. Phosphorus was determined using a spectrophotometer at a wavelength of 882 nm according to the method [7].

2.8. Available Potassium

Available soil potassium was extracted using a flame photometer as mentioned in [7].

2.9. Statistical Analysis

The three experiments were designed according to the completely randomized design (CRD)[8] The averages of the treatments were compared using the least significant difference (LSD) and under the probability level (0.05).

3. Results and Discussion

3.1. Total Bacteria

Table (2) showed the effect of different levels of cadmium significantly in inhibiting the total bacterial numbers, as the control factor gave the highest average of 46.58×10^{-6} CFU ml⁻¹, while *Bacillus subtilis* gave the lowest average of 43.33×10^{-6} CFU ml⁻¹. We note from the same table that the concentration of 75 excelled to control treatment by giving the highest value, which reached 51.00×10^{-6} CFU ml⁻¹, while the concentration of 0 *Bacillus subtilis* gave the lowest value, which reached 37.33×10^{-6} CFU ml⁻¹, and the concentration of 25 in *Bacillus cereus* bacteria gave the same value, which reached 37.33×10^{-6} CFU ml⁻¹. *Bacillus* bacteria have the ability to secrete enzymes, so they have the ability to modify their environment in a self-beneficial way, as well as produce resistant internal spores to maintain themselves in unfavorable conditions, thus reducing the effect of heavy elements [9].

Table 2. Effect of different levels of cadmium and bacterial inoculation on the total bacterial numbers ($\times 10^{-6}$ CFU) in the soil.

Cd concentration	B.0	B. subtilis	B. cereus	Average
0	50.00	37.33	46.67	44.67
25	47.33	50.33	37.33	45.00
50	38.00	46.00	50.33	44.78
75	51.00	39.67	49.00	46.56
LSD _{C*B}		3.01		N.S
average bacteria	45.83	43.33	46.58	
LSD _B		1.51		

3.2. Plant Height

Table (3) shows the effect of different levels of cadmium on plant height, as the *Bacillus subtilis* bacteria treatment gave the highest average, reaching 28.46 cm plant⁻¹, and the lowest average in the control treatment, reaching 23.58 cm plant⁻¹. We note from the same figure that the concentration of 50 mM exceeded, reaching 29.50 cm plant⁻¹, while the concentration of 75 mM in control treatment gave the lowest concentration, reaching 22.83 cm plant⁻¹. This may be due to the harmful effect of cadmium on the plant cell metabolism and its accumulation within the tissues, which reached the stage of toxicity, as it is noted that the plant height decreases when the cadmium concentration increases, and that cadmium can inhibit plant growth when exposed to high concentrations of it. This is due to many mechanisms such as inhibition of photosynthesis and its toxic effects on plant tissues. Exposure to cadmium may also lead to changes in the structure of plants, which may affect their overall growth and thus their height [10].

Table 3. Effect of different levels of cadmium and bacterial inoculation on the height of barley plants (cm.plant⁻¹).

Cd concentration	B.0	B. subtilis	B. cereus	Average
0	23.00	28.17	27.17	26.11
25	23.00	28.00	27.50	26.17
50	25.50	29.50	27.17	27.39
75	22.83	28.17	27.33	26.11

LSD _{C*B}		1.09		0.63
average	27.29	28.46	23.58	
LSD _B		0.54		

3.3. Dry Weight of the Vegetative Growth

Table (4) showed the effect of different levels of cadmium on the dry weight of the vegetative part, as the *Bacillus subtilis* bacteria coefficient gave the highest average, reaching 63.06 g plant⁻¹, and the lowest average in control treatment, reaching 44.02 g plant⁻¹. We note from the same figure that the concentration of 75 mM in *Bacillus subtilis* exceeded, reaching 83.78 g plant⁻¹, while the concentration of 0 mM in the *Bacillus subtilis* coefficient gave the lowest concentration, reaching 36.19 g plant⁻¹. This is attributed to the fact that increased levels of cadmium lead to damage to the physiological processes of the plant and the occurrence of physiological stress resulting from the heavy metal, and the first symptoms of the toxic effect appear in the form of inhibition of growth through changing the catalytic function of enzymes and damage to the cell membranes, which harms their selective permeability. Also, there is a disturbance and imbalance in the process of photosynthesis and the accumulation and transfer of nutrient ions, in addition to affecting the hormonal balance in the cell and causing damage to the stomatal complex and the rate of respiration [11], and thus affecting the weight of the plant.

Table 4. Effect of different levels of cadmium and bacterial inoculation on the dry weight of the vegetative group (g plant⁻¹).

Cd concentration	B.0	B. subtilis	B. cereus	Average
0	51.73	36.19	40.95	42.95
25	56.25	83.76	51.88	63.96
50	25.54	48.53	49.86	41.31
75	42.54	83.78	53.44	59.92
LSD _{C*B}		1.04		0.60
average	49.03	63.06	44.02	
LSD _B		0.52		

3.4. Dry Weight of the Root System

Table (5) showed the effect of different levels of cadmium on the dry weight of the roots, where the *Bacillus subtilis* bacteria coefficient gave the highest average, reaching 7.21 g plant⁻¹, and the lowest average in control treatment, reaching 2.79 g plant⁻¹. We note from the same table that the concentration of 25 mM in *Bacillus subtilis* was superior, reaching 7.95 g plant⁻¹, while the concentration of 50 mM in control treatment gave the lowest concentration, reaching 2.58 g plant⁻¹. Cell division may stop rapidly due to increased levels of cadmium and the formation of binuclear cells, causing chromosomal abnormalities during the metaphase stage [12]. Cadmium may cause oxidative stress through its effect on the formation of oxidative free radicals (ROS), in addition to disrupting antioxidant systems that remove the toxic effect. ROS is very dangerous due to its interaction with fats, proteins and nucleic acids, which causes an increase in lipid peroxidase, leakage from membranes, inactivation of enzymes and DNA damage. As a result, it will affect the viability of the plant cell [13] and thus affect the root weight.

Table 5. Effect of different levels of cadmium and bacterial inoculation on the dry weight of the root system (g plant⁻¹).

Cd concentration	B.0	B. subtilis	B. cereus	Average
0	3.51	6.71	5.81	5.34
25	2.70	7.95	7.29	5.98
50	2.58	7.23	6.88	5.56

75	2.35	6.93	7.85	5.71
LSD _{C*B}		0.56		0.32
average	6.96	7.21	2.79	
LSD _B		0.28		

3.5. Available Nitrogen in the Soil

Table (6) showed the significant effect of different levels of cadmium on nitrogen N, where the *Bacillus cereus* bacteria gave the highest average, which reached 10.70 mg kg⁻¹, and *Bacillus subtilis* bacteria gave the lowest average, which reached 6.70 mg kg⁻¹. We note in the same table that the concentration of 50 mM in *Bacillus cereus* bacteria exceeded the highest value, which reached 14.10, while the concentration of 75 mM in *Bacillus subtilis* bacteria gave the lowest concentration, which reached 4.90 mg kg⁻¹. The reason for this may be due to the overlapping effect of bacterial vaccines in increasing the availability of nutrients in general and nitrogen in particular, through improving plant growth and forming a dense root system with high absorption capacity [14].

Table 6. Effect of different levels of cadmium and bacterial inoculation on available nitrogen in soil (mg kg⁻¹).

Cd concentration	B. cereus	B. subtilis	B.0	Average concentrations
0	8.40	7.80	6.18	7.46
25	14.00	7.70	6.30	9.33
50	14.10	6.40	6.90	9.13
75	6.30	4.90	7.59	6.27
LSD _{C*B}		0.12		0.07
average bacteria	10.70	6.70	6.74	
LSD _B		0.06		

3.6. Available Phosphorus in Soil

Table (7) showed the significant effect of different levels of cadmium on the concentration of phosphorus P, as the comparison factor gave the highest average of 14.55 mg. km⁻¹, while *Bacillus cereus* gave the lowest average of 8.87 mg. km⁻¹. We note from the same table that the concentration of 75 mM in the *Bacillus subtilis* bacteria factor was superior by giving the highest value of 30.63 mg. km⁻¹, while the concentration of 0 in the comparison factor gave the lowest value of 0.26 mg. km⁻¹. This can be attributed to the role played by *Bacillus* bacteria in dissolving the phosphate originally fixed in the soil and increasing its availability and converting it into an active form ready for absorption by the plant [15,16].

Table 7. Effect of different levels of cadmium and bacterial inoculation on available phosphorus in the soil (mg kg⁻¹).

Cd concentration	B.0	B. subtilis	B. cereus	Average concentrations
0	0.26	0.32	0.41	0.33
25	14.28	12.73	2.05	9.69
50	19.32	0.41	14.39	11.37
75	24.35	30.63	18.60	24.53
LSD _{C*B}		0.20		0.12
average bacteria	8.87	11.02	14.55	
LSD _B		0.10		

3.7. Available Potassium in the Soil

Table (8) showed the significant effect of different levels of cadmium on potassium K, where the *Bacillus subtilis* bacteria coefficient gave the highest average, which reached 95.68 mg kg⁻¹, and the control gave the lowest average, which reached 76.85 mg kg⁻¹. We note in the same table that the concentration of 50 mM in *Bacillus cereus* bacteria exceeded the highest value, which reached 124.08 mg kg⁻¹, while the concentration of 0 in control treatment gave the lowest concentration, which reached 64.29 mg kg⁻¹. The reason may be due to the role played by *Bacillus subtilis* bacteria in increasing the availability of potassium as a result of its secretion of organic acids and reducing the pH and converting it into a form more available for absorption by the plant, as well as releasing potassium and converting it from unavailable sources to sources that can be absorbed by the plant, which was confirmed by [17].

Table 8. Effect of different levels of cadmium and bacterial inoculation on available potassium in the soil (mg kg⁻¹).

Cd concentration	B.0	B. subtilis	B. cereus	Average
0	64.29	118.47	83.41	88.72
25	85.14	101.70	98.66	95.17
50	75.18	90.10	124.08	96.45
75	82.79	72.44	73.83	76.36
LSD _{C*B}		0.77		0.44
average bacteria	95.00	95.68	76.85	
LSD _B		0.38		

3.8. Cadmium Concentration in Soil

Table (9) showed the significant effect of different levels of cadmium on the percentage of cadmium in the soil, as the *Bacillus subtilis* bacteria gave the highest average of 3.927 mg kg⁻¹, while *Bacillus subtilis* bacteria gave the lowest average of 1.740 mg kg⁻¹. From the same table, we note that the concentration of 75 mM *Bacillus subtilis* gave the highest value, reaching 12.296 mg kg⁻¹, while the concentration of 0 mM *Bacillus cereus* gave the lowest value, reaching 0.057 mg kg⁻¹. It was noted that there was a decrease in cadmium concentrations, and the reason for this may be due to the role played by bacteria and their secretions in modifying solubility and reducing pH, which contributes to improving the bioremediation process, which encouraged the creation of suitable conditions for its readiness in the soil. This is consistent with what [18] reached, which shows that the high concentration of cd in the soil is due to the existence of a high correlation between the rate of concentration of heavy elements in the soil and their concentration in plants growing under contaminated soil conditions.

Table 9. The effect of different levels of cadmium and bacterial inoculation on the concentration of cadmium in the soil (mg kg⁻¹).

Cd concentration	B.0	B. subtilis	B. cereus	Average
0	0.097	0.066	0.057	0.073
25	1.128	2.451	0.402	1.327
50	1.188	0.896	2.104	1.396
75	4.547	12.296	2.971	6.604
LSD _{C*B}		0.020		0.011
average bacteria	1.383	3.927	1.740	
LSD _B		0.010		

Conclusions

- The use of bio-inoculation (*Bacillus subtilis* and *Bacillus cereus*) had an effective role in absorbing heavy elements from the soil and increasing the availability of essential elements for the plant, which contributes to reducing environmental pollution and reducing production costs.
- The bacterial inoculation has a significant positive effect on most barley growth parameters and some soil characteristics.
- Barley has a role in removing heavy elements (cadmium) from the soil.

References

- [1] Tran ,N.H., M. M. Reinhard and K. H. Gin . 2018 . Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review Water research . 133: 182-207.USA.
- [2] Al-Zubaidi, Muhammad Malik Hamid (2020). Description of adsorption and reverse adsorption of cadmium in some calcareous soils of Iraq. PhD thesis, College of Agricultural Engineering Sciences, University of Baghdad.
- [3] Al-Hayani, Diaa Al-Din Aftan Hamad Abfan. (2010). Isolation and identification of some types of *Bacillus* SPP bacteria from Ramadi city hospitals and study of some histopathological changes resulting in rabbits infected with *Bacillus Cereus*. University of Anbar - College of Education for Pure Sciences - Department of Life Sciences.
- [4] Al-Amiri, Murtadha Ali Salman (2020). The effect of different salt concentrations of irrigation water and adding biofertilizer on the growth of soil bacteria and barley plant, Master's thesis, Department of Soil and Water, College of Agriculture, University of Kufa.
- [5] Subhi Ali Al-Sheikh. (2019). University of Aleppo - Faculty of Agriculture, Department of Field Crops, The effect of planting methods and the distance between rows on the growth, development and yield.
- [6] Al-Sahouki, Madhat Majeed. 1990. Yellow corn, its production and improvement. Ministry of Higher Education and Scientific Research. University of Baghdad.
- [7] Page, A.L.; R.H. Miller and D.R. Keeney. (1982). Methods of soil analysis. Part (2) 2nd .ed. Agronomy series 9. Amer. Soc of Agron. Madison .
- [8] Khashea Mahmoud Al-Rawi and Abdul Aziz Mohammed Khalaf Allah. Title, Design and Analysis of Agricultural Experiments. Place, Mosul. Publisher, University of Mosul. Date, 1980.
- [9] Allard, S. M., Walsh, C. S., Wallis, A. E., Ottesen, A. R., Brown, E. W., & Micallef, S. A. (2016). *Solanum lycopersicum* (tomato) hosts robust phyllosphere and rhizosphere bacterial communities when grown in soil amended with various organic and synthetic fertilizers. *Science of the Total Environment*, 573, 555-563.
- [10] Abdul Latif, Ali Akram. 2016. The effect of adding sludge on soil and plant pollution with lead and cadmium. Master's thesis. Al-Qasim Green University_ College of Agriculture.
- [11] Sayyad, G., Afyuni, M., Mousavi, S. F., Abbaspour, K. C., Richards, B. K., & Schulin, R. (2010). Transport of Cd, Cu, Pb and Zn in a calcareous soil under wheat and safflower cultivation—a column study. *Geoderma*, 154(3-4), 311-320.
- [12] Pal, S., Pal, P. B., Das, J., & Sil, P. C. (2011). Involvement of both intrinsic and extrinsic pathways in hepatoprotection of arjunolic acid against cadmium induced acute damage in vitro. *Toxicology*, 283(2-3), 129-139.
- [13] Puerta, V. L., Pereira, E. I. P., Wittwer, R., Van Der Heijden, M., & Six, J. (2018). Improvement of soil structure through organic crop management, conservation tillage and grass-clover ley. *Soil and Tillage Research*, 180, 1-9.
- [14] Jing, Y. D., He, Z. L., & Yang, X. E. (2007). Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *Journal of Zhejiang University Science B*, 8(3), 192-207.



- [15] Abduljaba, R. A. and Sameera F.M. (2017). Estimation of Metal Accumulation by Bioconcentration Factor Through The Determination of some Heavy Metals in Soil and Common Plants in Different Habitats in Kirkuk City.
- [16] Margaret, L. C.; S. G. Kristina; J. M Arcega, R. Norbertine, G. L. Cabigao, and S.U. Sia,. (2016). Isolation and Identification of Heavy Metal- Tolerant Bactria from an Industrial Site as a Possible Source for
- [17] Al-Radhi, Hadeer Adnan Saleh, Hassan Ali Abdul-Ridha and Hamid Ali Hadwan (2018). The effect of the locally produced Bacillus subtilis Bacillus megaterium biofertilizer from both Solanum bacteria and imported on the growth and yield of potatoes Bacillus mucilaginosus and tuberosum L. Iraqi Journal of Desert Studies (2) 53-65
- [18] Abduljaba, R. A. and Sameera F.M. (2017). Estimation of Metal Accumulation by Bioconcentration Factor Through The Determination of some Heavy Metals in Soil and Common Plants in Different Habitats in Kirkuk City.

Requirements for Implementing Total Quality Management in Training Extension Centers in Iraq

Ihsan Radhe Hassuni¹ and Bayan Abdul-Jabbar Ridha²

^{1,2}Department of Agricultural Extension and Technology Transfer, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.

¹E-mail: ihsan.radi2209@coagri.uobaghdad.edu.iq

²E-mail: bayan.reda@coagri.uobaghdad.edu.iq

Abstract. The research seek to determine the requirements for the application of total quality management in the training extension centers in Iraq, To achieve the research objectives, a questionnaire was developed based on relevant literature, previous studies, and expert opinions. The questionnaire consisted of 58 items divided into 4 fields ,The study was conducted on all (15) governorates of Iraq except the Kurdistan region, and a random sample of them was withdrawn by 50% and by (8) governorates: (Kirkuk, Baghdad, Diyala, Holy Karbala, Al-Najaf Al-Ashraf, Qadisiya, Dhi Qar, Basra), and the research sample included (149) respondents, Data was collected through the questionnaire and personal interviews conducted between 9/7/2023-5/9/2023, and The results showed that there are requirements that must be met to apply total quality management in training extension centers, as the requirement of the communication system came in the first place with a weighted mean of (4.221) degrees, and a percentage weight of (84.426) degrees, while the requirement to train employees in the training extension centers, it came in the last order with a weighted mean of (4.14) degrees and a percentage weight of (82.803) degrees, and The researchers recommended that the agricultural extension and training department take these requirements into account and work to provide them when applying total quality management.

Keywords. Training employees, Extension Centers, Total Quality.

1. Introduction

Attention to rural development has become a necessary requirement that the majority of countries, whether developed or developing, seek to achieve [1]. and rural development is one of the most important means to advance the agricultural sector [2]. agricultural extension is one of the main organs to achieve rural development, as it represents the active element in bringing about the desired changes, and developing positive trends through its various educational and cultural activities [3]. and aims to bring about behavioral changes in the components of the human behavior of the farmers, as well as the transfer of modern agricultural technologies and innovations and their application in their farms to keep pace with global development [4]. and plays vital roles in serving the extensions in all areas of their agricultural and animal activities and serving their organizations by meeting their knowledge, information, skill and technical needs to manage their agricultural activities properly and sustainability, as they facilitate their access sources of the knowledge.[5],[6]. as it acts as a link between them and research centers, as

it transfers their problems to research centers to find solutions to them and then transfers research results (solutions) to them[7]. The training extension centers are one of the formations of the Agricultural Extension and Training Department, which works to develop the capabilities of farmers, livestock breeders and agricultural employees by holding training courses for them, as well as developing the agricultural cooperative movement in the country[8].

Despite the importance of training extension centers in Iraq and their role in achieving rural development, the reality indicates the weakness of this role [9]. and the weakness of the role of the extension system in general [10], as many studies and research have indicated significant weaknesses and deficiencies in its performance. Reda and Hassouni also pointed out the poor performance of training extension centers [11]. Twayej and Al-Taiy mentioned in their study that there is a deficiency in the centers' performance Training extension [12]. In order to improve the reality of training extension centers and improve the quality of their services, a number of entrances must be applied, including the entrance to total quality management, as Al-Taiy stressed that one of the proposed strategies for agricultural extension reform in Iraq is to spread the culture of total quality and its importance and need in the agricultural sector in general and its extension system in particular [13]. Ridha and others also recommended in their study the need to pay attention to and improve the work of extension and training centres [14]. Al-Taiy and others summarized that one of the most important basic requirements for improving the quality of the extension service is the development or adoption of quality standards and their application in every element or process in the extension system or service provided [15], and Naji and Hussein recommended the need to apply total quality in the extension work [16] and Naji and Johi found in their study the existence of the necessary qualifications to apply the approach of total quality management in the extension management at various levels[17].

Total quality management is a common philosophy and culture and it is part of an integrated, comprehensive and interconnected process that requires all employees in the organization to carry out their responsibilities to the fullest, in order to constantly improve and develop quality[18]. and many quality studies confirmed that the application of total quality management has positive repercussions on the performance of the organizations that apply it, including the study of Saeed[19], Al-Khatib and Al-Haidari[20], Abdel Dayem [21], and Al-Fakiki and Al-Shuhani [22], as all these studies confirmed that the application of the method of total quality management in organizations contributes significantly to their survival and continuity by providing satisfactory and distinguished services, with the highest quality and at the lowest costs, as well as contributing to moving away from traditional and routine administrative methods.

With regard to the application of total quality management in the extension training centers, there are a set of benefits that accrue to the extension centers as a result of its application, the most important of which is improving the competitive position of the extension centers, raising the degree of satisfaction of the extensions, improving the quality of services provided, reducing the cost of the services provided, increasing the rate of response speed to the changes that occur in agricultural extension, providing extension services correctly[23].

In view of the importance of applying total quality management in training extension centers, and in order to be able to achieve the required role and responsibility with regard to rural development goals, this requires providing all the necessary requirements for the successful application of total quality management in it. Therefore, this research came to answer the following question:

What are the requirements for the application of total quality management in training extension centers in Iraq?

1.1. Research Aims

- Determining the requirements for the application of total quality management in training extension centers in Iraq.
- Arranging the fields of requirements for applying total quality management in training extension centers, represented by: (Commitment and support of the organization's higher management extension for the application of total quality management ,Focus on the target audience ,training employees in training extension centers, Communication System).

1.2. Research Hypothesis

- The respondents' approval of all fields of requirements for applying total quality management in training extension centers

2. Material and Methods

2.1. Research Methodology

In order to achieve the objectives of the research, the descriptive approach was used, which aims to study a certain phenomenon as it exists in reality and is interested in describing it accurately, so this approach is suitable to reach detailed data and facts about (requirements for the application of total quality management in training extension centers in Iraq).

2.2. Research Population and Sample

The research community included all the governorates of Iraq (15) governorates except the Kurdistan Region and all the training extension centers in these governorates, which numbered (16) extension center and all the extension farms of these centers, which numbered (59) farms, and the Department of Extension and Agricultural Training, and a random sample was selected by 50% of the governorates of Iraq and by (8) governorates: (Kirkuk, Baghdad, Diyala, Holy Karbala, Al-Najaf Al-Ashraf, Qadisiyah, Dhi Qar, Basra). Training in the governorates covered by the research sample, represented by (directors of training extension centers and their assistants, numbering) (16) directors, and all agricultural extensionists, numbering (37) agricultural extensionists. And all employees in the extension farms of the extension centers, represented by (officials of extension farms, numbering (29) officials, and all agricultural extension employees numbering (48) agricultural extensionists. And the employees in the Department of Agricultural Extension and Training, represented by (the Director General of the Department and his assistant and the heads of the departments of the Department of Management of Centers and Farms Extension, the Department of Manpower Development, and the Department of Planning and Monitoring and their assistants) and their number (8) respondents, as well as all agricultural extension employees in these sections and the number of (11) agricultural extensionists, thus the total number of respondents (149) respondents.

2.3. Data Collection Tool

For the purpose of achieving the objectives of the research, a questionnaire was prepared as a tool to collect data from the respondents, and the questionnaire included building a scale of requirements for the application of total quality management in the training extension centers, and the process of building the scale went through a series of stages:

- Preparing the scale in its initial form: In the view of the literature related to the subject of research and previous studies and the opinions of experts and specialists in the field of total quality management, and agricultural extension, the scale of the requirements for the

- application of total quality management in the training extension centers was prepared in its initial form, which included four fields : (Commitment and support of the higher management of the extension organization to apply total quality management, focus on the target audience, training employees in training extension centers, communication system) The items constituting these fields amounted to (61) items distributed over the fields previously mentioned above.
- Development of the scale: The fields and items were presented in their initial form to a group of experts specialized in the field of agricultural extension and management, numbering (27) experts, through a questionnaire to indicate the degree of their approval of the fields and items of each field in the view of the scale of approval be of three levels: agree, agree with the amendment, disagree.
 - Determining the criterion of expert approval (cutting threshold) on the fields and items of the scale: The threshold of cutting was determined by 75% or more in the degree of expert approval for the fields and items of the proposed questionnaire to remain in its final form.
 - Calculating the means of the scores of the experts' approval on the fields and items of the scale: Estimated weights were given to the levels of the approval scale as follows: (Agree = 2), (Agree with amendment = 1), (Disagree = 0) and thus the degree of the scale ranged between (0-2) degrees, and the means were calculated by calculating the total scores obtained on the number of experts.
 - Preparing the scale in its final form: In view of comparing the mean scores of expert approval on the fields and items of the questionnaire with the threshold of cutting to prepare the questionnaire in its final form, all fields and items have achieved (75%) and more, as the threshold of cutting for the scale of the requirements for the application of total quality management in the training extension centers reached (94.64%), as item (10) was deleted from the field of commitment and support of the higher management of the extension organization, item (10) was deleted from the Focus on the target audience, and item (5) was deleted from the field of training employees in training extension centers, and thus the number of items of the scale became (58) items distributed on (4) fields, and thus the final version of the requirements scale was come out Applying total quality management in training extension centers.

2.4. Questionnaire Reliability

To measure stability, a preliminary test of the pre-test questionnaire was conducted in June of 2023 on a sample of respondents in Babylon Governorate, consisting of all employees (managers and agricultural extensionists) in the training extension center and its extension farms, so the initial test sample reached (30) respondents, and the alpha cronbach equation was used to measure the stability of the requirements scale, which amounted to (0.84), and this value is scientifically acceptable.

2.5. Data Collection

Data was collected from the (149) respondents using a questionnaire form using a personal interview questionnaire, during the period 9/7/2023 - 5/9/2023.

2.6. Tabulation and Analysis of Data

The weights (5,4,3,2,1) were given to the levels of the scale of the requirements for the application of total quality management in the training extension centers (strongly agree, agree, somewhat agree, disagree, not agree at all) respectively, and to arrange the fields and items in

descending order , from the respondents' point of view, the weighted mean and percentage weight were used.

2.7. Statistical Methods

The statistical methods used in the analysis of research data are: weighted mean, percentage weight and alpha cronbach equation.

3. Results and Discussion

3.1. Determining the Requirements for the Application of Total Quality Management in Training Extension Centers in Iraq

3.1.1. Commitment and Support of the Higher Management of the Extension Organization to Apply Total Quality Management

The requirements related to the field (Commitment and support of the higher management of the extension organization to apply total quality management) of (17) requirements obtained weighted means located between (3.965-4.326) degrees, and percentage weights located between (79.29-86.51) degrees, as shown in Table (1).

Table 1. Distribution of respondents according to the items related to the field of Commitment and support of the higher management of the extension organization to apply total quality management.

Item number in the form	Items	Weighted mean	Percentage weight	Sequence
5	Supporting the higher management of the extension organization by providing material capabilities (devices and equipment... and others) that help to apply total quality.	4.326	86.51	1
11	The commitment of the higher management of the extension organization to achieve the principle of justice with all employees in the training extension centers.	4.267	85.33	2
16	Monitoring of the higher management of the extension organization on an ongoing basis to the training extension centers.	4.227	84.53	3
6	The higher management of the extension organization should establish a culture of total quality among all employees in the training extension centers.	4.191	83.82	4
2	The higher management of the extension organization should be committed to providing the necessary financial allocations for the application of total quality management.	4.185	83.7	5
15	Participation of the higher management of the extension organization in training courses related to total quality management to develop their capabilities in this field.	4.182	83.63	6
10	The higher management of the extension organization should work to improve the quality of services provided by the training extension centers to the targeted as part of	4.173	83.46	7

Item number in the form	Items	Weighted mean	Percentage weight	Sequence
	its vision and mission.			
4	The higher management should work to inform all employees of the objectives of the extension organization that it seeks to achieve through them.	4.17	83.4	8
12	The higher management of the extension organization should coordinate with the relevant local and international organizations to provide additional funding sources to support the training extension centers.	4.168	83.35	9
14	Supporting the higher management of the extension organization in coordination with university professors, experts and specialists in the field of total quality management.	4.153	83.06	10
9	The higher management of the extension organization should accept the fact that it is responsible for the quality of services provided to the targets	4.138	82.76	11
7	The higher management of the extension organization should rely on correct and reliable data in the decision-making process related to the application of total quality management.	4.137	82.75	12
13	The higher management of the extension organization should develop a vision and policy for total quality management to extension the employees.	4.121	82.41	13
17	Affiliation of the higher management of the extension organization to the membership of local and international total quality associations to provide them with the opportunity to learn and learn about professional experiences in this field and develop a network of relations with them.	4.078	81.56	14
8	The higher management of the extension organization should work on the principle of delegation of powers and facilitate the flow of information.	4.03	80.59	15
3	The higher management of the extension organization addressed the resistance to change for the departments of the training extension centers and their employees as a result of the application of total quality management.	3.997	79.93	16
1	The conviction of the higher management of the extension organization of the importance of applying total quality management.	3.965	79.29	17
	General rate	4.148	82.946	

With regard to the arrangement of the items related to the field of requirements (Commitment and support of the higher management of the extension organization to apply total quality management), it is clear from Table (1) that there is a convergence between the weighted means

of the items related to this field, however, the item { Supporting the higher management of the extension organization by providing material capabilities (devices and equipment... and others) that help to apply total quality in training extension centers.} It came in first place, with a weighted mean score (4.326) and a percentage weight of (86.51) scores. The reason for this can be attributed to the sample members' awareness of the importance of supporting the extension organization's higher management in providing devices, equipment, means of transportation, etc., as they are among the most important basic elements of the extension organization. The basic components of the successful application of total quality management in training extension centers are ranked first in terms of importance.

As for the item (the conviction of the higher management of the extension organization of the importance of applying total quality management in the training extension centers.), it came in the last order with a weighted mean of (3.965) degrees, and a percentage weight of (79.29) degrees, and the reason for this may be attributed to the belief of some respondents that the application of total quality management does not need the conviction of the higher management of the extension organization because the decision to apply total quality management in the extension organization, especially in the extension centers. Training is mostly done by the higher authorities in the Ministry of Agriculture, so the matter is mandatory and does not require the conviction of higher management in the extension organization, so this item came in the last order.

3.1.2. Focus on the Target Audience

The requirements related to the (Focus on the target audience) field, which are (12) requirements, obtained weighted means located between (4.128-4.284) degrees, and percentage weights located between (82.57-85.69) degrees, as shown in Table (2).

Table 2. Distribution of respondents according to items related to the focus on the target audience.

Item number in the form	Items	Weighted mean	Percentage weight	Sequence
1	The departments of the training extension centers encourage employees to perform all activities that work to achieve the satisfaction of the target audience.	4.284	85.69	1
5	Employees in the training extension centers were keen to treat the targeted people well and gain their trust.	4.258	85.17	2
7	Monitoring the complaints of the targeted people on an ongoing basis and work to find appropriate solutions to them.	4.249	84.98	3
12	The keenness of the employees in the training extension centers to achieve the satisfaction of the targeted people.	4.237	84.74	4
6	Ensure that services are provided to the targeted people at the right time and place.	4.233	84.67	5
2	Identify the reactions of the targeted about the quality of the services of provided to them to improve them.	4.187	83.75	6

3	Understand the current and future needs and desires of the targets, to be met through extension and training programs built according to total quality standards.	4.166	83.33	7
9	Taking into account the opinions and suggestions of the targeted to develop the work of the training extension centers.	4.159	83.19	8
8	The training extension centers should provide services that suit the social and economic conditions of the targets.	4.147	82.94	9
4	Survey studies should be conducted on an ongoing basis on the targeted people to determine their renewed needs for the services provided to them.	4.138	82.77	10
11	The departments of the training extension centers should believe in achieving quality by meeting the needs of the targeted.	4.13	82.6	11
10	To adopt objective measures to know the level of satisfaction of the targeted people with the quality of services provided to them	4.128	82.57	12
General rate		4.194	83.867	

With regard to the order of the items related to the field of requirements (Focus on the target audience), it is clear from Table (2) that there is convergence between the weighted means of the items related to this field, however, the item came (The departments of the training extension centers encourage employees to perform all activities that work to achieve the satisfaction of the target audience.) In the first order with a weighted mean (4.284) degrees, and a percentage weight of (85.69) degrees, and the reason for this may be attributed to the respondents' sense of the importance of this item, because achieving the satisfaction of the targeted is the main goal and the intended goal that the total quality management seeks to achieve.

As for the item (To adopt objective measures to know the level of satisfaction of the targeted people with the quality of services provided to them.), it came in the last order with a weighted mean of (4.128) degrees, and a percentage weight of (82.57) degrees, and the reason for this may be attributed to the belief of some respondents in the difficulty of setting objective measures to know the level of satisfaction of the targeted people with the quality of services provided to them, as well as the difficulty of the possibility of conducting or applying them in practice. , so this item came in the last order compared to the rest of the items.

3.1.3. Training Employees in the Training Extension Centers

The requirements related to the field of (training employees in the training extension centers) of (16) requirements obtained weighted means located between (3.976-4.255) degrees, and percentage weights located between (79.51-85.09) degrees, as shown in Table (3).

Table 3. Distribution of respondents according to the items related to the field of training employees in training extension centers.

Item number in the form	Items	Weighted mean	Percentage weight	Sequence
12	The managers of the training extension centers should be keen to develop and train their employees on an ongoing basis to ensure the ability to achieve quality continuously.	4.255	85.09	1
7	Providing sufficient financial allocations to train and develop the capabilities of employees in training extension centers in the field of total quality management.	4.252	85.03	2
8	The administrations of the training extension centers should send their employees abroad to participate in training courses for total quality.	4.217	84.34	3
15	Using external expertise specialized in the field of total quality to train and develop the capabilities of employees in training extension centers.	4.174	83.47	4
13	To develop a specialized unit to develop the capabilities of employees in the field of total quality management.	4.169	83.38	5
6	The need to develop training programs that suit and benefit employees in training extension centers in developing and improving their performance	4.165	83.3	6
11	Monitoring and evaluation of the performance of employees in the training extension centers should be carried out after the end of their training process in the field of total quality management.	4.161	83.21	7
14	The date of the training program for employees in the training extension centers shall be determined according to their engagements and job burdens.	4.152	83.04	8.5
16	Continuous improvement and development of the quality of training programs for employees in training extension centers.	4.152	83.04	8.5
3	Preparing a detailed plan to identify the training needs of employees in the field of total quality.	4.12	82.39	10
9	The need to use a variety of training methods to train employees in training extension centers, such as brainstorming, problem solving, and others.	4.103	82.06	11
5	The departments of the training extension centers should Reward employees Outstanding	4.1	82	12

Item number in the form	Items	Weighted mean	Percentage weight	Sequence
	who participated in training courses related to total quality management.			
10	The necessity of conducting the evaluation process for the training programs prepared for employees in the training extension centers after their completion to ensure their quality.	4.089	81.77	13
2	Work on developing the capabilities and knowledge of employees on the importance of total quality, its objectives.	4.086	81.72	14
4	The resources and capabilities available to the training extension centers should be used efficiently to provide training programs in the field of total quality management for their employees.	4.075	81.49	15
1	The need to make training in the field of total quality management mandatory for all employees.	3.976	79.51	16
	General rate	4.14	82.803	

With regard to the order of the items related to the field of requirements (training of employees in the training extension centers), it is clear from Table (3) that there is a convergence between the weighted means of the items related to this field, however, the item (The managers of the training extension centers should be keen to develop and train their employees on an ongoing basis to ensure the ability to achieve quality continuously) came in the first order with a weighted mean of (4.255) degrees, and a percentage weight (85.09). The reason for this may be attributed to the awareness of the departments of the training extension centers of the importance of continuous training and its role in improving the competencies and performance of employees, especially since the needs, desires and expectations of the targeted people change, which requires that the employees be qualified and always ready to keep pace with these changes, and thus provide satisfactory and quality services to the targeted people constantly.

As for the item (the need to make training in the field of total quality management mandatory for all employees in training extension centers), it came in the last order with a weighted mean of (3.976) degrees, and a percentage weight of (79.51) degrees, and the reason for this may be attributed to the fact that the training process for employees in training extension centers is a routine and binding procedure when any development work is to be applied, especially the application of total quality management, so this item came in the last order.

3.1.4. Communication System

The requirements related to the field (communication system) of (13) requirements obtained weighted means located between (4.111-4.283) degrees, and percentage weights between (82.21-85.66) degrees, as shown in Table (4).

Table 4. Distribution of respondents according to the items related to the field of the communication system.

Item number in the form	Items	Weighted mean	Percentage weight	Sequence
1	There should be an effective two-way communication system, whether between the departments of the training extension centers and the employees or between the centers and outside.	4.283	85.66	1
10	Providing all material necessities to support the quality of the communication process in the training extension centers.	4.28	85.59	2
3		4.267	85.34	3
	The use of modern information and communication technology in the work of training extension centers.			
8		4.262	85.24	4
	Provide sufficient financial allocations to establish effective communication systems .			
7	Overcoming obstacles (physical, technical, social.. etc) that hinders the process of communication with the targets.	4.249	84.97	5
	Providing an integrated database within the training extension centers that is updated periodically in a way that ensures the safety of the decisions taken.	4.233	84.65	6
5				
2	To have a communication system capable of delivering accurate information about the achievements of employees to apply quality as soon as possible.	4.227	84.53	7
11	The need to conduct an assessment of the extent to which the training extension centers use various methods and means of communication when transferring and exchanging information with the targets.	4.21	84.2	8
6	The communication system should be able to deliver information and orders correctly and usefully and as soon as possible	4.209	84.17	9
9	Following a clear communication policy that makes the employees fully aware of the concept of total quality.	4.2	83.99	10
13	The need to conduct a continuous evaluation process for the communication process in the training extension centers, to detect errors and deviations early and thus achieve the desired goal of the communication process.	4.178	83.56	11
4	The need for continuous communication with the targeted to know the degree of their	4.171	83.42	12

Item number in the form	Items	Weighted mean	Percentage weight	Sequence
	satisfaction with the quality of services and to ensure an immediate response to address their complaints, if any.			
12	Conducting an evaluation of the communication process through feedback from the employees of the training extension centers and the targeted.	4.111	82.21	13
	General rate	4.221	84.426	

With regard to the order of the items related to the field of requirements (communication system), it is clear from Table (4) that there is convergence between the weighted means of the items related to this field, however, the item (There should be an effective two-way communication system, whether between the departments of the training extension centers and the employees or between the centers and outside.) came in the first order with a weighted mean (4.283) degrees, and a percentage weight (85.66). The reason for this may be attributed to the importance of this item in the success of the application of total quality management, because through the process of two-way communication, instructions and directives for total quality management are transferred from the departments of the extension centers to the employees, and at the same time receiving inquiries, obstacles and problems facing employees in the application process, as well as the importance of communication with the relevant external departments to benefit from their experiences in this field

As for the item (conducting an evaluation of the communication process through feedback from employees in the training extension centers and the targeted), it came in the last order with a weighted mean of (4.111) degrees, and a percentage weight of (82.21) degrees, and the reason for this may be attributed to the belief of some respondents that most of those in charge of evaluating the communication process in the training extension centers do not have sufficient information and experience to conduct this process correctly and to achieve the desired goal, And different methods in the evaluation process, so this item came in the last order.

3.2. Arranging the Fields of Requirements for Applying Total Quality Management in Training Extension Centers

As for the arrangement of the fields of the requirements for the application of total quality management in the training extension centers (4) fields, the results of the research showed that the weighted means of the fields of requirements ranged between (4.14-4.221) degrees, with percentages weight between (82.803-84.426)degrees, as shown in Table (5).

Table 5. Distribution of respondents according to the requirements of the application of total quality management in training extension centers.

Item number in the form	Items	Weighted mean	Percentage weight	Sequence
4	Communication System	4.221	84.426	1
2	Focus on the target audience	4.194	83.867	2

1	Commitment and support of the higher management of the extension organization to apply total quality management	4.148	82.946	3
3	training employees in training extension centers	4.14	82.803	4
	General rate	4.175	83.511	

It is clear from Table (5) that the field of requirements (communication system) came in the first order with a weighted mean (4.221) degrees, and a percentage weight (84.426) degrees. The reason for this can be attributed to the great importance shown by the respondents to the communication system field as one of the important requirements for applying total quality management in training extension centers, as it is the basis for transferring information and extension within training extension centers. As well as communicating with relevant external parties.

As for the requirements of (training employees in the training extension centers), it came in the last order with a weighted mean of (4.14) degrees, and a percentage weight of (82.803) degrees, and the reason for this may be due to the belief of some respondents that the training of employees in training extension centers is one of the routine procedures carried out by the centers to develop the capabilities of their employees in line with modern technological and administrative changes, especially in The application of total quality management remained.

In view of the above-mentioned values of the weighted averages of the sample members' answers, we accept the hypothesis that the sample members agree on all fields of the requirements for applying total quality management in training extension centers.

Conclusions

In view of the results of the research, we conclude the following:

- The agreement of all respondents on the items and fields of the requirements for the application of total quality management in the training extension centers, and this indicates the need to provide these requirements and ensure their readiness before starting the application of total quality management.
- All respondents emphasize the importance of all fields of the requirements for the application of total quality management in the training extension centers, represented in (commitment and support of the higher management of the extension organization to apply total quality management, Focus on the target audience, training employees in training extension centers, communication system) with greater attention by them to the field of the communication system compared to the rest of the fields.

Recommendations

In view of the results and conclusions, the researchers recommend that decision makers in the Department of Agricultural Extension and Training should taking these requirements into consideration and working to provide them when applying total quality management and attention to all the fields that it includes, with a greater focus on the field of the communication system before starting the application process.

References

- [1] Haskah, S. A., Al-Hamdany, H. S., & Amin, H. M.(2023). The Opinion of Agricultural Extension Agents on the Importance of Participating in Extension Activities and Procedures Provided by the

- Agricultural Extension Organization in the Republic of Iraq, IOP Conf. Series: Earth and Environmental Science, 1214 (2023) 012054, doi:10.1088/1755-1315/1214/1/012054.
- [2] Naji, A. A., & Ibrahim, A. K.(2020). The Role of Extension Training in Developing the Capabilities of Agricultural Employees in the Sustaining field of Agricultural natural resources in the province of the Central Region of Iraq .Plant Archives, Vol (20), 1461-1466.https://www.plantarchives.org/SPL%20ISSUE%2020-2/237__1461-1466_.pdf.
- [3] Al-Saadi, A. H ., & Al-Badri, A.A.(2022). The reality of the extension services provided to workers in the production of vegetables in the farms belonging to the two holy shrines of Husseiniya and Abbasiya in the Holy province of Karbala, Iraqi Journal of Agricultural Sciences.53(3), 685-697.<https://doi.org/10.36103/ijas.v53i3.1579>.
- [4] Majd, Z. H., Heikal, S.A., & Abdullah, H.A.(2022). Problems facing the application of Total quality standards in agricultural extension management and proposals to solve them in the Republic of Iraq. Journal of the Scientific Society for Agricultural Extension.26(3),1-22. DOI: 10.21608/mgiz.2022.267126.
- [5] Khalaf, A. G., & Al-Mashhadani, A. L. (2023). Requirements for Using Digital Agricultural Extension in Providing Extension Service from Viewpoint of Agricultural Extension Agents, IOP Conf. Series: Earth and Environmental Science 1259 (2023) 012134 IOP Publishing doi:10.1088/1755-1315/1259/1/012134.
- [6] Al-Taïy, H. K., Al-Salhi, A.Th., & Al-mashadani, A.L.(2020). Development Extension Service to meeting The Requirements of Buffalo Breeders in Iraq. Iraqi Journal of Agricultural Sciences.51(1),432-442 .<https://doi.org/10.36103/ijas.v51i1.942>.
- [7] AL-Mashhadani, M. A., & Sbeih, F. E.(2022). Improving some coordination process domains between the Extension Department and research centers in the Baghdad governorate. International Journal of Health Sciences.6(56),5629-5649.<https://doi.org/10.53730/ijhs.v6nS6.10873>
- [8] Abdul Rahman, A. L .(2017). Extension centers and farms, a point of cultural radiation, a lecture at the extension training center in Diyala Governorate .pp (1-25).
- [9] Al-Hafidh, F. S., & Al-Taïy, H.k.(2022). Suggested visualization for some quality elements of extension service for vegetable farmers from their viewpoint in the governorate of Baghdad, Iraqi Journal of Agricultural Sciences.53(5), 1203-1211.<https://doi.org/10.36103/ijas.v53i5.1634>.
- [10] Al-Salhi, A. T. H. (2020). Extension activities provided to rice farmers in raising the awareness for compost manufacture in al-diwanayah province, Iraqi Journal of Agricultural Sciences .51(6),1678-1683.<https://doi.org/10.36103/ijas.v51i6.1196>.
- [11] Reda, b. A., & Hassouni ,A. R.(2015). The problems facing training extension centers and ways to develop them in Iraq from the point of view of their employees. Iraqi Journal of Agricultural Sciences.46(4),621-628.<https://ijasj.net/ijas/download/0f14e4ef3ed2185f>.
- [12] Twayej ,S.A., & Al-Taïy, H .K. (2022). Activities carried out by extension farms providing extension services to the farmers of the central euphrates provinces. Iraqi Journal of Agricultural Sciences.53(6) , 1418- 1426.<https://doi.org/10.36103/ijas.v53i6.1657>.
- [13] Al-Taïy, H.K. (2014). Reform of agricultural extension systems in Iraq. Iraqi Agriculture Research Journal, Iraqi Ministry of Agriculture.19(7),204-216.<https://ijarmoa.gov.iq/wp-content/uploads/2022/06/>.
- [14] Ridha, B. A., Hassuny, A .R ., & Al-Salhi, A.Th .(2020). Evaluation of Training Courses for Agricultural Employees Conducted by the Training and Rehabilitation Department Belonging to the Extension and Training of Agricultural in Iraq. Plant Archives.20 (1),155-162.[http://www.plantarchives.org/20-1/155-162%20\(5404\).pdf](http://www.plantarchives.org/20-1/155-162%20(5404).pdf).
- [15] Al-Taïy, H. K., Naji, A. A., & Reda, B. A.(2021). A proposed introduction to agricultural extension in Iraq to better respond to the needs of farmers in addressing their challenges. Al-Muthanna Journal of Agricultural Sciences.8 (3),1-13.<https://search.emarefa.net/detail/BIM-1350719>.
- [16] Naji, A .A., & Hussein, S .R.(2021). Evaluation the Performance of Senior Management for the Extension Organization in view of the Total Quality standards from the standpoint of Agricultural

- Extension employees in Governorates of Baghdad and Diyala .Iraqi Journal of Agricultural Sciences.52(4),1008-1018.<https://doi.org/10.36103/ijas.v52i4.1412>.
- [17] Naji, A. A., & Johi, A. S.(2014). The Performance of Extension Management under Total quality standards from the point of view of Agricultural Employees working in Agricultural Extension and Vegetable growers in the Governorates of the Central Region of Iraq . Journal of Economic and Administrative Sciences.20 (75),100-116. <https://doi.org/10.33095/jeas.v20i75>.
- [18] Sharif, N. M ., & Mohieddin, S. S.(2021). Developing educational philosophy according to Total quality standards. Journal of Literature, Issue (137), 215-234.<https://doi.org/10.31973/aj.v1i137.1615>.
- [19] Saeed, A. S.(2013). A vision and model - a proposal for psychological extension and educational extension under the Total Quality Management System. Journal of Educational and Psychological Research, Issue(39),122-143.<https://jperc.uobaghdad.edu.iq/index.php/jperc/article/view/519/435>.
- [20] Al-Khatib, S.K ., & Al-Haidari, D. A.(2017). Application of Total quality management requirements and their relationship to organizational performance from the point of view of the internal customer (comparative study) Iraqi Journal of Market Research and Consumer Protection.9(2),54-72.<https://jmracpc.uobaghdad.edu.iq/index.php/IJMRCP/article/view/30/25>.
- [21] Abdel Dayem, A. A .(2021). The effect of intellectual capital on the Total quality management of higher education and scientific research institutions in Baghdad. Journal of Economic and Administrative Sciences.27 (127), 75-97.<https://doi.org/10.33095/jeas.v27i127.2138>.
- [22] Al-Fakiki, A. F., & Al-Shuhani, A. Z.(2016). Total quality management in the Qur'an - the story of the Prophet of God, Joseph, peace be upon him, as an example. Iraqi Journal of Market Research and Consumer Protection.8 (1), 340-366.<https://www.iasj.net/iasj/download/045c33878f7add24>.
- [23] Abed, L. T. A. (2017). Building a measure for applying the principles of total quality management and a measure for codifying the stages of applying total quality management in agricultural extension centers. Journal of Agricultural Economics and Social Sciences.8 (12), 931-939.https://jaess.journals.ekb.eg/article_37032_659f67a54f4cff14a2963f0ffa327921.pdf.

Assessing the Effectiveness of Inoculating *Mycorrhiza* and *Trichoderma* Fungus, Adding Seaweed Extract and Interfering with Their Growth in Olive Seedlings

Noor Muneam Fadhil¹, Najwan Abbas Mohammed² and Enas Ammar Mohammed³

¹ Department of Animal Production, Collage of Agriculture, University of Diyala, Diyala, Iraq.

² Biotechnology Department, Collage of Science, University of Diyala, Diyala, Iraq.

³ Collage of Medicine, University of Diyala, Diyala, Iraq.

¹E-mail: noor.muneamfadhil@uodiyala.edu.iq

²E-mail: najwanabbas@uodiyala.edu.iq

³E-mail: enas.a@uodiyala.edu.iq

Abstract. The experiment was carried out in the Baquba nursery of the Diyala Agriculture Directorate the on olive seedlings. 240 of 2 years old seedlings were selected to study Evaluation of the effectiveness of bio inoculation with a fungus *VAM Glomus mosseae* and the fungus *Trichoderma harzianum*. The addition of seaweed extract and their interaction in the growth of olive seedlings a Nepali variety. the seaweed extract was added to (Algex Spanish) in a concentration of (1 to 1.5 kg ha⁻¹) according to the manufacturer's instructions. The design of the randomized complete block design (R.C.B.D) was employed with three replications. The least significant difference (L.S.D) was used to define the difference between the means. At the (0.05) level, the results demonstrated the following. A higher percentage of infection with mycorrhiza fungi alone or in conjunction with the *Trichoderma* fungus and seaweed, it reached (27.80%, 32.60%, 42.20%, 51.50%). In comparison with the control treatment, the intervention treatment was superior to inoculation with two fungi *Mycorrhiza*, *Trichoderma* and seaweed extract on all treatments in vegetative traits (Height of plants, diameter of seedlings, leaf area, dry weight of shoots, dry weight of roots, chlorophyll The total reached (27.2 cm, 5.03 mm, 8.07 dm², 88.68 g, 37.23 g, 87.30 mg, g) respectively compared with the control treatment.

Keywords. Olive seedlings, Seaweed extract, *Mycorrhiza*, *Trichoderma* spp.

1. Introduction

Microbial inoculants are one of the achievements of biotechnology is the isolation, purification and characterization of microorganisms Various vaccines are added to the plant growth medium in aim to increase the readiness to absorb nutrients. As the microbial content changes in the rhizosphere, its success depends on the efficiency of the organism used [2]. Bio fertilizers are generated by multiplying isolated microorganisms on suitable fields and then transporting them

from there. Carry and preserve in proper conditions until employed as root, seed, or soil pollen [7].

The long-living, multifunctional tree crop of Olive is significant not only as a source of table olives and oil, but also as an influence on human health and rural living [19]. and enhances olive plant growth (20), absorption of nutrients [21], physiological parameters [22], Microbial inoculation impacts the plant host via a variety of processes. Using bio fertilizers is a cost-effective way for protecting the plant host from infections, creating growth stimulants, increasing nutrient uptake, and fixing atmospheric nitrogen. It is safe to use because it reduces the need for chemical fertilizers by at least 25%. In addition to its role in reducing environmental pollution problems [1]. It is one of the living organisms that used bio fertilizer *mycorrhiza* fungi, the term mycorrhiza is used to describe the symbiotic association between certain fungi and the roots of numerous plant species in natural environments. It's a symbiotic relationship that isn't entirely successful, the host plant's growth and physiological characteristics, and its resistance to multiple diseases and illnesses are enhanced other properties are improved. many environmental factors increase Such as freezing, drought, salinity, water stress tolerance, and this state of coexistence remains throughout the life of the plant [3]. And the absorption of nutrients such as phosphorus, nitrogen, and potassium [18].

The reason is that fungal hyphae penetrate deeper into the earth than plant roots. Fungi absorb more efficiently than plant roots, and they obtain carbohydrates. Mycorrhizae help improve soil composition. Fungi operate as binding materials, collecting soil particles and enhancing aeration and the circulation of water and nutrients inside it. *Trichoderma* is a parasitic fungus. This fungus was utilized as a bio pesticide through its metabolic secretions, which provide the plant host resistance to some pathogens in the soil. It plays a role in the absorption of nutrients such as nitrogen, phosphorus, and sulfur [9].

Seaweed plant extracts are organic sources used in agricultural production and are harmless to human and animal health and the environment. It leads to improving the physical, chemical and biological properties of the soil, its ability to retain moisture and the activity of microorganisms. It also contains macro and micro nutrients. [10] found in a study they spraying seaweed extract on the leaves of olive trees improves their vegetative characteristics (number of leaves, leaf area, number of branches, length of branches). These organisms participate in types of interactions, including (Micro-plant), which have positive effects on the growth of the plant host and stimulate these organisms together [16,17].

Arbuscular *mycorrhizal* (AM) fungi are prevalent in the soil, and arbuscular *mycorrhizal* (AM) plants often have increased growth compared to non-*mycorrhiza* (NM) plants. This beneficial effect on plant growth is primarily due to high concentrations of nutrients, such as phosphate, zinc, copper and iron. [25]. Aside from the effect of *mycorrhiza* fungi on nutrient uptake, other positive aspects of *mycorrhiza* fungi include increased plant tolerance to drought [23], salt stress [25], as well as resistance to pathogens [24], alleviation of the oxidative stress, and increased antioxidant responses [26]. This study aimed to investigate the impact of inoculation with mycorrhiza and *Trichoderma* fungi. The individual effects of seaweed extract and mycorrhiza fungi, *Trichoderma*, on the growth of slow-growing olive seedlings, the Nibali variety.

2. Material and Methods

A field experiment was conducted at the in the Baquba nursery of the Diyala Agriculture Directorate. 240 of 2 years old seedlings were selected to study to evaluate the effectiveness of biological pollination. fungus VAM *Glomus mosseae* and the fungus *Trichoderma harzianum* the addition seaweed extract and its interference with the growth of olive seedlings was used as a mycorrhiza fungus vaccine (*Glomus mosseae*) Consisting of (spores + infected roots + dry soil).

This inoculum was obtained from the College of Science / University of Baghdad. The inoculum was examined for the presence of fungal spores by wet sieving and filtering only [12]. At a rate of (35 grams per) plastic bag, which was added under the seeds of olive seedlings, the Chinese commercial vaccine was used as a vaccine for the fungus, (*T. harzianum*) At a rate of (5 grams) per bag, noting that (1gram) of of *Trichoderma* challenge pesticide contains (1×10^9) Spore was added to the plastic anvils and under the roots of the olive seedlings (according to the manufacturer's instructions, China). the seaweed extract was added Algex Spanish made, in quantities of (1 to 1.5 kg ha^{-1}) According to the manufacturer's instructions, the soil of the anvils was prepared and the soil was sterilized using a sterilizer (Basamid Grana) The active ingredient in the sterilizer is Dazomet (98 %) (DMTT) It was added at a rate of (50 grams) per square meter in the form of granules, which were scattered and mixed with the soil by stirring, then moistened with water. Plastic anvils (15 cm diameter) were filled with soil Sterilized at a rate of (750 gm) soil per bag and (250 gm) organic fertilizer with anvils. Olive seedlings were planted on sterilized soil and watered with water.

Seaweed extract was added at a rate of (0.6 g) for one anvil adding it with water. A Randomized complete block design was used (R.C.B.D) The experiment contained the (8) coefficients that were produced from the interaction between two factors Fungal inoculation agent, adding seaweed extract, Distribute and repeat each treatment three times. it consisted (24) experimental units and each unit contained 10 plants at 2 years old. Bringing the number of plants to 240. And (0.4) space between two plants, After 30 days, 10 plants from each experimental unit. are randomly collected to study structural features. The SPSS program in (Windows 2000) was used to perform statistical evaluations, with the least significant difference (LSD) at the (0.05) probability level used to assess significant differences between the mean numbers.

Table 1. Shows the distribution of the experimental parameters.

Transactions	Organic fertilization	
Comparison transaction	Microbial inoculation	Microbial inoculation + Algex seaweed extract
0.0 Control	Without Algex seaweed extract	(0.6) Algex Seaweed extract
	T 1	T2
<i>Mycorrhiza</i>	<i>Mycorrhiza</i> (35 g)	Algex Seaweed extract (10 ml) + <i>Mycorrhiza</i> (35g)
	T3	T4
<i>T. harzianum</i>	<i>T. Harzianum</i> (5 g)	Algex seaweed extract (10ml) + <i>T. harzianum</i> (5 g)
	T5	T6
<i>Mycorrhiza</i> + <i>Trichoderma</i>	<i>Mycorrhiza</i> (35g) + <i>T. harzianum</i> (5 g)	Algex seaweed extract (10 ml) + <i>Mycorrhiza</i> (35g) + <i>T. harzianum</i> (5 g)
	T 7	T 8

2.1. Microbiological Estimates

The estimated incidence of mycorrhiza infections is:

Incidence rates were estimated according to the method [13] The process of dyeing the roots was carried out by following the following steps:

- Root hairs were cut into small pieces long (1 cm).
- These hairs were washed from the remains of mud and dirt.
- A solution of potassium hydroxide (10%) KOH was added to it, then it was placed in a water bath at (90°C) for (10-15 minutes), then washed with normal water.
- The color is shortened using a solution of (10%) H₂O₂ for (15-60) seconds

- Hydrochloric acid (1%) HCL was added for 3 minutes.
- Acid fuchsine dye was added for (10-15 minutes) in a water bath at (90°C). The dye Acid fuchsin was prepared as in Table (2).
- Lactic acid was added to the model after extracting it from the dye.

Table 2. Shows the components of acid fuchsine dye.

Acetic acid (ml)	437.5
Glycerol (ml)	31.5
Distilled water (ml)	31.0
Acid fuchsine (g)	0.05

The percentage of infection was estimated by examining 30 pieces of the roots under an optical microscope, and the infection was calculated according to the equation [14].

$$\text{Percentage of roots infected with mycorrhiza} = \left[\frac{\text{(Number of infected pieces)}}{\text{(Total number of pieces)}} \right] \times 100$$

3. Results and Discussion

The results of Table (3) showed The roots of the olive seedlings in the comparison treatment were free of any fungal infection After (45 days) From agriculture (0.0), The reason is the effectiveness of the pesticide Dazomet (98%) In killing harmful and beneficial microorganisms in the soil This is consistent with his findings [6]. The results showed in Table (3) There was no infection with the mycorrhiza fungus *G. mosseae* in treatments inoculated with fungus *T. harzianum* Alone or mixed with seaweed extract This indicates the success of the sterilization process with the pesticide Dazomet. The table shows that the seaweed extract addition treatments individually were free of infection with mycorrhiza fungi.

It is clear from the results of Tables (3) and (4) Inoculation with a fungus *G. mosseae* It led to a moral increase The percentage of infected roots of olive seedlings (45) days of agriculture, at a rate of (%28.80) Compared to the control treatment (0.0), the cause of this is the successful implementation of the vaccination campaign and the effectiveness of the vaccine used to cause injury. This concord with [26]. This was reflected in the vegetative characteristics of olive seedlings, respectively (Plant height 17.4 cm), stem diameter (3.55 mm), area of one leaf (7.12 dm²), dry weight of vegetative shoot (70.18 g), dry weight of root shoot (28.54 g), total chlorophyll (75.90 mg g), The reason is due to the increase in the readiness of nutrients such as phosphorus, nitrogen, potassium, and Rare elements, then their absorption by the plant and the increase in vegetative characteristics, respectively [4].

The results of Tables (3) and (4) showed that the treatments inoculated with fungus *T. harzianum* alone or mixed with seaweed extract significantly increased the vegetative characteristics of olive seedlings, respectively, (Plant height 15.8, 22.6 cm), stem diameter (3.03, 3.93 mm), area of one leaf (6.88, 7.33 dm²), dry weight of vegetative shoot (68.73, 69.16 g), dry weight of root shoot (26.91, 30.78 g), total chlorophyll (71.60, 80.50 mg g) respectively. The reason is due to the increase in the availability of nutrients such as phosphorus, nitrogen, potassium, and Rare elements, then their absorption by the plant and the increase in vegetative characteristics [11].

Table 3. The effect of the two fungi *G. mosseae* and *T. harzianum* and seaweed extract in the percentage of *mycorrhiza* infection of olive seedlings after (45) days of planting.

Transactions	Without seaweed extract	With seaweed extract	Average
Without treatment	0	0	0
<i>Mycorrhiza</i> treatment	27.80	42.20	35
Treatment of <i>Trichoderma</i>	0	0	0
<i>Mycorrhiza</i> + <i>Trichoderma</i> treatment	32.60	51.50	42.05
Average	15.10	23.42	23.72
L.S.D. values at 0.05 probability level	L.S.D FOR FUNGL = 1.5711	L.S.D FOR HUMIC ACID = 0.8879	L.S.D FOR INTERAZTION = 1.7012

Between Tables (3) and (4) the addition of the *Mycorrhiza* fungus increased the percentage of infected seaweed by over 50%. (42.20%). The reason for this is attributed to the nutrient medium that the seaweed extract plays in the growth and activity of microorganisms in the soil, especially fungi. Because it prefers organic and acidic media, It increases the soil's ability to retain water and increases the readiness of nutrients, which then increase their absorption by the plant Inside the plant, as well as increasing the surface area for absorption of nutrients as a result of mushroom hyphae expanding, which was reflected in increasing the vegetative characteristics of olive seedlings, respectively Plant height (25.8 cm), stem diameter (4.38 mm) , area of one leaf (7.70 dm²) , dry weight of vegetative shoot(80.69 g), dry weight of root shoot (38.22 g), total chlorophyll (83.42 mg g) , The cause is the increased readiness of nutrients like phosphorus, nitrogen, potassium, and Rare Elements, then their fixation by the plant and increase in vegetative attributes, respectively [5].

The results of Tables (3) and (4) showed that inoculation with the *G. mosseae* fungus and its interaction with the fungus *T.harzianum* , To an increase in the rate of infection with *mycorrhiza* fungi by a rate of (32.60%) compared to the measurement factors (0.0) Table (3), The reason for this is due to the positive interaction between the *mycorrhiza* and *Trichoderma* fungi, and this is consistent with the results reached by [15]. This increased the vegetative nature of the seedlings' scions. plant height (19.3 cm), stem diameter (3.66 mm), area of one leaf (7.06 dm²) dry weight of the vegetative shoot (71.16 g), dry weight of the root shoot (30.44 g) , total chlorophyll(74.90 mg g) Respectively, the reason is due to the increase in the readiness of nutrients such as phosphorus, nitrogen, potassium, and Rare elements, then their absorption by the plant and the increase in vegetative characteristics, respectively as a result of the absorption of nutrients and the increase in root and shoot growth .

It is noted from Tables (3) and (4) that the interaction of the two fungi *G. mosseae*, *T. harzianum* and the seaweed extract increased the percentage of infection of the roots of Olinda seedlings with *mycorrhiza* fungi, this increased prevalence was significant. (51.50%). Significant increase in the vegetative properties of seedlings of olives. plant height (27.2 cm), stem diameter (5.03 mm), area of one leaf (8.07 dm²), dry weight of the vegetative shoot (88.68 g), dry weight of the root shoot (37.23 g), total chlorophyll (87.30 mg g) respectively, the cause is attributed to the beneficial interaction between the two fungi that increased the area's capacity to process nutrients and absorb them. and the role of the seaweed extract in providing the nutritional medium that stimulated the growth and activity of fungi in the soil, which led to an increase in the growth of the root and shoot system, which reflected positively on the vegetative growth of olive seedling.

Table 4. Shows The effect of bioinoculation with the two fungi *G.mosseae* and *T.harzianum* and the seaweed extract and the interaction between them on the growth of olive seedlings.

Adjectives / Transactions	The increase in plant height is cm	Increase leg diameter mm	The area of one sheet is dm ²	Dry weight of shoots (g)	Dry weight of root mass (g)	chlorophyll Total
Comparison	12.2	2.65	6.02	63.51	23.43	66.96
Seaweed extract	19.7	4.03	8.16	73.96	32.57	77.50
Mycorrhiza fungus	17.4	3.55	7.12	70.18	28.54	75.90
Extract+	25.8	4.38	7.70	80.69	38.22	83.42
Mycorrhiza	15.8	3.03	6.88	68.73	26.91	71.60
Trichoderma	22.6	3.93	7.33	69.16	30.78	80.50
Extract	19.3	3.66	7.06	71.16	30.44	74.90
+Trichoderma	27.2	5.03	8.07	88.68	37.23	87.30
Mycorrhiza +						
Trichoderma						
Average	2. 72	0.49	2.09	4.89	1.76	4.42
coefficients						
L.S.D = 0.05						

Conclusions

The results obtained suggested that the interaction between the fungi *G. mosseae* and *T. harzianum* and the Seaweed extract had an effect on the plants. had a positive effect. was positive and had a significant effect on the growth of slow-growing olive seedlings.

References

- [1] Al-Tamimi, Fares Muhammad Suhail (2000). The role of mycorrhiza fungi *G.mosseae* In the growth of wheat and maize plants. Master's thesis - College of Agriculture - University of Baghdad.
- [2] Al-Haddad, Muhammad Al-Sayyid Mustafa (1988). The role of bio fertilizers in reducing agricultural costs, reducing environmental pollution, and increasing crop productivity. Faculty of Agriculture - Ain Shams University National training course on bio fertilizer production. The Hashemite Kingdom of Jordan 16-5/21/1998.
- [3] Al-Samarrai, Ismail Khalil Salman. Nariman Daoud (2004). The effect of fertilization with phosphate and inoculation with mycorrhiza fungi on the growth and yield of tobacco plants. Iraqi College of Agricultural Sciences - Volume (35) Issue (6).
- [4] Al-Ani, Muhjin Aziz Mustafa (1993). The role of life technology in growing wheat and soybean crops using mycorrhiza fungi. Doctoral thesis - College of Agriculture and Forestry - University of Mosul.
- [5] Al-Salhi, Qais Jamil, Tahani Jawad Muhammad Ali (2016). Response of local orange trees to mycorrhizal fungi and spraying with Putrescine. *Al-Furat Journal of Agricultural Sciences* 8 (1) 7-12.
- [6] Abbas, Hafez Ibrahim (2002). Encouraging the growth of tomato plants, *Lycopersicon esculentum*, by inoculating them with two types of mycorrhiza fungi. *Journal of Agricultural Sciences*, Volume (7), Issue (7), pp. 74-82, June 2002.
- [7] Mahmoud, Saad Ali Zaki, Abdel Wahab Muhammad, and Muhammad Al-Sadiq Muhammad (1988) *Land Microbiology*. Second edition, Cairo - Anglo Library.

- [8] Elad, Y.; David, D.R; Levi, T; Kapat, a ; Kirshner, B ; Curvrin, E. and Levine, A. (1999) *Trichoderma harzianum* T39. mechanisms of biocontrol of foliar pathogens. pages 459- 467.
- [9] Wajahatulla Khan. Vsha.P.RayirathSowmyalakshmi, Subramanian Mundaya, N .Jithesh,Prasanth Rayorath and D. Mark Hodges . 2009. Seaweed Extractsas Biostimulants of plant growth and development. *J. plant growth Regul* 28: 386-399.
- [10] Al-Shammari,M.F.(2013). Effect of Inoculation *Trichoderma* spp and Organic Fertilizer application on citrus Rootstocks growth. M.Sc. Thesis, College of Agriculture, University of Diyala, Iraq .
- [11] Gerdeman, J.W.and T.H. Nicolson, 1963 .Sports of Mycorrhizal end ogoe species extracted from soil by wet sieving and decating . *Transactions of the British Mycological Society*, 46;235.
- [12] Kormanik, P.P,W.C.Bryan , and R.C.Schultz,1980. Procedures and equipment for staining large number of plant root samples for endomycorrhizal assay. *Canadian Journal of Microbiology*, 26: 536- 538.
- [13] Nicolon, T.H.1972. Quoted by AL-zacho, J.M. B.Sc. Biology, 1990. college of science. Mosul University.
- [14] Mc Alliste, C.B, Carcia - Romeral. Godeas, A. and Ocampo, J.A. (1994). Interaction between *Trichoderma Koningii*, *fuarium solani* and *Glomus mosseae*: effect on plant growth arbuscular Mycorrhizas and the saprophytic. *Soil. Biol. Biochemman*, 26; 1363- 1367.
- [15] Al-Samarrai, Ismail Khalil and Al-Tai, Faza Mahmoud (2003a). The effect of the interaction between the type of mycorrhizal inoculum and the method of adding it to tomato plants grown in salted soil. *Journal of Agricultural Sciences – Volume (8), No (2)*.
- [16] Al-Samarrai, Ismail Khalil and Al-Tai, Faza Mahmoud (2003 b). The effect of salinity on the germination components of endophytic mycorrhizal spores. *Journal of Agricultural Sciences – Vol (34). No (4)*.
- [17] Sylvia, D.M; Alogeiy, A.K.; Chellemi, D.O. and Demchendo, 2001. Arbuscular mycorrhizal fungi influence tomato competition with bahia jress *Biology and fertility of soil* 34(6): 443-452.
- [18] Fabbri A, Lambardi M, Ozden-Tokatli Y. 2009. Olive Breeding. In: Jain SM, Priyadarshan PM, eds. *Breeding plantation tree crops: tropical species*. New York: Springer New York, 423_465.
- [19] Aganchich B, Wahbi S, Yaakoubi A, El-Aououad H, Bota J. 2022. Effect of arbuscular mycorrhizal fungi inoculation on growth and physiology performance of olive tree under regulated deficit irrigation and partial rootzone drying. *South African Journal of Botany* 148: 1_10DOI10.1016/j.sajb.03.051.
- [20] Rodrigues MÂ, Piroli LB, Forcelini D, Raimundo S, da Silva Domingues L, Cassol LC, Correia CM, Arrobas M. 2021. Use of commercial mycorrhizal fungi in stressfree growing conditions of potted olive cuttings. *Scientia Horticulturae* 275:109712 DOI10.1016/j.scienta.2020.109712.
- [21] Tekaya M, Mechri B, Mbarki N, Cheheb H, Hammami M, Attia F. 2017. Arbuscular mycorrhizal fungus *Rhizophagus irregularis* influences key physiological parameters of olive trees (*Olea europaea* L.) and mineral nutrient profile. *Photosynthetica* 55(2):308_316 DOI 10.1007/s11099-016-0243-5.
- [22] Ruiz-Sánchez M., Aroca R., Muñoz Y. et al. 2010: The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. – *J. Plant Physiol.* 167: 862-869.
- [23] Wehner J., Antunes P.M., Powell J.R. et al. 2010: Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity? – *Pedobiologia* 53: 197-201.
- [24] Porras-Soriano A., Sorano-Martín M.L., Porras-Piedra A., Azcon P. 2009: Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nurseryconditions. – *J. Plant Physiol.* 166: 1350-1359.
- [25] García-Sánchez M., Palm J.M., Ocampo J.A. et al. 2014: Arbuscular mycorrhizal fungi alleviate oxidative stress induced by ADOR and enhance antioxidant responses of tomato plants. – *J. Plant Physiol.* 171: 421-428.



- [26] Al-Samarrai, Ismail Khalil, Amer Wadih Abdel Karim and Abbas Jassim. 1993. The ability of V.A.M. fungi to increase the growth of corn plants and the amount of phosphorus absorbed from different phosphate fertilizers. Mesopotamia Agriculture Journal. 26 (1): 1-19.

Effect of Mycorrhizae Fungus, Seaweed Extract Spray, Amino Acid Arginine and Their Interaction on Vegetative Growth Characteristics of Sweet Corn

Aamal Abdulwahhab Abdul Kareem Al-Badri¹ and Ridha Mustafa Abdel Hussain Al-Ubaidy²

^{1,2}Department of Horticulture and Landscape, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.

¹E-mail: aamal.abd2305m@coagri.uobaghdad.edu.iq

²E-mail: ridha.mustafa@coagri.uobaghdad.edu.iq

Abstract. The study was conducted at one of the research stations affiliated to the Department of Horticulture and Landscape Engineering / College of Agricultural Engineering Sciences - University of Baghdad - Al-Jadriya for the spring season of 2024, to study the effect of mycorrhiza fungus and spraying organic nutrients and the amino acid arginine and their interaction on the vegetative growth characteristics of sweet corn. The experimental factors included studying three factors, the first factor was inoculating sweet corn seeds with mycorrhiza fungus, symbolized by M, and it was at two levels (M0 and M1), and the second factor was spraying with organic nutrients, symbolized by S, and it was at three concentrations (0, 1 and 2 ml L⁻¹), while the third factor represented the amino acid arginine, symbolized by G, and it was sprayed at three concentrations (0, 125 and 250 mg L⁻¹). A factorial experiment was conducted according to the RCBD design with a split-plot arrangement, and the results were analyzed according to Gen stat programs to calculate the least significant difference L.S.D at a probability level of 5%. The results of the experiment showed that The triple interaction between mycorrhiza fungi, seaweed extract and arginine acid at concentrations (M1 + S2 + G2) showed significant superiority in the studied traits represented by plant height 168.00 cm plant⁻¹, total number of leaves 15.00 leaves plant⁻¹, leaf area 606.0 cm² plant⁻¹, chlorophyll estimation in leaves 1078.3 mg g⁻¹ fresh weight, and dry weight of the vegetative group 105.33.

Keywords. Seaweed Extract Spray, Corn, Amino Acid.

1. Introduction

Corn is the third most important crop in the world after wheat and rice in terms of cultivated area and global production. Sweet corn (*Zea mays* L. var *saccharata*) has pale yellow kernels, sweet taste, soft texture, high moisture content, and high levels of sugar [1]. It is a source of carbohydrates, protein, vitamins, minerals, phenolic acids, carotenoids, and fiber. Regular consumption of sweet corn can reduce the severity of chronic diseases such as cardiovascular diseases, eye diseases, obesity, diabetes, and gastrointestinal diseases [2]. The role of bio fertilizers, including mycorrhiza fungi, plays an important role in improving soil fertility and increasing its availability of nutrients. Its importance is focused on its ability to fix symbiotic and

non-symbiotic atmospheric nitrogen in the soil through nitrogen-fixing microorganisms. The process of inoculation with mycorrhiza fungi highlights its importance in encouraging root growth in the early stages of plant growth, and increases the surface area of the roots. It also has the ability to dissolve unavailable phosphorus, such as phosphate rock phosphorus, which plants do not benefit from, into a ready-made formula that the plant can easily absorb [3]. [4] showed the use of mycorrhiza fungi on okra, Batira variety. Amino acids improve vegetative and chemical traits in plants such as bitter melon *Momordica charantia* L. [5]. The amino acid arginine is one of the most adaptable acids and is associated with the creation of important signal molecules to resist stress [6]. Spraying with arginine acid affects the increase in leaf area, number of leaves, dry weight of vegetative and root growth, and estimation of chlorophyll content in leaves and carbohydrates, in addition to its role as a plant growth stimulant and a rich source of nitrogen, which is important for the synthesis of important enzymes in physiological processes and proteins, especially growth, expansion, development and division processes in plant cells; because arginine acid is directly absorbed through the stomata to the outer guard cells [7]. Foliar spraying has an important role in improving plant growth and increasing its productivity by supplying it with its needs of various nutrients that contribute to many diverse physiological activities in plant [8]. As well as improving growth characteristics such as plant height, stem diameter, leaf area, and biological yield, as in the sunflower plant [9]. Therefore, it is necessary to provide foliar nutrients to help the plant regain growth and increase its early production, because plants are exposed to stress conditions, especially during the first period of growth, which causes damage to their roots, or due to heavy rains [10]. It has a significant and positive effect on growth indicators, yield and quantitative characteristics, as in spinach [11]. And in peas [12]. Due to the lack of studies on sweet corn, as it is an uncommon crop in Iraq, the study aimed to know the effect of the interaction of inoculation with mycorrhiza fungi and to determine the best concentration of organic nutrients and arginine acid on the vegetative growth characteristics of sweet corn.

2. Materials and Methods

The study was carried out in one of the research stations affiliated to the Department of Horticulture and Landscape Engineering / College of Agricultural Engineering Sciences - University of Baghdad - Al-Jadriya for the spring season of 2024, to study the effect of the interaction between the mycorrhiza fungus and spraying the seaweed extract and arginine acid on the vegetative growth characteristics of sweet corn. A factorial experiment was designed according to the RCBD design with a split-plot arrangement where the mycorrhiza fungi are within the main panels (the least important factor) and spraying the seaweed extract and arginine acids within the secondary panels (the most important) and with three factors. The first factor included inoculating sweet corn seeds with the mycorrhiza fungus vaccine at two levels and symbolized by (M0) and (M1) and the second factor was spraying the organic nutrient (seaweed extract and symbolized by S) and at three concentrations (0, 1, 2 ml. L⁻¹) and symbolized by S0, S1, (S2) and the factor The third spraying with arginine acid at three concentrations (0, 125, 250 mg. L⁻¹) and its symbol is G (G0, G1, (G2). The treatments were distributed into three replicates randomly, so we had 18 treatments (3 × 3 × 2). Sweet corn seeds were planted in the spring season in mid-March, and all soil service operations were carried out. The drip irrigation system was used, and the plants were fertilized with an amount of 25% of the recommended amount of chemical fertilization (Al-Bayati, 2023). Sweet corn seeds were inoculated with mycorrhiza fungi and 10 g was added at a pollination density of 50 spores.

3. Results and Discussion

3.1. Plant Height (cm plant^{-1})

Table (1) shows the spraying treatment for the triple interaction between all treatments, as all spraying treatments for the triple interaction took the highest values, reaching 136.67, 137.00, 138.67, 140.33, 143.67, 145.00, 145.33, 146.67, 147.00, 148.00, 148.67, 149.67, 150.67, 152.00, 154.00 and 168.00 cm plant^{-1} compared to the two triple interaction spray treatments (M0S0G0) and (M0S0G1) which recorded the lowest values of 134.00 and 135.33 cm plant^{-1} respectively.

Table 1. Effect of the response of mycorrhiza fungus and spraying with seaweed extract and arginine acid and their interaction on plant height (cm plant^{-1}) in sweet corn leaves for the spring season 2024 AD.

Mycorrhiza fungi (M)	Algae extract (S)	Arginine acid(G)			M*S	
		G ₀	G ₁	G ₂		
M ₀	S ₀	134.00	135.33	136.67	135.33	
	S ₁	137.00	138.67	140.33	138.67	
	S ₂	143.67	145.00	145.33	144.67	
M ₁	S ₀	146.67	147.00	148.00	147.22	
	S ₁	148.67	149.67	150.67	149.67	
	S ₂	152.00	154.00	168.00	158.00	
M*G	Average (M)					
	M0	138.22	139.67	140.78	139.56	
	M1	149.11	150.22	155.56	151.63	
S*G	Average (S)					
	S0	140.33	141.17	142.33	141.28	
	S1	142.83	144.17	145.50	144.17	
	S2	147.83	149.50	156.67	151.33	
Average (G)		143.67	144.94	148.17		
L.S.D 0.05						
M*S*G	S*G	M*G	M*S	G	S	M
1.572	1.112	0.908	0.908	0.642	0.642	0.524

3.2. Leaf Area ($\text{cm}^2 \text{ plant}^{-1}$)

Table (2) shows the spray treatment for the triple interaction between all treatments, as all spray treatments for the triple interaction took the highest values reaching 353.0, 374.0, 393.0, 397.9, 401.1, 407.2, 413.8, 429.5, 437.5, 447.1, 484.0, 525.0 and 606.0 $\text{cm}^2 \text{ plant}^{-1}$ compared to the spray treatments for the triple interaction (M0S0G0) and (M0S0G1) which recorded the lowest values reaching 306.9 and 322.3 $\text{cm}^2 \text{ plant}^{-1}$ respectively.

Table 2. Effect of the response of mycorrhiza fungus and spraying with seaweed extract and arginine acid and the interaction between them on the leaf area ($\text{cm}^2 \text{ plant}^{-1}$) in the leaves of sweet corn plants for the spring season 2024 AD.

Mycorrhiza fungi (M)	Algae extract (S)	Arginine acid(G)			M*S
		G ₀	G ₁	G ₂	
M ₀	S ₀	306.9	322.3	353.0	327.4
	S ₁	374.0	393.0	397.9	388.3
	S ₂	401.1	407.2	413.8	407.4
M ₁	S ₀	429.5	437.5	447.1	438.0
	S ₁	456.7	467.0	471.4	465.0
	S ₂	484.0	525.0	606.0	538.3
Average(M)					

M*G	M0	360.7	374.2	388.2	374.4	
	M1	456.7	476.5	508.2	480.5	
	Average(S)					
S*G	S0	368.2	379.9	400.1	382.7	
	S1	415.4	430.0	434.7	426.7	
	S2	442.6	466.1	509.9	472.9	
Average (G)		408.7	425.3	448.2		
L.S.D 0.05						
M*S*G	S*G	M*G	M*S	G	S	M
21.64	15.30	12.49	12.49	8.83	8.83	7.21

3.3. Estimation of Chlorophyll (mg g^{-1} fresh weight)

Table (3) shows the spray treatment for the triple interaction between all treatments, all spray treatments for the triple interaction took the highest values reaching 1078.3 mg g^{-1} fresh weight compared to the spray treatments for the triple interaction (M0S0G0) and (M0S0G1) which recorded the lowest values reaching 402.3 and 413.3 mg g^{-1} fresh weight respectively.

Table 3. Effect of the response of mycorrhiza fungus and spraying with seaweed extract and arginine acid and the interaction between them in estimating the percentage of chlorophyll pigment (mg g^{-1} fresh weight) in the leaves of sweet corn plants for the spring season 2024 AD.

Mycorrhiza fungi (M)	Algae extract (S)	Arginine acid(G)			M*S	
		G ₀	G ₁	G ₂		
M ₀	S ₀	402.3	413.3	423.3	413.0	
	S ₁	437.3	452.7	494.0	461.3	
	S ₂	544.7	576.3	601.0	574.0	
M ₁	S ₀	624.3	656.3	705.8	662.2	
	S ₁	724.7	746.3	820.3	763.8	
	S ₂	914.0	1005.3	1078.3	999.2	
Average(M)						
M*G	M0	461.4	480.8	506.1	482.8	
	M1	754.3	802.7	868.2	808.4	
Average(S)						
S*G	S0	513.3	534.8	564.6	537.6	
	S1	581.0	599.5	657.2	612.6	
	S2	729.3	790.8	839.7	786.6	
Average (G)		607.9	641.7	687.1		
L.S.D 0.05						
M*S*G	S*G	M*G	M*S	G	S	M
19.40	13.72	11.20	11.20	7.92	7.92	6.47

3.4. Total Number of Leaves

Table (4) shows the spraying treatment for the triple overlap between all treatments. All spraying treatments for the triple overlap took the highest values, reaching 10.00, 10.00, 10.66, 11.00, 11.00, 11.00, 12.00, 12.33, 13.00, 13.00, 13.00, 13.33, 14.00, 14.00, 14.00 and $15.00 \text{ plant leaves}^{-1}$, respectively, compared to the comparison treatment for the triple overlap (M0S0G0), which recorded the lowest value, reaching $9.00 \text{ plant leaves}^{-1}$.

Table 4. Effect of the response of mycorrhiza fungi and spraying with seaweed extract and arginine acid and the interaction between them on the total number of leaves of sweet corn plants for the spring season of 2024 AD.

Mycorrhiza fungi (M)	Algae extract (S)	G Arginine acid(G)			M*S	
		G ₀	G ₁	G ₂		
M ₀	S ₀	9.000	10.000	10.000	14.000	
	S ₁	10.667	11.000	11.000	14.000	
	S ₂	11.000	12.000	12.333	15.000	
M ₁	S ₀	13.000	13.000	13.000	13.000	
	S ₁	13.000	13.333	14.000	13.444	
	S ₂	14.000	14.000	15.000	14.333	
Average(M)						
M*G	M0	10.222	11.000	11.111	10.778	
	M1	13.333	13.444	14.000	13.593	
Average(S)						
S*G	S0	11.000	11.500	11.500	11.333	
	S1	11.833	12.167	12.500	12.167	
	S2	12.500	13.000	13.667	13.056	
Average (G)		11.778	12.222	12.556		
L.S.D 0.05						
M*S*G	S*G	M*G	M*S	G	S	M
0.3755	0.2655	0.2168	0.2168	0.1533	0.1533	0.1252

3.5. Dry Weight of the Vegetative Group (g)

Table (5) shows the spraying treatment for the triple interaction between all treatments, as the spraying treatment for the triple interaction took (the highest values and the most prominent of them were the spraying treatments for the interaction (M1S2G1) and (M1S2G2) which reached 97.33 and 105.33 g respectively compared to the comparison treatment (M0S0G0) which recorded the lowest value of 63.33 g.

Table 5. The effect of the response of the mycorrhiza fungus and spraying with seaweed extract and arginine acid and the interaction between them on the dry weight of the vegetative group (g) of sweet corn plants for the spring season 2024 AD.

Mycorrhiza fungi (M)	Algae extract (S)	Arginine acid (G)			M*S
		G ₀	G ₁	G ₂	
M ₀	S ₀	63.33	65.33	67.33	65.33
	S ₁	69.00	69.67	70.00	69.56
	S ₂	71.33	72.33	73.00	72.22
M ₁	S ₀	74.00	75.00	78.33	75.78
	S ₁	81.67	87.33	88.67	85.89
	S ₂	92.33	97.33	105.33	98.33
Average(M)					
M*G	M0	67.89	69.11	70.11	69.04
	M1	82.67	86.56	90.78	86.67
Average(S)					
S*G	S0	68.67	70.17	72.83	70.56
	S1	75.33	78.50	79.33	77.72
	S2	81.83	84.83	89.17	85.28
Average (G)		75.28	77.83	80.44	
L.S.D 0.05					

M*S*G	S*G	M*G	M*S	G	S	M
1.529	1.081	0.883	0.883	0.624	0.624	0.510

The results of Tables (1, 2, 3, 4, and 5) show significant differences between the spray treatments, and the reason may be attributed to the mycorrhiza fungus, as it improves soil properties, contributes to carbon recycling, and enhances the plant's ability to withstand biotic and abiotic challenges [13]. Recent studies have shown that mycorrhiza fungus hyphae increase the thickness of plant cell walls by increasing the lignin substance, and they also increase phenolic compounds and reduce the risk of water stress, thus leading to an increase in the chlorophyll content of leaves, an increase in the rate of photosynthesis and transpiration, and a reduction in the concentration of carbon dioxide between plant cells [14,15]. It was also found that there are some other compounds secreted by the mycorrhiza fungus that have a major and important role, as they work to improve crop growth such as plant height and leaf number, which results in an increase in leaf area and dry matter weight compared to the treatment not inoculated with the fungus [16]. Or the reason may be due to amino acids that play an important role in tolerating different conditions such as salinity, drought and high temperature, including arginine, in addition to its role in cell division and elongation, which results in plant height and increased chlorophyll content in the leaves [17]. The reason may be attributed to the amino acid arginine, as recent studies have shown that spraying with arginine affects the increase in leaf area, number of leaves, and estimation of chlorophyll content in leaves, in addition to its role as a plant growth stimulant and a rich source of nitrogen, which is important for the synthesis of enzymes important in physiological processes and proteins, especially growth, expansion, development, and division processes in plant cells because arginine acid is directly absorbed through the stomata to the outer guard cells [18]. The reason for the response of sweet corn to spraying with seaweed extract may be due to its contribution to increasing the vital activities of the plant and the absorption of nutrients. Seaweed also has a clear effect that increases the height of the plant through its effect on the cell protoplasm and cell wall, which enhances cell division and elongation, which results in the height of the plant and the activation of leaf tissues to divide and expand, thus increasing the leaf area, which results in a greater number of leaves [19,20].

Conclusions

We conclude that inoculation with the biofertilizer Mycorrhizae A, spraying with the amino acid arginine at a concentration of 250 mg L⁻¹, and spraying with seaweed extract at a concentration of 2 ml L⁻¹ led to a highly significant improvement in the vegetative growth characteristics of sweet corn plants.

References

- [1] Xiao, Y., Xie, L., Li, Y., Li, C., Yu, Y., Hu, J., & Li, G. (2024). Impact of low temperature on the chemical profile of sweet corn kernels during postharvest storage. *Food Chemistry*, 431, 137079. <https://doi.org/10.1016/j.foodchem.2023.137079>
- [2] Sheng, S., T. Li, and R. Liu. 2018. Corn phytochemicals and their health benefits. *Food Sci. Hum. Wellness* 7(3): 185- 195. doi: 10.1016/j.fshw.2018.09.003.
- [3] Sangeetha, J., Al-Tawaha, A. R. M., and Thangadurai, D. (Eds.). (2024). *Mycorrhiza Technology: Managing Plant Stress and Mitigating Climate Change Using Mycorrhizae*. CRC Press
- [4] Al-Samarai, M. M. A., Al-Ubaidy, R. M. A., & Al-Umrani, H. H. A. (2020). Response of *Abelmoschus esculentus* L. For inoculation with Mycorrhizae and Foliar Application with Bio stimulators and effect on Vegetative Growth Characters. *The Iraqi Journal of Agricultural Science*, 51 (Special Issue): 61-71.

- [5] Al-asadi, R. A. A. and Al-jebory, K. D. (2020). Effect of Spraying Amino Acids on Growth and Yield of Bitter Gourd Plant Genotypes *Momordica Charantia* L. and its Charantin Content. *Iraqi Journal of Agricultural Sciences*, 51: (4):991-1000.
- [6] Jin, X. (2022). Regulatory network of serine/arginine-rich (SR) proteins: the molecular mechanism and physiological function in plants. *International Journal of Molecular Sciences*, 23(17):1-13. <https://doi.org/10.3390/ijms231710147>.
- [7] Al-Ealayawi, Z. A. and A. F. Z. Al-Dulaimy (2023) The Impact of Spraying Marine Algae Extract, Arginine, and Brassinolide on Vegetative Growth Traits of Albion Strawberry Saplings. *IOP Conf. Series: Earth and Environmental Science* 1262 (042061): 1-13. doi:10.1088/1755-1315/1262/4/042061.
- [8] Al-Bayati ,WaSan Salih Mahdi. (2023) Efficiency of bacterial bio fertilization and spraying with magnesium and calcium on growth, yield and marketability of two sweet corn hybrids. PhD thesis, College of Agricultural Engineering Sciences. University of Baghdad. Republic of Iraq.
- [9] Al-Saedi, Maryam Ibrahim and Intisar Hadi Al-Halfi (2024). The effect of some algae and plant extracts on the growth and biological yield of sunflower. *Iraqi Journal of Market Research and Consumer Protection*, 16 (1): 111-122.
- [10] Al-Mashhadany, A. H., & Al-Mharib, M. Z. K. (2023). Effect of fertilizers starter solutions on growth and production of broccoli (*Brassica oleracea* var. *italica*). *Research on Crops*, 24(1): 119-122.
- [11] Al-Mharib, M.Z.K., Al-Ubaidy, R.M., Mohammed, M.M. (2022). Effect of spraying with folic acid and yeast extract on the growth, yield and calcium oxalate concentration of spinach (*Spinacia oleracea* L.). *Applied Ecology and Environmental Research*, 20(3): 2763-2768.
- [12] Mohammed, M. M., & Al-Ubaidy, R. M. (2020). Influence of yeast and intercropping system on growth and yield traits of pea. *International Journal of Agricultural and Statistical Sciences*, 16 (1): 1577-1580.
- [13] Khaliq, A., Perveen, S., Alamer, K. H., Zia, U. I. Haq, M., Rafique, Z., Alsudays, I. M., Althobaiti, A. T., Saleh, M. A., Hussain, S., and Attia, H. (2022) Arbuscular Mycorrhiza Fungi Symbiosis to Enhance Plant–Soil Interaction. *Sustainability*. 14 (13): 7840. <https://doi.org/10.3390/su14137840>
- [14] Sun, J., Yang, L., Yang, X., Wei, J., Li, L., Guo, E., and Kong, Y. (2021). Using spectral reflectance to estimate the leaf chlorophyll content of maize inoculated with arbuscular mycorrhiza fungi under water stress. *Frontiers in Plant Science*, 12, 646173.
- [15] Xie, W., Hao, Z., Zhou, J., Fu, W., Guo, L., Zhang, X., and Chen, B. (2023). Integrated transcriptomics and metabolomics reveal specific phenolic and flavonoid accumulation in licorice (*Glycyrrhiza uralensis* Fisch.) induced by arbuscular mycorrhiza symbiosis under drought stress. *Plant Physiology and Biochemistry*, 205, 108173.
- [16] Yang, L., Zou, Y. N., Tian, Z. H., Wu, Q. S., and Kuća, K. (2021). Effects of beneficial endophytic fungal inoculants on plant growth and nutrient absorption of trifoliate orange seedlings. *Scientia Horticulture*, 277, 109815.
- [17] Al-Hashimi, M. G., & Zeboon, N. H. (2023). The Response Of Some Growth Characters For Two Varieties Of Maize To Spraying With Vitamin B9 And E, And The Amino Acid Arginine. *Euphrates Journal of Agricultural Science*, 15(1): 325-325
- [18] Al-bayati, W. S. M., & Al-Juboori, A. W. (2023). Response Of Sweet Corn To Bacterial Bio fertilizers And Spraying With Mineral Nutrients: Response Of Sweet Corn To Bacterial Bio fertilizers And Spraying With Mineral Nutrients. *Iraqi Journal of Market Research and Consumer Protection*, 15(2): 162-172.
- [19] Bashir MA, Rehim A, Raza HMA, Zhai L, Liu H, Wang H (2021). Bio stimulants as plant growth stimulators in modernized agriculture and environmental sustainability. *Tech. Agric.* 11(3):27-45.
- [20] Abbas A (2017). Biochemical composition of some Syrian marine algal species of economic and medical importance. *Tishreen Univ. J. Biol. Sci. Ser.* 39(3): 22-41.

The Impact of Foliar Nutrition and Plant Waste on Yield Characteristics of Three Potato Cultivars (*Solanum tuberosum* L.)

Heba Ali Hussein¹, Haider Talib Hussein² and Ali Hassan Ali³

¹⁻³Department of Plant Production, Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University, Iraq.

¹E-mail: com.hba88@atu.edu.iq

²E-mail: com.had22@atu.edu.iq

³E-mail: com.ali22@atu.edu.iq

Abstract. Foliar nutrition and plant waste were tested on yield features and components of three potato cultivars in Al-Azzawiyah, Babylon Governorate, in the spring and autumn seasons of 2023–2024. The experiment included three factors: Synergy, Burren, and El-Mundo cultivars in the main plots. The sub-plots had four levels of plant waste (rice waste compost), while the sub-sub plots included three concentrations of foliar spray of the nutrient solution microponic. The experiment used split-split plots using a randomized complete block design (R.C.B.D) with three replicates. Burren cultivar outperformed Synergy and El-Mundo cultivars in the average number of marketable tubers (4.44, 6.80) tuber plant⁻¹, the weight of marketable tubers (113.55, 123.84) g, the yield of one marketable plant (505.2, 842.1) g plant⁻¹ and the marketable yield (26.94, 45.03) tons ha⁻¹ for both seasons. The treatment of adding rice waste compost + 50% NPK fertilizer had the highest rate number of marketable tubers (4.39, 6.97) tuber plant⁻¹, the weight of marketable tubers (112.70, 121.68) g, the yield of one marketable plant (495.0, 848.4) g plant⁻¹ and the marketable yield (26.40, 45.26) tons ha⁻¹ compared to control in both seasons. The foliar spray treatment with microponic nutrient solution at 200 ml 100 L⁻¹ water gave the highest average in the number marketable tubers (4.30, 6.84) tuber plant⁻¹, the weight of marketable tubers (111.43, 120.71) g, the yield of one marketable plant (480.8, 826.5) g plant⁻¹ and the marketable yield (25.64, 44.09) tons ha⁻¹ compared to control treatment and both seasons. All bi-interaction treatments outperformed control treatments in average yield attributes for both seasons. The triple interaction treatment between the Burren cultivar, rice waste compost + 50% NPK fertilizer and foliar spraying at 200 ml 100 L⁻¹ of water increased the average number of marketable tubers (5.23, 7.30) tuber plant⁻¹, the weight of marketable tubers (117.00, 129.96) g, the yield of one marketable plant (611.9, 948.7) g plant⁻¹, and the marketable yield (32.64, 50.68) tons ha⁻¹ compared to control treatment and for both seasons respectively.

Keywords. Potato, Cultivars, Foliar spraying, Yield.

1. Introduction

Potato *Solanum tuberosum* L. are among the most important vegetable crops in the world and belong to the Solanaceae family, which includes more than 2000 species and 90 genus , They rank fourth in the world in terms of cultivation, production and economic importance after wheat, corn and rice [1-3]. Potato contain proteins, vitamins, fibers, fats, energy, carbohydrates and minerals such as P, K, Fe, Ca, Mg and ascorbic acid. They are also rich in amino acids, as they contain 18 amino acids out of 20, especially lysine, which is lacking in cereal crops, giving them good nutritional value [4]. The area allocated for potato cultivation in Iraq in 2023 amounted to about (35.9) thousand dunums, and its total production amounted to (229.1) thousand tons, with a yield of 6380.8 kg dunum⁻¹ [5]. Among the methods adopted to increase production and improve the quality of potato is relying on cultivars with good specifications, high production, and suitability for environmental conditions [6]. The lack of interest in selecting high-quality genetic compositions and poor storage in warehouses that lack many of the specifications of ideal storage, in addition to the prevailing weather conditions during growth, are among the reasons that stand as an obstacle to the expansion of potato cultivation [7]. The increase in the rates of adding chemical fertilizers when growing vegetable crops compared to other crops, due to the possibility of growing them in more than one season per year, which leads to the exacerbation and increase of harmful effects on the environment, especially the residual effect of nitrates and oxalates, which are among the most dangerous compounds to human health, in addition to groundwater pollution and the decrease in organic matter in the soil and the decrease in the activity of beneficial microorganisms in it, causing a decrease in soil fertility [8,9]. Recently, there has been an adoption of a fertilization system that combines chemical and organic fertilization with the aim of determining the amount that these natural fertilizers compensate for chemical fertilizers and maintaining the sustainability of the balanced supply capacity of the soil and ensuring the sustainability of crop production [10,11]. The organic farming system is a low-cost and environmentally friendly production system in order to improve human and soil health, improve the environment and increase sustainable agriculture, as organic farming improves soil quality due to the use of organic fertilizer compared to the unbalanced and distinctive use of chemical agricultural inputs in terms of productivity [12,13]. Foliar nutrition is the process of supplying plants with nutrients by spraying them on the vegetative system, which has the ability to absorb them through the stomata in the leaves, stems, cell walls and membranes. The scientific basis for nutrient absorption is similar in principle to the process of nutrient absorption by the roots. The main step in this process is the transfer through the biomembranes of the cells based on the water potential difference or diffusion pressure between the spray solution and the leaf cells, It is considered foliar spray one of the most effective means of maintaining plant growth and productivity for a long time. Despite the occurrence of periods of stress and critical stages, foliar fertilization ensures the rapid correction of plant nutritional disorders, and eliminating manifestations of element deficiency, such as yellowing of leaves, spotting, and weak nodes [14-16]. All plants also need micronutrients in relatively low quantities compared to the main nutrients, so the deficiency of one or more of them will be a determining factor in the production and quality of that crop [17].

The study aimed to know the effect of foliar nutrition and the addition of plant waste on yield traits and its components for three cultivars of potato, also to determine the best cultivar, the best level of plant waste, and the best concentration of the nutrient solution , also to determine the best interactions between the factors of study.

2. Materials and Methods

The experiment was conducted in one of the agricultural fields in the Al-Azzawiyah region, about 40 km north of the center of Babylon governorate, located between longitude (44) and latitude (32) with the aim to know the role of foliar nutrition and plant waste on the yield characteristics of three cultivars of potato. in the spring season which started from 28/1 to 10/5/2023 and the autumn season from 9/15/2023 to 18/1/2024. The land was prepared for cultivation for both seasons by tillage it, then smoothing it with disc harrows, leveling it, and dividing it into three sectors. Each sector contains thirty-six experimental units for each, leaving a distance of 1 m between the experimental units and between one sector and another to prevent the transfer of fertilizer between treatments. Where cultivation was carried out on a row and on one side of the row an average of 3 rows for each experimental unit and the distance between one row and another is 75 cm, and the number of tubers for each row is 8 tubers on one side of the row at a distance of 25 cm between one tuber and another , where the number of tubers for one experimental unit is 24 tubers and total number of plants in the experiment 2592 plant ,at a rate of 108 experimental units with an area of 4.5 m².

Random samples were taken from the field soil before planting at a depth of 0-30 cm and from different locations. The samples were mixed and left to air dry, then ground and some of their physical and chemical properties were estimated in Table (1).

Table 1. Some of the physical and chemical properties of the field soil.

Chemical and physical properties	Spring season	Autumn season
pH	7.8	7.9
Ece (ds m ⁻¹)	2.8	3.1
Organic matter %	1.08	1.15
Nitrogen (mg kg ⁻¹)	19.7	23.5
Phosphorus (mg kg ⁻¹)	8.1	7.4
Potassium (mg kg ⁻¹)	158.9	162.7
CEC(centimoles kg ⁻¹ soil)	19.07	19.28
Bulk density(g cm ⁻³)	1.32	1.34
Sand %	59.5	61.0
Silt %	25.0	24.5
Clay %	15.5	14.5
Texture	Sandy loam	Sandy loam

The experiment was applied using the split-split plot system according to the randomized complete block design (R.C.B.D) with three replicates. The experiment included three factors:

The first factor includes cultivars and represents the main plots:

A1: Synergy

A2: Burren

A3: El-Mundo

The second factor is the addition of plant waste (rice waste compost) at four levels and represents the sub-plots, Table (2) and is as follows:

B1: Comparison NPK 15:15:15 fertilizer produced by the Russian company Ekim (according to the fertilizer recommendation 600 kg h⁻¹). [18]

B2: Adding rice waste compost (20 tons ha⁻¹).

B3: Adding rice waste compost + 25% NPK fertilizer.

B4: Adding rice waste compost + 50% NPK fertilizer.

Table 2. The physical and chemical properties of organic fertilizer.

Traits	pH	Ece dsm ⁻¹	Nitrogen %	Phosphorus %	Potassium %	Organic carbon %	O.M %	Humic acid%	Fulvic acid%
Values	7.43	3.04	1.12	1.01	0.61	44.5	56.2	14.2	7.1

The third factor is foliar spraying with nutrient solution (microponic containing microelements) produced by the Spanish company Artal and represents sub-sub plots with three concentrations Table (3) and includes:

C0: Spraying with distilled water only.

C1: Spraying with a concentration of 100 ml 100 L⁻¹ water.

C2: Spraying with a concentration of 200 ml 100 L⁻¹ water.

Table 3. The chemical properties of the nutrient solution microponic.

%Zn	%Mo	Mn%	%Fe	Cu%	B%
4.17	0.07	3.48	4.87	0.56	0.49

The decomposed organic fertilizer (rice waste compost) was used, which was brought from the organic fertilizer production project of the Najaf Agriculture Directorate, and was added to the experimental units during the preparation of the land for cultivation according to the procedures and mixed with the soil using a scratching machine. The microponic nutrient solution was sprayed 45 days after cultivation at a rate of three sprays, and the period between one spray and another was 15 days, with the addition of a spreading material (Al-Zahi) in an amount of 1 ml L⁻¹, The spraying process was conducted in the early morning.

2.1. Studied Traits

2.1.1. Quantitative Traits of Yield

- Number of marketable tubers (tuber plant⁻¹): It was calculated for five plants taken randomly from each experimental unit and the average was extracted after excluding damaged and deformed tubers whose diameter is less than 2.5 cm.
- Weight of marketable tubers (g): It was calculated by measuring the weight of tubers for the previous five plants and dividing by the number of tubers for the same plants for each experimental unit.
- The yield of one marketable plant (g plant⁻¹): It was calculated by multiplying the average number of tubers for the previous five plants × the average weight of the tuber for each experimental unit.
- The marketable yield (tons ha⁻¹): Calculated on the basis of the yield of one marketable plant × plant density (53333 plant ha⁻¹).

The results were analyzed according to the design followed, and the least significant difference test (L.S.D) was used to compare the averages at the probability level of 0.05 [19] and the Genstat program was used in the statistical analysis.

3. Results and Discussion

3.1. Number of Marketable Tubers (tuber plant⁻¹)

Table (4) shows that foliar spraying and adding plant waste of three potato cultivars and their interactions had a significant effect on the number of marketable tubers for both seasons. The Burren cultivar (A2) was significantly excelled in the number of marketable tubers in both

seasons, as it gave the highest average of (4.44, 6.80) tuber plant⁻¹, followed by El-Mundo cultivar (A3) which gave (3.97, 6.64) tuber plant⁻¹ while the Synergy cultivar (A1) gave the lowest average of (3.88, 6.55) tuber plant⁻¹ for both seasons respectively. The same table also indicates that adding plant waste has a significant effect on the number of marketable tubers, as the treatment of adding rice waste compost + 50% NPK (B4) fertilizer was significantly excelled, reaching (4.39, 6.97) tuber plant⁻¹ compared to control treatment (B1) which gave the lowest average of (3.83, 6.23) tuber plant⁻¹ for both seasons respectively. It is also noted from the same table that foliar spraying with the nutrient solution microponic at a concentration of 200 ml 100 L⁻¹ of water (C2) was significantly excelled, as it reached (4.30, 6.84) tuber plant⁻¹, while control treatment (C0) gave the lowest average, reaching (3.90, 6.51) tuber plant⁻¹, for both seasons respectively. The table also shows that bi-interactions between the experimental factors had a significant effect on this trait, as the interaction between the cultivars and plant waste (rice waste compost) gave a significantly excelled. The interaction between the Burren cultivar and the treatment of adding rice waste compost + 50% NPK fertilizer (A2B4) was excelled and gave the highest average of (4.77, 7.09) tuber plant⁻¹, compared to control treatment with the Synergy cultivar (A1B1), which gave the lowest average of (3.61, 6.12) tuber plant⁻¹ for both seasons respectively. The interaction between the cultivars and foliar spraying had a significant effect, as the interaction of the Burren cultivar and foliar spraying at a concentration of 200 ml 100 L⁻¹ of water (A2C2) was excelled and reached (4.77, 6.97) tuber plant⁻¹, compared to control treatment with the Synergy cultivar (A1C0), which gave the lowest average of (3.76, 6.42) tuber plant⁻¹, for both seasons.

The interaction between plant waste and foliar spraying showed a significant effect, as the interaction of the treatment of adding rice waste compost + 50% NPK fertilizer and foliar spraying at a concentration of 200 ml 100 L⁻¹ of water (B4C2) was excelled and gave the highest average of (4.61, 7.14) tuber plant⁻¹, compared to control treatment (B1C0), which recorded the lowest average of (3.63, 6.04) tuber plant⁻¹ for both seasons. The interaction between the three factors had a significant effect, where the interaction between the Burren cultivar and the addition of rice waste compost + 50% NPK fertilizer and foliar spraying at a concentration of 200 ml 100 L⁻¹ of water (A2B4C2) was excelled and gave the highest average of (5.23, 7.30) tuber plant⁻¹ compared to control treatment of the Synergy cultivar (A1B1C0) which gave the lowest average of (3.50, 5.87) tuber plant⁻¹ for both seasons respectively.

Table 4. The effect of foliar spraying and the addition of plant waste of three potato cultivars and their interactions on the number of marketable tubers (tuber plant⁻¹) during the spring and autumn seasons 2023-2024.

Plant waste B	Sprayin g C	Spring season				Autumn season			
		Cultivars			Plant waste × Sprayin g	Cultivars			Plant waste × Sprayin g
		A 1	A 2	A 3 El-		A 1	A 2	A 3 El-	
		Synerg y	Burre n	Mund o		Synerg y	Burre n	Mund o	
B1	C0	3.50	3.77	3.63	3.63	5.87	6.23	6.03	6.04
	C1	3.53	4.03	3.77	3.78	6.07	6.33	6.23	6.21
	C2	3.80	4.50	3.93	4.08	6.43	6.47	6.40	6.43
B2	C0	3.67	4.18	3.77	3.87	6.50	6.57	6.53	6.53
	C1	3.70	4.50	3.83	4.01	6.53	6.73	6.57	6.61
	C2	3.80	4.60	4.00	4.13	6.60	6.97	6.80	6.79
B3	C0	3.73	4.20	3.87	3.93	6.57	6.80	6.63	6.67
	C1	4.00	4.50	4.03	4.18	6.57	7.07	6.70	6.78
	C2	4.20	4.73	4.23	4.39	6.80	7.13	7.07	7.00

	C0	4.13	4.33	4.00	4.15	6.73	6.87	6.80	6.80
B4	C1	4.17	4.73	4.27	4.39	6.93	7.10	6.87	6.97
	C2	4.27	5.23	4.33	4.61	7.03	7.30	7.10	7.14
L.S.									
D		0.170			0.101		0.183		0.102
0.05									
average cultivars		3.88	4.44	3.97		6.55	6.80	6.64	
L.S.									
D		0.045					0.105		
0.05									
	Cultivars × Plant waste				average Plant waste	Cultivars × Plant waste			average Plant waste
	B1	3.61	4.10	3.78	3.83	6.12	6.34	6.22	6.23
	B2	3.72	4.42	3.87	4.00	6.54	6.76	6.63	6.64
	B3	3.98	4.48	4.04	4.17	6.64	7.00	6.80	6.82
	B4	4.19	4.77	4.20	4.39	6.90	7.09	6.92	6.97
L.S.									
D		0.099			0.063		0.133		0.071
0.05									
	Cultivars × Spraying				average Spraying	Cultivars × Spraying			average Spraying
	C0	3.76	4.12	3.82	3.90	6.42	6.62	6.50	6.51
	C1	3.85	4.44	3.98	4.09	6.53	6.81	6.59	6.64
	C2	4.02	4.77	4.13	4.30	6.72	6.97	6.84	6.84
L.S.									
D		0.078			0.050		0.110		0.047
0.05									

3.2. Weight of Marketable Tubers (g)

Table (5) shows that foliar spraying and adding plant waste of three potato cultivars and their interactions had a significant effect on the weight of marketable tubers for both seasons. The Burren cultivar (A2) was significantly excelled in the weight of marketable tubers in both seasons, as it gave the highest average of (113.55, 123.84) g, followed by El-Mundo cultivar (A3) which gave (109.25, 117.80) g, while the Synergy cultivar (A1) gave the lowest average of (104.81, 114.18) g for both seasons respectively. The same table also shows that adding plant waste has a significant effect on the weight of marketable tubers, as the treatment of adding rice waste compost + 50% NPK fertilizer (B4) was significantly excelled, reaching (112.70, 121.68) g compared to control treatment (B1) which gave the lowest average of (104.83, 112.66) g for both seasons respectively. The table also indicates that foliar spraying with the nutrient solution microponic at a concentration of 200 ml 100 L⁻¹ water (C2) was significantly excelled, reaching (111.43, 120.71) g, while control treatment (C0) gave the lowest average of (106.45, 116.00) g for both seasons respectively. It is also noted from the table that bi-interactions between the experimental factors had a significant effect on this trait, where the interaction between the cultivars and plant waste (rice waste compost) gave a significantly excelled. The interaction between the Burren cultivar and the treatment of adding rice waste compost + 50% NPK fertilizer (A2B4) was excelled and gave the highest average of (115.85, 127.42) g compared to control treatment with the Synergy cultivar (A1B1) which gave the lowest average of (100.36, 106.83) g for both seasons respectively. The interaction between cultivars and foliar spraying had a significant effect, as the interaction of the Burren cultivar and foliar spraying at a concentration of

200 ml 100 L⁻¹ water (A2C2) was excelled and reached (114.99,125.62) g compared to control treatment with the Synergy cultivar (A1C0) which reached (101.73,111.67) g for both seasons respectively. The interaction between plant wastes and foliar spraying showed a significant effect, as the interaction of the addition of rice waste compost + 50% NPK fertilizer and foliar spraying at a concentration of 200 ml 100 L⁻¹ water (B4C2) was excelled and gave the highest average of (114.42,123.10) g compared to control treatment (A1C0) which recorded the lowest average of (99.65,107.93) g for both seasons respectively. The interaction between the three factors had a significant effect, as the interaction between the Burren cultivar and the addition of rice waste compost + 50% NPK fertilizer and foliar spraying at a concentration of 200 ml 100 L⁻¹ water (A2B4C2) was excelled and gave the highest average of (117.00, 129.96) g compared to control treatment of the Synergy cultivar (A1B1C0) which gave the lowest average of (96.23,102.33) g for both seasons respectively.

Table 5. The effect of foliar spraying and the addition of plant waste to three potato cultivars and their interactions on the weight of marketable tuber (g) during the spring and autumn seasons 2023-2024.

Plant waste B	Spraying C	Spring season				Autumn season			
		Cultivars			Plant waste × Spraying	Cultivars			Plant waste × Spraying
		A 1 Synergy	A 2 Burren	A 3 El-Mundo		A 1 Synergy	A 2 Burren	A 3 El-Mundo	
	C0	96.23	104.37	98.35	99.65	102.33	114.33	107.13	107.93
B1	C1	101.11	110.62	104.18	105.30	106.05	119.92	111.88	112.62
	C2	103.45	113.00	110.43	108.96	111.51	121.65	117.92	117.03
	C0	102.18	112.15	107.18	107.17	113.85	122.74	116.23	117.61
B2	C1	103.97	113.98	107.68	108.54	117.37	124.67	118.07	120.03
	C2	107.11	114.13	110.78	110.67	119.39	125.01	119.07	121.16
	C0	103.51	114.26	108.22	108.66	112.68	122.54	118.51	117.91
B3	C1	104.85	115.73	111.84	110.81	114.16	126.13	121.60	120.63
	C2	107.62	115.98	111.96	111.85	117.46	125.57	121.08	121.37
	C0	104.60	113.88	110.95	109.76	116.90	123.99	118.78	119.89
B4	C1	110.41	116.87	114.33	113.87	117.75	128.27	119.99	122.00
	C2	111.19	117.00	115.06	114.42	117.68	129.96	121.65	123.10
L.S.D 0.05		1.779			1.003	2.064			1.237
average cultivars		104.81	113.55	109.25		114.18	123.84	117.80	
L.S.D 0.05		0.886				0.767			
		Cultivars × Plant waste			average Plant waste	Cultivars × Plant waste			average Plant waste
	B1	100.36	109.66	104.47	104.83	106.83	118.74	112.41	112.66
	B2	104.54	113.62	108.45	108.87	116.94	124.10	117.87	119.64
	B3	105.33	115.27	110.82	110.47	114.91	124.77	120.41	120.03
	B4	108.76	115.85	113.50	112.70	117.39	127.42	120.22	121.68
L.S.D 0.05		1.141			0.618	1.500			0.936
		Cultivars × Spraying			average Spraying	Cultivars × Spraying			average Spraying
	C0	101.73	111.40	106.23	106.45	111.67	121.00	115.32	116.00
	C1	105.32	114.25	109.62	109.72	113.94	124.80	118.04	118.93
	C2	107.39	114.99	111.91	111.43	116.47	125.62	120.03	120.71

L.S.D
0.05

1.001

0.501

0.950

0.524

3.3. The Yield of One Marketable Plant (g plant^{-1})

Table (6) shows that foliar spraying and adding plant waste of three potato cultivars and their interactions had a significant effect on the yield of one marketable plant for both seasons. The Burren cultivar (A2) was significantly excelled in the yield of one marketable plant in both seasons, as it gave the highest average of (505.2,842.1) g plant^{-1} , followed by El-Mundo cultivar (A3) which gave (434.8,782.9) g plant^{-1} , while the Synergy cultivar (A1) gave the lowest average of (406.5,747.9) g plant^{-1} for both seasons respectively. The same table also indicates that adding plant waste has a significant effect on the yield of one marketable plant, as the treatment of adding rice waste compost + 50% NPK fertilizer (B4) was significantly excelled, reaching (495.0,848.4) g plant^{-1} , compared to control treatment (B1), which gave the lowest average of (402.3,702.0) g plant^{-1} for both seasons. It is also noted from the same table that foliar spraying with the nutrient solution microponic at a concentration of 200 ml 100 L^{-1} of water (C2) was significantly excelled, reaching (480.8,826.5) g plant^{-1} , while control treatment (C0) gave the lowest average of (415.8,755.8) g plant^{-1} for both seasons. The table also shows that bi-interactions between the experimental factors had a significant effect on this trait, as the interaction between the cultivars and plant waste (rice waste compost) gave a significantly excelled, The interaction between the Burren cultivar and the treatment of adding rice waste compost + 50% NPK fertilizer (A2B4) was excelled and gave the highest average of (552.6,903.4) g plant^{-1} , compared to control treatment with the Synergy cultivar (A1B1), which gave the lowest average of (362.3,653.8) g plant^{-1} , for both seasons respectively. The interaction between the cultivars and foliar spraying had a significant effect. The interaction of the Burren cultivar and foliar spraying at a concentration of 200 ml 100 L^{-1} of water (A2C2) was excelled, reaching (548.5,875.6) g plant^{-1} , compared to control treatment with the Synergy cultivar, reaching (382.5,716.9) g plant^{-1} , for both seasons respectively. The interaction between plant waste and foliar spraying showed a significant effect, as the interaction of adding rice waste compost + 50% NPK fertilizer and foliar spraying at a concentration of 200 ml 100 L^{-1} of water (B4C2) was excelled and gave the highest average of (528.3,879.9) g plant^{-1} compared to control treatment ((B1C0) which recorded the lowest average of (362.8,653.0) g plant^{-1} for both seasons respectively. The interaction between the three factors also had a significant effect, as the interaction between the Burren cultivar and adding rice waste compost + 50% NPK fertilizer and foliar spraying at a concentration of 200 ml 100 L^{-1} of water (A2B4C2) was excelled and gave the highest average of (611.9,948.7) g plant^{-1} compared to control treatment of the Synergy cultivar (A1B1C0) which gave the lowest average of (336.8,600.7) g plant^{-1} for both seasons respectively.

Table 6. The effect of foliar spraying and the addition plant waste of three potato cultivars and their interactions on the yield of one marketable plant (g plant^{-1}) during the spring and autumn seasons 2023-2024.

Plant waste B	Sprayin g C	Spring season				Autumn season			
		Cultivars			Plant waste × Sprayin g	Cultivars			Plant waste × Sprayin g
		A 1	A 2	A 3 El-		A 1	A 2	A 3 El-	
		Synerg y	Burre n	Mund o		Synerg y	Burre n	Mund o	
B1	C0	336.8	394.5	357.0	362.8	600.7	712.3	646.0	653.0
	C1	356.9	445.8	393.8	398.8	643.7	759.1	697.0	699.9
	C2	393.1	508.5	434.0	445.2	717.0	787.1	754.7	752.9
B2	C0	375.0	468.8	403.7	415.8	740.0	806.4	759.0	768.5

Plant waste B	Spraying C	Spring season			Plant waste × Spraying g	Autumn season			Plant waste × Spraying g
		Cultivars				Cultivars			
		A 1 Synergy	A 2 Burren	A 3 Elm Mundo		A 1 Synergy	A 2 Burren	A 3 Elm Mundo	
B3	C1	384.7	512.9	412.4	436.7	766.4	839.0	775.7	793.7
	C2	407.0	525.0	443.1	458.3	788.0	871.3	809.7	823.0
	C0	386.1	479.9	418.8	428.3	740.3	833.3	785.7	786.5
	C1	419.4	520.8	450.7	463.6	750.0	891.7	814.7	818.8
	C2	452.0	548.6	473.6	491.4	798.7	895.3	856.0	850.0
B4	C0	432.0	493.1	443.8	456.3	786.7	851.8	807.7	815.4
	C1	460.4	552.8	488.2	500.4	816.0	909.7	824.3	850.0
	C2	474.8	611.9	498.2	528.3	827.3	948.7	863.7	879.9
L.S. D 0.05		20.10			12.23		28.93		15.74
average cultivars		406.5	505.2	434.8		747.9	842.1	782.9	
L.S. D 0.05		4.09					18.13		
Cultivars × Plant waste					average Plant waste	Cultivars × Plant waste			average Plant waste
B1	B1	362.3	449.6	394.9	402.3	653.8	752.8	699.2	702.0
	B2	388.9	502.2	419.7	436.9	764.8	838.9	781.5	795.1
	B3	419.2	516.4	447.7	461.1	763.0	873.4	818.8	818.4
	B4	455.7	552.6	476.7	495.0	810.0	903.4	831.9	848.4
L.S. D 0.05		13.22			8.63		21.72		11.11
Cultivars × Spraying					average Spraying	Cultivars × Spraying			average Spraying
C0	C0	382.5	459.0	405.8	415.8	716.9	801.0	749.6	755.8
	C1	405.4	508.1	436.3	449.9	744.0	849.9	777.9	790.6
	C2	431.7	548.5	462.2	480.8	782.7	875.6	821.0	826.5
L.S. D 0.05		8.39			5.56		18.41		7.16

3.4. The Marketable Yield (tons ha⁻¹)

Table (7) indicates that foliar spraying and adding plant waste of three potato cultivars and their interactions have a significant effect on the marketable yield for both seasons. The Burren cultivar(A2) was significantly excelled in the marketable yield in both seasons, as it gave the highest average of (26.94, 45.03) tons ha⁻¹, followed by Elmundo (A3) cultivar, which gave (23.19, 41.75) tons ha⁻¹, while the Synergy (A1) cultivar gave the lowest average of (21.68, 39.89) tons ha⁻¹ for both seasons respectively. The same table also shows that adding plant waste has a significant effect on the marketable yield, as the treatment of adding rice waste compost + 50% NPK fertilizer (B4) was significantly excelled, as it reached (26.40,45.26) tons ha⁻¹ Compared to control treatment (B1) which gave the lowest average of (21.45,37.59) tons ha⁻¹ for both seasons. It is also noted from the same table that the foliar spray with the nutrient solution

microponic at a concentration of 200 ml 100 L⁻¹ of water (C2) was significantly excelled, as it reached (25.64,44.09) tons ha⁻¹, while control treatment (C0) gave the lowest average of (22.17, 40.31) tons ha⁻¹ for both seasons. The table also shows that bi-interactions between the experimental factors had a significant effect on this trait, as the interaction between the cultivars and plant waste (rice waste compost) gave a significantly excelled, as the interaction between the Burren cultivar and the treatment of adding rice waste compost + 50% NPK fertilizer (A2B4) was excelled and gave the highest average of (29.47,48.21) tons ha⁻¹ compared to control treatment with the Synergy cultivar (A1B1)) gave the lowest average of (19.32,34.87) tons ha⁻¹ for both seasons respectively. The interaction between the cultivars and foliar spraying had a significant effect, as the interaction of the Burren cultivar and foliar spraying at a concentration of 200 ml 100 L⁻¹ water (A2C2) was excelled and amounted to (29.25,46.72) tons ha⁻¹ compared to control treatment with the Synergy cultivar (A1C0)) amounted to (20.40,38.23) tons ha⁻¹ for both seasons respectively. The interaction between plant waste and foliar spraying showed a significant effect, the interaction of adding rice waste compost + 50% NPK fertilizer and foliar spraying at a concentration of 200 ml 100 L⁻¹ of water (B4C2) was excelled and gave the highest average of (28.18, 46.96) tons ha⁻¹, compared to control treatment (B1C0), which recorded the lowest average of (19.35, 34.83) tons ha⁻¹ for both seasons respectively. The interaction between the three factors had a significant effect, as the interaction between the Burren cultivar and the addition of rice waste compost + 50% NPK fertilizer and foliar spraying at a concentration of 200 ml 100 L⁻¹ of water (A2B4C2) was excelled and gave the highest average of (32.64,50.68) tons ha⁻¹ compared to control treatment of the Synergy cultivar (A1B1C0) which gave the lowest average of (17.96, 32.03) tons ha⁻¹ for both seasons respectively.

Table 7. The effect of foliar spraying and the addition of plant waste to three potato cultivars and their interactions the marketable yield (tons ha⁻¹) during the spring and autumn seasons 2024-2023.

Plant waste B	Sprayin g C	Spring season			Plant waste × Sprayin g	Autumn season			Plant waste × Sprayin g
		Cultivars				Cultivars			
		A 1 Synerg y	A 2 Burre n	A 3 El- Mund o		A 1 Synerg y	A 2 Burre n	A 3 El- Mund o	
B1	C0	17.96	21.04	19.04	19.35	32.03	37.99	34.46	34.83
	C1	19.04	23.78	21.00	21.27	34.33	41.82	37.18	37.78
	C2	20.96	27.12	23.15	23.74	38.24	41.99	40.25	40.16
B2	C0	20.00	25.00	21.53	22.18	39.47	43.01	40.48	40.99
	C1	20.52	27.36	21.99	23.29	40.87	44.75	41.37	42.33
	C2	21.70	27.99	23.63	24.44	42.03	46.47	43.18	43.89
B3	C0	20.59	25.59	22.33	22.84	39.48	44.45	41.91	41.95
	C1	22.37	27.78	24.04	24.73	40.00	47.55	43.45	43.67
	C2	24.10	29.26	25.26	26.21	42.60	47.75	45.66	45.33
B4	C0	23.04	26.30	23.67	24.34	41.95	45.43	43.08	43.49
	C1	24.55	29.48	26.04	26.69	43.52	48.52	43.96	45.33
	C2	25.32	32.64	26.57	28.18	44.12	50.68	46.06	46.96
L.S. D 0.05		1.072			0.652		1.645		0.893
average cultivars		21.68	26.94	23.19		39.89	45.03	41.75	
L.S. D 0.05		0.218					1.022		

Cultivars × Plant waste				average Plant waste	Cultivars × Plant waste			average Plant waste
B1	19.32	23.98	21.06	21.45	34.87	40.60	37.29	37.59
B2	20.74	26.79	22.38	23.30	40.79	44.74	41.68	42.40
B3	22.36	27.54	23.88	24.59	40.69	46.58	43.67	43.65
B4	24.31	29.47	25.43	26.40	43.20	48.21	44.37	45.26
L.S.								
D	0.705			0.460	1.205			0.606
0.05								
Cultivars × Spraying				average Spraying	Cultivars × Spraying			average Spraying
C0	20.40	24.48	21.64	22.17	38.23	42.72	39.98	40.31
C1	21.62	27.10	23.27	23.99	39.68	45.66	41.49	42.28
C2	23.02	29.25	24.65	25.64	41.75	46.72	43.79	44.09
L.S.								
D	0.448			0.297	1.045			0.419
0.05								

The results of Tables (4-7) showed a significant increase in yield traits and its components between the potato cultivars used in the study. This significant increase may be due to genetic differences between the cultivars, as yield traits are controlled by genetic genes [20]. These results are consistent with [21-23], who noted significant differences between potato cultivars in terms of yield traits, depending on the genetic capacity of the cultivars and the environmental conditions prevailing in the region during the growth and production season. As for the plant waste treatments, the treatment of adding rice waste compost + 50% NPK (B4) fertilizer had a significant effect on traits and components of the potato plant. This increase may be due to the role of organic and chemical fertilizers, as these fertilizers improve the physical and chemical properties of the soil when they decompose in the soil, in addition to providing the plant with the necessary nutrients, which leads to increased vegetative growth and activity, in addition to increasing the number and weight of tubers, which leads to increased plant yield and marketable yield [24-26]. Or it may be due to the role of organic fertilizer in providing nutrients and increasing their availability and absorption through the roots, which led to an increase in vegetative growth traits as a result of increasing the photosynthesis process and increasing the carbohydrates formed in the leaves and then transferring them to the tubers, thus leading to an increase in their number and weight and positively reflected on the yield of a single plant and the marketable yield. This result is consistent with [27-32] who showed that the type and level of added fertilizer affects the physical and chemical properties of the soil and thus encourages vegetative growth, which is reflected in an increase in the yield and its components. As for the foliar spray treatments with the nutrient solution microponic at a concentration of 200 ml 100 L⁻¹ water (C2), it had a significant effect on traits of the yield and its components of the potato plant. This increase in traits of the yield is due to the fact that this nutrient contains important microelements and their effect on increasing the production of proteins and activating enzymes and carbohydrates in the leaves of the plant and then transferring them to the tubers, which leads to increased stimulation of cell elongation and division [33,34]. These results are consistent with [35-38].

Conclusion

This investigation has revealed that the cultivar Burren outperformed the cultivars El-Mundo and Synergy in terms of yield characteristics and its components. The addition of rice waste compost

+ 50% NPK fertilizer and the treatment of foliar spraying with microponic nutrient solution at a concentration of 200 ml 100 L⁻¹ water resulted in a substantial increase in yield characteristics. The interaction between the three factors also had a substantial impact, as the cultivar Burren and the treatment of adding rice waste compost + 50% NPK foliar spraying at a concentration of 200 ml 100 L⁻¹ water was superior in terms of yield characteristics and its components for both seasons.

References

- [1] Naz, F.; Ali, A., Iqbal, Z., Akhta, N., Asghar, S., & Ahmad, B. (2011). Effects of different levels of NPK fertilizers on the proximate composition of potato crop at Abbottabad. *Sarhad Journal of Agriculture*. 27(3):353-356.
- [2] Gehan, A.E. (2013). Effect of organic, nitrogen and potassium fertilization treatments on growth, yield and chemical contents of two cultivars of potato *Solanum tuberosum* L., Alexandria Sci. Exchange J. 34(4): 369-381.
- [3] Al-Taey, D.K.A., Hussain, A. J., & Kadhum, H. J. (2023). Bentonite Impact on Soil Properties and Biological Activity in the Face of Drought: A Review. 4th International Conference of Modern Technologies in Agricultural Sciences. IOP Conf. Series: Earth and Environmental Science. 1262 (2023) 042058. doi:10.1088/1755-1315/1262/4/042058
- [4] Fernández-López, J., Botella-Martínez, C., de Vera, C. N.R., Sayas-Barberá, M. E., Viuda-Martos M., Sánchez- Zapata, E., & Perez-Álvarez, J .A.(2020). Vegetable soups and creams: Raw materials, processing, health benefits, and innovation trends. *Plants*, 9(12), 1769.
- [5] Central Organization of Statistics. (2023). Annual Statistical Abstract. Ministry of Planning - Directorate of Agricultural Statistics. Department of Publishing and Public Relations. Baghdad. Republic of Iraq.
- [6] Taha, F. A. A. (2007). Effect of potassium fertilizer and soil cover on three cultivars of potato *Solanum tuberosum* L. grown in Basra Governorate. Master's thesis. College of Agriculture. University of Basra. Republic of Iraq.
- [7] Matar, H. M. , Mahmoud, S. A. W., & Ramadan. A. F. (2012). Effect of treatment with gibberellin and licorice extract on growth and yield of potato. *Diyala Journal of Agricultural Sciences*. 4 (1): 220-234.
- [8] Othman, J. Y.(2007). Study of the effect of using organic fertilizers in potato cultivation and production as a contribution to clean organic production. Master's thesis. Department of Horticulture. Faculty of Agriculture. Tishreen University. Republic of Syria.
- [9] Hasan, A.A. , Aubied, I.A., & Al-Taey, D.K. A. (2023). The Effect of Organic Amendments and Minerals on Increasing Soil Fertility (A review). IOP Conf. Series: Earth and Environmental Science. 1262 (2023) 042012. doi:10.1088/1755-1315/1262/4/042012
- [10] Chen, J. H. (2006). the Combined Use of Chemical and Organic Fertilizers and/or Biofertilizer for Crop Growth and Soil Fertility. International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use, (October), 8(4): 1–11.
- [11] Al-Taey , D.K.A. , Al-ftlawi, S. S., & Sahib, M. R.(2022). Effect of Zytonic-M, PalmWASTES Compost and NPK on the Growth and Yield of Tomato under Salt Stress Conditions. *Int. J. Agricult. Stat. Sci.*, 18(2): 829-836. DocID: <https://connectjournals.com/03899.2022.18.829>
- [12] Aulakh, C. S., Sharma, S., Thakur, M., & Kaur, P.(2022). A review of the influences of organic farming on soil quality, crop productivity and produce quality. *Journal of Plant Nutrition*, 1-22.
- [13] Jordan, C. F.(2022). Can Organic Agriculture Feed the World?. In *Evolution from a Thermodynamic Perspective*. Springer, Cham. (pp. 315-318).
- [14] El-Ramady, H., & Alshaal, T.(2017). Foliar application: from plant nutrition to biofortification. *Environment, Biodiversity and Soil Security*, 1: 71-83.
- [15] Fernández, V., & Bahamonde, H. A. (2020). Advances in foliar fertilizers to optimize crop nutrition. In *Achieving sustainable crop nutrition*. Burleigh Dodds Science Publishing. (p.p): 719-744.

- [16] Zaki, M.H.(2017). Foliar fertilization. Agricultural Research Center. Article available on the Egyptian Agriculture Network at the website: <https://www. Agriculture Egypt. com/ Agenda/Articles/464/ foliar spray>.
- [17] Al-Salmani, H. K., Abbas , J. M., & Sarhan, I. A. (2011). Response of Abu Ghraib-3 bread wheat to foliar feeding with iron and zinc. Iraqi Journal of Agriculture (Special Issue) 5:16.
- [18] Al-Zubaidi, S. Z. A .(2000). Determination of nitrogen, phosphorus and potassium balance of potato *Solanum tuberosum* L. in sedimentary soil. PhD thesis. Department of Soil and Water Sciences. College of Agriculture. University of Baghdad. Republic of Iraq.
- [19] Al-Rawi, K. M., & Khalaf Allah, A.A. M. (1980). Design and analysis of agricultural experiments. College of Agriculture. University of Mosul. Republic of Iraq.
- [20] Al-Jumaili, M. S. A., & Al-Mohammed, O. H. M.(2018). Response of two potato cultivars for spraying with some nutrients for the spring season. Journal of Education and Scientific Studies , 1(13): 43-58.
- [21] Al-Shammari, O. H. M. K .(2024). Effect of biofertilization and spraying with nanofertilizer on the growth and yield of three cultivars of potato *Solanum tuberosum* L., PhD thesis. Al-Furat Al-Awsat Technical University. Al-Mussaib Technical College. Republic of Iraq.
- [22] Al-Janabi, S. H. M .(2023). Response of two potato cultivars *Solanum tuberosum* L. to organic fertilizer and spraying with zinc sulfate. Master's thesis. College of Agriculture - University of Anbar. Republic of Iraq.
- [23] Jalal, V. J., Al-Mohammadi ,O. H. M., & Mohammed, K. A. (2022). The effect of using NPK nano compound fertilizer technology on the growth traits and yield of two potato cultivars grown in the autumn seasons. Journal of Kirkuk University for Agricultural Sciences. 13 (3): 230-239.
- [24] Abdelrazzag, A .(2002). Effect of chicken manure. sheep manure and inorganic fertilizer on yield and nutrients uptake by onion. Pakistan journal of Biological Sciences, 5 (3): 266 - 268.
- [25] Al-Zuhawi, S. M. A .(2007). The effect of different organic fertilizers and soil cover on the growth, production and quality of potato. Master's thesis. College of Agriculture. University of Baghdad. Republic of Iraq.
- [26] Al-Muhammadi, O. H. M. (2009). Use of animal fertilizers and whey as a method of organic agriculture and its effect on the growth and production of potato. PhD thesis. College of Agriculture. University of Baghdad. Republic of Iraq.
- [27] Shabana, M. M. A., El-Naqma ,K. A. A., Zoghdan, M. G., & Khalifa, R. M.(2023). Potassium Humate and Silicate Combined with Compost Application to Reduce the Harmful Effects of the Irrigation Water Salinity on Potato Plants and on the Soil Available Nutrient NPK. Journal of Soil Sciences and Agricultural Engineering. Vol. 14 (3):103 – 112.
- [28] Salamba, H.N, Malia, I.E., & Ardan, M.(2021). The Effectiveness of Rice Straw Based Compost on Potato Production as a Basis of Organic Farming System in North Sulawesi Indonesia. E3S Web of Conferences. 232, 03016.p:1-8.
- [29] Al-Bayati, H. J.M., Salem ,A. M., & Al-Sheikh, M. A. A. (2020). Effect of organic and chemical fertilization on the growth and yield of three local potato cultivars. Diyala Journal of Agricultural Sciences. Proceedings of the Fourth Scientific Conference and the First International Agricultural Research Conference. Volume 12 (Special Issue): 571-584.
- [30] Al-Taey, D. K. A., AL-Azawi, S. S. M., Al-Shareefi, M. J. H., & Al-Tawaha, A. R. (2018). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce *Lactuca sativa* var. Parris Island . Research on Crops.19 (3) : 441-449. DOI : 10.31830/2348-7542.2018.0001.14
- [31] Al-Taey, D.K.A., Al-Naely, I J.C.,& Kshash, B.H.(2019). A study on effects of water quality, cultivars, organic and chemical fertilizers on potato *Solanum tuberosum* L. growth and yield to calculate the economic feasibility. Bulgarian Journal of Agricultural Science, 5 (6) 1239 -1245.
- [32] Majoul, A. K.,& Jassim, H. M. (2018). Organic wastes and biological factors and their effect on some indicators of growth and productivity of potato *Solanum tuberosum* L., Karbala Journal of Agricultural Sciences. (1)5: 49-64.



- [33] Al-Dulaimy, A.F.Z., Alalaf, A.H., Al-Hayali, R.E.Y., & Al-Taey, D.K.A.(2023). Flowers and fruits abortion in fruit trees ... Causes and solutions: A review. IOP Conference Series: Earth and Environmental Science. 1158(4), 042010.
- [34] Unlu, H. Oz., Unlu, H., Karakurt ,Y., & Padem, H.(2011). Changes in fruit yield and quality in response to foliar and soil humic acid application in cucumber. Scientific Research and Essays .6(13):2800-2803
- [35] Al-Jburi, S.M.Y., & Al-Shammari ,M. F.(2022). The impact of zinc, calcium, and sorbitol spraying on some characteristics of the potato plant *Solanum tuberosum* L., Euphrates Journal of Agriculture Science,14 (3): 156-165.
- [36] Al-Dulaimy, A.F.Z., Alani, M.H.I., Awad, I.M., Khudair, R.I.A, Salim, H.A., &Jasem, S.M .(2021). Response of *Gazania* plants *Gazania Splendens* L. to seaweed extract (Tecamin Algae) and nano stimulator (Proteck CalBor). IOP Conference Series: Earth and Environmental Science.,910(1), 012052.
- [37] Al-Dulaimi, N. H. A., & Al-Amri, N. J. K.(2020). Stimulation growth and yield of potato by buttonwood pruning residues and spraying several micronutrients. Iraqi Journal of Agricultural Sciences. 51(4):1048-1057.
- [38] Al-Bayati, H. J. M., & Ali, K. R.(2019). Effect of soil mulching and spraying with zinc sulphate on the growth and yield of potato *Solanum tuberosum* L. Actrice cultivar. Rafidain Journal of Agriculture. Proceedings of the Third International Agricultural Conference. Additional Issue 47(2): 101-111.

Sustainability of Potato Farming under Drought Conditions

Suha Khudhair Abbas Al-Ameri¹ and Ayad Waleed A. ALjuboori²

^{1,2}Department of Horticulture and Landscape Engineering, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.

¹E-mail: Soha.Abbas2305p@coagri.uobaghdad.edu.iq

²E-mail: Ayad.waleed@coagri.uobaghdad.edu.iq

Abstract. To improve growth and production under drought conditions, it is necessary to understand the physiological, chemical, biological, molecular, and genetic foundations. Potatoes are crops that consume large amounts of water; insufficient and irregular water availability will directly or indirectly affect the yield. Water deficiency impacts the absorption of essential nutrients needed to complete the plant's life cycle and affects photosynthesis by reducing leaf area, impacting chlorophyll pigments, and influencing the opening and closing of stomata and hormonal balance within plant. Furthermore, inadequate irrigation during tuber formation and filling will negatively affect productivity and quality. also water stress conditions requires studying various factors and agricultural practices that improve growth and production conditions by adopting sustainable and successful methods to overcome stress conditions, including good management of organic and bio-nutrients, modern irrigation systems, and other factors.

Keywords. Organic fertilizer, Bio-nutrients, *Solanum tuberosum* L., Water stress.

1. Sustainable Agriculture

Agricultural development is defined as "a set of methods that play an important role in influencing economic structure to improve agricultural production in terms of quality and quantity. This is achieved through changes in production methods and means, relying on organization of production"[1].

Sustainable food production and environmental stewardship are critically important and will require a unified approach to food safety and consumer health, as humans, animals, and the environment are all closely interconnected. Agricultural advancement has become part of the modern era, necessitating the identification of suitable alternatives to traditional fertilizers while reducing harmful environmental impacts. This transition from conventional to modern agriculture involves employing advanced techniques to minimize nutrient loss and the productivity gap, aiming to achieve sufficient production and improve quality standards to meet the demands of a growing population [2].

To achieve sustainable agricultural development, optimal use of natural resources (plant and animal) from living natural resources, human resources, capital resources, and required technical resources is necessary. Methods or plans may directly improve the efficiency, effectiveness, and management of agricultural development. Pursuing sustainable agricultural development

positions the agricultural sector as a driving force for the economy and a supporter of economic development [3].

Soil is closely linked to sustainable agriculture, as the diversity and activity of microorganisms in the soil are the main components of soil health. Agricultural sustainability is defined as the ability of crops to produce food continuously without environmental degradation.

Fungi, blue-green bacteria, and beneficial nematodes enhance water use efficiency, nutrient availability to plants, production of plant hormones, soil nutrient cycling, and plant resistance to environmental conditions [4].

2. Introduction

Potato (*Solanum tuberosum* L.) one of Solanaceae family it is fourth most important crop after wheat, maize, and rice, and it has a significant impact on long-term food security. It serves as an alternative to grains in addressing food shortages, especially in developing countries, and is a primary source of starch with a wide range of industrial, both food and non-food, applications [5]. Potatoes are an energy source due to their high carbohydrate and protein content and are rich in vitamins B and C, fat-soluble vitamins, and bioactive components such as polyphenols and flavonoids. They also contain essential minerals like potassium, phosphorus, copper, iron, zinc, magnesium, and manganese [6]. These components contribute to the quality traits of potatoes, including external attributes like tuber appearance and internal characteristics such as dry matter and starch content, integration of these qualities depends on their structure.

Potato plants are notably affected by stress conditions as they are moderately sensitive to water stress. Most cultivated potato varieties have a shallow and less extensive root system, which makes them vulnerable when soil moisture content drops below 50% of field capacity. Irrigation water management is crucial for sustainable cultivation of potatoes. Insufficient irrigation water affects the growth, yield, and quality of potatoes. Water stress or inadequate irrigation leads to increased soil temperature and salinity, resulting in reduced nutrient absorption by plant. This diminishes tuber quality, causing deformities and smaller tuber sizes [7].

Environmental stresses are the primary cause of crop losses worldwide, reducing productivity by 50%. The issues of water scarcity and salinity are often interlinked and affect vast areas globally. This interdependence is exacerbated under hot climate conditions with saline irrigation water, as increased evaporation leads to higher soil salinity. Water is one of the five essential factors for life on Earth, alongside energy, air, minerals, and chlorophyll. Irrigated land accounts for about 15% of the world's agricultural areas and produces approximately 35% of the global food supply [8].

Water stress is a type of abiotic stress that affects plant growth and productivity. It is defined as the plant's inability to absorb water naturally and can occur due to an imbalance between the water content in soil and amount of water required by the plant. This stress affects the plant's physiological, chemical, and phenotypic processes, including photosynthesis, respiration, transpiration, and nutrient absorption, thereby impacting plant growth [9-11].

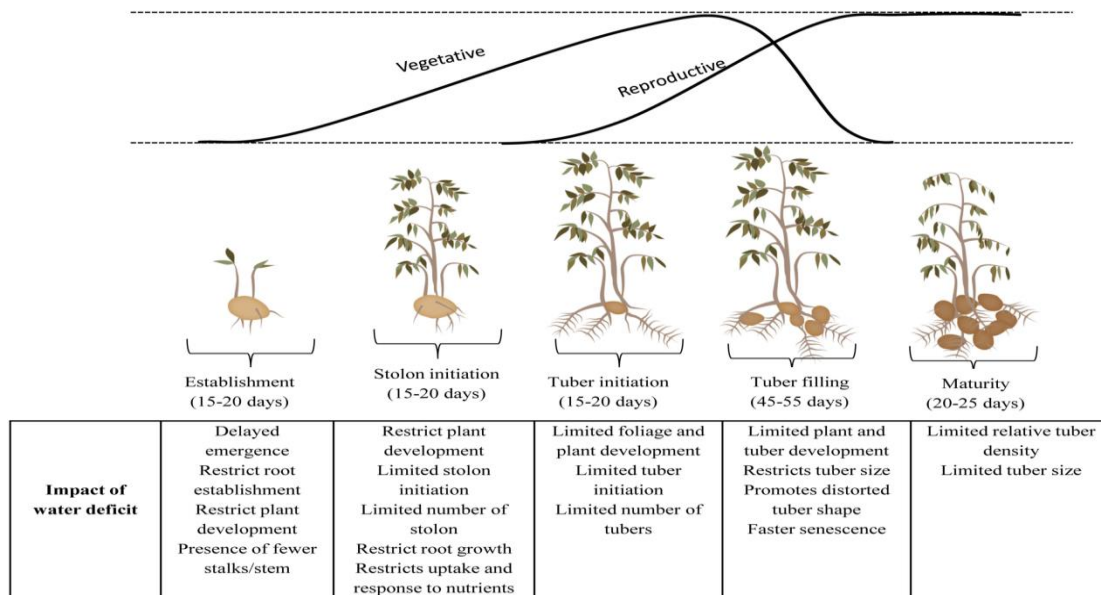
The duration of irrigation affects the physiological activity of potatoes, leading to reduced yields [12]. Water deficit has a negative impact on different stages of potato growth (germination, vegetative growth, tuber formation, and quality), adversely affecting plant development.

Below is a figure illustrating effect of water stress on various growth stages of potatoes.

[13]. found that water stress significantly affects the different growth stages of potato plants.

Water stress affects the morphological and physiological traits of potatoes and impacts various stages of their growth, resulting in reduced yield and its components [14]. Increased water stress leads to elevated ethylene accumulation in plants due to increased respiration and decreased water availability, affecting leaf area [15]. Water stress is considered one of the most severe

environmental pressures affecting plant growth and productivity [16]. Soil moisture deficit in the root zone below optimal levels affects plant growth, productivity, and quality [17]. The causes of water stress include decreased germination percentage, leaf area, and stem number [18]. Organic matter plays a crucial and significant role in soil ecosystems, enhancing various soil properties. It has beneficial effects on the physical, chemical, and biological characteristics of soil, which in turn influence soil productivity [19]. Organic matter is particularly important for supplying nutrients such as nitrogen, phosphorus, and sulfur, among others, and enhances soil biological activity by providing essential carbon for metabolic processes. The compounds released from organic matter during decomposition processes in soil contribute significantly to soil fertility [20].



Organic matter increases soil water retention capacity, attributed to its ability to hold water and enhance water-holding capacity [21]. Both organic matter and plant roots are crucial factors in soil structure formation and stability, improving soil structure, total porosity, and soil water retention capacity [22]. The organic matter content in soil significantly affects its available water capacity, with higher organic matter content increasing water storage capacity [23]. Organic matter plays a critical role in increasing soil's surface area and improving soil structure by enhancing inter-porosity, resulting in changes in soil bulk density, porosity, and pore size distribution [24]. These combined factors contribute to enhancing soil's ability to retain water, its available water content, and its water conductivity [25].

Organic fertilizers distinguish themselves from chemical fertilizers due to several advantages that make them more desirable for use. They are cost-effective and contribute to improving soil structure, texture, and aeration, thereby enhancing soil water retention capacity and stimulating root growth. Organic fertilizers, sourced from both plant and animal origins, enhance soil organic matter content, positively impacting optimal plant growth and improving production quality standards [26]. Addition of organic fertilizer (vermicompost) increased soil's readily available nitrogen and phosphorus content [27]. Using rice residues increased starch content in tubers by 14.33% and dry matter content by 20.75% [28]. Vermicompost enhanced potato characteristics such as plant height, aerial stem count, total green weight, tuber count per plant, single plant

yield, total yield, dry matter percentage, starch content, and protein content [29]. Application of organic fertilizers to sweet potatoes improved specific traits in tubers such as protein, fiber, and vitamin A content [30].

Adding rice residues as organic fertilizer to potato plants increased plant height and leaf area [31]. Incorporating Conocarpus plant residues at a rate of 15 tons per hectare enhanced vegetative growth rate, leaf number, chlorophyll content, yield, and protein content [32]. Soil addition of cow manure residues at 5% of soil weight increased vegetative growth parameters such as plant height, leaf number, leaf area, aerial stem count, and total green weight. It also increased NPK ratio in leaves, tuber count, marketable tubers, total yield, marketable total yield, starch content, and specific density [20]. Using a mixture of palm frond residues, rice residues, and humic acid increased leaf area of potato plants, while rice residues increased plant height and marketable yield [33]. Organic fertilization increased average tuber yield by 32.8% and 41.9% under water stress conditions [34]. Organic fertilizer enhanced potato yield under water stress by 21 tons per hectare and increased chlorophyll content to 12 mg/g, showing significant mitigation of drought stress effects [35].

Under water stress conditions, the use of organic fertilizer (vermicompost) on Spunta variety potatoes increased growth, yield, components, and quality, showing superior values across all studied traits [36].

The application of organic fertilizer (humic acid) under water stress for potato crops improved stress resistance characteristics, highlighting its effectiveness in mitigating water stress effects [37].

3. The Role of Biofertilizers in Reducing the Effects of Water Stress

Biofertilizers refer to communities of microorganisms living in the soil that effectively contribute to activating and stimulating biological reactions. Through these reactions, they facilitate the fixation or enhancement of nutrient availability for plants in a beneficial manner. Biofertilization has gained popularity in recent years across various crop cultivations by introducing efficient and beneficial microorganisms, such as bacteria, fungi, or both. These organisms play crucial roles in promoting the growth of beneficial relationships with plants, enhancing nutrient supply, and producing hormones and antibiotics.

Studies have shown that inoculation with mycorrhizae improves plant growth and yield by facilitating nutrient uptake, modifying soil reactions, and enhancing the diversity of microorganisms in the root zone. This also increases the plant's tolerance to drought, salinity, and resistance to pathogens [38]. Similarly, inoculating potatoes with mycorrhizae has positively impacted plant growth by increasing leaf number, plant height, tuber weight, and marketable yield [39].

Plant Growth-Promoting Microorganisms (PGPMs) include bacteria and fungi that live freely or symbiotically in association with plant roots. They play a crucial role by providing essential substances to host plants through increased availability of nutrients, enhancing uptake of plant nutrients, regulating the production of plant hormones, and improving plant tolerance to environmental stresses [40,41]. These microorganisms contribute significantly to sustainable agriculture by promoting plant growth and productivity in various ecological conditions.

Some common PGPMs used commercially include growth-promoting bacteria like *Azospirillum* and mycorrhizal fungi. Bacteria belonging to the genus *Azospirillum* are known to enhance plant growth by nitrogen fixation, production of plant hormones (including auxins, cytokinins, gibberellins, abscisic acid, ethylene, and salicylic acid), phosphate solubilization, and stimulation of root growth [42,44]. These microorganisms play a significant role in promoting plant health and productivity in agricultural settings.

Soil inoculation with mycorrhizal fungi has enhanced the availability of N, P, and K elements in potato crops [45]. Inoculation of potatoes with *Trichoderma harzianum* has increased root growth characteristics (root length, fresh and dry root weight) and vegetative growth (plant height, leaf number, and dry weight of aboveground parts), as well as single plant yield [46]. Trihalomethanes aid in plant resistance to biotic stresses and regulate stomatal conductance, water use efficiency, and tolerance to stresses by protecting membranes and proteins from deterioration [47]. The application of polymers and mycorrhizal fungi has led to improvements in all vegetative and physiological indicators of potato growth, yield, its components, chemical properties, and quality [48].

The use of biofertilizers under water stress at different irrigation stages for potato plants and their inoculation with *Azotobacter*, *Azospirillum*, and *Bacillus* resulted in an increase in total production by 19.26% in both seasons. Additionally, protein production, starch content, dry tuber weight, and the uptake of nitrogen, phosphorus, and potassium were higher [49].

The application of biofertilizers improved tuber yield under water stress conditions, with the highest photosynthetic rate achieved through mycorrhizal addition under irrigation conditions. Mycorrhiza played an effective role under water stress [50].

Rhizobacteria, which enhance plant growth, represent a group of microorganisms that play a crucial role in improving plant growth and controlling plant diseases under various water stress conditions. They may enhance biotic and abiotic stress tolerance in crops/plants to combat plant pathogens and sustain global food supplies sustainably [51]. The impact of biofertilizers under water stress conditions for potato crops has been significant in mitigating drought effects and maintaining higher growth, physical and chemical properties, dry matter production, leaf area, number of tubers, tuber weight, yield, chlorophyll content, proteins, and total sugars [52].

Conclusions

- Adding organic fertilizers, whether animal-based or plant-based, under stressful conditions improved all indicators of vegetative growth and yield.
- Application of biofertilizers under stress conditions led to improvements in both quantitative and qualitative traits.
- Addition of organic fertilizers enhanced soil properties.

Reference

- [1] Mahrous, Ayman. (2022). Rural productive activities and their role in achieving sustainable development, applied to Al-Ahsa Oasis in the Kingdom of Saudi Arabia. *Arab Geographic Journal*, 53(80), 167-200.
- [2] Caradonia,F.; D.Ronga; A.Tava and E.Francia.2022. Plant biostimulants in sustainable Potato Production: an overview. *Potato Research*.65(1):83-104.
- [3] Mahmud, S, F.2021.Opportunities and challenges of sustainable agricultural development in Iraq. *iJournals : International Journal of social Relevance & Concern (IJSRC)* ISSN-2347-9698 .9(1):1-14.
- [4] Tahat,M. M., M. Alananbeh, K., A. Othman, Y., & I. Leskovar, D. 2020. Soil health and sustainable agriculture. *Sustainability*, 12(12), 4859.
- [5] Ahmed,S.; X.Zhou; Y.Pang; L.Jin and J.Bao.2018. Improving Starch-Related Traits in Potato Crops: Achievements and Future Challenges.*Starch- Stärke*. 70(9-10): 1700113.
- [6] Choi,I.; J.Chun; H.S.Choi; J.Park; N.G.Kim; S.K.Lee; C.H.Park; K.H.Jeong; J.W.Nam; J.Cho and K.Cho.2020.Starch Characteristics, Sugars and Thermal Properties of Processing Potato(*Solanum tuberosum* L.) Cultivars Developed in Korea. *American Journal of Potato Research*.97(3):308-317.

- [7] Wagg, C., Hann, S., Kupriyanovich, Y., & Li, S. (2021). Timing of short period water stress determines potato plant growth, yield and tuber quality. *Agricultural Water Management*, 247, 106731.
- [8] Ayad, W.A. ALjuboori and Ahmed A.A. Al-Arazah .2021. Effect of Water Stresses and Genotypes on the Growth and Yield of Muskmelon (*Cucumis melo* L.) Indian Journal of Ecology . 48 Special Issue (13): 101-103.
- [9] Rahman, I.M.D.; Z.A. Begum and H. Hasegawa. 2016. Water Stress. IntechOpen. Pub. Croatia.
- [10] Boscaiu, M. and A. Fita. 2020. Physiological and molecular characterization of crop resistance to abiotic stresses. *Agronomy*, 10(9):1308.
- [11] Farooqi, Z. U. R.; M. A. Ayub; M. Z. Ur Rehman; M. I. Sohail; M. Usman; H. Khalid and K. Naz. 2020. Regulation of drought stress in Plants. In *Plant Life under changing environment*. Academic Press. 77- 104.
- [12] Badr, M. A., El-Tohamy, W. A., Salman, S. R., & Gruda, N. (2022). Yield and water use relationships of potato under different timing and severity of water stress. *Agricultural Water Management*, 271, 107793.
- [13] Bidiegwu, J , G.Bryan, H.G.Jones, and A.Prashar .2015. Coping with drought :stress and adaptive responses in potato and perspectives for improvement
- [14] Obidiegwu, J.; G. Bryan; G. Jones and A. Prashar. 2015. Coping with drought: stress and adaptive responses in potato and perspectives for improvement. *Front. Plant Sci.* 6:542.
- [15] Al-Muntafiqi, Haider Nasser Hussein. (2016). Role of selenium and brassinolide in growth and active compounds of coriander (*Coriandrum sativum* L.) exposed to water stress. PhD Thesis, College of Education, Ibn Al-Haitham, University of Baghdad, Iraq.
- [16] Brodersen, C.R.; A. B. Roddy; J.W. Wason and A.J. McElrone. 2019. Functional status of xylem through time. *Annu. Rev. Plant Biol.*, 70, 407–433.
- [17] Elemike, E.E.; I.M. Uzoh; D.C. Onwudiwe and O.O. Babalola. 2019. The role of nanotechnology in the fortification of plant nutrients and improvement of crop production. *Appl. Sci.*, 9, 499.
- [18] Dayok, S. T.; P. L. Agber and F. D. Uges. 2019. Assessment of supplementary irrigation rate on productivity of potato (*Solanum tuberosum* L.) in Kuru, Jos, Northern Guinea Savanna, Nigeria. *International Journal of Scientific and Research Publications*, 9(3):546-566.
- [19] Valarini , P.J. ; G. Curaqueo , A. Seguel , K. Manzano , R. Rubio , P. Cornejo and F. Borie. 2009. Effect of Compost Application on some Properties of a Volcanic soil from central south chile. *Chilean Journal of Agricultural Research* 69 (3) : 416-425.
- [20] Shayaa, A. H and W. A. Hussein .2019. Effect of neem leaves extract and organic fertillzer in the productivity and quality of two potato cultivars. *Iraqi Journal of Agricultural Sciences* 50(1): 275-285.
- [21] Al-Janabi, Mohammed Ali Aboud. (2005). Evaluation of drip irrigation for onion (*Allium cepa* L.) crop under mulching and organic matter application. Master's Thesis, Soil Department, College of Agriculture, University of Anbar, Iraq.
- [22] Balík, J., Kulháněk, M., Černý, J., Sedlář, O., Suran, P., Asrade, D.A. 2022. The Influence of Organic and Mineral Fertilizers on the Quality of Soil Organic Matter and Glomalin Content. *Agronomy*, 12, 1375. <https://doi.org/10.3390/agronomy12061375>
- [23] Azawi, Rashid Abdullah, and Maher Saleh Mohammed Al-Qaisi. (2021). Study on the agricultural exploitation in some biological characteristics and the biological activity of Entisols and Aridisols horizons in Salah al-Din governorate. *Kirkuk University Journal for Agricultural Sciences*, Volume 12, Issue 2, Pages 134-147.
- [24] Al-Maharbi, Mohammed Zaidan Khalaf. (2014). Effect of irrigation levels and organic matter on growth, yield, and quality of sweet pepper under organic farming system. PhD Thesis, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.
- [25] Wang , F.L. and A.K. Alva. 1999. Transport of soluble organic and inorganic carbon in sandy soils under nitrogen fertilization . *Can. J. Soil* , 79 : 303-310.
- [26] Assefa, S. and S. Tadesse .2019. The principal rose of organic fertilizer on soil properties and agricultural productivity – A review. *Agri Res & Tech: Open Access J.*22(2): ISSN : 2471-6774.

- [27] Al-Mamori, H A. and H, A. Abdul-Ratha.2020.Effect of addition of vermicompost , Bio and mineral fertilizer on the availability of some nutrient in soil and potato yield . Iraqi Journal of Agricultural Sciences:51(2):644-656.
- [28] Bayati, Hussein Jawad Mahram, Abdul Allah Mohammed Salem, and Mohannad Aqeel Ahmed Al-Sheikh. (2020). Effect of organic and chemical fertilization on growth and yield of three local potato varieties. Diyala Journal of Agricultural Sciences, Volume 12, Special Issue of the Fourth International Conference on Agricultural Research: 14-1.
- [29] Al-Maamouri, Haider Abbas Fadel. (2020). Effect of integrated organic, mineral fertilizers, and vermicompost on nitrogen and phosphorus availability in soil and growth and yield of potatoes (*Solanum tuberosum* L.). PhD Thesis, Department of Soil and Water Resources, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.
- [30] Kareem, I ., E, A. Akinrinde., Y, Oladosu., E, K. Eifediyi .,S, Y. Abdulmalik .,S, Y. Alasinrin ., S, A, Kareem., O.F. Adekola.2020. Influence of Organic, Inorganic and Organo-Mineral Fertilizers on Yield and Quality of Sweet Potato (*Ipomoea batatas*). J. Appl. Sci. Environ. Manage. 24 (1) 111-118
- [31] Najem, N. H; F. H. Al-sahaf and H. J. Albayati .2020. The effect of organic residues and spraying of potassium and zinc on some growth characteristics and yield of potato. Plant Archive.20(2):131-139.
- [32] Al-Dulaimi, N. H. A. and N. J. K. Al-Amri .2020. Stimulation growth and yield of potato by buttonwood pruning residues and spraying several micronutrients. Iraqi Journal of Agricultural Sciences 51(4) : 1048-1057.
- [33] Majoul, Abbas Khudhair, and Hanin Mohammed Jasim. (2018). Organic residues and biological factors and their impact on some growth and productivity indicators of potatoes (*Solanum tuberosum* L.). Karbala Journal of Agricultural Sciences, Volume 16, Issue 1: 1.
- [34] Masood, T. K., Ati, A. S., & Hammadi, Q. O. (2023). Effect of Water Stress and Levels of Bio-Organic Fertilizers on Water Productivity and Potato *Solanum tuberosum* L. Yield. *Basrah Journal of Agricultural Sciences*, 36(2), 134-143.
- [35] Yahyaabadi, M., & Dehghani, M. (2021). Effect of drought stress and consumption of organic fertilizers on yield and yield components of potato (Case Study of Isfahan Province). *Iranian Journal of Soil and Water Research*, 52(2), 483-495.
- [36] El-Nabi, A., El-Shal, Z. S. A., Doklega, S., & Abdel Razek, M. E. A. (2022). Effect of water deficit, organic fertilization and some soil additions on potato productivity. *Journal of Plant Production*, 13(8), 611-619.
- [37] Hasan, H., Dais, M., & Suleiman, S. (2023). Leaves attributes and water use efficiency of potatoes grown under water stress after cycocel, salicylic acid, and humic acid sprays. *DYSONA-Applied Science*, 4(2), 62-70.
- [38] Liu, N., C, Shao ., H, Sun ., Z , Liu ., Y, Guan ., L , Wu and B, Zhang . 2020 . Arbuscular mycorrhizal fungi biofertilizer improves American ginseng (*Panax quinquefolius* L.) growth under the continuous cropping regime . Journal Elsevier Geoderma , (363) : 114155
- [39] Osman, Janan, Mohammed Khreibeh, and Shurooq Barbahan. (2020). Effect of soil inoculation with Arbuscular Mycorrhizal Fungi (AMF) on morphological and yield traits of *Solanum tuberosum* L. planted in spring season. Tishreen University Journal, Biological Sciences Series, Volume 44(3): 295-308.
- [40] Batista BD, Singh BK. 2021. Realities and hopes in the application of microbial tools in agriculture. *Microb Biotechnol*. 14(4):1258–1268. <https://doi.org/10.1111/1751-7915.13866>
- [41] Lopes MJS, Dias-Filho MB, Gurgel ESC. 2021. Successful plant growth-promoting microbes: Inoculation methods and abiotic factors. *Front Sustain Food Syst*. 5. <https://www.frontiersin.org/articles/10.3389/fsufs.2021.606454>.
- [42] Fukami J, Cerezini P, Hungria M. 2018. Azospirillum: Benefits that go far beyond biological nitrogen fixation. *AMB Express*. 8:73. <https://doi.org/10.1186/s13568-018-0608-1>.
- [43] Zeffa DM, Perini LJ, Silva MB, de Sousa NV, Scapim CA, de Oliveira ALM, do Amaral J_unior AT, Azeredo Goncalves LS. 2019. Azospirillum brasilense promotes increases in growth and

- nitrogen use efficiency of maize genotypes. PLoS One. 14(4):e0215332. <https://doi.org/10.1371/journal.pone.0215332>.
- [44] Wasan, S. M. K and A.W. A. Al-Juboori. 2023.Effect of Bio fertilizers and Spraying with Magnesium and Calcium on Vegetative Growth Indicators of Sweet Corn IOP Conf. Series: Earth and Environmental Science 1225 012031
- [45] Saad, S. A. H and E, A. Salem.2020. Studies of Water Needs of Potatoes According to Climatic Conditions Cultivated in Bioinoculated Sandy Soil.9(4): 796-811.
- [46] Kumar , S., R, Chandra ., L, Behera., C, Keswani and E, Sansinenea.2021. Elevation of Systemic Defense in Potato Against Alternaria Solani by a Consortium of Compatible Trichoderma Spp . Research Article : <https://doi.org/10.21203/rs.3.rs-409856/v1>. p : 1-20.
- [47] Delorge, I.; M. Janiak; S. Carpentier and P. V. Dijck. 2014. Fine tuning of trehalose biosynthesis and hydrolysis as novel tools for the generation of abiotic stress tolerant plants. Frontiers in Plant Science . Plant Physiology. 5 : 147.
- [48] Tuman, Sabreen Sabah. (2021). Effect of polymers and mycorrhizal fungi on growth and yield of potatoes under different levels of water stress. PhD Thesis, College of Agriculture, University of Kufa, Iraq.
- [49] Zahran, M. M. A. A., Hefzy, M., & Mostafa, H. H. (2020). Enhancing the productivity of potato crop under drought stress by using some biological treatments. *Int. J. Environ*, 9(2), 83-103.
- [50] Nemati, A., Aboutalebian, M. A., & Chaichi, M. (2024). Mitigating the effects of water-deficit stress on potato growth and photosynthesis using mycorrhizal fungi and phosphate-solubilizing bacteria. *The Journal of Agricultural Science*, 1-13.
- [51] Mehmood, S., Muneer, M. A., Tahir, M., Javed, M. T., Mahmood, T., Afridi, M. S., ... & Chaudhary, H. J. (2021). Deciphering distinct biological control and growth promoting potential of multi-stress tolerant *Bacillus subtilis* PM32 for potato stem canker. *Physiology and Molecular Biology of Plants*, 27, 2101-2114.
- [52] Batool, T., Ali, S., Seleiman, M. F., Naveed, N. H., Ali, A., Ahmed, K., ... & Mubushar, M. (2020). Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. *Scientific Reports*, 10(1), 16975.

An Investigation of the Type of Bacteria Found in the Dust of Iraq's Al-Diwaniyah City

Haneen Fahim Jaber¹ and Saba Abdulameer Kadhim Al-Ziadi²

^{1,2}Department of Ecology, College of Science, University of Al-Qadisiyah, Al-Qadisiyah, Iraq.

¹E-mail: honyfahem@gmail.com

²E-mail: saba.abdulameer@qu.edu.iq

Abstract. The recurrence of dust in Iraq, especially in the city of Al-Diwaniyah, which suffers from frequent high levels of dust in the atmosphere recently. Given the significant environmental and health impacts of these events on Iraq in general and on the city of Diwaniyah in particular, Dust samples were collected in October 2023 for four areas in the city of Al- Diwaniyah, including the University district, Um Al-Khail area, Al-Urouba area, and Al- Jezair area. Bacterial species belonging to the genus *Bacillus* were isolated and identified based on the *16Sr RNA* gene and the following species were obtained: *Bacillus pumilus*, *Bacillus altitudinis*, *Bacillus amyloliquefaciens*, *Virgibacillus carmonensis* and *Bacillus stratospheric*, all isolates were registered in the NCBI gene bank website where the results of the genetic tree analysis showed a convergence between our local *Bacillus* spp isolates and the global isolates registered within (NCIB).

Keywords. *Bacillus* spp , Dust, Al-Diwaniyah city, Microbial air pollution.

1. Introduction

Dust storms are strong, turbulent winds that lift dust particles to very high altitudes and travel distances that may reach hundreds or thousands of kilometers [1]. This occurs in areas with weak soil, devoid of vegetation, little moisture and rain, so the raised sand grains may form a cloud of sand that blocks the sun and changes environmental standards, aerosols and climate and affects public health, especially for people who suffer from respiratory problems in areas where these winds blow [2]. The dust may be organic, such as in the burning of coal, herbs, animal and poultry waste, and waste recycling plants, or it may be inorganic, such as cement and mining plants, which pose a great danger to public health because they contain heavy elements or it may be a mixture of both. One of the negative effects of dust storms is that they strip the earth of its best components, which are light-weight and small organic particles rich in plant nutrients, which are the main component of the surface layers of soil, thus leaving it worthless agricultural, storms also affect all sectors of land, air and sea transport because they obstruct vision, which results in disrupting people's interests and causing them huge economic losses. They also cover plants, which affects the main vital processes such as transpiration and photosynthesis. Not to mention that they cover sidewalks, homes and all life facilities, which requires double the effort of service departments and citizens to clean them, it has also been noted that dust affects seas, lakes and rivers, as it causes changes in their chemical, physical and biological nature such as salinity, acidity, color, taste and the type of living organisms that inhabit them, especially coral reefs,

which are considered the lungs of the earth [3]. Perhaps the most dangerous aspect is what dust particles carry with them of bacterial and fungal pathogens, as their sizes, light weights and surface topography help them stick to dust particles and move with them thousands of kilometers [4]. As for bacteria, their small size and light weight facilitate their mixing with dust particles and transporting them to distant places, especially since these microscopic organisms form dormant spores that resist difficult conditions, and once they reach the appropriate place, they begin the germination process. These spores sometimes have thorns, holes, or small hairs that increase the chance of their transmission by dust. Among the bacterial species that have been recorded as a microbial load are *Staphylococcus aureus*, *P. aeruginosa*, *Mycobacterium tuberculosis*, and many other species that are considered to be the causes of various diseases [5].

2. Materials and Methods

2.1. Sample Collection

Dust samples were collected in October 2023 for four areas in the city of Al-Diwaniyah, including University district, Um Al-Khail area, Al-Urouba area, and Al- Jezair area and were collected according to the method of reference [6].

2.2. Preparation of Dust Dilutions

According to reference [7] dust dilutions were prepared for cultivation on the bacterial medium by spreading on plates, where 1 g of dust was weighed and added to 10 ml of distilled water. This is considered the stock solution, then 1 ml of this solution was taken and added to 9 ml of Normal saline to become the concentration 10^{-1} . The dilution process continued until we obtained a concentration of 10^{-5} , then the plates were placed in the incubator at 37°C for 24 hours.

2.3. Microscopic Diagnosis of Bacterial Isolates

The characteristics of the cells were studied under the microscope after staining with Gram stain, including the shape of the cells and their clustering, then they were diagnosed based on what [8] stated.

2.4. Genomic DNA Extraction

The bacterial DNA was extracted using the DNA extraction by the Min Kit ForGen. Then the polymerase chain reaction was performed using the Gotaq@Green master mix kit to extract the *16Sr RNA* gene according to the instructions of the manufacturer (promga/USA). The concentration of the extracted DNA was confirmed using the Nano drops spectrophotometer. The polymerase chain reaction amplification was performed using a thermal cycler to amplify the *16Sr RNA* gene using the following primer from (promga/USA).

Table 1. Primers used in this study.

Gene Name	Primer sequences (5' 3')	Product Size(pb)	References
<i>16Sr RNA</i>	F 5' AGAGTTTGATCCTGGCTCA 3' R 5' GGTACCTTGTTACGACTT 3'	1500bp	25

2.5. Thermal Cycles for the PCR Test (conditions Thermocycler PCR)

The test was performed using a thermocycler PCR device and according to the *16Sr RNA* gene The primer was used.

Table 2. Optimal conditions for thermal cycles of PCR reaction.

PCR Step	Temperature	Cycle	Time
Initial denaturation	95c	1	5 min
Denaturation	95c		1min
Annealing	53c	40	1min
Extension	72 C ⁰		3 min
Final Extension	72 C ⁰	1	10 min
Hold	4 C ⁰	-	forever

2.6. PCR Products were Subjected to Agarose Gel Electrophoresis

A 1.5% agarose gel was made using IX TBE, and was melted for 15 min at 100 °C in an Agarose water bath, and then cooled to 50 °C. Then, the Agarose gel solution was mixed with 3 µl of ethidium bromide dye. After adding the gel solution to the tray and placing the comb properly. The comb was carefully removed from the plate after allowing it to solidify for fifteen minutes at room temperature. After the gel tray was fixed in the electrophoresis chamber, IX TBE buffer was added to it. Each well received 10µl of PCR product and 3 µl of 100 base pair ladder were added to the first well. 80 V for 65 min was activated. PCR products were visualized using an ultraviolet light device [9].

3. Results and Discussion

3.1. Isolation and Identification

Samples were collected from the dust of Al-Diwaniyah city for four regions for the purpose of isolating pathogenic bacteria present in the dust of Al-Diwaniyah city in October 2023, 77 bacterial isolates were isolated, including 22 bacterial isolates from the University neighborhood area, 18 bacterial isolates from the Al-Urouba area, 17 bacterial isolates from the Um Al-Khail area, and 20 bacterial isolates from the Al-Jezair area , that is, the total number of bacterial isolates was 77 isolates from the air of Diwaniyah city, and the University neighborhood area was the highest percentage in the number of bacterial isolates, 22 isolates at a rate of 28.57. it is followed by the Al- Jezair region with 20 islets and a percentage of 25.97, then comes the Al-Uruba region with 18 islets and a percentage of 23.37, and the Um Al-Khail region with 17 islets and a percentage of 22, as shown in Table (3) and Table (4).

Table 3. Number of bacterial isolates distributed according to isolation areas.

Isolation areas	No. Isolation	Percentage%
University district	22	28.57%
Al-Urouba district	18	23.37%
Umm Al-Khail district	17	22%
Al- Jezair district	20	25.97%
Total	77	100%

Table 4. Number of bacterial genera isolated from dust of Al-Diwaniyah city areas.

Isolates	Study areas			
	Al-Urouba district	Um Al-Khail district	Al-Jezair district	University district
<i>Bacillus amyloliquefaciens</i>	0	7	0	0
<i>Bacillus altitudinis</i>	5	0	0	0
<i>Bacillus Stratospheric</i>	0	5	0	0
<i>Bacillus pumilus</i>	5	0	0	0
<i>Bacillus altitudinis</i>	0	0	13	0
<i>Virgibacillus carmonensis</i>	0	0	7	0
<i>Bacillus pumilus</i>	5	0	0	0
<i>Bacillus pumilus</i>	0	0	0	22
<i>Bacillus altitudinis</i>	3	0	0	0
<i>Bacillus pumilus</i>	0	5	0	0
Total	18	17	20	22

3.2. Molecular Diagnosis of Bacterial Isolates

3.2.1. Detection of 16SrRNA Gene for Bacterial Isolates

In the current study, ten bacterial isolates were selected. After extracting the genomic DNA and examining the gel using the ultraviolet unit, DNA bands with a molecular size of 1500 pb base pairs appeared for the bacterial isolates as shown in Figure (1).

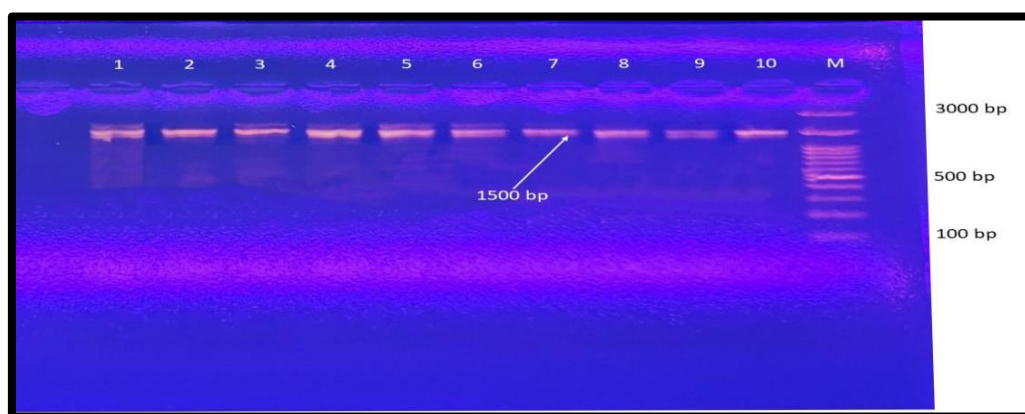


Figure 1. Electrophoresis of 1.5% agarose gel. Output of amplification of 16Sr RNA gene at 80 V for 65 min with a volume of 1500 pb base pair (1-10) Selected bacterial isolates containing 16Sr RNA gene M: Size guide. DNA ladder (100 base pairs).

The results of molecular diagnosis of bacterial isolates used in the current study using the *16Sr RNA* gene showed that all isolates possess this gene in their genetic form. After diagnosis using the *16Sr RNA* gene, it is one of the accurate diagnoses of bacterial species due to the presence of a fixed sequence for each type of bacterial species, due to the stability of the sequences of this gene, it played an important role in the classification of primitive organisms and molecular diagnosis [10].

Molecular diagnosis of bacterial isolates is important and has very accurate and sensitive results in the field of diagnosis. The *16Sr RNA* gene was historically the most widely used for the purpose of diagnosis, due to its high ability to possess a number of moderate gene copies attributed to the bacterial species that possess it, where all these bacterial genes were found, even those prone to mutation, due to the *16Sr RNA* gene containing nitrogenous bases that provide information or instructions on any bacteria that contain them, which ultimately facilitates the diagnosis of these bacterial species to which they belong. The great development in molecular biology has been exploited in many modern, rapid and advanced techniques such as detecting genetic relationships between isolates as well as genetic mutations [11].

The sequencing technique was used for the diagnosed isolates after completing the PCR examination and the examination results were sent to Macrogen Company in South Korea to conduct the nitrogenous bases sequence using the AB DNA sequencing device, then the results were compared with the bacterial sequence registered in the NCBI Gene Bank website using the MEGA10 program , as shown in Table (5) which shows the results of registering our local bacterial isolates globally to draw the genetic tree of bacteria *Bacillus* spp.

Table 5. Serial number and names of local isolates on the NCBI website.

Areas	Isolates name	Serial number
Al-Urouba	<i>Bacillus stratosphericus</i>	PP601379
	<i>Bacillus pumilus</i>	PP621477
	<i>Bacillus pumilus</i>	PP621506
	<i>Bacillus altitudinis</i>	PP621728
Um Al-Khail	<i>Bacillus amyloliquefaciens</i>	PP601312
	<i>Bacillus altitudinis</i>	PP621187
	<i>Bacillus pumilus</i>	PP621722
Al-Jezair	<i>Bacillus altitudinis</i>	PP621478
	<i>Virgibacillus carmonensis</i>	PP621564
University	<i>Bacillus pumilus</i>	PP621726

The results of recording local bacterial isolates isolated from dust globally (as shown in the table) showed the appearance of the following bacterial species: *Bacillus pumilaus* ,*Bacillus altitudinis*, *Bacillus amyloliquefaciens* , *Virgibacillus carmonensis* and *Bacillus stratospheric*. They were all Gram-positive and the genetic tree of these species was drawn. These results were consistent with [12]. Who found that the predominant bacteria isolated from dust are Gram-positive bacteria.

The results showed that the genetic tree of *Bacillus amyloliquefaciens* isolated from dust was similar to global isolates registered within (NCBI) with convergence rates ranging between 97%, 88% and 95%, and the isolates included:

MK120015.1 *Bacillus* sp. (in: Bacteria) strain 2SS1 16S ribosomal RNA gene Turkey
 .LN867273.1 *Bacillus* sp. 0913CV7GTS2 partial 16S rRNA gene isolate 0913CV7GTS2 Spain.
 MN100143.1 *Bacillus amyloliquefaciens* strain C13 16S ribosomal RNA gene Brazi.
 OP175964.1 *Bacillus amyloliquefaciens* strain DRDOBU048 16S ribosomal RNA gene India.
 OQ702658.1 *Bacillus subtilis* subsp. *subtilis* strain MBRrH9 16S ribosomal RNA geneIndonesia.
 MW380509.1 *Bacillus subtilis* strain SEM11H4 16S ribosomal RNA gene China. MW673667.1
Bacillus subtilis strain BK-3 16S ribosomal RNA gene India. MK823975.1 *Bacterium* strain
 BS0787 16S ribosomal RNA gene China. JX297473.1 *Bacillus licheniformis* strain CSP-002 16S
 ribosomal RNA gene India. The closest bacteria to isolation were from China and India, with a
 percentage of approximately 97%, as shown in Figure (2).

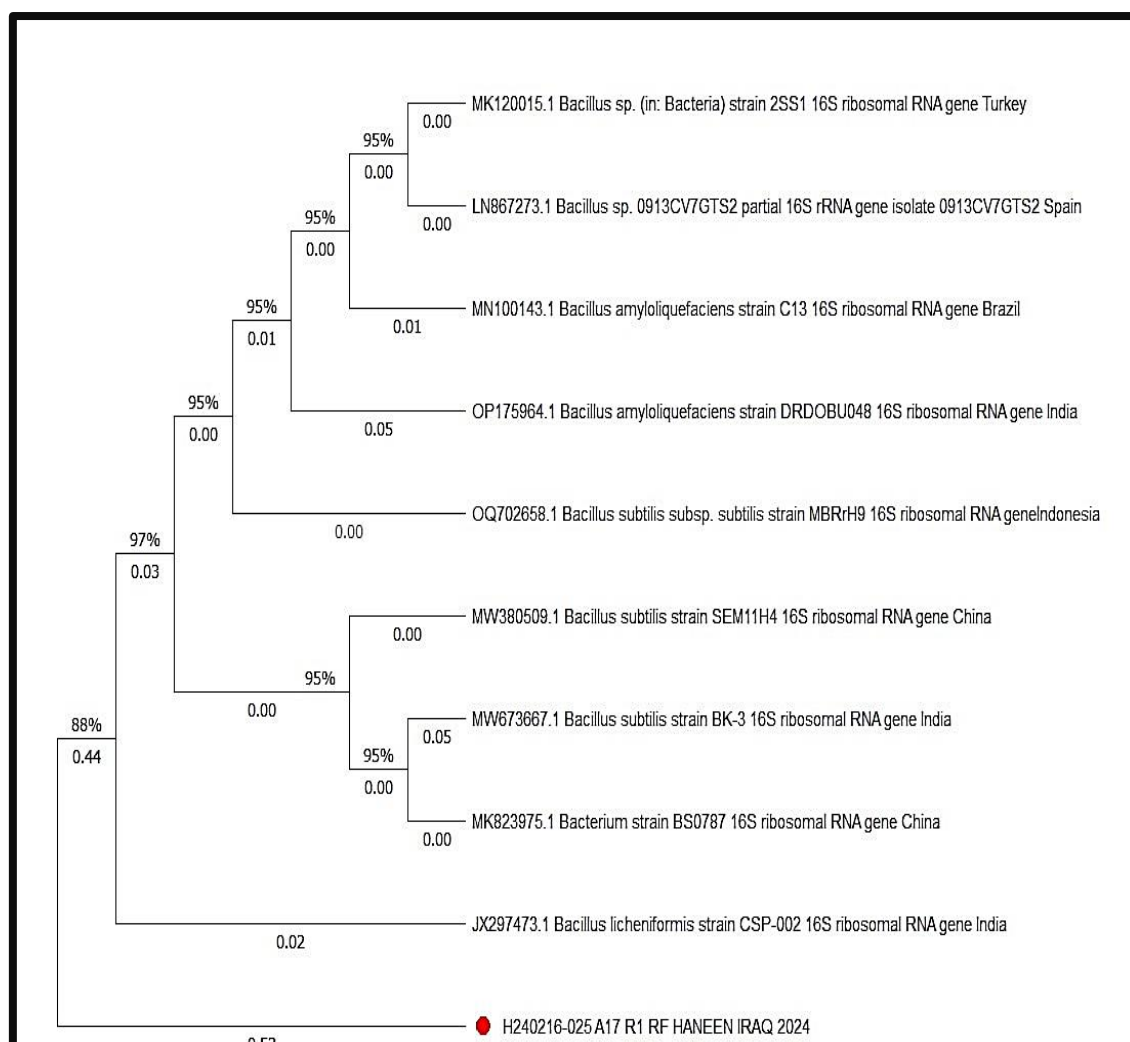


Figure 2. The genetic tree of *Bacillus amyloliquefaciens* isolated from dust.

The results showed that the genetic tree of *Bacillus stratosphericus* isolated from dust was similar to global isolates registered within NCBI with a convergence percentage ranging between 85, 86, 64, 87, 90, and 82. The isolates included :MT310711.1 *Bacillus altitudinis* strain SC109 16S ribosomal RNA gene China. PP125677.1 *Bacillus altitudinis* strain MCCB 446 16S ribosomalRNA gene India OQ597191.1 *Bacillus altitudinis* strain STENUS3 [14] 16S

ribosomalRNA gene India. KY622390.1 *Bacillus altitudinis* strain LA311 16S ribosomal RNA gene USA. MN910298.1 *Bacillus altitudinis* strain LL4 16S ribosomal RNA gene China. OR220840.1 *Bacillus altitudinis* strain NIOSV141 16S ribosomal RNA gene India. OR220827.1 *Bacillus altitudinis* strain NIOSV34 16S ribosomal RNA geneIndia. OL589129.1 *Bacillus altitudinis* strain BLU 16S ribosomal RNA gene Iran. It was most similar to the two Indian isolates by 90%, as shown in Figure (3).. OR220840.1 *Bacillus altitudinis* strain NIOSV141 16S ribosomal RNA gene India. OR220849.1 *Bacillus altitudinis* strain NIOSV187 16S ribosomal RNA gene India

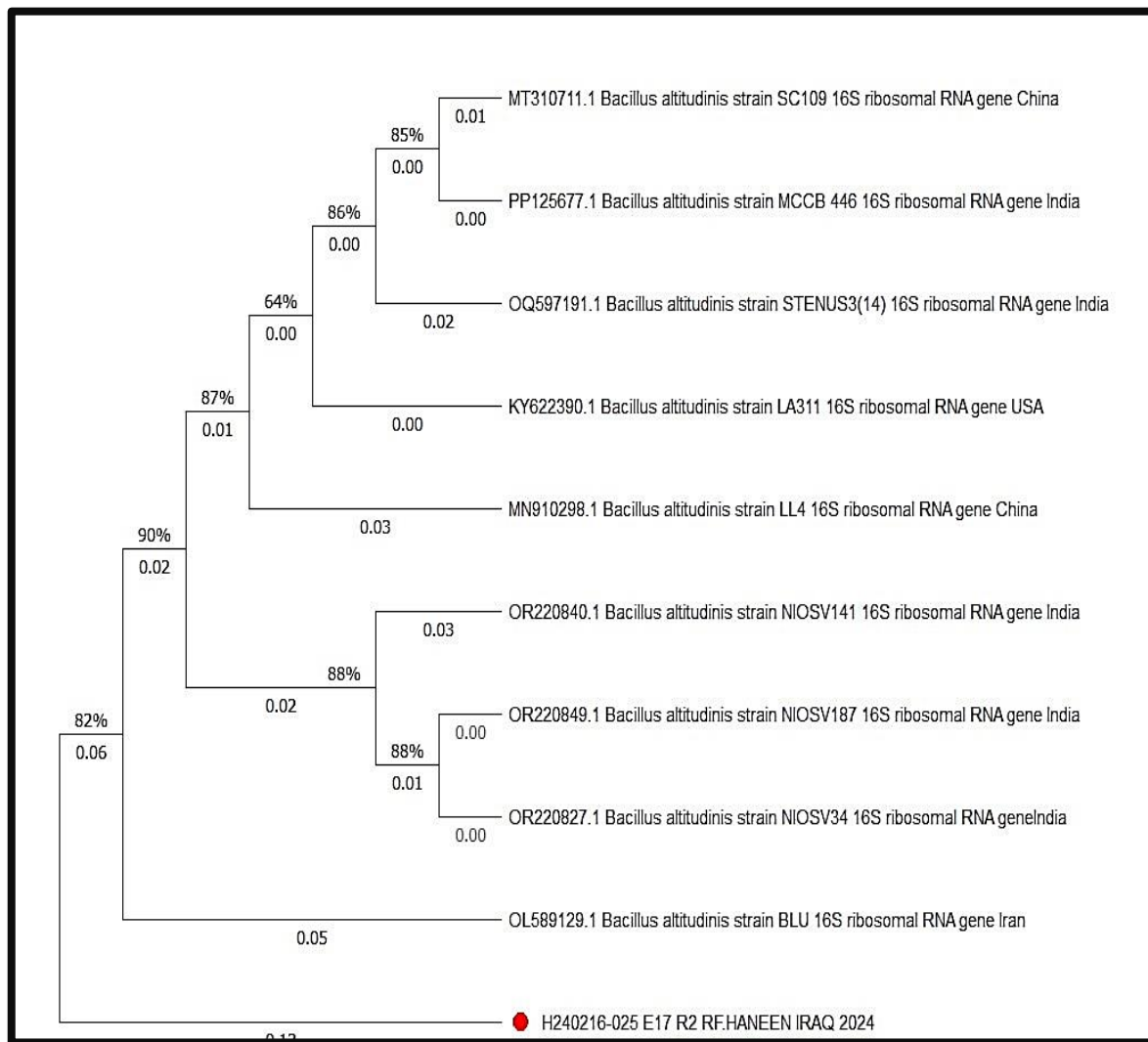


Figure 3. The genetic tree of *Bacillus stratosphericus* isolated from dust.

The results showed that the genetic tree of *Bacillus altitudinis* isolated from dust was similar to global isolates registered within (NCBI) with a convergence percentage ranging between 94, 93, and 73, and the isolates included: MT310686.1 *Bacillus altitudinis* strain SC5 16S ribosomal RNA gene China. OR392883.1 *Bacillus* sp. (in: firmicutes) strain nsu-1 16S ribosomal RNA gene China. OR353534.1 *Bacillus altitudinis* strain GPD 16S ribosomal RNA gene China. KY621527.1 *Bacillus pumilus* strain IP14 16S ribosomal RNA gene China. KC692172.1 *Bacillus*

pumilus strain ML211 16S ribosomal RNA gene Italy. OP185262.1 *Bacillus pumilus* strain BC-13 16S ribosomal RNA gene China. KF814106.1 *Bacillus pumilus* strain Ljb-10 16S ribosomal RNA gene China. KF933667.1 *Bacillus pumilus* strain HNS70 16S ribosomal RNA gene China. MH261104.1 *Bacillus pumilus* strain MGB3102 16S ribosomal RNA gene China. It was most similar to Chinese isolation. MT310686.1 *Bacillus altitudinis* strain SC5 16S ribosomal RNA gene China and Italian isolation. KC692172.1 *Bacillus pumilus* strain ML211 16S ribosomal RNA gene Italy.

At a rate of nearly 94%, as shown in Figure (4).

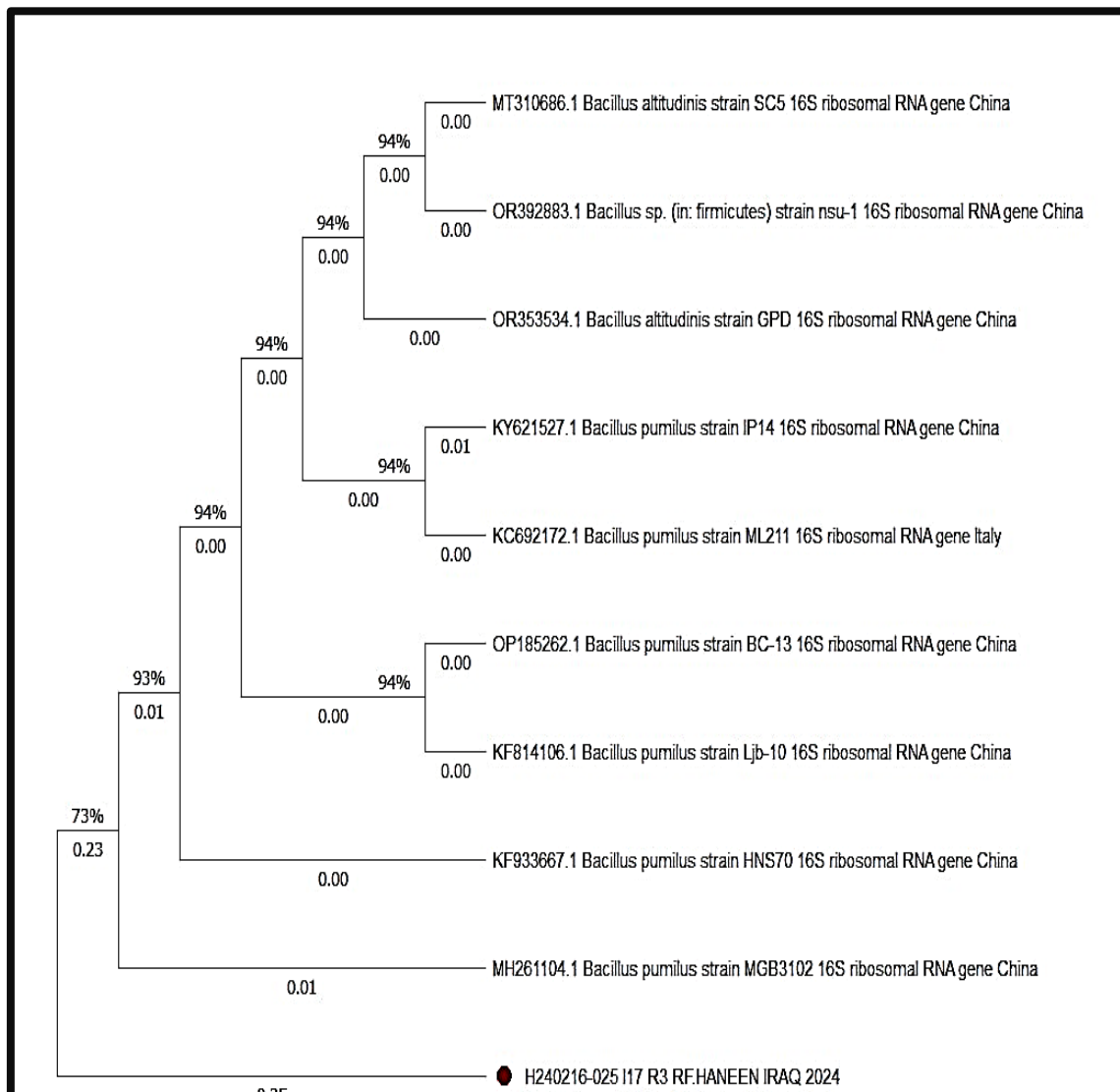


Figure 4. The genetic tree of *Bacillus altitudinis* isolated from dust.

The results showed that the genetic tree of *Bacillus pumilus* isolated from dust was similar to global isolates registered within NCBI with a convergence rate ranging between 96, 97, 99, and 100, and the isolates included: MG818474.1 *Bacillus pumilus* strain Kh16 16S ribosomal RNA gene Iran. MG818473.1 *Bacillus pumilus* strain Kh5 16S ribosomal RNA gene Iran. HQ647270.1

Bacillus pumilus strain T37 16S ribosomal RNA gene China. OL629593.1 *Bacillus pumilus* strain ASB27 16S ribosomal RNA gene India. OQ931913.1 *Bacillus safensis* strain EBCH3 16S ribosomal RNA gene Uzbekistan. KJ524515.1 *Bacillus pumilus* strain BF17 16S ribosomal RNA gene Turkey. HQ858059.1 *Bacillus pumilus* strain MB3 NIOT 16S ribosomal RNA gene India. HM011259.1. *Bacillus pumilus* strain OTN5G 16S ribosomal RNA gene India. OQ676638.1 *Bacillus pumilus*. strain cwf25 16S ribosomal RNA gene India , it was similar to turkish isolates.. KJ524515.1. *Bacillus pumilus* strain BF17 16S ribosomal RNA gene Turkey . HM011259.1 *Bacillus pumilus* strain OTN5G 16S ribosomal RNA gene India. 00% from India as shown in Figure (5).

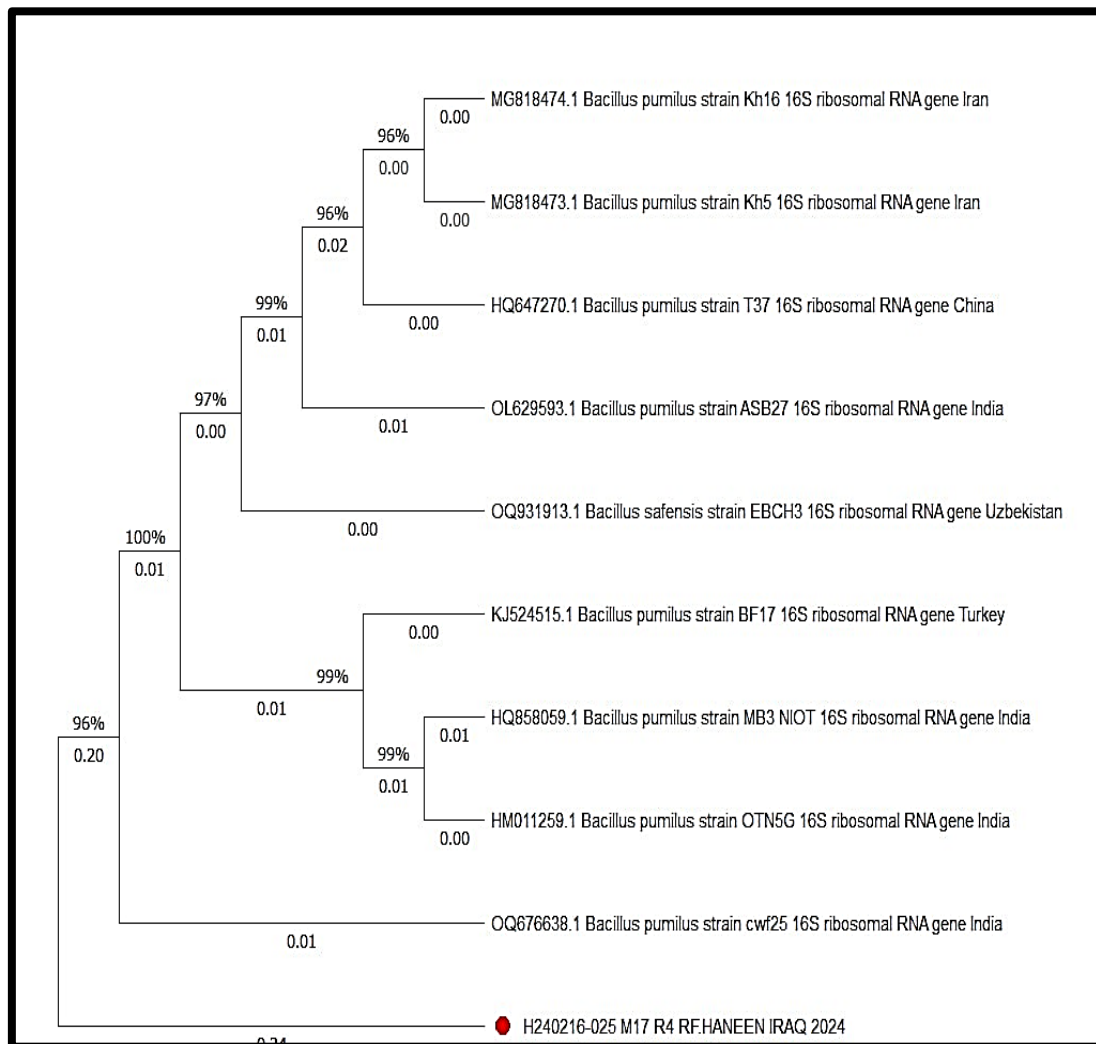


Figure 5. The genetic tree of *Bacillus pumilus* isolated from dust.

The results showed that the genetic tree of *Bacillus altitudinis* isolated from dust was similar to global isolates registered within (NCBI) with a convergence rate ranging from 98.94 and the isolates included: EF032675.1 *Bacillus pumilus* strain AU18 16S ribosomal RNA gene India KT965085.1 *Bacillus pumilus* strain JeYg5 16S ribosomal RNA gene Algeria KM226921.1 *Bacillus pumilus* strain PPL-SC6 16S ribosomal RNA gene Republic of Korea KT036668.1 *Bacillus pumilus* strain NDY-10 16S ribosomal RNA gene China HQ164544.1 *Bacillus pumilus*

strain sk-8 16S ribosomal RNA gene China OR125667.1 *Bacillus australimaris* strain SBI 16S ribosomal RNA gene India LC010678.1 *Bacillus pumilus* gene for 16S ribosomal RNA strain: JKCM-Q-1B Japan. OR272012.1 *Bacillus altitudinis* strain VUMS1 16S ribosomal RNA gene Malaysia. EU620415.1 *Bacillus pumilus* strain S8-07 16S ribosomal RNA gene India. The closest resemblance to the isolate from China, Korea and Japan was about 98% as shown in Figure (6).

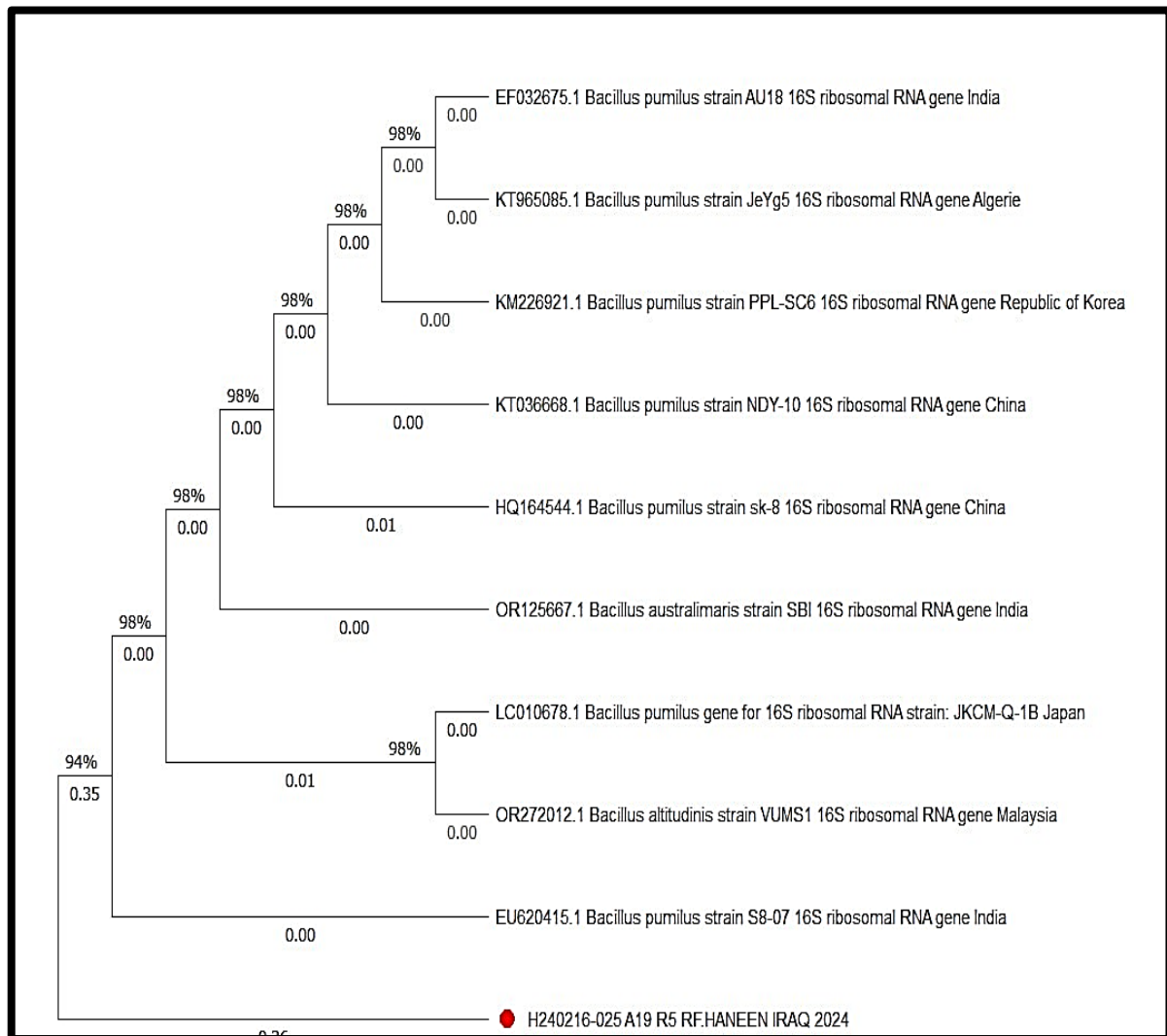


Figure 6. The genetic tree of *Bacillus altitudinis* isolated from dust.

The results showed that the genetic tree of *Virgibacillus carmonensis* isolated from dust was similar to global isolates registered within (NCBI) with a convergence rate ranging from 98, 93, 97, 71, 96 and included the isolates.: OM536059.1 *Virgibacillus necropolis* strain XB47 16S ribosomal RNA gene China OM536058.1 *Virgibacillus necropolis* strain *Virgibacillus carmonensis* strain 215-4.1-LH-Heh(s)-10 16S ribosomal RNA gene Spain EF010642.1 *Virgibacillus carmonensis* strain 264-3.2-LH-H16(s)-09 16S ribosomal RNA gene Spain AJ316303.1 *Virgibacillus carmonensis* partial 16S rRNA gene type strain LMG 20966T BELGIUM NR 025481.1 *Virgibacillus carmonensis* strain LMG 20964 16S ribosomal RNA USA MF682964.1 *Virgibacillus carmonensis* strain LM2315 16S ribosomal RNA gene Republic

of Korea MT516476.1 *Virgibacillus* sp. strain L15 16S ribosomal RNA gene China. The closest resemblance was to the isolate from Spain. EF010649.1 *Virgibacillus carmonensis* strain 215-4.1-LH-Heh(s)-10 16S ribosomal RNA gene Spain. LMG 20964 16S ribosomal RNA USA with a percentage of approximately 97%, as shown in Figure (7).

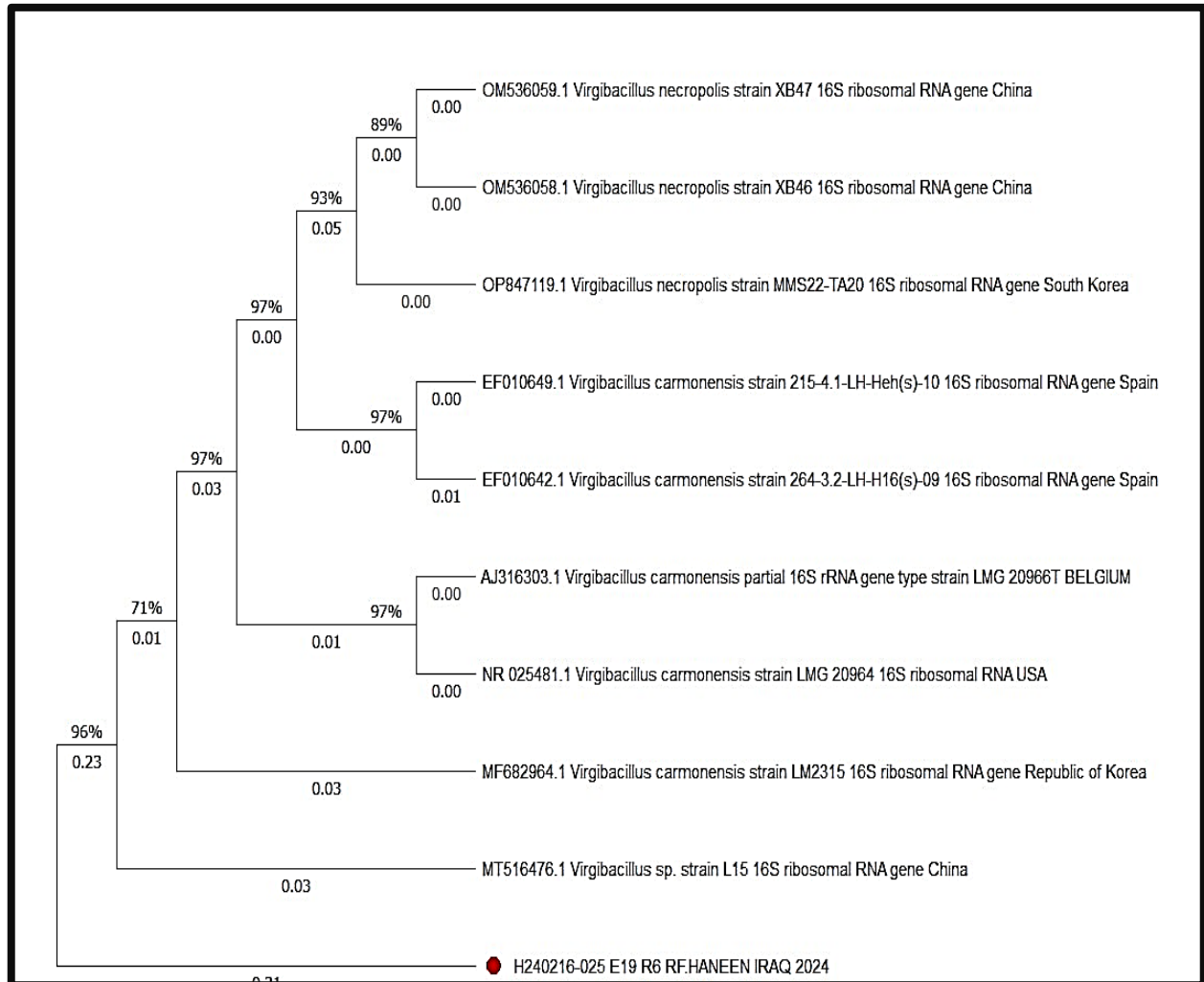


Figure 7. The genetic tree of *Virgibacillus carmonensis* bacteria isolated from dust.

The results of the genetic tree of *Bacillus Pumilus* isolated from dust showed a similarity with global isolates registered within (NCBI) with a convergence percentage ranging from 98.95 and the isolates included: OR570804.1 *Bacillus pumilus* strain LJ-6 16S ribosomal RNA gene partial sequence China. KT986126.1 *Bacillus pumilus* strain Lmb061 16S ribosomal RNA gene partial sequence China. KX344000.1 *Bacillus pumilus* strain G44 16S ribosomal RNA gene partial sequence India. KP992917.1 *Bacillus pumilus* strain xqq17 16S ribosomal RNA gene partial sequence China. KX343944.1 *Bacillus pumilus* strain PD5 16S ribosomal RNA gene partial sequence India. KY078813.1 *Bacillus pumilus* strain Rml7 16S ribosomal RNA gene partial sequence Malaysia. PP278051.1 *Bacillus pumilus* strain IIIH 16S ribosomal RNA gene partial sequence Nigeria. KY515400.1 *Bacillus pumilus* strain R184 16S ribosomal RNA gene partial sequence USA. HQ858059.1 *Bacillus pumilus* strain MB3 NIOT 16S ribosomal RNA gene

partial sequence inda The closest resemblance to the isolate from India Malaysia was 98% as shown in Figure (8).

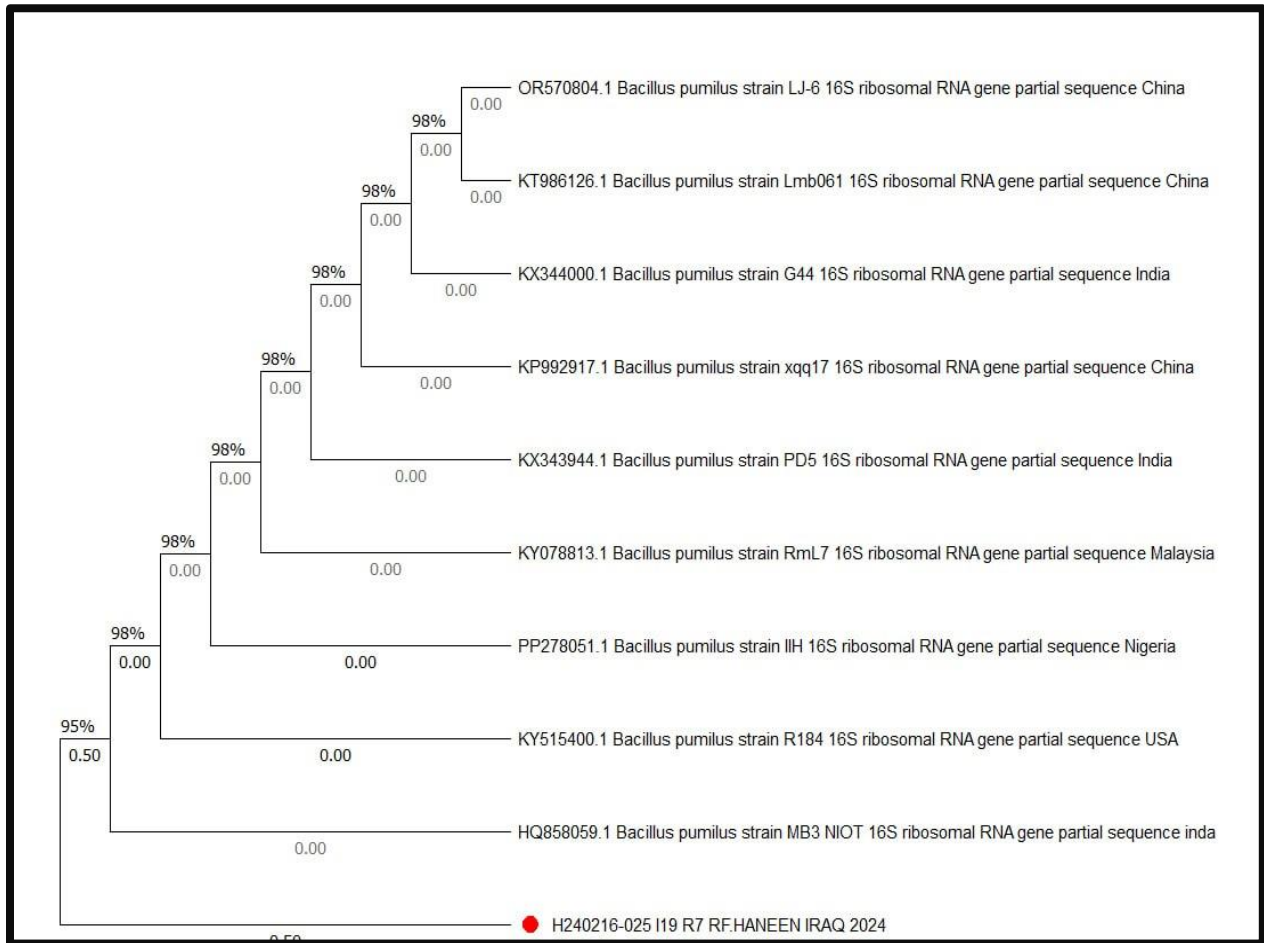


Figure 8. Genetic tree of *Bacillus pumilus* isolated from dust.

The results of the genetic tree of *Bacillus pumilus* isolated from dust showed similarity with global isolates registered within ((NCBI with a convergence percentage ranging from 88, 89, 74, 67, 81 and the isolates included: AB301019.1 *Bacillus pumilus* gene for 16S rRNA partial sequence strain: GH19 Japan MG546214.1 *Bacillus pumilus* strain JMB004 16S ribosomal RNA gene India FJ743437.1 *Bacillus pumilus* strain VIT BP 16S ribosomal RNA gene India AM778191.1 *Bacillus pumilus* partial 16S rRNA gene strain DURCK14 INDIA GQ285084.1 *Bacillus pumilus* strain SD8B2 16S ribosomal RNA gene India KF705249.1 *Bacillus pumilus* strain P12 16S ribosomal RNA gene India MF476191.1 *Bacillus pumilus* strain GoM 4 16S ribosomal RNA gene India MG738314.1 *Bacillus pumilus* strain ASSIUT 3 16S ribosomal RNA gene Egypt It was most similar to the isolate from India and China with a percentage of approximately 98% as shown in Figure (9).

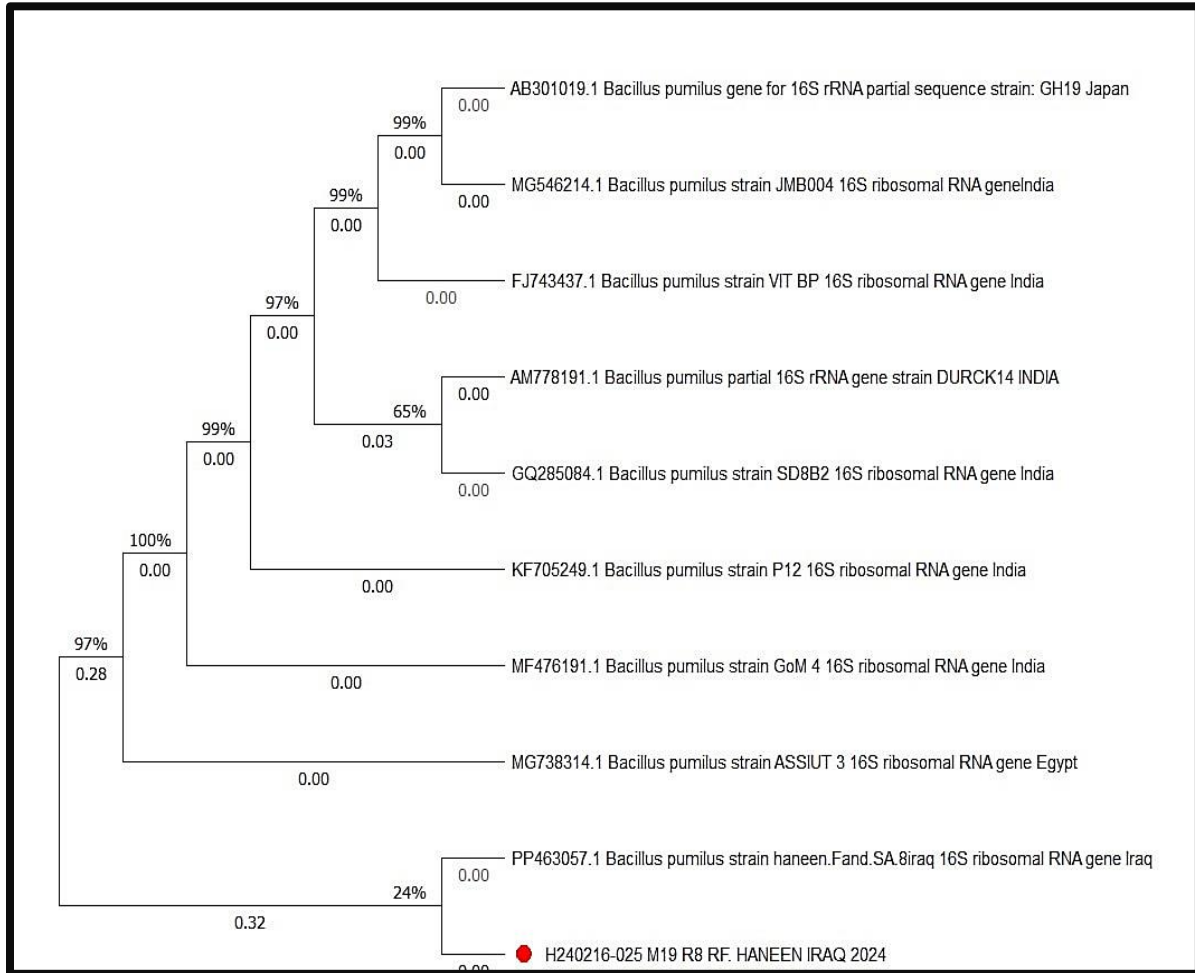


Figure 9. The genetic tree of *Bacillus pumilus* isolated from dust.

The results of the genetic tree of *Bacillus altitudinis* isolated from dust showed a similarity with global isolates registered within (NCBI) with a convergence percentage ranging between 88, 89, 74, 67, and 81, and the isolates included. OR594203.1 *Bacillus altitudinis* strain SS24 16S ribosomal RNA gene China. KU898276.1 *Bacillus altitudinis* strain 10 16S ribosomal RNA gene China OQ382865.1 *Bacillus altitudinis* strain J1 16S ribosomal RNA gene China MF954002.1 *Bacillus altitudinis* strain BCZ2 16S ribosomal RNA gene India MT310686.1 *Bacillus altitudinis* strain SC5 16S ribosomal RNA gene China MK521051.1 *Bacillus altitudinis* strain HTI 9 16S ribosomal RNA gene Malaysia OQ195705.1 *Bacillus altitudinis* strain AS K1(sw) 16S ribosomal RNA gene India. MH261001.1 *Bacillus altitudinis* strain MGB2029 16S ribosomal RNA gene China. MK386767.1 *Bacillus altitudinis* strain CCBDa-6 16S ribosomal RNA gene Colombia. It was most similar to the isolate from China and India with a percentage of approximately 89%, as shown in Figure (10).

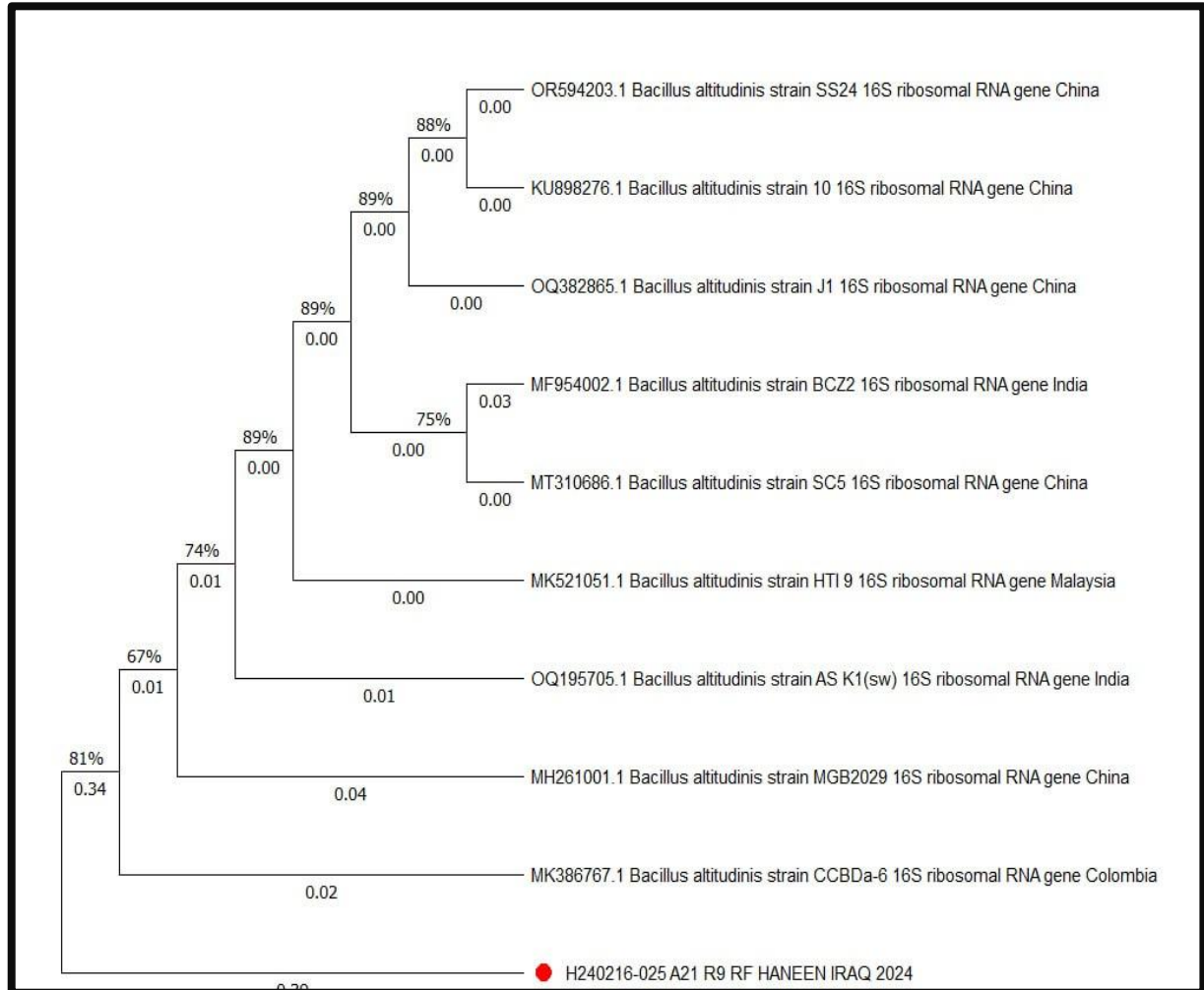


Figure 10. Genetic tree of *Bacillus altitudinis* isolated from dust.

The results of the genetic tree of *Bacillus pumilus* isolated from dust showed similarity with global isolates registered within (NCBI) with a convergence rate ranging between 95, 80, 85 and the isolates included: MZ712989.1 *Bacillus altitudinis* strain ABT AC37 16S ribosomal RNA gene India. MT184826.1 *Bacillus pumilus* strain NWPZ-14 16S ribosomal RNA gene India. MT126245.1 *Bacillus pumilus* strain SYA4 16S ribosomal RNA gene China. MT318666.1 *Bacillus pumilus* strain AS8 16S ribosomal RNA gene China. JX987716.1 *Bacillus pumilus* strain E14 16S ribosomal RNA gene China. OQ824998.1 *Bacillus pumilus* strain ALP-14 16S ribosomal RNA gene China. ON678270.1 *Bacillus pumilus* strain Y-814 16S ribosomal RNA gene India. KX905145.1 *Bacillus pumilus* strain AM08 16S ribosomal RNA gene Mexico. KM066948.1 *Bacillus pumilus* strain PJRB5 16S ribosomal RNA gene India. The closest resemblance to the isolate from China and India was approximately 95%, as shown in Figure (11).

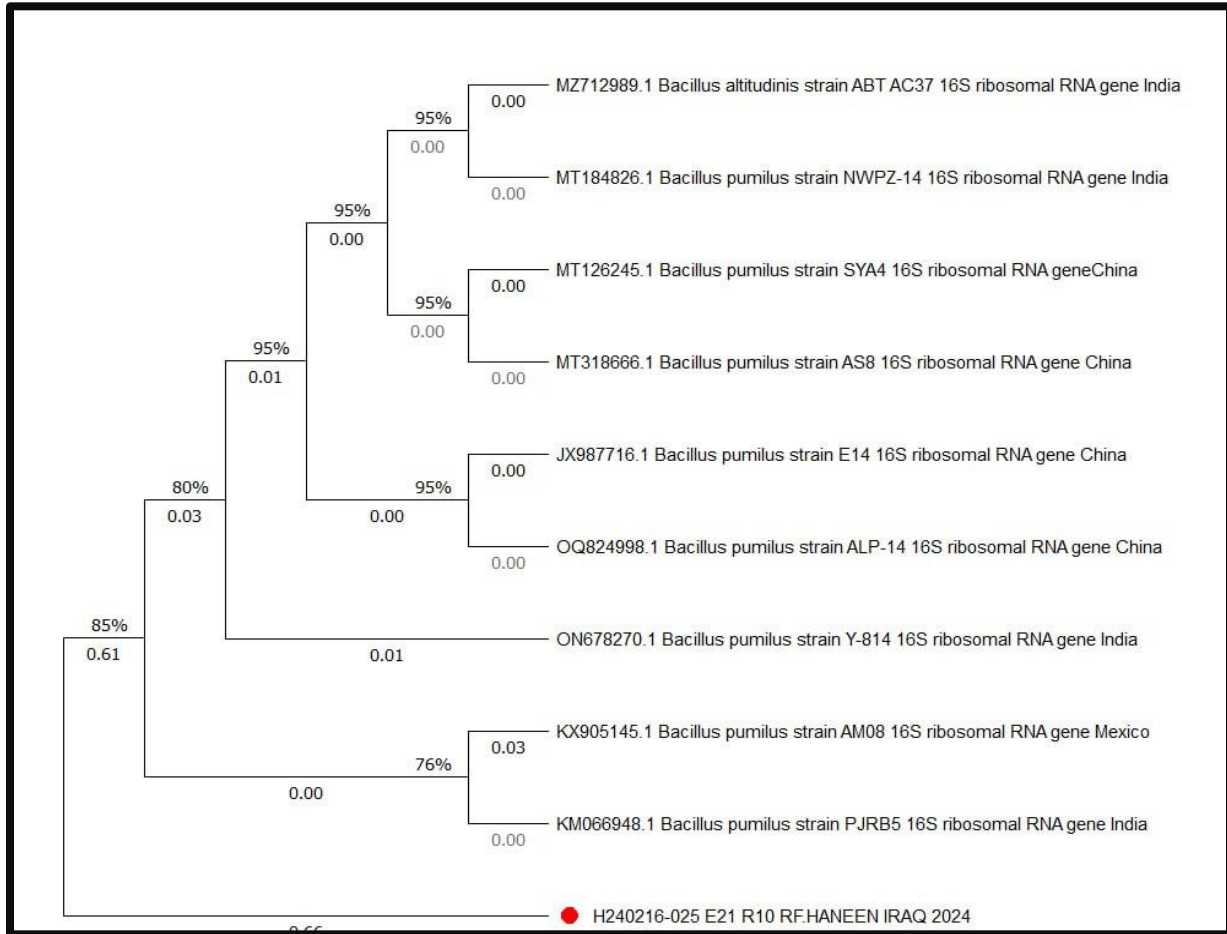


Figure 11. Genetic tree of *Bacillus pumilus* isolated from dust.

4. Discussion

Bacterial species belonging to the genus *Bacillus* were isolated from the dust of Al-Diwaniyah city / Iraq. The species *Bacillus pumilus*, *Bacillus amyloliquefaciens*, *Virgibacillus carmonensis*, *Bacillus altitudinis* and *Bacillus stratospheric*, were isolated. *Bacillus* bacteria are common organisms that live in soil and may frequently contaminate foods, including dairy products, meat, baby foods, rice dishes, vegetables, spices and grains. The ability of *Bacillus* bacteria to form spores enhances the survival of individuals of this genus during food processing treatments, such as pasteurization of milk or juice, and in many cases, in home-cooked meals [13].

At least one member of this genus, *Bacillus cereus*, is a known food poisoning organism and may cause illness due to the fact that it produces either heat-stable emetic toxins (cereulide) commonly found in fried and cooked rice and pasta or heat-sensitive diarrheal enterotoxins (HBL, NHE, and BcET) mostly found in dairy products and meat [14].

In Asia, the emetic form of food poisoning caused by *B. cereus* is more commonly reported than the diarrheal form, which is most common in Europe and North America [15].

Bacillus pumilus is a pathogenic strain of *B. cereus* that isolates humans and this study is consistent with that of [16], who isolated it from soil and found it to cause skin infections.

Bacillus pumilus has been observed in human pneumonia and antibiotic-associated enteritis in Ginea pigs [17].

However, there are several reports of *Bacillus pumilus* causing diseases in pear, apple, peach and olive according to a study [18]. *B. altitudinis* was first isolated by [19], while analyzing air samples for microbial communities from high altitudes [20].

The PGPR activity of *B. altitudinis* has recently been reported by other authors where the bacteria not only promoted growth but also suppressed root pathogens [21]. The ability of bacterial inoculants to increase plant growth, accelerate seed germination, improve seedling emergence, responses to external stressors, protect plants from diseases, and root growth pattern has also been reported in previous studies by [22] *B. amyloliquefaciens* is a versatile and beneficial bacterium of great importance in many industries due to its ability to produce a range of enzymes and antimicrobial compounds. It is a biocontrol agent in agriculture, promoting plant growth and protecting crops from pathogens. Its ability to produce enzymes such as amylases, proteases, and lipases has led to its widespread use in food and beverages, contributing to processes such as fermentation, baking, and brewing. Furthermore, *B. amyloliquefaciens* has applications in biotechnology, where it is used as a host for the production of heterologous proteins and as a source of valuable biomolecules.

The diverse applications of *B. amyloliquefaciens* highlight its importance in promoting sustainable and environmentally friendly solutions across multiple sectors [23].

Conclusion

We conclude from this study that the dust that the air of Diwaniyah city is exposed to due to its lack of vegetation cover, which leads to the displacement of the surface layer of soil and the microbes it carries, which were isolated from the dust belong to the genus *Bacillus* sp. Among the isolated species is *Bacillus pumilus*, which is the most widespread in the university district. This is attributed to the environmental conditions in the region and is less widespread in the Umm Al-Khail area. This is consistent with the study conducted by [24], which isolated *Bacillus pumilus* from dust. These bacteria have a high resistance to harsh environmental conditions such as lack or unavailability of nutrients.

References

- [1] Hudson, P. (2003). Applying the lessons of high risk industries to health care. *BMJ Quality & Safety*, 12(suppl 1), i7-i12 .
- [2] Goudie, A. S. (2009). Dust storms: Recent developments. *Journal of environmental management*, 90(1), 89-94.
- [3] Al-Hamidawi, A. (2014). Assessment of radiation hazard indices and excess life time cancer risk due to dust storm for Al-Najaf, Iraq. *WSEAS Trans. Environ. Dev*, 10, 312.
- [4] Liang, P.-S., Nicolini, A. M., Ogden, K. L., & Yoon, J.-Y. (2015) . Use of Biosensors in secondary education classrooms. *Transactions of the ASABE*, 58(2), 181-190 .
- [5] Al-Dabbas , M.; Abbas , M.A. and Al-Khafaji , R.(2012) : Dust storms loads analysis – Iraq. *Arabian Journal of Geosciences*. 5 : 121-131
- [6] Achudume ,A.C. and Oladipo, B. O. (2009): Effects of dust storm on health in the Nigerian environment . *Biology and edicine* :p21-27
- [7] Barrie, L.A.(2008):WMO sand and dust storm , Warning Advisory and Assessment system (SDS-WAS): Implementation Plan (2009- 2013. Research Department , Atmospheric Research and Environment Branch , World Weather Research Division , WMO secretariat.
- [8] Al-Qasir, Muhammad K. K. (2018). Estimation of the level of some toxic pollutants in suspended particles in the atmosphere of the city of Diwaniyah – Iraq. PhD thesis, College of Education, University of Al-Qadisiyah.

- [9] Geo. F. Brooks, Karen C. Carroll, Janet S. Butel, Stephen A. Morse, and Timothy A. Mietzner. (2013). Jawetz, Melnick, & Adelberg's Medical Microbiology .Twenty-Sixth Edition
- [10] MacFadden ,J. (2000). Biochemical test for identification of medical Bacteria . 3rdEd. The Williams and Wilkins-Baltimor. USA
- [11] Liu , Z.j. and Cordes, J. F. (2004).DNA marker technologist and their. Pereira, F., Carneiro, J., Matthiesen, R., van Asch, B., Pinto, N., Gusmao, L., & Amorim, A. (2010). Identification of species by multiplex analysis of variable-length sequences. Nucleic acids research, 38(22), e203-e203
- [12] Shirayama, M., Seth, M., Lee, H. C., Gu, W., Ishidate, T., Conte Jr, D., & Mello, C. C. (2012). piRNAs initiate an epigenetic memory of nonself RNA in the *C. elegans* germline. Cell, 150(1), 65-77.
- [13] Ranjbar, R., Karami, A., Farshad, S., Giammanco, G. M., & Mammina, C. (2014). Typing methods used in the molecular epidemiology of microbial pathogens: a how-to guide. The new microbiologica, 37(1), 1-15.
- [14] Leppänen , K. Hanna; Täubel , Martin ; Jayaprakash , Balamuralikrishna ; Vepsäläinen , Asko ; Pasanen , Pertti; and Anne Hyvärinen. (2017). Quantitative assessment of microbes from samples of indoor air and dust 1 , Journal of Exposure Science and Environmental Epidemiology .28(3)
- [15] Schaad N.W., Jones J.B., Chun W. (2001): Laboratory Guide For Identification of Plant Pathogenic Bacteria. St. Paul, American Phytopathological Society Press: 398. Sczerbin-Porfinenko A.D. (1963): Bacterial diseases of forest Trees. Moscow, Goslesbumizdat: 148
- [16] Granum, P. E. 2001. *Bacillus cereus*, p. 373–(381) In M.P. Doyle, L.R. Beuchat, and T. J. Montville (ed.), Food microbiology: fundamentals and frontiers, 2nd ed. ASM Press, Washington, D.C.
- [17] BERNSTEIN, D.I., LUMMUS, Z.L., SANTILLI, G., SISKOSKY, J., BERNSTEIN, I.L.: Machine operator's lung: A hypersensitivity pneumonitis disorder associated with exposure to metalworking fluid aerosols. Chest 108, 636–641 (1995)
- [18] BROPHY, P.F., KNOOP, F.C.: *Bacillus pumilus* in the induction of clindamycin-associated enterocolitis in guinea pigs. Infect Immun 35, 289–295 (1982).
- [19] Saleh O.I., Huang P.Y., Huang J.S. (2006): *Bacillus pumilus*, the cause of bacterial blotch of immature balady peach in Egypt. Journal of Phytopathology, 145: 447–453.
- [20] Galal A., El-Bana A., Janse J. (2006): *Bacillus pumilus*, a new pathogen on mango plants. Egyptian Journal of Phytopathology, 34: 17–29.
- [21] Gopalakrishnan, S., Pagidi, H., Bandru, K.K., Iyer, G.K.K. 18. Et al., 2010. Evaluation of bacteria isolated from rice 19. Rhizosphere for biological control of charcoal rot of 20. Sorghum caused by *Macrophomina phaseolina* (Tassi) Goid.
- [22] Rozenfeld V.V. (2008): Epiphyte and Endophyte Microflora Scotch Pine Seeds in Kyiv Polissia Region. [Ph.D. Thesis.] Kiev, National Agricultural University of Cabinet of Ministry Of Ukraine: 160.
- [23] Rinu, K., Pandey, A., 2009. *Bacillus subtilis* NRRL B-30408 2. Inoculation enhances the symbiotic efficiency of Lens 3. Esculentia Moench at a Himalayan location. J. Plant Nutr. 4.
- [24] Zalila-Kolsi, I.; Kessentini, S.; Tounsi, S.; Jamoussi, K. Optimization of *Bacillus amyloliquefaciens* BLB369 Culture Medium by Response Surface Methodology for Low Cost Production of Antifungal Activity. Microorganisms 2022, 10, 830.
- [25] *Bacillus aerius* sp. Nov., *Bacillus aerophilus* sp. Nov., 16. *Bacillus stratosphericus* sp. Nov. and *Bacillus altitudinis* sp. Nov. 'Soil Sci., 172, 134–139.

Studying the Possibility of Growing the Reishi Mushroom, *Ganoderma* spp. , on Agricultural Waste and Testing its Effect in Protecting Cucumber Plants from Some Pathogenic Fungi

Ahmad Shehab Ahmad Al-Kaabi¹ and Ahmad Mohammed Hussien²

¹Ministry of Agriculture, Department of Agricultural Extension and Training, Najaf, Iraq.

² Department of Plant Protection, College of Agriculture, University of Kufa, Najaf, Iraq.

¹E-mail: Ahmad.shehab.ahmad1@gmail.com

²E-mail: Ahmadm.tabra@uokufa.edu.iq

Abstract. The study is conducted in laboratories of Plant Protection Department, College of Agriculture - University of Kufa, in 2023-2024. The study aimed to grow three types of *Ganoderma* fungus on agricultural waste (wheat straw, Corn cobs , sawdust, rice hull, wild reeds and conocarpus) in order determine the optimal medium for germinating *Ganoderma* fungi on those agricultural wastes in vegetative reproduction. In addition to, studying the antagonistic and parasitic ability of these three species against the pathogenic fungi, *Rhizoctonia solani*, and *Fusarium oxysporum* isolated from cucumber plant , and testing the effect of the filtrates of these fungi on seed germination rates as well as protecting the seedlings from rot. The results indicate varying growth rates among the agricultural media for growing *Ganoderma resinaceum*, *Ganoderma applanatum*, and *Ganoderma lucidum*. Corn cob medium has the highest growth rate of 10 cm during the 10-day germination period, followed by wheat straw medium and wild reeds, and conocarpus medium is the least favorable for fungal growth among all fungi. The results of the study indicate that *Ganoderma* fungus displayed antagonistic properties against fungi isolated from cucumber. *Ganoderma resinaceum* and *Ganoderma applanatum* fully covered the dish area, preventing pathogenic fungi growth. However, *Ganoderma lucidum* covered two-thirds of the dish and *Rhizoctonia solani* covered the remaining third. The results indicated that , the two pathogenic fungi , *Rhizoctonia solani* and *Fusarium oxysporum* were impacted by the fungi's filtrates as *G.applanatum* shows the greatest inhibition rate towards both pathogenic fungi, reaching a growth rate of 3 cm. Similarly, *G.resinaceum* also inhibited both fungi by 3 cm. Regarding fungi, *G.lucidum* has the lowest inhibition rate among the two pathogenic fungi, with an inhibition rate of 3.5 cm. The results indicate that *Ganoderma* species play a role in boosting cucumber seedling growth, with all three species of the fungus enhancing seed growth significantly. Specifically, *G.resinaceum* treatment had the highest seed germination rate at 96.65% and lowest seedling mortality rate at 0.33%. Next was *G.lucidum* treatment with 90% germination rate and 10% seedling mortality rate, followed by *G.applanatum* treatment with 86.6% germination rate and 1.33% seedling mortality rate.

Keywords. *Rhizoctonia solani*, *Ganoderma applanatum*, Rot seeds, Rot root.

1. Introduction

Biocontrol methods are seen as safe and eco-friendly practices, and are among the most promising, widely embraced, and secure approaches in crop management. Among these successful agents are some extracts of fleshy fungi, as some basidiomycetes contain compounds that are anti-bacterial, anti-fungal and anti-viral [1]. Especially, extracts of *Ganoderma* fungus, which is one of the types of fungi that contains inhibitory substances in addition to its many benefits in the field of health [2].

Ganoderma fungus is rich in bioactive compounds like fibers, polysaccharides, triterpenoids, oligosaccharides, peptides, proteins, alcohols, and phenols, along with mineral elements such as zinc, copper, iodine, selenium, germanium, iron, and various vitamins, as well as amino acids. It also includes numerous other substances like carbohydrates, glycosides, and Phenolic substances, Tannins. These substances and compounds possess numerous healing qualities for various illnesses, in addition to having antibacterial properties [3]. The growing of *Ganoderma* started in 1969 in China and later gained popularity in Asian nations, particularly Japan and Korea, by inoculating one-meter-long natural tree stems and burying them in the soil [4].

Afterward, the fungus was grown in numerous farming environments at a low cost, utilizing different agricultural wastes such as sawdust, tree branches which all contain a complex composition of cellulose and lignin.

Shomali et al. [5] mentioned that fruiting body of *G.adspersum* contains high concentrations of phenols. Alves et al. [6] identified twenty-three phenolic compounds extracted from different fleshy fungus species, demonstrating inhibitory effects on various pathogenic organisms. The protein gandermin isolated from the fruiting bodies of the mushroom is found to have antifungal effects against *Botrytis cinerea*, *Fusarium oxysporium*, and *Physalo sporapiricola* [7]. Furthermore, the hot aqueous extract of the fungus and the polysaccharides extracted, using the gas chromatography technique, were shown to have an inhibitory effect on bacterial plant pathogens such as *Erwinia carotovora* bacterium and fungal pathogens such as *Penicillium digitatum* fungi and *Botrytis cinerea* [8].

2. Materials and Methods

2.1. *Ganoderma* spp. Isolates

The isolates of *Ganoderma lucidum* fungus is obtained from the Ministry of Agriculture, Agricultural Protection Department, National Center for Organic Agriculture, and they are originally brought from Fujian Agriculture and Forestry University | JUNCAO Research /China. The two isolates of *Ganoderma resinaceum* and *Ganoderma applanatum* were obtained by Dr. Mahmoud from the Ministry of Agriculture, Agricultural Protection Department, and these two isolates are registered in the World Gene Bank under (*Ganoderma applanatum* strain Has.AA-6 (ON834523.1) and *Ganoderma resinaceum* strain Has.AA-8 (ON834527.1) respectively [9].

2.2. Sample Collection

Dried wheat straw, yellow corn cob, and rice hulls were obtained from fodder production stores and rice milling machines. Sawdust was obtained from wooden door companies, while wild reeds, cynodon dactylon, and conocarpus were gathered from farms in Najaf province. Plant parts were pulverized using an electric blender to get dry plant powder, then stored in paper bags at room temperature in the laboratory until needed. 100 grams of agricultural waste were placed in an electric oven at 50°C and dried until all moisture was eliminated [10].

After the plant waste was prepared, 30 gm was placed into lab glass test tube, length 10cm, and then sterilized using an autoclave. The plant waste was separately inoculated after being

moistened by 60% with a 0.5 cm diameter disk containing a 5-day-old colony of *Ganoderma* spp . The efficiency of waste in promoting mycelium growth of *Ganoderma* spp is evaluated using [11] method. The media was incubated at 30°C with three replicates for each treatment , a transparent measuring ruler was used to measure the distance (cm) traveled by the mycelium along the medium 10 days post-inoculation. The growth depth was measured every two day, and the optimal medium was identified 10 days post-inoculation.

2.3. Isolating Fungi from the Greenhouse

The results of isolation from the roots of cucumber plants infected with wilt showed a number of fungal species, namely *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Macrophomina*, *Mucor*, *Penicillium*, *Rhizoctonia*, *Rhizopus*, and *Trichoderma*. The presence of these fungi on the surface of cucumber roots or in the soil surrounding the root may have a positive or negative role in plant growth. Therefore, the pathogenicity test of these fungi was conducted to determine their effect on the germination of cucumber seeds and the growth of its seedlings.

2.4. Testing the Pathogenicity of Fungi Isolated on Cucumber Seeds

P.D.A culture medium was added to Petri dishes, followed by placing *Ganoderma resinaceum*, *Ganoderma applanatum*, *Ganoderma lucidum*, *Rhizoctonia solani*, and *Fusarium oxysporum* in the center of each dish. These samples were obtained from pure cultures that were 7 days old, with three replicates per fungus, then, these dishes were incubated at 25 ± 2 °C. Three days later, 10 cucumber seeds per plate were placed in a circular pattern, each 1 cm away from the dish edge. The dishes were sterilized with a 6% commercial sodium hypochlorite solution for two minutes and rinsed multiple times with sterile distilled water to eliminate any residue from the sterilization process . Then , the dishes were placed on filter paper to remove free water , also three additional dishes with P.D.A culture media were prepared with 10 cucumber seeds per plate were placed without the fungus as a comparison treatment. Then , all the dishes were incubated again at 25 ± 2 °C, and after 14 days, the percentage was calculated for germinating and rotten seeds and infected and healthy seedlings.

2.5. Studying of the Effect of the Filtrate of Three Types of *Ganoderma reishi* Mushroom on the Fungal Growth of Pathogenic Fungi

Potato Dextrose (P.D.) was prepared consisting of 200 gm potato extract and 20 gm sugar per liter of water .This mixture was then placed into 250 ml conical flasks, filling each flask with 100 ml of the solution. It underwent sterilization in an autoclave at a temperature of 121 °C and a pressure of 15 Pa/N for a duration of 20 minutes. After cooling the flasks down , each was inoculated with a 0.5 cm diameter disc of the P.D.A. medium containing 7-day-grown *Ganoderma resinaceum*, *Ganoderma applanatum*, and *Ganoderma lucidum* fungi .

The flasks were left to incubate at a temperature of 25°C for a period of two weeks, with the additional step of shaking them every 2-3 days. Following 28 days of incubation, the fungal extract underwent filtration via Whatman No.1 filter paper and was sterilized using a fine filter with 0.22 millimicron diameter holes. 5 ml of filtrate from *Ganoderma resinaceum*, *Ganoderma applanatum*, and *Ganoderma lucidum* fungi was placed into Petri dishes with a 9 cm diameter, followed by the addition of P.D.A. Three replicates were conducted for every treatment and the media , after solidified ,was inoculated with 0.5 cm Petri dish of P.D.A , in the center of each dish , and 7-day -old *Rhizoctonia solani* and *Fusarium solani*, pathogenic fungi, were already germinated on those Petri dish of P.D.A .

Fungal growth was determined by calculating the average of two perpendicular diameters passing through the center of the dishes once the fungi growth had reached the dish edge in the control treatment.

2.6. The Percentage of Germination of Cucumber Seeds with Fungus Filtrate from Three Types of Ganoderma Mushroom.

The filtrate from *Ganoderma resinaceum*, *Ganoderma applanatum*, and *Ganoderma lucidum*, prepared in the previous paragraph and sterilized using a fine filter, was used. 5 ml of the filtrate was added to Petri dishes containing sterile filter paper, on which ten sterilized seeds from each cucumber plant were placed. Three replicates were made for each treatment with a control treatment, where 5 ml of sterile distilled water was added instead of the filtrate. The dishes were placed in incubator at a temperature of 27 ± 2 °C for ten days, after that, the percentage of germination of seeds and rotten and healthy seeds was calculated.

2.7. Testing the Antagonistic Ability of Three Types of Ganoderma reishi Mushroom on P.D.A Medium Against a Number of Isolated Fungi

The antagonistic ability of *Ganoderma resinaceum*, *Ganoderma applanatum* and *Ganoderma lucidum* against the pathogenic fungi, *Rhizoctonia solani* and *Fusarium oxysporum* was tested by dual culture in Petri dishes containing sterilized P.D.A. The 9 cm petri dish containing (P.D.A.) was divided into two equal parts, and the center of the first part of the dish was inoculated with the *Ganoderma* fungus, by taking a 0.5 cm disk from the three-day-old fungi culture. While the center of the second part was also inoculated with a matching disk of pathogenic fungi, three days old, to be tested against this fungus. The experiment was carried out with three replicates. The dishes were placed in an incubator at a temperature of 25 ± 2 °C for a period of three days. The antagonistic ability was estimated according to the 5-grade-scale [12].

2.8. Point Description

- Reishi mushroom covers the entire surface of dish without allowing the isolated fungus to grow.
- Reishi mushroom covers two-thirds of the dish, and isolated fungus covers the remaining third of the dish.
- Reishi mushroom covers half of the dish, and isolated fungus cover the other half of the dish.
- Reishi mushroom covers a third of the dish, while isolated fungus covers the remaining two-thirds of the dish.
- The isolated fungus covers the entire plate.

3. Results and Discussion

3.1. The Effect of Isolated Fungi on Cucumber Seed Germination and Seedling Growth

The results of this experiment revealed significant differences in the impact of the isolated fungi on various traits examined, including seed germination rates. The results of Table (1) illustrates an increase in germination rates in the treatment of *C. globosum*, *A. niger*, and *Penicillium sp. T. harzianum* to 83.33, 93.33, 86.76 and 80.83.33%, respectively, compared to 80% for the comparison treatment. While the *F. oxysporum* fungi and *R. solani* show a significant reduction in the seed germination rates, reaching 13.33 for each of them. Regarding the remaining fungi, the majority of them had a detrimental impact on the rate of germination.

Table 1. Effect of some fungi isolated from the roots of cucumber plants and from the soil surrounding the root on the percentage of cucumber seed germination.

Fungus	Germination %
Control	80
<i>Alternaria alternata</i>	40
<i>A.solani</i>	73.33
<i>Aspergillus fumigatus</i>	66.67
<i>A.niger</i>	83.33
<i>A. flavus</i>	20
<i>A.silvra</i>	76.67
<i>A.terreus</i>	73.33
<i>Chaetomium globosum</i>	86.67
<i>Cladosporium Cladosporioides</i>	43.33
<i>C.herbarium</i>	53.33
<i>Fusarium oxysporum</i>	13.33
<i>Macrophomina phaseolina</i>	46.67
<i>Penicillium sp.</i>	83.33
<i>Rhizoctonia solani</i>	13.33
<i>T.harzianum</i>	80
L.S.D	14.05

The results of this experiment aligned with multiple studies showing that various fungi, like *T. harzianum*, *C. globosum*, *A. niger*, *Penicillium*, and non-pathogenic *F. oxysporum* isolates, are effective in enhancing plant growth indicators, such as cucumber seed germination rates [13]. The reason was attributed to the ability of these fungi to secrete substances that regulate plant growth, or their ability to dissolve some poorly soluble nutrients such as zinc, copper, iron, and manganese ions, thus making them more available to the plant. It may also be due to the ability of the fungi to secrete antibiotics that reduce the density of plant pathogenic fungi or protect the seeds from parasitism by pathogenic fungi [14]. While the study shows the negative effect of the two fungi *F. oxysporum* and *R. solani* on the plants. The reason may be attributed to the ability of these fungi to secrete hydrolytic enzymes or secrete metabolic substances that have a toxic effect on the plant [15]. In light of the results achieved by this experiment, the fungi *R. solani* and *F. oxysporum* were selected to complete the rest of the experiments.

3.2. Isolation, Phenotypic and Microscopic Diagnosis of *Ganoderma* spp.

Plant Diseases Laboratory - Department of Plant Protection / University of Kufa provides three varieties of reishi mushrooms: *Ganoderma resinaceum*, *Ganoderma applanatum*, and *Ganoderma lucidum*. The mushroom culture stands out with its vivid white color in PDA, and when sections of the modern mushroom colony are prepared and examined under the microscope, filaments in the mushroom are separated by transverse and branched walls.

Large chlamydospores are found at the tips of the branches, along with basidiospores when examining a swab taken from the holes in the fruiting body. These spores have an oval shape (elliptical in shape) and when viewed under the microscope, they seem to have a smooth wall both on the exterior and interior, along with an inner wall containing small, delicate spiny protrusions. The phenotypic properties used to diagnose *Ganoderma* spp are consistent with a number of taxonomic studies of this mushroom [16-18].

3.3. Determining the Efficiency of Agricultural Waste in Fleshy Fungi Growth Under Laboratory Conditions and its Effect on Fungal Growth 10 Days After Inoculation

Table (2) displays how efficient agricultural waste is at accelerating mycelium growth. The data presented in the table demonstrates how different types of media can impact the growth of four varieties of fleshy fungi. Media made from Corn cob resulted in the highest growth rate, significantly the highest of all other types of media with a growth rate of 9.6 cm. Then , it was followed by wheat straw medium and wild reed medium, growth reaching (7.5) and (7.1) cm, respectively. The results were similar to the results of [19] when growing *Pleurotus ostreatus* on sawdust and Corn cob, the Corn cob medium was significantly higher than the sawdust and rice straw media concerning the number of days required for mycelium to grow and spread. The table indicates that every fungus exhibited low growth in both sawdust and conocarpus waste, with growth rates of 3.3 cm and 3.2 cm in the two media, respectively. The rest of the media demonstrated moderate growth for all types of fungi. The weak growth of mycelium on sawdust and conocarpus waste can be attributed to the fact that these wastes contain highly effective antifungals because they consist of semi-alkaline substances [20].

The table shows that the highest rate of fungal growth was in *G. resinaceum*, it reached 6.1, and therefore there are significant differences in the rest of the fungi. The result also indicated that the most effective interference for promoting growth was found to be between corn cob medium and all the fungi, while the least effective one was between sawdust medium and *G. applanatum*.

Table 2. Efficiency of agricultural wastes in fungal growth of three types of reishi mushrooms under laboratory conditions and their effect on fungal growth 10 days after inoculation.

	<i>G.applanatum</i>	<i>G. resinaceum</i>	<i>G. lucidum</i>	Effect rate of media type
sawdust	3.8	4.6	4	3.8
wheat straw	7.3	7.7	7.3	7.5
conocarpus	3	3.5	2.6	3.3
Cynodon dactylon waste	3.8	6.1	3.3	4.8
rice hull	4.6	4.3	5.5	4.9
Corn cob	9.8	10	9.6	9.6
wild reeds	6.9	7.5	6.7	7.1
Effect rate of fungus type	5.6	7.1	6.3	
L.S.D	1.9=interference 0.7=fungi 0.9=wastes			

Corn cob media is a very promising option which can be utilized due to its availability as agricultural waste, ability to support the growth of fleshy fungi, and high capacity to retain water [21]. This is why it is a great option compared to conventional ones , as corn cob media is known for its nutritious elements for fungi [22]. This result aligns with what [23] indicated that materials rich in cellulose, such as corn cobs and wheat and rice straw, can support the growth of mycelium of fleshy fungi. The results are also consistent with fact that the species of fleshy fungi are known by containing many antimicrobials, antioxidants, and effective biological substances, and with a high ability to colonize and rapidly grow mycelium on various cultural media. Mycelium includes wide-ranging antimicrobial compounds [24].

3.4. Testing the Antagonistic Ability of Three Types of Ganoderma Mushroom Against Fungi Isolated from Cucumber Plants

The study results presented in Table (3) demonstrated the high antagonistic ability of the reishi mushroom against isolated fungi, as *G.applanatum* fungus covered the entire dish area, preventing other fungi from growing, and *G.resinaceum* fungus also covered the entire dish area, preventing the two pathogenic fungi from growing. Moreover, *G. lucidum* fungus covered the

entire dish without allowing *F. oxysporum* to grow at all, and the pathogenic fungus, *R. solani*, covered two-thirds of the dish and the fungus covered the remaining third of the dish's area. The results are consistent with many studies that indicated the effectiveness of species of *Ganoderma* spp as antifungals [25], which is due to their content of antifungal compounds such as Phenols, Terpenoids, Flavonoids, lignin, alkaloids, and steroids [26].

The fungus's ability to inhibit may come from its production of hydrolytic enzymes such as Chitinase, Glucanase, and Protase, which can decompose cell wall and thus inhibit the growth of pathogenic fungi, or it may be due to the fungus containing many compounds of a non-enzymatic nature. In addition to, many compounds that have the ability to inhibit pathogenic fungi, when fungus contains many compounds such as alkaloids, phenols, glycosides, and flavonoids, which have several mechanisms for inhibiting the pathogen, for example, flavonoids can inhibit the growth of fungi by affecting the permeability of the cell membrane, the hydroxyl group in flavonoids causes a change in the transport of nutrients, which may cause toxic effects on pathogenic fungi [27].

Table 3. Antagonistic ability of three types of *Ganoderma* mushroom against fungi isolated from cucumber plants according to Bell scale.

Fungi isolated from cucumber plants	<i>G.applanatum</i>	<i>G.resinaceum</i>	<i>G. lucidum</i>
<i>R.solani</i>	1	1	2
<i>F.oxysporum</i>	1	1	1
L.S.D. 0.05	0.50		

3.5. The Effect of Filtrates of Three Types of Reishi Mushrooms in Inhibiting Pathogenic Fungi

The results of Table (4) show the effect of the filtrate of fleshy fungi on the diameter of the fungal colonies of the pathogenic fungi, *R.solani* and *F. oxysporum*. The results indicate that the highest inhibition rate of growth in the diameter of fungal colonies occurred in the filtrate of the fungi, *G.applanatum* and *G.resinaceum*, as the average colony diameter reached 3 cm, with a significant difference from the rest of the fungal filtrate, and compared with the control treatment in which the average colony diameter reached 9 cm. Followed by *G. lucidum* fungal filtrate, in which the average colony diameter of the two pathogenic fungi reached 3.5 cm.

Regarding the interference between the filtrate of *G. applanatum* and the pathogenic fungus, *R. solani*, had the highest inhibition rate, with the fungal colony's growth reaching 2.9 cm, higher than all other interferences except for the one between the filtrate of *G. applanatum* and the pathogenic fungus *F. oxysporum*, which reached a colony diameter of 3.1 cm.

Table 4. The effect of filtrates from three types of reishi mushrooms in inhibiting pathogenic fungi.

	<i>R.solani</i>	<i>F. oxysporum</i>	Rate of Fungi
<i>G.applanatum</i>	2.9	3.1	3
<i>G.resinaceum</i>	3	3	3
<i>G. lucidum</i>	3.6	3.5	3.5
comparison	9	9	9
rate of pathogenic fungi	4.5	4.5	
L.S.D	fleshy fungi=0.2	pathogenic fungi=0.1	interference =0.2

The reason for the inhibitory properties of *Ganoderma* spp fungal filtrate could be due to the presence of specific compounds in this Basidiomycota fungus that have antifungal, antiviral, and antibacterial effects. *Ganoderma* spp is a fungus that contains over 400 compounds with biological effects, including steroids, triterpenes, phenols, and alkaloids. Those compounds are secondary metabolic compounds that the fungus does not need for its growth and activity, and to

which most of the biological antifungal, antibacterial, and antiviral properties are attributed [28,29].

Moreover, it contains many bio inhibitory proteins, including Lactins, Laccase, and Heamolysin, which have many applications in medical fields. The inhibitory effect may also be due to the mushroom *Ganoderma* spp containing the ganodermin protein, which is one of the types of antifungal protein, which is due to its antifungal effect, such as *Botrytis cinerea*, *Fusarium oxysporum*, *Pysalo sporapiricola*,

Additionally, the ability of the fungus *Ganoderma* spp to inhibit pathogenic fungi may be due to the presence of volatile oils, a product of secondary metabolism that has inhibitory effects on the fungi [30,31], Besides, the inhibitory effect may be due to the presence of phenolic compounds, terpenes, and other compounds with the inhibitory effect of fungi, as terpenes possess antifungal properties, and the most important terpenes are (ganoderic acids, ganodereni, ganoderols, and lucideric acids) [32].

3.6. The Effect of Types of Reishi Mushrooms and Pathogenic Fungi on the Growth of Cucumber Seeds in Petri Dishes

The data in Table (5) indicates that fleshy fungi significantly contribute in the growth of cucumber seedlings. All fleshy fungi tested show positive effects on cucumber seed growth, with the *G.resinaceum* fungus treatment resulting in the highest rates of germination of cucumber seeds 100%, and seedling mortality 0%. Similar results were also observed with the comparison treatment, followed by the *G.applanatum* fungus treatment, with a germination rate of 100% and a seedling mortality rate of 0%. *G. Lucidum* fungus treatment achieves a germination rate of 90% and a seedling mortality rate of 10%. The filtrate treatment of *Ganoderma* spp fungus may achieve the highest result because it helps stimulate the vegetative growth of the tested cucumber varieties. This may be due to the presence of growth stimulants, such as some enzymes, which help provide more readily available elements to the plant, as well as the possibility of producing plant hormones among the metabolic substances present in the mushroom filtrate. These results are consistent with previous studies that emphasized the role of fungi and their filtrates in stimulating plant growth [33,34].

Table 5. Effect of types of *Ganoderma* spp and the two Pathogenic fungi on the growth of cucumber seeds in petri dishes.

	Germination %	Seedling mortality
Comparison	100	0
<i>G.applanatum</i>	100	0
<i>G.resinaceum</i>	100	0
<i>G. Lucidum</i>	90	10
<i>R.solani</i>	0	100
<i>F. oxysporum</i>	0	100
L.S.D.	0.85= germination	0.85= Seedling mortality

The data in the table indicates that both pathogenic fungi, *R. solani* and *F. oxysporum*, did not achieve any growth rate of cucumber seedlings, with a germination rate of 0 and a seedling mortality rate of 100%. This is due to genetic variation, as the high genetic variation between *Fusarium* species may constitute a major obstacle to tomato production in the world [35]. The reason for the variation in the pathogenicity of different fungal isolates may be due to differences in pathogenicity factors in the isolates. The variation in the pathogenicity of various fungal isolates could be attributed to differences in pathogenicity factors such as enzyme secretion (such

as pectinase, cellulase, and protease) that decompose plant cell walls, along with variations in toxin secretion such as fusaric acid [36].

3.7. The Effect of *Ganoderma* spp Filtrate on the Germination Rate of Cucumber Seeds and the Length and Weight of Seedlings

Table (6) indicates clear significant differences between the fungal filtrate and its effect on the germination and mortality rates of cucumber seedlings. The germination and seedling mortality rates were significantly the highest, among other treatments, in the two filtrate treatments of *G.applanatum* and *G.resinaceum* reaching 100% germination rate and 0% seedling mortality rate. The germination rate was 100% and seedling mortality rate was 0% in the control treatment. Followed by the treatment of the filtrate, *G.Lucidum*, the germination rate is 93.33 and the seedling mortality rate is 6.66. Seedling length rate is significantly higher than to the two treatments of the filtrates, *G. applanatum*, and *G.resinaceum*. Moreover, Seedling length rate ranges from 14.6 and 15 cm, respectively.

After that, *G.Lucidum* fungus treatment, the seedling length rate is 12.3, and in the pure treatment, the seedling length rate is 10 cm. In seedling weight, the two treatments of the fungal filtrate, *G.applanatum* and the fungal filtrate *G.resinaceum* were significantly higher, as the seedling weights are 1489 and 1508 mg, respectively. Then, the treatment of the fungal filtrate *G.Lucidum*, the seedling weight is 1170 mg and the control treatment, the seedling weight is 977.3 mg.

The positive effect of *Ganoderma* mushroom's filtrate and extract could be attributed to its rich content of active compounds like phenols, which are crucial for plant development processes like cell division and regulation of growth hormones. These compounds enhance shoot system size, photosynthesis efficiency, and nutrient absorption in plants. This demonstrates the important role of phenolics in increasing plant height and increasing yield [37]. The high results of filtrate and extract of *Ganoderma* spp, is also due to the fact that this fungus helped increase the plant's readiness with nutrients, especially nitrogen and phosphorus, as the nitrogen element is one of the most important elements that enter into the construction of the chlorophyll molecule. Through the construction of porphyrin-ring, which is essential in the synthesis of chlorophyll, as well as the formation of some amino acid that help in the formation of chloroplasts [38], and this reflects positively on the chlorophyll content of leaves.

Table 6. Effect of filtrate from types of *Ganoderma* spp on germination rate of cucumber seeds and length and weight of the seedling.

	Germination	mortality seedling rate	Seedling length	Seedling weight
Distilled water	100	0	10	977.3
<i>G.applanatum</i>	100	0	14.6	1489
<i>G.resinaceum</i>	100	0	15	1508
<i>G.Lucidum</i>	93.33	6.66	12.3	1170
L.S.D	6.6	6.6	1.81	361.4

Conclusions

The results from isolates taken from the roots of cucumber plants revealed the existence of numerous fungal species, with *R. solani* and *F. oxysporum* being the most pathogenic, both showing a germination rate of 13.13%. In contrast, the reishi mushroom species had a germination rate ranging from 90-96.6%. The results showed that the best medium for the growth of the reishi mushroom is corn cob, as the growth rate is 9.6 cm, and that the least medium is wood sawdust, the growth rate is 3.2 cm.

The results also showed the high antagonism ability of the reishi mushroom species against the two pathogenic fungi *R. solani* and *F. oxysporum*, all the fungi had antagonism ability reaching 1, that is, they completely covered the dish, except for *G. lucidum* fungus with *R. solani* fungus, which had an antagonism ability reaching 2. The results demonstrated that the filtrate from the reishi mushroom species effectively hinders the growth of pathogenic fungi by participating in inhibiting the growth of the mycelium of these fungi. The results also demonstrated the effectiveness of reishi mushroom filtrate in preventing rot in cucumber seedlings, while also aiding in boosting germination rate and enhancing seedling length and weight.

References

- [1] Yang, X.; Sun, S.; Chen, Q.; Zhang, Z.; Wang, J.; Liu, Y.; Wang, H. A (2022). Polysaccharide of *Ganoderma lucidum* Enhances Antifungal Activity of Chemical Fungicides against Soil-Borne Diseases of Wheat and Maize by Induced Resistance. *Agriculture*, 12, 55. <https://doi.org/10.3390/agriculture12010055>
- [2] Čör Andrejč, d., knez, ž. & knez marevci, m. (2022). Antioxidant, antibacterial, antitumor, antifungal, antiviral, anti-inflammatory, and neuro-protective activity of *Ganoderma lucidum*: An overview. *Frontiers in Pharmacology*, 2757.
- [3] Vapottič, J., Barišić, D., Kobal, I., & Lulić, S. (2007). Radioactivity and radon potential of the terra rossa soil. *Radiation measurements*, 42(2), 290-297.
- [4] Liang, D., Yong, T., Chen, S., Xie, Y., Chen, D., Zhou, X., ... & Zuo, D. (2018). Hypouricemic Effect of 2, 5-Dihydroxyacetophenone, a Computational Screened Bioactive Compound from *Ganoderma applanatum*, on Hyperuricemic Mice. *International journal of molecular sciences*, 19(5), 1394.
- [5] Bijalwan, a., bahuguna, k., vasishth, a., singh, a., chaudhary, s., tyagi, a., thakur, m., thakur, t., dobriyal, m. & kaushal, r. (2020). Insights of medicinal mushroom *Ganoderma lucidum*: prospects and potential in India. *Biodivers Int J*, 4, 202-9.
- [6] Shomali, N., Onar, O., Karaca, B., Demirtas, N., Cihan, A. C., Akata, I., Yildirim, O., (2019). Antioxidant, Anticancer, Antimicrobial, and Antibiofilm Properties of the Culinary-Medicinal Fairy Ring Mushroom, *Marasmius oreades* (Agaricomycetes). *International Journal of Medicinal Mushrooms*, 21(6):571 – 582
- [7] Alves, M.J., Ferreira, I.C., Froufe, H.J., Abreu, R.M.V., Martins, A. and Pintado, M., (2012.) Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. *Journal of applied microbiology*, 115 (2), pp.346-357.
- [8] Wang H, Ng TB. (2006). Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom *Ganoderma lucidum*. *Peptides*. 27:27–30.
- [9] Bai, D.; Chang, N.-T.; Li, D.-H.; Liu, J.-X.; You, X.-Y. (2008). Antiblastic Activity of *Ganoderma lucidum* Polysaccharides. *Acta Agric. Boreali Sin.* 23, 282–285
- [10] Mahmoud, Mahmoud Adel Saadi. 2023. The effectiveness of some compounds extracted from the fungus *Ganoderma* spp. In combating the pathogenic fungi *Fusarium oxysporum* and *Alternaria tenuissima* on tomato plants. Doctoral thesis. College of Agriculture. Tikrit University. Republic of Iraq
- [11] Al-Rikabi, Firas Ali Ahmed. (2007). The effect of vegetative growth extraction of some weeds on pathogenic fungi on tomato roots and on the biocontrol fungus *Trichoderma harzianum* Rifai. Master's thesis, University of Kufa - College of Agriculture.
- [12] Sharma, V.R.; S. R. Sharma and Kumar. S. 2006. Effect of supplementation and cultivation containers on the productivity of *Flammulina velutipes*. *Mushroom Research* 15:129-134.
- [13] Bell, D. K., H. D. Wells and Markham, C. R. 1982. In vitro antagonism of *Trichoderma* species against six fungal Plant Pathogens. *Phytopathology*. 72 (4) : 379 – 382.
- [14] Ozbay, N.; and S. E. Newman. 2004. *Fusarium* crown and root rot of tomato and control methods. *Plant Pathology Journal*, 3(1), 9-18.

- [14] Larena, I.; P, Sabuquillo; P, Melgarejo and De Cal, A. 2003. Biocontrol of fusarium and verticillium wilt of tomato by *Penicillium oxalicum* under greenhouse and field conditions. *J Phytopathol.* 151(9):507–512.
- [15] Inoue, I.; F. Namiki and Tsuge, T. 2002. Plant colonization by vascular wilt fungus *Fusarium oxysporum* requires FOW1, ageneencoding amitochondrial protein .*The Plant Cell, American Societyof Plant Biologists* 14:1869-1883.
- [16] Kaur G, A Singhm and G Dhingra. (2017).Diversity of the genus *Ganoderma* in Punjab (India). *Mycobiota*; 7, 25-49
- [17] Chen, B., Binrong, K., Ye, L., Jin, S., Jie, F., Zhao, L., and Wu, X.(2017) . Isolation and varietal characterization of *Ganoderma resinaceum* from areas of *Ganoderma lucidum* productionin China. *Scientia Horticulturae*, 224:109-114.
- [18] Hapuarachchi K .K, SC Karunarathna, P., Phengsintham, H Yang, P Kakumyan, KD Hyde and T Wen. *Ganodermataceae (polyporales)*. (2019).Diversity in Greater Mekong Subregion countries (China, Laos, Myanmar, Thailand and Vietnam). *Mycosphere*; 10, 221-309.
- [19] Sitaula, H. P., R., Dhakal, D. C., Geetesh, and D., Kalauni.2018. Effect of various substrates on growth and yield performance of oyster mushroom (*Pleurotus ostreatus*) in chitwan, Nepal.*International Journal of Applied Sciences and Biotechnology*, 6(3), 215-219.
- [20] Indravathi, G., R., S.,Reddy and P. S.,Babu .2016. *Albizia amara*-A Potential Medicinal Plant: A. *International journal of Science and Research*, 5(3), 621-7.
- [21] Samuel, A. A. and T., L., Eugene .2012. Growth performance and yield of oyster mushroom (*Pleurotus ostreatus*) on different substrates composition in Buea South West Cameroon. *Science journal of biochemistry*, 2012
- [22] Kumla, J., N. Suwannarach, K. Sujarit, W. Penkhrue, P. Kakumyan , K. Jatuwong and S. Lumyong.2020. Cultivation of mushrooms and their lignocellulolytic enzyme production through the utilization of agro-industrial waste. *Molecules*, 25(12), 2811.
- [23] Balakrishnan, B. and Nair ; M.C .1995. Production Technology of Oyster mushroom (*Pleurotus spp.*) *Advances in Howb. Mush. Vol. 13: 109-116.*
- [24] Vamanu, E. 2012 . *In Vitro* antimicrobial and antioxidant activities Varley, H. Gowenlock, A.H. and Bell, M. 1980 . *Practical Clinical Biochemistry*. Fifth Edn., London: William Heinemann Medical Books Ltd.
- [25] Tamilselvan N. , Rajesh K. (2019) Antimicrobial Efficacy of Medicinal Mushroom *Ganoderma lucidum*.*International Journal of Trend in Scientific Research and Development*, Vol:3 3 :2456-6470.
- [26] Hidayat, F.R., Sukiman, Hidayati, E., Sarkono, Suryadi, B. F., Faturrahman., (2020). Effectiveness of Ethanol Extract from 3 *Ganoderma* Species from Lombok in Inhibition Growth of *Candida albicans* and *Cryptococcus neoformans*. *Journal of Science and Science Education (JoSSEd)*, 1(1): 35-40.
- [27] Effendy, L., (2013). Potensi Antijamur Kombinasi Ekstrak Etanol Daun Sirih Merah (*Piper crocatum* Ruiz & Pav.) dan Kelopak Bunga Rosella (*Hibiscus sabdariffa* Linn.) terhadap *Candida albicans*. *Jurnal Ilmiah Mahasiswa University Surabaya*. 2(1):1-10.
- [28] Bishop, K. S., Kao, C. H., Xu, Y., Glucina, M. P., Paterson, R. R. M., & Ferguson, L. R. (2015). From 2000 years of *Ganoderma lucidum* to recent developments in nutraceuticals. *Phytochemistry*, 114, 56-65.
- [29] Chaturvedi, V. K.; Agarwal, S.; Gupta, K. K.; Ramteke, P. W.; & Singh, M. P. (2018). Medicinal mushroom: boon for therapeutic applications. *3 Biotech*, 8(8).
- [30] Zuzarte, M. and Ligia, S. (2015) .*Essential Oils Chemistry*. Springer International Publishing Switzerland. P:19-28.
- [31] Dhifi,W.,Sana, B.,Sabrine, J., Nada, B., and Wissem, M. (2016) .Essentialoils chemical characterization and investigation of some biological activities : A Critical Review. *J.med. icines*,Vol:4 (2) 1-16.
- [32] Baby, S. , Johnson, A.J., Govindan, B.,(2015). Secondary metabolites from *Ganoderma*, *Phytochemistry* 114 .66–101.



- [33] Hassan A. Abdulkareem and Maryam Hamid Nasir (2014) Effect of interaction between biocontrol fungus *Trichoderma harzianum* and mycorrhizal fungus *Glomus mossae* in fusarium wilt disease on some pepper cultivars. *Journal of Tikrit University For Agriculture Sciences*. 14(3):116-129.
- [34] Hassan A. Abdulkareem and Maryam Hamid Nasir (2014) Effect of interaction between biocontrol fungus *Trichoderma harzianum* and mycorrhizal fungus *Glomus mossae* in fusarium wilt disease on some pepper cultivars. *Journal of Tikrit University For Agriculture Sciences*. 14(3):116-129.
- [35] Akbar, A.; Hussain, S.; Uiah, K.; Fahim, M. and Ali, G. (2018). Detection, virulence and genetic diversity of *Fusarium* species infecting tomato in Northern Pakistan. *PLoS ONE* 13(9).
- [36] Mezzomo, R., Rolim, J. M., Santos, Á. F. D., Poletto, T., Walker, C., Maciel, C. G., & Muniz, M. F. B. (2019). Aggressiveness of *Fusarium oxysporum* and *Fusarium solani* isolates to yerba-mate and production of extracellular enzymes. *Summa Phytopathologica*. 45(2):141-145.
- [37] Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M. and Zheng, B., (2019). Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules*, 24(13), p.2452.
- [38] Abbas, Jamal Ahmed and Aidan, Asmaa Thamer. (2017) The role of soil inoculation with the fungus *Trichoderma harzianum* and soil mulching in. *Kufa Journal for Agricultural Sciences*, 9(2).

Bioaccumulation of the Concentration of some Heavy Metals in, Spring Water in Al-Shinafiyah District in Al-Diwaniyah Governorate - Iraq

Shahad Jassim Mohammed¹ and Haider Mashkooor Hussein²

^{1,2} Department of Environment, College of Science, University of Al-Qadisiyah, Al-Qadisiyah, Iraq.

¹E-mail: shhdjasm653 @qu.edu.iq

²E-mail: haider.mashkooor.h@qu.edu.iq

Abstract. This study was conducted in the city of Al-Shinafiya - in Al-Diwaniyah Governorate, southern Iraq. This study aims to measure some of the physicochemical characteristics of spring water quarterly basis . As well as measuring the concentration of heavy metals (cadmium, zinc, copper and zinc) in water during the summer, and indicating the extent of the effect and suitability of spring water for human use . water samples were collected from 3 springs (S1-S2-S3) for the year 2024, based on isotopic measurements. Through this study, some physical properties were measured, such as temperature, turbidity, and some chemical properties (pH, electrical conductivity, total hardness, sodium absorption rate (SAR) and the concentration of each of (SO₄, Cl, Mg, Ca, Na, K) of spring water. A comparison of the water quality with internationally approved values was conducted to indicate its suitability for human use and irrigation. The study showed that the water temperature ranges between (21.00-27.17°C), the air temperature ranges between (31.67-41.00°C), the pH ranges between (6.92-7.14), and the electrical conductivity measurement ratio ranges between (4.24-5.61 MS\CM). The results showed that the total solids ranged between (3628.33 - 4051.33 Mg/L), the turbidity ranged between (2.75 - 22.27 NTU), the percentage of dissolved oxygen ranged between (7.50-8.63 Mg/L), and the vital oxygen requirement (1.5 - 16 Mg/L). The total base ranges between (104.33-130.67 Mg/L). The total hardness is between (2452.67 - 2524.67 Mg/L), calcium is between (484.33 - 534.00 Mg/L), magnesium is between (291.09 - 318.03 Mg/L), and chloride is (746.00 - 1284.53 Mg/L). Nitrates ranged between (16.534 - 29.892 Mg/L), phosphate (0.041 - 0.197 Mg/L), and nitrite (0.152 - 0.616 Mg/L). Minerals were also measured for the water.

Keywords. Heavy metals , Groundwater , Pollution , Spring water.

1. Introduction

Water is considered one of the basic pillars that support all human, plant and animal life forms and is obtained from major natural sources, including surface water (lakes, rivers, streams, etc.) and groundwater (well water and spring water), more than one billion people in the world do not have access to safe drinking water, and three billion lack access to basic sanitation services. About five million die annually from water-related diseases [1].

In the hydrological cycle, less than 0.1% of metals are actually dissolved in water and more than 99.9% are stored in soil and sediments. Heavy metals are considered among the most important main pollutants of water sources springs on the surface of the Earth, and sometimes form a waterway or land Wet or puddle. The importance of water comes from the fact that it is used in drinking, agriculture, industry, and urban development to preserve fresh water resources, which is one of the causes of common problems in countries of the world, and requires the development of programs and studies to monitor these water sources from pollution.

Groundwater is considered one of the most important parts of water resources, as it constitutes 71.7% of the world's drinking water. Groundwater includes well and spring water, which originates mainly from rainwater and irrigation water that drains into the ground and is stored below its surface in non-porous layers to form reservoirs of water. Subsurface area [2].

Water has a large and important role. Water is necessary for life on Earth. The fresh water supply makes up less than 1% of the water available on Earth and human life depends primarily on it. Water quality is important for health and economic development, and factors such as seasons, human influences, and natural processes affect the chemical composition of water, drinking water is generally obtained from surface water, such as lakes, rivers, and streams, and groundwater, such as spring water and wells. A spring is a natural flow of water from an underground source to the surface of the earth.

Springs generally occur where the Earth's surface and impermeable aquifers intersect the water table. [3]. The spring being pushed to the surface could be the result of a confined water reservoir where the recharge area of the spring water level is located at a higher elevation than the outlet elevation. The spring water discharge fluctuates seasonally, it depends mainly on the pattern of rainfall in the recharge area and the difference in the amount of rainwater that is able to infiltrate the ground. Pollution of the environment by heavy metals tends to occur in areas that are highly urbanized, industrialized or that are systematically supplied with pollutants, for example by rivers carrying Mineral-rich suspensions [4].

Concentrations of heavy metals in water and air can be identified, however increasing environmental degradation is also affecting remote areas, including national parks. The heavy elements are those whose atomic number is more than 20 and whose density is greater than 5 g/cm³.

They can move between different parts and parts of the environment, they come from nature and can be found in many places, and they constantly change from one place to another. One place to another and from one form to another. Micronutrients are essential components of plant and animal diets, micronutrients such as (Cu, Zn, Fe, Mn, Co, Mo, Cr, Se) and macronutrients such as (Ca, Mg, Na, P, and S) are essential components required in milligram or microgram amounts per day that require biological and metabolic processes that Macronutrients identify a wide range of dangerous non-essential heavy metals, such as (Pb, Cd, Hg, and Ni) but in larger amounts (100 mg or more per day [5].

Heavy metal pollution comes from a wide range of sources, including the oil and gas industry, oil refineries, iron and steel mills, glass and aluminum manufacturers, tanning plants, fertilizer, pesticide, and gasoline companies, and many other types of businesses and industries, heavy metals may enter water supplies as a result of pollution resulting from industrial or consumer waste, and the contribution of liquid waste resulting from industrial processes in raising the concentrations of elements in the water. Heavy metals are widespread in riverine ecosystems and are affected to varying degrees by physical and chemical factors and the characteristics of the biomass within the water body.

Which therefore plays a role, as heavy metals are distributed in the water between the dissolved and partial phases, and they are also absorbed on the surfaces of suspended materials and bottom

sediments, and all of these solutions can affect the concentration of heavy metals in suspended materials and the presence of living organisms in the sediments, and these elements are Part of how the concentration, circulation, movement between layers of water, and diffusion of these elements are regulated [6].

1.1. Study Area: Shenafiya City

An Iraqi city and district in the Diwaniyah Governorate in southern Iraq. It is about 70 kilometers west from the center of the Diwaniyah Governorate. It is about 245 kilometers away from Baghdad to the south. Its borders are in contact with the Muthanna Governorate from the southeast, about 70 kilometers, and from the west, about 20 kilometers from the Al-Qadisiyah district. To Najaf Governorate.

The Euphrates River passes near it. The study area is located between longitude ($44^{\circ}50' - 44^{\circ}30.5'$) and latitude ($31^{\circ}40' - 31^{\circ}10'$) and is confined between the cities of Al-Shenafiya and Samawah. Where the course of the Euphrates River, in its two parts between Shinafiya and Samawah, approaches the borders of the plateau, the distance between them sometimes reaching a few kilometers (KM7-5). The region covers a group of formations that begin with the Al-Rass Formation and end with the Euphrates Formation. The region is characterized by a hot, dry climate in the summer and a cold, humid climate in the winter, depending on the station. Weather conditions in Diwaniyah Governorate: The average annual temperature is 24° .

The annual relative humidity rate (40%-35%), the dryness coefficient (30%-35%), the annual rate of evaporation (3500 mm), and the annual rainfall rate (75-100 mm), and one of the most important sources of water in this region is the river Al-Shinafiyah, Al-Qadisiyah River, and Al-Haffar River, in addition to many artesian wells, numbering 29 wells, and also the presence of natural resources, including springs, which number 24, as well as springs, which number about 13 springs located in the city of Al-Asr, which are (Ain Hajjah Sukkar, Ain Al-Qaim, Umm Ghaila, Al-Gharia, Umm Al-Wawiyah, Umm Ghous, Zaidan, Druze, Al-Majhoul, Al-Tarouk, Zaher, Daoud Al-Alawi, Umm Thakkar), and (10 springs) in the Al-Rukbaniyah area, which are (Al-Shuwayridh, Mashar, Jassum, Ubaid, Umm Qasir, Suniha, Wawi Al-Hamza, Sandan, Al-Aboud, and Al-Hassouni). There is one water spring located in the Marsh Hor Allah area called Al-Awaina, and there is an oil refinery located in it, which is far from the center. City 15 km.

The area is considered to be of protected agricultural importance, as the water of the Thirsty River and the water of some springs and semi-deep wells are exploited to grow barley and some vegetables.

1.2. Study Sites

In this Current Study, Three Sampling Sites were chosen as follows:

1.2.1. The First site: Ain Al-Qaim

Al-Qaim Spring: This spring is located in the Al-Asr area, about 14 km from the center of Al-Shinafiya district. It is located at approximately ($31^{\circ} 35' - 22.36'$) north ($44^{\circ} 32' - '33.02''$) east. The depth of this spring is about 1 meter and extends, and the source of water from these springs reaches a distance of 7 km, and is characterized by biological diversity.



Figure 1. The first site showing the location of the Al-Qaim water spring.

1.2.2. The Second site: Ain Jassoum

It is located in the village of (Albu Halil), about 20 km from the center of Al-Shinafiya. It is located at about (35°,31') north and (44°,32') east. Its depth is about (7 m) and it is characterized by the presence of plants such as reeds and palm trees.



Figure 2. The second site, Jassoum water spring.

1.2.3. The Third Site: Ain Dahak

Ain Dahak is located in the village of Buhlil. It is about 25 meters away from the center of Shinafiya. It is located at about (21°.30') north to (35°.41') east. Its depth is about (10 metres). It is characterized by It is used by the residents of this region for drinking, irrigation, and many other uses.



Figure 3. The third site, Dahak water spring.

1.3. Collection of Samples

Samples 3-were collected in the morning, starting at the first site and ending at the third site, quarterly for a full year during the summer of 2023 and ending in the spring of 2024, three times for each season, as follows: Pumping the spring water for approximately 10 minutes to get rid of stagnant or polluted water: then, water samples were collected directly using 5-liter polyethylene containers with the minimum possible air space to preserve the sample's properties during transportation, taking into account washing the containers with sample water three times before filling them, and conducting chemical, physical, and heavy metal (HMs) tests.

1.4. Physical and Chemical Factors of Water

1.4.1. Physical Factors of Water

The physical characteristics of water play a major role in the revival of that water. Physical factors play an important role in the chemical interactions of the aquatic environment, as well as affecting the metabolism of those organisms and their ability to compete with each other [7]. Physical and chemical factors are a measure to determine water quality. The physical and chemical properties of water play an important role in determining the quality and suitability of water and give an idea about the water content of organic and inorganic elements and compounds, such as temperature, which affects the course of chemical reactions and metabolic processes in the water environment and its solubility [8]. Among the important physical characteristics of water are temperature and the water load of suspended materials of organic and mineral origin, which determine the quality of water in terms of its suitability for irrigation . While other physical properties such as density, hardness, surface tension, etc. are often not taken into consideration. Chemical properties of irrigation water, when judging the quality of irrigation water, special importance is given to its dissolved components, especially the dissolved salts in irrigation water. It greatly determines the suitability of water for irrigating various agricultural crops [9]. It was found that throwing waste into spring water in the city of Shinafiya increases the turbidity values. Physical and chemical factors also include pH, suspended and dissolved solids, electrical conductivity, dissolved oxygen, and physical factors such as the amount of light transmittance, thermal distribution, flow speed, and sedimentation rates.

Turbidity is a measure of the amount of suspended particles in water. Their quantity in water sources is determined by a number of factors, including the characteristics of the area, such as its geology and human development. While dissolved oxygen (O.D.) in water is one of the factors affecting the water quality of any water source and its concentration in the water is controlled by more than one factor, the most important of which is water temperature.

Electrical conductivity is considered one of the important factors in environmental studies to determine water quality. It means the ability of water to transmit electrical current and is directly affected. Total solids and total suspended solids are mostly composed of inorganic substances such as (calcium, magnesium, potassium, sodium, carbonates, bicarbonates, chlorides and sulfates), in addition to dissolved organic materials and salts [10]. (BOD) is defined as the amount of oxygen required by microscopic organisms to decompose organic materials present in water under aerobic conditions during a specific period of time and under specific temperatures for one liter of water, while the chemical oxygen demand is the amount of oxygen consumed by oxidizing chemical compounds that oxidize organic materials without the intervention of microorganisms, and organic and inorganic materials are oxidized in an acidic medium and by potassium bichromate, Cr_2O_7 [11].

1.4.2. Chemical Factors of Water

One of the most important chemical properties of water, which was addressed through reviewing research and studies, and among the most important, which was addressed through this study:

The most important of them is the sodium ion, which comes into the water through the weathering of rocks containing a high percentage of sodium ions, such as clay minerals. Sodium salts are characterized by their extreme dissolution in water and do not precipitate easily, and a large amount of Na can be removed by ion exchange, while the magnesium ion is a major component of ferromagnetic minerals, as it is found in igneous rocks, it participates with calcium as one of the causes of hardness in water. Despite the high solubility of compounds containing magnesium, its concentration in natural water is less than the concentration of calcium, as it is smaller in size than calcium and sodium and tends to combine with water [12].

The concentration of potassium ion is lower in natural water than sodium, as most potable water contains less than (10) parts per million, as potassium ion is more stable than sodium ion due to its high resistance to chemical weathering factors and the ease of absorption from clay minerals.

Chlorides in the water of Laayoune are mainly attributed to weathering processes that occur in rocks and soil and are transferred to the water of Laayoune through filtration, where water seeps from beneath the soil and bypasses its layers and rocks, leading to the absorption of chlorides and an increase in the chloride concentration of (250 mg/L) in Water changes the taste of water [13].

Also, sulfate concentrations in drinking water are more than (400 mg/L), which can lead to cases of diarrhea and digestive system disturbance. The main source of sulfate ions in water are solutions of sulfate minerals that are present in sedimentary rocks as a result of the oxidation of sulfur minerals, and sulfates are one of the important components of hardness. Water after calcium and magnesium, and their concentrations above 500 mg/L cause the water to taste bitter.

Nitrates occur in nature, either from organic or inorganic sources, such as waste discharge, artificial fertilizers, and animal waste. A high concentration of nitrates in water supplies beyond permissible limits locally and globally is considered toxic to living organisms, and physiologists, doctors, and veterinarians agree [14].

1.5. Heavy Metals

heavy metals, whose density exceeds 5 mg/cm³ in water, and they have negative effects on human, animal and plant health, including heavy elements (lead, cadmium, arsenic, selenium, zinc).

Pollution of systems with heavy metals has become a common environmental problem, and water contamination with heavy metals comes from various sources, which may be natural, resulting from weathering of soil and rocks, or from human sources resulting from the disposal of industrial waste.

Zinc and cadmium are considered among the most dangerous elements that cause problems in agricultural production, as well as in the disposal of solid and toxic waste. Household and factory waste can lead to soil contamination with heavy elements, whether thrown or buried in the soil and thus transferred to the water [15].

Concentrations of free zinc ions are usually low due to the dominance of poorly soluble metals within the reaction degree range of natural waters (18). The most important sources of zinc ions are minerals. Zinc precipitates when the pH value is between (7-9) in the form of zinc sulphate (ZnSO₄).

Copper is a metal that is a good conductor of electricity and a good conductor of heat. It is also an essential element for all body systems to survive, as it is found in all body tissues. It is important in maintaining nerve cells and the immune system, and plays a role in energy production. Lead is a toxic metal found naturally in the Earth's crust. Its widespread use has led to widespread

environmental pollution and caused major public health problems in many parts of the world [16].

2. Materials and Methods

2.1. Temperature

It was measured using a mercury thermometer, the temperature of both water and air in the field, the temperature of the air in the shade, while the mercury tip of the thermometer was immersed in the water sample for several minutes and the reading of the thermometer was recorded.

2.2. Electrical Conductivity (EC)

Was measured using a German-made manual EC device, measured in units (microsiemens.cm). Field-measured electrical conductivity was measured and the readings were adjusted to the temperature at 25°C by projecting the readings onto the standard table and according to the equation:

$$EC_{25} = EC_t \times Ft$$

2.3. pH

pH was measured in the laboratory using a pH meter device, OAKTON type, made in Singapore, after regulating the device with pH buffer solutions (PH 4, pH 7, pH 9) [17].

2.4. Turbidity

The turbidity was measured directly upon arrival to the laboratory using a turbidity measuring device (Turbidity Meter, Model 20220 LaMotte) after calibrating it with standard solutions, where the samples were shaken well, and the measuring cell was filled until the mark marked on it was reached.

Taking into account not to leave traces of fingers on the measuring cell. This can be done by wiping the cell using a cloth to avoid the formation of air bubbles and ensure the stability of the device. Express the result in units (NTU).

Dissolved oxygen in water was measured using the Azide modification method. Water samples were stabilized in the field and after being transported to the laboratory, 1 ml of concentrated sulfuric acid (H₂SO₄) was added.

Then it was shaken up and down to homogenize the sample, after which a specific volume of the sample was taken and drops of starch indicator were added to change the color of the sample to blue and it was flushed against a standard sodium thiosulfate solution (N0.025) while continuing to calibrate and stir the sample until the blue color disappeared, and the concentration of the dissolved oxygen was calculated. According to the following equation [18]:

$$DO \text{ mg}$$

$$L = V \times N \times eq. wt \times 100$$

2.5. Biochemical Oxygen Requirement

(BOD) was calculated using the method of measuring dissolved oxygen, after placing unfixed and opaque bottles in temperature conditions (25 °C) for five days, then determining the dissolved oxygen (5DO) and expressing it in mg/L according to what Stated in accordance with the following equation:

$$BOD5 \text{ mg/l} = DO \text{ mg/l} - DO5$$

2.6. Bicarbonate and Basic Alkaline Ions

They were measured by taking a known volume of two drops of Orange Methyl Index, which turns the color of the aqueous sample yellow, and then titration is done with sulfuric acid (H₂SO₄) until the color of the sample changes from yellow to reddish orange, indicating the end of the test. The reaction point, the basicity, is from the following equation:

$$T. \text{ Alk mg.l}^{-1} = (V \times N \times EQ.WT \times 1000) / (\text{ml of Sample})$$

$$\text{HCO}_3 \text{ mg.l}^{-1} = (V \times N \times 61 \times 1000) / (\text{ml of Sample})$$

2.7. Salinity (%)

The salinity of the water was estimated based on the electrical conductivity values E and C expressed in parts per thousand according to what was mentioned and according to the equation [19]:

$$\text{Salinity} = E.C \times 0.64/1000$$

2.8. Calcium Hardness

Was measured by taking a known volume of the water sample and to make the pH = 12, drops of sodium hydroxide (N1) NaOH solution were added to it, then a little meroxide indicator was added and it was triturated against a diaminetetraacetic acid solution of the standard sodium salt (EDTA2Na) N0.02) while stirring using a glass rod until the color changes from pink to violet, and calculate the calcium hardness concentration from the following equation:

$$\text{Ca.H (mg.l) CaCO}_3 = (V \times N \times EQ.WT \times 1000) / (\text{ml of Sample})$$

2.9. Magnesium Hardness

Was calculated as mentioned in (APHA, 1998) from the difference between chelate hardness and calcium hardness as shown in the following equation:

$$\text{Mg. H mg.l}^{-1} \text{ as CaCO}_3 = T.H - \text{Ca.H}$$

2.10. Concentration of Sodium and Potassium Ions

The concentration of both sodium ions and potassium ions was measured using a Japanese-made flame emission spectrophotometer (photometer Flame 7PFP), and then the readings were compared with the standard curve values and the results were recorded in mg.

2.11. Concentration of Chloride Ions

The concentration was measured using the Mhor method by taking a known volume of the water sample, and adding drops of potassium chromate indicator (4CrO₂K) to it until the color of the sample became yellow, and then titrated against the standard silver nitrate solution (N0.0141) until it changed Color from yellow to Red wine, and a Blank was done in order to correct the results according to the concentration from the following equation [20]:

$$\text{Cl-mg.l}^{-1} = (V \times N \times EQ.WT \times 1000) / (\text{ml of Sample})$$

2.12. Concentration of Sulfate Ions

The concentration of sulfate ions was measured using a method. Turbid matrix: It was done by taking a known volume of the well-filtered aqueous sample and diluting it with distilled water according to the sulfate concentration.

Then (2 ml) of the reagent Conditional solution and (1 ml) of the barium chloride solution were added with mechanical stirring using a magnetic stirrer device at a constant speed using the magnetic stirrer for one minute.

The models are then measured using a Turbidity Digital Labtech meter device with a Planck operation to obtain accurate results, and the concentration is calculated in mg.l after comparing the device's readings with the standard curve.

2.13. Concentration of Phosphate Ions

To measure the phosphate ion, the mixed solution method was adopted according to (APHA, 2017) using a spectrophotometer at a wavelength of (885 nm). The results were expressed in units of micrograms of phosphorus atom/liter.

$$PO_4 = ABC^*$$

Nitrates were estimated according to the Ultra Violet Screening Method by taking a known volume of the well-filtered water sample, then adding (1 ml) (HCl) to it and shaking well to homogenize the sample, and then measuring it using a UV Spectrophotometer (of Japanese origin) at wavelengths (220 and 275). Nanometer) taking into account the plank work to correct the readings, then compare with the standard curve, and the concentration is calculated in (mg.l) [21].

2.14. Total Heavy Metals in Water in HMs Total

Dry (100-1000 ml) of the water sample at a temperature of (80°C), transfer the remaining solution after evaporation to a Teflon container, add (6 ml) a mixture of concentrated hydrochloric acid HCL and nitric acid 3HNO in a ratio of 1:1, and heat the mixture to dryness at a temperature of 80°C. ,

The addition was repeated in the same proportion to a mixture of hydrofluoric acid (HF) and perchloric acid (4HClO) in the amount of 4 ml, while continuing to heat until dry. The precipitate was dissolved with dilute hydrochloric acid (N0.5) in the amount of 20 ml and left for 10 minutes, then separated by centrifugation for 20 minutes at a speed of 3000 r/min.

The solution is then transferred to a 25 ml volumetric bottle, and the sediment is washed with distilled water and added to the volumetric bottle, and the volume is supplemented to 25 ml with distilled water free of ions (APHA-2017). The samples are kept until they are examined with a flame atomic absorption spectrophotometer (Absorption Atomic Flame) and the results are expressed. In units of micrograms/liter [22].

3. Results and Discussion

3.1. Physical and Chemical Factors

The results of the current study, which was conducted on the study sites during the summer, showed that different concentrations of physical and chemical factors were recorded. It is noted from the results that the pH values were within the permissible range, reaching (6.92 - 7.14), as the permissible limits were between (6.5 - 8.5).

The reason for this is the presence of suspended materials such as clay, organic materials, and total dissolved solids. Through the results of the analysis, we notice an increase in the temperature of both air and water during the study sites, and the highest air levels were recorded during the third study site, where it reached (41.00 C).

As for the water temperature, it reached its highest rate in the first locations (27.17 C). The reasons for the high temperature are attributed to the fact that Iraq's climate is hot and dry during

the summer, in addition to desertification. It is also noted that these springs have a high percentage of salinity, which leads to an increase in the percentage of electrical conductivity [23]. While water is considered a good conductor of electricity and therefore it is classified as salty water, the EC percentage (NUT5.61) reached the highest values across the study sites, and therefore it cannot be used for drinking and mowing the lands because salts lead to reducing the fertility of the land. Salinity increases as it approaches feeding areas [24].

With regard to total dissolved salts, which is considered the main factor in determining the suitability of drinking water, it is noted from the recorded results that the values of dissolved salts increased in the study sites during the summer, and they reached their highest values in the first site, where they reached (4051.33).

The reasons for their increase are due to the use of fertilizers on the grazing lands adjacent to the springs, in addition to the presence of salts as a result of natural factors. [25] With regard to turbidity, it represents the quality and purity of the water. The recorded results showed high turbidity values in the third study sites, reaching (22.27). The reason for this is due to dissolved solids in addition to the waste of human use, fertilizers, and the dredging of agricultural lands adjacent to the springs.

It is noted in Table No. (1) that the total base values are high, reaching (130.67). This is due to the decrease in oxygen concentration, as well as the increase in temperature, which leads to the decomposition process, as well as the nature of the rocks, while in some study sites, a high percentage of hardness was recorded, reaching (2524.67) in the first site. The reason for this is due to the pollutants represented by human and industrial waste that are added to it, as well as rainwater, which leads to the erosion of the soil adjacent to the river [26].

The study recorded a high concentration of calcium in some study sites, where it reached its highest level in the third site, where it reached (534.00). The reason for this is the falling rain and the drifts occurring from neighboring areas.

The low concentration of calcium in some study sites is due to the lack of water and the lack of solubility of calcium carbonate. The results of the study showed an increase in the concentration of magnesium in some study sites during the summer, reaching (318.03) in the first site.

The reason for this is due to the decomposition of the photosynthesis process in plants, which means its liberation as the main factor in the photosynthesis process. As for the decrease occurring in some study sites other than the summer season, the reason is due to the large presence of sulphates, which causes the deposition of magnesium ions as well as the flowering of aquatic plants.

The results showed that the chloride ion concentration is increasing throughout the study sites, as listed in Table No. (1), and the reason is due to household waste water and waste, increased water evaporation, and an increase in the amount of dissolved salts and minerals. It decreases in some study sites, and the reason for this is due to the rise in water levels and an increase in the drainage plant [27].

While the results showed that the nitrite ion decreased in some locations, the reason for this is due to the high values of solvent oxygen due to low temperatures.

The recorded results showed that the concentration of nitrate ions recorded high rates in some of the study sites during the summer, where the highest rates reached (29.892) in the third site as a result of the increase in their concentration, animal waste and waste, in addition to the agricultural fertilizers used in agriculture. All of the above contribute to increasing the concentration of nitrate ions, while the results recorded an increase in phosphate levels during the summer in the third site, reaching (0.197), the reason for this is the sediments present at the bottom of the eyes [28].

3.2. Heavy Metals in Water

Some heavy elements were measured in spring water, including copper, and the results recorded the highest value of copper concentration in the first location during the summer, at a rate of (mg/L 0.1134).

The results showed a decrease in copper values in spring water. The reasons for this decrease are attributed to its presence in a small percentage, in addition to the difficulty of its dissolution or mixing with other compounds such as copper sulphide and other compounds, which leads to its deposition in moderate water. The results of the current study recorded the highest value of zinc concentration in the second location during the winter season. Summer at a rate of (mg/L 0.0720), the results of the current study showed that zinc values were high in some study sites. The reason for this is the high percentage of waste in these springs, in addition to the solubility of zinc ions in the water, as the water of these springs is characterized as alkaline [29].

The results of the study showed that lead concentrations were at their highest values at the third water source during the summer, at a rate of (0.7132 mg/L). The results of the study showed variation in lead values across the study sites. The reason for this is due to the difference in sources of pollution and the nature of the surrounding area, and that the high concentration of lead in some The study sites are due to excessive use of chemical fertilizers and waste and increased solubility.

Table 1. Tests of physical and chemical factors of water for the study sites during the summer.

Tests	S1	S2	S3
Water temperature	27.17	21.00	21.17
Air temperature	31.67	37.33	41.00
PH	7.14	7.1	6.92
DO	7.50	7.63	8.63
BOD	2.04	1.47	14
EC	5.54	4.24	5.61
TDS	4051.33	3127.63	3628.33
TUYS	2.75	6.30	22.27
Total Alk	130.67	113.00	104.33
TH	2524.67	2452.00	2483.33
Ca	484.33	518.00	534.00
Cl	1284.53	746.00	944.23
Mg	318.03	299.19	291.09
NO ₃	16.534	29.705	29.892
PO ₄	0.041	0.057	0.197
NO ₂	0.622	0.152	0.616
Salinity%	3.13	2.48	3.10

The results of the study showed that the highest concentration of cadmium was at the second site during the summer with a value of (mg/L 0.0155). It is considered one of the most important heavy elements and is known as a flammable bluish-white metallic element. Cadmium pollution is a source of concern today due to various human activities such as burning waste. Smelting, use of phosphate fertilizers, and tobacco smoke [30].

Table 2. Heavy metal tests for study sites during the summer.

Tests	S1	S2	S3
Copper	0.1134	0.0759	0.0929
Zinc	0.0140	0.0720	0.0657
Lead	0.6672	0.0156	0.7132
Cadmium	0.0003	0.0155	0.0053

Conclusion

According to the research, the acceptable ranges for water temperature, air temperature, pH, and electrical conductivity measurement ratio are (21.00-27.17°C, 31.67-41.00°C, 6.92-7.14), and (4.24-5.61 MS\CM, respectively). The total solids ranged from 3628.33 to 4051.33 Mg/L, the turbidity from 2.75 to 22.27 NTU, the percentage of dissolved oxygen from 7.50 to 8.63 Mg/L, and the essential oxygen demand from 1.5 to 16 Mg/L, according to the data. The range of total base is 104.33 to 130.67 milligrams per liter. The magnesium concentration ranges from 291.09 to 318.03 mg/L, the total hardness from 2452.67 to 2524.67 Mg/L, the calcium concentration from 484.33 to 534.00 Mg/L, and the chloride concentration from 746.00 to 1284.53 Mg/L. The concentrations of nitrates, phosphates, and nitrites were measured in millimg/L, uMg/L, and mMg/L, respectively. The water was also tested for minerals.

References

- [1] Boyd, R. S. (2010). Heavy metal pollutants and chemical ecology : Exploring new frontiers. J. Chem. Ecol., 36:46-58
- [2] Sharma P et Dubey RS (2005). Lead toxicity in plants."Brazilian Journal of Plant Physiology 17: 35-52 .
- [3] Harry, T., Asuaiko, E., Akata, N., & Akpan, N. (2020). 1. Department of Geology, Akwa Ibom State University, Ikot Akpaden, AKS 2. Diviem Nigeria Ltd, Victoria Island, Lagos Nigeria.
- [4] Abdur-Rahman, S. O. (2007). Nitrogen reduction in Lulea Kommun wastewater effluent. M.sc. thesis. Environmental engineering-Lulea university.
- [5] Culha, S. T.; Bat, L.; Culha, M.; Efendioglu, A.; Andac, M. B .and Bati, B. (2007). Heavy metals levels in some fishes and mollusks from Sinop Peninsula of the southern Black sea, Turkey. Rapp . comm. Int. Mer. Medit, 38:323.
- [6] Matrood, M. J., & Hussein, H. M. (2021, June). A preliminary ecological analysis of spring water in Al-Shanafiyah District, Al-Qadisiyah Province ‘Southern Iraq. In IOP Conference Series: Earth and Environmental Science (Vol. 790, No. 1, p. 012004). IOP Publishing doi:10.1088/1755-1315/790/1/012004
- [7] Khudair, B. H. (2013). Assessment of water quality index suitability of the Tigris River of drinking water within Baghdad city, Iraq. J. Engineer., 19(6), 764-774.
- [8] Anyanwu·E. D., & Onyele, O. G. (2018). Occurrence and concentration of heavy metals in a rural spring in South-eastern Nigeria. Journal of Applied Sciences and Environmental Management, 22(9), 1473-1478.DOI:https://dx.doi.org/10.4314/jasem.v22i9.19
- [9] Walna·B., & Siepak, M. (2012). Heavy metals: their pathway from the ground·groundwater and springs to Lake Góreckie (Poland). Environmental monitoring and assessment, 184, 3315-3340 DOI/10.1007 s10661-011-2191-7
- [10] Avdullahi, S., Fejza, I., Tmava, A., Rama, M., & Hetemi, M. (2013). Assessment of heavy metal in the water springs, Stan Terg, Kosovo. International Journal of Engineering, 2(4), 2305-8269
- [11] Madan, R. Chaudhry, S. Sharma, M. and Madan, S. (2018) Determination of water quality index of Indraprastha estate region and the vicinity area in Delhi, India. ESSENCE Int. J. Env. Rehab. Conserv., IX (1)100-89 :DOI: 10.31786/09756272.18.9.1.126
- [12] Ibrahim, R. K., Hayyan, M., AlSaadi, M. A., Hayyan, A., & Ibrahim, S. (2016). Environmental application of nanotechnology: air, soil, and water. In Environmental Science and Pollution Research .https://doi.org/10.1007/s11356-016-6457-z
- [13] Kasamatsu, Y. et al. 2022 ‘Coprecipitation with samarium hydroxide using multitracer produced through neutron-induced fission of 235U toward chemical study of heavy elements’, Applied Radiation and Isotopes, 179, p. 110006 .Available at: <https://doi.org/10.1016/j.apradiso.2021.110006>.

- [14] M. Hussein, "A study of some physical and chemical properties of Al-Shanafiya Spring water in alDiwaniya governorate-Iraq," Al-Kufa Univ. J. Biol., vol. 4, no. 2, 2012. <http://www.uokufa.edu.iq/journals/index.php/ajb/index>
- [15] Al-Abadi, A. M., Fryar, A. E., Arjan A. Rasheed, A. A. & Pradhan, B(2021) . Assessment of groundwater potential in terms of the availability and quality of the resource: a case study from Iraq. Environmental Earth Sciences. 80:426. <https://doi.org/10.1007/s12665-021-09725-0>
- [16] Aiyesanmi, A. F., Oladele, M. F., Adelodun, A. A., Idowu, G. A. (2020) .Speciation and bioavailability studies of toxic metals in the alluvial soil of Onukun River floodplain in Okitipupa, South- western Nigeria. Environ Qual Manage.30:131–143. DOI: 10.1002/tqem.21720.
- [17] Adimalla, N. (2019). Controlling factors and mechanism of groundwater quality variation in semiarid region of South India: an approach of water quality index (WQI) and health risk assessment (HRA). Environ Geochem Health. <https://doi.org/10.1007/s10653-019-00374-8> (0 1 234 567 89 0),-vo IV () 0 123 45 869 7 (),-v o IV)
- [18] Prasad, S. and Lall, R. 2022 _Zinc-curcumin based complexes in health and diseases: An approach in chemopreventive and therapeutic improvement', Journal of Trace Elements in Medicine and Biology, 73, p. 127023. Available at :<https://doi.org/10.1016/j.jtemb.2022.127023> .
- [19] ARKOÇ, O. (2016). Application of Water Quality Index with the Aid of Geographic Information System in Eastern Thrace to Assess Groundwater Quality. Geological Engineering J./Jeoloji Mühendisliği Dergisi., 40(2):189-207.www.werathan.com
- [20] Marčiulionienė, D. et al. 2017 _137Cs and plutonium isotopes accumulation/retention in bottom sediments and soil in Lithuania: A case study of the activity concentration of anthropogenic radionuclides and their provenance before the start of operation of the Belarusian Nuclear Power Pla', Journal of Environmental Radioactivity, 178–179, pp. 253–264. Available at : <https://doi.org/10.1016/j.jenvrad.2017.07.024> .
- [21] Shabbir, Z. et al. 2020 _Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment', Chemosphere, 259, p. 127436 .Available at : <https://doi.org/10.1016/j.chemosphere.2020.127436>.
- [22] Albert, H.A. et al. 2021 _Influence of biochar and soil properties on soil and plant tissue concentrations of Cd and Pb: A meta-analysis', Science of The Total Environment, 755, p. 142582. Available at :<https://doi.org/10.1016/j.scitotenv.2020.14258>
- [23] Hui Y. H. et al., Handbook of food products manufacturing: health, meat, milk, poultry, seafood, and vegetables, vol. 2. John Wiley & Sons, 2007 .
- [24] Marković, S., Vučković, B., Nikolić-Bujanović, L., Kurilić, S. M., Todorović, N., Nikolov, J., ... & Đokić, B. (2020). Heavy metals and radon content in spring water of Kosovo. Scientific reports, 10(1), 10359
- [25] Federation W. E. and Association A. P. H., "Standard Methods for the examination of water and wastewater," Am. Public Heal. Assoc. Washington, DC, USA, 2005 .
- [26] Lind O. T., Handbook of common methods in limnology. The CV Mosley Company, 1979 .
- [27] Maiti S. K., Handbook of Methods in Environmental Studies, 1: Water and Wastewater Analysis . ABD Publishers, 2004 .
- [28] Al- Janabi, Z.Z.; Al- Kubaisi, A.A.; Al- Obaidy, A.M.J. (2015). Applied of CCME water quality index for protection of aquatic life in the Tigris River with in Baghdad City. J. Al- Nahrain University, 18 (2), 99-107.
- [29] Salman, J.M.; Abd- Al- Hussein, N.A.; Al- Hashimi, O. (2015). Assessment of water Quality of Hilla River for drinking water purpose by Canadian Index (CCME WQI). Internat. J.Recent Scient. Research. 6(2), 2746-2749.
- [30] Hosam, E.A.F.; Hamuda, B.; Patko, I. (2012). Ecological monitoring of Danube water quality in Budapest Region. American J. Environmental Sci., 2(3), 202-211 .
- [31] Khudair, B. H. (2013). Assessment of water quality index suitability of the Tigris River of drinking water within Baghdad city, Iraq. J. Engineer., 19(6), 764-774.

Improved Efficiency of Actara and Movento Insecticidal by Silica Oxide Nanoparticles to Control *Bemisia tabaci*

Raneen Mohammed Ali¹ and Baida Mohsen Alshukri²

^{1,2}Faculty of Agriculture, University of Kufa, Najaf, Iraq.

¹ E-mail: raneenr.almuusawi@student.uokufa.edu.iq

² E-mail: baidaa.alshukri@uokufa.edu.iq

Abstract. The white fly, *Bemisia tabaci*, is the most dangerous pest of agricultural crops in greenhouses worldwide, and has now been recorded on all continents of the world. In this work, a series of field and laboratory experiments were conducted to evaluate the efficiency of silicon oxide nanoparticle (SiO-NPs) and Actara/Movento pesticides on the nymphs of *B. tabaci* on Cucmber, *Solanum lycopersicum* L. The results of the laboratory study showed that the corrected percentage of mortality of the 3rd and 4th instar nymphs of *B. tabaci* when applying silica oxide nanoparticles at concentrations of 25, 50 and 75 ppm were 39, 49 and 52% mortality respectively. The results also confirmed that loading SiO-NPs at 25, 50 and 75 ppm with Actara pesticide at the recommended concentration caused mortality of 77, 78 and 82% respectively. In another laboratory experiment the use of SiO-NPs 25, 50 and 75 ppm with Movento at the recommended concentration affected nymphs by 54, 67.5 and 73.3%, mortality, respectively after 72 hours of treatment. while the Actara and Movento treatments gave nymphal mortalities of 60 and 42%, respectively. In the greenhouse experiment, the SiO- Actara treatment recorded the lowest numerical density of nymph, which was 0.0 nymph/leaf, and the average nymphal density decreased as a result of the treatments SiO –Movento, Actara, Movento , SiO NPs, Actara and Movento to 2.3, 1.3, 5.7 and 2.7 nymph/leaf respectively, compared to the control treatment which reached 73 nymph/leaf at 10 day post-treatment. These results suggest that the application of SiO-NPs at 75 ppm can be used as a way to improve the pesticide's action to control whitefly.

Keywords. Whitefly, Silica Oxide Nanoparticles, Actara, Movento.

1. Introduction

The cotton whitefly is an economically harmful insect that infects many plant families. The whitefly *Bemisia tabaci* (Gennadius), which belongs to the family Aleyrodidae and the order Hemiptera, was first identified in 1889 on tobacco plants, so this species was called at that time the tobacco whitefly *Aleyrodes tabaci* [1]. Whiteflies are a major pest of vegetable crops and greenhouse crops, because multiple plant hosts, rapid reproduction and high fertility, as well as their resistance to many pesticides [2,3]. The damage caused by this insect comes through its direct feeding, as both nymphs and adults insert their piercing and sucking mouthparts into the plant tissues and suck plant sap from the green parts, causing yellowing of the leaves [4]. The nymphs also inject enzymes during feeding, which changes the physiology of the crops and thus delays the ripening of the fruits [5]. As for its indirect damage, it is represented by its secretion of

honeydew (sugar waste), which hinders the process of photosynthesis, and it also affects the processes of transpiration and respiration through the adhesion of dust to it, in addition to the fact that it promotes the growth of sooty mold on the leaves and fruits [6,7]. Whiteflies are considered efficient vectors of plant pathogenic viruses as a result of feeding on infected plants and transferring them to healthy plants, where they transmit more than 350 types of viruses [8]. For this reason, *B. tabaci* has been classified as one of the most harmful pests to agriculture worldwide [9]. Chemical control has a fundamental and important role in reducing crop damage by getting rid of pests that infect them. Farmers in most countries often rely on chemical pesticides to control whiteflies due to the rapid appearance of control results and their effective contribution to increasing productivity. Due to their low price [10]. Nanotechnology has intervened to help develop agricultural crops free from insect pests and diseases [11]. In many studies, nano-silica has been used as an insecticide. Nanosilica is used in two ways: either directly in the field, and it played a major role in controlling insects [12] or as carriers and nano-spreaders mixed with commercial pesticides to enhance them, increase their spread and effectiveness, and increase the fluidity of their penetration into the tissues of the insect body or the plant host [13]. The aims of this study are devoted to examining the efficacy of SiO-NP with Actara pesticide or with Movento pesticide against *B. tabaci* and evaluated their enhanced insecticidal activity.

2. Material and Method

2.1. Preparation of Nano-Silica Oxide SiO-NPs

Nano-silica oxide SiO-NPs powder (0.1 g) was taken and dissolved in 3 ml of hydrochloric acid which was at a concentration of 10%. the mixture was then placed on a magnetic stirrer for 45 minutes at a temperature of 60 ° C for the intent of dissolution, then the solution was topped off to 1 liter by adding distilled water. To make sure that the mixture is homogenized [14], we placed it on a magnetic stirrer for another 30 minutes. The concentration of the prepared solution is 100 ppm, and from it the concentrations of 25 ppm, 50 and 75 were prepared by dilution with deionized water.

2.2. Preparation of the Nano- Silica Oxide SiO-NPs Loaded on the Pesticides Actara and Movento

The nano silica oxide particles were loaded on the pesticide by preparing 1000 ml of the nano silica oxide particles solution as mentioned in the above paragraph and at a concentration of 100 ppm. The concentration of 75 ppm was prepared by adding 250 ml of distilled water to 750 ml of the solution at 100 ppm. The mixture was placed in a magnetic stirrer for 30 minutes at a temperature of 27 °C. 0.75 ml/L of Movento pesticide was added to prepare the nano silica oxide and Movento pesticide compound. As for Actara 25% pesticide, 0.2 g/L was added to the nano silica oxide solution, then the mixture was placed in a magnetic stirrer for 30 minutes at 27°C. The solution was fermented for one hour under laboratory conditions, then The solution was moved to the water bath for 1 hour at 50°C. For the rest of the concentrations used in the experiment, the same process was performed [15].

2.3. Laboratory Experiment

Testing the effect of nano-silica oxide SiO-NPs on the mortality rates of nymphs of the cotton whitefly *B. tabaci*. Fresh and healthy leaf of cucumber crop was placed on small plastic boxes with a diameter of 9 cm and a height of 10 cm were prepared and a layer of medical cotton was placed at the bottom of the box and a quantity of distilled water was placed, then a circular filter paper with the same diameter as the box was placed on top on it. Then the third and fourth instar

nymphs were transferred on the leaves placed top of the filter paper. Each concentration was applied in five replicates each consisting of 10 nymphs of the cotton whitefly. Small plastic boxes with a diameter of 9 cm and a height of 10 cm were prepared and a layer of medical cotton was placed at the bottom of the box and a quantity of distilled water was placed, then a circular filter paper with the same diameter as the box was placed, then the cut cucumber leaves containing the nymphs were placed inside the small plastic box and closed and transferred to the incubator. At a rate of 5 replicates for each concentration after the treatment was done by spraying nano-silica oxide at concentrations of 25, 50, 75 ppm at a volume of 1 ml/replicate using a hand sprayer [16]. The control treatment included two treatments, the first, nymphs sprayed by water with Hcl 10% and the second. nymphs sprayed by insecticide. The boxes were placed in the incubator at a temperature of 2 ± 26 °C and a relative humidity of 5 ± 65 %. Then, the mortality rates were taken after 24, 48 and 72 hours of treatment, respectively. The second bioassay the nymphs of *B. tabaci* were treated with SiO-NPs loaded Actara and the third bioassay the nymphs of *B. tabaci* were treated with SiO-NPs loaded Movento at the same conditions.

2.4. Greenhouse Experiment

Testing the effect of the pesticides Actara and Movento and nano silica oxide and their combinations on the population density of the nymph of the white cotton fly *B. tabaci* on the Super Faris F1 hybrid for the cucumber crop. The experiment was conducted in greenhouse (90 m²) at Abu Sakhir District, Najaf Governorate, Iraq. The greenhouse was divided into three blocks; each block was divided into 7 experimental units. Each experimental unit contained fourth cucumber plants, with a distance of 30 cm between plants. The experiment was conducted according to a Complete randomized block design in the greenhouse, which began planting Super Faris F1 hybrid cucumber seeds on 1/17/2024. The germination rate was 100%. The house was provided with sticky traps for the purpose of reducing the pest density. All agricultural service, irrigation and fertilization operations were carried out according to a well-studied scientific plan. A 20-liter backpack sprayer was prepared to carry out the spraying process for the treatments that was carried out on 4/19/2024, as the spraying included complete coverage of the plant. To study the effect of the different treatments on the insect's roles, 3 leaves were taken from each plant from the upper, middle and lower parts, so the sample size for the experimental unit became 12 leaves, which were placed in bags marked with the treatments, then brought to the laboratory and the effect of the treatments on the role of the insect's nymph was calculated under a light microscope and a lens with a magnification power of 2X. The sampling process continued one day before the control and after the treatment on days 1, 3, 7, 10 and 14 day. The experimental units were treated with the following treatments: 1 -Actara 25% pesticide 0.2 g/liter of water 2- Movento pesticide 0.75 ml/liter of water 3- SiO-NPs 75 PPM 4- the composite of SiO-NPs at 75ppm with Actara 0.2 g/liter 5 - the composite of SiO-NPs at 75ppm with Movento 0.75 ml/liter 6- Actara+ Movento pesticide 0.75 ml/liter of water + 0.2 g/liter of water 7- Control water + HCL 3 ml/liter.

2.5. Statistical Analysis

Laboratory experiments were analyzed according to the statistical program Prism version 7 program. The percentage of mortality was corrected for mortality according to the Abbott equation [17]. As for the field experiments, they were designed as factorial experiments according to the randomized block design RCBD and the data were analyzed using the statistical software Genstat 12 and the least significant difference L.S.D was adopted at the probability level of 0.05 to compare the averages of the results.

3. Results and Discussion

3.1. Examination of Nanoparticles of Nanosilica Oxide Loaded on the Pesticides Actara and Movento

3.1.1. Scanning Electron Microscope (SEM)

The microscopic examination images to determine the average diameter of nanosilica oxide particles loaded on the pesticide Actara and silica oxide loaded on the pesticide Movento showed that the average diameter of nanosilica oxide particles loaded on the pesticide Actara was 0.42 nm (Figure 1A) and loaded on the pesticide Movento was 0.71 nm (Figure 1B).

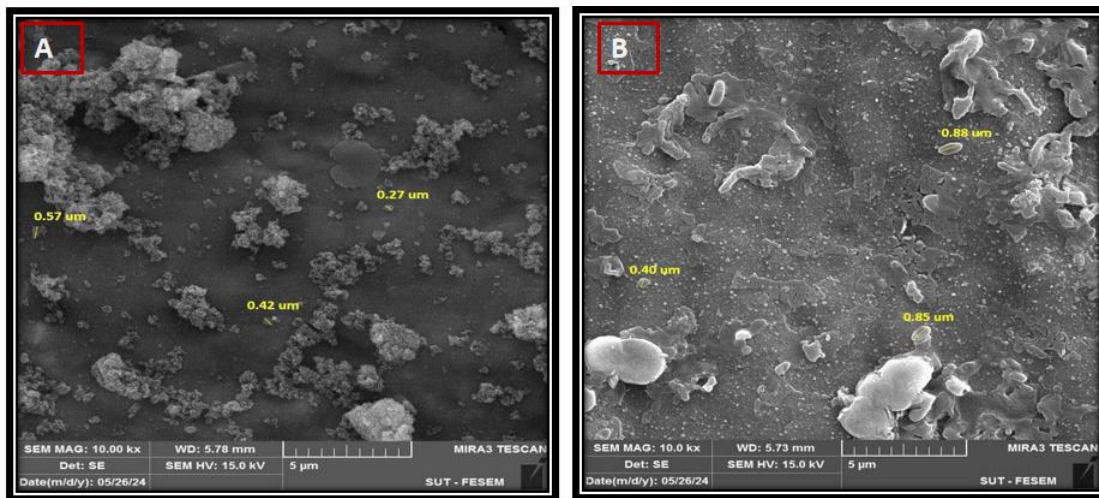


Figure 1. Scanning electron microscope showing the size of silica oxide nanoparticles loaded on the pesticide (A) Actara (B) Movento.

3.2. Laboratory Studies

3.2.1. Testing the Effect of Different Concentrations of Silica Oxide Nanoparticles on the Third and Fourth Instar Nymphs of *B. tabaci*

The results showed that the highest mortality percentages of silica oxide nanoparticles on the third and fourth instar nymphs of *B. tabaci* at the 75 ppm, was reached 52% (Figure 2). As for the factor of the effect of the time period, as for the time period factor, the 72 hours period was significantly superior to the 24 and 48 hours, as the mortality percentages reached 39, 49 and 52% at concentrations of 25, 50 and 75 ppm, respectively (Figure 5C). The results show that there are significant differences (Log-rank, $\chi^2 = 29.89$, $P < 0.0001$) between the treatments of different concentrations of nano-silica oxide and the control treatment, Ctr-, where the mortality percentages in the control treatment was only 8%. The effectiveness of nano-silica oxide on the white cotton fly *B. tabaci* is attributed to the absorption of the nanoparticles the waxy layer in the cuticle wall of the insect, which exposes the insect to the risk of dehydration leading to death [18].

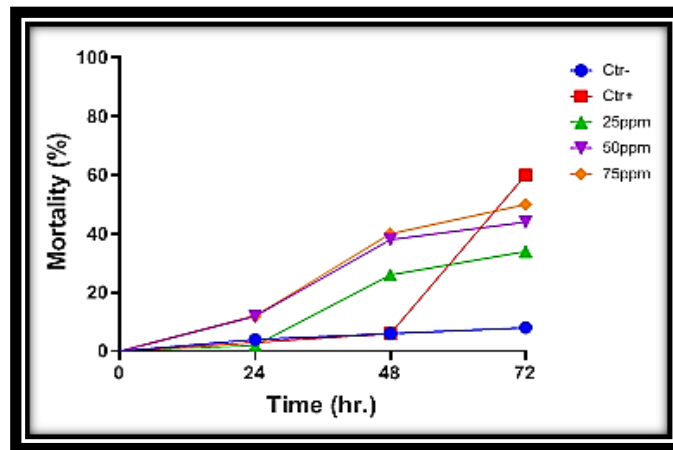


Figure 2. Mortality curves of *B. tabaci* nymphs spray by different concentrations of silica oxide nanoparticles ($p < 0.05$). Ctr- = nymphs sprayed by water+Hcl 10%, Ctr+ = nymphs sprayed by Actara.

3.2.2. Testing the Effect of Different Concentrations of Silicon Oxide Nanoparticles Loaded on Actara at the Recommended Concentration on the Third and Fourth Instar Nymphs of *B. tabaci*

The results showed that the highest mortality percentage was 82% at 75ppm of SiO-NPs loaded on the pesticide Actara (Figure 3). as for the time period factor, the 72 hours period was significantly superior to the 24 and 48 hours, as the mortality percentages reached 77, 78 and 82% at concentrations of 25, 50 and 75 ppm, respectively (Figure 5D). The results show that there are significant differences (Log-rank, $\chi^2 = 71.54$, $P < 0.0001$) between the treatments of different concentrations of nano-silica oxide loaded on the Actara pesticide at the recommended concentration and the control treatment Ctr-, where the mortality percentages reached 8%, Figure (A-8-4). Also, using the Actara pesticide alone at the recommended concentration gave a mortality percentage of 60% (Figure 5E) This is a result of the synergistic action between the pesticide and the nanoparticles. The toxic effect of nanoparticles depends on their size, physicochemical properties, ability to bond and act as a catalyst for the formation of toxic substances [19].

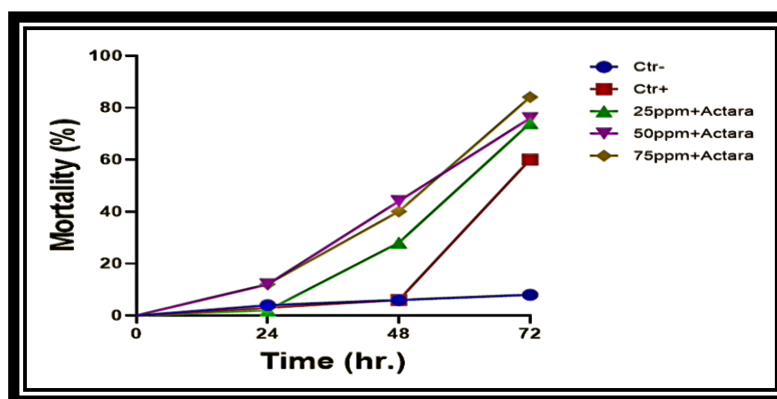


Figure 3. Mortality curves of *B. tabaci* nymphs spray by different concentrations of silica oxide nanoparticles loaded on Actara. with the recommended concentration ($p < 0.05$). Ctr- = nymphs sprayed by water+Hcl 10%, Ctr+ = nymphs sprayed by Actara.

3.2.3. Testing the Effect of Different Concentrations of Nano-Silica Oxide Particles Loaded on Movento at the Recommended Concentration on the Third and Fourth Instar Nymphs of *B. tabaci*

The results showed that the highest effect rate of the factor of different concentrations of nano-silica oxide loaded on Movento pesticide at the recommended concentration was at the concentration of 75 ppm, as the mortality rate reached 77% (Figure 4). as for the time period factor, the 72 hours period was significantly superior to the 24 and 48 hours, as the mortality percentages reached 61, 70 and 77% at concentrations of 25, 50 and 75ppm respectively (Figure 5B). The results showed that there were significant differences.) (Log-rank, $\chi^2 = 52.81$, $P < 0.0001$) between the treatments of different concentrations of nano-silica oxide loaded on Movento pesticide at the recommended concentration and the control treatment Ctr- where the mortality rate in the control treatment reached 8%. Also, using Movento pesticide alone at the recommended concentration gave a mortality rate of 42% (Figure 5 F). It is worth mentioning that adding silica oxide nanoparticles with the pesticide Movento gave a positive synergistic effect, it was concluding from this that their combined action increased the mortality rate of nymphs of the white cotton fly *B. tabaci*. The pesticide Movento contains the active ingredient spirotetramat, which acts as an inhibitor of Acetyl-CoA carboxylase (ACC), which prevents the biosynthesis of fats in insects [20].

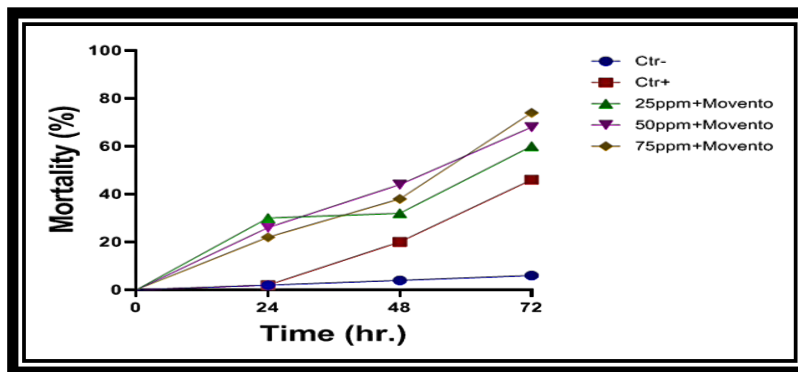


Figure 4. Mortality curves of *B. tabaci* nymphs spray by different concentrations of silica oxide nanoparticles loaded on Movento. with the recommended concentration ($p < 0.05$;) Ctr- = nymphs sprayed by water+Hcl 10%, Ctr+ = nymphs sprayed by Movento.

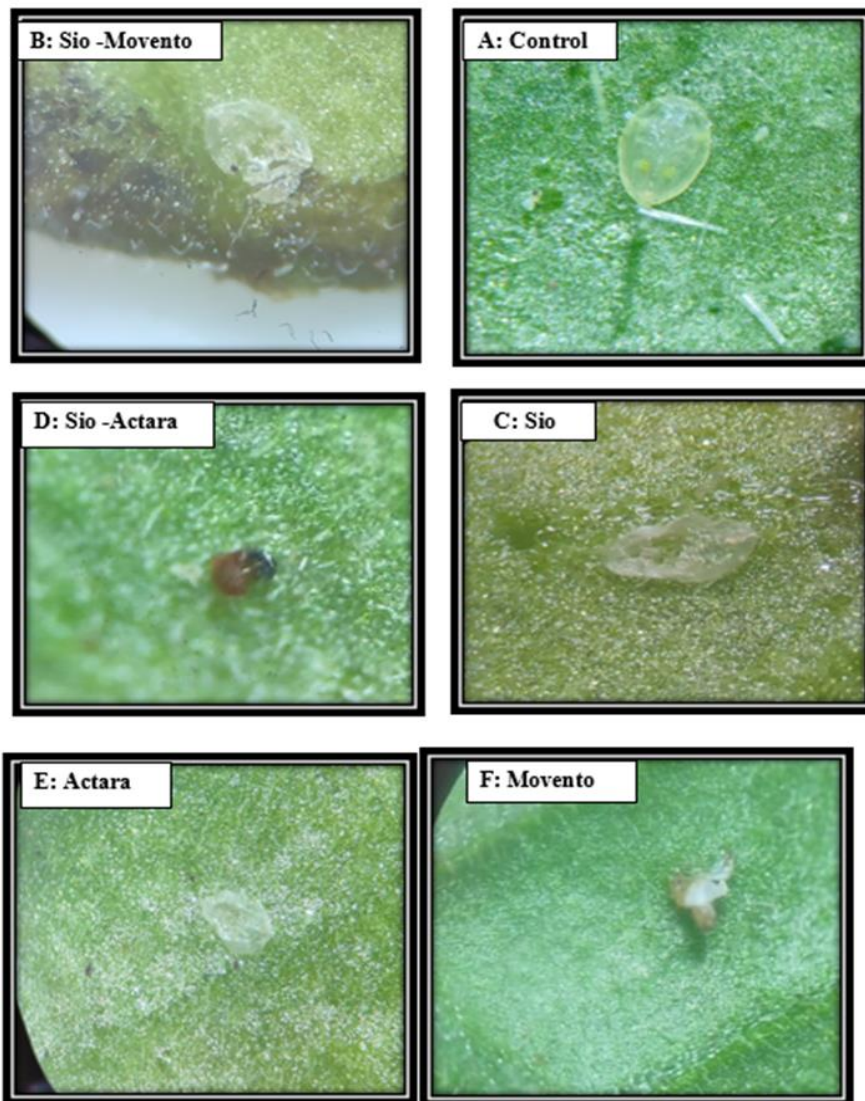


Figure 5. Nymphs of the cotton whitefly A: untreated nymph. B: nymph treated with Movento loaded with silica nanoparticles. C: nymph treated with silica nanoparticles. D: nymph treated with Actara loaded with silica nanoparticles. E: nymph treated with Movento. F: nymph treated with Actara.

3.2.4. Field Evaluation of the Effectiveness of the Pesticides Actara, Movento and SiO-NPs and Their Combinations on the Population Density of the Nymphs of the Cotton Whitefly *B. tabaci*.

The results of Table (1) indicate the effect of the treatments of each of the pesticides Actara, Movento, SiO-NPs and their combinations on the numerical density of the number of nymphs density of the cotton whitefly *B. tabaci* in the field on the cucumber crop, as the treatment of the pesticide Actara loaded with silica nanoparticles caused a clear decrease in the numerical density of the average number of nymphs, which amounted to 5.9 nymphs/leaf, which differs significantly from the treatments of Actara and SiO-NPs, which recorded 9.3 and 11.9

nymph/leaf, respectively. As for the treatment of the pesticide Sio-Movento, it recorded a numerical density of nymphs was 9.2 nymph/leaf, which differs statistically significantly from the treatment of the pesticide Movento, SiO-NPs, Actara and Movento, which amounted to 14.7, 11.7 and 10.5 nymph/leaf, respectively. In the presence of sticky traps and that all treatments differ significantly from the control treatment. As for the days, the lowest number of nymphs was on 10th day after treatment and the number of nymphs density was 12.7 nymph / leaf, while the highest rate of nymphs was on the first day after treatment, which reached 22.2 nymph / leaf, and significant differences were observed in the number of density between the days in which the readings were taken except for 3rd and 14th, where there were no significant differences between them. Regarding the interaction, the lowest number of nymphs of the white cotton fly *B. tabaci* was recorded in the interaction treatments of both the Sio-Actara and the Actara treatment on 10th day, as it reached 0.0 and 2.3 nymph / leaf, respectively. It is worth noting that all treatments recorded a significant decrease in the number of nymphs of the white cotton fly *B. tabaci* compared to the control treatment.

Table 1. Effect of the two pesticides Actara, Movento, Sio and their combinations on the population density of the nymphs of the white cotton fly *B. tabaci*. in the greenhouse.

Treatment	Before treatment	Average density of nymphs of <i>B. tabaci</i> (nymph / leaf)						Treatment rate
		1	3	5	7	10	14	
Actara	44	18.7	12.3	9	5.7	1.3	8.7	9.3
Movento	44	22.7	18	15.3	12.7	5.7	14	14.7
SiO NPs	44	21	15.3	11.5	7.3	3.7	12.7	11.9
Actara-Sio	44	11.7	6.3	5.6	5	0	6.7	5.9
Movento-Sio	44	16	13.3	9.6	6	2.3	7.7	9.2
Actara+Movento	44	20.7	13.7	10.2	6.7	2.7	8.7	10.5
Control	44	44.7	57.3	61.6	66	73	80	63.8
Days rate	44	22.2	19.5	17.6	15.6	12.7	19.8	
L.S.D	0.971 treatment		Time 0.82			interaction 2.17		

The SiO- Actara treatment was more efficient in reducing the population density than Movento and Actara alone or SiO-NPs alone due to the synergistic effect of the two substances together. The results show that SiO-NPs had an effect on all nymphal stages and that nanoparticles may enter the plant through the stomata or stick to the base of the hairs on the leaf [21]. Uzu [22] stated that plant cells take nanoparticles and transfer them to the wood and bark of the plant [23]. Thus, it can be concluded that the nanomaterial works as a contact and systemic pesticide at the same time. It was clear that all treatments recorded a significant decrease in the numerical density of *B. tabaci* nymphs compared to the control treatment, and decrease in the number of nymphs in the Actara treatment compared to the Movento pesticide. This is due to the active ingredient of the Actara pesticide, as it is more efficient than the active ingredient of the Movento pesticide. As for the effect of nano-silica oxide particles, it has the ability to absorb water present in the fatty layer in the body wall, which causes dehydration [18]. In this regard, El-Smahy [24] reported that a concentration of 100 ppm of silica nanoparticles was able to reduce the number of *T. absoluta* larvae on tomato crops by 84% after 15 days of field spraying. In this field, Shnawa and Alshukri [25] stated that nano-silica oxide loaded with the pesticide Oxymatrine had of *T. absoluta*, the mortality of 4th instar larva of *T. absoluta* was 100%, while the treatment with the pesticide Oxymatrine alone recorded a 97%, and the treatment with nano-silica oxide alone recorded a 94% after 7 days of treatment on the tomato crop in the field.

Conclusions

This study can be considered the first effort of using silicon oxide nanoparticles to improve the efficiency of Actara and Movento pesticides against *Bemisia tabaci*.

References

- [1] Gennadius, P. (1889). Disease of tobacco plantations in the Trikonion. The aleurodid of tobacco. *Ellenike Georgia*, 5:1-3.
- [2] Hidayat, P., Bintoro, D., Nurulalia, L. and Basri, M. (2018). Species, Host Range, and Identification Key of Whiteflies of Bogor and Surrounding Area. *J. HPT Tropika*. Vol.18, No.2:127-150.
- [3] Soumia, P.S., Guru Pirasanna Pandi, G., Krishna, R., Ansari, W.A., Jaiswal, D.K., Verma, J.P. and Singh, M. (2021). Whitefly-transmitted plant viruses and their management. In *Emerging Trends in Plant Pathology* (pp. 175-195). Springer, Singapore.
- [4] Gangwar R, Gangwar C (2018) Lifecycle, distribution, nature of damage and economic importance of whitefly, *Bemisia tabaci* (Gennadius). *Acta Sci Agric* 2(4):36–39
- [5] Smith, H.A. and Krey, K.L. (2019). Three release rates of *Dicyphus hesperus* (Hemiptera: Miridae) for management of *Bemisia tabaci* (Hemiptera: Aleyrodidae) on greenhouse tomato. *Insects*. 10(7):213.
- [6] Perring TM, Stansly PA, Liu TX, Smith HA, Andreason SA (2018) Whiteflies: biology, ecology, and management. In: Wakil W, Brust GE, Perring TM (eds) *Sustainable management of arthropod pests of tomato*, 1st edn. Academic Press, Cambridge, pp 73–110.
- [7] Sonalkar, V.U. (2020). Biochemicals in cotton hybrids and varieties and their correlation with sucking insect pests. *Journal of Current Microbiology and Applied Sciences*. 9(1):1172-1183. of
- [8] Li, R., Huang, X., Xu, X., Wang, S., Yang, Y., Di, N., Li, H. and Tian, L., 2024. *Bemisia tabaci* infection of tomato plant alters bumblebee foraging behaviour. *Journal of Pest Science*, 97(2), pp.767-776.
- [9] Abubakar, M., Koul, B., Chandrashekar, K., Raut, A., & Yadav, D. (2022). Whitefly (*Bemisia tabaci*) management (WFM) strategies for sustainable agriculture: a review. *Agriculture*, 12(9), 1317.
- [10] Reddy, G.V., Antwi, F.B., Shrestha, G. and Kuriwada, T., (2016). Evaluation of toxicity of biorational insecticides against larvae of the alfalfa weevil. *Toxicology Reports*, 3, pp.473-480.
- [11] Gogos A, Knauer K, Bucheli TD (2012) Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities. *J Agric Food Chem* 60:9781–9792
- [12] Magda, S., & Hussein, M. M. (2016). Determinations of the effect of using silica gel and nano-silica gel against *Tuta absoluta* (Lepidoptera: Gelechiidae) in tomato fields. *J Chem Pharm Res*, 8(4), 506-512.
- [13] Ziaee, M. and Babamir-Satehi, A., (2020). Characterization of nanostructured silica as carrier for insecticides deltamethrin, pyriproxyfen, and chlorpyrifos and testing the insecticidal efficacy against *Trogoderma granarium* (Coleoptera: Dermestidae) larvae. *Journal of Economic Entomology*, 113(1), pp.511-517.
- [14] Al-Esawy, M. T., Alshukri, B. M., & Kahdum, B. J. (2021). Carbon nanotubes as control agents against the Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *Revista Brasileira de Ciências Agrárias*, 16(4), 1-9.
- [15] Jameel, M. S., Noqta, O. A., Dheyab, M. A., Aziz, A. A., & Mehrdel, B. (2020). Synthesis and coating methods of biocompatible iron oxide/gold nanoparticle and nanocomposite for biomedical applications. *Chinese Journal of Physics*, 64, 305-325.
- [16] Ardalan, R. B., Jamshidi, N., Arabameri, H., Joshaghani, A., Mehrinejad, M., & Sharafi, P. (2017). Enhancing the permeability and abrasion resistance of concrete using colloidal nano-SiO₂ oxide and spraying nanosilicon practices. *Construction and Building Materials*, 146, 128-135.
- [17] Abbot, C.G., (1925). Solar variation and forecasting. *Smithsonian Miscellaneous Collections*.
- [18] Derbalah, A. S., Morsey, S. Z., & El-Samahy, M. (2012). Some recent approaches to control *Tuta absoluta* in tomato under greenhouse conditions. *African Entomology*, 20(1), 27-34.



- [19] Klaine, S.J., Alvarez, P.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., McLaughlin, M.J. and Lead, J.R.,(2008). Nanomaterials in the environment: behavior, fate, bioavailability, and effects. *Environmental Toxicology and Chemistry: An International Journal*, 27(9), pp.1825-1851.
- [20] Mao, L., & Henderson, G. (2007). Antifeedant activity and acute and residual toxicity of alkaloids from *Sophora flavescens* (Leguminosae) against formosan subterranean termites (Isoptera: Rhinotermitidae). *Journal of economic entomology*, 100(3), 866-870.
- [21] Eichert, T., Kurtz, A., Steiner, U., & Goldbach, H. E. (2008). Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water-suspended nanoparticles. *Physiologia plantarum*, 134(1), 151-160.
- [22] Uzu, G., Sobanska, S., Sarret, G., Munoz, M., & Dumat, C. (2010). Foliar lead uptake by lettuce exposed to atmospheric fallouts. *Environmental science & technology*, 44(3), 1036-1042
- [23] Raliya, R., Nair, R., Chavalmane, S., Wang, W. N., & Biswas, P. (2015). Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (*Solanum lycopersicum* L.) plant. *Metallomics*, 7(12), 1584-1594.
- [24] El-Samahy, M., El-Ghobary, A. M., & Khafagy, I. F. (2014). Using silica nanoparticles and neem oil extract as new approaches to control *Tuta absoluta* (meyrick) in tomato under field conditions. *Int. J. Plant Soil Sci*, 3(10), 1355-1365.
- [25] Shnawa, U.M. and Alshukri, B.M.,(2022). Enhanced Insecticidal Activity of Orizon and Oxymatrine by Silica Oxide Nanoparticles to control *Tuta absoluta* (Lepidoptera: Gelechiidae). *Ann. For. Res.*, 65(1), pp.2186-2200.

Susceptibility of Two Cucumber Hybrids to Infection with Whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrididae)

Raneen Mohammed Ali¹ and Baida Mohsen Alshukri²

^{1,2}Faculty of Agriculture, University of Kufa, Kufa, Najaf, Iraq.

¹E-mail: raneenr.almuusawi@student.uokufa.edu.iq

²E-mail: baidaa.alshukri@uokufa.edu.iq

Abstract. The results of the greenhouse experiment indicated the sensitivity of two cucumber hybrids (SAYFF and Super Faris) to the Whitefly, *Bemisia tabaci* from the average numerical density of eggs and nymphs, the hybrid Super Faris recorded the highest infection, which amounted to 30 egg/leaf and 30 nymph/leaf, while the hybrid SAYFF recorded the least average population density of eggs and nymphs of with an average of 23 egg/leaf and 22 nymph/leaf. This is because Super Faris contains the highest content of chlorophyll and moisture percentage of 2.56 mg/g and 90%, respectively, while the SAYFF hybrid recorded the lowest chlorophyll content and moisture percentage of 2.43 mg/g and 87%, respectively. Anatomical characteristics of the cucumber hybrids revealed variability in the thickness of the epidermis and cortex, measured before inducing infection, showing a significant difference of 48.94 between the SAYFF and Super Faris hybrids. The Super Faris hybrid recorded the smallest average thickness for both the epidermis and cortex at 101.48 micrometers, while the SAYFF hybrid's thickness averaged 134.5 micrometers. Additionally, the Leaf area results for both cucumber hybrids (SAYFF and Super Faris) showed variability in leaf area measurements the Super Faris hybrid significantly outperforming with an average leaf area of 300 cm² compared to the SAYFF hybrid's average leaf area of 285 cm².

Keywords. Whitefly, Chlorophyll Content, Leaf Area, Leaf Area.

1. Introduction

The whitefly *B. tabaci* was described over a century ago and has since become one of the most dangerous pests for agricultural crops, especially in subtropical and tropical regions, as well as in greenhouses worldwide [1]. It has now been recorded on all continents except Antarctica. The most preferred hosts for this pest include tomatoes, potatoes, peppers, sweet potatoes, watermelons, lettuce, cucumbers, eggplants, okra, and various weeds, with eggplants, potatoes, peanuts, soybeans, tobacco, and cotton being the most favored hosts for this pest [2]. The whitefly is characterized by features and capabilities that make it a major pest on vegetable crops and greenhouse crops, including its broad host range, rapid reproduction, and high fertility, as well as its resistance to many pesticides [3,4]. Whiteflies cause severe damage and economic losses to agricultural crops [5]. The damage from this pest occurs through direct feeding, as both nymphs and adults insert their piercing-sucking mouthparts into plant tissues and extract sap from green parts, leading to yellowing of leaves [6]. Moreover, nymphs inject enzymes during feeding

that alter the physiology of crops, thereby delaying fruit maturation [7]. Indirectly, the damage manifests through the secretion of honeydew (sugary waste), which hinders photosynthesis and affects transpiration and respiration processes by facilitating dust accumulation on the leaves, in addition to promoting the growth of sooty mold on leaves and fruits [8,9]. The cucumber varieties have shown considerable variation in morphological and physiological traits and their ability to withstand environmental conditions in the regions where they are cultivated, which is a prerequisite for the successful cultivation of vegetable crops, including cucumber. Thus, selecting the suitable variety is one of the keys to the success of vegetable crop production for most farmers. The study aimed to sensitivity test some hybrids of cucumber approved for cultivation in Iraq by the whitefly *B. tabaci* and the study some phenotypic and biochemical characteristics that were the cause of this difference in sensitivity to whitefly infection

2. Materials and Methods

*2.1. Greenhouse Cultivation to Test the Susceptibility of Cucumber Hybrids to *B. tabaci* Infestation*

On January 15, 2024, cucumber hybrids seeds of SAYFF and Super Faris hybrids were sown in two plastic trays, each containing 200 cells, filled with peat moss. After watering the peat moss to stabilize it, a seed was placed in each cell and watered regularly until germination. After 20 days, the seedlings reached approximately 15 cm in height, then transform to greenhouse (25 m²). The experimental greenhouse was located at the research station of the Faculty of Agriculture at the University of Kufa. Irrigation was carried out according to a scientific plan, the experimental design consisted of three replicates for each hybrid (Figure 1), which were 25 m², containing 40 plants of each hybrid. All necessary agricultural practices, such as irrigation and fertilization, were applied consistently. Samples were taken randomly from each replicate, 5 plants for each replicate and 5 leaves for each plant. The leaves were placed in 2 kg plastic bags, then those leaves were transferred to the laboratory for the purpose of examining them and counting the numbers of nymphs and eggs. The process was repeated every week for five weeks.



Figure 1. The greenhouse planted with two hybrids of cucumber.

2.2. Determination of Total Chlorophyll Content in Leaves

Chlorophyll content was estimated by collecting the leaf from five randomly selected plants for each experimental unit. The leaves were washed with water, dried, and 0.5 g of fresh leaf tissue was ground with 10 ml of 85% acetone. The solution was filtered using Whatman No.1 filter paper, and the chlorophyll was extracted again until the leaf tissue turned white. The total volume of the filtrate was adjusted to 20 ml with acetone [10] and the chlorophyll content was measured using a spectrophotometer at wavelengths of 645 nm and 663 nm. The following equation was used to calculate the total chlorophyll content:

$$\text{Total Chlorophyll} = 20.2 \times D(645) + 8.02 \times D(663) \times VW \times 1000$$

Where:

D(663) = Absorbance at 663 nm.

D(645) = Absorbance at 645 nm.

V = Final extraction volume (20 ml).

W = Weight of leaf tissue (0.5 g).

2.3. Measurement of the Total Moisture Content in the Leaves

The fresh weight of 10 leaves from each cucumber hybrid (SAYFF and Super Faris) was determined using a precision electronic balance. The samples were then dried using an oven at 70°C for 3 days, after which the dry weights were recorded. The moisture content was calculated using the following equation [11]:

$$\text{Moisture (\%)} = (\text{Fresh Weight of Leaves} - \text{Dry Weight of Leaves} / \text{Fresh Weight of Leaves} \times 100$$

2.4. Measurement of Leaf Area

Leaf area was measured using the method described by [12]. Random samples of 30 fully grown leaves from each hybrid were collected from three rows, with 10 leaves from each row. The leaf area was determined using the ImageJ software.

2.5. Measurement of Leaf Epidermis and Cortex Thickness

The anatomical study was conducted in the laboratories of the Faculty of Science at the University of Kufa. Fresh leaf samples were collected from the cucumber hybrids. Hand sectioning was performed according to the method of [13]. Three leaves from each hybrid (SAYFF and Super Faris) were randomly selected and preserved in glass jars filled with FAA solution (formalin-acetic acid-alcohol). The leaf petioles were sectioned transversely, and the fresh sections were treated in a series of ethanol and xylene washes. The cross-sections were examined using an electron microscope at the laboratories of the Faculty of Science, University of Kufa

2.6. Statistical Analysis

Results were statistically analyzed by using An ANOVA analysis. The averages were compared using the lowest significant difference LSD at a probability level of 0.05 by using software GenStat12.

3. Results and Discussion

3.1. Testing the Susceptibility of Cucumber Hybrids to *Bemisia tabaci* Infestation

The results, as shown in Table (1), indicate significant differences in the population density of whitefly eggs and nymphs on the cucumber hybrids tested (SAYFF and Super Faris). The SAYFF hybrid recorded an average density of 23 eggs/leaf and 22 nymph/leaf, while the *Super Faris* hybrid recorded an average density of 30 eggs/leaf and 30 nymph/leaf. The least significant difference (L.S.D) was 3.633 at a 0.05 probability level. The variation in susceptibility between the cucumber hybrids is attributed to differences in structural, morphological, and biochemical traits such as leaf carbohydrate content and nitrogen levels, which increase tissue softness, making the plant more susceptible to insect penetration [14].

Table 1. Number density of the average number eggs and nymphs of *B. tabaci* on the leaves of two hybrids of the cucumber: Super Faris and SAYFF.

Plant	The numerical density of the average number of insects (eggs or Nymphs / leaf)											
	1 st week		2 nd week		3 rd week		4 th week		5 th week		Average	
	Eg g	Nymp h	Eg g	Nymp h	Eg g	Nymp h	Eg g	Nymp h	Eg g	Nymp h	Eg g	Nymp h
Super Faris	6	4	23	22	30	33	43	44	48	48	30	30
SAYFF	4	2	20	16	26	27	32	33	33	34	23	22
L.S.D (0.05)	3.633											

3.2. Total Chlorophyll Content in Cucumber Hybrid Leaves

The results, shown in Figure (2), indicate a significant difference in total chlorophyll content between the cucumber hybrids tested. The Super Faris hybrid had a higher average chlorophyll content of 2.56 mg/g, while the SAYFF hybrid recorded a lower average chlorophyll content of 2.43 mg/g and the correlation coefficient was 0.31 between the number of *B. tabaci* insects and the total chlorophyll content in leaves (Table 2). This suggests that whiteflies are more attracted to plants with higher chlorophyll content, as these plants offer more nutritious food sources [15,16].

3.3. Epidermal and Cortex Thickness of Cucumber Hybrid Leaves

The anatomical analysis of the cucumber hybrid leaves, as shown in Figure (3), revealed significant differences in epidermal and cuticle thickness between the hybrids. The Super Faris hybrid had a thinner epidermis and cuticle, with an average thickness of 101.48 micrometers, while the SAYFF hybrid had an average thickness of 134.5 micrometers (Figure 4) as the correlation coefficient for the relationship between the thickness of the cortex and the numerical density of the insect *B. tabaci* was - 0.45, and it is a negative correlation, (Table 3). Thicker leaves act as a physical barrier, making it harder for the whiteflies to penetrate and feed on the plant tissue [17].

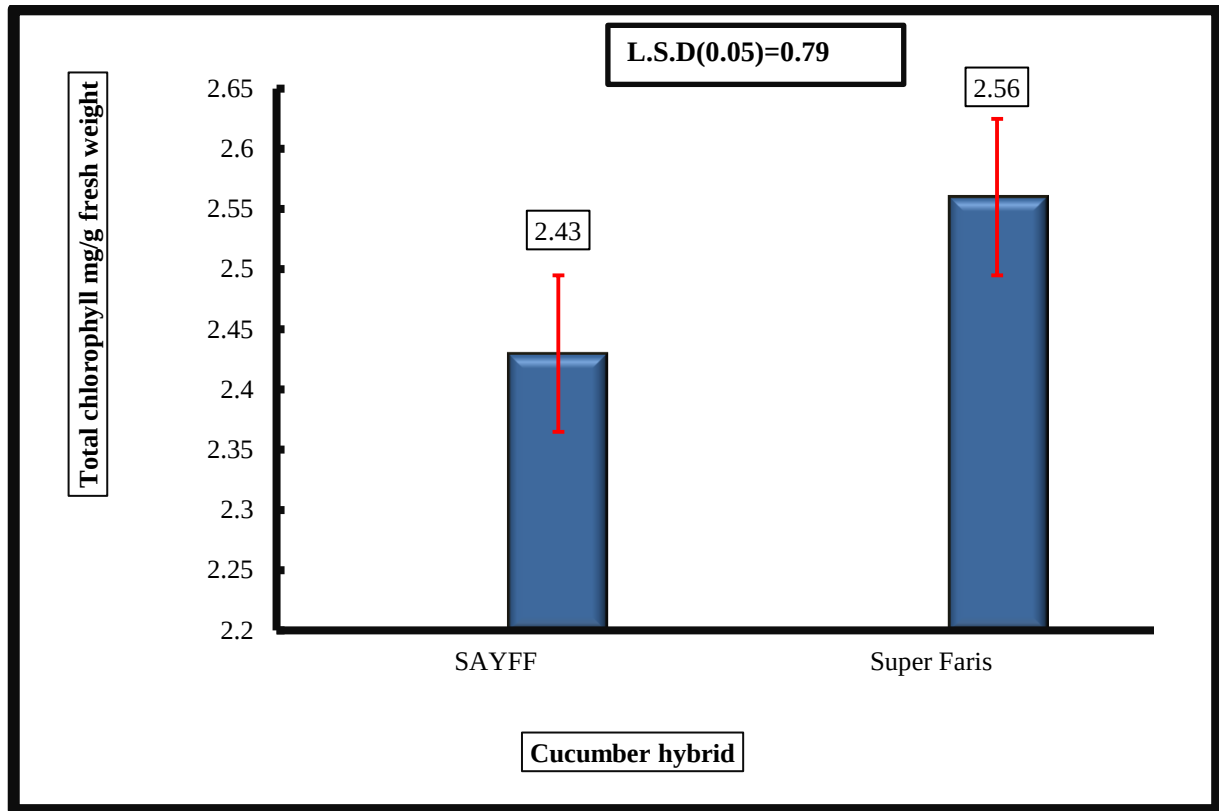


Figure 2. Total chlorophyll of two hybrids of cucumber Super Faris and SAYFF.

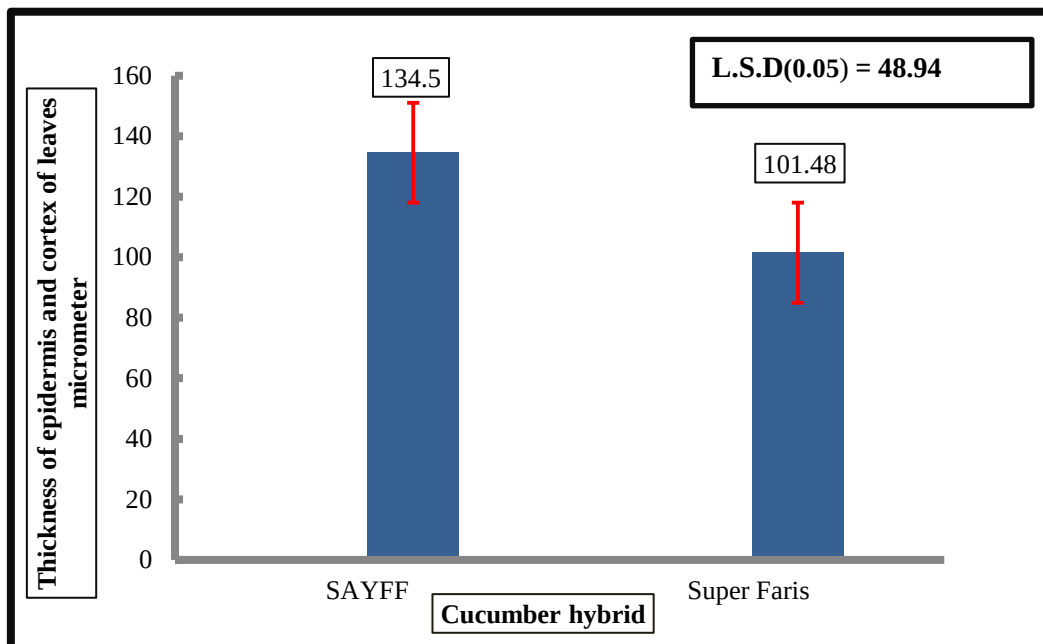


Figure 3. Thickness of epidermis and cortex of leaves of two hybrids of cucumber Super Faris and SAYFF.

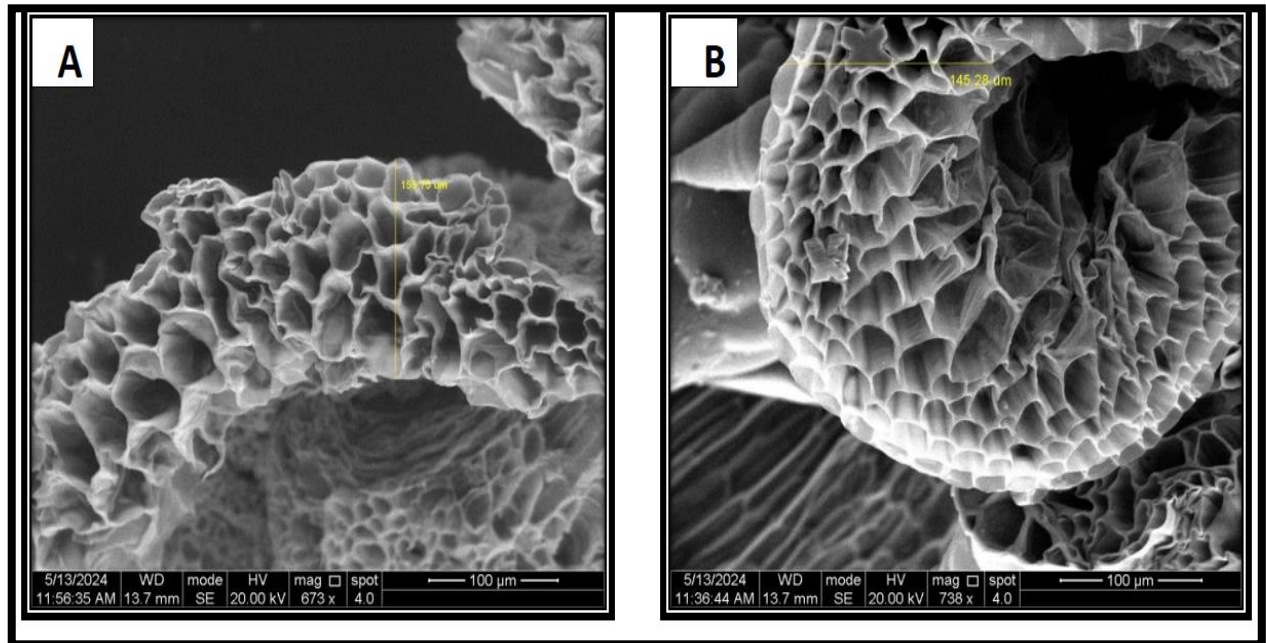


Figure 4. Cross-section of leaves of Cucumber Hybrids: Super Faris (A), SAYFF (B).

3.4. Total Leaf Moisture Content of Cucumber Hybrids

The results shown in Figure (5) indicated that significant differences in the total moisture content of the cucumber hybrid leaves. The Super Faris hybrid had the highest moisture content, reaching 90%, while the SAYFF hybrid had a lower moisture content of 87% and the average correlation coefficient for the relationship between the percentage of moisture and the numerical density of the insect was 0.24, which is a positive correlation (Table 3). Plants with higher moisture content are more susceptible to infestation, as the softer tissues are more easily penetrated by the insects [18].

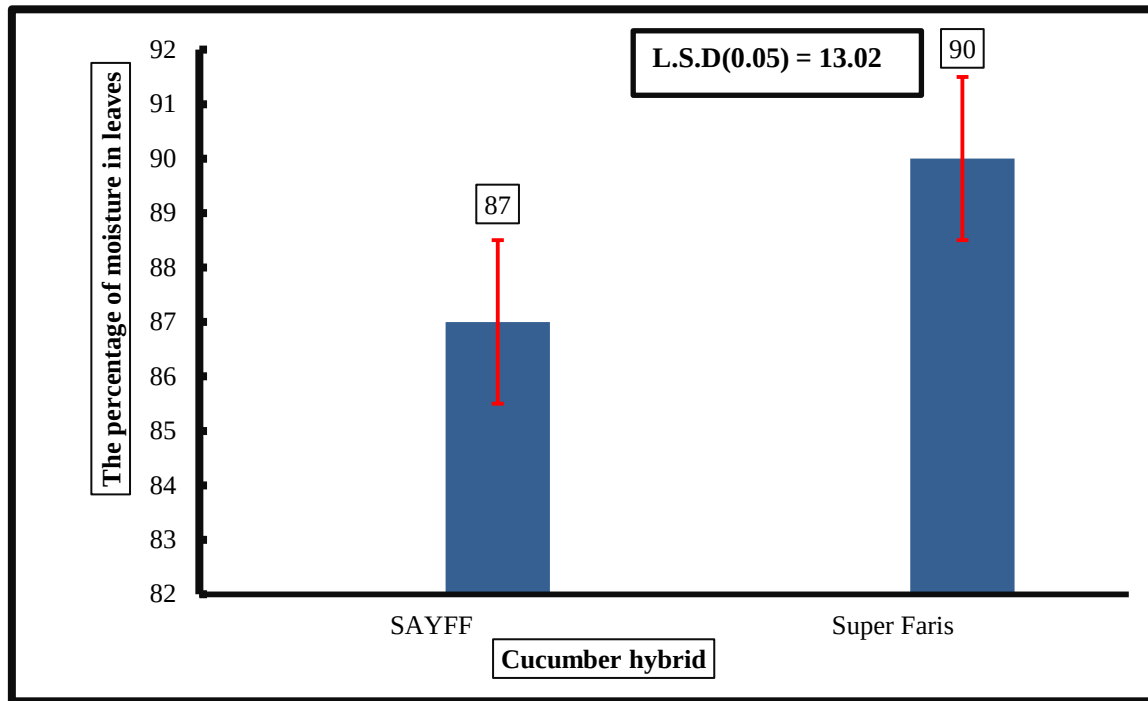


Figure 5. Percentage of moisture in the leaves of two hybrids of cucumber Super Faris and SAYFF.

3.5. Leaf Area of Cucumber Hybrid

The results for leaf area, shown in Table (2), indicate significant differences between the cucumber hybrids. The Super Faris hybrid had a larger average leaf area of 300 cm², while the SAYFF hybrid had an average leaf area of 285 cm², the average correlation coefficient for the relationship between area leaf and the numerical density of the insect was 0.71, and it is a strong positive correlation, (Table 3). Larger leaves provide more surface area for the insects to feed and move on, increasing the likelihood of infestation [19].

Table 2. Leaf area for two hybrids of Cucumber: Super Faris and SAYFF.

Hybrids	Leaf area (cm ²)
SAYFF	285
Super Faris	300
L.S.D 0.05	17.39

Table 3. Correlation coefficient for some phenotypic and physiological traits in comparison with the numerical density of *B. tabaci*.

Adjective	Correlation coefficient	Link strength
Epidermis and Cortex thickness	-0.456	negative correlation
Leaf area	0.716	Strong positive correlation
Moisture percentage	0.248	positive correlation
Amount of chlorophyll	0.319	g positive correlation

Conclusion

The results of the study indicate, based on the population density of whitefly nymphs and eggs, that the *Super Faris* hybrid of cucumber is more susceptible to infection due to its biochemical and morphological characteristics.

References

- [1] Islam, M. T., & Ren, S. X. (2009). *Effect of Sweetpotato Whitefly Bemisia tabaci (Homoptera: Aleyrodidae) Infestation on Eggplant (Solanum melongena L.) Leaf*. Journal of Pest Science, 82(3), 211-215.
- [2] Chakraborty, P., & Ghosh, A. (2022). *Topical Spray of dsRNA Induces Mortality and Inhibits Chilli Leaf Curl Virus Transmission by Bemisia tabaci Asia II 1*. Cells, 11(5), 833.
- [3] Hidayat, P., Bintoro, D., Nurulalia, L. and Basri, M. 2018. Species, Host Range, and Identification Key of Whiteflies of Bogor and Surrounding Area. J. HPT Tropika.Vol.18, No.2:127-150
- [4] Soumia, P.S., Guru Pirasanna Pandi, G., Krishna, R., Ansari, W.A., Jaiswal, D.K., Verma, J.P. and Singh, M. 2021. Whitefly-transmitted plant viruses and their management. In Emerging Trends in Plant Pathology (pp. 175-195). Springer, Singapore
- [5] Sani, I., Ismail, S.I., Abdullah, S., Jalinas, J., Jamian, S. and Saad, N., 2020. A review of the biology and control of whitefly, Bemisia tabaci (Hemiptera: Aleyrodidae), with special reference to biological control using entomopathogenic fungi. Insects, 11(9), p.619.
- [6] Gangwar, R. K., & Gangwar, C. (2018). *Lifecycle Distribution, Nature of Damage, and Economic Importance of Whitefly Bemisia tabaci (Gennadius)*. Acta Scientifica Agric, 2(4), 36-39.
- [7] Smith, H. A., Nagle, C. A., & Evans, G. A. (2014). *Densities of Eggs and Nymphs and Percent Parasitism of Bemisia tabaci (Hemiptera: Aleyrodidae) on Common Weeds in West Central Florida*. Insects, 5(4), 860-876.
- [8] Perring TM, Stansly PA, Liu TX, Smith HA, Andreason SA (2018) Whiteflies: biology, ecology, and management. In: Wakil W, Brust GE, Perring TM (eds) Sustainable management of arthropod pests of tomato, 1st edn. Academic Press, Cambridge, pp 73–110
- [9] Solanki, V., Verma, J. P., & Singh, M. (2021). *Whitefly-Transmitted Plant Viruses and Their Management*. In Emerging Trends in Plant Pathology (pp. 175-195). Springer
- [10] Lorenzen, C.J., 1967, December. Vertical distribution of chlorophyll and phaeo-pigments: Baja California. In Deep Sea Research and Oceanographic Abstracts (Vol. 14, No. 6, pp. 735-745). Elsevier
- [11] Horwitz, W., 1982. Evaluation of analytical methods used for regulation. Journal of the Association of Official Analytical Chemists, 65(3), pp.525-530.
- [12] Stawarczyk, M., & Stawarczyk, K. (2015). Use of the ImageJ program to assess the damage of plants by snails. Chemistry-Didactics-Ecology-Metrology, 20(1-2), 67-73.
- [13] Al-Khazraji, T. O. and F. M. Aziz (1990). Practical plant anatomy and microscopic preparations. Salah al-Din University, Ministry of Higher Education and Scientific Research, Iraq.
- [14] El-Fakharany, S. K. (2018). *Effect of Different Host Plants on the Onion Thrips Thrips tabaci Lind. and the Land Snail Monacha spp. Population Abundance According to Intercropping some Vegetable Crops and the Final Yield*. Journal of Plant Protection and Pathology, 9(3), 195-200.
- [15] Shadchina, D. M. (1995). *Correlation Between Chlorophyll Content and Growth Parameters in Plants*. Physiology and Biochemistry of Cultivated Plants, 27(2), 75-81.
- [16] Amaliotis, D., Therios, I., & Karatissiou, M. (2004). *Effect of Nitrogen Fertilization on Growth, Leaf Nutrient Concentration, and Photosynthesis in Three Peach Cultivars*. International Symposium on Irrigation of Horticultural Crops, 449, 529-534.
- [17] Kaur, H., Kumar, V., Pathak, D., & Sangha, M. K. (2024). *Antibiosis Mechanism and Bases of Resistance to the Whitefly Bemisia tabaci in Upland Cotton Introgression Lines*. Phytoparasitica, 52(2), 42.
- [18] Shibuya, T., Hirai, N., Sakamoto, Y., & Komuro, J. (2009). *Effects of Morphological Characteristics of Cucumis sativus Seedlings Grown at Different Vapor Pressure Deficits on*



- Initial Colonization of Bemisia tabaci (Hemiptera: Aleyrodidae). Journal of Economic Entomology, 102(6), 2265-2267.*
- [19] Bayoumy, M. H., Awadalla, S. S., El-Gendy, M. A., & El-Lawatay, N. E. (2017). *Comparative Morphology and Chemical Composition of Plant Leaf and Their Relation with Population Density of Certain Piercing-Sucking Insect Pests. Journal of Plant Protection and Pathology, 8(2), 31-37.*

The Effect of NPK Nano Elements and Amino Spray on some Physiological, Chemical and Production Characteristics of Date Palm Fruits *Phoenix dactylifera* L. Sayre Cultivar

Asmaa Rashid Saqr¹ and Batool Hanoon Falih Al-Zubaidy²

^{1,2} Department of Horticulture and Landscape Engineering, College of Agriculture and Marshlands, University of Thi-Qar, Iraq.

¹E-mail: Asmaa.post2021@utq.edu.iq

²E-mail: Batool-z@utq.edu.iq

Abstract. During the growing season (2023), the experiment was conducted in a private orchard in Thi Qar Governorate. The objective was to study the effects of spraying fruits with different concentrations of NPK Nano elements (0, 75, and 150 mg L⁻¹) and Amino Spray (0, 2, and 4 ml L⁻¹) as well as their interactions. The experiment followed a Completely Randomized Block Design Three 8-year-old trees of the Al-Sayer cultivar. Each treatment was replicated three times, and the process of spraying the fruits began 21 days after pollination and after the completion of fruit set, with 6 sprays, and the period between one spray and the next was 10 days, alternating until the fruits completed their growth and began their coloring phase. The results showed that spraying the fruits with NPK Nano elements at a concentration (150 mg L⁻¹) had a highly significant effect, as it achieved the highest averages for the dry matter percentage (65.01%), bunch weight (7.73 kg), and total yield (61.08 kg), compared to the control treatment, while the lowest averages, with a significant difference, were recorded for both the percentage of fruit drop (21.80%) and the water content (34.99%) compared to the control treatment. In terms of the spraying treatment with Amino Spray at a concentration of 4 ml L⁻¹, it had a very noticeable impact. The averages for fruit drop and water content percentages were the lowest, at 23.89% and 38.50%, respectively. On the other hand, the percentage of dry matter was 61.15%, bunch weight was 6.31 kg, and total yield was 60.028 kg, which was significantly higher than the control treatment. Compared to the control treatment, the interaction treatment involving 150 mg L⁻¹ of NPK Nano elements and 4 ml L⁻¹ of Amino Spray significantly reduced fruit drop and water content by 21.07% and 32.05%, respectively, while simultaneously increasing dry matter by 67-95% and bunch weight and total yield by 7.86-70.74%, with the latter two variables outperforming the former.

Keywords. NPK Nano, *phoenix dactylifera* L, Bunch weight, Total yield.

1. Introduction

Date palms, or *phoenix dactylifera* L., are evergreen fruit trees that have been known to humans for a long time. Date palms are grown extensively in tropical and subtropical regions, from latitudes 10–30 north to 20 south of the equator. One cultivar that stands out in Iraq is the Al-

Sayer, which is known for its commercial importance. The semi-dry fruits of this cultivar are sweet and delicious, with a good texture and an early ripening characteristic [1].

Traditional chemical fertilizers cause pollution in water, air, and soil, in recent years, many efforts have been made to reduce this problem by producing new fertilizers in improved forms and in different ways. Nanotechnology is a useful method in the fields of fertilization because it reduces the amount of chemical fertilizers used and increases the ability of the plant to absorb and store it inside itself for a longer period to improve crop quality and increase productivity [2].

The plant needs large quantities of NPK compound fertilizer, which is one of the necessary and important nutrients for the purpose of increasing plant growth, photosynthesis processes, and many different functions. Nanotechnology has helped regulate the release of nutrients from fertilizers, especially nitrogenous and phosphate compounds, as nutrient loss is reduced by washing or stabilization which leads to their slow absorption by the plant, and also avoids the interaction of nutrients with soil, living organisms and water [3].

Nano fertilizers are characterized by their small size and high surface area, which leads to an increase in the absorption surface, as Nano fertilizer particles are among the most widespread and widely used particles as a result of the positive effect of these fertilizers in improving plant growth and performance. At present, the use of fertilizers that contain NPK elements is one of the processes necessary to improve production quantitatively and qualitatively as it provides the nutrients necessary for plant growth and development [4].

Spraying bio stimulants containing amino acids is one of the necessary methods to reduce environmental pollution and achieve sustainable agriculture. These bio stimulants, which contain amino acids and organic materials, improve the metabolism of fertilizers, increase the absorption of water and nutrients, increase yields, and have a positive effect on plant growth [5].

Due to the lack of studies on the effect of Nano-fertilizers and Amino spray on Al-Sayer date palm trees, this study came with the aim of knowing the effect of Nano-fertilizers and Amino spray using different concentrations of them and determining the optimal level for each of them in improving some physiological and chemical characteristics and yield.

2. Materials and Methods

During the 2023 growing season, this experiment was conducted at a private orchard in the Muhammadiyah region, which lies next to the Euphrates River and is part of the Thi Qar Governorate in the Nasiriyah District. The experiment followed a Completely Randomized Block Design. Three 8-year-old trees of the Al-Sayer cultivar were chosen for their comparable vegetative growth. Each tree had nine fruit bunches remaining. One factor was a 20:20:20 ratio of NPK Nano fertilizer, with three concentrations (0, 75, 150) mg L⁻¹. The second factor was an amino spray, with three concentrations (0, 2, 4) ml L⁻¹. Three replicates were used for each treatment. The study factors were sprayed six times after fruit set was complete on 5/2/2023, with a 10-day interval between each spray.

2.1. Studied Characteristics

The measurements were taken in the Rutab stage, and the end of the field experiment was in August 2023, while the end of the laboratory analyzes was in October 2023.

2.1.1. Percentage of Fruits Drop (%)

After (90) days of pollination, the following equation was used to calculate the percentage of fruit drop for all treatments, in accordance with what was described [6].

Percentage of fruits drop = (Number of empty scars / (The number of empty scars + The number of present fruits) × 100)

2.1.2. Percentages of Water Content and Dry Matter (%)

The water content of the fruits in the Rutab stage was estimated by weighing (25) g of the core for each replicate randomly, they were cut using a knife and then placed in a vacuum convection oven at a temperature of 70 °C for a period of 72 hours until the weight was constant, after which the samples were weighed with a sensitive balance to calculate the percentage of water content and dry matter according to the following equations:

Water content (%) = ((fresh sample weight - dry sample weight) / fresh sample weight) × 100

Dry matter (%) = (dry sample weight/ fresh sample weight) × 100

2.1.3. Bunch Weight (Kg)

The average bunch weight of each palm tree was calculated by dividing the total yield of each palm tree by the number of bunches.

2.1.4. Total Yield (Kg)

The amount of total yield was calculated in the Rutab stage after harvesting the fruits for each palm tree separately, and then it was weighed with a field balance, after which the total yield rate was extracted.

3. Results and Discussion

3.1. Percentage of Fruit Drop (%)

In Table (1), we can see that there was a highly significant relationship between the concentration of NPK Nano-elements sprayed onto fruits and the percentage of fruit drop; for example, the control treatment had the highest average of 29.64 percent, while the concentration treatment at 150 mg L⁻¹ had the lowest average of 21.80%.

Spraying fruits with varying concentrations of Amino Spray considerably decreased the proportion of fruit drop, according to the data. In terms of average percentage, the control treatment came in at 26.74% while the concentration therapy (4 ml L⁻¹) came in at 23.89%.

Table 1. Effect of spraying fruits with NPK Nano elements and Amino spray and their interactions on the percentage of fruit drop (%).

NPK Nano elements mg L ⁻¹	Amino Spray ml L ⁻¹			NPK Average
	0	2	4	
0	31.80	29.37	27.75	29.64
75	25.97	23.81	22.84	24.21
150	22.47	21.87	21.07	21.80
Amino Spray Average	26.74	25.02	23.89	
L.S.D. ≤ 0.05				
	NPK	Amino Spray	Interactions	
	0.438	0.438	0.759	

Some of the interaction treatments between the concentrations of NPK Nano elements and Amino Spray were very effective in lowering the fruit drop percentage; for example, the control treatment had the highest average concentration (31.8%) and the interaction treatment with 150

mg L⁻¹ NPK Nano elements and 4 mL L⁻¹ Amino Spray had the lowest average concentration (21.07%).

3.2. Percentage of Water Content (%)

Table (2) shows that the statistical analysis revealed that spraying fruits with NPK Nano-elements significantly reduced their water content in the rutab stage. The average was 47.07% for the control treatment and 34.99% for the concentration therapy (150 mg L⁻¹).

When compared to the control group's average water content percentage of 43.51%, the Amino Spray treatment at 4 ml L⁻¹ resulted in a much lower value of 38.85% for the rutab stage fruits.

With a concentration of 150 mg L⁻¹ of NPK Nano elements and 4 ml L⁻¹ of Amino Spray, the interaction treatment had the lowest average of 32.05% compared to the control treatment's 48.65%. This was largely due to the decrease in the percentage averages of water content.

Table 2. Effect of spraying fruits with NPK Nano elements and Amino spray and their interactions on the percentage of water content (%).

NPK Nano elements mg L ⁻¹	Amino Spray ml L ⁻¹			NPK Average
	0	2	4	
0	48.65	47.32	45.25	47.07
75	44.04	41.46	39.25	41.59
150	37.83	35.10	32.05	34.99
Amino Spray Average	43.51	41.29	38.85	
L.S.D. ≤ 0.05				
	NPK	Amino Spray	Interactions	
	0.610	0.610	1.056	

3.3. Percentage of Dry Matter (%)

With a concentration of (150 mg L⁻¹) producing the greatest average of (65.01%), compared to the control treatment's lowest average of (52.93%), the statistical analysis in Table (3) demonstrated that spraying fruits with NPK Nano-elements has a highly significant effect by increasing the dry matter percentage averages of the fruits in the rutab stage.

Comparing the findings of the concentration treatment (4 ml L⁻¹) to the control treatment (56.49%), the concentration treatment produced a higher average of (61.15%), indicating that Amino Spray considerably raises the average percentage of dry matter in fruits.

Interaction therapy with (NPK Nano elements at a concentration of 150 mg L⁻¹ and Amino Spray at a concentration of 4 ml L⁻¹) produced the greatest average dry matter percentage of 67.95%, in contrast to the control treatment's lowest average of 51.35%.

Table 3. Effect of spraying fruits with NPK Nano elements and Amino spray and their interactions on the percentage of dry matter (%).

NPK Nano elements mg L ⁻¹	Amino Spray ml L ⁻¹			NPK Average
	0	2	4	
0	51.35	52.68	54.75	52.93
75	55.96	58.54	60.75	58.41
150	62.17	64.9	67.95	65.01
Amino Spray Average	56.49	58.71	61.15	
L.S.D. ≤ 0.05				
	NPK	Amino Spray	Interactions	
	0.610	0.610	1.056	

3.4. Bunch Weight (Kg)

The statistical analysis in Table (4) showed that spraying fruits with NPK Nano elements significantly increased bunch weight averages. The concentration treatment, with 150 mg L⁻¹, outperformed the control treatment, which recorded the lowest average of 4.46 kg, and achieved the highest average of 7.37 kg.

The control treatment had the lowest average bunch weight of 5.31 kg, whereas the treatment that involved spraying fruits with Amino Spray at a concentration of 4 ml L⁻¹ had a highly significant effect on boosting bunch weight averages, recording the highest average of 6.31 kg.

The statistical analysis table showed that the interaction treatments significantly increased bunch weight averages. The group that performed best was the one that combined NPK Nano elements at 150 mg L⁻¹ with Amino Spray at 4 ml L⁻¹; this group achieved an average of 7.86 kg, which was higher than all the other interaction treatments and the control group, which recorded an average of 4.17 kg.

Table 4. Effect of spraying fruits with NPK Nano elements and Amino spray and their interactions on Bunch weight (kg).

NPK Nano elements mg L ⁻¹	Amino Spray ml L ⁻¹			NPK Average
	0	2	4	
0	4.17	4.48	4.74	4.46
75	4.93	5.66	6.32	5.64
150	6.84	7.41	7.86	7.37
Amino Spray Average	5.31	5.85	6.31	
L.S.D. ≤ 0.05				
	NPK	Amino Spray	Interactions	
	0.112	0.112	0.194	

3.5. Total Yield (Kg)

According to Table (5), there was a highly significant increase in total yield when fruits were sprayed with NPK Nano-elements. The control treatment recorded the lowest average yield of 40.15 kg, while the greatest average yield was 61.082 kg at a dosage of 150 mg L⁻¹.

All of the study's findings are laid forth in one convenient table. A substantial increase in total yield was seen when fruits were treated with varying amounts of Amino Spray. As an illustration, the control treatment produced an average of 44.262 kg, but the treatment with a concentration of 4 ml L⁻¹ produced an average of 60.028 kg. The study factors' interaction treatments considerably enhanced total yield, as the control treatment produced the lowest average of 37.494 kg and the interaction treatment involving NPK Nano elements at a concentration of 150 mg L⁻¹ and Amino Spray at a concentration of 4 ml L⁻¹ produced the highest average of 70.740 kg.

Table 5. Effect of spraying fruits with NPK Nano elements and Amino spray and their interactions on total yield (kg).

NPK Nano elements mg L ⁻¹	Amino Spray ml L ⁻¹			NPK Average
	0	2	4	
0	37.494	40.320	42.636	40.150
75	44.370	56.731	66.708	55.936
150	50.922	61.584	70.740	61.082
Amino Spray Average	44.262	52.878	60.028	

	L.S.D. ≤ 0.05	
NPK	Amino Spray	Interactions
1.0510	1.0510	1.8204

The results in Tables (1, 2, 3, 4 and 5) showed the significant effect of spraying fruits with NPK Nano elements and Amino Spray in increasing the averages of the physiological, chemical, and production traits studied. Nano fertilizers have a high ability to penetrate the cellular membranes of plant cells, which leads to the activation of physiological processes, food making and metabolism, and thus increases cell division and number [7], and thus fruit drop will decrease. These results are consistent with the findings of [8,9], when they studied the NPK Nano elements on the Khastawi cultivar .

The effect of NPK Nano elements in increasing the percentage of dry matter may be due to the role of nitrogen, which affects plant growth by regulating the action of plant hormones such as auxins and gibberellin, or to the role of phosphorus, which affects the plant by controlling the increase in dry weight [10].

The increase in bunch weight averages and total yield as a result of treatment with NPK Nano elements may be attributed to the role of these elements in biosynthesis processes and the activation of enzymes that stimulate the growth processes that take place in both the ovary that produces fruits and the embryo that forms seeds. Thus, these elements contribute to increasing the rate of fruit set, growth and development up to the final maturity stage, which is reflected in increased production by increasing the number and weight of fruits on the bunch and thus increasing the total yield of the palm [11]. Or the reason for the increase in the amount of yield may be due specifically to phosphorus, which is considered one of the main components of the plant cell. It is also included in the structure of the cell nucleus protein, without which cell division cannot occur, in addition to the fact that it plays an essential role in the production of fruits [12]. This study agrees with what was found to him [13,14] during their studies on the effect of NPK Nano elements on date palms.

The effect of Amino Spray in reducing the percentage of fruit drop may be due to the role of its components in activating dissolved mineral and organic substances and their transfer to the fruits, in addition to their role in breaking down and decomposing organic substances such as pectin and hemicellulose, which help in adhesion and cohesion of cell walls, in addition to the means of activating hydrolytic enzymes [11]. This is consistent with a study [15]. on date palms, Shwaithi cultivar. the decrease in water content in the fruit at the rutab stage may be due to biological processes associated with the ripening of the fruit, such as the rapid loss of water, which occurs in conjunction with an increase in the accumulation of total solved solids in the fruit [16].

The primary ingredient in Amino Spray is a mix of amino acids, which play an important role in a variety of physiological and biochemical processes. These include protein and carbohydrate building, carbon metabolism stimulation, and the production of numerous enzymes and enzymatic companions. As a result, plants grow better and have a higher percentage of dry matter when treated with Amino Spray [17]. This study's findings corroborate those of the date palm investigations conducted by [18,19].

The Amino Spray's components work to supply the set fruits with water and food, especially protein materials to build new cells in the plant tissues. They also facilitate the flexibility and elasticity of the new cells to help them grow and elongate by filling them with water and food. This increases their size, the intensity of their attachment to the bunch, and prevents them from falling, which positively affects the increase in the weight of the bunch and total yield. The results of this study agree with those of [20].

Conclusions

The physiological, chemical, and production features that were evaluated were significantly improved when the fruits of Al-Sayer date palm trees were sprayed with NPK Nano elements at a dosage of 150 mg L⁻¹ and Amino Spray at a concentration of 4 ml L⁻¹, either alone or in combination.

References

- [1] Al-Jubouri , H. J. (2002). The importance of palm trees *Phoenix dactylifera* L. in the State of Qatar, proceedings and activities of the national training course on applications of plant tissue culture in improving agricultural production. Publications of the Agricultural Development Organization (21-27) January. Doha, Qatar, P:1-25
- [2] Al-Hchami, Salah H. J. and T.K.Alrawi. (2020). Nano fertilizer, benefits and effects on fruit trees: a review, *Plant Archives*.20 (1) P :1085-1088.
- [3] Liu, X.; Feng, Z.; Zhang, S.; Zhang, J.; Xiao, Q. and Wang, Y. (2006) . Preparation and testing of cementing nano-subnano composites of slow or controlled release of fertilizers. *Sci. Agric. Sin.*, 39Pp 1598- 1604
- [4] Shareef, H.J.,(2011). Effect of spraying with Urea and NPK on Production of Date Palm (*Phoenix dactylifera* L.) cv. Khidrawi. *Basrah J. Date Palm Res.* 10: 34-39.
- [5] Kowalczy, K. K.; T. Zielony. And M. Gajewski (2008). Effects of amino plant and asahi on yield and quality of lettuce grown on rock wool. *Biostimulators* .In *Modern Agriculture Vegetable crops*. 35-43.
- [6] Al-Asadi , A. D. K. (2009). The effect of pollen cultivar and bagging periods on some physiological, physical and chemical characteristics of date palm fruits *Phoenix dactylifera* L., Al-Mayasi and Al-Khadrawi cultivars. Master's thesis - College of Agriculture - University of Basra - Iraq.
- [7] Nair, S.H.; B.G.Nair; T.Maekawa; Y. Yoshida and D. S.Kumar.(2010). Nanoparticulate material delivery to plants. *Plant Science*, 179: 154- On the date palm .23-25 march.Pp:196-201.
- [8] Jubeir, S. M., and Ahmed, W. A. (2019). Effect of nano fertilizers and application methods to yield characteristics of date palm. *Plant Archives*, 19(1)Pp1881-1886.
- [9] Al-Zubaidi , S. M. J. (2018). The effect of fertilizers and methods of adding them on vegetative growth and yield of date palms, Khastawi cultivar . Doctoral dissertation , College of Agricultural Engineering Sciences, University of Baghdad , Iraq : 121 p
- [10] Zaid , A. .(2002). Date Palm Cultivation . Food and Agriculture Organization of he United Nation (FAO) , Rome , Italy .
- [11] Taiz , L. and E. Zeiger .(2002). *Plant Physiology*. 3rd ed. , Sinauer Associates, Inc. , Publishers , Sunderland , Massachusetts . Pp:623
- [12] Rotaru, V. (2010). The effects of phosphorus application on soybean plants under suboptimal moisture conditions. *Lucrări Științifice*. 53(2)Pp 27–30.
- [13] Abd- ElRahman, A., and Abd-Elkarim, N. A. (2022). Effect of nano N fertilizer on growth, fruiting and the fruits nutritive value of zaghoul date palm. *SVU-International Journal of Agricultural Sciences*, 4(1), 124-134.
- [14] El-salhy, A. M., Al-Wasfy, M. M., Badawy, I. F. M., Gouda, F. M., and Shamroukh, A. A. (2021). Effect of nano-potassium fertilization on fruiting of Zaghoul date palm. *SVU-International Journal of Agricultural Sciences*, 3(1)Pp 1-9.
- [15] Kazem ,Z.F., and Al-Asadi,A.D.,(2024) .The effect of the growth stimulant Bio20 and thinning on some physiological and physical characteristics of date palm fruits (*phoenix dactylifera* L.) the Al-Shwaithi cultivar growing in Thi –Qar Governorate, Thi- Qar University journal of Agricultural Sciences , 13(1)Pp 130-139
- [16] Al-Taha, A. H. and Taein , D. A. (2011). A comparative study of the growth and maturity of date palm fruits, Al-Shwaithi cultivar, grown in the Basra and Thi Qar regions. *Agricultural Science Studies* 38(2,1) 1-12.



- [17] Khalil,A.A.;E.A.M. Osman and F.A.F.Zahran(.2008) . Effect of amino acids and micron utrient foliar applicationon growth , yield and its components andchemical characteristics .J.Agric-Sci.Mansoura Univ.33(4)Pp3143-3150
- [18] Lafta , A. Y. (2013). The effect of bagging periods and spraying with plant extracts on some physical and chemical characteristics of date palm Phoenix dactylifera L. , Al-Sayer cultivar fruits. Master Thesis. College of Agriculture - University of Basra .
- [19] Al-Sardah , A. S. M. (2014). The effect of spraying with Fetrilon Combi 2 fertilizer and the two application times on some physical and chemical characteristics and yield of date palm Phoenix dactylifera L. , Al-Halawi cultivar. Master's thesis , College of Agriculture , University of Basra : 88 p
- [20] Al-Taha , A. H. M. and Al-Mubarak , N. R. (2016). The effect of spraying with Kelpak seaweed extract and NPK complex fertilizer on the quantitative concentration of nitrogen, phosphorus and potassium in the leaves and fruits of the date palm Phoenix dactylifera L., Barha cultivar. Journal of Basra Research (Sciences), 40 (1) : 65 – 84 .

Producing Single Hybrids with Early Flowering of Maize that are Suitable for Fall Season (Agronomic Traits)

Rafid Kamil Odhaib¹ and Wajeeha Abed Hassan²

^{1,2}Crops Sciences Department, College of Agriculture Engineering Sciences,
University of Baghdad, Baghdad, Iraq.

¹E-mail: rafed.kamel2206p@coagri.uobaghdad.edu.iq

²E-mail: wajeeha@coagri.uobaghdad.edu.iq

Abstract. A field experiment was carried out in the spring season (2023) by planting seeds of 13 pure inbred lines (to select six early inbreds) of maize. They were planted on 12-m-long rows with 3 rows for each of the inbreds. When the plants reached the flowering stage, they were selected. The six early inbreds were for the purpose of crossed by half-diallel crosses program between them to obtain 15 single hybrids according to the second method of [1]. $n(n-1)/2$, and in the fall season (2024), single hybrids (15) were planted with their parent and compared to the local variety Al Maha in three cultivation sites (Baghdad, Diyala, and Wasit) according to a design using a randomized complete block design (RCBD) to evaluate Its performance in some growth characteristics. The results showed that the Diyala site was significantly superior to the Baghdad and Wasit sites in terms of tasseling and silking characteristics (52.386 and 55.068 days) and plant and ear height (179.10 and 93.38 cm), while the Diyala site recorded the highest mean for the number of effective leaves (11.843 leaves per plant). Parent 6 outperforms with the fewest days for tasseling and silking (53.167 and 56.000 days) and the Parent 5 was superior in terms of the highest mean stem diameter (27.833 mm). Among the single hybrids, the hybrid (2×3) was superior with the fewest days for tasseling and silking (50.33 and 52,583 days), and the single hybrid (5×6) with the highest mean stem diameter characteristic (29.00 mm), and the hybrid (1×5) with the highest number of effective leaves. (13,333 leaves⁻¹).

Keywords. Maize, Single hybrids, Early flowering, Inbred lines.

1. Introduction

Maize (*Zea mays* L.) is one of the most important strategic crops for its use in human nutrition and its use in the production of poultry feed, and the vegetative part of it is used in animal feed, especially cows, due to its high protein content. This crop is also used in many important industries such as oil and starch. Maize is characterized by its high productivity and its ability to adapt to a wide range of different environmental conditions, as it is cultivated well in most regions of the world [2], the fact that maize contains male and female inflorescences separate from each other distinguishes it from many other crops due to the ease of performing the multiplication process in it and the fact that it is a cross-pollinated crop, which made many researchers resort to using it in their scientific experiments and producing new genetic combinations with the aim of increasing yields and improving many characteristics. Important for

plant breeders, such as early flowering and improving other growth characteristics, which thus contribute to increasing the plant's yield [3].

diallel crosses breeding is one of the most important breeding methods through which new single hybrids are produced by crossbreeding between genetically diversity inbreds [4,5]. The scientist [6] proposed the use of single hybrids because of the hybrid vigour they provide with high stability, as they result from the crossing of two genetically diversity inbreds. In order to develop a successful single hybrid, the strong effects of the hybrid must be increased, as it is expected to obtain favorable results when testing and selecting pure inbreds Genetically diversity and superior in their ability to combine, which is the key to producing good hybrids [7].

Many studies have been conducted to evaluate different genotypes of maize, including a study conducted by [8] on three genotypes of maize, which indicated that there are significant differences between the three genotypes, as the Sagunto genotype was superior in recording the highest mean for the height trait. The plant reached 169.47 cm, while the Abcaro genotype excelled, giving it means of 57.33 days and 61.67 days for the characteristics of the number of days of flowering, tasseling and silking, the third genotype (Cadis) had the highest stem diameter with a mean of 20.05 mm. [9] conducted half-diallel crosses between five inbreds of maize, through which they found significant differences recorded by the single hybrids, as the hybrid (ZM6 × BK121) excelled with the best significant means for the number of days of silking traits, amounting to 54.33 days.

In a study to evaluate the performance of triple and single hybrids and their inbreds of maize and compare them with the synthetic variety Ibaa 5018, [10] noted the presence of significant differences between the genotypes. The CDCN5 inbred outperformed the rest of the inbreds with the highest means for ear height and number of leaves per plant reaching 116.10 cm and 16.33 leaves sequentially. Confirmed by [11] in their study to evaluate the performance of five genotypes of maize and compare them with the local variety, there were significant differences between the genotypes for all the studied traits, as the variety DKC 6315 recorded its superiority in terms of plant height, ear, and number of leaves per plant reaching 230.5 cm, 123.07 cm, and 13.33 Leaf⁻¹. The results of [12] indicated that there were highly significant differences for the genotypes (parents and the hybrids resulting from them), and the earliest hybrid BK104) × (ABS6) with the lowest number of days for tasseling and silking amounted to 54.37 and 57.70 days, while the hybrid (Ibn-27 × BK104) the highest plant height reached 203.1 cm. According to [13] [14] in their study to evaluate the performance of five triple hybrids of maize and compare them with the local variety (Fajr 1), showed that the triple hybrid (ZM43WIZE × ZM49W3E) × ZM60 excelled with the lowest number of days for tasseling (55.25 days), and the triple hybrid (ZM43WIZE × ZM19) × ZM60 recorded the highest number of days for silking (55.25 days). The highest mean plant height was 212.1 cm.

The study aims to evaluate the performance of six inbreds Pure lines maize and its single hybrids resulting from half-diallel crosses and comparing them with the local variety Al-Maha in some growth traits in three different cultivation sites in Iraq.

2. Materials and Method

The study included six pure inbred lines of maize : (1) ART-A-23, (2) NAD-H-905, (3) NAD-H-26, (4) ART-B-17, (5) AM-80 and (6) NAD-H-706. The six inbreds were grown in the spring season (2023) at Research Station A of the College of Agricultural Engineering Sciences - University of Baghdad. (Al-Jadriya), and upon reaching the flowering stage. The inbred lines were crossed by half-diallel crosses program between them to produce 15 single hybrids.

2.1. Field Managements

The land used for all experiments was prepared by carrying out all agricultural operations, including plowing, harrowing, and leveling, according to recommendations. It was divided into furrows with a length of 12 m. The distance between one furrow and another was 0.75 m and between one plant and another was 0.25 m. It was fertilized with triple super phosphate fertilizer (46%P₂O₅). 200 kg P₂O₅ ha⁻¹ in one batch before planting, and nitrogen fertilizer. 350 kg N ha⁻¹ in the form of urea (46% N) in three batches, the first two weeks after germination, the second in the elongation stage, and the third at the beginning of the flowering stage, in addition to carrying out all agricultural operations, including irrigation, hoeing and weeding, and control of the corn stem borer insect was carried out (*Sesamia crinita*) by feeding the growing tips of plants with granulated diazinon pesticide (10% active ingredient) at a rate of 6 kg. /ha⁻¹, added in two batches, the first when the plant reaches a height of 20 cm and the second two weeks after the first batch.

2.2. First Season (Spring 2023)

Seeds of 13 pure inbred lines (to select six early inbred) were planted on a furrow 12 m long, but with 3 furrows for each of the inbred, on 21/3/2023. the inbred lines were crossed by half-diallel crosses program between them to produce 15 single hybrids. According to Griffing's second method (1956) $n(n-1)/2=6(6-1)/2=15$, when plants arrive to the flowering stage, the early inbreds were selected and the female ears were wrapped in paper bags before the emergence of the silking to obtain the required pollination and to avoid open pollination between the inbreds. The male tassels were also wrapped in paper bags one day before the pollination process between the pure inbreds, and the next day they were collected. Pollen from plants and the female ears of the inbreds that were ready to receive the pollen were pollinated with them. This process was continued until all the required crosses between the pure inbreds were performed. The six inbreds were self-pollinated for the purpose of multiplying their seeds for planting in subsequent seasons.

2.3. Second Season (Fall 2024)

All genotypes included in the experiment, which represent the single hybrids, their parent, and the control variety (Al-Maha) were planted to evaluate some growth characteristics in three planting sites: Baghdad, Diyala, and Wasit, during the fall season on 25/7/2024 using the Randomized Complete Block Design (RCBD) with four replicates, 88 experimental units for each planting site. Planting was done in furrows as in the first season, at a rate of 3 seeds in one hole were reduced to one plant two weeks after germination, and all agricultural operations were carried out for the soil and the crop, including plowing, smoothing, leveling, irrigation, hoeing, weeding, and weed control, as mentioned above. When flowering was completing in all the plants, 5 plants were chosen randomly from each experimental unit (leaving Peripheral plants) to measure growth characteristics: number of days of tasseling and silking, plant height, ear height, stem diameter, and number of leaves.

2.4. Statistical Analysis

Statistical analysis was conducted for all of the studied traits according to analysis of variance (ANOVA) according to the Randomized Complete Block Design (RCBD) with four replicates for the three sites. Significance was tested with the F test at the probability level of 0.05, and the means were compared using the least significant difference (LSD) at the probability level of 0.05, using the Genestat 2014 program.

3. Results and Discussion

3.1. Number of Days from Planting to 50% Tasseling (Day)

The mean table (1) shows that the trait was highly significantly affected by the differences in planting sites and genotypes and the interaction between them. It is noted that the number of days to tasseling was significantly affected by the difference in planting location and the earliness of the plants of the second site (Diyala). reached 50% tasseling with a number of days of 52,386 days, followed by the first site (Baghdad) with a number of days of tasseling of 52,977 days, and the third site (Al Kut), whose plants were late in reaching tasseling by a number of days of 53,091 days, did not differ from it. Regarding the early Diyala site, with a number of days amounting to 0.705 days, the reason for the early maize plants in reaching tasseling in the Diyala site is due to the deficiency of accumulated thermal units in the plant. These results agreed with [15] who found a difference in the number of days to tasseling depending on Agriculture site.

The results of Table 1 indicate that the genotypes (single hybrids and their parents, the control variety Al-Maha) differed significantly among them in reaching 50% tasseling. Parent 6 was the first of the parents to reach tasseling with a number of days of 53,167 days, and parent 4 did not differ from them by a number of days of 53,333 days, and these two parents were earlier with mean number of days of 3,000 and 2,834 days compared to the control variety (Al-Maha), whose plants flowered for a number of days that reached 56,167 days. As for the last parents to reach flowering, they were parents 2 and 5, with a number of days that reached 54,667 days for both of them, and also earlier than the control variety.

Of the single crosses, the hybrid (2×3) was the earliest by a number of days amounting to 50.333 days, and the hybrids (1×3) and (2×4) were not significantly different from it by a number of days amounting to 50.833 days for both of them, and the three crosses were earlier by a mean of 5.834, 5.334 and 5.334. days for the control variety, while the hybrids were the last to reach tasseling the hybrid (2×5) with a number of days reached 54,667 days, and it is also earlier than the control variety, whose plants flowered for a number of days that reached 56,167 days, and it differed significantly from all the hybrids and their parents. The reason for the difference between plants in achieving tasseling between the hybrids, their parents, and the control variety is that the genetic structures vary among them depending on the morphological characteristics and the extent of their response to the environment in terms of temperature and the length of the lighting period, leading to a difference in the number of days required to achieve tasseling, and this result is consistent with The findings of [16] are also consistent with what found by [17], who found that the triple hybrids differed significantly among them in reaching tasseling.

The results of Table 1 also show that the interaction was significant between the sites and genotypes (the hybrids, their parents, and the control variety) and that the response of the genotypes and the control variety to the different planting sites was in the direction of decrease and a reduction in the number of days to reach 50% tasseling at the Diyala site compared to the Baghdad and Wasit sites, which came after it. In terms of flowering, it is noted that parent 6 at the Diyala and Wasit sites is earlier with the lowest number of days, reaching 53.00 days for both sites. It did not differ significantly at the Baghdad site, which flowered half a day after them. As for the hybrids, the hybrid (2 x 3) was the earliest with the least number of days for tasseling, which reached 49,500 days at the Diyala site, and it did not differ significantly from the Wasit site, with a number of days that reached 50,000 days. The Baghdad site did not differ from Wasit site, with a number of days of 50,500 days, the hybrid (2×3) is earlier in flowering than the variety control with a number of days of 5,500, 5,500, and 7,500 days at the locations of Diyala, Baghdad, and Wasit, respectively, compared to the control variety.

Table 1. Mean number of days to tasseling for single hybrids and their inbred lines of maize for the fall season for three locations (Baghdad, Diyala, and Kut).

Single hybrids and their inbred lines	Baghdad location	Diyala location	Wasit location	Mean
1	55.000	53.000	54.500	54.167
2	55.000	54.500	54.500	54.667
3	54.000	53.500	55.50	54.333
4	53.500	53.000	53.500	53.333
5	55.500	54.500	54.000	54.667
6	53.500	53.000	53.000	53.167
(1×2)	52.000	51.000	51.500	51.500
(1×3)	51.500	50.500	50.500	50.833
(1×4)	51.000	52.000	51.500	51.500
(1×5)	51.500	50.500	52.500	51.500
(1×6)	53.000	53.500	52.500	53.000
(2×3)	50.500	49.500	50.000	50.333
(2×4)	50.000	52.000	50.500	50.833
(2×5)	55.500	54.500	54.000	54.667
(1×6)	53.000	53.500	54.000	53.500
(3×4)	52.000	49.500	51.500	51.000
(3×5)	51.500	52.000	53.000	52.167
(3×6)	51.500	50.000	51.500	51.000
(4×5)	53.500	52.000	53.500	53.000
(4×6)	53.000	52.000	54.000	53.000
(5×6)	53.500	53.500	54.000	53.667
control variety (Al-Maha)	56.000	55.000	57.500	56.167
L.S.D0.05		0.972		
Mean	52.977	52.386	53.091	0.561
L.S.D0.05		0.325		

3.2. Number of Days from Planting to 50% Silking (Day)

Table (2) indicates that there are significant differences due to the influence of planting sites, genotypes, and the interaction between them, as the second site (Diyala) recorded the least number of days to reach 50% silking, amounting to 55,068 days, followed by the site The third (Wasit) had a number of days to flowering that reached 55,705 days, which did not differ significantly from the first site (Baghdad), which needed the most days to reach 50% silking , amounting to 55,920 days, lagging behind the early Diyala site by 0.852 days. These results are consistent with what was found by [18] who found significant differences in this trait due to the effect of cultivation sites.

The results of Table (2) showed that there were significant differences in the effect of genotypes (single hybrids, their parents, and the control variety) in the number of days to reach 50% silking . The parent 6 recorded the least number of days to reach 50% silking , amounting to 56,000 days, superior to all parents and the control variety (Al-Maha), which was 6,500 days late, flowered by 62,500 days, and The parent 5 was the last of the parents to reach silking , with a number of days of 58,167 days, superior to the control variety, which was 4,333 days later in reaching silking .

The two single hybrids (2×4) and (3×6) were earlier, with the least number of days required to reach 50% silking, amounting to 52,500 days for both of them. The single hybrid (2×3) did not differ significantly from them, with a number of days amounting to 52,583 days, the control

variety (Al-Maha) was late, took 62,500 days to reach silking , 10 days late compared to the hybrids (2×4) and (3×6), and 9,917 days for the hybrid (2×3), and the hybrid (2×5) was the last of the single hybrids to arrive to the silking took 57.00 days, which was also earlier than the control variety (Al-Maha), which was significantly later than all the parents and their single hybrids. These results agreed with [19] and [20] who indicated that there were significant differences between the genotypes in trait of the number of days from planting until 50% silking.

The same results in Table 2 indicated a significant interaction between locations and genotypes (single hybrids, their parents, and control variety) The genotypes recorded in response to the planting sites showed a decrease in the number of days of silking at the Diyala site compared to the Wasit and Baghdad sites, which flowered later. The earliest parents were 1 (at the Diyala site) and 6 (at the Wasit site) with less the number of days reached 55,500 days for both of them, and they differed significantly from the rest of the parents at the rest of the sites, with the exception of parent 6 at the Baghdad site, which did not differ significantly and recorded a number of days of 56.00 days, while the control variety (Al-Maha) at the Wasit site lagged behind the earliest parents with a number of days of 9,500 days to arrive to the flowering stage, the table shows that the hybrids (2×3) and (3×6) were earlier than their parents and the control variety, and were earlier by the least number of days to reach the silking stage, amounting to 51,500 days for both of them at the Diyala site, compared to the control variety, which was later than them with a number of days of 10,500, 9,000, and 13,500 days to reach the silking stage at the Baghdad, Diyala, and Wasit sites, respectively. The hybrid (2×5) at the Baghdad site was the last of the single hybrids to reach flowering, 58.00 days late, but it was earlier than Control variety (Al-Maha) in all sites.

Table 2. Mean number of days to silking for single hybrids and their inbred lines of maize for the fall season for three locations (Baghdad, Diyala, and Kut).

Single hybrids and their inbred lines	Baghdad location	Diyala location	Wasit location	Mean
1	58.500	55.500	57.000	57.000
2	58.500	57.000	57.000	57.500
3	58.000	57.000	57.500	57.500
4	57.500	57.500	57.500	57.500
5	58.500	57.500	58.500	58.167
6	56.000	56.500	55.500	56.000
(1×2)	54.500	53.000	53.000	53.500
(1×3)	54.500	52.000	53.000	53.167
(1×4)	54.000	55.500	54.500	54.667
(1×5)	54.500	54.000	55.000	54.500
(1×6)	54.000	55.500	54.000	54.500
(2×3)	52.750	51.500	53.500	52.583
(2×4)	52.000	53.500	52.000	52.500
(2×5)	58.000	56.500	56.500	57.000
(2×6)	56.500	56.000	57.000	56.500
(3×4)	54.500	52.500	53.000	53.333
(3×5)	54.000	54.000	55.500	54.500
(3×6)	53.000	51.500	53.000	52.500
(4×5)	55.000	53.500	54.500	54.333
(4×6)	57.500	55.000	57.000	56.500
(5×6)	56.500	56.000	56.000	56.167
control variety (Al-Maha)	62.000	60.500	65.000	62.500

Single hybrids and their inbred lines	Baghdad location	Diyala location	Wasit location	Mean
L.S.D0.05		0.918		
Mean	55.920	55.068	55.705	0.530
L.S.D0.05		0.226		

3.3. Plant Height

It is clear from the means table (3) that the plant height characteristic was highly significantly affected by the differences in planting sites, genotypes, and their interactions. It was found that the Diyala site excelled significantly and achieved the highest mean plant height of 179.10 cm, followed by the Baghdad and Wasit sites, which did not differ significantly between them with two means they reached 174.77 cm and 174.17 cm, respectively. This is consistent with the findings of [21-23], who found a difference in plant height when they studied several genotypes in different cultivation sites.

It is noted from the above-mentioned table that there are highly significant differences under the influence of genotypes (single hybrids, their parents, and the control variety), as the control variety (Al-Maha) outperformed the parents with a mean plant height of 241.13 cm, and among the parents, it outperformed the parent 5, which reached a height of 169.67 cm, and did not differ. Significantly higher than parent 2, with a plant height of 166.67 cm, while parent 3 recorded the lowest mean for the trait compared to the control variety and the rest of the parents, which reached 163.67 cm. The decrease in the height of the plants of the inbreds is due to the decrease in concentration of the pairs of genes responsible for plant height. It was noted from the same table that the superiority of the control variety (Al-Maha) was significantly superior to the single hybrids resulting from cross inbreds, and it was superior to the single hybrids hybrid (2×3), which recorded a mean plant height of 182.83 cm. It was not significantly different from the single hybrid (2×5), with a mean of 180.33 cm. This result was consistent with the results of [17,24,25]. As for the interaction between the cultivation sites and the different genotypes (single hybrids, their parents, and the control variety), a highly significant effect was observed towards an increase in most of the genotypes at the Diyala cultivation site, with the exception of some genotypes at the Wasit and Baghdad sites. It was superior to the parents, parent 2, with the highest response amounting to 12.00 cm depending on the planting location, from Baghdad to Wasit, the plant height increased from 161.00 to 173.00 cm depending on the location. Among the single hybrids, the hybrid (5×6) excelled with the highest response to the change in locations, reaching 16.50 cm from the Wasit location to the Diyala location, and the response of the control variety reached 13.40 cm the two planting sites were from Wasit to Baghdad, and it was superior to the rest of the genotypes (parents and hybrids) with mean plant height of 248.45 cm, at the Baghdad site.

Table 3. Mean of Plant height for single hybrids and their inbred lines of maize for the fall season for three locations (Baghdad, Diyala, and Kut).

Single hybrids and their inbred lines	Baghdad location	Diyala location	Wasit location	Mean
1	166.50	169.00	159.00	164.83
2	161.00	166.00	173.00	166.67
3	162.50	170.00	158.50	163.67
4	162.50	168.00	165.00	165.17
5	171.50	173.50	164.00	169.67
6	164.50	173.50	160.00	166.00
(1×2)	171.50	179.00	172.50	174.33
(1×3)	171.00	175.00	173.50	173.17

(1×4)	180.50	180.50	171.00	177.33
(1×5)	175.50	183.00	179.50	179.33
(1×6)	175.50	177.00	173.50	175.33
(2×3)	175.50	185.50	187.50	182.83
(2×4)	167.00	172.25	167.50	168.92
(2×5)	181.50	186.00	173.50	180.33
(2×6)	173.00	180.50	185.00	179.50
(3×4)	169.50	178.50	168.50	172.17
(3×5)	175.50	176.50	180.00	177.33
(3×6)	170.50	179.00	176.25	175.25
(4×5)	171.00	176.00	173.50	173.50
(4×6)	175.00	168.50	169.00	170.83
(5×6)	175.50	183.00	166.50	175.55
control variety (Al-Maha)	248.45	239.90	235.05	241.13
L.S.D0.05		5.57		
Mean	174.77	179.10	174.17	3.22
L.S.D0.05		2.33		

3.4. The Ear Height

The means table (4) shows that the cultivation sites, genotypes, and the interaction between them had a highly significant effect on the height of the ear. The Diyala site was significantly superior to this characteristic with a mean height of 93.38 cm compared to the other sites. It was followed significantly by the Wasit site with mean of 91.97 cm, it differed from them both significantly, the Baghdad site had the lowest mean ear height of 89.88 cm. Similar results were obtained by [188,22] as they found an increase in ear height due to the influence of different agricultural sites. The results of Table 4 indicate that the genotypes (single hybrids and their parents and the control variety Al-Maha) differed significantly from each other in the ear height characteristic of maize. The control variety achieved the highest mean for the trait, reaching 137.13 cm, superior to the rest of the genetic combinations. Of the parents, parent 1 outperformed with the highest height of 88.50 cm, and parent 3 was the lowest in height at 81.67 cm. From the single hybrids, the hybrid (2×6) was superior and recorded a mean ear height of 100.17 cm. It was not significantly different from the single hybrid (2×3) which achieved a mean of 99.00 cm. The rest of the single hybrids with lower means for the trait, were the lowest in height of the hybrid (3×6) with a mean of 82.50 cm. This wide range in ear height between the genotypes reflects the genetic differences between them. This result is consistent with the findings of [19,26] in their study of hybrids resulting from maize crosses.

The results also indicated that the interaction between planting sites and genotypes was significant in terms of the cob height of yellow maize. The effect of the three sites on the genotypes in the mean ear height varied, with the Diyala site achieving the highest values for these means the interaction with the majority of the genotypes, while the Baghdad site recorded the highest ear height for the control variety (Al-Maha) reached 143.90 cm, which did not differ significantly from the Diyala site for the same variety, with a mean of 141.90 cm. Of the hybrids, the hybrid (2×3) was superior at the Diyala site, with a mean of 101.50 cm, and it did not differ at the Wasit site, with a mean of 100.50 cm. A similar study was obtained by [22] that the interaction between planting sites and genotypes led to variation in the ear height characteristic of maize.

Table 4. Mean of ear height for single hybrids and their inbred lines of maize for the fall season for three locations (Baghdad, Diyala, and Kut).

Single hybrids and their inbred lines	Baghdad location	Diyala location	Wasit location	Mean
1	87.00	83.50	95.00	88.50
2	82.00	85.50	91.50	86.33
3	78.00	84.00	83.00	81.67
4	79.50	84.50	88.00	84.00
5	86.00	87.50	84.50	86.00
6	83.00	82.50	84.50	83.33
(1×2)	81.00	92.00	97.75	90.25
(1×3)	82.50	94.50	93.50	90.17
(1×4)	93.00	97.50	94.00	94.83
(1×5)	90.00	100.50	87.50	92.67
(1×6)	95.00	94.50	91.50	93.67
(2×3)	95.00	101.50	100.50	99.00
(2×4)	81.50	93.50	95.50	90.17
(2×5)	84.50	103.00	92.00	93.17
(2×6)	100.00	92.00	108.50	100.17
(3×4)	89.00	95.00	82.50	88.83
(3×5)	92.50	82.50	92.50	89.17
(3×6)	80.00	82.50	85.00	82.50
(4×5)	94.00	86.50	82.00	87.50
(4×6)	98.50	98.00	86.00	94.17
(5×6)	81.50	91.50	82.50	85.17
control variety (Al-Maha)	143.90	141.90	125.60	137.13
L.S.D0.05		6.48		
Mean	89.88	93.38	91.97	3.74
L.S.D0.05		1.11		

3.5. Stem Diameter

The results of the means table (5) show that there are highly significant differences between the genotypes and the interaction between them and the planting sites, while the planting sites did not significantly affect the stem diameter characteristic. The means table (5) shows that, all parents outperformed the control variety, and the highest was parent 4, with the highest mean of 27.833 mm. Parents 3 and 6 did not differ significantly from it, with means reaching 27.167 mm and 27.000 mm. These parents outperformed the control variety, which recorded the lowest mean for this trait, amounting to 20.583 mm, with a difference of 7.250 mm, 6.584, respectively. Among the single hybrids, the hybrid outperformed (5×6) with the highest mean of 29.00 mm, and the hybrids (1×5), (1×6), (3×5), (3×6), and (4×5) did not differ significantly from it, with means of 28.833, 28.333, 28.500, 28.500, and 28.500 mm for each of them, respectively, outperforming the control class by a difference of 8.250, 7.750, 7.917, 7.917, and 7.917 mm. This is consistent with what was found by [8,25] said that the genotypes varied in their performance and recorded significantly different means in the stem diameter of maize.

The same results showed that the interaction between planting sites and genotypes had a highly significant effect on the stem diameter of maize. The results of the table showed that the increase in stem diameter was in the direction of increase in the Baghdad site, where most genotypes excelled in giving the highest means for the trait, followed by the Wasit site and then the Diyala

site. The two genotypes (1×5) and (5×6) outperformed by a mean of 30,500 mm each in the Baghdad and Diyala sites, respectively, and the rest of the single hybrids came after them in all agricultural sites with highly significant means compared to the control variety, which recorded the smallest stem diameter in the Baghdad sites, Diyala and Wasit reached 22.750 mm, 21.500 mm, and 17.500 mm respectively.

Table 5. Mean of Stem diameter for single hybrids and their inbred lines of maize for the fall season for three locations (Baghdad, Diyala, and Kut).

Single hybrids and their inbred lines	Baghdad location	Diyala location	Wasit location	Mean
1	27.000	26.500	27.000	26.833
2	25.500	26.500	26.500	26.167
3	27.000	27.500	27.000	27.167
4	28.000	27.000	28.500	27.833
5	27.000	26.000	26.500	26.500
6	25.500	27.500	28.000	27.000
(1×2)	27.500	27.000	29.500	28.000
(1×3)	29.000	25.500	27.500	27.333
(1×4)	28.500	27.000	26.500	27.000
(1×5)	30.500	28.000	28.000	28.833
(1×6)	27.500	28.000	29.500	28.333
(2×3)	27.500	25.000	27.500	26.667
(2×4)	27.500	26.000	28.500	27.333
(2×5)	26.500	28.500	27.500	27.500
(2×6)	26.500	27.000	29.000	27.500
(3×4)	26.000	29.500	27.000	27.500
(3×5)	29.000	28.000	28.500	28.500
(3×6)	29.500	29.000	27.000	28.500
(4×5)	28.000	29.000	28.500	28.500
(4×6)	26.500	25.500	27.500	26.500
(5×6)	27.000	30.500	29.500	29.000
control variety (Al-Maha)	22.750	21.500	17.500	20.583
L.S.D0.05		1.688		
Mean	27.261	27.091	27.386	0.975
L.S.D0.05		ع.غ		

3.6. Number of Effective Leaves

The results showed in the means table (6) that planting sites and genotypes and their interaction had a highly significant effect on the number of effective leaves. The Wasit site had the highest effect on this characteristic, amounting to 11.843 leaves per plant, and the Diyala site did not differ significantly from it in the number of effective leaves amounted to 11.800 leaves per plant, while the Baghdad site gave the lowest number of effective leaves per plant, amounting to 11.466 leaves per plant. These results are consistent with [27] found significant differences in the number of maize leaves in different planting sites.

It is also noted from the previously mentioned table that the genotypes (single hybrids, their parents, and the control variety) showed highly significant effects on the number of effective leaves. The hybrid (1×5) outperformed and gave the highest number of leaves, amounting to 13,333 leaves per plant, followed by the hybrid (4×6) with a number of leaves of 12,800 leaves per plant, and did not differ significantly from the hybrids (1×2), (1×6), (3×4), (3×5), (3×6), and

(5×6) and the control variety. With a number of leaves of 12.383, 12.600, 12.500, 12.383, 12.500, 12.533 and 12.750 leaves per plant, respectively. These results agreed with the findings of [28-31].

The results of Table (6) them show that the interaction between the planting sites and the genotypes (single hybrids, their parents, and the control variety) was significant, and was in the direction of an increase in the number of leaves in the Wasit site, where most of the genotypes recorded an increase in the number of leaves, followed by the Diyala site and then the Baghdad site, where it participated the hybrid (1 × 5) in the Wasit site with the control variety in the Diyala site had the highest value of the trait reaching 13,700 and 13,750 leaves per plant, respectively.

Table 6. Mean number of effective leaves for single hybrids and their inbred lines of maize for the fall season for three locations (Baghdad, Diyala, and Kut).

Single hybrids and their inbred lines	Baghdad location	Diyala location	Wasit location	Mean
1	10.300	10.500	9.900	10.233
2	9.900	10.100	10.000	10.133
3	9.800	10.000	11.000	10.267
4	10.700	11.100	11.200	11.000
5	10.400	11.700	11.500	11.200
6	10.600	11.200	10.300	10.700
(1×2)	12.150	12.600	12.400	12.383
(1×3)	11.400	11.600	12.400	11.800
(1×4)	11.300	11.000	11.800	11.367
(1×5)	13.100	13.200	13.700	13.333
(1×6)	12.200	13.100	12.500	12.600
(2×3)	11.000	11.100	11.800	11.300
(2×4)	11.200	12.000	11.500	11.567
(2×5)	10.300	11.250	10.400	10.650
(2×6)	11.800	11.000	11.200	11.333
(3×4)	11.800	12.700	13.000	12.500
(3×5)	11.500	12.600	13.050	12.383
(3×6)	11.800	12.600	13.100	12.500
(4×5)	12.700	11.500	12.200	12.133
(4×6)	13.100	12.400	12.900	12.800
(5×6)	12.300	12.600	12.700	12.533
control variety (Al-Maha)	12.900	13.750	11.600	12.750
L.S.D0.05		0.890		
Mean	11.466	11.800	11.843	0.514
L.S.D0.05		0.145		

Conclusion

When comparing tasseling and silking characteristics (52.386 and 55.068 days), plant and ear height (179.10 and 93.38 cm), and the number of effective leaves per plant (11.843), the Diyala site was found to be significantly better than the Baghdad and Wasit sites. When comparing the number of days it takes to tassele and silk a plant, Parent 6 comes out on top with 53.167 days, whereas Parent 5 has the largest mean stem diameter at 27.833 mm. The hybrid with the shortest time to tasseling and silking (50.33 and 52,583 days, respectively) was the 2×3 hybrid. The single

hybrid with the largest mean stem diameter (29.00 mm) and the most effective leaves was the 1×5 hybrid. with 13,333 leaves.

Reference

- [1] Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. of Biol. Sci.* 9: 463-93.
- [2] Tesfaye, S., H. Zeleke and D. Abakemal. 2019. Combining ability of highland adapted maize (*Zea mays* L.) inbred lines for grain yield and yield related traits under optimum and low nitrogen conditions. *African Science*, 13(5): 125-137.
- [3] Shull, G. H. 1910. Hybridization methods in corn breeding. *Journal of Heredity*, 1(2): 981-07.
- [4] Wuhaib, K. M., W. A. Hassan and B. H. Hadi .2017. Genotypic, phenotypic correlation and path coefficient in maize. II- yield and yield components. *Iraqi Journal of Agricultural Sciences*. 48(3):885-891.
- [5] Odhaib, R.K. and W.A. Hassan.2024. Breeding Double Hybrids of Maize with Early Flowering and Physiological Maturity. *IOP Conf. Series: Earth and Environmental Science*. Doi: 10.1088/1755-1315/ 1371/5/ 052078.
- [6] Jones, D. F. 1918. The effect of inbreeding and cross breeding upon development. (in *Connecticut Agric. Exp. Stn. Bull.* 207). P. 5-100.
- [7] Hallauer, A. R. and B. Miranda. 1982. *Quantitative Genetics in Maize Breeding*. Ames: Iowa State University Press.
- [8] Al-Shumary, A. M. J. Haithum. A. A. and Alabdulla. S. A. 2019. effect of spraying concentrations of integrated nano-fertilizer on growth and yield of genotypes of corn (*Zea mays* L.). *Al-Muthanna J. For Agric Sci.* 7(2): 114-121.
- [9] Abdul-Hamed, Z.A., M.H. Amoon. 2020. Performance and hybrid vigor for product hybrids using half diallel in maize. *Journal of Education Studies* 16 (6):147-154.
- [10] Almousawi, S. H. T., W. A. Hassan. 2021. Evaluating the performance of triple crosses and their parents of maize under two plant densities. *Al-Muthanna J. For Agric Sci.* 8(2): 233-245.
- [11] Hadi, B.H. and W.A. Hassan.2021. Evaluating the performance of introduced varieties of Maize (*Zea mays* L.) and estimating some genetic parameters. *International Journal of Agricultural and Statistical Sci.* 17(1): 85-91. DOI/03899.2021.17.85.91.
- [12] Al-Mohammady, H.S.H. 2022. Estimation of some genetic parameters of maize using half diallel cross under different levels of salicylic. Ph.D. Dissertation, University of Anbar/ College of Agriculture. Pp:182.
- [13] Abed, N.Y., B. H. Hadi, W. A. Hassan and K. M. Wuhaib . 2017. Growth trait's and yield evaluation of Italian maize inbred lines by full diallel cross. *The Iraqi Journal of Agricultural Sciences*. 48(3):773-781.
- [14] Al-Amili, M.A.Q., W. A. Hassan. 2024. Evaluation the performance of three-way crosses of maize under different sowing dates in fall season. *Journal of modern and Heritage Science*. 12(1): 31-42.
- [15] Kazem, K. A. and W. A. Hassan. 2020. 'Evaluation of the performance of introduced varieties of maize under different planting dates and environments. *Plant archives*, 20(2), pp.2036-2045.
- [16] Hadi, B.H. Hassan, W.A., Alshugeairy, Z.K., and Alogaidi, F.F., 2023. Estimating genetic parameters of maize hybrids and parents under different plant densities (Combining ability for yield and some other traits for maize *Zea mays* L.). *Revista Bionatura J.* 8(1) pp.87.
- [17] Al-Mousawi, S. H. and W. A. Hassan. 2020. Evaluation of the performance of triple, single Crosses and their inbred of maize under two plant population. *Plant Archives*. 20 (1): 1705-1716. Doi/366089861.
- [18] Kazem, K. A. and W. A. Hassan. 2021. Some genetic parameter for maize under different planting dates and environments (Some genetic parameter LAI, TDM, DTM, CGR). *Al-Muthanna J. For Agric Sci.* 8(2): 108-112.
- [19] Al-Falahy, M. A. H., Kanosh, K. H. and Al-Zubaidy. 2019. Heteosis, combining ability and their relation to the genetic distance between maize lines and their half diallel hybrids for grain yield per plant and some of its components. *Mesopotamia Joournal of agriculture*. 47(1): 501-520.

- [20] Abdel-Amir, A. N. and B. H. Hadi. 2018. Evaluate the performance of double, single hybrids and inbreds of maize under different plant densities and estimate the percentage of heterosis and hybrid vigor ((yield and its components)). *Euphrates J. Agric. Sci.* 10 (2): 60-79.
- [21] Al-Nassery, A. s., Sedeeq. A. Q. and Al-Janabi. M. A. 2016. effect of spring cultivars and fertilization on growth and yield of corn (*Zea mays* L.). *J. Tikrit Univ. for Agric. Sci.* 16 (3): 1-13.
- [22] Kakarash, S. A., Mustafa. K. M. Mohammed. M. S. and Al-Falahy. M. A. and Sadalla. H. A. 2019. Homoeostasis and genotypic resultant studies for hybrids of maize. *Mesopotamia Joournal of agriculture.* 47(3): 165-173.
- [23] Lawand, F. M. Towfiq. S. I. and Abdulkhaleq. D. A. 2022. gene action of some agronomic traits in maize by half diallel cross at two location in sulaimani- iraq. *Iraqi Joournal of agriculture scinces.* 53(2): 329-340.
- [24] Hamood., J. A. 2019. half diallel crossing among maize inbred lines and their evaluation under defferent nitrogen levels. . Ph.D. Dissertation, University of Baghdad /College of Agriculture Enginnering Science. Pp:131.
- [25] Taha, A.A., M.J. Al-Layla and K.S. Abdulla. 2019. effect of humic acid and plant density on growth and yield of tow varieties of maize (*Zea mays* L.) 1-field traits. *Inter. Sci. International Scientific conference for Agricultural Sciences.* 871-887.
- [26] Al-Joboory, A. H. A. and Al-Gaisi. W. H. M. 2017. Estimate of combining ability in maize corn (*Zea mays* L.) using (Line×Tester) method. *Tikrit J. Agric. Sci.* 17(3): 80-95.
- [27] Younis, S. A., Al-Hasan. A. M. 2018. effect of sowing dates in growth charcter, silage andgrain yield of of (*Zea mays* L.). *Mesopotamia Joournal of agriculture.* 46 (1): 169-174.
- [28] Hadi, B. H. 2018. Estimation gene effects based on joint scaling test and some genetic parameters in four maize crosses 1-yield components. *The Iraqi Journal of Agricultural Sciences.* 49(2):188-193.
- [29] Altaweel, M. S. and Yousif, Q. A. 2020. study of genetic parameters using half diallel cross in maize (*Zea mays* I.). *Mesopotamia Journal of Agriculture,* 48(4): 11-20.
- [30] Brto, E.F., and B.H. Hadi.2023. Some Genetic Parameters of Double Crosses under Different Planting Dates Suitable to the Middle Region of Iraq. 4th International Scientific Conference of Agriculture, Environment and Sustainable Development, ISAESC 2023Al Diwaniyah17 May 2023through 18 May . IOP Conference Series: Earth and Environmental Science.1259(1):1-9.
- [31] Rezgar, I.S. and M.A. Hussain.2024. Heterosis and genetic parameters for yield and yield components in maize using half diallel cross. *The Iraqi Journal of Agricultural Sciences.*..55(5):1859-1869.

Studying of Some Chemical Properties of the Soil and Evaluating Its Suitability for Growing Potatoes, Sustaining the Soil in the Lands of Ibrahimiyya District/Babylon Governorate Using Geospatial Technologies

Amaal Abdullah Oleiwi Al-Masodi¹ and Amal Radhi Jubier²

¹College of Agriculture, Al-Qasim Green University, Babylon, Iraq.

²Department of Soil Sciences and Water Resources, College of Agriculture, Al-Qasim Green University, Babylon 51013, Iraq.

²E-mail: amelradha@agre.uoqasim.edu.iq

Abstract. This study was conducted in Al-Ibrahimiyya district in Al-Qasim district, Babylon province, located in the central region of Iraq, extending between longitudes 44°37'29.206" - 44°38'42.149" east and latitudes 32°18'31.143" - 32°19'27.166" north. Six pedons and Thirty drilling hole locations were identified with an auger machine to a depth of 0 - 40 cm. and their coordinates were determined using a GPS device., and samples were obtained from each location to conduct the necessary laboratory analyses on them. The results of the chemical properties showed that the soil pH values were neutral tending to basicity, with low salinity in the peduncle and soil locations, while the organic matter content in the soil was low and gradually decreased with depth, ranging between 2.02 - 13.67 g kg⁻¹, and the soil organic carbon content ranged between 1.17 - 7.95 g kg⁻¹, and the values of the exchange capacity of positive ions were consistent with the organic matter content in this soil, as well as with the tissue type and clay content in it, as its content ranged between 12.65 - 23.32 Cmol+Kg⁻¹. The results of the carbonate mineral content showed high values, ranging between 159.15-211.20 g kg⁻¹, as its content increased with depth, and was inversely proportional to the gypsum content, which ranged between 1.43-8.65 g kg⁻¹ in these soils. As for the percentage of exchangeable sodium (ESP), it was observed that it varied between decrease and increase in the soils of the study area.

Keywords. Chemical properties, Evaluation, Suitability, Soil sustainability, Potato cultivation.

1. Introduction

The success of the agricultural production process and maintaining soil fertility requires a comprehensive, applied view to preserve the soil as an important food source to provide the plant with the necessary nutrients because it is a major source of natural wealth and has no alternative to produce what humans need in terms of food and many other necessary materials, especially in light of the increase in population and the pressures it exerts on agricultural lands[1]. The soil must be exploited according to its productive capacity and its characteristics must be preserved, and full benefit when exploiting it requires the application of scientific field methods and

procedures in order to achieve sustainable agricultural production [2]. At the forefront of these methods is the implementation of soil survey work with the provision of the necessary recommendations on how to use and maintain soil units and determine the suitability of each soil unit for different purposes and the appropriate crop in the case of agricultural purposes [3]. The assessment of land suitability is of great importance to identify suitable and appropriate lands for developing agricultural systems and irrigation practices, and assessing their suitability for specific use is closely related to the different soil properties by increasing the productivity of the land unit agriculturally and protecting it from degradation processes [4]. The emergence of modern programs and methods recently contributed to the processes of revealing the issues that hinder the soil and its suitability for agriculture and determining its productive capacity. Due to the lack of studies on the assessment of the lands of the study area (Dublah) and the potato crop, which is one of the important crops in terms of nutrition, as it occupies the fourth place after wheat, corn and rice, and the first place in energy production and the second in protein production, and because the productivity of this crop in Iraq is high, the research was directed to achieve the following aim: -Evaluation of the suitability of soils and climate for potato crops using geospatial technologies and mapping modeling.

2. Materials and Methods

2.1. Location

The study area was chosen in Al-Ibrahimiyya (Al-Dublah) district, which is one of the newly established districts that administratively belongs to Al-Qasim District in Babylon Province . It is the link between the city of Hillah on the one hand and the city of Hashimiyah on the other hand. It is located between longitudes 37' 29".206 440 to 440 38' 42".149 east and between latitudes 31".143 18' 32" to 320 19' 27.166" north .

2.2. Field Procedures

Based on the field information obtained from the people of the region, a field visit was made to the Ibrahimiyyah district, and then the area was irregularly interconnected and Six pedons thirty locations were identified with a drilling rig (uger) at a surface depth of 40-0 cm, and the salinity, texture, color and carbonate mineral content were examined. After that, the pedons representing the region were identified and their horizons were described in a fundamental morphological description according to the principles stated in [5]. The coordinates of the location and pedons were determined using a GPS device. Then, samples were obtained from each location and each horizon and stored in special bags, numbered and brought to the laboratory to conduct the necessary laboratory analyses.

2.3. Laboratory Procedures

2.3.1. Chemical Estimates

- pH: The soil reaction in the saturated paste extract was estimated using a Phmeter device according to the method of [6].
- Electrical conductivity (ECe): The electrical conductivity (ECe) was estimated in the saturated soil paste extract by EC-meter according to the method given in [6].
- Sodium (Na): It was estimated in the extract by flame spectroscopy [6].
- Exchangeable sodium percentage (ESP): The exchangeable sodium percentage was estimated according to the method given in (Richards).

- Soil organic matter (SOM): The soil organic matter was estimated by wet digestion method according to the method [5] given in [6].
- Organic carbon (OC): Its estimation is closely related to the organic matter in the soil.
- Cation exchange capacity (CEC): The CEC was determined using ammonium acetate NH₄ OAC (1N) at pH = 7.0 according to the method given in [7]
- Carbonate minerals and gypsum: The concentrations of calcium and magnesium were determined by titration with EDTA-Na, 0.01N according to the method described in (Jackson, 1958), and gypsum was determined by the precipitation method as given in [6].
- Office procedures: At this stage, a file was created in Excel and then called into the GIS program to correct the coordinates and convert them to the UTM metric system, to create a Shepfail and cut off the study area. After that, maps of the soil characteristics suitable for potato cultivation were produced according to the Inverse Distance Weighting (IDW) method.

3. Results and Discussion

3.1. Soil pH Reaction

The results of Table (1) and (2) showed that the values of soil pH reaction in the study area ranged between 7.49-7.00, as the lowest value was in the C2 horizon of Pedon 1 and Pedon 5 and the C3 horizon of Pedon 3, and in locations 6, 13 and 22, while the highest value was in the C3 horizon of Pedon 4, and the reason for this is attributed to the fact that Iraqi soils are neutral to slightly basic due to their containing high amounts of carbonate minerals that are characterized by the ferrous property, which affects the soil reaction.

Table 1. Chemical traits of the soil pedons of the study area.

pedon	Horizon	pH	ECe dSm ⁻¹	Na ⁺ Meq L ⁻¹	ExNa ⁺	ESP %	CaCO ₃	gypsum g Kg ⁻¹	SOM	O.C	CEC Cmol ⁺ Kg ⁻¹
P1	Ap	7.18	3	8.23	1.66	7.99	180.24	8.23	10.2	5.93	20.77
	C1	7.23	3.22	8.78	1.84	8.89	180.46	8.26	6.28	3.65	20.7
	C2	7	4.16	9.05	1.3	7.01	180.9	8.31	3.42	1.99	18.54
	C3	7.11	4.29	9.12	2.23	12.03	18.93	8.32	3.4	1.98	18.53
P2	Ap	7.35	4.1	10.11	2.51	13.49	185.11	4.5	8.95	5.2	18.6
	C1	7.4	4.18	10.24	2.52	14.41	193.47	4.76	4.26	2.48	17.49
	C2	7.37	4.36	11.2	2.44	14.77	192.56	4.77	3.81	2.22	16.52
	C3	7.38	4.61	11.31	2.6	17.13	200.2	4.77	2.02	1.17	15.18
P3	Ap	7.22	3.27	12.32	2.87	17.14	181.42	3.58	9.39	5.46	16.74
	C1	7.31	3.35	11.06	3.05	16.5	200.09	3.67	6.41	3.73	18.48
	C2	7.31	3.44	8.87	2.04	15.22	207.82	4.23	4.18	2.43	13.4
	C3	7	3.67	7.45	2.12	16.76	211	4.28	3.06	1.78	12.65
P4	Ap	7.17	3.95	7.89	2.15	9.63	184.17	2.54	11.3	6.57	22.33
	C1	7.28	4.02	10.03	3.01	13.5	188.1	2.78	7.63	4.44	22.3
	C2	7.45	4.13	10.13	3.96	19.2	200.02	3.9	4.19	2.44	20.62
	C3	7.49	4.33	10.13	3.1	15.47	211.2	3.94	3.62	2.1	20.04
P5	Ap	7.37	2.52	9.42	1.85	8.59	196.04	4.19	10.08	5.86	21.53
	C1	7.1	3	9.52	1.93	9.6	200.13	4.48	6.85	3.98	20.11
	C2	7	3.28	8.07	2	10.99	201.05	4.62	4.27	2.48	18.2
	C3	7.29	3.5	8.96	2.12	11.64	201.66	4.69	4.11	2.39	18.22
P6	Ap	7.23	4.23	9.42	3.41	14.59	178.45	7.61	10.23	5.95	23.37
	C1	7.4	4.61	9.85	3.94	18.71	17913	7.29	5.4	3.14	21.06
	C2	7.41	4.62	10.74	3.82	21.01	180.83	8.3	4.14	2.41	18.18
	C3	7.39	4.76	10.15	4.04	22.38	182.1	8.12	3.45	2.01	18.05

Table 2. Chemical properties of the drilling soils of the study area.

Location	pH	ECe dSm ⁻¹	Na ⁺ Meq L ⁻¹	ExNa ⁺ L ⁻¹	ESP %	CaCO ₃	Gypsum g Kg ⁻¹	SOM	O.C.	CEC Cmol+ Kg ⁻¹
1	7.19	4.43	8.86	3.14	17.33	169.53	1.56	13.67	7.95	18.12
2	7.3	4.7	8.47	3.04	17.48	169.42	1.58	7.91	4.6	17.39
3	7.45	5.11	9.05	4.15	22.52	170	1.43	8.11	4.72	18.43
4	7.39	5.18	9.22	4.01	19.59	175.91	2.74	10.13	5.89	20.47
5	7.06	3.2	7.59	2.2	12.68	164.87	1.87	9.02	5.24	17.35
6	7	3.28	8.6	2.46	13.44	172.88	4.76	6.95	4.04	18.29
7	7.09	3.56	8.48	3.88	21.11	172.91	2.11	6.03	3.51	18.38
8	7.16	4.06	9.1	3.51	19.39	175.2	3.27	7.1	4.13	18.1
9	7.18	4.4	10.24	3.02	14.02	198.95	3.96	10.62	6.17	21.54
10	7.43	4.33	9.77	3.32	14.25	190.19	3.26	8.41	4.89	23.3
11	7.41	4.42	9.86	3.18	17.28	181.57	2.08	6.3	3.66	18.4
12	7.44	4.2	9.93	3.34	18.74	182.61	3.1	7.28	4.23	17.82
13	7	3.22	6.98	2.57	14.96	166.3	7.7	11.12	6.47	17.18
14	7.31	3.8	7.53	2.68	14.05	181.42	6.74	12.77	7.42	19.07
15	7.11	4.17	9.15	3.63	15.57	183.5	5.35	11.42	6.64	23.32
16	7.32	4.33	10.4	3.12	14.11	180.53	4.86	8.37	4.87	22.11
17	7.3	4.18	7.32	3.03	17.19	170.18	2.47	7.17	4.17	17.63
18	7.12	3.5	6.89	3.15	14.38	198.2	4.3	10.25	5.96	21.9
19	7.4	3.19	10.27	2.23	10.46	181.06	5.13	10.16	5.91	21.32
20	7.42	3.82	5.95	2.18	10.63	200.18	6.43	9.5	5.52	20.51
21	7.39	4.2	10.18	3.02	16.19	205.71	2.86	8.16	4.74	18.65
22	7	4.12	10.24	3.13	14.78	184.06	3.45	13.02	7.57	21.18
23	7.12	5.3	9.77	4.2	20.91	176.84	3.03	11.6	6.74	20.09
24	7.08	4.18	8.3	3.18	18.12	159.15	2.29	6.82	3.97	17.55
25	7.11	5.38	7.19	3.91	22.28	174.81	3.78	7.8	4.53	17.55
26	7.04	4.32	8.3	3.48	19.51	185.9	3.9	7.32	4.26	17.84
27	7.27	3.12	2.77	1.95	8.78	177.13	2.87	10.11	5.88	22.2
28	7.23	3.85	2.82	1.54	7.02	160.83	8.65	11.04	6.42	21.94
29	7.08	3.21	2.39	1.87	8.05	195.43	5.3	9.18	5.34	23.22
30	7.35	4.3	8.06	3.11	15.45	180.07	7.48	10.23	5.95	20.13

The results of Table (3) and Figure (1) showed that the spatial distribution of soil reaction values in the study area was distributed in three ranges, as the range 7.30-7.20 occupied the largest area of 1376.31 hectares and a percentage of 78.14%, while the smallest area was occupied by the range 7.20-7.00, which amounted to 134.91 hectares and a percentage of 7.66%, that the difference in spatial distribution is due to the variation in the soil content of carbonate minerals, and these results are consistent with what was obtained by[8] . As for its suitability for potato cultivation, it falls within the appropriate category, as it was given an estimate ranging between 85-74.5, so soil reaction does not constitute a determining factor for potato cultivation in the soils of the study area.

Table 3. Area and percentage of soil reaction in the soils of the study area.

Ranges	Area (hectare)	Percentage%
7.20-7.00	134.91	7.66
7.30-7.20	1376.31	78.14
7.50-7.30	250.02	14.20
Sum	1761.24	100

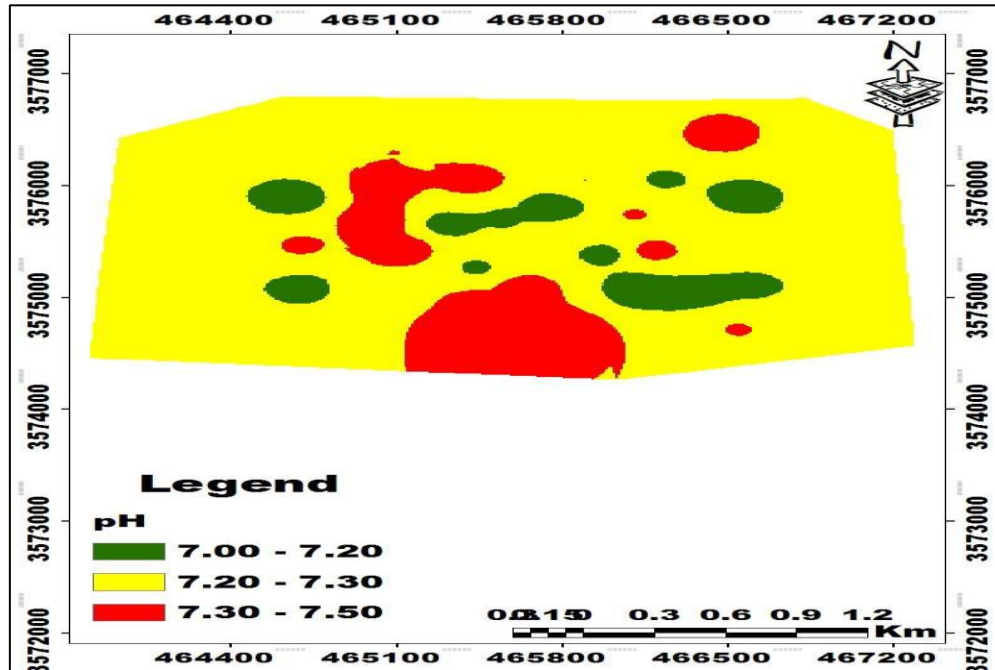


Figure 1. Spatial distribution of soil reaction in the study area.

3.2. Electrical Conductivity E_{ce}

The results of Table (1) and (2) showed that the values of electrical conductivity E_{ce} representing soil salinity ranged between 5.38-2.52 dSm^{-1} , as the lowest value was in the Ap horizon of Pedon 5 and the highest value was in location 25, as a decrease was observed in them in the soils of the region, and the reason is due to its agricultural exploitation, which causes a decrease in soil salinity, washing, dissolving and descending to the bottom of the soil. The results of Table (4) and Figure (2) indicated that the spatial distribution of electrical conductivity in the soils of the study area was distributed according to two classes, the slightly saline class within the range 4 - 0 dSm^{-1} occupied the largest area amounting to 1163.65 hectares and a percentage of 66.07%, while the other class is moderately saline within the range 8 - 4 dSm^{-1} occupied the smallest area amounting to 597.59 hectares and a percentage of 33.93% of the total area, as for its suitability, it falls within the suitable and moderately suitable class S2, and salinity did not constitute a determining factor for potato cultivation and growth in most locations, but it was a determining factor in other locations, as it was given an estimate ranging between 85.5 - 58.4, and these results are consistent with what was obtained by [9, 10] . Table (4) Area and percentage of electrical conductivity in the soils of the study area .

Table 4. Area and percentage of electrical conductivity in the soils of the study area.

Ranges dSm^{-1}	Area (hectare)	Percentage%
4 - 0	1163.65	66.07
8 - 4	597.59	33.93
Sum	1761.23	100

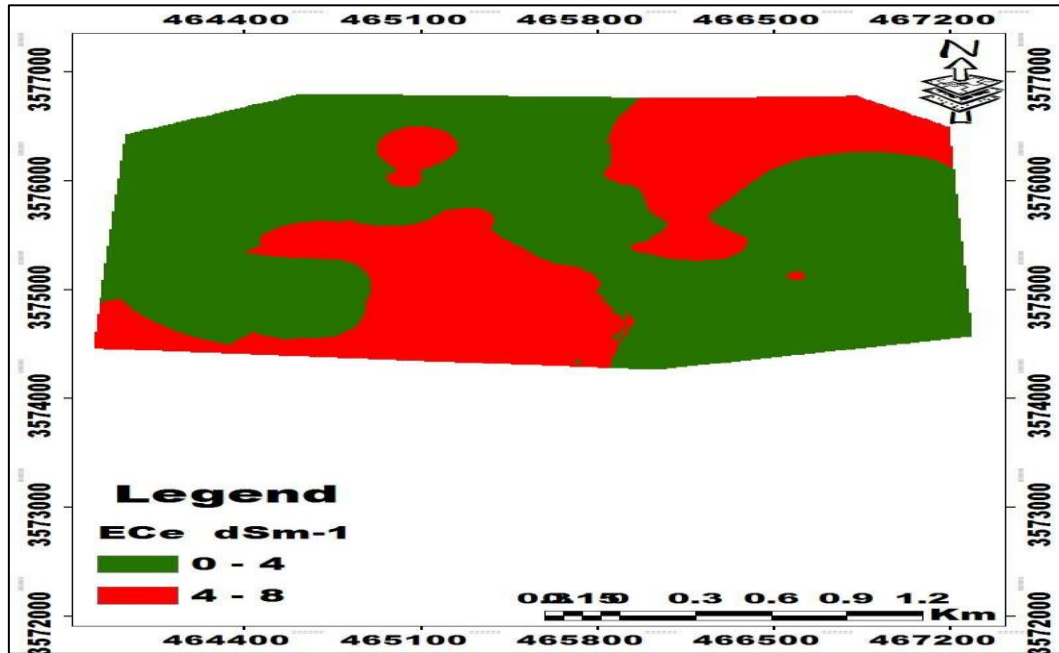


Figure 2. Spatial distribution of electrical conductivity in the soils of the study area.

3.3. The Exchanged Sodium Percentage ESP

The results of Table (1) and (2) indicate that the exchanged sodium ratio ESP ranged between 22.52-7.01%, as the lowest ratio was in the C3 horizon of Pedon 1 and the highest ratio was in location 3. The exchanged sodium ratio varied between low and high, and the reason is due to the variation in the values of exchanged sodium and the exchange capacity of positive ions CEC. Also, the soils with a high ratio do not turn into alkaline soils due to the high values of soil reaction and the presence of carbonate minerals in them.

The results of Table (5) and Figure (3) showed that the spatial distribution of the exchanged sodium ratio ESP was distributed in two ranges. The range 25-15% occupied the largest area, amounting to 971.17 hectares, with a percentage of 59.66%, while the range 15-0% occupied the smallest area, amounting to 791.06 hectares, with a percentage of 40.34%. As for the suitability for growing potatoes, it was within the degree of very suitability and suitability. The estimate ranged between 97.19-88.09, so it did not constitute a specific factor in all the soils of the study area.

Table 5. Area and percentage of exchangeable sodium in the soils of the study area.

Ranges dsm ⁻¹	Area (hectare)	Percentage%
15-0	971.17	66.07
25-15	791.06	33.93
Sum	1762.23	100

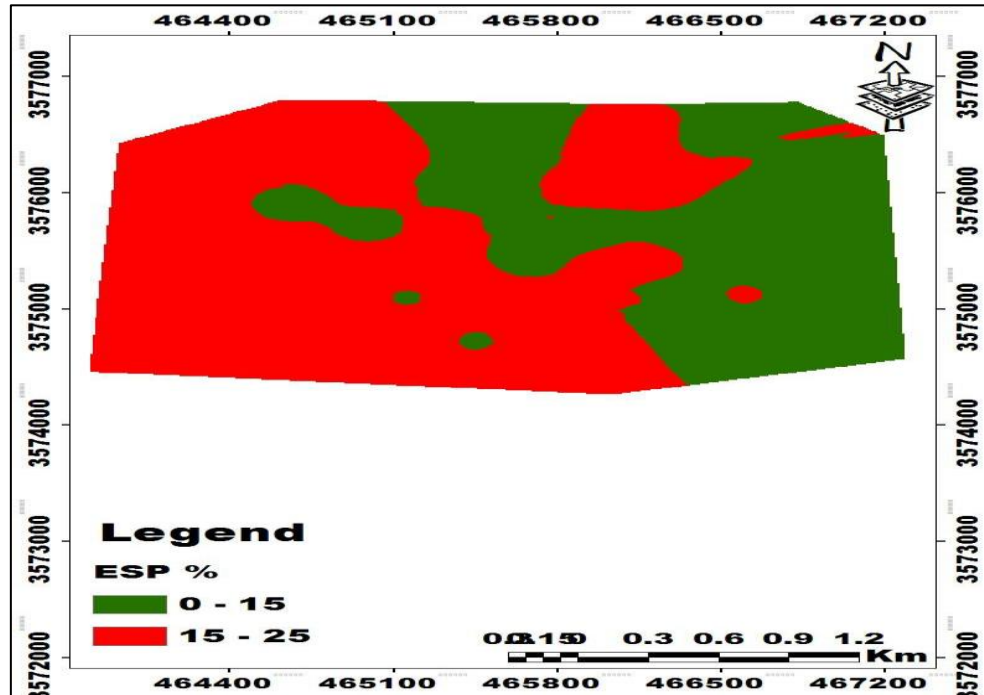


Figure 3. Spatial distribution of the exchangeable sodium ratio in the soils of the study area.

3.4. Carbonate Minerals CaCO_3

The results of Table (1) and (2) indicate that the content of carbonate minerals in the soils of the study area ranged between 211.20-159.15 g kg^{-1} , as the lowest content was at location 24, and the highest content was in the C3 horizon of Pedon 4, as the variation in the distribution of carbonate minerals in the study soils is noted and the reason is attributed to agricultural operations, especially in the surface horizons, as well as the fact that the sedimentary origin materials are rich in carbonates due to the nature of the limestone deposits in the study area, high temperatures, and lack of sufficient moisture.

The results of Table (6) and Figure (4) showed that the spatial distribution of carbonate minerals content in the soils of the study area was distributed in two ranges, the range 150-200 g kg^{-1} occupied the largest area of 1225.33 hectares and a percentage of 69.57%, while the range 200-250 g kg^{-1} occupied the smallest area of 535.90 hectares and a percentage of 30.43% of the total area, which confirms the importance of agricultural exploitation of the lands and not leaving them without cultivation. As for the suitability for planting potatoes, it was of a suitable and very suitable degree according to the S1 variety, as it was given an estimate ranging between 88.92-83.57, so carbonate minerals did not constitute a determining factor for planting potatoes in the soils of the study area.

Table 6. Area and percentage of carbonate minerals in the soils of the study area.

Ranges g kg^{-1}	Area (hectare)	Percentage%
200-150	1225.33	69.57
250-200	535.90	30.43
Sum	1761.23	100

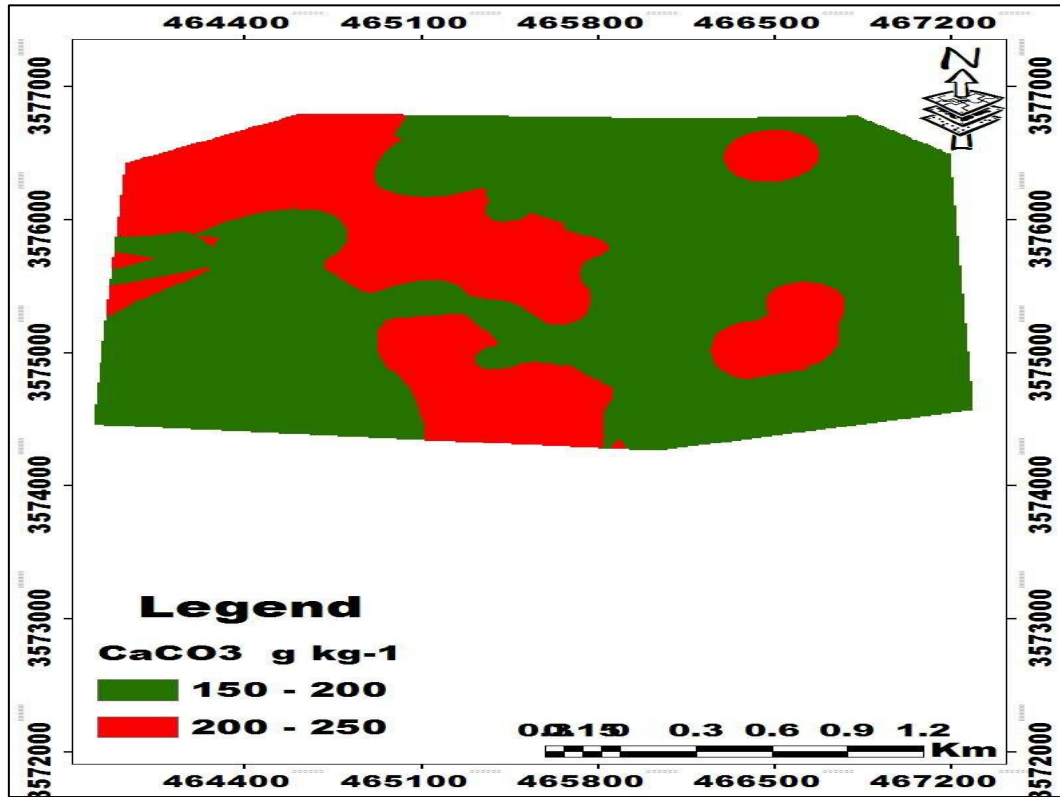


Figure 4. Spatial distribution of carbonate minerals in the soils of the study area.

3.5. Gypsum

The results of Table (1) and (2) showed that the gypsum content in the soils of the study area ranged between 8.65-1.43 g kg⁻¹, as the lowest content was at location 3, and the highest content was at location 28 of the drilling locations. The gypsum content of the soils of the study area was low and gypsum did not appear at a high content in the soil. The reason is due to the solubility of this compound and its washing as a result of agriculture due to the irrigation process or rain, in addition to the cumulative preference of carbonate minerals compared to hydrated calcium sulfate [11]. The results of Table (7) and Figure (5) showed that the spatial distribution of gypsum content in the soils of the study area was distributed in two ranges, the range 5.0-1.5 g kg⁻¹ occupied the largest area of 1494.77 hectares and a percentage of 84.87%, while the range 8.5-5.0 g kg⁻¹ occupied the smallest area of 266.46 hectares and a percentage of 15.13% of the total area of the study area. It is noted that most of the pedons and drilling locations recorded low values of gypsum, especially in the surface horizons, and it increases in the subsurface horizons, which is attributed to soil management operations because they are agricultural soils and that gypsum is a rapidly soluble salt, so it moves from top to bottom, in addition to the inverse relationship between the content of carbonate minerals and the content of gypsum in the soil, which was confirmed by [12] As for the suitability of gypsum for potato cultivation, it was of a very suitable degree S1, as it was given an estimate ranging between 99.86-99.23, which means that gypsum does not constitute a limiting factor for potato cultivation in the soil.

Table 7. Area and percentage of gypsum content in the soils of the study area.

Ranges g kg ⁻¹	Area (hectare)	Percentage%
5.0-1.5	1494.77	84.87

8.5-5.0	266.46	15.13
Sum	1761.23	100

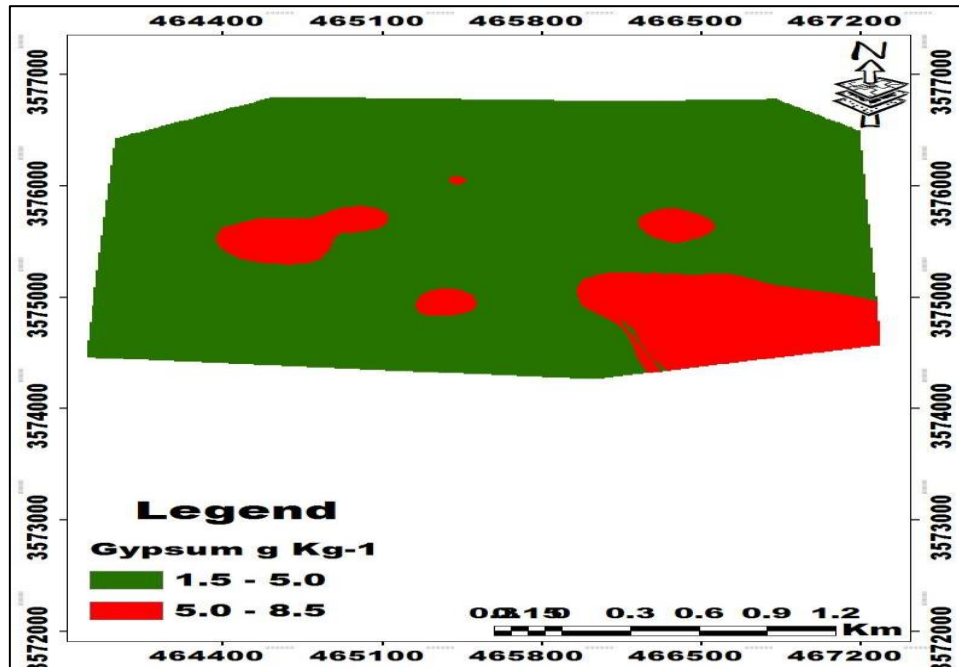


Figure 5. Spatial distribution of gypsum content in the study area.

3.6. Organic Matter in the Soil

The results of Table (1) and (2) showed that the organic matter content in the soil in general was low except in the surface horizons as a result of the cultivation process and the roots of the plants have a relatively high content, as its content in the soils of the region ranged between 13.67-2.02 g kg⁻¹, as the lowest content was in the C3 horizon of the bed 2, because the organic matter in the soil gradually decreases with depth as well as the distribution of the roots and the texture of this horizon is of a sandy clay type, while the highest content was in location 1 of the drilling locations.

The results of Table (8) and Figure (6) showed that the spatial distribution of organic matter content in the soil was distributed in two ranges, the range 10.0-5.0 g kg⁻¹ occupied the largest area of 1387.60 hectares and a percentage of 78.79%, while the range 15.0-10.0 g kg⁻¹ occupied the smallest area of 373.63 hectares and a percentage of 21.21% of the total area of the study area. The difference in the distribution of organic matter in the soil is due to the dry climatic conditions prevailing in the region, such as high temperatures and low rainfall, which help in the occurrence of oxidation processes and burning and losing organic matter in it, which was confirmed by [13].

Table 8. Area and percentage of organic matter content in the soils of the study area.

Ranges g kg ⁻¹	Area (hectare)	Percentage%
10.0-5.0	1387.60	78.79
15.0-10.0	373.63	21.21
Sum	1761.23	100

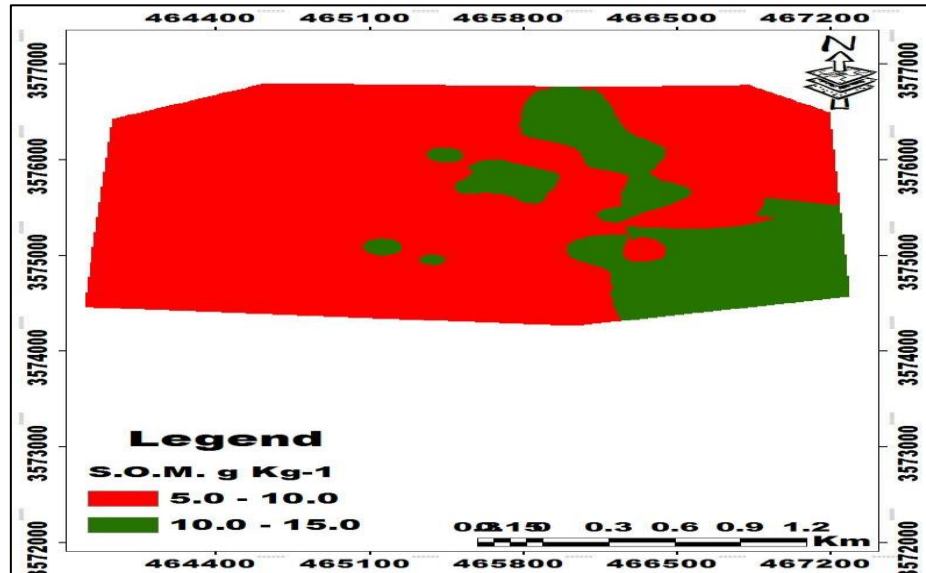


Figure 6. Spatial distribution of organic matter content in the soils of the study area.

3.7. Organic Carbon

The results of Table (1) and (2) indicate that the organic carbon content in the soils of the study area ranged between 7.95-1.17 g kg⁻¹ the largest area reached 1389.13 hectares and a percentage of 78.87%, while the range 8.0-5.50 g kg⁻¹ occupied the smallest area reached 372.10 hectares and a percentage of 21.13% of the total area of the study area, and these values are consistent with the results of the values of organic matter in the soil because the organic matter results from the product of organic carbon in the soil 1.72, as for the suitability for growing potatoes, it was between moderate suitability and low suitability, as an estimate was given ranging between 85-55.74, as organic carbon is a determining and important factor for growing potatoes in the soils of the study area [14].

Table 9. Area and percentage of organic carbon in the soils of the study area.

Ranges g kg ⁻¹	Area (hectare)	Percentage%
5.50 - 3.50	1389.13	78.87
8.0 - 5.50	372.10	12.13
Sum	1761.23	100

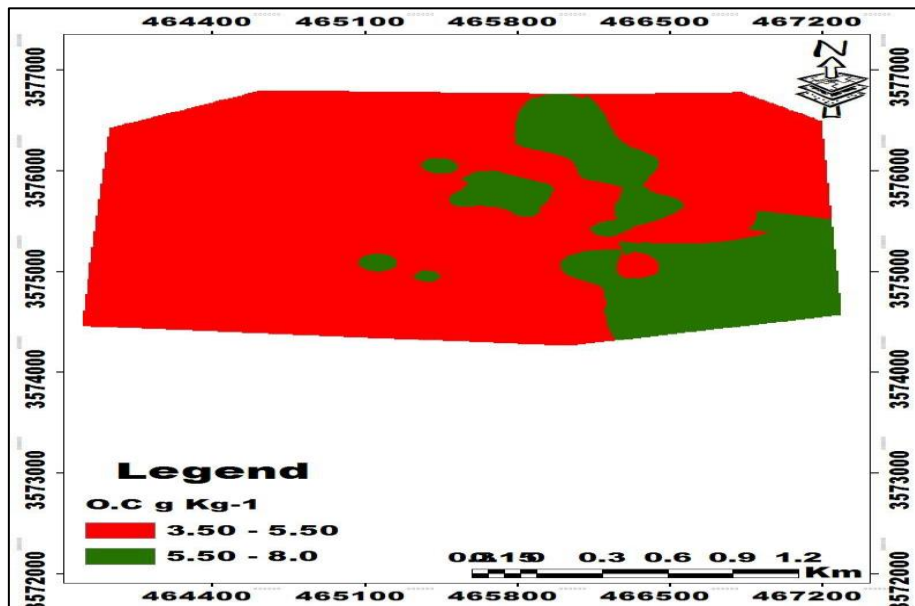


Figure 7. Spatial distribution of organic carbon content in the soils of the study area.

3.8. Cation Exchange Capacity CEC

The results of Table (1) and (2) showed that the values of the exchange capacity of positive ions in the soils of the study area ranged between 23.32-12.65 $\text{Cmol} \cdot \text{Kg}^{-1}$, as the lowest value was in the C3 horizon due to the low content of this horizon of organic matter and clay and its high content of carbonate minerals and the texture was classified as Sand Clay Loam, while the highest content was in location 15 of the drilling locations because these locations are surface soil locations and have a good organic matter content and clay content and its texture is classified as Silt Clay Loam and its content of carbonate minerals is relatively low.

The results of Table (10) and Figure (8) showed that the spatial distribution of the exchange capacity in the study area was distributed within the range 16-20 $\text{Cmol} \cdot \text{Kg}^{-1}$, which occupied the largest area of 1167.08 hectares and a percentage of 66.27%, and the range 20-24 $\text{Cmol} \cdot \text{Kg}^{-1}$, which occupied the smallest area of 594.15 hectares and a percentage of 33.73% of the total area of the study area. It is noted from the results that the values of the exchange capacity of positive ions are related to the content of clay and organic matter in the soil, as their values increase with the increase in the content of clay and organic matter, which was confirmed by [14,15,16] As for the suitability for growing potatoes, it was of the S1 class, very suitable and suitable, as it was given an estimate ranging between 94.15-85.46, so the exchange capacity of positive ions does not constitute a determining factor for growing potatoes.

Table 10. Area and percentage of positive ion exchange capacity in the study area.

Ranges $\text{Cmol} \cdot \text{Kg}^{-1}$	Area (hectare)	Percentage%
20-16	1167.08	66.27
24-20	594.15	33.73
Sum	1761.23	100

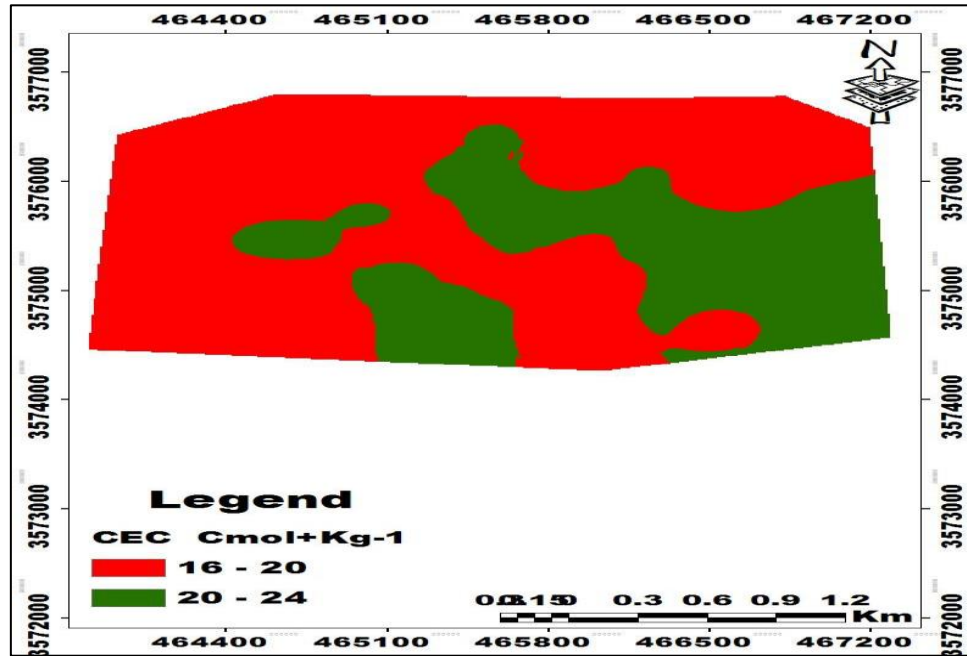


Figure 9. Spatial distribution of ion exchange capacity in the soils of the study area.

Conclusions

Some chemical properties were a determining factor in potato cultivation, including electrical conductivity and organic carbon.

References

- [1] AL-Taey, D. K. A., S. S. M. AL-Azawi, M. J. H. AL-Shareefi, and A. R. AL-Tawaha. (2018). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (*Lactuca sativa* var. Parris Island) Research on Crops.19 (3) : 441-449. DOI : 10.31830/2348-7542.2018.0001.14
- [2] Abbas A.K. , H. A. Mohammed., D.K. A. Al-Taey., R. A. Al-Khafajy., S. S. Mahmood and M. F. Alsaffar.2024. The Effect of NaCl Treatment on the Nutritional Balance of Wheat under Salt Stress Conditions. IOP Conf. Series: Earth and Environmental Science 1371 (2024) 052090. doi:10.1088/1755-1315/1371/5/052090.
- [3] Hamad , Abdul - Ghafour Ibrahim ,Amal Radhi Jubeir and Mohammed Sallal Oliwi (2021). SPATIAL DISTRIBUTION OF THE WESTERN JADWAL SOILS PROPERTIES AND SUITABILITY EVALUATION FOR WHEAT CROP CULTIVATION BY GEOMATICS TECHNOLOGY.Iraqi Juornal of Agricultural Sciences .vol52(3). <https://doi.org/10.36103/ijas.v52i3.1363..>
- [4] Sys C., Van Ranst E., and Deadeye J. (1991). Land evaluation, part II. Methods in land evaluation. Agriculture publications No.7, General Administration for Development Cooperation. Brussels, Belgium pp. 70-76.
- [5] Soil Science Division Staff .(2017). Soil survey manual. C. -8 Ditzler.k. Scheffe,and H.C.Monger(eds). USDA Handbook 18. GovermentPrinting Office. Washington. D.C.
- [6] Richards ,L.A (ed)(1954) . Diagnosis and improvement of saline and alkali Soils Handbook No.60.
- [7] Black , A. and Walkely. (1934) . An examination of the degtjare methods for determining soil orgaic matte rand aproposed modification of the chromic acid titration methods. Soil Science . 37:29- 38.

- [8] Jackson, M. L. (1958). Soil chemical analysis prentice Hall. 10.Inc., Englewood Cliffs, NJ, 498, 183-204
- [9] Al-Shammari, Hussein Ali Abdel and Jubier, Amal Radhi. (2024). Evaluating the Suitability of Soil in Al-Khairat District for Growing Forage Crop Alfalfa. IOP Conference Series Earth and Environmental Science 1371(5):052068. DOI:10.1088/1755-1315/1371/5/052068.
- [10] Al-Alwani, Omar Karim Obaid, (2010). Classification and evaluation of the suitability of some desert lands (35 km oasis) under irrigated agriculture conditions. Master's thesis - College of Agriculture - University of Anbar
- [11] Idan, Marwan Khaled and Oleiwi, Mohammed Sallal. (2024). Effect of Inoculation with Mycorrhizae, Phosphorus Levels, and Humic Acids on some Components of Wheat Yield. IOP Conference Series: Earth and Environmental Science., Volume 1371, Issue 5, id.052067, 10 pp. DOI: 10.1088/1755-1315/1371/5/052067
- [12] Al-Taie, Ahmed Samir. ((2013. Pedogenic distribution of iron, manganese, zinc and copper in soils (Aridisols) from northern Iraq. Master's thesis, College of Agriculture, University of Baghdad, Iraq
- [13] Jubier, Amal Radhi, Ail, Mustafa Thabit. (2023). The effect of agricultural exploitation and physiographic location on the soil particle size distribution for soils in Diyala Governorate. Bionatura. 2023, 8(4). <http://dx.doi.org/10.21931/RB/CSS/2023.08.04.23>.
- [14] Hepper, E.N., Buschiazzi, D.E., Hevia, G. G., Urioste, A., & Antón, L. (2006). Clay mineralogy, cation exchange capacity and specific surface area of loess soils with different volcanic ash contents. Geoderma, 135, 216-223.
- [15] Gaeid, Fatima Ibrahim., Oleiwi, Mohammed Sallal and Jubier, Amal Radhi (2023). The Effect of the Vertical Distribution of Nutrients for some Soils in Babylon Governorate. IOP Conference Series: Earth and Environmental Science., 2023, 1262(8), 082033. doi:10.1088/1755-1315/1262/8/082033.
- [16] Jaber, Zeina Kareem and Oleiwi, Mohammed Sallal. (2023). The Effect of Biofertilizers, Phosphorus Sources and Vermicompost on the Preparation of Bacterial and Fungal Cells for the Two Phases of Vegetative Growth and Flowering of Potato Plants. IOP Conference Series: Earth and Environmental Science, Volume 1262, Sustainability of Soil and Water Resources, 1262 082040. DOI 10.1088/1755-1315/1262/8/082040.

The Role of Organic Matter on Phenolic Compounds in Vegetable Plants

Hiba Miaad Abd Zaid Al-Fatlawi¹ and Mohammed Zedan Khalaf Al-Mharib²

^{1,2} Department of Horticulture and Landscape Engineering, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.

¹E-mail: heba.miad1105a@coagri.uobaghdad.edu.iq

²E-mail: mohammed.z@coagri.uobaghdad.edu.iq

Abstract. The quality characteristics of vegetable crops are enhanced and the phenolic compounds are increased by the addition of organic detritus. Phenolic compounds are characterized by an aromatic ring that contains one or more hydroxyl groups. They are secondary plant compounds that share the presence of one or more phenolic groups in their structural composition. Phenolics serve numerous functions in the process of growth. These compounds have been created as natural defense molecules in plants to combat stress, viral assaults, and fungal infections. They enhance the rigidity of the cell wall and the mechanical resistance of plants by increasing lignin. It contains a variety of compounds, flavors, and pigments that safeguard the plant from fungi, bacteria, and viruses. Flavonoids, flavanols, anthocyanins, and betacyanins are the most significant forms of phenols, which are divided into two categories: simple and multiple.

Keywords. Phenolic aromatic ring, Simple and multiple phenols, Anthocyanins and betacyanins.

1. Introduction

Organic fertilizers are a critical tool for preserving soil productivity, as they provide a variety of essential nutrients and micronutrients, and they also enhance soil preparedness. They contribute to the enhancement of the soil's physical and chemical characteristics. Additionally, they promote the activity of a variety of soil organisms, thereby increasing biological diversity. This, in turn, aids in the preservation of soil and the increase of production. In an effort to mitigate environmental pollution and the high financial costs associated with mineral fertilizers, countries worldwide have shifted to organic fertilization or reduced their use [1], from the other hand, organic fertilizers and pesticides are one of the pillars of sustainable agriculture. They are a comprehensive system that depends on managing and sustaining the natural system of the environment by not using materials manufactured from pesticides and chemical fertilizers and replacing them with what is natural and available in nature, such as animal and plant waste, as well as improving their characteristics. Physical soil such as permeability, porosity, air movement, root spread, and moisture retention Increasing the efficiency of water use [2,3], increasing the exchange capacity, and reducing the deposition of elements, which reflects positively on the characteristics of the soil and thus its effect on the plant, as it works to provide the plants with the nutritional elements it needs for its growth [4-7].

The use of chemical fertilizers may pose potential risks to the quality of the soil because they may cause environmental pollution and the death of organisms in the soil and contribute to global warming and depletion of the ozone layer. Therefore, organic fertilization is the best alternative for farmers from an economic and environmental perspective to develop sustainable agriculture [8,9]. [10] The additional organic matter in the soil enhances enzymatic activity by introducing materials that microorganism enzymes decompose and preparing basic materials for chemical reactions that produce enzymes. This is evident in the activity and vitality of the soil. the plant and subsequently increase the nutrients within the plant [11-13]. One environmentally responsible method for recycling waste and boosting crop output is to make use of organic wastes as bio stimulants for the creation of secondary chemicals[14,15].

Because these compounds increase the resistance of the cell wall and the mechanical resistance of plants, the addition of organic waste results in an increase in the amount of phenolic compounds, which are considered to be natural defense molecules in environmental conditions, poor transportation and storage conditions, damage caused by insects and pathogens, and stresses caused by metals, ultraviolet radiation, and pesticides. [16,17], we say. Included in this are waste products from fruits and vegetables as well as their origins. The incorporation of bioactive compounds that include antioxidant and antibacterial characteristics results in an improvement in quality for the reason that these compounds contain a high phenolic content. It is [18]. Increasing the amount of thiocyanate (S–CN), isothiocyanate (N=C=S), and amine (N–H) was the consequence of the addition of animal and plant wastes, which were represented by potato peels, banana peels, orange peels, egg shells, cow bones, chicken bones, and fish bones [19] The article aims to increase antioxidant phenols in vegetable plants by adding organic matter .

2. Phenolic Compounds

Plant secondary metabolites are biologically active natural products with therapeutic and nutritional value and have tremendous health benefits against various diseases. These substances may include vitamins, minerals, fiber, essential fatty acids, essential amino acids, and phytochemicals such as terpenoids, nitrogen-containing compounds, and phenolic compounds [20].

The term phenolic compounds includes a wide range of secondary plant compounds that have an aromatic ring in their structural structure. Phenolic compounds are plant chemicals that have an aromatic benzene ring, meaning that phenol is a hexagonal ring bearing one or more hydroxyl groups. Phenols are characterized by their hydrophilic property and tend to be dissolved in water with one hydroxyl group, which is secreted by plants in response to biological stress. In addition to this, the health role of phenols and their importance in the productivity and quality of plants, and they have a set of biological properties that also affect the health of farmers. Phenolic compounds are considered promising secondary metabolic compounds in eliminating the causes and effects of various diseases such as skin aging, wound healing, burns, and life-threatening diseases such as cancer [21].

3. General Properties of Phenols

- Phenols have the ability to form complex compounds with protein through hydrogen bonds between the phenolic OH and the OH or N of proteins H - bond Intermolecular, and this is the basis of the tanning process [22].
- Phenols are very sensitive to oxidation enzymes, especially the phenolase enzyme, as phenol is quickly oxidized to a polymer, as in the case of discoloration of apples when cut in half after a short period. Therefore, attention must be paid to this point when

- extracting phenols, as they must be treated with ethanol or alcohol. Boiling methanol to prevent enzymatic oxidation and this method should be performed routinely [23,24].
- Phenols form coordination bonds (chelation) with iron and chromium, so ferric chloride is used to detect phenols [25].
 - Phenolic compounds are a large group of secondary plant compounds (more than 30,000 compounds) that have multiple functions in the growth, development and defense of plants and include signaling molecules, pigments and flavors that work to attract or repel insects and produce many compounds that protect the plant against fungi and bacteria. And pathogenic viruses [26,27]. In addition to the importance of flavonoids and flavonoids' anti-cancer activity [28,29].
 - It is usually found in the vacuoles of cells combined with sugars in the form of glycosides. The latter is a union between a sugar molecule called a glycon, and it is usually glucose with another non-sugar molecule called an aglycon, which is a phenol, terpene, or alkaloid molecule [30].

Phenols are divided into two groups:

3.1. Simple Phenols

Simple phenols are an aromatic ring to which one or more hydroxyl (OH) groups are directly attached. The aromatic ring is a benzene (hexagonal) ring. Thus, phenol is similar in many ways to aliphatic alcohols, in which the hydroxyl group is attached to the carbon chain. Sometimes several different groups are associated with the phenolic compound, such as OH, COOH, and CH₃ [31]. They are also called aromatic compounds because of their distinctive smell, and they are also called closed cyclic compounds. The presence of the aromatic ring makes the hydrogen in the phenolic hydroxyl unstable, thus making the phenol a weak acid.

3.1.1. Locations of Simple Phenols

In high-end and low-end plants, such as ferns, mosses, seaweeds, and many microorganisms, most of them are not found free inside the plant cell, but are found bound to one or several molecules of lipopolysaccharides by a sugar ester bond with one of the OH or COOH groups to form glycolipids, which are stored in the vacuoles. of the cell [32]. Most amino acids, such as tryptophan, tyrosine, and phenylalanine, are classified as closed-cyclic organic phenolic compounds due to the similarity of the method of metabolism of these amino acids to phenolic compounds [33].

3.2. Polyphenols

3.2.1. Classification of Polyphenols

Flavonoids were divided on the basis of the association of alternative groups into 15 groups, the most important of which are: Flavone, Flavonol, Flavanone, Isoflavone, Chalcone, Aurone, Anthocyanin and Betacyanine [34,35]. These groups exist in a free form, known as aglycons, or linked in the form of glycosides, that is, their structure contains sugar units. The sugar can be mono- or disaccharide, and more than two mono-sugar units may be included in its structure. The sugar unit may be linked to an oxygen atom or an OH group, or directly linked to one of the atoms. Carbon aromatic ring. Most of the sugars that participate in the structural structure of flavonoids are: Glucose, Galactose, Arabinose, Rhamnose, and Xylose [36].

3.2.1.1. Flavone Group

The compounds of this group are characterized by the absence of any group linking to the middle ring in the common structural structure. One or more groups of OH, CH₃, or sugar groups are linked to the A and B rings, or one of them, with glycosidic bonds such as Apigenin, Chrysin, Luteolin, and Baicalin. The compounds of this group are similar to the Anthocyanine group, except the middle ring of its compounds is characterized by containing oxygen. They are pigments that cause the flowers to be yellow and are anti-inflammatory, anti-oxidant, and anti-bacterial [37].

3.2.1.2. Flavonol Group

Its compounds are characterized by the presence of an OH group in the position of carbon atom number 3 of the middle ring in the common structural structure. The common structural structure forms a basic nucleus for many compounds such as Quercetin, Campiferol, Myricetin, Galangin, and Fisetin. These compounds are found in green tea, broccoli, onions, and garlic [38,39].

3.2.1.3. Flavanone Group

Its compounds are characterized by the saturation of the 3-carbon atom in the middle ring in the common structural structure. An example is the compound Eriodictyol, Hesperitin, and Naringenin, which are found in citrus fruits, especially grapefruit.

3.2.1.4. Isoflavone Group

This group is closely related to the structural composition of the Flavone group of compounds, and differs in its structure from it only in the connection of the B ring to the position of carbon atom number 3 of the middle ring in the common structural structure. Among the compounds belonging to this group are Genistein, Daidzein, Glycitein, and Formononetin, which are frequently found in soybeans. Isoflavones are not widespread. In nature, unlike the flavones and flavonols groups that are widely spread, in general, flavonoids are found abundantly in seeds and work to increase the content of total protein and hemocyanin in the blood lymph, and alkaline phosphatase activity is significantly enhanced [40,41].

3.2.1.5. Chalcone Group

The compounds of this group are distinguished by containing only two rings, A and B. As for the central ring, it is in the common structural structure. The most important compounds of this group are Butein and Okanin [42].

3.2.1.6. Aurone Group

This group is characterized by a central ring that includes two carbon atoms and an oxygen atom, while the third carbon atom of the central ring remains outside to connect the two central rings, ring A on the one hand, and ring B on the other hand, in the common structural structure. The most important compounds of this group are Sulphuretin and Maritimetin.

3.2.1.7. Anthocyanin Group

The compounds of this group are distinguished by their central ring and the absence of an O₂ group on carbon atom number 4. Rather, there is an OH group in the position of carbon atom number 3 and two other hydroxyl groups in the position of carbon atom number 5 and 7 on ring A in the common structural structure. The compounds of this group are colored dyes. Red, purple, or blue, it is found in flowers, fruits, leaves, roots, and stems in the form of compounds dissolved

in The cellular juice of the epidermal cells in the vegetative parts of leafy plants. These pigments are not found in algae and gymnosperms. These pigments are linked to the sugar molecule by glycosidic bonds, and when the sugars are removed from them, the remaining part is called anthocyanidins. The anthocyanins differ among themselves in the groups linked to the B ring [43].

The true color of this group depends on the substituted groups present in ring B. When CH₃ groups are present, as in the Peonidin dye, the anthocyanins are colored red, while when other types of phenolic compounds are attached as substituted groups, the dye appears blue. The pH of the cell sap also affects the color of the anthocyanins, as they are red. It is purple in acidic media, while in basic media it is blue. It performs the function of attracting birds and insects to facilitate the pollination process. It also works to resist diseases because it has an antioxidant role [44,45].

3.2.1.8. Betacyanins Group

The compounds of this group are characterized by containing N in their structural structure, which are red pigments found in the roots of beets, beets, and red radishes. The most important type of these compounds that were isolated from the roots of beets and beets is the pigment Betanin, which is considered an antioxidant and increases with the addition of organic matter [46,47]. These compounds are not affected by the pH of the medium in which they are found, but they are similar to Anthocyanin and Flavones in their decomposition, producing sugar and a colored substance [48].

4. The Effect of Organic Matter on Phenolic Compounds in Vegetables

The demand for organic vegetables has increased all over the world, which has led to the growth of organic agriculture as a result of increased awareness and consumer interest in environmentally friendly organic agricultural products, their medicinal benefits, and their concentration of minerals and chemical compositions, most notably phenolic compounds, which are biologically active compounds that are most common in the diet, such as flavonoids and flavanones. And flavanols, isoflavones, anthocyanidins, and antioxidants [49].

Vegetables are a good source of food and due to their health benefits and nutritional content, vegetables are commonly available and have great application in the management of chronic diseases such as; Coronary heart disease, stroke, cancer, diabetes, and many medical purposes, controlling excess weight and obesity due to the high fiber content present. And many phenolic compounds; Catechin, rutin, naringin, naringenin, chlorogenic acid, caffeic acid, p-coumaric acid, sinapic acid and anthocyanins. Health-conscious consumers generally focus on antioxidant ability, phenolic content, vitamins and carotenoids Polyphenols with antioxidant activity depend on cultivar, stage of maturity and parts of the fruit. Conduct an experiment on eggplant plants A combination of organic waste, represented by sewage sludge, horse and chicken waste, vermicompost, and biochar, was added, and when eggplant fruits were analyzed, an increase in total phenols and vitamin C was found [50,51].

In a study conducted on watercress plants treated with cow manure, an increase in the content of phenolic compounds and antioxidants with medical efficacy was observed against some life-threatening diseases such as heart disease, Hardening of blood vessels , diabetes, high blood pressure, and obesity [52,53] . Organic tomatoes also showed the highest value of phenolic compounds, including flavonoids and antioxidants [54]. Adding organic matter from decomposed plant waste below 5°C for 49 days increased the broccoli plant's content of phenolic compounds and isothiocyanates [55]. Cruciferous family plants (cauliflower, broccoli, and broccoli) were also recorded when treated with organic matter resulting from waste. Plant remains of leaves and stems that contain high-value biomolecules Such as biologically active proteins and plant

metabolites, they contain the highest percentage of flavonoids, anthocyanins, carotenoids and tocopherols, which are powerful anti-inflammatory and anti-cancer agents [56]. Adding wild seaweed to carrots resulted in enhanced anti-inflammatory, antioxidant and health-promoting flavones [57].

The use of green manure and vermicompost to fertilize broccoli plants increased its content of total phenols [58]. A study was also conducted on broccoli plants that were treated with three levels of humic acid and garlic extract, which led to an increase in the content of total phenols and flavonoids in the leaves [59]. Adding humic acid and moringa leaf extract increased antioxidants in broccoli [60].

The use of two yeast strains, *Candida guilliermondii* and *Rhodotorula mucilaginosa*, improved the biosynthesis and accumulation of phenolic compounds in lettuce plants [61]. While treatment with yeast extract and algae extract showed an increase in total phenolic compounds and total flavonoids that have a medicinal effect as antioxidants against free radicals in watercress [62].

Treatment with Black Soldier fly waste showed the highest values of seedling height, stem diameter, number of leaves, length and width of the first real leaf, and the content of flavonoids in the Lahana plant increased [63].

A study on the addition of corn bran waste showed an effective importance in enhancing the phenolic content and antioxidant activity [64]. The effect of wastewater and biochar on phenols, flavonoids, and polyphenols in corn plants [65]. In an experiment conducted on the cabbage plant, treatment with organic fertilizers represented by biochar was superior compared to chemical fertilizers, as there was a significant difference in the products of photosynthesis and total phenols. [66].

Treatment with poultry and livestock waste increased the content of total phenols and antioxidants in ginger [67]. The use of organic fertilizers containing organic nitrogen had significant effects on the quality of tomato fruits, such as the concentrations of vitamin C, lycopene, phenols, flavonoids, sucrose, and fructose [68].

Seaweed containing 19 fatty acids successfully stimulated the synthesis of phenolic compounds along with defense enzymes peroxidase and polyphenol oxidase and enhanced the synthesis of defense enzymes and phenols in tomato plants [69]. The results of adding organic poultry waste to chili pepper plants showed a significant effect on flavonoids, phenols, and total carotenoids [70]. Results showed increased levels of flavonoids in onion plants when treated with seaweed extracts of humic and fulvic acids [71].

Treating broccoli plants with a combination of humic acid and moringa leaf extract led to an increase in the indicators of phenolic compounds (free and bound) and the stimulation of enzymes derived from phenols (catalase and peroxidase) that are highly effective against biotic and abiotic stresses [72]. Beetroot is grown for its high nutritional value and good flavour, and can often be processed in different ways. Adding poultry waste and vermicompost to red beet plants resulted in many compounds, such as ascorbic acid, carotenoids, phenolic acids and flavonoids, as well as high levels of betalain pigments, red betacyanins, red and purple and yellow betaxanthin [73].

The results showed that poultry waste and seaweed extract in watermelon plants from two organically grown cultivars (New Dragon and Golden Delight) increased antioxidants, lycopene content, and other physicochemical properties [74]. In this study, the addition of biological waste led to the generation of a large proportion of secondary compounds that are anti-inflammatory, anti-oxidant, heart disease-preventive, and anti-cancer, with an emphasis on the importance of incorporating watermelon into regular diets to improve the health of watermelon *Citrullus lanatus* [75].

Plants of the Cucurbitaceae family, especially watermelon *Citrullus lanatus* and watermelon *Cucumis melo*, provide significant amounts of mineral carotenoids and phenolic compounds,

which contribute to their antioxidant activity. In seeds that are usually discarded as plant waste. In the present study, seeds were separated, dried, ground, and extracted using ethanol to investigate total phenolic content (TPC), flavonoid content (TFC), carotenoid content (TC) content, and total antioxidant activity (TAA). In addition, the antimicrobial activity and response of organic pumpkin crop (*Cucurbita moscata* Duch) to the addition of Organic waste, such as compost, manure, and types of green manure, which was reflected in antioxidant levels [76,77].

Conclusions

The content of total phenols in vegetable crops increases as a result of the addition of a variety of organic waste. These phenols, which include flavonoids, flavanols, anthocyanins, and betacyanins, are crucial for the improvement of human health, the strengthening of the immune system, and the resistance to diseases. Additionally, they are important as natural defense molecules in plants against fungal infections, viral attacks, and stresses. These compounds are lignin, which is responsible for the mechanical resistance and rigidity of plant cell walls.

References

- [1] Shah, Z. H., H. M. Rehman., T. Akhtar., H. Assamadany., B. T. Hamooh., T. Mujtaba., I. Daur., Y. Al Zahrani ., H. A .S., Al Zahrani., S. Ali., S. H. Yang and G. Chung. 2018. H Substances Determining potential molecular regulatory processes in plants. *Frontiers in plant Science* 263(9):1-12 .
- [2] Ati A .S Alsahaf F. H ., and Zedan M. 2015. Effect of Irrigation Levels and Organic Matter on Water use Efficiency of Chilli Pepper. *International Journal of Engineering Research & Technology (IJERT)* , ISSN: 2278-0181.
- [3] Hasan A.A. , Aubied I.A. and D.K. A. Al-Taey. (2023). The Effect of Organic Amendments and Minerals on Increasing Soil Fertility (A review). *IOP Conf. Series: Earth and Environmental Science*. 1262 (2023) 042012. doi:10.1088/1755-1315/1262/4/042012
- [4] AL Katia F .H .A . AL 1 and Mohammed Z .K .ALMharib2 .(2020). Effect of addition of organic and chemical fertilization and spraying of alpha tocopherol on the growth and yield of tomato (*Plant Archives* 20 (1 :)163-168 e-ISSN:2581-6063(online), ISSN:0972-5210.
- [5] AL-Lamy H. A. Hussein1and Mohammed Z. K. AL-Mharib2. (2021). The Response of Cauliflower Growth and Yield to Organic and Chemical Fertilizers Application and Spraying With Salicylic Acid. *Annals of R.S.C.B.*, 25 (6 :)9483 - 9491<http://annalsofscb.ro>.
- [6] Al-Sahaf F. H. M. Z . AL-Mharib, and A.H. Mahmood(2018) .. Growth and production of three potato cultivars as affected by organic foliar nutrition .*Al-Kufa Journal*.
- [7] Al-Falahi, M. N. A., K. H. Al-Dulaimi. , E. T. A. Ghani , D.K. A. Al-Taey and K. J. Farhan.2022. EFFECT OF HUMIC ACIDS AND THE AMOUNT OF MINERAL FERTILIZER ON SOME CHARACTERISTICS OF SALINE SOIL, GROWTH AND YIELD OF BROCCOLI PLANT UNDER SALT STRESS CONDITIONS. *Agraarteadus*. 32 (1):11–20. <https://doi.org/10.15159/jas.22.24>
- [8] Chuan-Chuan, N., G, Peng-dong ., W, Bing-qing ., L, Wei-peng ., J, Ni-hao and C, Kun-zheng.(2018). Impacts of chemical fertilizer reduction and organic amendments supplementation on soil nutrient enzyme activity and heavy metal content. *Journal of Integrative Agriculture*. 16(8): 1819-1831.
- [9] Al-Taey DKA, Al-Janabi AH, and Rachid AM.2019. Role of Additive in Mitigation of the Negative Effects of Saline Water on Cabbage (*Brassica oleracea* var. *Capitata* L. *Plant Archives* Vol. 19,Supp 1 : 78-85
- [10] Ayilara, M. S ., O, S. Olanre., O, O. Babalola and O, Odeyemi .(2020). Waste management through composting: challenges and potentials. *Sustainability*: 1-23.
- [11] AL-Khazraji H. I, N. S. Abed¹, M. Ze.n AL-Mharib², S. AL-Darraji S. .(2018). Reef Amirich) in the population density of *Bemisia tabaci* (Genn.) and *Thrips tabaci* (L.) on cucumber .*Journal of*

- Biodiversity and Environmental Sciences (JBES) 12 : (2)11-18 ISSN: 2220-6663 (Print) 2222-3045 (OEffect of potassium fertilization and organic nutrient, <http://www.innspub.net>
- [12] Devi, C. and M. Khwairakpam . (2020). Bioconversion of Lantana camara by vermicomposting with two different earthworm species in monoculture. *Bioresource technology. Journal Pre-Proofs*, (296):122308.
- [13] Al-Dulaimy, A.F.Z., B. M. Abed , G.I. Abd and D. K. A. ALTaey.2024. Response of Gazania (Gazania splendens L.) Plants to Foliar Application with Bio Health and Salicylic Acid on some Growth and Flowering Traits. *IOP Conf. Series: Earth and Environmental Science* 1371 (2024) 042007. doi:10.1088/1755-1315/1371/4/042007
- [14] Zhang, F., Liu, Y., Liang, Y., Dai, Z., Zhao, Y., Shi, Y., ... & Ahammed, G. J. (2024). Improving the Yield and Quality of Tomato by Using Organic Fertilizer and Silicon Compared to Reducing Chemical Nitrogen Fertilization. *Agronomy*, 14(5), 966.
- [15] AL-Taey, D. K. A., S. S. M. AL-Azawi, M. J. H. AL-Shareefi, and A. R. AL-Tawaha. (2018). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (*Lactuca sativa* var. Parris Island) *Research on Crops*.19 (3) : 441-449. DOI : 10.31830/2348-7542.2018.0001.14
- [16] Khanday, A. H., Badroo, I. A., Wagay, N. A., & Rafiq, S. (2024). Role of Phenolic Compounds in Disease Resistance to Plants. In *Plant Phenolics in Biotic Stress Management* (pp. 455-479). Singapore: Springer Nature Singapore.
- [17] Xu, X., Bi, R., Song, M., Dong, Y., Jiao, Y., Wang, B., & Xiong, Z. (2024). Organic substitutions enhanced soil carbon stabilization and reduced carbon footprint in a vegetable farm. *Soil and Tillage Research*, 236, 105955.
- [18] Aqilah, N. M. N., Rovina, K., Felicia, W. X. L., & Vonnie, J. M. (2023). A review on the potential bioactive components in fruits and vegetable wastes as value-added products in the food industry. *Molecules*, 28(6), 2631.
- [19] Islam, M. R., Wang, Q., Guo, Y., Wang, W., Sharmin, S., & Ebere Enyoh, C. (2023). Physico-Chemical Characterization of Food Wastes for Potential Soil Application. *Processes*, 11(1), 250.
- [20] Bordoloi, J., Kalita, J., & Manna, P. (2024). Formulation and Challenges Involved in Developing Plant/Phytochemical-Based Nutraceuticals. In *Formulations, Regulations, and Challenges of Nutraceuticals* (pp. 29-53). Apple Academic Press.
- [21] Rathore, D., Naha, N., Singh, S. (2024). Phenolic Compounds and Nanotechnology: Application During Biotic Stress Management in Agricultural Sector and Occupational Health Impacts. In: Lone, R., Khan, S., Mohammed Al-Sadi, A. (eds) *Plant Phenolics in Biotic Stress Management*. Springer, Singapore. https://doi.org/10.1007/978-981-99-3334-1_21
- [22] Liu, C., Girard, A. L., Hartel, R. W., and Bolling, B. W. (2024). Improved analysis of grape seed extract by liquid chromatography–high resolution mass spectrometry (LC-HRMS) reveals that proanthocyanidin-protein interaction mechanisms in cream depend on degree of polymerization. *Food Chemistry*, 139432.
- [23] Blaschek, L., & Pesquet, E. (2021). Phenoloxidasases in plants—how structural diversity enables functional specificity. *Frontiers in plant science*, 12, 754601.
- [24] Kesharwani, V., Kabra, S., Semwal, B. C., & Saini, D. (2024). Neuroprotective Effects of Flavonoids. In *Advances in Flavonoids for Human Health and Prevention of Diseases* (pp. 95-123). Apple Academic Press.
- [25] Qiu, H., Ni, W., Yang, L., and Zhang, Q. (2022). Remarkable ability of Pb (II) capture from water by self-assembled metal-phenolic networks prepared with tannic acid and ferric ions. *Chemical Engineering Journal*, 450, 138161.
- [26] Ofoedum, A. F., Uyanwa, N. C., Chikelu, E. C., Iroagba, L. N., Ugwoezuonu, J. N., Anaeke, E. J. and M. U. (2024). Primary and Secondary Metabolites as Products of Microbial Metabolism: Uses and Application in Foods, Pharmaceutical and Allied Industries. A Review. *European Journal of Applied Science, Engineering and Technology*, 2(2), 4-16.

- [27] Shafiq, N. E., Mahdee, A. F., & Mohammed Hasan, Z. Y. (2024). Leaf Extracts of *Moringa oleifera* Cultivated in Baghdad: Characterization and Antimicrobial Potential against Endodontic Pathogens. *The Scientific World Journal*, 2024.
- [28] Salman, Z. O., Alwash, B. M., & Kadhim, E. J. (2024). Isolation and Identification of Flavonoid Compounds from *Euphorbia Milii* Plant Cultivated in Iraq and Evaluation of its Genetic Effects on Two Types of Cancer Cell Line. *Baghdad Science Journal*.
- [29] zuhair Abdul-lalil, T. (2024). Ultrasound-Assisted Extraction of Fennel Leaves: Process optimization, Thin Layer Chromatography and Cytotoxic Activity of Ethanolic Extract. *Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512)*, 33(1), 94-103.
- [30] Bartnik, M., & Facey, P. (2024). Glycosides. In *Pharmacognosy* (pp. 103-165). Academic Press.
- [31] Zhou, Y., Klinger, G. E., Hegg, E. L., Saffron, C. M., & Jackson, J. E. (2022). Skeletal Ni electrode-catalyzed CO cleavage of diaryl ethers entails direct elimination via benzyne intermediates. *Nature Communications*, 13(1), 2050.
- [32] Sadeghi, A., Rajabiyan, A., Nabizade, N., Meygolinezhad, N., & Ahmady, A. Z. (2024). Seaweed-derived phenolic compounds as diverse bioactive molecules: A review on identification, application, extraction and purification strategies. *International Journal of Biological Macromolecules*, 131147.
- [33] Clifford, M. N., Ludwig, I. A., Pereira-Caro, G., Zeraik, L., Borges, G., Almutairi, T. M., ... & Crozier, A. (2024). Exploring and disentangling the production of potentially bioactive phenolic catabolites from dietary (poly) phenols, phenylalanine, tyrosine and catecholamines. *Redox Biology*, 103068.
- [34] Gogoi, M. (2021). *Exotic Natural Dye of North East India* (Vol. 1). Sarv Bhasha Trust.
- [35] Pathak, D., & Mazumder, A. (2024). Potential of Flavonoids as Promising Phytotherapeutic Agents to Combat Multidrug-Resistant Infections. *Current Pharmaceutical Biotechnology*.
- [36] Bartnik, M., & Facey, P. (2024). Glycosides. In *Pharmacognosy* (pp. 103-165). Academic Press.
- [37] Chagas, M. D. S. S., Behrens, M. D., Moragas-Tellis, C. J., Penedo, G. X. M., Silva, A. R., & Gonçalves-de-Albuquerque, C. F. (2022). Flavonols and Flavones as Potential anti-Inflammatory, Antioxidant, and Antibacterial Compounds. *Oxidative medicine and cellular longevity*, 2022, 9966750. <https://doi.org/10.1155/2022/9966750>
- [38] Oh, W. Y., Ambigaipalan, P., & Shahidi, F. (2021). Quercetin and its ester derivatives inhibit oxidation of food, LDL and DNA. *Food chemistry*, 364, 130394.
- [39] Guan, Q., Tang, L., Xu, M., Zhang, L., Huang, L., & Khan, M. S. (2024). Comparison of binding sites and affinity of flavonol–Cu (II) complexes with the same parent nucleus: Synthesis, DFT prediction, and coordination pattern. *Food Chemistry*, 442, 138453.
- [40] Messina, M., Messina, V., & Nagata, C. (2024). Perspective: Observational studies involving low soy intake populations have limited ability for providing insight into the health effects of soybean isoflavones. *Advances in Nutrition*, 100210.
- [41] He, L., Wang, H., Li, E., Huang, Q., Wang, X., Qiao, F. & Chen, L. (2024). Effects of soy isoflavones on growth performance, antioxidant capacity, non-specific immunity and lipid metabolism of juvenile Chinese mitten crab, *Eriocheir sinensis*. *Aquaculture*, 581, 740470.
- [42] Abid, I., Moslah, W., Cojean, S., Imbert, N., Loiseau, P. M., Chamayou, A., & Baltas, M. (2024). The Synthesis of 2'-Hydroxychalcones under Ball Mill Conditions and Their Biological Activities. *Molecules*, 29(8), 1819. <https://doi.org/10.3390/molecules29081819>
- [43] Alappat, B., and Alappat, J. (2020). Anthocyanin pigments: Beyond aesthetics. *Molecules*, 25(23), 5500.
- [44] Dudek, A., Spiegel, M., Strugała-Danak, P., & Gabrielska, J. (2022). Analytical and theoretical studies of antioxidant properties of chosen anthocyanins; a structure-dependent relationships. *International Journal of Molecular Sciences*, 23(10), 5432.
- [45] Omer, G. A., & Mohammed, L. Y. (2024). Total Phenolic, Flavonoids and Vitamin C Contents with Antioxidant Activity of *Urtica dioica* L. Leaves Growing in Zakho, Kurdistan Region-Iraq. *Baghdad Science Journal*, 21(5), 1592-1592.

- [46] Sadowska-Bartos, I., & Bartosz, G. (2021). Biological Properties and Applications of Betalains. *Molecules* (Basel, Switzerland), 26(9), 2520. <https://doi.org/10.3390/molecules26092520>
- [47] Yasaminshirazi, K., Hartung, J., Fleck, M., & Graeff-Hoenninger, S. (2020). Bioactive compounds and total sugar contents of different open-pollinated beetroot genotypes grown organically. *Molecules*, 25(21), 4884.
- [48] Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & nutrition research*, 61(1), 1361779. <https://doi.org/10.1080/16546628.2017.1361779>
- [49] Hurkul, M. M., Phenolic Cetinkaya, A., Kaya, S. I., Yayla, S., & Ozkan, S. A. (2024). Investigation of Health Effects of Major Compounds in Foods: Extraction Processes, Analytical Approaches and Applications. *Critical Reviews in Analytical Chemistry*, 1-35.
- [50] Antonious, G. F., Turley, E., Mishra, B., Heist, Q., Upadhyaya, Y., Trivette, T., & Nkuwi, L. (2020). Characterization of eggplant grown in animal manure amended soil. *International journal of environmental health research*, 30(5), 492-503.
- [51] Mansurat, A. I., Dandago, M. A., & Diya'udeen, H. B. (2023) Phytochemical Contents and Antioxidant potentials of Eggplants from Kano State, Nigeria: *Journal / Dutse Journal of Pure and Applied Sciences* / Vol. 9 No. 1a
- [52] Hata, F. T., da Silva, D. C., Yassunaka Hata, N. N., Pavinatto, M. D. S., de Queiroz Cancian, M. A., Macedo, R. B., ... & Spinosa, W. A. (2024). Using Bokashi and Cow Urine as Organic Low-Cost Amendments Can Enhance Arugula (*Eruca sativa* L.) Agronomic Traits but Not Always Total Polyphenols and Antioxidant Activity. *Horticulturae*, 10(2), 155.
- [53] Daphedar, A. B., Khan, S., Kakkalamel, S., & Taranath, T. C. (2024). Plant Phenolics Compounds and Stress Management: A Review. *Plant Phenolics in Biotic Stress Management*, 481-502
- [54] de Lima, D. P., Júnior, E. D. S. P., de Menezes, A. V., de Souza, D. A., de São José, V. P. B., da Silva, B. P., ... & de Carvalho, I. M. M. (2024). Chemical composition, minerals concentration, total phenolic compounds, flavonoids content and antioxidant capacity in organic and conventional vegetables. *Food Research International*, 175, 113684.
- [55] Salas-Millán, J. Á., Conesa-Bueno, A., & Aguayo, E. (2024). A novel antidiabetic lactofermented beverage from agro-industrial waste (broccoli leaves): Process optimisation, phytochemical characterisation, and shelf-life through thermal treatment and high hydrostatic pressure. *Food Bioscience*, 103999.
- [56] Shinali, T. S., Zhang, Y., Altaf, M., Nsabiyeze, A., Han, Z., Shi, S., & Shang, N. (2024). The Valorization of Wastes and Byproducts from Cruciferous Vegetables: A Review on the Potential Utilization of Cabbage, Cauliflower, and Broccoli Byproducts. *Foods*, 13(8), 1163.
- [57] Szczepanek, M., Pobereżny, J., Wszelaczyńska, E., & Gościnną, K. (2020). Effect of biostimulants and storage on discoloration potential of carrot. *Agronomy*, 10(12), 1894.
- [58] Tascı, F. G., & Kuzucu, C. O. (2023). The effects of vermicompost and green manure use on yield and economic factors in broccoli. *Horticulturae*, 9(3), 406.
- [59] Hilo, B. R., Ali, B. A. A., & Jasim, H. M. (2023, July). Effect of Foliar Fertilization with Humic Acid and Garlic Extract on Growth and Yield of Broccoli and its Content of some Secondary Metabolites. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1213, No. 1, p. 012020). IOP Publishing.
- [60] Kibar, H., Kibar, B., & Turfan, N. (2024). Exogenous citric acid, salicylic acid, and putrescine treatments preserve the postharvest quality and physicochemical properties of broccoli (*Brassica oleracea* L. var. *italica*) during cold storage. *Food Science & Nutrition*, 12(3), 1686-1705.
- [61] Berrios, D., Nahuelcura, J., González, F., Peña, F., Cornejo, P., Pérez-Navarro, J., ... & Ruiz, A. (2024). The Biosynthesis, Accumulation of Phenolic Compounds and Antioxidant Response in *Lactuca sativa* L. Plants Inoculated with a Biofertilizer Based on Soil Yeast and Iron Nanoparticles. *Plants*, 13(3), 388.

- [62] . Al-Mousawi, Z. J., Salloom, Y. F., & Abdul-Qader, Z. M. (2024). Evaluation of foliar spray with extract of marine algae and yeast and mowing date on growth, yield, and active components of watercress. *iraq journal of agricultural sciences*, 55(1), 459-469
- [63] González-Lara, H., Parra-Pacheco, B., Gudiño-Calixto, A., Feregrino-Perez, A. A., & García-Trejo, J. F. (2024). Effect of Using Black Soldier Fly Larvae Thermocomposted Frass as a Germination Substrate on Phytotoxicity, Germination Index, Growth, and Antioxidants Content in Kale (*Brassica oleracea*).
- [64] Akbari, M., Gómez-Urios, C., Razavi, S. H., Khodaiyan, F., Blesa, J., & Esteve, M. J. (2024). Optimization of solid-state fermentation conditions to improve phenolic content in corn bran, followed by extraction of bioactive compounds using natural deep eutectic solvents. *Innovative Food Science & Emerging Technologies*, 103621.
- [65] Shirzad, H., Siavash Moghaddam, S., Rahimi, A., Rezapour, S., Xiao, J., & Popović-Djordjević, J. (2024). Combined Effect of Biological and Organic Fertilizers on Agrobiological Traits of Corn (*Zea mays* L.) under Wastewater Irrigation. *Plants*, 13(10), 1331.
- [66] Wang, J., Zhai, B., Shi, D., Chen, A., & Liu, C. (2024). How Does Bio-Organic Fertilizer Combined with Biochar Affect Chinese Small Cabbage's Growth and Quality on Newly Reclaimed Land?. *Plants*, 13(5), 598.
- [67] Poozesh, V., & Amirahmadi, A. (2024). The Impact of Organic and Inorganic Fertilizers on Some Phytochemical and Antioxidant Properties in Zingiber Officinale Rosc. *Journal of Chemical Health Risks*.
- [68] Zhang, X., Yin, J., Ma, Y., Peng, Y., Fenton, O., Wang, W., ... & Chen, Q. (2024). Unlocking the potential of biostimulants derived from organic waste and by-product sources: Improving plant growth and tolerance to abiotic stresses in agriculture. *Environmental Technology & Innovation*, 103571.
- [69] Chanthini, K. M. P., Deva-Andrews, A., Kirupaanntha-Rajan, P., Almutairi, B. O., & Senthil-Nathan, S. (2024). Systemic defense induced by fatty acid compounds from marine macroalgae, *Chaetomorpha antennina* in tomato (*Solanum lycopersicum*) plants alters the susceptibility of the polyphagous agricultural pest, *Spodoptera litura* Fab. *Chemical and Biological Technologies in Agriculture*, 11(1), 1-17.
- [70] Kusumiyati, K., Ramadhani, R., Maulana, Y. E., & Hamdani, J. S. (2024). Effect of Poultry Manure and Ethephon on Growth, Yield, and Quality of Cayenne Pepper (*Capsicum frutescens* L.) 'Ratuni UNPAD'. *AGRIVITA Journal of Agricultural Science*, 46(1).
- [71] Vojnović, Đ., Maksimović, I., Tepić Horecki, A., Milić, A., Šumić, Z., Žunić, D., ... & Ilin, Ž. (2024). Biostimulants Improve Bulb Yield, Concomitantly Affecting the Total Phenolics, Flavonoids, and Antioxidant Capacity of Onion (*Allium cepa*). *Horticulturae*, 10(4), 391.
- [72] Radi, A. A., Abdel-Wahab, D. A., Hamada, A. M., & Farghaly, F. A. (2023). The Impact of Humic Acid and Moringa Treatments on Enhancing Arsenic Tolerance in Broccoli Plants: Modulation of Sulphur Components and Enzymatic Antioxidant Defense. *Journal of Soil Science and Plant Nutrition*, 23(4), 6721-6737.
- [73] Kondal, P., Kaur, R., Singh, N., Maurya, V., Sharma, A., & Kumar, R. (2024) Effect of organic and inorganic fertilizers on the growth, yield and quality of beetroot *International Journal of Research in Agronomy* 7(2): 180-186 (*Beta vulgaris* L.). DOI: <https://doi.org/10.33545/2618060X.2024.v7.i2c.305>
- [74] Dalorima, T., Shehu, I. B., Bunu, A., & Kyari, Z. Towards a (2023). Sustainable Fertilization: The Physiochemical Properties of Red and Yellow-Fleshed Cultivars of Watermelon Fruits in the Sudan Savanna Zone of Nigeria. *International Academic Journal of Agriculture & Agribusiness Research* ISSN: 5280-5269 Volume 8, Issue 2 P :01- 16 February, DOI: 222394283425227821
- [75] Meghwar, P., Saeed, S. M. G., Ullah, A., Nikolakakis, E., Panagopoulou, E., Smaoui, S., & Tsoupras, A. (2023). Health Promoting Properties and Applications of Nu-Trients and Bioactives of Watermelon and Its By-Products. Preprints. <https://doi.org/10.20944/preprints202312.1677.v1>



- [76] Hussain, A., Akram, S., Siddique, T., Yaqub, S., Fatima, H., Arif, M. R., ... & Shehzad, A. (2023). Exploration of two Cucurbitaceae fruit (muskmelon and watermelon) seeds for presence of phytochemicals, and antioxidant and antimicrobial activities. *Turkish Journal of Agriculture-Food Science and Technology*, 11(s1), 2493-2498.
- [77] Castronuovo, D., De Feo, V., De Martino, L., Cardone, L., Sica, R., Caputo, L., ... & Candido, V. (2023). Yield Response and Antioxidant Activity of Greenhouse Organic Pumpkin (*Cucurbita moschata* Duch.) as Affected by Soil Solarization and Biofumigation. *Horticulturae*, 9(4), 427.

Performance Evaluation of the Varieties of *Sorghum Bicolor* L. Moench and the Estimation of Some Genetic Indicators Based on the Effect of NPK Nanofertilizer

Mohammed Natiq Ismail Al-Shindah¹ and Wael Mustafa Jassim²

^{1,2}Department of Field Crops, College of Agriculture, Tikrit University, Tikrit, Iraq.

¹E-mail: mnatiq67@gmail.com

²E-mail: Wael.mustafa@tu.edu.iq

Abstract. A field experiment was conducted at the Field Crops Department Research Station affiliated to the College of Agriculture / Tikrit University for two spring and autumn seasons in 2022 and spring 2023 with the aim of evaluating the performance of seven cultivars of Sorghum (Alkhair, Rabih, Inqath, Giza, Sudani, Bohuth 70 and Liulo) and estimating some of their genetic indicators under the influence of four concentrations of NPK compound nanofertilizer (0, 100, 200, 300 ppm) according to a factorial experiment with a complete randomized block design and three replicates. The most important results were: 1- The concentration of nanofertilizer 200 ppm achieved the highest rate for the following traits: number of heads per plant, weight of 500 grains, grain yield per plant, total yield and biological yield, while the concentration of 100 ppm significantly outperformed the number of grains per head and yield efficiency in the two years of the study. The efficiency trait did not differ Fertilization was significant between concentrations of 100, 200, 300 ppm. 2- Plants of the (Giza) cultivar recorded the highest rate for the following traits: weight of 500 grains, individual and total grain yield, and biological yield, while plants of the (Liulo) cultivar excelled in the traits of number of heads per plant, number of grains per head, and fertilization efficiency, while plants of the Rabih and Bohuth 70 cultivars excelled in yield efficiency in the two years of the study, and plants of the Rabih cultivar excelled in leaf area. 3- The interaction between the two study factors had a significant effect on the studied traits, where the interaction between Giza plants and the concentration of 100ppm recorded the highest rate of 500-grain weight and crop growth rate, while the Giza cultivar with the concentration of 200ppm gave the highest rate of biological yield, while the combination of the Liulo cultivar with the concentration of 100ppm was distinguished by giving the highest traits of individual and total grain yield and fertilization efficiency.

Keywords. Sorghum, Cultivars , NPK compound nanofertilizer.

1. Introduction

Sorghum (*Sorghum bicolor* L. Moench) is considered one of the important cereal crops, as it ranks fifth in the world after wheat, rice, yellow corn and barley in terms of cultivated area and production [1]. It is a C4 plant and is characterized by anatomical and morphological traits that make it one of the most important cereal crops that can be successfully cultivated in large areas of

the world due to its ability to withstand heat and lack of water, as well as its high physiological efficiency in producing dry matter [2]. The importance of the crop lies in its use for multiple purposes, including the use of its grains as food for humans in the manufacture of bread and pastries that are widely popular, and in the manufacture of high-protein biscuits after fortifying them with wheat flour. As for its nutritional importance for animals, its grains are used as a basic and important material in the manufacture and preparation of concentrated feed for poultry due to the high percentage of protein in it, in addition to being provided as green fodder or silage for livestock. Sorghum grains are used as a raw material in the manufacture of oils, candles and dyes [3]. Sorghum has recently been considered one of the important sources and raw materials necessary for the production of biofuel by using starch, sugar and organic matter found in the plant. [4], and the area planted with this crop in Iraq is estimated at (7,058) dunums, with a productivity of (13,319 tons[5]. It is better and necessary to reduce the loss of nutrients in fertilization and work to increase crop productivity by adopting new methods with the help of nanotechnology and nanomaterials [6]. This is done by using alternative fertilizers to traditional fertilizers, which are environmentally friendly and very effective, called nano fertilizers. As a result of improving the efficiency of using nano-fertilizers, this led to the manufacture and development of nano-fertilizers, which can be more soluble, effective, and faster in penetrating and assimilation in plant tissues than regular fertilizers [7] The use of nano-fertilizer technology is witnessing widespread use in the agricultural sector due to its effective and positive impact in improving plant growth [8].

2. Materials and Methods

2.1. Experiment Location and Soil Analysis

A field experiment was conducted at the Field Crops Department Research Station at the College of Agriculture / Tikrit University, located north of Tikrit city in Salah al-Din Governorate, which is located at longitude 43° east and latitude 34° north. The experiment was conducted on three crops, the first crop was spring on 3/15/2022, the second crop was autumn on 7/1/2022, and the third crop was spring on 3/15/2023 with the aim of evaluating the performance of Sorghum cultivars and estimating some genetic indicators under the influence of the NPK compound nano fertilizer. Before starting cultivation, random samples were taken from the field soil at a depth of 30 cm to study the chemical and physical properties and were analyzed in the Soil and Water Department laboratory at the College of Agriculture, Tikrit University. The results were as follows:

Table 1. Analysis of the physical and chemical properties of the soil at the experimental location.

Traits	Values	Units
pH	7.17	PH
Electrical conductivity (EC)	2.22	ds/m ⁻¹
Available nitrogen (N)	48	mg.kg ⁻¹
Available phosphorus (P)	6.8	mg.kg ⁻¹
Available potassium (K)	93	mg.kg ⁻¹
Calcium carbonate (lime)	160	g.kg ⁻¹
Organic matter (O.M)	1.12	g.kg ⁻¹
Sand	%60	g.kg ⁻¹
Silt	%25	g.kg ⁻¹
Clay	%10	g.kg ⁻¹
Texture	Sandy loam	-----

2.2. Experimental Parameters

The experiment included (28) experimental units for each The result of the combination of the first factor represented by four concentrations of the nano-complex fertilizer (NPK) (0ppm, 100ppm, 200ppm, 300ppm) and the second factor represented by seven cultivars of Sorghum (Table 2).

Table 2. Cultivars used in the study.

Cultivar symbol	Cultivar name	Origin
V1	Alkhair	Iraqi cultivar
V2	Rabih	Introduced to Iraq in 1998 and improved by selection in the White Maize Development Project of the General Authority for Agricultural Research / Ministry of Agriculture
V3	inqath	Introduced from Sudan to Iraq in 2001 and improved by selection in the White Maize Development Project of the General Authority for Agricultural Research / Ministry of Agriculture
V4	Giza	Cultivar introduced to Iraq from Egypt
V5	sudani	Commercial cultivar imported from Sudan
V6	Bohuth 70	Introduced from Sudan and registered and approved in 2016 by the Iraqi Ministry of Agriculture as a high-yielding cultivar in green fodder
V7	Liulo	Iraqi cultivar

2.3. Agricultural Operations

The land was plowed and smoothed by disc harrows and the field was divided into three replicates and each replicate into four sectors according to the levels of the nano-compound fertilizer to be sprayed on the experimental plants, which are four levels (0, 100, 200, 300ppm). Each sector contains seven experimental units according to the number of cultivars included in the study. Each experimental unit contained 4 lines, the length of the line was 3 m and the distance between one hole and another was 20 cm. The distance between one line and another was 70 cm. Phosphate fertilizer was added in the form of triple superphosphate (45%P₂O₅) mixed with the soil in one batch before planting at a level of 100 kg P.ha⁻¹. As for nitrogen fertilizer, it was added at a level of 100 kg N.ha⁻¹ in the form of urea fertilizer (46%N) in two batches, the first after planting and the second after (40) days of planting [5] and the first spring crop was planted on 3/15/2022, and the second autumn crop on 7/1/2022, while the third spring crop was planted on 3/15/2023. After planting, the experiment's drip irrigation system was designed and connected to ensure that water reaches all holes equally. The experiment was lightly irrigated to avoid seed drift, and other irrigations were given as needed. After germination, the plants were thinned and one plant was kept, and the holes that failed to germinate were patched. Manual weeding of the field was conducted several times during the growing season as needed. The granular diazinon pesticide (10%) was used as an effective material to control the corn stalk borer (*Sesamia cretica*). It was added by feeding on the seedlings at a rate of 6 kg.ha⁻¹ in two stages, the first after 20 days of planting and the second after 20 days of the first control [8]. The experimental plants were sprayed foliarly with nano fertilizer at a rate of two sprays and at four levels in each spray according to the study plan, where the first spray was after (40) days of planting and the second after (50) days of the first spray. The spray levels were (0, 100, 200, 300ppm). The spraying process was conducted using a 10-liter backpack sprayer. The plants of control treatment were sprayed with distilled water only, while the plants of the second treatment were added 100, 200 and 300ppm of the compound nano fertilizer after dissolving each of them in 10 liters of distilled water, and the Zahi substance was added as a spreading material for all

levels of the nano fertilizer included in the experiment. The compound nano fertilizer (NPK 20:20:20) was obtained from the Iranian company (Sodour Ahrar Shargh), and the first crop was harvested on 8/30/2022 and the second crop on 11/15/2022, while the third crop was harvested on 9/1/2023.

2.4. Studied Traits

The following traits were studied by taking random samples consisting of ten plants recorded as an average randomly determined from the two middle lines of each experimental unit to measure each trait except for flowering, which was taken from the top of the experimental unit's base. The studied traits, the method of calculating them and the unit of measurement are:

2.4.1. Number of Heads in the Plant (plant head⁻¹)

The number of heads in each plant of the sample was calculated and the trait rate was found.

2.4.2. Number of Grains in the Head (grain. head⁻¹)

The number of grains was manually calculated in each plant of the random sample after ripening and then its average was taken.

2.4.3. Weight of 500 Grains (g)

500 grains were taken from the sample grains of ten plants and then weighed on a sensitive scale and their average was calculated.

2.4.4. Individual Plant Yield (gm. plant⁻¹)

The weight of the grains of ten plants was taken for each experimental unit and the average was extracted for them.

2.4.5. Total Grain Yield (ton ha⁻¹)

The weight of the grains of the plants was taken from the two middle lines of each experimental unit and the area they occupy was determined and then the result was converted to (ton ha⁻¹).

2.4.6. Biological Yield (gm. plant⁻¹)

Each plant was weighed completely from the three middle lines with the heads for each experimental unit at the stage of full maturity and dried in an electric oven at a temperature of (65 C) for 48 hours as a first stage, and then dried at a temperature of (105 C) for 3 hours until its weight was fixed [10].

2.5. Statistical Analysis

The statistical analysis was conducted for each trait according to the randomized complete block design (R.C.B.D) with three replicates according to a factorial experiment, and the means of each trait were compared using Duncan's multiple range test.

3. Results and Discussion

3.1. Analysis of Variance

The results of the analysis of variance in appendices (4,3,2) represented by the mean squares for the spring and autumn crops in 2022 and the spring crop in 2023 showed a significant effect at the level (1%) of spraying with nano-compound fertilizer and cultivars and the interaction between them on all the studied traits.

3.1.1. Number of Heads Per Plant (plant head⁻¹)

The plants that were treated with a concentration of 300 ppm of nano-compound fertilizer gave the highest rates for the trait number of heads per plant for the years 2022 and 2023 and reached (1.45 and 1.52, 1.44 heads. plant⁻¹) and did not differ significantly from the trait rates at a concentration of 200 ppm and control treatment was the lowest for the trait rates reaching (1.24 and 1.32 and 1.23 plant head⁻¹).

The Liulo cultivar recorded a significantly excelled on all cultivars with rates of (1.74, 1.82 and 1.73 head.plant⁻¹), while the Sudani cultivar had the lowest rates of the trait with rates of (1.08, 1.16 and 1.08 head.plant⁻¹). The reason for the variation of cultivars is attributed to environmental influences and the extent of response to them by genotypes, as well as the variation between genotypes in the ability to grow under the influence of environmental factors in a better way than the other genotype, which has a lower ability to do so. This is consistent with what [10,11,12]. The interaction between the Liulo cultivar and the nano-compound fertilizer at concentrations of 200 ppm and 300 ppm outperformed all other combinations with rates of (1.95, 2.04 and 1.94 head . plant⁻¹) at concentration 200 ppm (2.01, 2.10 and 2.00 head. plant⁻¹) at concentration 300 ppm. This is due to the excelled of the Liulo cultivar's response, which has a genetic nature that gave it a greater response to the two concentrations of nano-compound fertilizer, which had an effect in increasing the rate of the trait for that interaction, while the interaction between the Sudani cultivar and the nano-compound fertilizer at concentration 300 ppm was the lowest for the trait and recorded (1.01, 1.06 and 1.00 head. plant⁻¹) in the two years of the study, respectively, and did not differ significantly from some other interactions.

Table 3. The effect of spraying with nano-compound fertilizer, cultivars and the interaction between them on the number of heads in the plant (plant head⁻¹).

Spring session 2022						autumn session 2022						Spring session 2022					
Cultivars	Concentrations					Cultivars	Concentrations					Cultivars	Concentrations				
	0ppm	100 ppm	200 ppm	300 ppm	Average cultivars		0ppm	100 ppm	200 ppm	300 ppm	Average cultivars		0ppm	100 ppm	200 ppm	300 ppm	Average cultivars
Alkhair	1.34	1.06	1.09	1.21	1.11	Alkhair	1.40	1.15	1.18	1.26	1.25	Alkhair	1.33	1.05	1.08	1.20	1.17
	fg	k	jk	hi	d		fg	jk	jk	hi	d		fg	jk	jk	hi	d
inqath	1.53	1.37	1.71	1.41	1.51	inqath	1.60	1.40	1.78	1.53	1.58	inqath	1.52	1.33	1.69	1.41	1.51
	cd	fg	b	cde	b		cd	fg	b	cde	b		cd	fg	b	cde	b
Liulo	1.26	1.73	1.95	2.01	1.74	Liulo	1.32	1.81	2.04	2.10	1.82	Liulo	1.25	1.72	1.99	2.01	1.73
	gh	3	5	1	4		gh	1	4	0	82		gh	2	4	0	73
	i	b	a	a	a		i	b	a	a	a		i	b	a	a	a
Rabih	1.27	1.46	1.44	1.56	1.44	Rabih	1.33	1.53	1.51	1.63	1.50	Rabih	1.26	1.45	1.44	1.51	1.42
	gh	cde	de	c	c		gh	cde	def	c	c		gh	cde	def	c	c
Bohuth 70	1.04	1.09	1.29	1.16	1.11	Bohuth 70	1.15	1.11	1.35	1.23	1.23	Bohuth 70	1.03	1.08	1.28	1.11	1.14
	k	jk	gh	ij	d		k	jk	gh	ij	d		k	jk	gh	ij	d
Giza	1.23	1.71	1.41	1.76	1.53	Giza	1.29	1.79	1.46	1.84	1.59	Giza	1.22	1.70	1.39	1.71	1.52
	hi	b	ef	b	b		gh	b	ef	b	b		gh	b	ef	b	b
sudani	1.03	1.05	1.24	1.01	1.08	sudani	1.13	1.15	1.30	1.06	1.16	sudani	1.02	1.04	1.23	1.00	1.08
	k	k	hi	k	e		k	jk	ghi	k	e		k	k	ghi	k	e

average	1.2	1.3	1.4	1.4	average	1.	1.4	1.5	1.5	average	1.	1.3	1.4	1.4
Concentra	4	5	4	5	Concentra	32	3	2	2	Concentra	23	4	3	4
tions	c	b	a	a	tions	c	b	a	a	tions	c	b	a	a

3.1.2. Number of Grains in the Head (head grain⁻¹)

The results of Table (4) indicated that there were significant differences between the plants that were sprayed with the compound nano fertilizer, as the plants that were sprayed with a concentration of 100 ppm gave the highest rates for the trait and for the spring and autumn crops of 2022 and the spring crop of 2023, amounting to (3590.96, 3883.47 and 3689.30 head grain⁻¹), respectively, while control treatment showed the lowest rates for the trait, which were (3446.85, 3726.76 and 3541.25 head grain⁻¹), followed by the rates of the trait at concentrations of 200 and 300 ppm, which did not differ significantly between them. The reason for the increase in the number of grains in the head when sprayed with the compound nano fertilizer is likely that the fertilizer works to increase the efficiency of the photosynthesis process and thus increase its products, which provides a suitable opportunity to reduce the abortion of flowering by reducing the state of competition between them, as well as On the role of fertilizer in increasing pollen production as well as activating enzymes related to the production of nucleic acids in cells and protein metabolism, in addition to the role played by the components of the nano-complex fertilizer such as potassium in the process of hormonal balance, which positively affects the process of formation and growth of flowers [13]. This confirms what [14,15], have reached. Table (4) showed significant differences between the cultivars in the trait of the number of grains per head, as the Liulo cultivar gave the highest rates of all the cultivars included in the experiment, reaching (4065.47, 4395, and 4176.81 grains. head⁻¹), while the Sudani cultivar gave the lowest rates for the trait, reaching (2832.20, 3062.08, and 2909.76 grains. head⁻¹). The differences between the cultivars in the number of grains per head are due to the increase in the amount of nutrients available to the plant during the flowering period, which positively affected the storage of dry matter, which increases the flowering plants in the head at the expense of increasing the vegetative mass of the plant. This is consistent with what was mentioned by [10,12,16,17]. The interactions between the concentrations of nano-fertilizer and the cultivars recorded significant differences, as the plants of the Sudani cultivar that were sprayed with a concentration of 100 ppm recorded the lowest rate of the trait, reaching (2691.85, 2910.33 and 2765.57 grains head⁻¹) for the study years 2022 and 2023, while the interaction between the concentration of the compound nano-fertilizer NPK at a concentration of 100 ppm and the Liulo cultivar gave the highest rates of the trait, reaching (4264.07, 4610.33 and 4380.85 grains head⁻¹).

Table 4. The effect of spraying with compound nano-fertilizer and the cultivars and the interaction between them on the number of grains in the head (grain head⁻¹).

Spring session 2022							autumn session 2022							Spring session 2022										
Cultivars	Concentrations						Cultivars	Concentrations						Cultivars	Concentrations						Cultivars			
	0ppm	100	ppm	200	ppm	300		Average	0ppm	100	ppm	200	ppm		300	Average	0ppm	100	ppm	200		ppm	300	Average
	cultivars							cultivars								cultivars								cultivars
Alkhair	372					378		402					408		382						388			
	4.0	386	368	384			6.6	418	398	415		7.1	6.0	397	378	395	388							
	9	6.36	3.04	7.27	0.1	9	7	0.33	2.00	9.67	7	8	2.25	3.91	2.63	3.72								
	ef	cd	f	d	b		fg	cd	gh	de	b	ef	c	fg	cd	b								

	336				344		363			372		345					
	2.9	341	339	361	4.2		6.3	368	366	390	3.9	5.0	350	348	370	353	
inqath	6	0.00	3.55	0.37	2	inqath	3	6.67	9.00	3.67	2	6	3.39	6.49	9.25	8.55	
	jk	ij	ij	gh	e		jk	j	j	hi	e	ij	i	i	gh	d	
	368				406		398				439		378				
	1.5	426	418	412	5.4		0.3	461	452	446	5.5	2.3	438	430	424	417	
Liulo	4	4.07	7.41	8.85	7	Liulo	3	0.33	7.33	4.33	8	6	0.85	2.09	1.93	6.81	
	f	a	b	b	a		gh	a	b	b	a	fg	a	ab	b	a	
	324				349		351				378		333				
	8.9	377	340	355	6.6		2.6	407	368	384	0.5	7.9	387	350	365	359	
Rabih	7	2.33	8.00	7.24	4	Rabih	7	8.67	4.67	6.00	0	5	5.64	1.33	4.66	2.40	
	lm	e	ij	h	d		lm	ef	j	i	d	kl	de	i	h	c	
	392				374		423				404		402				
	0.9	393	366	344	1.5	Bohuth	9.3	425	396	372	5.4	8.3	403	377	353	384	
Bohuth	70	5	2.08	9.60	3.46	70	3	1.33	7.67	3.33	2	3	9.77	0.10	7.76	3.99	
	c	c	fg	i	c		cd	c	gh	j	c	c	c	fg	i	b	
	330				325		357				352		339				
	6.2	320	330	322	9.3		4.6	345	357	348	4.0	6.8	328	339	331	334	
Giza	5	0.00	7.06	4.24	9	Giza	7	9.67	5.67	6.00	0	0	7.64	7.63	2.54	8.65	
	kl	m	kl	m	f		kl	m	kl	lm	f	jk	l	jk	kl	e	
	288				283		311				306		296				
	3.2	269	296	278	2.2		7.3	291	320	301	2.0	2.1	276	304	286	290	
sudani	0	1.85	6.43	7.31	0	sudani	3	0.33	7.33	3.33	8	6	5.57	7.67	3.64	9.76	
	o	q	n	p	g		o	q	n	p	g	n	p	m	o	f	
	344						372					354					
averag	6.8	359	351	351		averag	6.7	388	380	379		1.2	368	361	361		
e		0.96	6.44	4.11		e		3.47	1.95	9.47		5	9.30	2.74	0.35		
Concen	5	a	b	b		Concen	6	a	b	b		c	a	b	b		
trations	c					trations	c										

3.1.3. Weight of 500 Grains (g)

Plants sprayed with a concentration of 200 ppm of NPK nano-compound fertilizer outperformed the rest of the spray levels included in the experiment, as they gave rates of (14.13, 15.28 and 14.52 g) Table (5), followed by the trait rates at a concentration of 100 ppm, while control treatment plants gave the lowest trait rates of (12.97, 14.03 and 13.33 g) for the study years 2022 and 2023, and this is due to the role of nano-compound fertilizer in raising the efficiency of photosynthesis and increasing the accumulation of manufactured materials, which increases the fullness of the grains and thus increases their weight. This result is consistent with what [18] reached.

The seven Sorghum cultivars included in the experiment differed significantly, as the Giza cultivar outperformed the rest of the cultivars included by giving the highest rates for the trait, reaching (16.72, 18.09 and 17.19 g) for the fall and spring 2022 and spring 2023 crops, while the Alkhair cultivar plants gave the lowest rates, reaching (11.06, 11.96 and 11.37 g). This may be due to the excelled in the leaf area trait, Table (5), which helped fill the grains with nutrients manufactured during the photosynthesis process, which has an effect on increasing their weight, as well as reducing the number of grains in the head, Table (5). This is consistent with what was

found by [19,10,12] . As for the interactions between the study factors, the interaction between the concentration of Nano fertilizer 100 ppm and 200 ppm with Giza cultivar gave the highest rates of the trait reaching (17.18, 18.59 and 17.66 g) and (17.16, 18.56 and 17.64 g) for the two concentrations respectively, while the lowest rates of the trait were for the interaction between control treatment and Alkhair cultivar at rates of (10.81, 11.69 and 11.11 g) for the years 2022 and 2023 respectively.

Table 5. The effect of spraying with compound nano fertilizer and cultivars and the interaction between them in the weight of 500 grains (g).

Spring session 2022						autumn session 2022						Spring session 2022					
Concentrations						Concentrations						Concentrations					
Cultivars	0ppm	100 ppm	200 ppm	300 ppm	Average cultivars	Cultivars	0ppm	100 ppm	200 ppm	300 ppm	average cultivars	Cultivars	0ppm	100 ppm	200 ppm	300 ppm	Average cultivars
Alkhair	10.81	11.05	11.36	11.01	11.06	Alkhair	11.69	11.95	12.29	11.91	11.96	Alkhair	11.11	11.36	11.68	11.32	11.37
	o	no	mn	no	f		o	no	mn	no	f		o	no	mn	no	f
inqath	13.52	14.26	15.38	13.74	14.23	inqath	14.63	15.43	16.64	14.86	15.39	inqath	13.90	14.66	15.81	14.12	14.62
	hi	g	d	h	c		ghi	f	d	g	c		ghi	f	d	g	c
Liulo	12.99	13.49	13.27	12.99	13.18	Liulo	14.05	14.59	14.36	14.05	14.26	Liulo	13.35	13.87	13.64	13.35	13.55
	jk	hi	hij	jk	d		ijk	ghi	ghi	ijk	d		ijk	ghi	g-j	ijk	d
Rabih	14.40	15.07	14.77	14.64	14.72	Rabih	15.58	16.30	15.98	15.84	15.92	Rabih	14.80	15.49	15.18	15.05	15.13
	fg	de	ef	efg	b		f	de	ef	ef	b		f	de	ef	ef	b
Bohuth 70	10.63	11.76	12.60	13.12	12.03	Bohuth 70	11.50	12.72	13.63	14.19	13.01	Bohuth 70	10.93	12.09	12.95	13.49	12.36
	o	m	kl	ij	e		o	m	kl	hij	k		o	m	kl	h-k	e
Giza	16.00	17.18	17.16	16.55	16.72	Giza	17.31	18.59	18.56	17.90	18.09	Giza	16.45	17.66	17.64	17.01	17.19
	c	a	a	b	a		c	a	a	b	a		c	a	a	b	a
sudani	12.42	13.57	14.35	12.82	13.29	sudani	13.44	14.68	15.52	13.87	14.38	sudani	12.77	13.95	14.75	13.18	13.66
	l	hi	fg	jkl	d		l	gh	f	jkl	d		l	gh	f	jkl	d
average Concentrations	12.97	13.77	14.13	13.55		average Concentrations	14.03	14.89	15.28	14.66		average Concentrations	13.33	14.15	14.52	13.93	
	d	b	a	c			d	b	a	c			d	b	a	c	

3.1.4. Individual Plant Yield ($g\ plant^{-1}$)

The results of Table (6) showed that the plants that were sprayed with nano-compound fertilizer at a concentration of 200 ppm recorded the highest rates of the trait, reaching (98.57, 106.59 and 101.29 $g\ plant^{-1}$) for the study years 2022 and 2023, respectively, with an increase of 12.5% over control treatment, which recorded the lowest rates of the trait, reaching (87.64, 94.77 and 90.06 $g\ plant^{-1}$). This excelled is attributed to the fact that the concentration of 200 ppm recorded the highest rate of 500-grain weight, Table (6), which is considered one of the important components of the yield and had a major impact in increasing the rate of the trait at the third concentration.

The results showed that the Sudani cultivar achieved the lowest rates of the trait in the spring and autumn seasons of 2022 and spring 2023, reaching (75.02, 81.13 and 77.09 $g\ Plant^{-1}$) While Giza plants excelled with the highest rates of (107.11, 115.83 and 110.07 $g\ plant^{-1}$) and did not differ significantly from the Liulo cultivar. The reason for the variation of the genetic composition

among them in this trait is attributed to the efficiency of the genetic compositions in benefiting from environmental factors, as well as the high fertilization rate at the appropriate temperature for them [11] and the increase in the weight of the grain, Table (6). Many researchers have shown the variation of cultivars and genetic compositions among them in the trait of the individual plant yield, including[12,20,21,22]. The results of Table (6) showed that the trait rates for the interaction between the two factors differed significantly, as the combination of 100 ppm nano fertilizer concentration with the Liulo cultivar recorded the highest trait rates, reaching (114.13, 123.42 and 117.28 g plant⁻¹), which is due to the excelled of that combination in the number of heads per plant and the number of grains per head, Tables (3 and 4), while control treatment with the Sudani cultivar recorded the lowest trait rates, reaching (87.64, 94.77 and 90.06 g plant⁻¹).

Table 6. Effect of spraying with compound nano fertilizer and cultivars and their interaction on individual plant yield (g plant⁻¹).

Spring session 2022						autumn session 2022						Spring session 2022					
cultivars	Concentrations				average cultivars	cultivars	Concentrations				average cultivars	cultivars	Concentrations				average cultivars
	0ppm	100ppm	200ppm	300ppm			0ppm	100ppm	200ppm	300ppm			0ppm	100ppm	200ppm	300ppm	
Alkhair	80.1	84.jk	90.hi	82.kl	84.e	Alkhair	87.l	90.k	97.ij	89.kl	91.e	Alkhair	82.l	86.k	92.ij	84.kl	86.e
inqath	89.80	94.89	101.17	100.09	96.49	inqath	97.11	102.61	109.41	108.24	104.34	inqath	92.28	97.51	103.96	102.85	99.15
Liulo	94.72	114.13	111.06	105.35	106.32	Liulo	102.43	123.42	120.10	113.93	114.97	Liulo	97.34	117.28	114.13	108.26	109.25
Rabih	93.22	110.17	101.24	104.72	102.34	Rabih	100.81	119.14	109.48	113.24	110.67	Rabih	95.79	113.21	104.04	107.61	105.16
Bohuth 70	80.34	92.37	89.71	88.53	87.74	Bohuth 70	86.88	99.89	97.01	95.74	94.88	Bohuth 70	82.56	94.92	92.19	90.97	90.16
Giza	103.80	107.80	111.44	105.40	107.11	Giza	112.25	116.57	120.51	113.98	115.83	Giza	106.67	110.78	114.52	108.31	110.07
sudani	71.08	71.68	85.03	72.30	75.02	sudani	76.87	77.51	91.95	78.19	81.13	sudani	73.04	73.66	87.38	74.30	77.09
average Concentrations	87.64	96.44	98.57	94.15		average Concentrations	94.77	104.29	106.59	101.81		average Concentrations	90.06	99.11	101.29	96.75	

3.1.5. Grain Yield (ton ha⁻¹)

The treatment of spraying with nano-compound fertilizer at a concentration of 100ppm and 200ppm on Sorghum plants recorded the highest rates of grain yield for the study years 2022 and 2023, as their rates reached (5.82, 6.30 and 5.99 tons ha⁻¹) and (5.85, 6.34 and 6.02 tons ha⁻¹) for the two concentrations, respectively, while control treatment for the study years 2022 and 2023 gave the lowest rates of (5.26, 5.70 and 5.42 tons ha⁻¹). This is due to the effective role of the nano-compound fertilizer in increasing the concentration of materials and elements inside the cells and increasing their activity, which was evident through the increase in the number of grains per head, Table (5) and the weight of the grain, Table (6), which increased the yield of the individual plant, Table (7), which had a direct impact on increasing the yield for concentrations of 100ppm and 200ppm. Many researchers have mentioned the variation of the trait on the crops of

white and yellow corn when treated with different concentrations of nano-fertilizers, including [9,23,24]. The results of Table (7) showed significant differences between the cultivars included in the experiment, as the two cultivars Giza and Liulo achieved the highest averages for grain yield, reaching (6.46, 6.99, and 6.65 tons ha⁻¹) and (6.36, 6.88 and 6.54 tons ha⁻¹) respectively, compared to the Sudani cultivar, which achieved the lowest rates for the trait, reaching (4.46, 4.83, 4.59 tons ha⁻¹). The reason for the excelled of the Giza cultivar is due to its excelled in grain weight and individual plant yield, Table (6 and 7). This is consistent with what was reached by [25,26] who indicated that the cultivars differ among themselves in total grain yield. The combinations of 100ppm nano fertilizer concentration with Liulo cultivar plants recorded the highest rates of the trait for the study years 2022 and 2023, rates of (6.82, 7.38 and 7.02 tons ha⁻¹), which did not differ significantly from some combinations, due to their excelled in the trait of the individual yield and some components of the yield affecting it, while the concentration of 300 ppm nano fertilizer with the Sudani cultivar recorded the lowest rates of interaction, reaching (4.23, 4.59 and 4.36 tons ha⁻¹).

Table 7. The effect of spraying with compound nano fertilizer and cultivars and their interaction on grain yield (ton ha⁻¹).

Spring session 2022						Autumn session 2022						Spring session 2022					
Cultivars	Concentrations					Cultivars	Concentrations					Cultivars	Concentrations				
	0ppm	10ppm	20ppm	30ppm	Average cultivars		0ppm	10ppm	20ppm	30ppm	Average cultivars		0ppm	10ppm	20ppm	30ppm	Average cultivars
Alkhair	4.77 i	5.0 6	4.9 6	5.0 2	4.95 e	Alkhair	5.17 l	5.4 8	5.3 7	5.4 4	5.36 e	Alkhair	4.91 l	5.2 1	5.1 1	5.1 7	5.10 e
inqath	5.39 h	5.7 6	6.1 9	5.8 8	5.80 c	inqath	5.84 ij	6.2 4	6.7 0	6.3 7	6.29 c	inqath	5.55 ij	5.9 3	6.3 7	6.0 5	5.97 c
Liulo	5.67 fgh	6.8 2	6.5 9	6.3 5	6.36 a	Liulo	6.14 f-i	7.3 8	7.1 3	6.8 8	6.88 a	Liulo	5.83 f-i	7.0 2	6.7 8	6.5 4	6.54 a
Rabih	5.54 gh	6.7 4	5.9 7	6.1 7	6.11 b	Rabih	6.01 ghi	7.3 0	6.4 6	6.6 8	6.61 b	Rabih	5.71 ghi	6.9 4	6.1 4	6.3 5	6.28 b
Bohuth 70	4.94 i	5.4 8	5.4 8	5.3 5	5.31 d	Bohuth 70	5.35 l	5.9 4	5.9 3	5.8 0	5.75 d	Bohuth 70	5.08 l	5.6 4	5.6 4	5.5 1	5.47 d
Giza	6.27 bcd	6.5 2	6.7 3	6.3 2	6.46 a	Giza	6.79 bcd	7.0 6	7.2 8	6.8 5	6.99 a	Giza	6.45 bcd	6.7 1	6.9 2	6.5 1	6.65 a
sudani	4.24 j	4.3 3	5.0 5	4.2 3	4.46 f	sudani	4.60 m	4.6 9	5.4 6	4.5 9	4.83 f	sudani	4.37 m	4.4 6	5.1 9	4.3 6	4.59 f
average Concentrations	5.26 c	5.8 2	5.8 5	5.6 2		average Concentrations	5.70 c	6.3 0	6.3 4	6.0 9		average Concentrations	5.42 c	5.9 9	6.0 2	5.7 8	

3.1.6. Biological Yield (g plant⁻¹)

The results of Table (8) showed that the trait rates of plants sprayed with a concentration of 200 ppm of the nano-compound fertilizer were superior to the rest of the plants sprayed with other fertilizer concentrations at rates of (280.88, 303.63 and 288.51 g plant⁻¹) and an increase of 15% over the control treatment plants that gave the lowest trait rates of (244.55, 264.35 and 251.19 g plant⁻¹) for the study years 2022 and 2023. This excelled is due to the increase in the trait rates of plant height, leaf area, stem diameter and individual plant yield Tables (4, 5, 6 and 12) at a

concentration of 200 ppm, which had a significant impact on increasing the plant's biological yield. Many researchers have shown, including [27,28,29]. The cultivars had a significant effect on the biological yield trait, as the Giza cultivar gave the highest rates of the trait for the spring and autumn 2022 and spring 2023 seasons, reaching (314.52, 339.99 and 323.06 g plant⁻¹), while the Sudani cultivar recorded the lowest rates of the trait, reaching (179.25, 193.77 and 184.12 g plant⁻¹). The reason for the excelled of the Giza cultivar in the biological yield trait is due to the rate of the plant height and individual plant yield traits, Tables (4 and 12), which led to an increase in the biological yield of the plant. Many researchers have mentioned these differences in the biological yield trait between the cultivars , including[30,31,12] . The results of Table (8) showed that the interaction between the levels of the compound nano fertilizer and the Sorghum cultivars included in the study was significant, as the combination of nano fertilizer concentration 100 ppm and 200 ppm with the Giza cultivar achieved the highest rates for the trait for the study years 2022 and 2023 at rates of (329.18, 355.84 and 338.12 g plant⁻¹) and (326.92, 353.40 and 335.80) for the two concentrations, respectively, which did not differ significantly from some other combinations. This may be attributed to the high rate of the trait of plant height and 500-grain weight, Tables (4 and 7) for that combination, while the lowest rate was for control treatment combination with the Sudani cultivar at rates of (157.34, 170.08 and 161.61 g plant⁻¹) for the study years.

Table 8. The effect of spraying with nano-compound fertilizer, cultivars and the interaction between them on the biological yield (g plant⁻¹).

Spring session 2022						autumn session 2022						Spring session 2022					
Cultivars	Concentrations				Average cultivars	Cultivars	Concentrations				Average cultivars	Cultivars	Concentrations				Average cultivars
	0pp m	100 PP m	200 PP m	300 PP m			0pp m	100 PP m	200 PP m	300 PP m			0pp m	100 PP m	200 PP m	300 PP m	
Alkhair	210.71	235.50	251.79	247.03	236.26	Alkhair	227.78	254.58	272.18	267.04	255.39	Alkhair	216.43	241.90	258.63	253.74	242.68
	k j i i f	l k j j f	l k j j f														
inqath	250.85	278.38	295.23	270.81	273.82	inqath	271.17	300.93	319.14	292.75	296.00	inqath	257.66	285.94	303.25	278.17	281.26
	i g ef h d	j gh ef hi d	j gh ef hi d														
Liulo	277.77	317.64	311.60	310.12	304.28	Liulo	300.27	343.37	336.84	335.24	328.93	Liulo	285.32	326.27	320.06	318.54	312.55
	g b c cd b	gh b bc bc b	gh b bc bc b														
Rabih	280.63	305.13	293.98	299.22	294.74	Rabih	303.36	329.85	317.79	323.46	318.61	Rabih	288.25	313.42	301.97	307.35	302.75
	g d ef e c	g cd ef de c	g cd ef de c														
Bohuth 70	239.38	267.77	289.39	281.34	269.47	Bohuth 70	258.77	289.46	312.83	304.13	291.30	Bohuth 70	245.88	275.04	297.25	288.98	276.79
	j h f g e	k i f g e	k i f g e														
Giza	295.14	329.18	326.92	306.83	314.52	Giza	319.05	355.84	353.40	331.68	339.99	Giza	303.16	338.12	335.80	315.16	323.06
	ef a a cd a	ef a a cd a	ef a a cd a														
sudani	157.34	181.61	197.08	180.73	179.25	sudani	170.08	196.41	213.23	195.37	193.77	sudani	161.61	186.63	202.61	185.64	184.12
	n m l m g	o n m n g	o n m n g														
average Concentrations	244.55	273.61	280.88	270.87		average Concentrations	264.35	295.78	303.63	292.81		average Concentrations	251.19	281.05	288.51	278.23	
	d b a c	c b a b	c b a b														

Conclusions

- The concentration of nano fertilizer 200 ppm showed the highest rates for most of the growth and yield traits studied.
- The cultivar (Giza) was distinguished by giving the highest rates for the traits: weight of 500 grains, individual plant yield, grain yield and biological yield.
- The combination of nano fertilizer concentration 200 and 300 ppm with the cultivar (Giza) outperformed the rest of the combinations by giving the highest rates for most of the studied traits, which indicates the response of the cultivar to spraying at the above concentrations and giving it the highest rates for the studied traits, so it can be recommended to adopt this combination in agriculture and agricultural research.

Recommendations

In light of these results of the study, I recommend:

- Adopting the concentration of nano fertilizer 200 ppm to give it the highest growth and yield rates for the studied traits.
- Recommendation to plant the (Giza) cultivar and then the (Liulo) cultivar for their high growth and productivity. 3- Adoption of the combination of nano-compound fertilizer with a concentration of 200 and 300 ppm with the Giza cultivar as the best combination to give it high rates for most of the traits included in the research

References

- [1] Al-Ani, Alaa Abdul-Ghani Hussein Shuwaish, 2011. Effect of foliar feeding with zinc and potassium fertilization on growth traits, yield and quality of two cultivars of Sorghum. Master's thesis, College of Agriculture, University of Anbar. P. 79.
- [2] Abu Dahi, Yousef. 2004. The relationship between soil, water and plants. University of Baghdad. College of Agriculture. Department of Soil and Water Sciences.
- [3] Rampho, E. T. 2005. National herbarium, Pretoria, South Africa .
- [4] Henzel, D. Bob. 2007. Strategy for the global ex situ conservation of sorghum genetic diversity. (GRDC) .Australia. [http:// dx.doi.org/10.1016 /j.scitotenv.2015.01.104](http://dx.doi.org/10.1016/j.scitotenv.2015.01.104)..
- [5] Ministry of Agriculture, 2006. Guidelines for the cultivation and production of white sorghum. General Authority for Agricultural Guidance and Cooperation, White Sorghum Research Development Project. Guidance Bulletin No. 19.
- [6] DeRosa, M.C., Monreal, C., Schnitzer, M., Walsh, R. and Sultan, Y. (2010) Nanotechnology in Fertilizers. Nature Nanotechnology, 5, 91 <https://doi.org/10.1038/nnano.2010.2>.
- [7] Rameshaiah, G.N. and Jpallavi, S. 2015. Nano fertilizers and nano sensors an attempt for developing smart agriculture. International Journal of Engineering Research and General Science, 3(1): Pp 314320.
- [8] Drostkar ,E.; R. Talebi and H. Kanouni. 2016. Foliar application of Fe, Zn and NPK nanofertilizers on seed yield and morphological traits in chickpea under rainfed condition. J. of Res. in Ecology. 4(2): 221228..
- [9] Al-Younes, Abdul Hamid Ahmed. (1993). Production and improvement of field crops. Ministry of Higher Education and Scientific Research, Iraq, p. 469.
- [10] Al-Thuwaini, Abdullah Amer Saleh. 2018. Evaluation of selection evidence based on the productive effects of Sorghum Sorghum bicolor L using biofertilizer in gypsum soils, Master's thesis, Tikrit University, College of Agriculture.
- [11] Marai, Manal Ali and Imad Khalaf Khader. 2020. Effect of different levels of humic acid on the growth traits and biological yield of several genotypes of white sorghum Sorghum bicolor L.. Proceedings of the 8th International Conference. College of Agriculture. Tikrit University.
- [12] Al-Douri, Afrah Abdul Karim Hussein. 2022. Thermal collection of the flowering window for planting dates of Sorghum cultivars and estimation of growth and productivity indicators and

- some genetic parameters. PhD thesis. College of Agriculture. Department of Field Crops. Tikrit University
- [13] Atiya, Hatem Jabbar and Khadir Abbas Jadoua. 1999. Plant growth regulators theory and application, Directorate of Dar Al-Kutub for Printing and Publishing. Baghdad
- [14] Al-Shammari, Anhar Mahmoud Jaaz, Haitham Abdul Salam Ali, and Sondos Abdul Karim Al-Abdullah. 2019. (Zea mays L.) The effect of spraying integrated nano-fertilizer on the growth and yield of yellow corn genotypes. *Jornal of AlMuthanna for Agricultural Sciences*, 7(2).
- [15] Al-Shammari, Anhar Mahmoud Jaaz. 2018. Response of three genetic compositions of yellow corn to spraying with levels of nano-fertilizer. Master's thesis.; College of Agriculture, University of Basra
- [16] Al-Mahasneh, Hussein. 2017. Evaluation of the performance of some genotypes of white sorghum (*Sorghum bicolor* L. Moench) under rainfed and irrigated cultivation conditions. *Journal of Al-Muthanna University for Agricultural Sciences*, Issue 2, 169186.
- [17] Aboud, Nihad Muhammad and Muaz Aziz Hassan Al-Hadith and Baraa Hamid Saleh. 2017. Effect of biostimulant concentrations on qualitative and quantitative traits of three cultivars of Sorghum. *Anbar Journal of Agricultural Sciences*. 15 (2):442430.
- [18] Yassin, Labib Ibrahim and Nazim Younis Abdul. 2017. Effect of planting dates on the traits and components of the yield of two cultivars of white sorghum, *Al-Furat Journal of Agricultural Sciences*, 9(4):942952.
- [19] Nihaba, Raed Saleh 2004 Effect of plant distribution on the growth and grain yield of three cultivars of white sorghum *Sorghum bicolor* L, Master's thesis, College of Agriculture, University of Baghdad
- [20] Al-Dawghi, Kifah Abdul-Ridha and Kazem Hassan Hudhaili and Dhurgham Subaih Kareem. 2013. The effect of spraying iron on some growth and yield traits of three cultivars of white sorghum. *Thi Qar Journal of Agricultural Research* Volume 2 Issue (2) 2013 University of Basra / College of Agriculture / Department of Field Crops.
- [21] Salama, Mahmoud Abbas Abdul (2008) Response of white sorghum *Sorghum bicolor* (L.) moench to nitrogen fertilization. *Iraqi Journal of Desert Studies* 1(1) 11_17
- [22] Al-Dawghi, Kifah Abdul-Ridha Jassim 2001 Response of two cultivars of white sorghum (*Sorghum bicolor* (L. Moench) to the date of addition and the amount of nitrogen fertilizer Master's thesis College of Agriculture University of Basra
- [23] Valadkhan, M.; K.H. Mohammadi., and N. M.T. Karimi. 2015. Effect of priming and foliar application of nanoparticles on agronomic traits of chickpea. *Biological Forum*, 7(2):599602.
- [24] Gommaa, M.A.; F.L.Radwan; E.E.Kandil and D.H.H.ALChallabi. 2017. Comparison of some new maize hybrids response to mineral fertilization and some nano fertilizers, *Elexandria Sci.Exchange J*.38 (3):506514..
- [25] Hadili, Kazem Hassan and Iman Alaa Eldin Ghazi. 2015. Effect of biofertilization on the yield of two cultivars of white sorghum. *Al-Qadisiyah Journal of Agricultural Sciences*, 5(2).
- [26] Aswad, Ibrahim Khalil and Mohsen Ali Ahmed Al-Janabi and Labid Sharif Mohammed. 2019. Response of some growth traits and yield components in two Sorghum cultivars to the effect of the type of addition and irrigation periods in gypsum soil. *Annals of Agricultural Sciences*, Mushtaher, 57 (2).
- [27] Janmohammadi, M., Navid, A., Segherloo, A. E., & Sabaghnia, N. (2016). Impact of nanochelated micronutrients and biological fertilizers on growth performance and grain yield of maize under deficit irrigation condition. *Biologija*, 62..(2)
- [28] Ali, N. S., and H. W. A. AlJuthery. "The application of nanotechnology for micronutrient in agriculture production." *The Iraqi Journal of Agricultural Science* 48.4 (2017): 984..
- [29] Khoshvaghti, H.and Taj Bakhsh, M. (2022). The effect of foliar application of nano and amino acid fertilizers on some biochemical properties of grain and yield of corn. *Journal of Crops Improvement*,24 (1): 252237.



- [30] Mohammed, Saja Abdullah. 2017. Effect of spraying levels of potassium on the growth traits of the yield and its components of two cultivars of white sorghum *Sorghum bicolor* L.. Master's thesis, College of Agriculture. Department of Field Crops. Al-Muthanna University.
- [31] Yassin, Labib Ibrahim. 2018. Effect of planting dates on growth traits and yield of two Sorghum cultivars . Master's thesis. Agricultural Sciences – Field Crops. University of Baghdad

Evaluation of some Vegetative Growth Traits of Tomato Lines and Their Individual Hybrids Under the Effect of Spraying with Nano-Calcium

Aziz Mahdi Abd Al-Shammari¹ and Ahmed Amer Murad²

^{1,2}College of Agriculture, University of Diyala, Diyala, Iraq.

¹E-mail: Azizmabd@uodiyala.edu.iq

²E-mail: ahmed.amir.morad92@gmail.com

Abstract. The field study was conducted during the two agricultural seasons from August 2022 to June 2024 in the Baqubah Nursery affiliated with the Diyala Agriculture Directorate to study and evaluate some vegetative growth traits of tomato lines and their individual hybrids under the influence of calcium spraying. The first season included conducting a genetic fingerprint test and determining the genetic dimension of the twenty pure tomato lines imported from the University of California in Davis, USA, CM Rick Tomato Genetics Center (TGRC). Using ten primers from and through the application of Simple Sequence Repeats technology (SSR). In light of the results of the genetic fingerprint test and the evaluation of the genetic dimension by the cluster diagram, 6 pure lines with the highest genetic dimension were selected, which are the following: LA3695 (2), LA2804 (9), LA4355 (10), LA4449 (11), LA2970 (13), LA0806 (15), to be grown in a greenhouse and entered into a semi-reciprocal breeding program. The second season included comparing the genetic compositions (six parents and fifteen hybrids) under three levels of calcium spraying (no spraying, ordinary calcium spraying, nano calcium spraying) using protected cultivation according to the RCBD design and using the split-split plot design system more than once (Split-Split Plot Design) with three replicates. The results were statistically analyzed using the SAS program, and the averages were compared according to the Duncan multiple range test at a probability level of 0.05. The results were as follows: There was a significant effect of the genetic compositions, as parent 1 succeeded in the trait of plant length and calcium percentage in leaves. Parent 5 excelled in the trait of number of leaves and plant chlorophyll content. Parent 6 excelled in the total leaf area of the plant. While the hybrid 6×5 excelled in the trait of plant length, leaf area, plant chlorophyll content, and calcium percentage in leaves. The hybrid 5×1 excelled in the trait of number of leaves.

Keywords. Tomato lines, Individual hybrids, Calcium.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown, produced, and consumed vegetable crops in the world. It is an herbaceous plant belonging to the Solanaceae family. Its importance in the nutritional value of its fruits, which are used in cooking, making paste, tomato salad, and juice. It is an important source of vitamins A, C, and E, potassium, folic acid, lycopene, and fiber [1]. Tomatoes in the world are diverse and different in color, shape, and taste.

This may be natural or the result of hybridization, as they are found in several colors, including pink, red, orange, yellow, purple, green, and black. Lycopene and beta-carotene pigments cause the common red color of tomatoes. The intensity of the fruit's color depends on the relative concentration of the two pigments. In normal red tomatoes, the beta-carotene pigment does not have any effect despite its presence because its concentration is not sufficient to affect the lycopene pigment. The lycopene pigment disappears completely in both yellow and orange fruits, while beta-carotene increases in them several times. As for green tomatoes, they remain green when ripe and do not contain the pigment that turns them red. Black tomatoes are a type of tomato that is characterized by its dark color, with varying degrees of color depending on the intensity of direct sunlight in the area where it is grown, and its content of anthocyanin pigment, which is an antioxidant [2,3]. At the beginning of the last century, tomato breeding and improvement programs began, primarily through the selection of good genetic compositions resulting from natural and artificial pollination, or through genetic mutations. These programs led to the development of new varieties and hybrids, which were characterized by early maturity, high productivity, fruit quality, multiple colors, and resistance to environmental and biological stresses. Determining the genetic dimension between strains is one of the requirements of plant breeders in implementing their research in order to develop distinctive hybrids by crossbreeding these strains. Therefore, estimating the genetic dimension and its degree is positively correlated to the development of distinctive hybrids, and the diversity of genetic sources for strains or varieties provides basic information for plant breeders and benefits them in designing genetic breeding and improvement programs [4]. Reciprocal crossbreeding is one of the best mating designs used for hybridization between different genetic compositions, whether they are pure strains or open-pollinated varieties, as it ensures the discovery of all possible hybrid combinations and enables the researcher to determine which parents are most likely to be compatible with each other by guessing a set of genetic parameters. This way, the best hybrids with the desired traits are found [5]. The interaction of many environmental factors such as temperature, lighting, and nutritional factors that have a direct and overlapping effect on the genetic aspect of plant growth makes it difficult for plant breeders to distinguish the degree of genetic relative between the studied compositions, but this problem can be overcome as a result of the great development that has occurred in the field of molecular genetics, which depends on the study of DNA, which has had a positive impact on traditional breeding programs. With the emergence of biotechnologies, the study of the genetic dimension has become more accurate and in the shortest time to reach the best results. One of the most important and most widely used technologies is the Simple Sequence Repeats (SSR) technology, which is one of the most important accurate and rapid molecular indicators with a great ability to determine genetic variation between multiple genetic compositions and has been widely used in the field of finding genetic fingerprints and drawing genetic maps [6,7]. The use of nano-applications in the agricultural process leads to improving production in quantity and quality, as it contributes to increasing the productive efficiency of the cultivated area when sprayed on the green part by increasing the activity of carbon metabolism processes, increasing crop resistance to diseases, maintaining the required characteristics of agricultural crops, and increasing the active substances in the plant [8]. Currently, there are more than 800 nano-fertilizer products worldwide, the active substance of which is produced with nano-specifications to cover the needs of plants [9]. Calcium is a major nutrient that has many physiological functions that are important for plant growth and development in both stressful and non-stressful conditions [10]. It has a dual function, as it is an essential component of the structure, cell walls, and membranes. In addition to its structural role, its main function is its ability to act as a second messenger in a variety of physiological processes, such as growth and fertilization of pollen grains within cells, absorption, distribution, and storage in the plant.

Therefore, calcium must be available to the plant in sufficient quantities to regulate these processes [11-14]. Calcium deficiency symptoms often occur in young tissues such as leaves and young fruits due to decreased resupply from old to young tissues, which leads to deficiency symptoms in young leaves and meristematic tissues, and deficiency of this element can cause blossom end rot disease of tomato fruits. The objective of the study was to evaluate the effect of genetic compositions and calcium on some growth traits of tomatoes.

2. Materials and Methods

The study was implemented at the College of Agriculture, University of Diyala, for the period from August 2022 to June 2024, as genetic tests were carried out in the laboratories of the private Wahj Al-Dana Company, Baghdad, Karrada, to determine the six plants required to carry out the hybridization process (genetic dimension) through the genetic fingerprint of the studied samples (pure tomato lines) and drawing the cluster diagram. While field experiments were carried out in the fields of the government nursery affiliated with the Diyala Agriculture Directorate, Baqubah District, Diyala Governorate for the two seasons. The study involved three essential stages, starting with studying the genetic dimension to determine the six pure lines, then hybridization to produce hybrids, and finally comparing the hybrids and spraying them with calcium of both types.

2.1. Sources of Pure Tomato Lines

The twenty pure tomato parental lines were imported from the CM Rick Tomato Genetics Center (TGRC), University of California in Davis, USA (<http://tgrc.ucdavis.edu>). The study included finding the genetic fingerprint and determining the genetic dimension of the 20 pure tomato lines studied: LA2374 (1), LA3695 (2), LA0797 (3), LA2973 (4), LA2972 (5), LA1996 (6), LA4026 (7), LA 4657 (8), LA2804 (9), LA4355 (10), LA4449 (11), LA0279 (12), LA2970 (13), LA 0030 (14), LA0806 (15), LA2377 (16), LA 2971 (17), LA2661 (18), LA4345 (19), LA4452 (20).

2.2. First Agricultural Season (2022) Reciprocal-Half-Crossing Between Pure Tomato Lines

In light of the results of the DNA test (genetic fingerprint) that was previously conducted, six pure genetically distant lines were selected bearing the following codes: LA3695 (2), LA2804 (9), LA4355 (10), LA4449 (11), LA2970 (13), and LA0806 (15). These selected lines will be coded to represent the parents involved in the hybridization (P1, P2, P3, P4, P5, P6). This selection based on genetic variation allows for high genetic diversity in plant breeding programs. The seeds of the selected pure lines were planted based on the genetic dimension between them in a private nursery on 15/10/2022 in sterile cork plates. After all the seeds germinated and were verified, service operations of irrigation, fertilization, and control were applied until 5-6 true leaves of the plant (seedling) appeared, and then they were transferred to the permanent field (a plastic house with a width of 9 meters and a height of 3.20 meters) located in the Baqubah Governmental Nursery on 15/11/2022. After preparing the soil of the plastic house by plowing, smoothing, and making terraces, it was divided into six terraces. The one terrace contains two planting lines; the distance between one line and another is 40 cm, and between one plant and another is also 40 cm within one terrace. Chemical and organic fertilizers and preventive pesticides (fungicide and insecticide) were added to the soil according to the recommended quantities, and the soil was covered with black polyethylene [15,16]. The seedlings were planted on lines alongside the GR irrigation pipes in the shape of a duck's paw, with two irrigation lines for each terrace. Each pure line was planted with 30 plants. We continued to provide the plants with balanced fertilizers, nutrients, and humic, in addition to amino acids, according to scientific recommendations, while carrying out continuous control operations for the whitefly. When the

plants reached the flowering stage, they were introduced into a reciprocal-half-crossing program according to the second method of [17] to produce generation hybrids as shown in Table (1), by transferring pollen grains from the anther of a flower as a father that is open and fully developed physiologically to the stigma of another flower as a mother before it opens and is in the cream color stage, and after completing the neutering process on them (the neutering process of the flower of the mother plant) by removing the male parts (anthers), and then they were wrapped in tin bags or paper bags to ensure that pollen grains from another plant were not mixed, with marks indicating the parents involved in the hybridization (name of father and mother). This process continued daily for all the parents involved in the hybridization (the strains used as father and mother) and in one direction until the completion of the crossings. After ensuring the success of the hybridization and fruit setting process and monitoring the fruits until fully ripe, all the seeds were extracted and placed in paper bags, and all the information related to the hybridization was recorded on them. This process continued until a sufficient quantity of seeds was obtained. They were stored at a suitable temperature and humidity until used in the following season.

Table 1. Reciprocal-half crosses between pure lines of tomato.

Parents	P1	P2	P3	P4	P5	P6
P1	+	*	*	*	*	*
P2		+	*	*	*	*
P3			+	*	*	*
P4				+	*	*
P5					+	*
P6						+

2.3. Second Season (2023) Comparing Hybrids with Their Parents Under the Influence of Two Types of Calcium Fertilizer

The experiment was carried out in the plastic houses of the Baqubah Government Nursery, which is affiliated with the Diyala Agriculture Directorate. The second-season experiment included two factors: comparing the genetic compositions of the parents with their hybrids (the first factor) and the influence of spraying with two types of calcium fertilizer (the second factor). As for the foliar fertilization factor with calcium, there were the following levels: comparison (spraying with distilled water only), spraying with nano-calcium at a concentration of 2 g L⁻¹ (recommendation of the producing company) provided by the Iranian company, and spraying with ordinary calcium at a concentration of 2 g L⁻¹ (recommendation of the producing company) provided by the Spanish Disper company.

2.4. Agriculture

The seeds of the parents and hybrids were planted in cork plates on 9/8/2023 in one of the private nurseries in the Khan Bani Saad area. After reaching the stage of four to five true leaves, they were transferred to plastic houses on 17/10/2023. The area of the experimental unit was 3.584 meters with dimensions of 2.8 x 1.28 m, with 7 plants in the experimental unit, under the GR drip irrigation system. The field was irrigated before planting the seedlings to moisten the soil, and the seedlings, which were 10-15 cm long and 4-3 mm in diameter, were planted next to the irrigation pipes in the middle of the terrace, according to the split-plot system with a complete random block design (RCBD). To ensure ease of spraying, calcium fertilization levels (ordinary and nano) will occupy the main terraces, while the genetic compositions will be placed in the secondary terraces. The genetic compositions will consist of 21 (15 single hybrids with 6 parents), resulting in 63 treatments. Each treatment will be replicated three times, resulting in a total of 189 experimental treatments. Soil samples were taken before the planting experiment

from different locations in the field at a depth of 30 cm and were representative of the field soil and mixed well. It was analyzed in the Laboratory of Agricultural Research, Iraq (Table 2). The plants' spraying process began before the flowers appeared on the plant and continued for three sprays, with ten days between each spray. The plant service operations continued after planting, including irrigation and weeding as needed at regular times. A preventive program was followed to protect against insects and diseases until the fruits were harvested.

Table 2. The physical and chemical properties of soil.

Measurements	Value	Unit of measurement
Texture of soil	Sandy loam	-
Clay	156.70	%
Silt	284.30	%
Sand	559	%
Ph	7.49	-
Ec	1.41	ds.m ⁻¹
N	18.13	mg. kg ⁻¹
P	14.10	mg. kg ⁻¹
K	174.89	mg. kg ⁻¹
S	11.00	mg. kg ⁻¹
Organic matter	1.53	%
CaCO ₃	237.10	g. kg ⁻¹

2.5. Vegetative Growth Characteristics

Measurements were recorded for all plants in the experimental unit and in three replicates to include all of the following:

2.5.1. Plant Height (cm)

It was measured at the end of the growing season from the area of contact of the stem with the soil to the growing tip of the longest branch of the plant using a tape measure.

2.5.2. Number of Leaves (leaf plant⁻¹)

It was measured by counting the leaves of all plants in the experimental unit at the end of the growing season.

2.5.3. Plant Leaf Area (dm²)

The leaf area of the plant was estimated using the weight method (based on wet weight). Five fully expanded leaves were taken and weighed using a sensitive balance. Then, pieces of known area were taken from these leaves using a circular tube with a diameter of 2 cm². Then the cut pieces were weighed [18]. The area of a single leaf was calculated according to the following equation:

$$\text{Area of a single leaf (cm}^2\text{)} = (\text{weight of fresh leaves (g)} \times \text{area of cut discs (cm}^2\text{)}) / (\text{fresh weight of cut discs (g)} / 5)$$

Then the total leaf area of the plant was calculated as follows:

$$\text{Total leaf area (dm}^2\text{)} = \text{Area of a single leaf} \times \text{Number of leaves in the plant}$$

This method enables the estimation of the leaf area of the plant with high accuracy using the measured weight and area data.

2.5.4. Relative Chlorophyll Content (SPAD unit)

It was estimated using a chlorophyll meter type SPAD-502 as mentioned in [19].

2.5.5. Estimation of Calcium in Leaves (%)

Leaves samples from each experimental unit were dried in an electric oven for 72 hours until the weight stabilized. After drying, a sample of 0.2 g was taken from each experimental unit, and 1 ml of concentrated perchloric acid and 4 ml of concentrated sulfuric acid were added to it and placed on a hot plate to heat to complete the digestion process. Then the samples were placed in 50-ml glass bottles, and distilled water was added to complete the volume to the specified limit according to the method of [20]. Then the calcium in the leaves was estimated.

2.6. Statistical Analysis

The data were analyzed according to the split-plot design and the complete randomized design (RCBD). The Statistical Analysis System (SAS) program was used to analyze the data according to Duncan's multiple test to compare the means at the probability level (0.05). Then, the genetic analysis for the reciprocal-half cross was conducted by dividing the square means of the coefficients into their components from the data for all measured traits [21].

3. Results and Discussion

Figure 1 presented a cluster analysis diagram, also known as a relative tree, based on SSR-P markers for pure tomato lines.

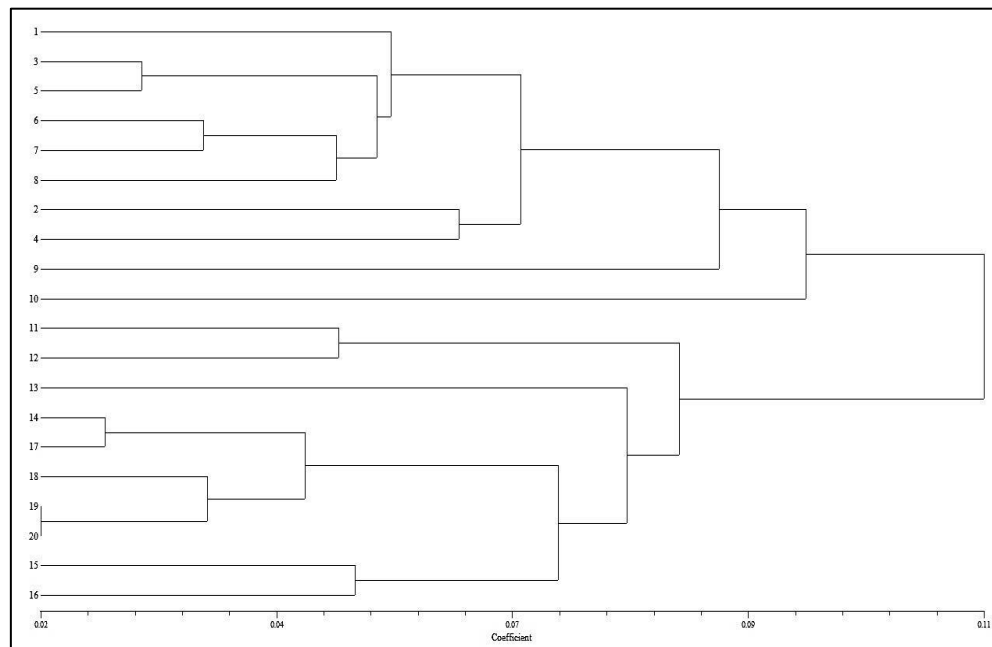


Figure 1. Cluster analysis diagram (relative tree) of pure tomato lines according to SSR-P markers.

3.1. Plant Length (cm)

The results of Table (3) showed significant differences in plant length between genetic compositions, as parent 1 recorded the highest value of 110.31 cm, while parent 2 recorded the lowest value of 74.31 cm. The difference between parents also had a significant effect on the

values of hybrids for the same trait, as the hybrid 6×5 achieved the highest value of 140.08 cm, while the hybrid 3×2 recorded the lowest value of 77.37 cm. Calcium, both ordinary and nano, had a significant effect on the trait, as the plants treated with Ca-nano gave the highest plant length of 113.4 cm compared to the plants treated with control, which recorded the lowest value of 100.7 cm. The interaction between the study factors significantly influenced the trait. The use of calcium in its two forms, ordinary and nano, resulted in a significant increase in the trait values for the genetic compositions under study. The plants of parent 1 and the Ca-nano treatment outperformed, recording the highest plant length of 115.53 cm, while the plants of parent 2 and the control treatment recorded the lowest value of 65.10 cm. The hybrid plants 1×5 and Ca-nano treatment recorded the highest plant length of 146.46 cm, while the hybrid plants 2×3 and the control treatment gave the lowest value of 64.100 cm.

Table 3. Effect of genetic compositions, calcium and their interaction on plant height (cm).

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	103.9 tuv	111.5 q	115.5 p	110.31 Y
2	65.10 $\bar{n}$	76.50 $\bar{l}$	81.33 $\bar{j}$	74.31 T
3	75.10 $\bar{m}$	83.30 $\bar{i}$	87.43 $\bar{g}$	81.94 R
4	82.80 $\bar{i}$	92.20 $\bar{cd}$	95.53 $\bar{a}$	90.17P
5	95.60 $\bar{a}$	104.4 stu	105.1 st	101.7 K
6	94.00 $\bar{b}$	100.5 x	105.7 rs	100.0 L
1×2	77.70 $\bar{kl}$	85.10 $\bar{h}$	89.16 $\bar{f}$	83.98 Q
1×3	110.7 q	119.8 mn	122.5 l	117.6 I
1×4	118.5 n	125.4 j	129.5 h	8124.4 F
1×5	128.0 i	139.3 d	146.4 a	137.9B
1×6	124.1 k	135.3 f	142.4 c	133.9 C
2×3	64.10 $\bar{n}$	78.30 $\bar{k}$	89.73 $\bar{f}$	77.37S
2×4	90.00 $\bar{fe}$	98.10 yz	102.3 w	96.81 N
2×5	120.8 m	117.1 o	119.2 n	119.0 H
2×6	116.7 op	129.6 h	138.7 d	128.4 E
3×4	96.90 Z- $\bar{A}$	103.1 uvw	106.6 r	102.2 K
3×5	116.6 op	125.4 j	123.7 kl	121.9G
3×6	85.80 $\bar{h}$	91.26 $\bar{de}$	96.30 $\bar{a}$	91.12 O
4×5	122.4 l	133.2 o	137.1 e	130.9 D
4×6	93.20 $\bar{bc}$	98.96 y	102.9 vw	98.35 M
5×6	133.3 g	142.6 c	144.3 b	140.0 A
Mean of calcium	100.7 C	109.1 B	113.4 A	

3.2. Number of Leaves

Parent 5 was significantly superior to all parents in the number of leaves, which reached 110.3 leaves plant⁻¹, while parent 2 recorded the lowest number of leaves, which reached 83.98 leaves plant⁻¹. As for the hybrids, the 1×5 hybrid outperformed all hybrids significantly by recording the highest value of 139.3 leaves plant⁻¹ compared to the 1×2 hybrid, which gave the lowest value of 83.15 leaves plant⁻¹. The Ca-nano treatment outperformed significantly, as it gave the highest rate for this trait of 115.4 leaves plant⁻¹ compared to the control treatment, which gave the lowest average of 98.59. The interaction between the two study factors affected the trait, as parent 5 and Ca-nano treatment gave the highest number of leaves of 118.5 leaves per plant⁻¹, while parent 2

and control recorded the lowest number of leaves of 70.66 leaves plant⁻¹. The hybrid 1×5 and Ca-nano treatment was distinguished by the highest number of leaves of 149.5 leaves plant⁻¹, compared to the plants of the hybrid 1×2 and the control treatment, which amounted to 75.53 leaves plant⁻¹ (Table 4).

Table 4. Effect of genetic compositions, calcium and their interaction on number of leaves.

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	88.70 $\overline{cd}$	96.06 YZ	103.4 QR	96.06 L
2	70.66 $\overline{i}$	87.10 $\overline{DE}$	94.20 $\overline{A}$	83.98 P
3	74.56 $z-\overline{a}$	104.6 PQ	112.2 L	103.7 J
4	80.76 $\overline{g}$	97.90 VWX	105.8 OP	94.82 M
5	99.83 VT	112.63 L	118.5 IJ	110.32 H
6	90.73 $\overline{b}$	97.30 WXY	101.0 ST	96.35 KL
1×2	75.53 $\overline{F}$	83.10 $\overline{h}$	90.83 $\overline{B}$	83.15 P
1×3	99.76 tv	107.9 MN	114.6 K	107.4 I
1×4	87.20 $\overline{de}$	99.13 UV	102.4 RS	96.25 KL
1×5	128.1 EF	140.5 B	149.5 A	139.3 A
1×6	119.9 I	127.1 F	132.8 CD	126.6 C
2×3	86.03 $\overline{e}$	92.90 $\overline{A}$	98.73 UVW	92.55 N
2×4	83.13 $\overline{f}$	89.06 $\overline{C}$	96.73 XY	89.64 O
2×5	115.2 K	127.2 F	131.2 D	124.5 D
2×6	108.7 M	115.2 K	122.0 H	115.3 EF
3×4	102.0 rs	117.5 J	128.1 EF	115.8 E
3×5	106.8 no	111.9 L	118.1 J	112.3 G
3×6	111.3 L	129.2 E	133.9 C	124.8 D
4×5	104.9 pq	114.4 K	124.8 G	114.7 F
4×6	87.80 cde	97.90 VWX	105.9 OP	97.21 K
5×6	128.8 EF	133.5 C	139.0 B	33.78 B
Mean of calcium	98.59 C	108.6 B	115.4 A	

3.3. Leaf Area

The results of Table 5 show that there are significant differences between the genetic compositions in leaf area, as parent 6 achieved the largest leaf area of 312.5 dm², while parent 2 recorded the smallest leaf area of 159.3 dm², and the genetic differences between the parents were reflected in their individual hybrids, as the hybrid 5×6 outperformed with the highest leaf area of 395.3 dm² compared to the hybrid 2×1, which recorded the lowest leaf area of 161.9 dm². The use of calcium in its two forms, ordinary and nano, led to a significant increase in leaf area, as the highest value of 300.2 dm² was recorded in the Ca-nano treatment compared to the control treatment, which recorded 249.8 dm². The interaction between the study factors had a significant effect on the leaf area of the plant, as the interaction of the Ca-nano treatment led to an increase in the leaf area regardless of the type of genetic composition, as the parent 6 and the hybrid 6×5 recorded the highest value of 350.4 and 457.9 dm², respectively, while the parent 2 and the hybrid 2×1 with the control treatment recorded the lowest value of 140.1 and 133.5 dm², respectively.

Table 5. Effect of genetic compositions, calcium and their interaction on leaf area (dm²).

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	196.7 <i>abc</i>	218.1 <i>z</i>	239.5 <i>vw</i>	218.1 M
2	140.1 <i>h</i>	164.9 <i>g</i>	173.0 <i>efg</i>	159.3 P
3	185.7 <i>b - f</i>	202.6 <i>a</i>	222.2 <i>yz</i>	203.5 N
4	163.6 <i>g</i>	181.1 <i>c - f</i>	188.9 <i>a - e</i>	177.9 O
5	286.6 <i>mno</i>	301.2 <i>lm</i>	330.4 <i>jk</i>	306.0 G
6	267.3 <i>p-s</i>	319.9 <i>k</i>	350.4 <i>e-i</i>	312.5 G
1×2	133.5 <i>h</i>	160.7 <i>g</i>	191.6 <i>a - d</i>	161.9 P
1×3	176.5 <i>d-g</i>	200.2 <i>ab</i>	303.3 <i>l</i>	226.7 L
1×4	225.6 <i>xyz</i>	243.7 <i>uvm</i>	259.5 <i>g-u</i>	242.9 K
1×5	358.2 <i>ef</i>	374.6 <i>cd</i>	413.9 <i>b</i>	382.2 B
1×6	347.2 <i>f-i</i>	362.9 <i>ef</i>	377.1 <i>cd</i>	362.4 C
2×3	170.3 <i>fg</i>	184.3 <i>b - f</i>	200.6 <i>ab</i>	185.1 O
2×4	252.3 <i>s-v</i>	263.4 <i>q-t</i>	274.2 <i>opq</i>	263.3 IJ
2×5	319.6 <i>k</i>	341.1 <i>g-j</i>	353.0 <i>e-h</i>	337.9 E
2×6	291.7 <i>lmn</i>	334.8 <i>ijk</i>	348.9 <i>e-i</i>	325.1 F
3×4	243.3 <i>uvw</i>	275.3 <i>opq</i>	303.0 <i>l</i>	273.9H
3×5	328.6 <i>jk</i>	351.2 <i>e-i</i>	365.0 <i>de</i>	348.3 D
3×6	235.3 <i>wxy</i>	250.1 <i>t-w</i>	296.3 <i>lmn</i>	260.6 J
4×5	327.2 <i>jk</i>	357.5 <i>efg</i>	374.3 <i>cd</i>	353.0 D
4×6	257.6 <i>r-u</i>	270.0 <i>pqr</i>	281.5 <i>nop</i>	269.7 HI
5×6	338.4 <i>hij</i>	389.7 <i>c</i>	457.9 <i>a</i>	395.3 A
Mean of calcium	249.8 C	273.7 B	300.2 A	

3.4. Chlorophyll Content

The results of Table 6 indicate that there are significant differences in the genetic compositions in the chlorophyll content, as parent 5 showed the highest concentration of 47.18 SPAD, while parent 4 recorded the lowest chlorophyll content of 40.57 SPAD, which reflected this difference in the performance of the parents significantly on the performance of their hybrids, as the hybrid 5×6 recorded the highest average of 59.54 SPAD units, while the hybrid 2×4 gave the lowest percentage of 42.12 SPAD. The use of nano-calcium significantly increased the percentage of chlorophyll, 50.98 SPAD, while the plants not treated with calcium (control) showed a decrease to 45.85 SPAD.

The interaction between genetic compositions and calcium fertilizer had a significant effect on the trait, as the plants of parent 5 and the Ca-nano treatment outperformed with the highest percentage of chlorophyll, reaching 50.10 SPAD, compared to the plants of parent 4 with the control, which recorded the lowest percentage, reaching 38.36 SPAD. The 6×5 hybrids were distinguished by the highest percentage of chlorophyll, reaching 63.50 SPAD with the Ca-nano treatment, while it decreased to 40.50 SPAD in the 2×4 hybrid with the control treatment.

Table 6. Effect of genetic compositions, calcium and their interaction on chlorophyll content (SPAD unit).

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	40.30 <i>de</i>	43.26 <i>x-b</i>	45.50 <i>s-w</i>	43.02 KLM
2	39.46 <i>ef</i>	41.20 <i>cde</i>	42.06 <i>z-d</i>	40.91 N
3	41.40 <i>bcd</i>	42.66 <i>y-c</i>	43.40 <i>x-a</i>	42.48 LM
4	38.36 <i>f</i>	40.90 <i>cde</i>	42.46 <i>y-c</i>	40.57 N
5	44.70 <i>u-x</i>	46.76 <i>q-t</i>	50.10 <i>mno</i>	47.18 HI
6	42.56 <i>y-c</i>	44.90 <i>t-x</i>	47.26 <i>p-s</i>	44.91 J
1×2	42.00 <i>z-d</i>	43.86 <i>v-z</i>	45.50 <i>s-w</i>	43.78 K
1×3	43.46 <i>x-a</i>	45.10 <i>t-x</i>	46.46 <i>r-u</i>	45.01 J
1×4	44.30 <i>v-y</i>	45.76 <i>s-v</i>	48.80 <i>nop</i>	46.28 I
1×5	53.36 <i>ij</i>	59.10 <i>cd</i>	61.86 <i>b</i>	58.11 B
1×6	52.60 <i>jk</i>	56.96 <i>ef</i>	60.20 <i>b c</i>	56.58 C
2×3	41.86 <i>a - d</i>	43.30 <i>x-b</i>	44.86 <i>t-x</i>	43.34 KL
2×4	40.50 <i>de</i>	42.16 <i>z-d</i>	43.70 <i>w-a</i>	42.12 M
2×5	50.76 <i>klm</i>	53.80 <i>ij</i>	56.06 <i>fgh</i>	53.54 E
2×6	49.60 <i>mno</i>	51.46 <i>klm</i>	57.90 <i>de</i>	52.98E
3×4	45.66 <i>s-v</i>	47.70 <i>pqr</i>	50.46 <i>lmn</i>	47.94 H
3×5	51.20 <i>klm</i>	54.86 <i>ghi</i>	58.90 <i>cd</i>	54.98 D
3×6	50.26 <i>l-o</i>	52.10 <i>jkl</i>	56.36 <i>efg</i>	52.91 E
4×5	48.90 <i>nop</i>	50.26 <i>l-o</i>	53.90 <i>ij</i>	51.02 F
4×6	47.06 <i>p-s</i>	48.50 <i>opq</i>	51.46 <i>klm</i>	49.01 G
5×6	54.50 <i>hi</i>	60.63 <i>bc</i>	63.50 <i>a</i>	59.54 A
Mean of calcium	45.85 C	48.34 B	50.98 A	

3.5. Calcium (%)

The results of Table 7 confirmed the existence of significant differences between the genetic compositions in the percentage of calcium in the leaves, as parent 1 achieved the highest percentage, reaching 1.212%, while parent 4 recorded the lowest value, reaching 0.828%. This variation between the parents affected the resulting hybrids, as the hybrid 5×6 recorded the highest value, reaching 1.692%, while the hybrid 2×4 recorded the lowest value, reaching 0.907%. The plants of the Ca-nano treatment outperformed with the highest value, reaching 1.351%, compared to the control plants, which reached 1.155%. The interaction also had a significant effect on the trait, as parent 1 recorded the highest percentage of 1.273% with the Ca-nano treatment, and the lowest percentage was for the interaction between parent 4 plants and the control treatment, which amounted to 0.776%, and the 6×5 hybrid was distinguished by the highest value of 1.836% with the Ca-nano treatment, compared to the 4×2 hybrid plants and the control treatment, which amounted to 0.833%.

Table 7. Effect of genetic compositions, calcium and their interaction on calcium in leaves (%).

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	1.156 <i>w</i>	1.206 <i>u</i>	1.273 <i>s</i>	1.212 J
2	0.860 <i>i</i>	0.906 <i>gh</i>	0.983 <i>e</i>	0.916 Q

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
3	0.943 $\bar{f}$	0.986 $\bar{e}$	1.076 z	1.002 P
4	0.776 $\bar{l}$	0.813 $\bar{k}$	0.896 $\bar{h}$	0.828 R
5	1.036 $\bar{bc}$	1.063 z- $\bar{a}$	1.146 wx	1.082 M
6	1.110 y	1.133 x	1.206 u	1.150 K
1×2	0.993 $\bar{e}$	1.023 $\bar{cd}$	1.133 x	1.050 N
1×3	1.016 $\bar{d}$	1.046 $\bar{ab}$	1.243 t	1.102 L
1×4	1.266 s	1.376 p	1.483 kl	1.375 H
1×5	1.470 l	1.586 g	1.770 c	1.608 C
1×6	1.513 ij	1.636 e	1.796 b	1.648 B
2×3	0.920 $\bar{g}$	0.953 $\bar{f}$	1.176 v	1.016 O
2×4	0.833 $\bar{j}$	0.873 $\bar{i}$	1.016 $\bar{d}$	0.907 Q
2×5	1.310 r	1.403 no	1.496 jk	1.403 G
2×6	1.343 q	1.433 m	1.523 i	1.433 F
3×4	1.113 y	1.146 wx	1.393 op	1.217 J
3×5	1.383 p	1.466 l	1.573 g	1.474 E
3×6	1.410 no	1.526 i	1.613 f	1.516 D
4×5	1.056 $\bar{a}$	1.096 y	1.316 r	1.156 K
4×6	1.213 u	1.323 r	1.416 mn	1.317 I
5×6	1.546 h	1.693 d	1.836 a	1.692 A
Mean of calcium	1.155 C	1.223 B	1.351 A	

The results of the statistical analysis showed significant effects of the genetic compositions for all the studied traits, as the high significance of the genetic compositions is clear evidence of the extent of the differences between them genetically, which required studying their genetic behavior and knowing the nature of the work of the genes that control the inheritance of the studied traits, and that these results are consistent with [22,23,24] during their study on tomato plants. The results of Tables 3, 4, 5, 6, and 7 showed differences between the genetic compositions of the studied traits. This may be attributed to the difference in the genes responsible for cell growth, division, and elongation for different genetic compositions and their interaction with environmental conditions, through their effect on growth hormones such as auxins and gibberellins in addition to other hormones, as well as the difference in the efficiency of carbon metabolism processes and the utilization of nutrients between genetic compositions and the extent of their interaction with environmental conditions, which affects vegetative growth traits positively or negatively [23,25,26,22]. The vegetative growth characteristics are mainly controlled by several genetic factors in combination with other environmental factors that affect the growth of these genetic compositions [27,28,26]. The difference in genetic compositions in plant height may be attributed to the number of nodes and the length of the internodes as a result of their differences in the primary and secondary genes [29] or to the difference in their content of auxins and gibberellin responsible for cell elongation and expansion [30]. This result is consistent with the results of [29,31], with a number of crosses outperforming the parents in plant height. The plant's ability to photosynthesis is also measured by its leaf area, and increasing it enhances photosynthesis. Increasing leaf area, within certain limits, leads to a relative increase in photosynthesis efficiency, which leads to improving most vegetative growth traits [32,23]. These results are consistent with what was found by [27,33,34]. The effect of calcium in raising the values of vegetative growth traits illustrates the importance of calcium, as calcium plays an

effective role in cell division and elongation, regulating the permeability of cell membranes, and the growth of meristematic tips responsible for new growth. It also activates some enzymes important for growth, such as Adenosine Tri Phosphate, ATPase, and Arginine Kinase, in addition to its role in increasing the efficiency of photosynthesis and thus the accumulation of carbohydrates, which leads to activating the vegetative growth of plants, thus improving most of the vegetative growth traits [35]. As for the increase in the percentage of calcium in the leaves with the increase in the concentration used, the reason for this may be due to the ease of absorption of calcium from the leaves when sprayed on the plant leaves. Calcium is also a weakly mobile element within plant tissues, so the largest proportion of it remains in the leaves and accumulates in them, or it may accumulate in the gaps in the form of calcium oxalate, or form chelating compounds in the protoplasm of cells). These results are consistent with [36].

Conclusion

The pure lines of colored tomatoes used in the study are characterized by a genetic dimension, and thus they can be used as parents in other breeding programs to improve growth and productivity traits of tomatoes. The 6×5 crossbreed was superior in plant height, leaf area, chlorophyll content, and leaf calcium percentage, and the 5×1 crossbreed was superior in total number of leaves. The use of nano-calcium at a concentration of 2 g L⁻¹ (recommended by the fertilizer producing company) improved most of the vegetative growth traits.

References

- [1] Perveen, R, Suleria HAR, Anjum FM, Butt MS, Pasha I and Ahmad S 2015. Tomato (*Solanum lycopersicum*) carotenoids and lycopenes chemistry; metabolism, absorption, nutrition, and allied health claims-a comprehensive review. *Critical Reviews in Food Science and Nutrition*. 55:919-929.
- [2] Lenucci M and Dalessandro G 2009. Carotenoid content during tomato (*Solanum lycopersicum* L.) fruit ripening in traditional and high-pigment cultivars. *Italian Journal of Food Science* 21(4).
- [3] Lenucci, MS, Cadinu D, Piro G and Dalessandro G 2006. Antioxidant Composition in Cherry and High-Pigment Tomato Cultivars. *J. Agric. Food Chem.*(54). 7.2613–2606 .
- [4] Kulus D 2022. Genetic diversity for breeding tomato. In: Priyadarshan P., Jain S.M. (eds) *Cash Crops*. Springer, Cham. https://doi.org/10.1007/978-3-030-74926-2_13
- [5] Al-Shammari, AMA 2005. Complete crosses and estimation of genetic parameters of some traits in tomato grown under plastic tunnels. PhD thesis. College of Agriculture. University of Baghdad. Ministry of Higher Education and Scientific Research. Republic of Iraq.
- [6] Ahmad, R, Anjum, MA and Balal, RM 2020. From markers to genome based breeding in horticultural crops: An Overview. *Phyton-International Journal of Experimental Botany*. 89(2):183-204.
- [7] Scarano, D, Rao, R, Masi, P and Corrado, G 2015. SSR fingerprint reveals mislabeling in commercial processed tomato products. *Food Control* 51:397–401.
- [8] Al-Moussawi, ANA. Al-Yasari, JWM 2016. The effect of adding potassium and calcium mixed in reducing salt stress for some wheat varieties and its relationship to some physiological and biochemical indicators. *Karbala University Scientific Journal* (1) (14).
- [9] Al-Rifai, FN 2016. "Basic concepts in nanotechnology" - Thi Qar University, College of Science. Issue (19 Dar Al-Kutub for Printing and Publishing.
- [10] White, PJ, and Broadley, MR 2003. Calcium in plants. *Annals of Botany* 92(4), 487-511.
- [11] Michard, E, Lima, PT, Borges, F, Silva, AC, Portes, MT., Carvalho, JE 2011. Glutamate receptor-like genes form Ca²⁺ channels in pollen tubes and are regulated by pistil D-serine. *Science* 332(6028), 434-437.

- [12] Monshausen, GB, Miller, ND, Murphy, AS, and Gilroy, S 2011. Dynamics dependent Ca²⁺ and pH signaling in root growth revealed by integrating high-resolution of auxin imaging with automated computer vision-based analysis. *The Plant Journal* 65(2), 309-318.
- [13] Ortiz-Ramírez, C, Michard, E, Simon, AA, Damineli, DSC, Hernández-Coronado, M, Becker, JD 2017. Glutamate receptor-like channels are essential for chemo taxis and reproduction in mosses. *Nature* 549, 91.
- [14] Zhang, S, Pan, Y, Tian, W, Dong, M, Zhu, H, Luan, S 2017. Arabidopsis CNGC14 mediates calcium influx required for tip growth in root hairs. *Molecular Plant* 10(7), 1006-1004 .
- [15] Saleh, HM and Salman IS 2010. Bulletin on recommended fertilizer additions according to available fertilizers for summer and winter crops. Ministry of Agriculture. Central Fertilizer Combinations Committee. Al-Farah Press. Baghdad.
- [16] Al-Shammari, AMA 2018. Effect of organic fertilization and breeding method on some quantitative yield traits of three genetic combinations of tomato (*Lycopersicon esculentum* Mill) grown under plastic house conditions. *Al-Rafidain Agriculture Journal*. Volume (46) Issue (2).
- [17] Griffing, B 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences* 9(4):463-493. <https://doi.org/10.1071/BI9560463>
- [18] Dovrnic, V 1965. *Lucravipactic de ampelographic E.Dielactica spedagogica Bucureseti R. S.Romania.*
- [19] Jemison, J and Williams M. 2006. Potato-Grain Study Project Report Water Quality Office. University of maine, Cooperation Extension. <http://www.umext.maine.edu>
- [20] Creaser, MS. and Parson JW 1979. Sulphoric, perchloric acid and digestion of plant material for the determination of Nitrogen, Phosphorus, Potassium, Calcium and magnesium . *Analytical Chemical Acta*. 109: 431-436.
- [21] Dean A, Voss D and Draguljić D 2017. Split-Plot Designs. In: *Design and Analysis of Experiments*. Springer Texts in Statistics. Springer, Cham, 703-764.
- [22] Islam, S, Hassan L and Hossain MA 2022. Breeding potential of some exotic tomato lines: a combined study of morphological variability, genetic divergence, and association of traits. *Phyton-International Journal of Experimental Botany*, 91(1): 97–114.
- [23] Hamdi, G J 2022. Estimation of genetic dimension and genetic parameters of tomato growth and yield traits by half-crossing under the influence of water stress. PhD thesis. College of Agriculture. University of Diyala.
- [24] Essa, BA, and Alwan, OK 2024. Impact evaluation combining ability of tomato hybrids and lines for production and fruit quality traits. *Agronomía Meso americana*, Volumen 35: Artículo. 57558-57558. doi.org/10.15517/am.2024.57558.
- [25] Al-Ubaidi, ARHH 2022. Derivation of single and triple hybrids in tomato And estimation of some genetic parameters in it. PhD thesis. College of Agriculture. University of Diyala.
- [26] El- Sappah, AH., Islam MM, Rather SA, Li J, Yan K, Xianming Z, Liang Y, Abbas M 2022. Identification of novel root-knot nematode (*Meloidogyne Incognita*) resistant tomato genotypes. *The Journal of Animal and Plant Sciences*. 33(1):1-17.
- [27] Lázaro, A 2018. Tomato landraces: An analysis of diversity and preferences. *Plant Genetic Resources: Characterization and Utilization*, 16(4):315-324.
- [28] Anuradha, B, Saidaiah P, Reddy KR, Sudini H, Geetha A 2021. Mean performance of 40 genotypes in tomato (*Solanum lycopersicum* L.). *International Journal of Chemical Studies*. 9(1):279-283.
- [29] Nezami, S, Nemati SH, Arouiee H, Kafi M 2019. Combining ability, heterosis and potency ratio analysis of earliness and important traits of tomato (*Solanum lycopersicum* L.). *Journal of Pharmacognosy and Phytochemistry*. 8(5):99-106.
- [30] Kiran, TP, Srinivasa V, Dushyanth DBM, Ganapathi M, Akshay A 2020. Assessment of genetic diversity in tomato (*Solanum lycopersicum*) under protected condition. *International Journal of Current Microbiology and Applied Sciences*. 9(10): 937-942.



- [31] Sirkeci, M, Tüzel Y, Öztekin GB, Aşçıoğlu T, Durdu T 2021. Response of local tomato genotypes to drought stress. *Acta Horticulturae*. 607-614.
- [32] Rowland, SD, Zumstein K, Nakayama H, Cheng Z, Flores AM, Chitwood DH, Maloof JN, Sinha NR 2020. Leaf shape is a predictor of fruit quality and cultivar performance in tomato. *New Phytologist*. 226: 851–865.
- [33] Murariu, OC, Brezeanu C, , Jităreanu CD, Robu T, Irimia LM, Trofin AE, Popa LD., Stoleru V, Murariu F, Brezeanu PM. 2021. Functional quality of improved tomato genotypes grown in open field and in plastic tunnel under organic farming. *Agriculture*. 11(7):1-15.
- [34] Athinodorou, F, Foukas P, Tsaniklidis G, Kotsiras A, Chrysargyris A, Delis C, Kyrtzis AC, Tzortzakis N, Nikoloudakis N 2021. Morphological diversity, genetic characterization, and phytochemical assessment of the cypriot tomato germplasm. *Plants*. 10(8):1698.
- [35] Barker, AV, Pilbeam AV 2015. *Handbook of Plant Nutrition*. 2nd ed. CRC Press. Boca Raton, FL, USA. pp. 537-564.
- [36] Al-Moussawi, HN 2020. The effect of salicylic acid and calcium on the growth and yield of tomatoes. Master's thesis, College of Agriculture. University of Baghdad. Iraq.

Effect of Ordinary and Nano Calcium on some Growth Characteristics of Tomato Lines and Their Individual Hybrids

Ahmed Amer Murad¹ and Aziz Mahdi Abd Al-Shammari²

^{1,2}College of Agriculture, University of Diyala, Diyala, Iraq.

¹E-mail: aalkurdi5@gmail.com

²E-mail: Azizmabd@uodiyala.edu.iq

Abstract. The study was carried out in the Baqubah Nursery, Directorate of Diyala Agriculture, during the seasons 2022-2024 to study and evaluate the effect of ordinary and nanocalcium on some growth characteristics of tomato lines and their individual hybrids. The study included comparing the genetic compositions (six parents and fifteen hybrids) under three levels of calcium spraying (no spraying, ordinary calcium spraying, nano calcium spraying) using protected cultivation according to the RCBD design and using the split-split plot design system more than once (Split-Split Plot Design) with three replicates. The results were statistically analyzed using the SAS program, and the averages were compared according to the Duncan multiple range test at a probability level of 0.05. The findings were as follows: There was a significant effect of the genetic compositions, as parent 5 succeeded in the trait of number of inflorescences and yield per plant. Parent 6 excelled in the trait of fruit set percentage and number of fruits. Parent 4 excelled in the number of flowers in the inflorescence. While the hybrid 5×6 excelled in the trait of number of inflorescences and total yield per plant. The hybrid 4×6 excelled in the trait of fruit set percentage and number of fruits per plant. The hybrid 1×4 excelled in the trait of number of flowers in the inflorescence.

Keyword. Tomato lines, Calcium, Individual hybrids.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the world's most popular food crops for growing, making, and eating [1]. Tomatoes vary in color, shape, and taste, influenced by natural factors or hybridization. They range from pink, red, and orange to yellow, purple, green, and black. The red color in tomatoes is mainly due to lycopene, while yellow and orange tomatoes have more beta-carotene. Green tomatoes remain green because they lack lycopene, and black tomatoes get their dark color from anthocyanin, an antioxidant, with sunlight affecting their color intensity [2,3]. In the early 20th century, tomato breeding programs began focusing on selecting desirable genetic traits from natural and artificial pollination, as well as genetic mutations. These efforts resulted in the creation of new tomato varieties and hybrids with early maturity, high productivity, improved fruit quality, various colors, and resistance to environmental and biological stresses. Understanding the genetic diversity between strains is crucial for breeders in developing unique hybrids. Estimating genetic differences helps in crossbreeding efforts, and the diversity of genetic sources provides essential information for designing successful breeding programs [4].

Reciprocal crossbreeding is an effective mating method used in hybridization between different genetic compositions, including pure strains and open-pollinated varieties. It helps identify all possible hybrid combinations and allows researchers to assess which parents are most compatible by estimating genetic parameters. This approach aids in selecting the best hybrids with desirable traits [5]. Environmental factors like temperature, lighting, and nutrition affect plant growth and make it challenging for breeders to assess genetic relationships between studied compositions. However, advances in molecular genetics, particularly DNA studies, have improved traditional breeding programs. The emergence of biotechnologies has made the study of genetic dimensions faster and more precise. One key technology is Simple Sequence Repeats (SSR), a widely used molecular marker that accurately identifies genetic variation, aids in creating genetic fingerprints, and helps draw genetic maps [6,7]. The use of nano-applications in agriculture enhances both the quantity and quality of production. When applied to plant foliage, nano-products improve carbon metabolism, boost disease resistance, maintain crop characteristics, and increase active substances in plants. Over 800 nano-fertilizer products are available globally, designed with nano-specifications to meet plant needs [8,9]. Calcium is a major nutrient that has many physiological functions that are important for plant growth and development in both stressful and non-stressful conditions [10]. It has a dual function, as it is an essential component of the composition, cell walls, and membranes. Calcium plays a dual role in plants: as a key structural component of cell walls and membranes, and as a second messenger in various physiological processes like growth, pollen fertilization, nutrient absorption, and storage. Adequate calcium is essential for regulating these processes. Calcium deficiency often affects young tissues, such as leaves and fruits, due to limited resupply from older tissues, leading to symptoms like blossom end rot in tomatoes [11,12,13,14]. The objective of the study was to evaluate the effect of genetic compositions and calcium on some growth traits of tomatoes.

2. Materials and Methods

The study took place from August 2022 to June 2024 at the College of Agriculture, University of Diyala. Genetic tests were done in the labs of the private Wahj Al-Dana Company in Baghdad, Karrada, to find the six plants needed to do the hybridization process (genetic dimension) through the genetic fingerprint of the studied samples (pure tomato lines) and making a tree diagram. Field experiments were carried out in the fields of the government nursery affiliated with the Diyala Agriculture Directorate, Baqubah District, Diyala Governorate for the two seasons. The study involved three essential stages, starting with studying the genetic dimension to determine the six pure lines, then hybridization to produce hybrids, and finally comparing the hybrids and spraying them with calcium of both types.

2.1. The Pure Tomato Lines

The twenty pure tomato parental lines were imported from the CM Rick Tomato Genetics Center (TGRC), University of California in Davis, USA (<http://tgrc.ucdavis.edu>). The study included finding the genetic fingerprint and determining the genetic dimension of the 20 pure tomato lines studied: LA2374 (1), LA3695 (2), LA0797 (3), LA2973 (4), LA2972 (5), LA1996 (6), LA4026 (7), LA 4657 (8), LA2804 (9), LA4355 (10), LA4449 (11), LA0279 (12), LA2970 (13), LA 0030 (14), LA0806 (15), LA2377 (16), LA 2971 (17), LA2661 (18), LA4345 (19), LA4452 (20).

2.2. The Reciprocal-Half-Crossing Between Pure Tomato Lines in the First Agricultural Season (2022)

In light of the results of the DNA test (genetic fingerprint) that was previously conducted, six pure genetically distant lines were selected bearing the following codes: LA3695 (2), LA2804)

(9), LA4355 (10), LA4449 (11), LA2970 (13), and LA0806 (15). These selected lines will be coded to represent the parents involved in the hybridization (P1, P2, P3, P4, P5, P6). This selection based on genetic variation allows for high genetic diversity in plant breeding programs. The seeds of the selected pure lines were planted based on the genetic dimension between them in a private nursery on 15/10/2022 in sterile cork plates. After all the seeds germinated and were verified, service operations of irrigation, fertilization, and control were applied until 5-6 true leaves of the plant (seedling) appeared, and then they were transferred to the permanent field (a plastic house with a width of 9 meters and a height of 3.20 meters) located in the Baqubah Governmental Nursery on 15/11/2022. After preparing the soil of the plastic house by plowing, smoothing, and making terraces, it was divided into six terraces. The one terrace contains two planting lines; the distance between one line and another is 40 cm, and between one plant and another is also 40 cm within one terrace. Chemical and organic fertilizers and preventive pesticides (fungicide and insecticide) were added to the soil according to the recommended quantities, and the soil was covered with black polyethylene [15,16]. The seedlings were planted on lines alongside the GR irrigation pipes in the shape of a duck's paw, with two irrigation lines for each terrace. Each pure line was planted with 30 plants. We continued to provide the plants with balanced fertilizers, nutrients, and humic, in addition to amino acids, according to scientific recommendations, while carrying out continuous control operations for the whitefly. When the plants reached the flowering stage, they were introduced into a reciprocal-half-crossing program according to the second method of [17] to produce generation hybrids as shown in Table (1), by transferring pollen grains from the anther of a flower as a father that is open and fully developed physiologically to the stigma of another flower as a mother before it opens and is in the cream color stage, and after completing the neutering process on them (the neutering process of the flower of the mother plant) by removing the male parts (anthers), and then they were wrapped in tin bags or paper bags to ensure that pollen grains from another plant were not mixed, with marks indicating the parents involved in the hybridization (name of father and mother). This process continued daily for all the parents involved in the hybridization (the strains used as father and mother) and in one direction until the completion of the crossings. After ensuring the success of the hybridization and fruit setting process and monitoring the fruits until fully ripe, all the seeds were extracted and placed in paper bags, and all the information related to the hybridization was recorded on them. This process continued until a sufficient quantity of seeds was obtained. They were stored at a suitable temperature and humidity until used in the following season.

Table 1. Reciprocal-half crosses between pure lines of tomato.

Parents	P1	P2	P3	P4	P5	P6
P1	+	*	*	*	*	*
P2		+	*	*	*	*
P3			+	*	*	*
P4				+	*	*
P5					+	*
P6						+

2.3. Comparing Hybrids with Their Parents Under the Influence of Two Types of Calcium Fertilizer in the Second Season (2023)

The experiment was carried out in the plastic houses of the Baqubah Government Nursery, which is affiliated with the Diyala Agriculture Directorate. The second-season experiment included two factors: comparing the genetic compositions of the parents with their hybrids (the first factor) and the influence of spraying with two types of calcium fertilizer (the second factor). As for the foliar fertilization factor with calcium, there were the following levels: comparison (spraying with

distilled water only), spraying with nano-calcium at a concentration of 2 g L⁻¹ (recommendation of the producing company) provided by the Iranian company, and spraying with ordinary calcium at a concentration of 2 g L⁻¹ (recommendation of the producing company) provided by the Spanish Disper company.

2.4. The Cultivation

The seeds of the parents and hybrids were planted in cork plates on 9/8/2023 in one of the private nurseries in the Khan Bani Saad area. After reaching the stage of four to five true leaves, they were transferred to plastic houses on 17/10/2023. The area of the experimental unit was 3.584 meters with dimensions of 2.8 x 1.28 m, with 7 plants in the experimental unit, under the GR drip irrigation system. The field was irrigated before planting the seedlings to moisten the soil, and the seedlings, which were 10-15 cm long and 4-3 mm in diameter, were planted next to the irrigation pipes in the middle of the terrace, according to the split-plot system with a complete random block design (RCBD). To ensure ease of spraying, calcium fertilization levels (ordinary and nano) will occupy the main terraces, while the genetic compositions will be placed in the secondary terraces. The genetic compositions will consist of 21 (15 single hybrids with 6 parents), resulting in 63 treatments. Each treatment will be replicated three times, resulting in a total of 189 experimental treatments. Soil samples were taken before the planting experiment from different locations in the field at a depth of 30 cm and were representative of the field soil and mixed well. It was analyzed in the Laboratory of Agricultural Research, Iraq (Table 2). The plants' spraying process began before the flowers appeared on the plant and continued for three sprays, with ten days between each spray. The plant service operations continued after planting, including irrigation and weeding as needed at regular times. A preventive program was followed to protect against insects and diseases until the fruits were harvested.

Table 2. The physical and chemical properties of soil.

Measurements	Value	Unit of measurement
Texture of soil	Sandy loam	-
Clay	156.70	%
Silt	284.30	%
Sand	559	%
Ph	7.49	-
Ec	1.41	ds.m ⁻¹
N	18.13	mg. kg ⁻¹
P	14.10	mg. kg ⁻¹
K	174.89	mg. kg ⁻¹
S	11.00	mg. kg ⁻¹
Organic matter	1.53	%
Caco3	237.10	g. kg ⁻¹

2.5. The Studied Traits

2.5.1. Number of Inflorescences

It was calculated for the seven plants in the experimental unit, and the average was calculated.

2.5.2. Number of Flowers in One Inflorescence

It was calculated according to the following equation:

$$\text{Number of flowers in one inflorescence} = \left(\frac{\text{Average number of total flowers in one plant}}{\text{number of inflorescences in one plant}} \right)$$

2.5.3. Percentage of Fruit Set (%)

It was calculated for each experimental unit according to the following equation:

$$\text{Percentage of fruit set} = \left(\frac{\text{Average number of fruits per plant in the experimental unit}}{\text{Average number of flowers for the same plant}} \right) \times 100$$

2.5.4. Number of Fruits

It was calculated for each experimental unit according to the following equation:

$$\text{Average number of fruits} = \left(\frac{\text{Number of fruits in the experimental unit}}{\text{Number of plants}} \right)$$

2.5.5. Plant Yield (kg)

It was calculated by taking the cumulative yield of the experimental unit and dividing by the number of plants and recording the average.

3. Results

Figure 1 presented a tree analysis diagram, also known as a relative tree, based on SSR-P markers for pure tomato lines.

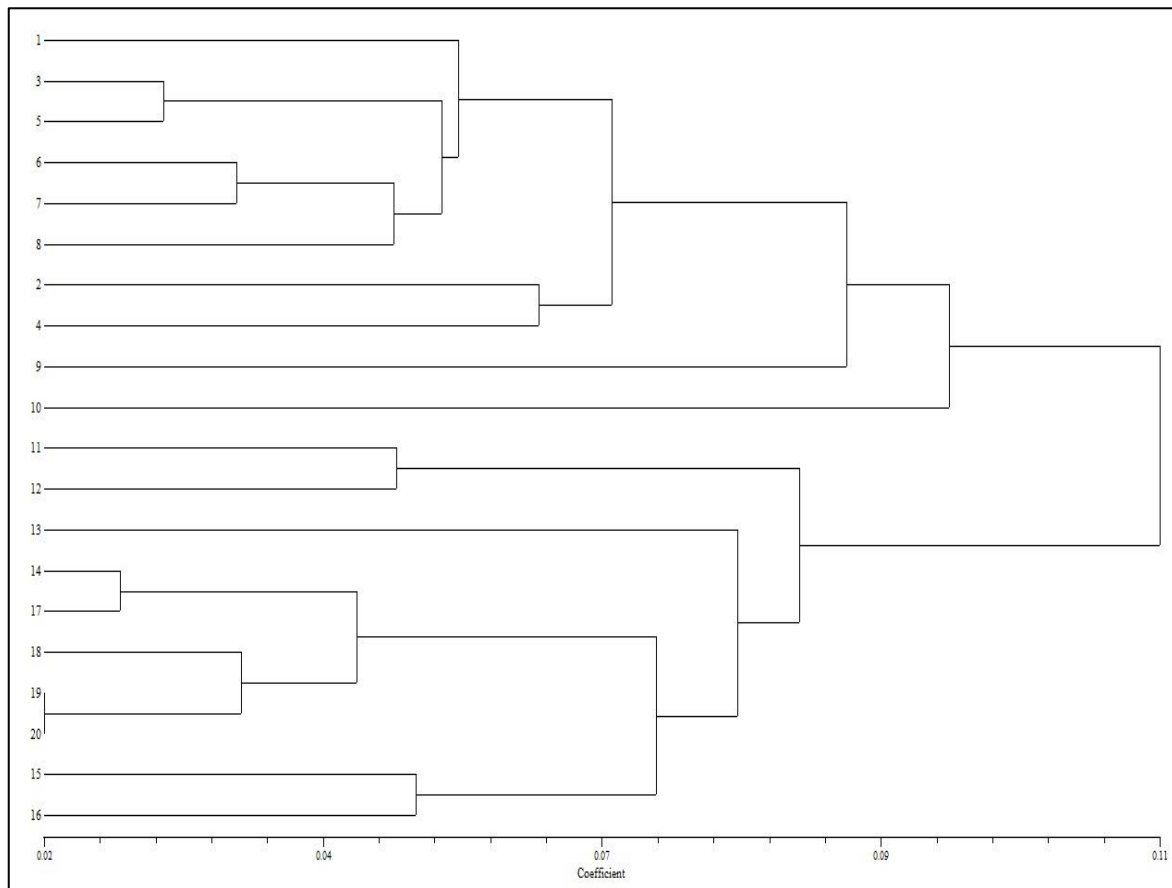


Figure 1. Tree analysis diagram (relative tree) of pure tomato lines according to SSR-P markers.

3.1. Number of Inflorescences

The results of Table (3) showed a significant effect of genetic compositions in the number of inflorescences, as parent 5 recorded the highest value of 28.33, while parent 4 gave the lowest value of 16.15. As for the individual hybrids, the hybrid 5×6 gave the highest value of 37.30, while it decreased in the hybrid 2×4 to 17.07. Calcium also had a significant effect on the number of inflorescences, as the genetic compositions recorded the highest number of 27.34 in the Ca-nano treatment, while it decreased to 23.21 in the control treatment. The interaction between the genetic compositions and calcium levels significantly influenced the trait, as the plants of parent 5 in the Ca-nano treatment gave the highest number of inflorescences, reaching 30.70, while the plants of parent 2 in the control treatment recorded the lowest number of inflorescences, reaching 16.35. As for the individual hybrids, the 1×5 hybrid under the Ca-nano treatment gave the highest number, reaching 49.04, while it decreased in the 2×4 hybrid to 15.54 under the control treatment.

Table 3. Effect of genetic compositions, calcium and their interaction on number of inflorescences in the plant.

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	19.32 q-t	21.37 nop	22.26 n	20.98 J
2	16.35 yz	18.15 t-x	18.92 stu	17.81 M
3	17.28 wxy	18.70 tuv	20.48 pq	18.82 L
4	14.04	16.37 yz	18.04 t-x	16.15 O
5	25.15 m	29.15 k	30.70 j	28.33 G
6	20.04 p-s	22.02 no	24.48 m	22.18 I
1×2	19.04 rst	20.92 op	21.18 nop	20.38 JK
1×3	17.48 v-y	19.32 q-t	20.15 p-s	18.98 L
1×4	16.92 y	18.04 t-x	18.70 tuv	17.89 M
1×5	37.71 d	43.48 b	49.04 a	43.41 A
1×6	31.18 ij	34.04 fg	36.32 e	33.85 D
2×3	18.37 t-w	20.32 pqr	21.15 nop	19.95 K
2×4	15.54 z	17.64 u-y	18.04 t-x	17.07 N
2×5	29.46 k	32.70 h	33.15 gh	31.77 E
2×6	26.66 l	30.92 j	31.04 j	29.54 F
3×4	18.04 t-x	20.37 pq	21.26 nop	19.89 K
3×5	32.37 hi	36.41 e	37.15 de	35.31 C
3×6	29.16 k	32.26 hi	34.92 f	32.11 E
4×5	24.40 m	26.32 l	28.70 k	26.47 H
4×6	24.45 m	26.92 l	28.48 k	26.62 H
5×6	34.48 f	37.37 de	40.04 c	37.30 B
Mean of calcium	23.21 C	25.85 B	27.34 A	

3.2. Number of Flowers in the Inflorescence

The results in Table (4) showed that parent 4 was the best among the parents for the number of flowers in the inflorescence, as it gave 5.908, while the parent 5 recorded the lowest number of 3.657. The single hybrid 1×4 gave the highest value of 5.765, while the hybrid 1×5 recorded the lowest number of flowers, which amounted to 2.819. The results of the same table showed the superiority of the control treatment in the number of flowers, which reached 4.634, which differed significantly from ordinary and nano calcium treatments, which reached 4.338 and 4.292, respectively. As for the interaction between calcium and genetic compositions, it had a significant

effect on the trait, as the plants of parent 4 at the control treatment gave the highest number of flowers, reaching 6.519, compared to parent 5 at the Ca-nano treatment. The hybrid 3×4 at the control treatment recorded the highest number of flowers, reaching 5.919, while it decreased to 2.516 flowers in the hybrid 1×5 at the Ca-nano treatment.

Table 4. Effect of genetic compositions, calcium and their interaction on number of flowers per inflorescence.

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	4.472 lm	4.249 n-q	4.178 pqr	4.299 G
2	5.419 e	5.065 gh	4.994 gh	5.159 D
3	5.383 e	5.117 fg	4.800 ij	5.100 D
4	6.519 a	5.814 bc	5.392 e	5.908 A
5	3.968 st	3.582 wxy	3.421 $\bar{z}b$	3.657 J
6	4.753 ij	4.472 lm	4.309 m-p	4.511 F
1×2	4.542 kl	4.318 m-p	4.719 j	4.526 F
1×3	4.700 jk	4.525 l	4.381 l-o	4.535 F
1×4	5.842 bc	5.697 cd	5.757 bcd	5.765 B
1×5	3.166 $\bar{c}$	2.776 $\bar{d}$	2.516 $\bar{e}$	2.819 M
1×6	3.730 uvw	3.508 $\bar{x}a$	3.384 $\bar{z}b$	3.541 K
2×3	5.191 f	4.899 hi	4.796 ij	4.962 E
2×4	5.824 bc	5.426 e	5.702 cd	5.651 C
2×5	3.731 uvw	3.456 $\bar{x}b$	3.557 w-z	3.581 JK
2×6	4.206 o-r	3.791 uv	3.381 tu	3.943 I
3×4	5.919 b	5.624 d	5.713 cd	5.752 B
3×5	3.624 vwx	3.367 $\bar{a}b$	3.524 $\bar{x}a$	3.505 K
3×6	4.171 pqr	3.841 tu	3.720 uvw	3.911 I
4×5	4.251 n-q	4.165 pqr	4.056 rs	4.157 H
4×6	4.398 lmn	4.098 qrs	4.200 o-r	4.232 GH
5×6	3.510 $\bar{x}a$	3.299 $\bar{c}$	3.182 $\bar{c}$	3.330 L
Mean of calcium	4.634 A	4.338 B	4.292 C	

3.3. Fruit Set Percentage (%)

It is clear from the data in Table (5) that there are significant differences between the genetic compositions in the trait of fruit set percentage, as the percentages ranged between 33.11% for parent 6 and 23.89% for parent 5, while for the individual hybrids, the hybrid 4×6 achieved the highest value of 35.65% and decreased to 21.62% in the hybrid 2×3. The fruit set percentage increased significantly when the plants were sprayed with calcium fertilizer, as the Ca-nano treatment gave the highest value of 29.02%, followed by ordinary calcium, which achieved 28.57%, compared to the control treatment (spraying with distilled water), which recorded 28.05%. The interaction between the study factors had a significant effect on the percentage of fruit set. Parent 6 and the Ca-nano treatment had the highest percentage of 34.21%, while the lowest value was 23.08% in parent 5 and the control treatment. As for the hybrids, the percentage ranged between 36.56% for the 3×4 hybrid with the control treatment and 21.62% for the 2×3 hybrid and the control treatment.

Table 5. Effect of genetic compositions, calcium and their interaction on fruit set percentage (%).

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	30.23 no	30.54 m	30.06 o	30.28 G
2	24.85 $\bar{c}$	26.37 $z\bar{a}$	26.19 $\bar{a}$	25.80 N
3	26.99 xy	28.42 u	28.99 s	28.13 K
4	30.82 l	31.73 ghi	31.92 g	31.49 D
5	23.08 $\bar{i}$	24.08 $\bar{e}$	24.53 $\bar{d}$	23.89 P
6	31.51 ij	33.61 f	34.21 e	33.11 B
1×2	27.16 wx	27.68 v	27.34 w	27.39 L
1×3	29.37 r	29.72 pq	31.59 hij	30.22 G
1×4	28.69 t	28.38 u	30.87 l	29.31 I
1×5	24.48 $\bar{d}$	25.63 $\bar{b}$	26.80 y	25.63 O
1×6	29.09 s	29.54 qr	31.02 kl	29.88 H
2×3	21.62 $\bar{k}$	22.33 $\bar{j}$	23.88 $\bar{e}f$	22.61 S
2×4	31.76 gh	30.82 l	29.09 s	30.55 F
2×5	22.51 $\bar{j}$	23.36 $\bar{h}$	23.61 $\bar{g}$	23.16 R
2×6	29.52 qr	29.85 p	31.21 k	30.19 G
3×4	36.56 a	31.44 j	30.37 mn	32.79 C
3×5	23.31 $\bar{h}$	23.82 $\bar{f}g$	23.63 $\bar{g}$	23.58 Q
3×6	29.12 s	31.55 hij	31.55 hij	30.74 E
4×5	26.26 $\bar{a}$	26.84 y	26.35 $\bar{a}$	26.48 M
4×6	35.58 c	35.36 d	36.01 b	35.65 A
5×6	26.57 z	28.97 s	30.31 n	28.62 J
Mean of calcium	28.05 C	28.57 B	29.02 A	

3.4. Number of Fruits Per Plant

It is noted from the results of Table (6) that there are significant effects of genetic compositions on the number of fruits, as parent 6 outperformed with the highest number of fruits, reaching 33.07, compared to parent 2, which recorded the lowest number, reaching 23.67, and the hybrid 4×6 also outperformed with the highest number of fruits, reaching 40.12, and the hybrid 2×3 with the lowest number of fruits, reaching 22.36. The effect of calcium, both ordinary and nano, was significant on the number of fruits, as the Ca-nano treatment achieved the highest number of fruits, reaching 32.10, compared to the control treatment, which recorded the lowest number, reaching 28.58. The interaction between calcium and genetic compositions had a significant effect on the number of fruits, as parent 6 under the Ca-nano treatment gave the highest number of fruits, reaching 36.10, compared to parent 2 under the control treatment, which recorded 22.02. The hybrid 4×6 and the Ca-nano treatment achieved the highest number of fruits, reaching 43.09, while the hybrid 2×3 and the control treatment gave the lowest number of fruits, reaching 20.62.

Table 6. Effect of genetic compositions, calcium and their interaction on number of fruits in the plant.

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	26.12 v	27.74 rst	27.96 q-t	27.27 K
2	22.02 $\bar{d}$	24.24 $yz\bar{a}$	24.75 yz	23.67 O
3	25.11 wxy	27.20 tu	28.50 pqr	26.93 K

4	28.19 qrs	30.69 klm	31.05 k	29.81 HI
5	23.04 $\overline{bc}$	25.16 wxy	25.77 vwx	24.65 N
6	30.02 lmn	33.10 i	36.10 fg	33.07 F
1×2	23.49 $\overline{ab}$	25.01 xyz	27.33 st	25.27 M
1×3	24.13 $\overline{za}$	25.99 vw	27.89 q-t	26.00 L
1×4	28.36 pqr	29.17 nop	33.24 i	30.25 H
1×5	29.24 nop	30.96 k	33.09 i	31.09 G
1×6	33.85 i	35.30 gh	38.15 d	35.76 D
2×3	20.62 $\overline{e}$	22.24 $\overline{cd}$	24.23 $\overline{yz\overline{a}}$	22.36 P
2×4	28.74 opq	29.50 mno	29.92 lmn	29.38 IJ
2×5	24.75 yz	26.42 uv	27.85 q-t	26.34 L
2×6	33.12 i	35.00 h	37.13 e	35.08 E
3×4	39.03 cd	36.02 g	36.89 ef	37.31 C
3×5	27.36 st	29.22 nop	30.95 k	29.17 J
3×6	35.43 gh	39.11 c	41.01 b	38.51 B
4×5	27.25 tu	29.43 mno	30.69 kl	29.12 J
4×6	38.27 cd	39.02 cd	43.09 a	40.12 A
5×6	32.17 j	35.74 gh	38.64 cd	35.51 DE
Mean of calcium	28.58 C	30.27 B	32.10 A	

3.5. Yield Per Plant (kg)

The data in Table (7) confirmed that parent 5 was distinguished by the highest yield per plant, reaching 3.621 kg, while parent 2 recorded the lowest total yield, reaching 2.213 kg. The hybrid 5×6 was significantly superior with the highest total yield per plant, reaching 5.866 kg, compared to the hybrid 1×2, which recorded the lowest total yield, reaching 2.216 kg. The nano calcium spray treatment was significantly superior and gave the highest average for this trait, reaching 4.094 kg compared to the control treatment, which gave the lowest average, reaching 2.976 kg. The results of the same table indicate that the interactions between the genetic compositions and the calcium spray treatments of the ordinary and nano calcium also had a significant effect, as the interaction between parent 5 and nano calcium spray achieved the highest yield per plant, reaching 4.351 kg plant⁻¹, while parent 2 under the control treatment gave the lowest yield, reaching 1.841 kg. The hybrid 5×6 with the nano calcium treatment was distinguished by the highest yield per plant, reaching 6.370 kg plant⁻¹, compared to the hybrid 1×2 plants in the F0 treatment, which gave 1.770 kg plant⁻¹.

Table 7. Effect of genetic compositions, calcium and their interaction on plant yield (kg plant⁻¹).

Genetic compositions	Calcium (Ca)			Mean of genetic compositions
	Ca (0 g L ⁻¹)	Ca (2 g L ⁻¹)	Ca-nano (2 g L ⁻¹)	
1	2.140 $\overline{hij}$	2.495 $\overline{d - g}$	3.150 wxy	2.595 K
2	1.841 $\overline{kl}$	2.214 $\overline{g - j}$	2.583 $\overline{c - f}$	2.213 N
3	2.059 $\overline{ijk}$	2.432 $\overline{e - h}$	3.114 xyz	2.535 KL
4	1.934 $\overline{jkl}$	2.307 $\overline{f - i}$	2.997 $\overline{yz\overline{a}}$	2.413 LM
5	3.170 wxy	3.340 u-x	4.351 mno	3.621 I
6	2.642 $\overline{b - e}$	3.095 xyz	3.604 stu	3.114 J
1×2	1.770 $\overline{l}$	2.138 $\overline{hij}$	2.739 $\overline{a - d}$	2.216 N
1×3	2.041 $\overline{i - l}$	2.402 $\overline{e - h}$	2.890 $\overline{y - b}$	2.445 KLM
1×4	2.076 $\overline{ijk}$	2.229 $\overline{g - j}$	2.843 $\overline{z - c}$	2.383 LM
1×5	3.930 pqr	5.165 e-h	5.553 cd	4.883 C

1×6	3.664 rst	4.888 hij	5.289d-g	4.614 DE
2×3	2.208 <i>gj</i>	2.403 <i>e – h</i>	2.828 <i>z-ĉ</i>	2.480 KLM
2×4	2.128 <i>h – k</i>	2.258 <i>ghi</i>	2.650 <i>b – e</i>	2.346 MN
2×5	3.311 vwx	4.184 op	4.533 k-n	4.010 H
2×6	3.479 s-v	4.570 klm	4.655 jkl	4.235 G
3×4	3.612 stu	3.983 pq	4.442 l-o	4.013 H
3×5	4.261 no	5.404 de	5.761 bc	5.142 B
3×6	3.732 qrs	5.084 fgh	5.461 d	4.759 CD
4×5	3.709 q-t	4.780 ijk	5.140 e-h	4.543 EF
4×6	3.429 t-w	4.739 jk	5.024 ghi	4.398 F
5×6	5.350 def	5.878 b	6.370 a	5.866 A
Mean of calcium	2.976 C	3.619 B	4.094 A	

4. Discussion

The findings of the study showed significant effects of the genetic compositions for all the studied characteristics, this may be attributed to the difference in the genes responsible for cell growth, division, and elongation for different genetic compositions and their interaction with environmental conditions, through their effect on growth hormones such as auxins and gibberellins in addition to other hormones, as well as the difference in the efficiency of carbon metabolism processes and the utilization of nutrients between genetic compositions and the extent of their interaction with environmental conditions, which affects vegetative growth traits positively or negatively [18-21]. The vegetative growth characteristics are mainly controlled by several genetic factors in combination with other environmental factors that affect the growth of these genetic compositions [22,23,20]. As the high significance of the genetic compositions is clear evidence of the extent of the differences between them genetically, which required studying their genetic behavior and knowing the nature of the work of the genes that control the inheritance of the studied traits, and that these results are consistent with [18,21,24] during their study on tomato plants. The effect of calcium in raising the values of vegetative growth traits illustrates the importance of calcium, as calcium plays an effective role in cell division and elongation, regulating the permeability of cell membranes, and the growth of meristematic tips responsible for new growth. It also activates some enzymes important for growth, such as Adenosine Tri Phosphate, ATPase, and Arginine Kinase, in addition to its role in increasing the efficiency of photosynthesis and thus the accumulation of carbohydrates, which leads to activating the vegetative growth of plants, thus improving most of the vegetative growth traits [25]. As for the increase in the percentage of calcium in the leaves with the increase in the concentration used, the reason for this may be due to the ease of absorption of calcium from the leaves when sprayed on the plant leaves. Calcium is also a weakly mobile element within plant tissues, so the largest proportion of it remains in the leaves and accumulates in them, or it may accumulate in the gaps in the form of calcium oxalate, or form chelating compounds in the protoplasm of cells). These results are consistent with [26].

Conclusion

Because the colored tomato lines that were used in the study are genetically pure, they can be used as parents in other breeding projects to accelerate tomato growth and increase yield. The 5×6 crossbreed was superior in number of inflorescences and yield per plant, and the 4×6 crossbreed was superior in fruit set percentage and number of fruits per plant, while the 1×4 crossbreed was superior in number of flowers in the inflorescence. The use of nano-calcium at a concentration of 2 g L⁻¹ improved most of the vegetative growth traits.

References

- [1] Perveen, R, Suleria HAR, Anjum FM, Butt MS, Pasha I and Ahmad S 2015. Tomato (*Solanum lycopersicum*) carotenoids and lycopenes chemistry; metabolism, absorption, nutrition, and allied health claims-a comprehensive review. *Critical Reviews in Food Science and Nutrition*. 55:919-929.
- [2] Lenucci M and Dalessandro G 2009. Carotenoid content during tomato (*Solanum lycopersicum* L.) fruit ripening in traditional and high-pigment cultivars. *Italian Journal of Food Science* 21(4).
- [3] Lenucci, MS, Cadinu D, Piro G and Dalessandro G 2006. Antioxidant Composition in Cherry and High-Pigment Tomato Cultivars. *J. Agric. Food Chem.*(54). 7.2613–2606 .
- [4] Kulus D 2022. Genetic diversity for breeding tomato. In: Priyadarshan P., Jain S.M. (eds) *Cash Crops*. Springer, Cham. https://doi.org/10.1007/978-3-030-74926-2_13
- [5] Al-Shammari, AMA 2005. Complete crosses and estimation of genetic parameters of some traits in tomato grown under plastic tunnels. PhD thesis. College of Agriculture. University of Baghdad. Ministry of Higher Education and Scientific Research. Republic of Iraq.
- [6] Ahmad, R, Anjum, MA and Balal, RM 2020. From markers to genome based breeding in horticultural crops: An Overview. *Phyton-International Journal of Experimental Botany*. 89(2):183-204.
- [7] Scarano, D, Rao, R, Masi, P and Corrado, G 2015. SSR fingerprint reveals mislabeling in commercial processed tomato products. *Food Control* 51:397–401.
- [8] Al-Moussawi, ANA. Al-Yasari, JWM 2016. The effect of adding potassium and calcium mixed in reducing salt stress for some wheat varieties and its relationship to some physiological and biochemical indicators. *Karbala University Scientific Journal* (1) (14).
- [9] Al-Rifai, FN 2016. "Basic concepts in nanotechnology" - Thi Qar University, College of Science. Issue (19 Dar Al-Kutub for Printing and Publishing.
- [10] White, PJ, and Broadley, MR 2003. Calcium in plants. *Annals of Botany* 92(4), 487-511.
- [11] Michard, E, Lima, PT, Borges, F, Silva, AC, Portes, MT., Carvalho, JE 2011. Glutamate receptor-like genes form Ca^{2+} channels in pollen tubes and are regulated by pistil D-serine. *Science* 332(6028), 434-437.
- [12] Monshausen, GB, Miller, ND, Murphy, AS, and Gilroy, S 2011. Dynamics dependent Ca^{2+} and pH signaling in root growth revealed by integrating high-resolution of auxin imaging with automated computer vision-based analysis. *The Plant Journal* 65(2), 309-318.
- [13] Ortiz-Ramírez, C, Michard, E, Simon, AA, Damineli, DSC, Hernández-Coronado, M, Becker, JD 2017. Glutamate receptor-like channels are essential for chemo taxis and reproduction in mosses. *Nature* 549, 91.
- [14] Zhang, S, Pan, Y, Tian, W, Dong, M, Zhu, H, Luan, S 2017. Arabidopsis CNGC14 mediates calcium influx required for tip growth in root hairs. *Molecular Plant* 10(7), 1006-1004 .
- [15] Saleh, HM and Salman IS 2010. Bulletin on recommended fertilizer additions according to available fertilizers for summer and winter crops. Ministry of Agriculture. Central Fertilizer Combinations Committee. Al-Farah Press. Baghdad.
- [16] Al-Shammari, AMA 2018. Effect of organic fertilization and breeding method on some quantitative yield traits of three genetic combinations of tomato (*Lycopersicon esculentum* Mill) grown under plastic house conditions. *Al-Rafidain Agriculture Journal*. Volume (46) Issue (2).
- [17] Griffing, B 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences* 9(4):463-493. <https://doi.org/10.1071/B19560463>
- [18] Hamdi, G J 2022. Estimation of genetic dimension and genetic parameters of tomato growth and yield traits by half-crossing under the influence of water stress. PhD thesis. College of Agriculture. University of Diyala.
- [19] Al-Ubaidi, ARHH 2022. Derivation of single and triple hybrids in tomato And estimation of some genetic parameters in it. PhD thesis. College of Agriculture. University of Diyala.



- [20] El- Sappah, AH., Islam MM, Rather SA, Li J, Yan K, Xianming Z, Liang Y, Abbas M 2022. Identification of novel root-knot nematode (*Meloidogyne Incognita*) resistant tomato genotypes. *The Journal of Animal and Plant Sciences*. 33(1):1-17.
- [21] Islam, S, Hassan L and Hossain MA 2022. Breeding potential of some exotic tomato lines: a combined study of morphological variability, genetic divergence, and association of traits. *Phyton-International Journal of Experimental Botany*, 91(1): 97–114.
- [22] Lázaro, A 2018. Tomato landraces: An analysis of diversity and preferences. *Plant Genetic Resources: Characterization and Utilization*, 16(4):315-324.
- [23] Anuradha, B, Saidaiah P, Reddy KR, Sudini H, Geetha A 2021. Mean performance of 40 genotypes in tomato (*Solanum lycopersicum* L.). *International Journal of Chemical Studies*. 9(1):279-283.
- [24] Essa, BA, and Alwan, OK 2024. Impact evaluation combining ability of tomato hybrids and lines for production and fruit quality traits. *Agronomía Meso americana*, Volumen 35: Artículo. 57558-57558. doi.org/10.15517/am.2024.57558.
- [25] Barker, AV, Pilbeam AV 2015. *Handbook of Plant Nutrition*. 2nd ed. CRC Press. Boca Raton, FL, USA. pp. 537-564.
- [26] Al-Moussawi, HN 2020. The effect of salicylic acid and calcium on the growth and yield of tomatoes. Master's thesis, College of Agriculture. University of Baghdad. Iraq.

Measuring Radioactive Pollution by ^{238}U , ^{232}Th , ^{40}K in Table Salt Using (HPGe)

Hanan Kaayem Ghazi¹ and Hadi D. Alattabi²

^{1,2}Department of Physics, College of Science, Wasit University, Wasit, Iraq.

¹E-mail: hananalkazaali@gmail.com

²E-mail: alattabih@uowasit.edu.iq

Abstract. In this work, the high-purity germanium detector (HPGe) was used to study the concentration of radioactivity of the ^{238}U , ^{232}Th , ^{40}K series in refined and unrefined table salt samples. The absorbed dose in the air was calculated and its value in the refined salt samples was (17.8920, 18.4957) (nGy/h), while in samples of unrefined salt was (21.0532, 21.1989) (nGy/h). The effective annual equivalent dose was (0.0315, 0.0309) (mSv/yr⁻¹) in refined table salt samples, and unrefined table salt samples was (0.0348, 0.0346) (mSv/yr⁻¹). The radioactive equivalent of radium in refined salt samples (33.9959, 32.8582) (Bq/kg), while in unrefined salt samples (42.0295, 39.8220) (Bq/kg). The risk standard was also calculated in the refined salt samples and unrefined salt samples, and its value was less than one to be within the permissible limits for the refined and unrefined salt samples. Radioactive concentrations of ^{238}U , ^{232}Th , ^{40}K , chains were also measured in samples of refined and unrefined table salt. The lowest radioactive concentration in the ^{238}U series was ^{214}Pb , which has an energy of 295.22(keV) in refined table salt. The lowest radiation concentration in the ^{232}Th series was ^{228}Ac , which has an energy of 911.07(keV) in refined table salt, equal 0.25 Bq/kg, for ^{40}K was seen to be equal to 289.82 ± 0.14 , 303.94 ± 0.01 (Bq/kg) in refined table salt and unrefined table salt respectively. All results were within permissible limits, meaning that salt in Iraq is safe for use and does not pose any danger.

Keywords. HPGe, Table salt, Radioactivity, Absorb Dose Rate, Hazard Index.

1. Introduction

Radionuclides are widely distributed between the atmosphere and the lithosphere of earth. Radionuclides are defined as materials that consist of unstable atoms [1]. These atoms may release energy; this emitted atomic energy is what we call radiation, Radiation exists in the form of small bundles of energy called photons, The earth and its inhabitants are exposed to radiation emitted from radioactive materials found in the crust of the earth, as well as from the sun, the radioactivity may be found in soils, rocks, water, and plants and other sources [2,3]. The three most common chains that occur in nature arise from decays of ^{238}U , ^{235}U , and ^{232}Th [4]; the quantity of terrestrial radiation varies across the world owing to differences in uranium and thorium concentrations in soil, as well as their progenies [5].

With the passage of time and the arms race that the world is witnessing and the effects of weapons on living and non-living organisms, the faces of a type of environmental pollution have emerged, which is radioactive pollution, which is defined as pollution resulting from the spread of

nuclear and atomic waste from the reactor or as a result of nuclear tests and the use of nuclear weapons in Wars, Radioactive contamination occurs when radioactive materials (solid, liquid, gaseous) are released or leaked from the containers they contain through holes or as a result of their explosion [6,7,8].

Salt is considered as an important food additive because it is used in all foods, and salt being free of radioactive materials is essential for human health. Therefore, through this study, natural radioactive materials will be detected in refined table salt and unrefined table salt, in this study, two samples of natural salt (S1,S2) and two samples of salt available in local markets were taken (S3,S4), and natural radioactive materials were detected using a high-purity germanium detector.

2. Equipment Used and Materials

In this research, a high gamma ray spectroscopy system was used to measure the concentration of radioactivity in samples with background radiation. The measurement system consists of a nuclear detector made of high-purity germanium (HPGe), with a crystal size of 96 cm³ and an energy separation capacity of 2.2 keV at the 1332 keV energy line for gamma rays emanating from the standard source of the radioactive cobalt isotope (Co⁶⁰) operates at an operating voltage of (3000V) and requires cooling during its operation, which requires the use of liquid nitrogen to cool the detector crystal well. The detector is surrounded by a main cylindrical shield of lead to reduce background sources of radiation from X-ray fluorescence. The detector is connected to successive parts. These are the primary amplifier of the electronic signal, then the main amplifier, then the electronic computer that includes a channel selector to record and analyze the gamma ray spectrum [9,10].

(Fig. 1) shows the high-purity germanium detector, the high-voltage feeder that supplies the detector with the voltage necessary to operate it, its ranges between (1500-5000 V), a pre-amplifier to extract the weak signal coming from the detector protect it from interference, a linear amplifier to exist the incoming signals from the primary amplifier, and a channel analyzer that records the entire spectrum at once, then translates the pulses arriving at it, putting them in the form of numbers, divides them, records each number in a special channel, stores it in its memory, and displays the entire spectrum on its display screen, and bulletproof barriers that are coated from the inside with a thin layer of cadmium and another layer these barriers reduce the contribution of background radiation resulting from radioactive sources present in the laboratory or from nature [11-13].



Figure 1. High Purity Germanium Detector (HPGe).

2.1. Sample Preparation

Each sample was placed individually in an electric oven at 104°C - 24h until it is dried to remove any remaining moisture. Then it was ground with a manual grinder or an electric grinder, and sieved with a manual sieve with holes whose diameter is 1 mm to remove impurities and to obtain homogeneous grains as shown in Fig (2). Then, special containers was weighed in which the samples will be placed using an electronic scale.

After the samples to be measured were prepared, and each sample is measured for 24 hours, where the sample pads were fixed around the detector crystal and surrounded by a lead shield (20 mm thick) lined with a thin layer of copper to absorb X-rays and other things.



Figure 2. Sample preparation of salt.

2.2. Dosage Calculation

2.2.1. Absorb Dose Rate in Air

To evaluate any radiation risk, it is necessary to calculate the percentage of the absorbed dose in the air resulting from the exposure of the soil to radionuclides, whether they result from natural concentrations of radionuclides present in table salt, such as uranium U^{238} , thorium Th^{232} , and the radioactive isotope of potassium K^{40} , or not. To find the rate of absorbed dose in salt samples, equation (1) was used [14].

$$D=0.462A_{\text{Ra}}+0.604A_{\text{Th}}+0.0417A_{\text{K}} \text{ (nGy/h)} \quad (1)$$

Where: D: is absorbed dose rate ratio, A_{Ra} : is radioactive concentration of radium, A_{Th} : is radioactive concentration of thorium, A_{K} : is radioactive concentration of potassium [14,15].

2.2.2. Annual effective Dose Equivalent (AEDE)

The annual equivalent dose is a radiation parameter used to judge the extent of health effects resulting from the absorbed dose [14]. It can be found using equation (2).

$$D_{\text{eff}}=D \times 1.23 \times 10^{-3} \text{ m Sv/y} \quad (2)$$

2.2.3. Radium Equivalent Activity

It is used to ensure the uniform distribution of natural radionuclides, namely radium- 226, thorium- 232, and potassium- 40, that are measured in units (Bq/kg). The maximum value

allowed for the equivalent effectiveness of radium is (370 Bq/kg), and it can be found from equation (3).

$$Ra_{eq} (BqKg^{-1}) = A_{Ra} + 1.43A_{Th} + 0.077A_K \quad (3)$$

Where: A_{Ra} : is radioactive concentration of radium, A_{Th} : is radioactive concentration of a series of thorium, A_K : is radioactive concentration of potassium [14,16].

2.2.4. Hazard Index

It is a radiation coefficient used to determine external and internal radiation risks. The internal risk standard (H_{in}) and the external risk standard (H_{ex}) were calculated using equations (4) ,(5), and must be less than one, which is the globally accepted limit by international institutions [14,15,17].

$$H_{ex} = (A_{Ra}/370) + (A_{Th} / 259) + (A_K/4810) \leq 1 \quad (4)$$

$$H_{in} = (A_{Ra}/185) + (A_{Th}/259) + (A_K/4810) \leq 1 \quad (5)$$

3. Results and Discussion

Table (1) shows the radioactive concentrations of the 238U series for refined salt samples and unrefined salt samples. We were noticed that the radioactive concentrations of the 238U series for refined salt samples were lower than the radioactive concentrations for unrefined salt samples, as that evident in (Fig. 3). The lowest radioactive concentration in this series was 214Pb. This has energy of 295.22 keV in refined table salt. Table (2) and Fig. 4 explain 232Th series radioactive concentrations for samples of refined table salt and unrefined table salt. Table (3) explain 40K radioactive concentrations for samples of refined table salt and unrefined table salt, The average specific activities of the natural radionuclides occurring in the salt samples were found ($^{238}U=11.91 \pm 1.85 Bq/Kg$), ($^{232}Th=3.79 \pm 0.26 Bq/Kg$), ($^{40}K= 91.3 \pm 0.14 Bq/Kg$) in salt refined, and ($^{238}U=8.49 \pm 0.23 Bq/Kg$), ($^{232}Th=4.54 \pm 0.36 Bq/Kg$), ($^{40}K=44.3 \pm 0.01 Bq/Kg$) in salt unrefined. The UNSCEAR (2008) recommended standard indicate that the world's average specific activity of Ra 226, 232Th, and 40K are 33 Bq/kg, 45 Bq/kg, and 420 Bq/kg, respectively.

Table 1. 238U series radioactive concentrations for samples of refined table salt and unrefined table salt.

Series	Sample	Radio Nuclide	Energy (KeV)	salt refined (Bq /kg)	Sample	Salt unrefined (Bq /kg)
238U	S1	^{226}Ra	185.99	7.89 ± 0.07	S3	22.60 ± 0.06
	S1	^{214}Pb	241.92	2.24 ± 0.08	S3	2.48 ± 0.32
	S2	^{214}Pb	295.22	0.11 ± 0.00	S4	1.98 ± 0.14
	S1	^{214}Pb	351.99	2.46 ± 0.18	S4	2.46 ± 0.03
	S1	^{214}Bi	609.32	2.26 ± 0.13	S3	3.20 ± 0.09
	S2	^{214}Bi	1120.18	4.86 ± 0.04	S4	9.30 ± 0.40
	S2	^{214}Bi	1238.11	7.18 ± 0.27	S4	27.9 ± 0.12
	S2	^{210}Pb	46.54	22.05 ± 0.34	S3	29.88 ± 0.34
	Weight mean			6.773 ± 0.4		8.49 ± 0.23

Table 2. ^{232}Th series, ^{40}K radioactive concentrations for samples of refined table salt and unrefined table salt.

Series	Sample	Radio Nuclide	Energy (KeV)	Salt refined (Bq /kg)	Sample	Salt unrefined (Bq /kg)
^{232}Th	S1	^{212}Bi	727.17	0.43 ± 0.00	S3	3.44 ± 0.01
	S2	^{212}Bi	1620.56	2.89 ± 0.95	S3	8.71 ± 0.86
	S1	^{212}Pb	238.63	2.36 ± 0.03	S3	2.57 ± 0.33
	S2	^{212}Pb	300.09	0.47 ± 0.00	S4	2.43 ± 0.31
	S2	^{228}Ac	338.40	1.81 ± 0.14	S4	1.77 ± 0.01
	S2	^{228}Ac	911.07	0.25 ± 0.00	S3	2.71 ± 0.53
	S1	^{228}Ac	964.60	4.79 ± 0.34	S4	5.51 ± 0.47
	S1	^{228}Ac	1587.90	4.36 ± 0.96	S4	5.95 ± 0.43
	S1	^{208}Tl	510.72	12.85 ± 0.22	S4	13.52 ± 0.12
	S1	^{208}Tl	583.14	0.75 ± 0.04	S3	1.84 ± 0.77
	S2	^{208}Tl	860.47	2.06 ± 0.07	S4	2.53 ± 0.53
	S1	^{208}Tl	2614.47	2.51 ± 0.03	S3	3.49 ± 0.06
	Weight mean			3.79 ± 0.26		4.54 ± 0.36

Table 3. ^{40}K radioactive concentrations for samples of refined table salt and unrefined table salt.

Series	Energy (KeV)	Sample	Specific Activity Bq/kg
^{40}K	1460.80 KeV	S1	91.3 ± 0.14
		S2	69.6 ± 0.17
		S3	74.2 ± 0.21
		S4	44.3 ± 0.012

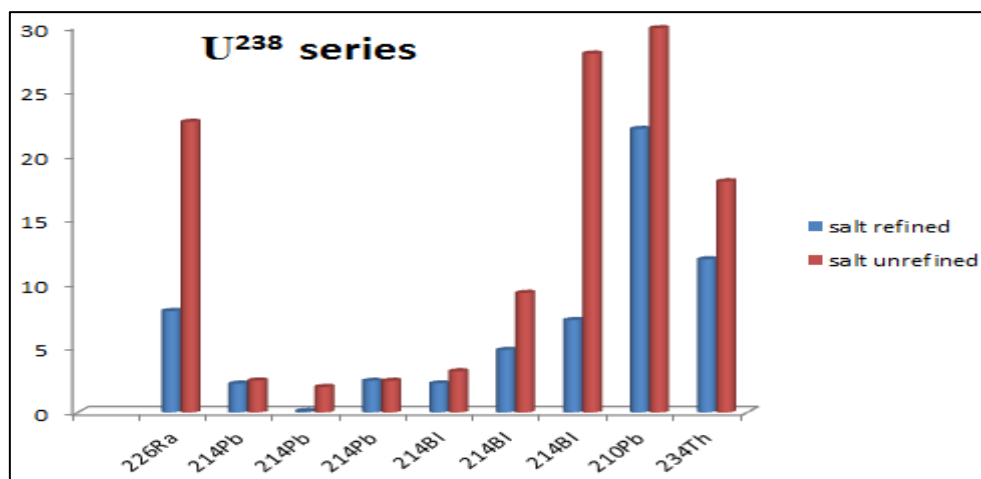


Figure 3. ^{238}U series radioactive concentrations for refined salt and unrefined salt samples.

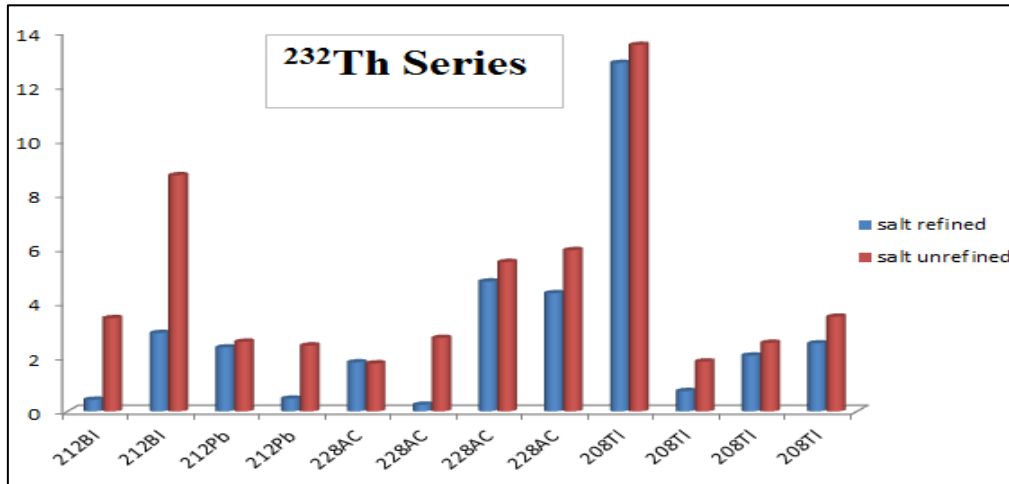


Figure 4. ²³²Th series radioactive concentrations for samples of refined table salt and unrefined table salt.

Through the use of equations (1, 2, 3, 4, 5) the results were presented in Table No. (4) and (Fig. 5) where the risk standard was <1 of all samples, (S1, S2) represents refined salt samples and (S3, S4) represent unrefined salt samples. Note that all the results obtained for refined salt samples are within the permissible limits.

Table 4. The radiation dose, the equivalent concentration of radium, the equivalent dose of radiation to which humans are exposed, and the internal and external risk standard for a sample of refined and unrefined table salt.

Sample	Ra _{eq} (Bq/kg)	D (nGy/h)	D _{eff} (mSv/y ⁻¹)	H _{ex}	H _{in}
S1	33.9959	18.4957	0.0315	0.0921	0.1110
S2	32.8582	17.8920	0.0309	0.0910	0.1085
S3	42.0295	21.1989	0.0348	0.1076	0.1365
S4	39.8220	21.0532	0.0346	0.107	0.1355

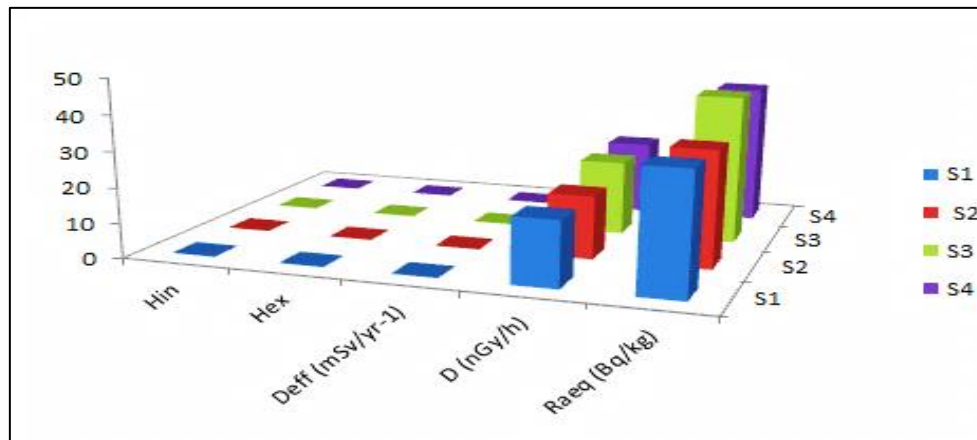


Figure 5. Radiation dose, radium equivalent concentration, radiation equivalent dose, and internal and external risk criteria for a sample of refined and unrefined table salt.

Conclusions

Different natural salt samples were collected and purified from different areas of Iraq. Values of radiological parameters were obtained using the available high resolution HPGe spectrometer. The average specific activities of the natural radionuclides occurring (^{238}U , ^{232}Th , ^{40}K) in the salt samples were found all being below the nationally accepted levels of 33Bq.kg^{-1} , 45Bq.kg^{-1} , and 420Bq.kg^{-1} , respectively. The results of the radiation indices showed that the concentrations of these radionuclides in all samples were seen to be within the allowable limits, which are less than unity. Measurements of radiation hazard indices showed that the concentrations of these isotopes in all samples are within the allowable limits internationally. From this study, we conclude that the Iraqi salts extracted from the sites and used in local markets are free of radioactive effects and suitable for human use and do not pose any danger to humans.

References

- [1] Bajoga, Abubakar D. Evaluation of natural and anthropogenic radioactivity in environmental samples from Kuwait using high-resolution gamma-ray spectrometry. University of Surrey (United Kingdom), (2016).
- [2] Mohammed, Aamir Abdullah. "Concentration measurements of radon, uranium and background of gamma rays in air at the university of Baghdad–Al-Jadiriya site." University of Baghdad, Ms. c thesis 46 (2013).
- [3] Aswood, M. S., Radiological assessment associated with vegetable farming in Cameron Highlands and Seberang Perai. Ph.D Thesis, College of Education, Malaysia University (2014).
- [4] Adelikhah, M., Imani, M., Hegedűs, M., & Kovács, T. Modelling of indoor external and internal exposure due to different building materials containing NORMs in the vicinity of a HNBRA in Mahallat, Iran. *Heliyon*, 8(2) (2022).
- [5] Brenner, D. J., & Hall, E. J.. Computed tomography—an increasing source of radiation exposure. *New England journal of medicine*, 357(22), (2007).
- [6] Prise, K. M., Schettino, G., Folkard, M., & Held, K. D. New insights on cell death from radiation exposure. *The lancet oncology*, 6(7), 520-528(2005).
- [7] Kamiya, K., Ozasa, K., Akiba, S., Niwa, O., Kodama, K., Takamura, N., & Wakeford, R.. Long-term effects of radiation exposure on health. *The lancet*, 386(9992), 469-478(2015).
- [8] Li, W., & Hudson, M. K. Earth's Van Allen radiation belts: From discovery to the Van Allen Probes era. *Journal of Geophysical Research: Space Physics*, 124(11), 8319-8351(2019).
- [9] Hossain, I., Sharip, N., & Viswanathan, K. K.. Efficiency and resolution of HPGe and NaI (TI) detectors using gamma-ray spectroscopy. *Sci. Res. Essays*, 7(1), 86-89. (2012)
- [10] Ouseph, P. Introduction to nuclear radiation detectors. Vol. 2. Springer Science & Business Media, (2012).
- [11] Carchon, R., et al. "Gamma radiation detectors for safeguards applications." *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment* 579.1 (2007)
- [12] Cooper, R. J., Amman, M., & Vetter, K. High resolution gamma-ray spectroscopy at high count rates with a prototype high purity germanium detector. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*, 886, 1-6(2018).
- [13] UNSCEAR, "Effects of Atomic Radiation to the General Assembly, in United Nations Scientific Committee on the Effect of Atomic Radiation. United Nations," New York, (2008).
- [14] Miglierini M., "Detectors of Radiation", E. Wigner Publisher on Reactor Physics Experiments Journal, Vol.2, No.4, pp.1-67, (2004).
- [15] Harb, S.R.M., "On the human radiation exposure as derived from the analysis of natural and man-made radionuclides in soils". Hannover: Universität (2004)



- [16] Stajić, Jelena, et al. "Radioactivity levels and health risks associated with Himalayan salt consumption." 2nd International Conference on Chemo and BioInformatics. University of Kragujevac, Institute for Information Technologies, (2023).
- [17] Jasim, Mahdi Hadi, Afrah Essa Ramadan, and Nessrian Ali Hussain. "Measurement and Analysis of natural radioactive elements in salty sediments from central and southern Iraq." Iraqi Journal of Science (2021).

Molecular Diagnosis and Genetic Divergence of Some Flax Genotypes (*Linum usitatissimum* L.)

Sawsan Fadhel Fawaz¹ and Jassim Jawad Jader Alnuaimi²

^{1,2}Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Najaf, Iraq.

¹E-mail: Sawsan.fadhel@uokerbala.edu.iq

²E-mail: alnuaimid92@atu.edu.iq

Abstract. This study aimed to molecularly diagnose eight flax genotypes (*Linum usitatissimum* L.) using polymerase chain reaction (PCR) technology and analysis of the nitrogenous base sequence of the *rbcl* gene region and determine the genetic divergence between these genotypes. DNA was extracted from the flax genotypes, and the *rbcl* gene region was amplified using polymerase chain reaction (PCR) technology and analysis of the nitrogenous base sequence of the amplified region. The results of the nitrogenous base sequence analysis showed clear differences in some sites of the nitrogenous base sequences of the genotypes used. The phylogenetic tree also revealed the presence of two distinct groups of genotypes, with great similarity between some structures in each group. The results of the comparison between the nitrogenous base sequences of the genetic constructs identified in this study and the nitrogenous base sequences of other flax genetic constructs previously registered at the National Center For Biotechnology Information (NCBI) showed that there is genetic divergence between the flax constructs used and those registered globally. These results indicate the importance of the *rbcl* gene region in revealing the genetic diversity among flax constructs, which can be exploited in breeding programs and improving the selection of constructs with desired traits.

Keywords. Genetic divergence, Flax genotypes, Polymerase chain reaction.

1. Introduction

Flax (*Linum usitatissimum* L.) belonging to the Linaceae family is one of the important agricultural crops globally and in the Arab world, and is grown to obtain its seeds rich in valuable oils and fibers. Flax is of great economic and nutritional importance, as the production of flax oil and fibers constitutes a basic pillar for many industries, while its seeds are a food source rich in unsaturated fatty acids, such as oleic acid (19-20%), linoleic (17-19%) and linolenic (45-60%), which makes them of high nutritional value and health benefits such as helping to treat cardiovascular diseases [1]. The cake (seed residue after oil extraction) is also used in animal feed and in organic industries [2]. The Food and Agriculture Organization (FAO) indicated for the year 2021 that the area of flax cultivation was about 241,103 hectares, with a productivity of 896,636.43 tons [3,4]. A disparity in global production was also noted, with Canada being the largest producing country, followed by China, India, America, Ethiopia and Egypt [3]. In Iraq, flax cultivation areas are still limited, due to several reasons, including the focus on cultivating other strategic crops, the lack of interest in this crop, and the absence of the necessary processing

industries. Despite the importance of flax, the classification of its species based on morphological characteristics faces major challenges, as phenotypic classification depends on studying the phenotypic characteristics of plants, but there is a great similarity between some of its species, which hinders the process of accurate classification. For example, it may be difficult to distinguish between similar species based on leaf shape, plant height or flower color only. This similarity is partly attributed to hidden genetic variation that cannot be easily observed based on phenotypic traits[5]. Therefore, phenotypic classification is considered insufficient to accurately classify flax species, as genetic variation that cannot be detected based on external appearance, and relying on phenotypic traits often leads to misclassification due to the overlap of these traits, in addition to being affected by many different environmental factors [6]. To overcome these limitations, molecular classification, using techniques such as polymerase chain reaction (PCR), is more accurate and efficient in diagnosing many plants [7]. PCR technology allows the amplification of specific parts of DNA, which allows the study of genetic variation between flax species with high accuracy. PCR technology has previously been used successfully in studying genetic diversity and classifying other plant species, including the previous flax [8,9]. Despite Phenotypic classification provides valuable information about flax, but the phenotypic similarity between some of its species limits the accuracy of classification. Therefore, this research aimed to use the polymerase chain reaction (PCR) technology and determine the sequence of the nitrogenous bases of the PCR-amplified products of the targeted gene region *rbcL*, to analyze the genetic variation between the different flax genetic structures and produce a more accurate and comprehensive classification, which helps to exploit it with high efficiency in crop improvement programs and develop new productive flax varieties.

2. Materials and Methods

2.1. DNA Extraction

DNA was extracted from eight genotypes (Table 1) using the kit provided by Zymo Research (DNA Plant/Seed Miniprep Kit, Cat. No. D6020, USA) and following the manufacturer's instructions. The concentration and purity of DNA were measured according to the procedure. The extracted DNA from the eight flax genotypes was then stored at -20°C until used in the polymerase chain reaction (PCR).

Table 1. Flax genotypes used in this study.

Cultivars Name	Origin	Source	Lineage
Indian	Indian	College of Agriculture - Tikrit University	I.1485 x Bombay
Polish	Polish	College of Agriculture - Salahaddin University	Imported
Syrian	Syrian	College of Agriculture - Salahaddin University	Imported
Giza 10	Egyptian	College of Agriculture - Cairo University	)Belinka (2E) $\times$ 1.2096
Sakha 5	Egyptian	College of Agriculture - Cairo University	)Belinka (R3) $\times$ 1.2569
Sakha 6	Egyptian	College of Agriculture - Cairo University	S.420 x bombay (I. USA)
Giza 11	Egyptian	College of Agriculture - Cairo University	Hera $\times$ 1.123
Giza 8	Origin	College of Agriculture - Cairo University	)Giza6 $\times$ Senta Catalina

2.2. Polymerase Chain Reaction (PCR)

The PCR test was performed using the kit (Maxime PCR PreMix (i-Taq), Cat. No. 25026) provided by iNtRoN, South Korea. A 25 μl PCR was prepared containing 1 μl each of the forward primer (*rbcLa-F-5'*- (ATGTCACCACAAACAGAGACTAAAGC-3')) and reverse primer (*rbcLa-R 5'*- GTAAATCAAGTCCACCACG -3') and 1 μl of extracted DNA. All the above components were placed in the tube provided by the manufacturer and the volume was made up

with nuclease-free water to 25 μ l. The DNA was amplified using the following PCR steps and conditions: Initial denaturation of DNA for 5 min at 98°C followed by 35 cycles each consisting of a final denaturation for 45 s at 94°C, ligation Primer annealing for 45 seconds at 55°C, followed by initial elongation of the PCR-amplified product for one minute at 72°C, and finally PCR was completed by final elongation at 72°C for five minutes.

2.3. Agarose Gel Electrophoresis

Agarose gel layer was prepared by taking one gram of agarose powder and dissolving it in 100 ml of 1×TBE (Tris boric acid EDTA buffer) until the mixture turned into a clear solution. Five microliters of Ethidium bromide dye were added after the solution temperature dropped to about 45°C. Agarose gel tray was prepared, containing a comb at one end, to make wells inside the agarose gel layer. Then, the melted agarose containing the ethidium bromide dye was poured and left to solidify at room temperature. After solidification, the comb was carefully removed and the tray was returned to its place in the electrophoresis tank. Then, 1×TBE buffer solution was added to the electrophoresis tank, covering the agarose gel layer to a height of about 70 mm. 10 μ l of the PCR product was added to each well of the previously prepared agarose gel layer. Five μ l of molecular-weight size marker was added to the well on the left side of the added samples for the purpose of determining the sizes of the PCR. The electrodes of the power supply were connected to the electrical current and operated at 150 mA for one hour. After the sample transfer process was completed, the agarose gel layer containing the PCR products was examined under UV transillumination and photographs were taken.

PCR analysis of double-stranded DNA bases For the purpose of identifying the genotypes of flax (1, 2, 3, 4, 5, 6, 7, and 8), the double-stranded DNA products of these genotypes were sent by PCR with primers rbcLa-F and rbcLa-R to Macrogen Company (South Korea) for the purpose of determining the nucleotide sequence in the forward and reverse directions of the double-stranded DNA products. All the nucleotide sequences were analyzed using BLAST (Basic Local Alignment Search Tool) program to compare them with the data available at the National Center for Biotechnology Information (NCBI) for the same plant and identified globally. Based on the sequences of nitrogenous bases of the identified genotypes, the phylogenetic tree was drawn by MEGA-X software [10].

3. Results and Discussion

3.1. Molecular Diagnosis of Some Genetic Structures of Flax Plant

The results of DNA extraction from the genetic structures of flax plant (*L. usitatissimum*) and amplification by polymerase chain reaction (PCR amplification) showed the possibility of amplifying nucleic acid products (PCR-amplified products) each of which is about 600 nitrogenous base pairs (Base pair, bp) (Figure 1).

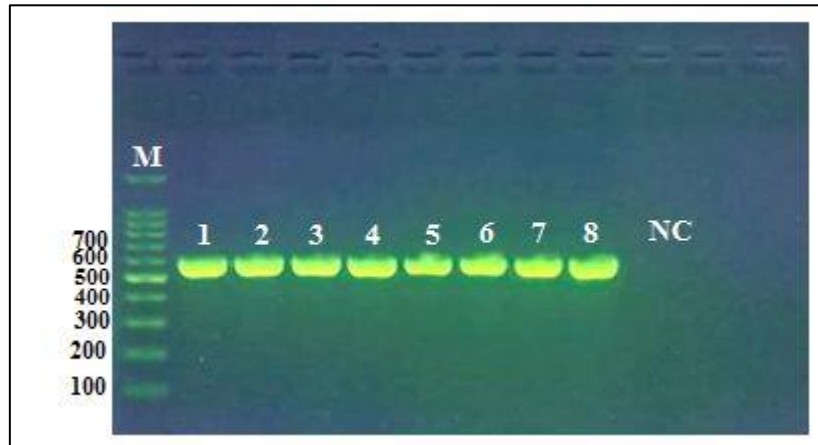


Figure 1. The 600bp-PCR products amplified by polymerase chain reaction (PCR) from the flax genetic constructs (1, 2, 3, 4, 5, 6, 7 and 8) used in this study. NC: Negative control carried out by polymerase chain reaction (PCR mixture) containing all reaction components except flax DNA. M: DNA ladder (Molecular-weight size marker) with the number of nitrogenous base pairs (bp) and the sizes of each fixed on the left side of the figure.

The results of the analysis of nucleotide sequences (Analysis and alignment of nucleotide sequences) of the doubled DNA products of the flax genetic structures showed differences in some sites of the nitrogenous bases in the doubled genetic region by the primers *rbcl-F* and *rbcl-R* Figure (1)

It was also shown through the alignment of the nucleotide sequences (Nucleotide sequences alignment) and using the MEGA X program that there was a difference in the similarity rates between the flax genetic structures, as it was found that the highest similarity rate reached 96% and was between genotypes 2 and 3 as well as 4 and 6, in addition to genotypes 6 and 7, while it was found that the lowest similarity rate was between genotypes 1 and 8. As for the similarity rates between the other structures, they ranged between 85%-95% Table (1) The genetic analysis tree also confirmed the existence of differences between the genetic structures, specifically in the *rbcl* genetic region (Figure (2) It can be concluded that these genetic structures are genetically different, in addition to the possibility of exploiting the *rbcl* gene region in the diagnosis or detection of genetic variations between the genetic structures of the flax plant.

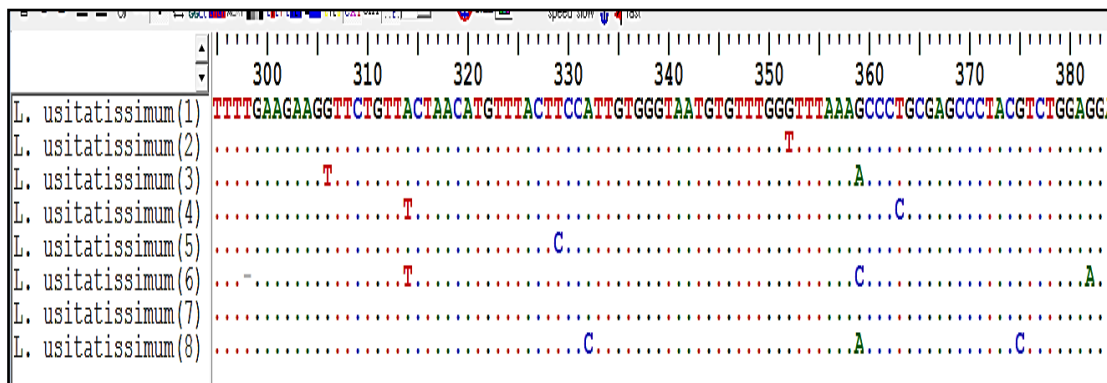


Figure 2. Similarity and difference in some regions of the sequence alignments of the DNA products (PCR-amplified products) amplified by the polymerase chain reaction (PCR) from the genetic structures of the flax plant (1-8). The points shown in the figure represent the similarity

with the genetic structures in the same nitrogenous base site. The numbers on the right side of the figure represent the genetic compositions (1- Indian), (-2 Polish), (3- Syrian), (4- Giza 10), (-5 Sakha 5), (6- Sakha 6), (7- Giza 11), and (8- Giza 8).

Table 2. Similarity ratios between the sequences of nitrogenous bases of the DNA products duplicated by polymerase chain reaction (PCR) from the genetic compositions of the flax plant used in this study.

Genotype number	1	2	3	4	5	6	7	8
1	-							
2	95	-						
3	95	96	-					
4	93	94		-				
5	93	94	95	95	-			
6	93	94	95	96	95	-		
7	93	94	95	96	96	96	-	
8	83	86	85	87	87	89	89	-

The results also showed that there is a similarity between the sequences of the nitrogenous bases of the double-stranded DNA products of the flax plant genetic compositions when compared with the sequences of the nitrogenous bases of the same plant genetic compositions previously registered at the National Center for Biotechnology Information (NCBI).

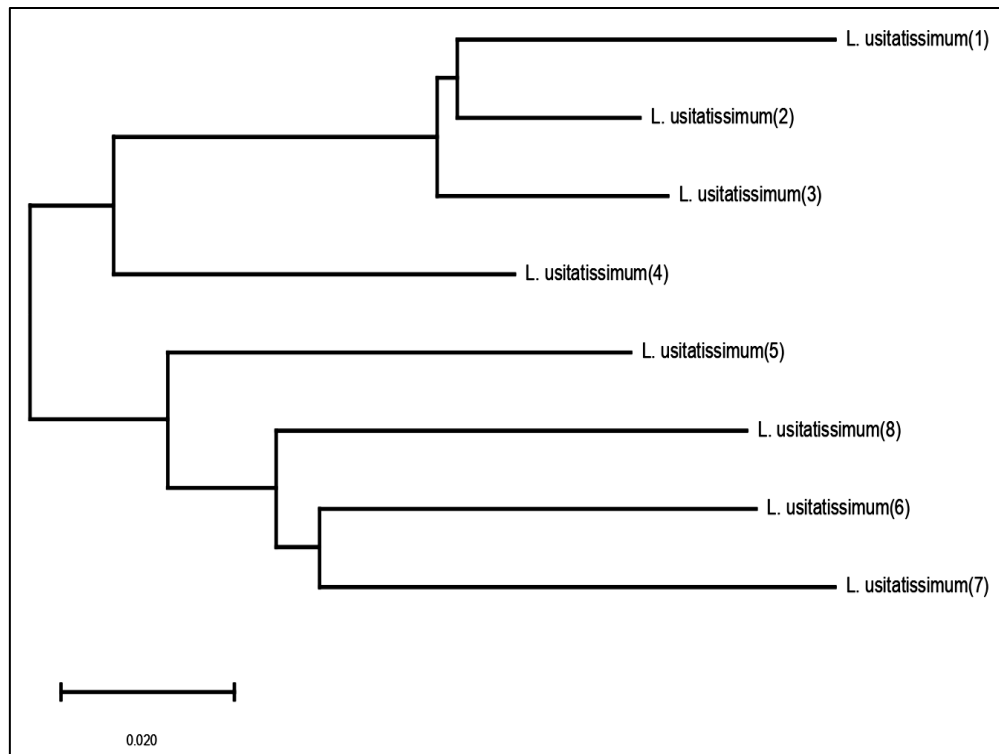


Figure 3. Neighbor-Joining tree shows the genetic relationship between the genetic structures of the flax plant (1-8) used in this study.

The results of the comparison between the genetic structures used in this study and the genetic structures of flax plants registered in the American National Center for Biotechnology Information (NCBI) showed a difference in the similarity percentages, the lowest of which was (94%) for the eight genetic structures when compared to the genetic structure diagnosed in the United States (JX664057, KU645802, and PP374794), Pakistan (MG946893), Canada (MG245934), and China (MT930609), while these genetic structures (1, 3, and 7) were the closest (98%) to the genetic structure registered in the United States (JX664057, KU645802, PP374794), Pakistan (MG946893), Canada (MG245934), and Germany (FJ169596). While genetic structures 2 and 4 gave 5 and 6 similarity ratios ranged between 96%-97% with other registered structures in America, Pakistan, Canada, China and Germany, including JX664057, MG946893, MT930609, MG245934 and FJ169596.

Table 3. Similarity ratios between the sequences of nitrogenous bases of the DNA products amplified by polymerase chain reaction (PCR-amplified products) from the flax genetic structures (1, 2, 3, 4, 5, 6, 7 and 8) used in this study, and the sequences of nitrogenous bases of the same genetic region (rbcl) for the genetic structures registered in the US National Center for Biotechnology Information (NCBI).

Origin	Nucleotide sequence similarity (%)	Deposit number (Accession No.)	Genotype	Origin	Nucleotide sequence similarity (%)	Deposit number (Accession No.)	Genotype
Pakistan	97	MG946893	1	USA	97	JX664057	1
	98	MG946893	2		98	JX664057	2
	98	MG946893	3		98	JX664057	3
	96	MG946893	4		96	JX664057	4
	96	MG946893	5		96	JX664057	5
	96	MG946893	6		96	JX664057	6
	98	MG946893	7		98	JX664057	7
	94	MG946893	8		94	JX664057	8
Germany	97	FJ169596	1	Canada	97	MG245934	1
	98	FJ169596	2		98	MG245934	2
	98	FJ169596	3		98	MG245934	3
	96	FJ169596	4		96	MG245934	4
	96	FJ169596	5		96	MG245934	5
	96	FJ169596	6		96	MG245934	6
	98	FJ169596	7		98	MG245934	7
	94	FJ169596	8		94	MG245934	8
USA	97	PP374794	1	China	95	MT930609	1
	98	PP374794	2		96	MT930609	2
	98	PP374794	3		96	MT930609	3
	96	PP374794	4		94	MT930609	4
	96	PP374794	5		94	MT930609	5
	96	PP374794	6		95	MT930609	6
	98	PP374794	7		94	MT930609	7
	94	PP374794	8		92	MT930609	8

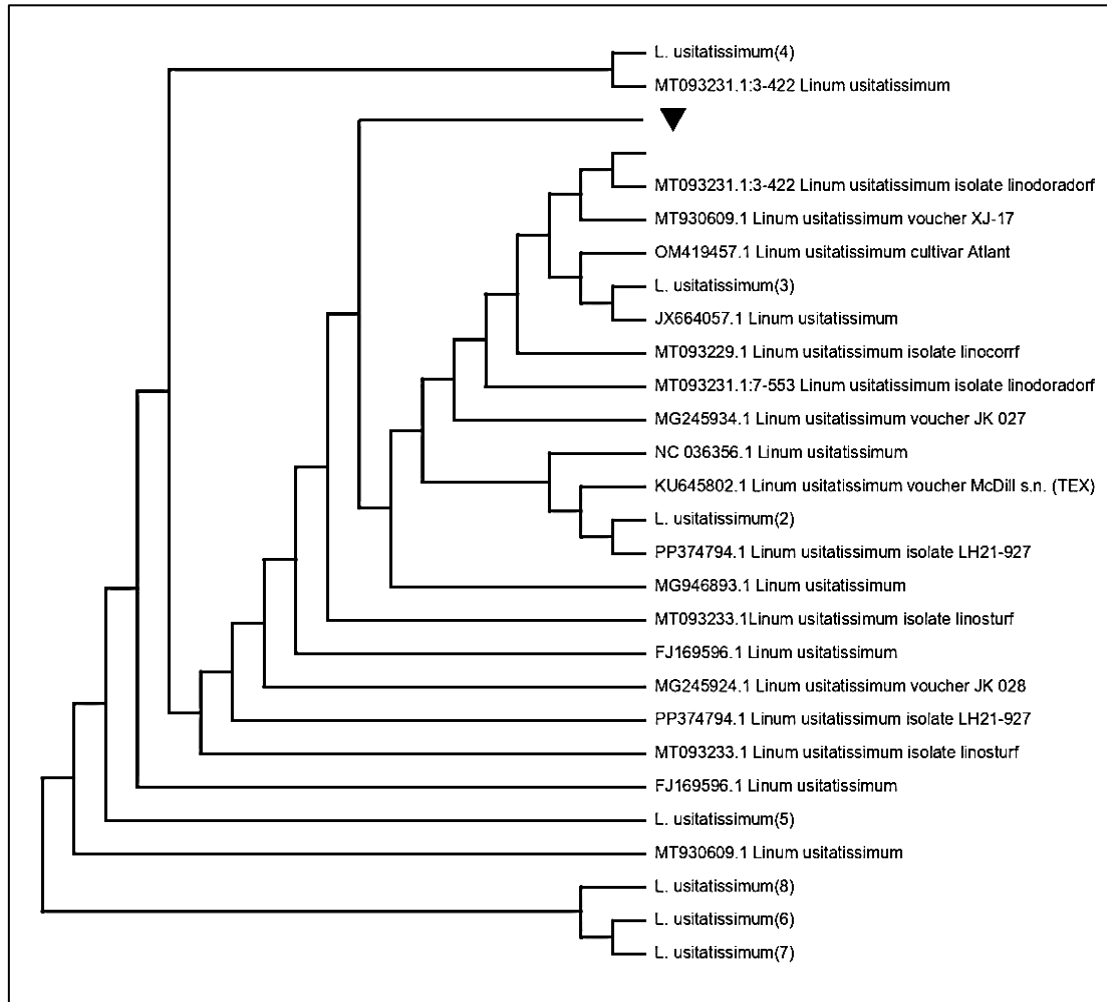


Figure 4. Neighbor-Joining tree shows the genetic relationship between the flax genetic structures (1, 2, 3, 4, 5, 6, 7, and 8) used in this study and other genetic structures registered at the National Center for Biotechnology Information (NCBI).

The polymerase chain reaction (PCR) technology and the determination of the nitrogenous base sequence (Nucleotide sequence) have been used to diagnose many living organisms, including fungi, viruses, and others [11]. This technology has also been used previously efficiently to diagnose many plants such as wheat, flax, and others [12,11]. The *rbcl* gene has also contributed efficiently to the diagnosis of many plant species such as Gabon red (*Anthocephalus macrophyllus*), Ojoba (*Simmondsia chinensis*), and potatoes at all different growth stages, as well as the detection of genetic variations between the genetic compositions of certain plant species [13,14,9] .. The *rbcl* gene has also been used to detect genetic variation in four types of black rice (*Oryza Sativa* L.) in Indonesia [15].

Conclusion

The results of this study and through the analysis of the sequence of nitrogenous bases of the *rbcl* gene region in some flax genetic compositions indicate the presence of genetic differences between these compositions, indicating that this gene region (*rbcl*) Variable between flax genetic

compositions and can be used in molecular diagnosis and detection of genetic changes between flax genetic compositions, which encourages their exploitation in selecting compositions with desired characteristics.

References

- [1] El- Gedwy, E.M.E., Hammam, G.Y., Allam, S.A., Mostafa, S.H., and Shimy, K. S. E. (2020) .Straw, seed yield and quality of three *Linum usitatissimum* L. Cultivars in relation to nitrogen fertilizer rate and plant density . *Asian Journal of Advances in Agricultural Research*, 14(4):8-29.
- [2] Mahmoud, Abdul Karim Khairallah and Abdul Samad Hashim Noman. (2024). Response of Four Flax Genotypes to Growth Traits, Seed Yield and Oil Yield under Different Seed Rates. *Anbar Journal of Agricultural Sciences*. Vol. 22 (1): 544-559.
- [3] Nimr, Rasool Hassan, 2023. Effect of Planting Distances and Weed Control on the Growth and Yield of Flax. Master's Thesis, College of Agricultural Engineering Sciences, University of Baghdad.
- [4] FAO.2021. Food and Agriculture Organization of the United Nation.
- [5] Jassim, Abdul Qader Hamidi, 2021. Genetic indicators of half-crosses and stability of flax (*linum usitatissimum*) genetic compositions under the influence of different planting dates. PhD thesis, College of Agriculture, Tikrit University.
- [6] Al-Taie, Ali Saleh Hassoun. 2022. Evaluation of flax genetic compositions and estimation of genetic parameters and active compounds under the influence of biofertilization. PhD thesis. Al-Musayyab Technical College. Middle Euphrates University.
- [7] Al-Obaidi, Imad Abdul Razzaq Wahib (2017). Genetic evaluation of the performance of wheat (*Triticum aestivum* L.) genetic compositions to tolerate salinity under the influence of kinetin. Master's Thesis, College of Agriculture - University of Anbar.
- [8] AL-Musawi, B. H., & AL-Abedy, A. N. (2020). genetic variability analysis of some selected rice (*Oryza sativa* L.) genotypes in Iraq. *International Journal of Agricultural and Statistical Sciences*. Vol, 16(1), 191-197.
- [9] Nderitu, K. W., Ager, E., Mecha, E., & Nyachio, A. (2023). DNA barcoding using ITS2 and RBCL markers for Solanaceae species identification. *East African Medical Journal*, 100(1), 5567-5574.
- [10] Kumar, S., Stecher, G., Li, M., Knyaz, G. and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, 35(6): 1547-1549.
- [11] AL-Abedy, A. N., Al-Fadhal, F. A., Kareem, M. H., Al-Masoudi, Z., & Al-Mamoori, S. A. (2018). Genetic variability of different isolates of *Rhizoctonia solani* Kühn isolated from Iranian imported potato tubers (*Solanum tuberosum* L.). *International Journal of Agriculture and Statistical Sciences*, 14(2), 587-598.
- [12] Bruno, M. C., Posik, D. M., Zappa, M. E., Baroni, V., Wunderlin, D., Giovambattista, G., & García, P. P. (2020). Genetic identification of flax, chia and sesame seeds in processed foods. *Food Control*, 118, 107374.
- [13] Bell, K. L., Loeffler, V. M., & Brosi, B. J. (2017). An *rbcL* reference library to aid in the identification of plant species mixtures by DNA metabarcoding. *Applications in plant sciences*, 5(3), 1600110.
- [14] Jayali, A. M., & Sukamto, N. H. (2019). The application of barcode DNA *rbcL* gene for identification of medicinal plants: red jabon and gofasa. In *Journal of Physics: Conference Series* (Vol. 1146, No. 1, p. 012030). IOP Publishing.
- [15] Basith, A., E.L. Arumingtyas and Widodo. 2021. Genetic Variation Analysis of Four Local Varieties of Indonesian Blak Rice (*Oryza sativa* L.) Based on Partially *rbcL* cpDNA Gene Sequence . *J.Exp. Life Sci*. Vol.(11) No.1.

Effect of Water Stress on the Growth of Several Flax Cultivars

Sawsan Fadhel Fawaz¹ and Jassim Jawad Jader Alnuaimi²

^{1,2}Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Najaf, Iraq.

¹E-mail: Sawsan.fadhel@uokerbala.edu.iq

²E-mail: alnuaimid92@atu.edu.iq

Abstract. A field experiment was conducted in Al-Hussainiya district, 20 km northeast of Karbala Province, during the winter season (2023-2024) to study the effect of water stress on several flax compositions. The experiment was applied in a split plot arrangement according to a randomized complete block design (RCBD) with three replicates. The main plots included three levels of water stress (control treatment depletion 50% of the available water, depletion 70% of the available water, depletion 90% of the available water), while the sub-plots included eight flax genetic compositions (Indian, Polish, Syrian, Giza10, Sakha5, Sakha6, Giza11, Giza8). The results showed that the Giza 11 cultivar was superior in height, dry weight of root and shoot system, and proline content (125.16 cm.plant⁻¹, 0.66 g.plant⁻¹, 157.11 g.plant⁻¹, 16.317 µmol/g fresh weight), respectively, and the Sakha 5 cultivar was superior in number of main branches and number of leaves (6.60 branches.plant⁻¹, 597.00 leaf.plant⁻¹), respectively. While the plants in the irrigation treatment (50% depletion of the available water) excelled in plant height, number of main branches, number of leaves, dry weight of the root and vegetative system as follows (129.93 cm. plant⁻¹ 5.975, branch. plant⁻¹, 613.3 leaf.plant⁻¹, 0.675, g. plant⁻¹, 152.87 g. plant⁻¹) respectively compared to the plants in the irrigation treatment (70% depletion of the available water) and the irrigation treatment (90% depletion of the available water).

Keywords. Growth, *Linum usitatissimum* L., Water stress.

1. Introduction

Flax (*Linum usitatissimum* L.) belongs to the Linaceae family and is diploid (2n = 30), self-pollinated, winter crops and is one of the oldest plant crops known to man, and has been cultivated since ancient times. Flax is grown in different parts of the world for the purpose of obtaining oil and fibers. The area planted with flax was estimated at about 241,103 hectares, and the productivity reached about 896,636.43 tons [1,2]. Canada leads the world, followed by China, India, America, Ethiopia and Egypt with about (34, 25.5, 9.8, 3.5, 2.2)% of global production, respectively, and there are other countries with less production FAO [1]. In Iraq, the areas planted with this crop are almost non-existent, for many reasons, the most important of which is the focus on producing strategic winter crops, the lack of interest of specialists in the productivity of this crop and the absence of specialized factories to extract oil and fibers from it. Flax seeds are classified as important functional foods due to their abundance of many nutrients compared to

other vegetable oils, such as unsaturated fatty acids and protein. Therefore, it is unique among oil seeds due to its high content of fixed oils of the fatty acid Alpha-Linolenic Acid (ALA), which ranges from (30-45)% of the oil, which constitutes about 57% of the total fatty acids. In some countries, its oil is used for industrial purposes, primarily due to its high content of linolenic acid, which ranges from (45-65)%, which gives it drying and rapid oxidation properties, making it suitable for the manufacture of many biodegradable products such as dyes and others. Flax seeds are also rich in soluble and insoluble dietary fiber, which makes them useful as a nutritional supplement [3]. Plants in general and flax in particular face the problem of water availability due to water scarcity and climate change, which is one of the most serious environmental challenges facing humanity, causing a shortage of water suitable for agriculture and more extreme weather pressures. It has become necessary to use modern scientific methods to fill the deficit in irrigation water and regulate or schedule crop irrigation to reach an ideal state and create a state of balance between water consumption and the amount of crop [4]. Flax is exposed to drought stress and high temperature. Drought stress greatly affects the growth of flax seedlings, flowering, seed development, and crop. Prolonged exposure to drought leads to a partial decrease in cellular oxygen, which leads to the production of active oxygen radicals (ROS). Rapid oxidation disrupts cell membranes and components and ultimately kills them. The accumulation of ROS increases cellular oxidative stress, which leads to the loss of economic yield, oil, fiber, and nutrients in flax [5].

2. Materials and Methods

2.1. Field Experiment Experimental Soil Location and Properties

A field experiment was carried out in the fields of Al-Hussainiya district, Karbala Province, to determine the performance of eight flax cultivars under the influence of water tension during the winter season 2023-2024. In loam clay silty soil, the relationship between the structural tension and the moisture content of a sample of field soil sifted with a sieve with a 2 mm pit diameter was estimated to estimate the soil's water retention capacity, as different tensions (33, 100, 300, 500, 1000 and 1500) kilopascals were applied to it, and the available water content of the soil was calculated from the difference in the volumetric moisture content at the field capacity and the wilting point.

2.2. Land Preparation and Agricultural Operations

The experimental land was plowed twice perpendicularly using a disc plough, then smoothing and leveling operations were conducted to prepare a suitable bed for the seeds. Then the land was divided according to the field plan for the experiment, which included 72 experimental units distributed in three replicates, with 24 experimental units per replicate, and the area of the experimental unit was 4 m² (2 × 2 m).

2.3. Experimental Design and Experiment Treatments

A factorial experiment according to RCBD design with split plot arrangement with three replicates included three treatments for adding irrigation water placed in the main plots, namely irrigation when 50%, 70%, 90% of the available water amount was depleted, while the sub plots included eight cultivars of flax, namely (Indian, Polish, Syrian, Giza 10, Sakha 5, Sakha 6, Giza 11, Giza 8).

2.4. Irrigation Method and Monitoring of Moisture Depletion of Treatments

The irrigation process was carried out by means of a gasoline-powered pump with a drain pipe installed with a meter to calculate the amount of water and connected to a network of plastic pipes distributed over the experimental units, as equal amounts of water were added to all plots upon planting.

2.5. Studies Traits

The following characteristics were studied for a random sample of (ten plants recorded as an average randomly selected from the two median lines of each experimental unit) as follows:

2.5.1. Plant Height (cm. plant⁻¹)

The average height of the main stem was measured from the soil surface to the top of the main stem at the harvest stage and for ten plants randomly taken from the median lines of each experimental unit.

2.5.2. Number of Green Branches (branch. plant⁻¹)

According to the average number of main lateral branches in the plant at the harvest stage and for ten plants randomly taken from the median lines of each experimental unit.

2.5.3. Number of Leaves (leaf plant⁻¹)

Calculating the average for the leaves of ten plants

2.5.4. Dry Weight of Vegetative Growth (g)

Calculated from the average of ten plants cut and dried naturally with continuous stirring until the weight stabilized.

2.5.5. The Dry Weight of the Root System (g)

Calculated as an average of the roots of ten plants randomly taken from the guarded midlines of each experimental unit. The roots were extracted using a root extractor, then washed well with water to get rid of the soil and air dried until the weight was constant and weighed with a sensitive balance.

2.6. Proline Content of the Leaves

2.6.1. Extraction Process

Amino acids were extracted according to the method presented by the scientist [6] where a weight of (3 g) was taken from the sample and placed in a volumetric bottle with a capacity of (25 ml) and (25 ml) of hydrochloric acid (1M) was added to it at a temperature of (55 C) for 3 hours, after which the sample was dried using a rotary evaporator and (5 ml) of sodium citrate pH 2.2 was added to it. The sample was filtered using a plastic filter (0.45um) and taken to the device to perform the injection process.

3. Results and Discussion

3.1. Effect of Cultivars, Irrigation Treatments and Their Interaction on Vegetative Growth Traits

3.1.1. Average Plant Height (cm)

The results of the statistical analysis show that there are significant differences between flax cultivars, irrigation treatments and their interaction in flax crop height.

The results of Table 1 showed significant differences between flax cultivars, as the Giza 11 cultivar excelled by giving the highest average plant height, reaching 125.16 cm, compared to the Sakha 5 cultivar, which gave the lowest average, reaching 90.73 cm. The difference in plant height is often related to the genetic nature of the cultivar and its response to environmental conditions and in terms of the effectiveness of dwarfing genes, which makes them different in height or the difference in the structures under study in their hormonal content, which led to a difference in their height in terms of the elongation of the internodes by competing for light [7]. This is consistent with what was found by [3,8,9] who indicated that flax cultivars differed in vegetative growth indicators, including plant height. The results in the table also indicate a significant effect of irrigation treatments on plant height, as the irrigation treatment (90% depletion of available water) gave the lowest average plant height of (93.02) cm, while the highest average height was reached in the control treatment (50% depletion of available water) of (129.93) cm. There are also significant differences between the two depletion treatments (50%, 70% of available water), as the average plant height was (110.06) cm for the irrigation treatment (70%). The reason for the decrease in plant height in the depletion treatment (90%) is attributed to the loss of turgor pressure in its cells, or the decrease in the ability of these cells to expand due to the increase in the hardness of their walls due to severe stress that is sufficient to stop plant growth [10]. The results agree with what was mentioned. Also, [11] found that drought tolerance genes in flax showed great variation in the phenotypic traits of flax cultivars, including plant height. The effect of interaction between the two study factors was significant on the average plant height, as Giza 11 cultivar in the control treatment (50% depletion of available water) achieved the highest average plant height, reaching (147.19) cm, while the lowest average for the trait was in the interaction (90% depletion of available water) and Sakha 5 cultivar, reaching (79.67) cm.

Table 1. Effect of irrigation levels, cultivars and interaction between them on the average plant height (cm).

Traits	Irrigation treatments			Average cultivars
	50%	70%	90%	
Indian	122.25	98.07	83.16	101.16
Polish	105.70	97.42	80.29	94.47
Syrian	131.21	116.66	90.57	112.98
Giza 10	143.49	117.76	99.73	120.33
Sakha 5	99.28	93.22	79.67	90.73
Sakha 6	146.83	119.27	100.97	122.36
Giza 11	147.19	119.26	109.03	125.16
Giza 8	142.97	118.81	100.74	120.84
Average irrigation treatments	129.93	110.06	93.02	
LSD irrigation= 1.049 •LSD Cultivars= 1.076 •LSD interaction= 1.902				

3.1.2. Average Number of Main Branches (*plant branch⁻¹*)

Branching is an important phenotypic component in the development of seed yield in flax seeds and one of the adaptive mechanisms to maintain the balance between sources and outlets. Increasing the availability of nutrients in the soil solution and their transfer within plant tissues and their accumulation in appropriate quantities encourages branching and stimulates their growth. Branches differ in their representative efficiency for physiological and morphological reasons in terms of the height of the branch, the location of connection to the stem, the number of leaves it carries and their distribution. Therefore, the effect is clear in the process of carbon representation and yield [12]. The results of the statistical analysis in Table (2) indicate that there is a significant difference between flax cultivars in the average number of branches .plant. Sakha 5 cultivar plants achieved the highest average for the trait, reaching 6.608 branches .plant⁻¹, compared to Giza 8 cultivar plants, which gave the lowest average number of branches, reaching 3.520 branches.plant⁻¹. The reason for the difference between cultivars in the number of main branches may be due to the variation in their content of internal plant hormones. This is consistent with what was concluded by [13-15] who indicated that flax cultivars vary in the number of main branches .plant as a result of the variation in their genetic composition and the variation in their response to environmental conditions. Irrigation treatments had a significant effect on the average number of main branches in the plant, as the irrigation treatment (90% depletion of available water) gave the lowest average of the trait, reaching 3.228 branches.plant⁻¹, while the control treatment plants (50% depletion of available water) gave the highest average of the trait, reaching 5.975 branches.plant⁻¹, which differed significantly from the irrigation treatment (70% depletion of available water), as it recorded an average number of main branches of 4.510 branches.plant⁻¹. The effect of the interaction between the two study factors was significant on the average number of main branches in the plant, as the Sakha 5 cultivar in the control treatment (50% depletion of available water) achieved the highest value of interaction, reaching 8.693 branches .plant⁻¹, while the Giza 8 cultivar achieved the lowest value of interaction when treated with irrigation (90% depletion of available water) of (2.397) branches.plant⁻¹.

Table 2. Effect of irrigation levels, cultivars and their interaction on the average number of main branches (plant⁻¹ branch).

Cultivars	Irrigation treatments			Average cultivars
	50%	70%	90%	
Indian	5.363	4.337	2.630	4.110
Polish	8.103	5.707	4.283	6.031
Syrian	5.817	4.150	3.393	4.453
Giza 10	5.077	3.440	2.530	3.682
Sakha 5	8.693	6.553	4.577	6.608
Sakha 6	4.777	3.750	2.463	3.663
Giza 11	5.397	4.553	3.553	4.501
Giza 8	4.577	3.587	2.397	3.520
Average irrigation treatments	5.975	4.510	3.228	
LSD irrigation= 0.1938 ,LSD Cultivars = 0.3092 ,LSD interaction= 0.5191				

3.1.3. Number of Leaves (*leaf.plant⁻¹*)

The results shown in Table 3 showed a significant effect between flax cultivars and irrigation treatments and their interaction in the average number of leaves (leaf.plant⁻¹), where the Sakha5 cultivar excelled in the average number of leaves, reaching 597.0 leaf.plant⁻¹, which did not differ significantly from the Polish cultivar, which gave an average number of leaves of 594.7

leaf.plant⁻¹, while the lowest average number of leaves was in the Giza8 cultivar, which achieved an average of 406.9 leaf.plant⁻¹, and did not differ significantly from the Sakha6 cultivar, which gave an average for the trait of 407.6 leaf.plant⁻¹. The reason for the variation in the average number of leaves between cultivars is due to the variation in their genetic composition and response to environmental conditions.

The results also showed a significant effect of irrigation treatments on the average number of leaves (leaf.plant⁻¹), as the irrigation treatment (90% depletion of available water) gave the lowest average number of leaves, reaching 330.7 leaf.plant⁻¹, while the control treatment achieved the highest average number of leaves, reaching 613.3 leaf.plant⁻¹, which also differed significantly from the irrigation treatment (70% depletion of available water), as it gave an average number of leaves, reaching 500.8 leaf.plant⁻¹. Water stress activates a group of hydrolytic enzymes as well as proteins. These enzymes break down large molecules such as proteins, starch, and nucleic acids into their structural units. These enzymes include Nucleases and Rib nucleases, which break down DNA and RNA. The increase in the activity of these enzymes is greatest in cases of severe stress. It is known that severe stress accelerates the death of tissues, especially leaves, which leads to their death and fall. Death is preceded by the breakdown of cell components and their transfer to new tissues before the leaf separates from the plant, [10]. The effect of the interaction between the two study factors was significant in the average number of leaves, as the Polish cultivar in the irrigation treatment (50% depletion of the available water) gave the highest value of interaction, reaching 741.7 leaf.plant⁻¹, which did not differ significantly from the Sakha5 cultivar, which gave an interaction of 733.0 leaf.plant⁻¹ compared to the Giza8 cultivar in the irrigation treatment (90% depletion of the available water), which gave the lowest value of interaction, reaching 246.2 leaf.plant⁻¹.

Table 3. Effect of irrigation levels, cultivars and their interaction on the number of leaves (leaf per plant⁻¹).

Cultivars	Irrigation treatments			Average cultivars
	%50	%70	%90	
Indian	558.5	488.3	318.7	455.1
Polish	741.7	604.1	438.3	594.7
Syrian	612.7	488.5	340.0	480.4
Giza 10	565.1	457.7	266.5	429.8
Sakha 5	733.0	626.0	432.0	597.0
Sakha 6	531.6	435.0	256.1	407.6
Giza 11	621.2	475.2	348.1	481.5
Giza 8	542.6	432.0	246.2	406.9
Average irrigation treatments	613.3	500.8	330.7	
LSD irrigation= 20.26 ‹LSD Cultivars= 25.14 ‹LSD interaction= 43.21				

3.1.4. Dry Weight of the Root System (g plant⁻¹)

The results show a significant difference between flax cultivars and irrigation treatments and their interaction in the average dry weight of the root system (Table 4). The Giza 11 cultivar outperformed by giving the highest average for the trait, reaching 0.668 g plant⁻¹, compared to the Sakha 5 cultivar, which gave the lowest average for the trait, reaching 0.512 g plant⁻¹ for the study season. The reason for the variation of cultivars in the dry weight of the root system is mostly related to the nature of the cultivar's growth and its ability to form a root system that is proportional to the vegetative system during the plant growth stages. This result is consistent with what [8,13,15] reached, that flax cultivars vary in the dry weight of the root system. The results also indicate that there is a significant effect of irrigation treatments on the dry weight of the root

system (g plant^{-1}), as the irrigation treatment (90% depletion of the available water) gave the lowest average dry weight of the root system, reaching (0.511) g plant^{-1} , while the control treatment (50% depletion of the available water) achieved the highest average, reaching (0.675) g plant^{-1} , which also differed significantly from the irrigation treatment (70% depletion of the available water), as the average of the trait reached (0.614) g plant^{-1} . The decrease in the dry weight of the root system in the 90% depletion treatment of available water is attributed to the decrease in the relative humidity rate near the root zone due to water stress, which leads to a decrease in the area of root spread and its depth, which reduces the weight of the roots. On the other hand, the plant works under conditions of low humidity to close the stomata, which reduces the representation of CO_2 , which results in a decrease in the amount of represented materials and the accumulation of dry matter, which is reflected in the amount of materials transferred to the roots, reducing their weight [16] This result is consistent with the results of [11] who found that drought tolerance genes in flax greatly affected the roots of flax cultivars and reduced their depth and spread in the soil, thus reducing the dry weight of the root system. The effect of interaction between the two study factors was significant in the average dry weight of the root system, as the Giza 11 cultivar in the irrigation treatment (50% depletion of the available water) gave the highest value of interaction, reaching $0.756 \text{ g plant}^{-1}$, compared to the Poloni cultivar in the irrigation treatment (90% depletion of the available water), which gave the lowest value of interaction, reaching (0.436) g plant^{-1} .

Table 4. Effect of irrigation levels, cultivars and their interaction on the dry weight of the root system (g).

Cultivars	Irrigation treatments			Average cultivars
	50%	70%	90%	
Indian	0.643	0.573	0.483	0.566
Polish	0.600	0.540	0.436	0.525
Syrian	0.736	0.680	0.573	0.663
Giza 10	0.663	0.613	0.513	0.596
Sakha 5	0.573	0.523	0.440	0.512
Sakha 6	0.703	0.646	0.530	0.626
Giza 11	0.756	0.683	0.566	0.668
Giza 8	0.723	0.653	0.550	0.642
Average irrigation treatments	0.675	0.614	0.511	
LSD irrigation= 0.0108 ·LSD Cultivars = 0.0130 ·LSD interaction= 0.0224				

3.1.5. Average Dry Weight of Vegetative Growth (gm plant^{-1})

The plant dry weight is the final result of the processes of absorption, transport, representation, carbon representation, and respiration. The dry matter in the plant is in the form of organic and mineral compounds, and the percentage of water and dry matter in plants, their organs, and tissues varies greatly Moawed, 2001.

The results of the statistical analysis in Table (5) show that there is a significant difference between flax cultivars in the average dry weight in the plant for the experimental season. The plants of the Giza 11 cultivar achieved the highest average for the trait, reaching (157.11) gm plant^{-1} , compared to the plants of the Indian cultivar, which gave the lowest average for the trait, reaching $129.50 \text{ gm plant}^{-1}$. The reason for this variation in the average dry weight is related to the genetic material of each cultivar and the difference in the genetic compositions of the cultivar. The difference between flax cultivars in the dry weight trait of the plant is consistent with [17], and the result is consistent The results in Table (5) indicate that irrigation treatments have a significant effect on the dry weight of the plant, as the irrigation treatment (90% depletion of

available water) gave the lowest average weight of vegetative growth, which amounted to 131.58 g plant⁻¹, while the control treatment (50% depletion of available water) achieved the highest average for the trait, which amounted to 152.87 g plant⁻¹, which also differed significantly from the irrigation treatment (70% depletion of available water), as it gave an average for the trait of 144.12 g plant⁻¹. The reason for the decrease in the dry weight of vegetative growth in the irrigation treatment (90% depletion of available water) is due to the decrease in the rate of vegetative growth in general due to water stress and because the plant forms and accumulates the components necessary for the production of fruits and seeds, the most important of which are carbohydrates during the vegetative growth stage in the leaves and stems, in addition to a reduction in the height, number and area of the leaves and stems, and then the movement of these components from their storage places in the leaves and stems To fruits and seeds and thus weight reduction [10]. The effect of the interaction between the two study factors was significant in the average dry weight of vegetative growth, as the Giza 11 cultivar in the irrigation treatment (50% depletion of available water) gave the highest interaction value of 168.07 g plant⁻¹, compared to other treatments, especially the Indian cultivar in the irrigation treatment (90% depletion of available water) which gave the lowest interaction value of 119.92 g plant⁻¹.

Table 5. Effect of irrigation levels, cultivars and their interaction on the dry weight of vegetative growth (g).

Cultivars	Irrigation treatments			Average cultivars
	50%	70%	90%	
Indian	138.28	130.31	119.92	129.50
Polish	156.59	146.27	133.86	145.57
Syrian	162.10	155.69	141.24	153.01
Giza 10	148.05	139.92	128..27	138.75
Sakha 5	140.42	131.13	121.40	130.98
Sakha 6	147.92	137.60	125.33	136.95
Giza 11	168.07	157.67	145.59	157.11
Giza 8	161.56	154.41	137.07	151.02
Average irrigation treatments	152.87	144.12	131.58	
LSD irrigation = 0.855 †LSD Cultivars= 1.743 †LSD interaction= 2.885				

3.1.6. Leaf Proline Content ($\mu\text{mol g}^{-1}$ fresh weight)

The results in Table (6) for physiological traits show a significant effect between flax cultivars on leaf proline content, as Giza 11 plants recorded the highest proline content of 16.317 $\mu\text{mol g}^{-1}$ fresh weight, compared to Sakha 5 cultivar, which recorded the lowest average proline content of 13.824 $\mu\text{mol g}^{-1}$ fresh weight, due to their genetic differences.

The results also showed in Table 6 that there were significant differences in irrigation treatments in leaf proline content, as the irrigation treatment (90% depletion of available water) achieved the highest average leaf proline content of 18.441 $\mu\text{mol g}^{-1}$ fresh weight, while the control treatment (50% depletion of available water) recorded the lowest average for the trait of 12.263 $\mu\text{mol g}^{-1}$ fresh weight, which also differed significantly from the irrigation treatment (70% depletion of available water) which achieved an average leaf proline content of 13.989 $\mu\text{mol g}^{-1}$ fresh weight. This increase may be attributed to the plant physiology in order to protect its cells from damage caused by water deficiency by contributing to the prevention of damage from ROS compounds under water deficiency conditions, [4]. The inhibition of the protein synthesis process due to the low water content of the plant led to an increase in the level of amino acids, especially the amino acid proline, which is a tight and cohesive water sheath or crust that works to resist changes under water stress, as its high content increases the plant's ability to absorb water by changing the

osmotic potential of the plant tissue, and the enzymatic activity of protein-degrading enzymes increases with the increase in water tension, such as proteinase enzymes, which causes a decrease in pH, and the accumulation of the amino acid proline increases to help reduce the osmotic potential of the plant tissue, as it works to store metabolic materials in the cells that regulate the osmotic potential to help increase the plant's ability to absorb water from the soil [18]. The result is consistent with what was found by [19], who found an increase in proline concentrations in flax with increasing water stress. The results of Table 10 showed a significant interaction between irrigation treatments and flax cultivars in leaf proline concentration, as Giza 11 cultivar in the irrigation treatment (90% depletion of available water) achieved the highest average leaf proline content of 21.770 $\mu\text{mol g}^{-1}$ fresh weight, compared to Sakha 5 cultivar in the control treatment (50% depletion of available water) which achieved the lowest average leaf proline content of 12.030 $\mu\text{mol g}^{-1}$ fresh weight.

Table 6. Effect of irrigation levels, cultivars and their interaction on leaf proline content ($\mu\text{mol g}^{-1}$ fresh weight).

Cultivars	Irrigation treatments			Average cultivars
	50%	70%	90%	
Indian	12.170	13.847	17.243	14.420
Polish	12.12	14.153	18.133	14.853
Syrian	12.123	13.683	16.950	14.252
Giza 10	12.233	13.973	17.907	14.704
Sakha 5	12.030	13.277	16.167	13.824
Sakha 6	12.427	14.040	20.113	15.527
Giza 11	12.497	14.683	21.770	16.317
Giza 8	12.347	14.257	19.243	15.282
Average irrigation treatments	12.263	13.989	18.441	
LSD irrigation = 0.1002 LSD Cultivars = 0.114 LSD interaction = 0.198				

Conclusions

Giza 11 cultivar and the Sakha 5 cultivar was excelled in all traits While the plants in the irrigation treatment (50% depletion of the available water) excelled in traits.

References

- [1] FAO.2021. Food and Agriculture Organization of the United Nation. FAO STAT. <https://www.fao.org/faostat/ar/#data/QCL/visualize>.
- [2] Nimr, Rasool Hassan, 2023. Effect of planting distances and weed control on the growth and yield of flax. Master's thesis, College of Agricultural Engineering Sciences, University of Baghdad.
- [3] Jassim, Abdul Qader Hamidi, 2021. Genetic indicators of semi-crosses and stability of flax (*linum usitatissimum*) genotypes under the influence of different planting dates. PhD thesis, College of Agriculture, Tikrit University.
- [4] Mahdi, Abdul-Khaq Saleh and Hussein Aziz Muhammad. 2009. Effect of moisture stress, seed hardening process and potassium fertilizer on quantitative and qualitative characteristics of yellow corn crop. Tikrit Journal. 9 (3): 79_90.
- [5] Dash,P.K., Rai, R., Pradhan, S.K., Shivaraj, S. M., Deshmukh, R., Sreevathsa, R. and Singh, N. K. (2023). Drought and oxidative stress in flax (*Linum usitatissimum* L.) entails harnessing non – canonical reference gene for precise quantification of qRT-PCR gene expression. *Antioxidants*,12(4) p.950.
- [6] Rasmus Dahl Lassen, Jan van Hecke, Henning Jørgensen, Christian Bukh, Birgit Andersen and Jan K. Schjoerring. (2018). High throughput analysis of amino acids in plant materials by single quadrupole mass spectrometry, Dahl Lassen et al. *Plant Methods* (2018) 14:8.

- [7] Gupta, M.; S. Kour; V. Gupta; R. Bharat and C. Sharma. 2017. Effect of different doses of fertilizers on yield and NPK uptake of linseed (*Linum usitatissimum* L.). Bangladesh J. Bot. 46(2): 575-581.
- [8] Al-Samarrai, Aws Ali Muhammad. 2019. Response of flax cultivars to organic fertilization from plant sources and salicylic acid. PhD thesis, College of Agricultural Engineering Sciences, University of Baghdad.
- [9] Al-Taie, Ali Saleh Hassoun. 2022. Evaluation of flax genetic structures and estimation of genetic parameters and active compounds under the effect of biofertilization. PhD thesis. Al-Musayyab Technical College. Middle Euphrates University.
- [10] Abu Jad Allah, Jaber Mukhtar. 2018. Plant physiology and molecular biology during water stress. Department of Botany - Faculty of Science / Damietta University. 63- 66.
- [11] Desta,D.S., 2019. Genomics of drought tolerance in flax (*Linum usitatissimum* L.) .Doctoral dissertation, University of Ottawa .
- [12] Moawed, E.A. 2001. Effect of microelements and row distance on the yield of some flax (*Linum usitatissimum* L.) cultivars. Egypt J. Appl. Sci. 16(6): 157-172.
- [13] Bakry, A. B.; M. Sh. Sadak; M. M. S. Abdallah; T. M. Abd El-Razik and M. G. Dawood. 2016. Maximizing the performance, productivity and quality traits of two flax cultivars by using some bio-fertilizers under newly reclaimed sandy soil. Res. J. Pharm., Biol. and Chem. Sci. 7(6): 429-441.
- [14] Kumar, S.; J. K. Singh and A. Vishwakarma. 2016. Effect of NPK levels and bio-fertilizers on quality parameters and seed yield of linseed (*Linum usitatissimum* L.) cultivars under irrigated condition. Intl. Quarterly J. Life Sci. 11(2): 1339-1343.
- [15] Al-Sudani, Ilaf Khader Faleh. 2018. Effect of biofertilization on seed yield and quality of flax oil. Master's thesis, College of Agricultural Engineering Sciences, University of Baghdad. A. S. 92.
- [16] Schachtaman, D.P. and J. Q.D. Goodger. 2009. Chemical root to shoot signaling under drought. Trends plant Sci. 13: 281- 287.
- [17] Bakry, B. A.; D. M. El-Hariri; M. Sh. Sadak and H. M. S. El-Bassiouny. 2012b. Drought stress mitigation by foliar application of salicylic acid in two linseed cultivars grown under newly reclaimed sandy soil. J. Appl. Sci. Res. 8(7): 3503-3514.
- [18] Anjum,N.A.,I.M. Aref. A.C. Duarte,E. Pereira, I.Ahmad and M.Iqbal.2014. Glutathione and proline can coordinately make plants withstand the joint attack of metal(loid) and salinity stresses.Front.plant Sci.,5(662):1-4.
- [19] Mahfouze, H., Mahfouze,S.,El-Enany,M. and Ottai,M., 2017. Assessment of flax cultivars for drought tolerance. Annu Res RevBio,21,pp.1-12.

Efficiency of Treatment Plant and Drinking Water Quality Assessment in Al-Numaniyah City, Iraq

Wisam Thamer Al-Mayah¹ and Wisam Basim Al-Tmemy²

¹Department of Basic Science, College of Dentistry, University of Wasit, Wasit, Iraq.

²Department of Safety and Health Environment, AL KUT University College, Wasit, Iraq.

¹E-mail: walmayah@uowasit.edu.iq

²E-mail: Pad.24.018@alkutcolleg.edu.iq

Abstract. This study aimed to know the purity and suitability of drinking water produced from the Al-Numaniyah city water treatment plant, and to compare the properties of this water with Iraqi and international standard drinking water, and to know the presence of potential pollution points. Physical, chemical and biological tests were conducted on drinking water, and monthly samples were taken from September 2022 to August 2023, for a period of one year. This study has found that: water temperature values ranged between (11-35) , turbidity values (2-68) NTU, Electrical conductivity (980-1375) $\mu\text{S cm}^{-1}$, and total dissolved solid (655-893) mg/L. It was found that the drinking water at Al-Numaniyah city studied was basophilic (pH =7.2-8.1) with free residual chlorine values recorded varied monthly (0.46-3.1) mg/L. It was evident the total hardness was high and ranged between (320-519) mg / L, and sulphate values ranged between (300-437) mg/L. The highest bacterial count was recorded at raw water (R) the during January 2023 (905 cell /1 ml), whereas the lowest value was found during April 2023 (Zero cell /100ml), whereas numbers of *E.coli* were (0-433) cell/100 ml. The results of the water quality index (AW-WQI) showed that all the collected drinking water samples were considered to be of poor quality (51-75) to very poor (76-100). The results showed that all the water quality parameters are exceed maximum permissible limit values recommended by World Health Organization (2017) and Iraqi drinking water standards (417/2011), except pH, EC and TDS. In terms of water quality index (AW-WQI), the results showed that poor quality (51 - 75) for all season according to the classification of Arithmetic Weighted Index except the winter is very poor (76-100).

Keywords. Water treatment plant, Drinking water, Raw water, Efficiency, WQI.

1. Introduction

Water is one of the basic natural resources that every living organism needs, in addition to that water represents one of the strategic dimensions in making life in all its forms and ensures its continuity and sustainability [1]. Al-Mayah [2] defined by the World Health Organization (WHO) that "any change in the natural, chemical and biological properties of water causes its state to change directly or indirectly so that the water becomes less suitable for natural uses intended for it, whether for drinking or for domestic, agricultural or other consumption.

In Iraq, the need for water resources is constantly increasing as a result of growth and population expansion in addition to the lack of rain, which led to water scarcity, and the conditions experienced by Iraq led to the deterioration of its quality and this water has become one of the important factors in the transmission of diseases 'as well as containing organic matter, which is a good food source for the growth and reproduction of microorganisms [3].

The sources of drinking water in most societies are surface water such as streams, rivers and lakes, and this water is always exposed to pollution, as it enters unwanted chemicals and microbes, causing a change in the quality of water and affecting its environment unevenly according to the concentrations of these materials and the number of microbes, so the balance in the environment of this water may be compromised as the same water is used for drinking and hygiene as well as used in agriculture, so drinking water becomes at great risk Unless treated hygienically and drinking water supplies are controlled with care and precision [4].

Many studies and scientific research have been conducted that dealt with the levels of water pollution in Iraq from various aspects the extent of its impact on human health and how to treat it and reduce its risks [5-7].

From the foregoing and the importance of drinking water and the lack of research and studies related to the quality of local domestic drinking water, this study came to aim at the following: Identifying the microbial content (bacteria) pathogenic in liquefied water, due to its wide spread and its importance in causing diseases in Wasit Provenance - Al-Numaniyah city. Estimating physical and chemical pollutants, identifying pollutants that affect water, and reaching conclusions and proposals that lead to maintaining water quality. Studying the qualitative and quantitative standards for drinking water pollution and their conformity with international and local standard specifications.

2. Materials and Methods

2.1. Description of Study Area

The Al-Numaniyah city is one of the cities of Wasit Provenance in Iraq, about 45 km north of Kut city, located on the banks of the Tigris River, about 219 km southeast of Baghdad Governorate as presented in Figure (1). There is a drinking water purification project in the city, which was established in 1972. It takes its water from the Tigris River, filters and purifies it, and delivers it to the city's neighbourhoods through the network.

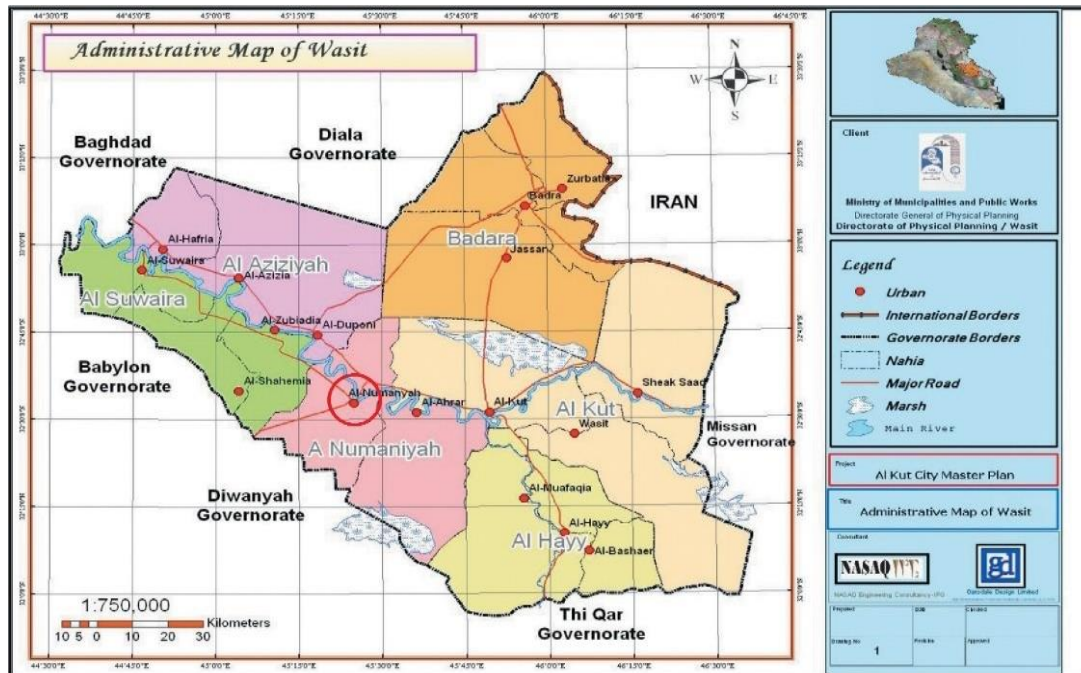


Figure 1: Map of location study area (Al-Numaniyah city).

2.2. Sampling

Water samples were collected during September 2022 to August 2023, at a rate of three groups of models per month, through all stages of filtering to homes, and each set of samples includes the following:

- Raw water (from the middle of the river) (S1).
- Water coming out of the filter complex (S2).
- Water for the first house in the network (S3).
- Water for the last house in the network (S4).

2.3. Collecting Water Samples

For the purpose of collecting water samples, two sets of bottles were used, the first group of 250 ml is intended for the collection of water samples for microbial tests, which are bottles with hermetic plugs containing 0.2 ml of sodium thiosulfate solution at a concentration of 10% to neutralize the effect of residual chlorine [8], sterilized by means of a separatist at a temperature of 121 °C for 30 minutes.

The second group is plastic bottles of 500 ml with a tight lid washed with soap and then diluted hydrochloric acid to remove sediment from the inner surface of the vial, and then was homogenized with distilled water three times to remove the effect of acid, and is intended for collecting water samples for the purposes of physical and chemical tests.

2.4. Physical and Chemical Tests

These examinations were carried out according to the working methods mentioned below:

- Temperature; Using a mercury thermometer by dipping it directly at the specified location for sampling and measuring the temperature after it is fixed by measuring Celsius [8].

- Hydrogen pH degree: Using a (pH) meter of the type (Hana) according to [8].
- Electrical conductivity: EC using the Portable Conductivity Meter of the Bischof L17 type according to [8].
- Dissolved solids (TDS): TDS can be calculated in terms of the electrical conductivity of water [8] as in the following equation

$$\text{TDS mg/l} = \text{E.C. } \mu\text{S/cm} \times 0.64$$

- Turbidity: Turbidity using the Turbidity Meter 355 according to [8].
- Residual chlorine. Based on the colour method and using the DPD Free, Total & Combined Chlorine Test Kit Model SL-26. According to [8].
- SO₄ sulphate: The sulfate was measured using the turbidity method and measured by a spectrophotometer with a wavelength of 450 nm and expressed in units (mg/L) according [9].
- Total hardness: Hardness was measured by the method of abrasion using (EDTA) according to [9].

2.5. Bacteriological Tests

2.5.1. Total Bacterial Count (TPC)

Calculation of the total count of bacteria Total Bacterial Count. The total number of bacteria in the water was calculated using the dish counting method by planting 1 ml of the original or diluted sample (1:10,1:100,1:1000), on the Nutrient agar medium by Power plate method incubated at 35 °C for 48 hours, then the number of colonies in 1 ml and if diluted and using the equation given in [10].

$$\text{CFU/ml} = (\text{No. of colony} \times \text{volume of sample}) / \text{Dilution}$$

2.5.2. *E. Coli* Bacteria

The total number of *E. Coli* bacteria was calculated by the most probable count method (MPN) given in [10] and according to a special table the most likely number of *E. coli* bacteria is estimated at 100 ml of the original sample. The confirmatory examination was carried out by inoculating three groups of tubes containing mono-concentration MacConkey broth by the loop through the work of secondary cultures of tubes with positive reactions from the previous examination and incubated at 44 C° for 24 hours and then the number of positive tubes was calculated and under a special table the most likely number of fecal coliform bacteria was found in 100 ml of the sample.

2.6. Calculate of Water Quality Index (AW-WQI)

Eight factors were used to calculate the water quality index for drinking purposes, which are water temperate, turbidity, electrical conductivity, total dissolved solid, free residual chlorine, total hardness, sulphates, and total bacterial count. To calculate the above quality indices, the Arithmetic Weighted Index method (AW-WQI) was used, where the water quality data were collected and arranged according to season, and the index values were calculated based on the following:

- First step: The quality rating scale (Q_i) is calculated for each parameter.

$$Q = [(V_a - V_i) / (V_s - V_i)] \times 100$$

Where:

V_a is actual value of the water quality factors (physical and chemical properties) that obtained from laboratory analysis as presented Table (3), V_i is ideal value of factors, all ideal values are zero except pH=7, and V_s is standard permissible values for drinking water quality as recommended by Iraqi and international standards Table (2).

- Second step: The relative weight (W_i) is calculated from the inverse proportional value of the standard specification (V_s) corresponding to each of the factors.

$$W_i = 1 / V_{\text{standard}}$$

Finally: The total water quality index (WQI) is calculated by assembling the qualitative classification (Q_i) with the relative weight (W_i) written by using the following equation:

$$WQI = \sum_{i=1}^n \left[Q_i W_i / \sum_{i=1}^n W_i \right]$$

The water quality index for optimal and accurate water use is discussed according to [11], Table (1).

Table 1: Water quality classification based on AW-WQI values.

Class	Water quality	AW-WQI rank
I	Excellent	0 -25
II	Good	26 - 50
III	Poor	51 - 75
IV	Very Poor	76 - 100
V	Unsuitable	>100

3. Results and Discussion

3.1. Water Quality Parameters (WQPs)

3.1.1. Water Temperature

The growth of microorganisms increases with the rise in water temperature and thus temperature plays an essential role in determining many of the physical, chemical and biological properties of water and water temperatures vary significantly because they are associated with climatic, geological and hydrological factors [12]. Table (2) show the temperature values during the study period, the temperature of the river water ranges between 13-32 C°, while drinking water had temperature values ranging between 10-30 C°, and it was noted that there were few site changes in water temperatures in different locations and during the same month, and this difference may be due to the time of collecting and examining samples. This study was similar to the results of Al-Mayah study [13] in which bacterial contamination of drinking water in Wasit governorate was studied.

3.1.2. Turbidity (NTU)

Turbidity is a measure of the degree of transparency of water and expresses the optical property of the dispersion and absorption of light during its passage through the water sample by suspended particles [14], and it is also responsible for protecting organisms from the effects of sunlight and toxic substances such as chlorine [15]. Table (2) shows the turbidity values during the study period, turbidity of river water ranging between (27-68) NTU, while drinking water values of turbidity ranging between (3-9) NTU. From comparing the results with the Iraqi drinking water standards for drinking water [16], which were determined at (5) NTU and the World Health Organization [15], which were determined at (5) NTU, Table (2) the majority of the samples studied exceeded these determinants.

3.1.3. Electrical Conductivity (EC)

The measurement of electrical conductivity is evidence of changes in the concentrations of total solids and concentrations of dissolved salts such as sulfate and chlorides salts, as well as changes in the concentrations of calcium, magnesium and other ions, as the conductivity of electrical conductivity is often related to the sum of solids dissolved in water [17] and is expressed in microcemics units per cm ($\mu\text{S. cm}^{-1}$). Table (2) show the values of electrical conductivity during the study period, the electrical conductivity of river water ranges between (980-1345) $\mu\text{S. cm}^{-1}$, while drinking water values of electrical conductivity ranged between (987-1369) $\mu\text{S. cm}^{-1}$. From comparing the results with the Iraqi standard determinants (2011) for drinking water, which were determined by (1500) $\mu\text{S. cm}^{-1}$ and the determinants of the World Health Organization (2017), which were determined by (2000) $\mu\text{S. cm}^{-1}$, we note that all the studied samples did not exceed the permissible limits.

3.1.4. Total Dissolved Solid (TDS)

Dissolved solids (TDS) are defined as all dissolved solids in water, whether ionized or non-solid and do not include suspended or colloidal substances [15]. The quantity of TDS and its concentration is important for the quality of water and depends on the validity of water for domestic, industrial, and agricultural purposes, and these salts have an impact on the appearance of an unpalatable taste that appears after the concentration of 600 mg / l, as we find that the values of TDS for raw water ranged between 655-862 mg /L. The results of drinking water ranged between 671-893 mg/L as presented in Table (2). The months with high concentrations of dissolved solids in their water models are rainy months and rainfall when it falls, especially in densely populated cities and industrial areas, carries with them pollutants in the atmosphere and soluble, and when these rains reach the rivers increase the concentrations of substances in the water [18].

3.1.5. Power Hydrogen (PH)

Iraqi and international specifications indicate that the value of pH should be between 6.5-8.5 for potable water [19]. In general, most of the Iraqi water tends to basal slightly because of the presence of carbonates and bicarbonates, and the pH values shown in Table (2) ranged between (7.3-8.1) for river water, while drinking water was pH values between (7.2-7.8). They are within the permissible limits. It is known that determining the pH value of the water entering the filter complexes is important because of its impact on the treatment process, especially in the case of high or low pH value, but the treatment process does not cause a change in the pH value except in the case of adding basic or acidic substances, and accordingly we find that the results of the table for pH values are close so as not to add acidic or basic substances during the treatment process.

3.1.6. Free Residual Chloride

The characteristic of chlorine survival in the water is a distinctive property that may make it chosen in the purification plants to ensure safe water during its passage through the distribution networks and until it reaches the consumer, and the filtration process does not work efficiently to remove bacteria and viruses due to the small size of these organisms, which requires the addition of chlorine to ensure their elimination [5].

The permissible limits of residual free chlorine concentrations are 0.3-2.0 mg/L, if this percentage decreases, the possibility of water contamination increases microorganisms, especially when there is leakage or cracks in the water network, and if the percentage of chlorine increases, this will be dangerous due to the combination of chlorine with organic compounds that give rise to compounds that have a health effect [20].

We note from the results of Table (2) that the concentration of free chlorine remaining ranged between (0.46-3.1) mg/L, which is outside the Iraqi and international standard specifications, and this threatens the possibility of biological contamination of water, as for the sites that recorded a concentration of less than 0.3 mg / l upon examination, this is caused either by adding low concentrations of chlorine or not adding it to the water, so this water coming out of this station is vulnerable to biological pollution.

3.1.7. Total Hardness (TH)

Hardness is one of the traditional measures of water quality and suitability for human use, hardness is not caused by the effect of one substance, but by various polyvalent metal ions, as the positive calcium and magnesium ions are the dominant ions. We note from Table (2) that the values of hardness ranged between 320-489 mg/L for raw water, while the results of drinking water have ranged values between 343-519 mg/L. This result showed that this water is very hard according to the classification [21]. The recorded increase in hardness values in the winter months may be due to the drift of pollutants into the river water from industrial waste near the river. Agricultural flows from neighbouring lands also led to high hardness rates in the studied drinking water (Al-Mayah, 2018). By comparing these results with the standard standards, it was found that some samples exceeded the permissible limits according to the Iraqi drinking water standards (417/2011), which were set at (500) mg/L, and the World Health Organization (2017), which set to (500) mg/L.

3.1.8. Sulphate (SO₄)

Table (2) show the sulphate values during the study period, sulphates for river water range between (300-410) mg/L, while drinking water sulphate values range between (320-437) mg/L, which did not match Iraqi drinking water standards (417/2011), and World Health Organization standards (2017). The reasons for the increase in sulphate concentrations in the water may be due to the gypsum nature of sedimentary soils, which are a direct source of sulphates dissolved in natural water and the nature of the agricultural area surrounding the river and its use of fertilizers, as these materials contain a good percentage of sulphates that eventually make their way to the river [22].

3.1.9. Total Bacterial Count (TBC)

The presence of bacteria in raw water naturally is very normal, as these are part of the living components of the ecosystem, and as their numbers increase and their types vary when there is any source of organic pollution that may benefit us in sterilization operations in purification plants, and it is possible to measure and know the degree of river nutrition through the presence of those bacteria in its waters, as these bacteria increase in rivers containing high concentrations of nutrients [23].

Aerobic bacteria are one of the most common pollutants in water, as their numbers in raw water models range between (153-905) cells / 1 ml, while drinking water was between (0-13) cells / 1 ml, and from Table (2), it turns out that it is the highest numbers during the month of January 2023 and the lowest during the month of November 2022, and this is consistent with [19][24], who confirmed higher rates of aerobic bacteria during the winter months than during the summer months. The total numbers of bacteria did not conform to the standard specifications for Iraqi drinking water [16] of 10 cells / 1 ml and the universal of 5 cells / 1 ml [15], and the presence of some types of aerobic bacteria resistant to chlorine may be due to the effectiveness of [25].

3.1.10. *Escherichia Coli* Bacteria

The concentrations of coliform bacteria in the raw river samples ranged between (275-433) cells / 100 ml, while drinking water was between (0-7) cells / 100 ml, the highest value was in January 2023 and the lowest in November 2022 as in Table (2). The reason for the increase in the number of fecal coliform bacteria and *E. Coli* bacteria may be due to the lack of control of the quantities of alum and chlorine doses added to the water in the station under study, and the addition of alum in an uncalculated manner based on the estimate may also contribute to the lack of quality of this station in reducing the numbers of these bacteria [26][27].

Table 2: Annual mean of the physical, chemical, and bacteriological values in the raw water and water network in the drinking water purification plant in the Al- Numaniyah city for the period from September 2022 to August 2023.

Seasons	Sites	W.T °C	Turb NTU	E.C µS/cm	TDS mg/L	pH	R.Chl mg/L	T.H mg/L	SO4 mg/L	TCP cell/1ml	E. Coli cell/100ml
Autumn 2022	S1	27	59	1230	784	7.4	-	411	345	153	275
	S2	25	2	1239	792	7.3	1.6	429	363	5	1
	S3	25	4	1280	819	7.5	0.9	435	357	6	3
	S4	24	8	1305	855	7.3	0.5	437	350	5	1
Winter 2023	S1	13	68	1345	862	8.1	-	489	410	905	433
	S2	13	9	1366	875	7.8	2.8	500	437	7	1
	S3	10	7	1369	877	7.4	0.81	503	431	9	3
	S4	13	9	1375	893	7.8	0.46	519	429	16	7
Spring 2023	S1	30	27	980	655	7.5	-	387	300	125	211
	S2	28	4	987	671	7.6	0.9	391	325	4	0
	S3	28	6	995	679	7.4	2.7	400	322	5	3
	S4	26	7	1100	782	7.5	0.9	407	320	8	3
Summer 2023	S1	32	49	1144	715	7.3	-	320	351	99	200
	S2	30	3	1169	734	7.5	2.9	343	366	5	0
	S3	31	5	1190	765	7.4	0.8	355	360	3	2
	S4	30	7	1195	788	7.2	0.7	364	358	6	4
						6.5					
WHO limits		25	5	2000	1500	-	2	500	250	10	0
						8.5					
						6.5					
Iraqi Criteria		25	5	1500	1000	-	2	500	250	10	0
						8.5					

3.2. *Water Quality Index (AW-WQI)*

The average value of the water quality index for all drinking water samples collected during four seasons is (63.4), which is equivalent to poor water quality as presented in Table (3) and Figure (2). Higher water quality index values were observed in drinking water samples collected in winter as presented in Table (3) compared to those of water samples collected in autumn, spring and summer as presented in Table (3) respectively. The observed high water quality index values are associated with high values of turbidity, dissolved solids, sulphate (NTU, TDS, SO4), and total bacterial count (TCP) measured in drinking water samples collected in January 2023. This may be due to an inadequate treatment system, poor water quality monitoring, and contamination in the different connections of networks.

Table 3. The AW-WQI values of the water produced by water treatment plant in Al-Numaniyah city.

WQPs / WQI Calculat e	Autumn 2022								AW- WQI Valu e	AW- WQI Ran k	Water Qualit y Rating
	W.T. °C	Turb · NTU	E.C μS/c m	TDS mg/ L	R.Chl · mg/L	T.H mg/ L	SO ₄ mg/ L	TCP cell/1m l			
V actual	25	4	1280	819	0.9	435	357	5	57.08	51- 75	Poor
QI	100	80	85.33	81.9	45	87	142. 8	50			
WI	0.04 7	0.236	0.001	0.00 1	0.590	0.00 2	0.00 5	0.118			
WIQI	4.72	18.88	0.07	0.10	26.54	0.21	0.67	5.90			
Winter 2023											
V actual	13	9	1369	877	0.81	503	431	9	80.70	76- 100	Very Poor
QI	52	180	91.26	87.7	40.5	100. 6	172. 4	90			
WI	0.04 7	0.236	0.001	0.00 1	0.590	0.00 2	0.00 5	0.118			
WIQI	2.45	42.47	0.07	0.10	23.89	0.24	0.81	10.62			
Spring 2023											
V actual	28	6	995	679	0.9	400	322	5	66.97	51- 75	Poor
QI	112	120	66.33	67.9	45	80	128. 8	50			
WI	0.04 7	0.236	0.001	0.00 1	0.590	0.00 2	0.00 5	0.118			
WIQI	5.29	28.31	0.05	0.08	26.54	0.19	0.61	5.90			
Summer 2023											
V actual	30	5	1190	765	0.8	355	360	5	59.75	51- 75	Poor
QI	120	100	79.33	76.5	40	71	144	50			
WI	0.04 7	0.236	0.001	0.00 1	0.590	0.00 2	0.00 5	0.118			
WIQI	5.66	23.59	0.06	0.09	23.59	0.17	0.68	5.90			

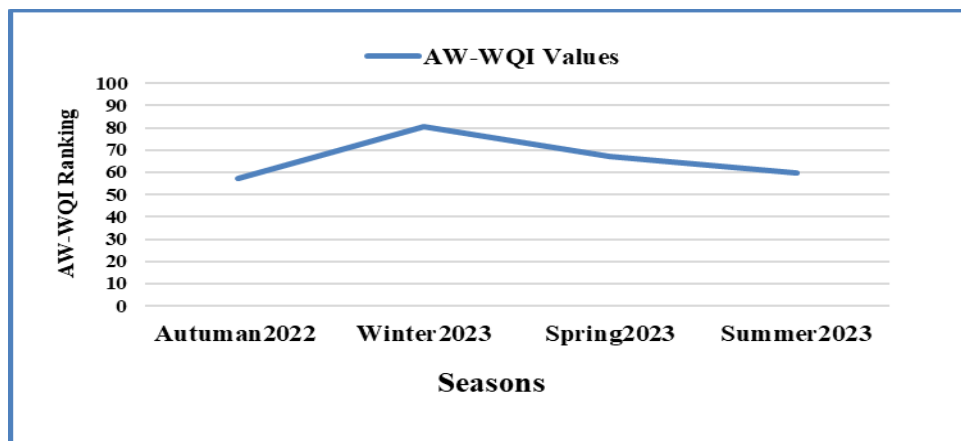


Figure 2. AW-WQI values during study period.

Conclusion

Based on the results, it was found that some of the investigated drinking water quality parameters (turbidity, SO₄, TH, TPC, E.Coli) were not within permissible limit values recommended by World Health Organization (2017) and Iraqi drinking water standards (417/2011). The removal efficiency of the treatment plant was at an unsatisfactory level, and the water quality index was classified as poor water quality. To properly treat drinking water and improve the treatment plant, proper pretreatment such as screening to reduce turbidity, proper chlorination of the drinking water system, and frequent monitoring and maintenance of the treatment plant system are required.

Acknowledgments

The authors are grateful to the owners of Sincere thanks and gratitude to the Central Health and Environment Laboratory in the Ministry of Health, Iraq for providing opportunity and support for conducting this research.

Reference

- [1] P. Li and J. Wu, (2019). "Drinking water quality and public health," Exposure and Health, vol. 11, no. 2, pp. 73–79, 2019.
- [2] Al-Mayah W. Th. (2018). Evaluating of Water Quality in Al-Gharraf River Southern of Iraq Using Different Environmental Indices, Remote Sensing Technique and Geographical Information System. Ph.D. Thesis, College of Science, University of Baghdad, Iraq: 43p.
- [3] A. S., Ahmed and H. A., Jabbar. (2023). Evaluation The Water Quality of Al-Rusafa Treatment Plant in Baghdad City / Al-Rusafa Side Using Several Water Quality Indices. 3C Tecnología. Glosas de innovación aplicada a la pyme, 12(1), 176-189. <https://doi.org/10.17993/3ctecno.2023.v12n1e43.176-189>.
- [4] Belay Desye, Biniam Belete, Zinabu Asfaw Gebrezgi, and Tsegaye Terefe Reda (2021). Efficiency of Treatment Plant and Drinking Water Quality Assessment from Source to Household, Gondar City, Northwest Ethiopia. Journal of Environmental and Public Health
- [5] Al-Tmemy. W. B. M. (2018). Evaluation of drinking water treatment plants in Wasit Province, depending on the chemical by-products resulting from chlorine disinfection. Ph.D. Thesis, College of Science, University of Baghdad, Iraq: 117p, 2018.
- [6] Ali H. M. ; Zageer D. and Alwash A. H. (2019). Performance Evaluation of Drinking Water Treatment Plant in Iraq. Orient. J. Phys. Sciences, Vol. 4(1) 18-29 (2019) 19.
- [7] Al-Mayah, W. Th. (2024). Ecological and Health Risks Assessment of Levels of Heavy Metals in Drinking Water of Baghdad City, Iraq. Asian J. Biol. Sci., 17 (4): 558-566, 2024.
- [8] APHA,AWWA and WFF (2015). Standard Methods for the Examination of Water and wastewater, 23th ed., edited by Eaton, A. D American Water Work Association and Water Environment Federation, USA.
- [9] EPA (Environmental Protection Agency), (2015). Parameters of Water Quality Interpretation and Standards. Ireland: Environmental Protection Agency. ,Ireland. www.epa.ie/whatwedo/advice/wastewater.
- [10] WHO, (World Health Organization), (2011) WHO Guidelines for Drinking-water Quality, 4th edition; WHO: Geneva, Switzerland.
- [11] Shweta, T., Bhavtosh, S., Prashant, S., Rajendra, D., (2013). Water quality assessment in terms of water quality index. Am. J. Water Res. 1 (3), 34–38.
- [12] Robinne F.N., Hallema D.W., Bladon K.D., Buttler J.M. 2020. Wildfire impacts on hydrologic ecosystem services in North American high-latitude forests: A scoping review. Journal of Hydrology 581(12), 22-31.
- [13] Al-Mayah, W. Th. (2021). Chemical and microbial health risk assessment of drinking water treatment plants in Kut City, Iraq. Materials Today: Proceedings, 42, 3062-3067.

- [14] Angela Casado, Primitivo Ramos, Jaime Rodríguez, Norberto Moreno & Pedro Gil,(2015), Feasibility of sulfide control in sewers by reuse of iron rich drinking water treatment sludge, *Water Research*, Volume 71, Pages 150-159.
- [15] WHO, World Health Organization. Guidelines for Drinking Water Quality, 4rd Edition, WHO, Geneva, 2017.
- [16] ICS, Iraqi Criteria and Standards of water's chemical limits/7.100.20 number 2270/14, first update 2011.
- [17] H.M. Rafique, Iftikhar Abbas, Muhammad A. Sohl, Ramoona Shehzadi, Shahid Mahmood Ramay, Muhammad Imran, Yousef Al-Zaghayer, Asif Mahmood & Maryia N. Sohl,(2014), Appraisal of drinking water quality of tehsil Jampur, Pakistan, *Desalination and Water Treatment* Volume 52, Issue 25-27,pages 4641-4648.
- [18] Wisam Thamer Al-Mayah & Wisam Basim Al-Tmemy (2024). Use Remote Sensing, GIS and Regression Analysis in Monitoring Water Quality of Al-Gharraf River Southern of Iraq. *Middle East Res J Biological Sci*, 4(4): 96-112.
- [19] Al-Mayah, W. Th. (2022). Evaluation of Efficiency of Drinking Water Treatment Plant in Al-Aziziyah City, Iraq. *Sci Res. Jr Agr Lf Sci*; Vol-2, Iss-4 (Jul-Aug, 2022): 1-7.
- [20] World Health Organization (2022). Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda. Geneva:World Health Organization; 2022. <https://www.who.int/publications/i/item/9789240045064>.
- [21] Lind, O.T. (1979). A handbook of Limnological methods C.V. Mosby, St. Louist 199pp.
- [22] Al-Mayah, W. Th. (2013). Effect of domestic sewage on water quality of Al-Gharraf River in Al-Haay city. M. Sc.Thesis. College of Science, University of Baghdad, Iraq, 2013.
- [23] Banda, R. K., Mubita, P., Moonga, G. & Meki, C. D. (2021). Bacteriological quality and heavy metal analysis of packaged water produced in Lusaka, Zambia and associated quality control measures. *Frontiers in Public Health* 9, 620700. <https://doi.org/10.3389/fpubh.2021.620700>.
- [24] Oumar Traoré; Nafissatou Ouédraogo; René Dembélé; Dissinviel Stéphane Kpoda; Courage Kosi Set soafia Saba; Isidore Juste Ouindgueta Bonkougou; Nicolas Barro (2023). Physicochemical and bacteriological quality and safety of packaged drinking water in Ouagadougou, Burkina Faso. *Water Supply* (2023) 23 (10): 4346–4356. <https://doi.org/10.2166/ws.2023.249>.
- [25] Zmirou, D., S.Rey, X.Courtois, J.P.Ferley, J.F.Blatier, P.Chevallier, J.Boudot, J.L.potelon, and R.Mounir. (2017) . Residual microbiological risk after simple chlorine treatment of drinking ground water in small community systems . *Eur. J. Public. Health*. 5: 75 – 81.
- [26] Al-Mayah, W. Th., Emad A. Mohammed Salih Al-Heety, Wisam Basim Al-Tmemy, et al. (2022). Evaluation of Efficiency of Water Treatment Plants in Baghdad City Using Comprehensive Pollution and Water Quality Indices. *AIP Conf. Proc.* 2398, 040053-1–040053-15; <https://doi.org/10.1063/5.0093956>.
- [27] WB Al-Tmemy, YF Alfatlawy, SH Khudair(2018)). Seasonal variation and modeling of disinfection by-products (DBPs) in drinking water distribution systems of Wassit Province Southeast Iraq. *Journal of Pharmaceutical Sciences and Research* 10 (12), 3393-3399. 2018.

Comparison Between the Growth Trait Performance of Awassi and Karakul Sheep

Zaid M. Jassim¹, Thamer R. S. Aljubouri² and Mohammed Baqur S. Al-Shuhaib³

¹⁻³Department of Animal Production, College of Agriculture, Al-Qasim Green University, Al-Qasim 51013, Babil, Iraq.

²E-mail: thamerradhi@agre.uoqasim.edu.iq

Abstract. A comparative analysis between two sheep breeds, Karakul and Awassi, was undertaken to understand their growth performance when kept under similar management and feeding conditions. The two-breeding sheep were kept in the same optimal environment in Al-Kafeel Company/Iraq. Preference was given to the measurement of six key growth traits that favor sheep husbandry: body weight, body length, wither height, shoulder height, and circumferences of chest and abdomen. Measurements were made 24 times over a one-year period on 140 sheep (70 from each breed). Consequently, it was found that the Karakul sheep type had better performance than Awassi sheep in most of the growth performance parameters. Several disparities were observed on the body weight and body size of the two breeds. The study also considered the influence of age and sex of the dam on growth traits of the lambs. This observed that the lambs produced by older ewes had better growth rates right from birth up to about 3 months of age and after that, there was no significant difference in the performance of the various groups of lambs. At six months, the study recorded a sex effect in that the male lambs recorded better growth rates as compared to female lambs. This suggests that the Karakul sheep, a native breed, have a genetic predisposition for meat production. Furthermore, the research places emphasis on the importance of not only the dam's age but also her sex as significant factors in the growth of their progeny, which must be given careful consideration in both breeding and management practices. If sheep breeders heed these lessons, they can expect to make growth-related decisions that will elliptically benefit not just them, but also the state and national economies in the long run.

Keywords. Breed comparison, Dam age, Growth indices, Sex influence, Sheep.

1. Introduction

Global agriculture industry is depend on sheep farming for its wool and meat product. Sheep rearing in arid and semi-arid regions of the world play a key role in life support to millions people economically. The domestication of the sheep and their over one billion populations worldwide provide many critical forms part of our biological fabric, for example meat, milk wool as well leather; they define village Europe [1]. Sheep are not only economically important but have cultural and traditional importance in many societies. In response to the growing global population, agriculture faces an enormous challenge to improve productivity and efficiency in its systems with few corners where greater pressure is exerted than on livestock production. Consequently, enhancing growth traits in sheep has become an important target for improved

meat production given that it is the main economic trait involved with regards to sheep farming [2]. Body weight, body length, wither height, rump height is the important indicator of growth which shows health and meat production tendency in animal. These traits do not only help determine the market value of these sheep but also their adaptability in different environments and reproductive performance. Accordingly, growth traits are the primary target for genetic selection and breeding programs designed to improve productivity and sustainability of sheep farming enterprises [3].

There are many breeds in the Middle East, among them are Karakul and Awassi that both have unique traits which position them ideally in the respective agriculture system in the region [4]. The Karakul, hailing from the harsh environs of Central Asia (arid to semi-arid regions), is one breed well known for its native ability. Karakul sheep are historically farmed for their fur, which is used to create karakul (textile), a type of coat that forms the basis of many traditional hats and coats. The pelts are of high value but Karakul sheep was used also for meat and wool, being a multipurpose breed suitable to be included in mixed farming systems [5]. The Awassi breed is one of the world's oldest and most prolific sheep breeds, with well known Middle Eastern origins [6]. Awassi are renowned for a great dairy breed, and their milk provides higher yield than almost any other sheep. Their milk is used to make classic dairy products like cheese and yoghurt, both of which are important parts of Middle Eastern diets [7]. Awassi sheep are also best-suited for tropical climates, thus more suitable in the conditions with intense environmental pressure [8]. A large number of breeding programs have been conducted for both Karakul and Awassi sheep to produce better pelts in the case of Karakul or improve milk production in those Awassi breed as well [9]. On the other hand, the growth traits of these breeds, which are particularly important to meat production, have been little studied in a comparative context.

As such, growth traits have always been major parameters for assessing meat production potential of an animal – one among the topmost economical trait considered in sheep farming [10]. Growth traits such as body weight are the main contributors to an increase in market value of animals, and the efficiency with which feed is converted into muscle mass. The body length, wither height, rump height, chest circumference and abdominal perimeters as other growth traits bring more information about the entire aspect regarding conformation and welfare of sheep [11]. In sheep breeding, selecting animals with superior growth traits can lead to significant improvements in flock productivity and profitability [12]. The selection of animals with improved growth traits is a course of action executed in sheep breeding that has the potential to result in noteworthy gains for productivity and profit from flocks.

Several reports have also shown the genetic background of growth traits in sheep [13], indicating that such characters may be improved substantially by selection [14]. Growth traits are, however controlled by environmental attributes in addition to these genetic factors) that include nutrition, management and climatic variables. From this it can be seen that the study of growth traits is a complicated, but very important area within different breeds and under various conditions [15].

There are numerous factors influencing the growth traits of sheep, which includes genetics, environmental conditions and management practices. Genetics itself is one of the most important factors, as certain breeds are genetically predisposed to a better growth performance [16]. For example, the Karakul and Awassi breeds have usually been selected for hardiness and adaptation in arid conditions or harsh environments [17]. Genetic differences among these breeds may also involve growth traits, which reinforces the importance of a comparative analysis between them [18].

Growth traits are also highly influenced by environmental factors such as feed quality, grazing conditions and climate. Sheep raised with better forage conditions are considered more lightly in the kind of foraging environments thus their growth performance would be likely to increase than

those grown under harsher situations [19]. Furthermore, the expression of growth traits can be modulated by management practices such as weaning time or supplementation strategies and health care. It is well known that the growth traits of sheep are affected by dam age and sex. Older dams are also known to produce lambs with significantly better early growth traits. But as the lambs get older, this advantage may decline. For example, male lambs usually grow more rapidly than females do, especially after weaning, because of hormonal differences and different muscle development. Therefore, it is critical to demonstrate the effects of these environmental factors in order that breeding and management practices can be optimized for growth traits in sheep [20]. In the available literature, plenty of studies have been documented on growth traits in sheep breeding, but no significant data for Awassi and Karakul breeds seems to be reported. Existing reports on growth traits in sheep were breed specific or carried out under general context without considering the particular genetic and environmental factors that may affect such phenotype. Hence, the objectives of this study were to perform detail comparative analysis on growth traits between Karakul and Awassi sheep to understand to what extent these differences are noticeable. This study will assess the superior growth performance under similar environmental and management conditions by using different growth traits like body weight, body length, wither height, rump height, chest circumference, and abdominal circumference. Furthermore, the current research will investigate how dam age and sex affects these growth traits that can be included for further parameter optimization in productivity of this breeds.

2. Materials and Methods

2.1. Study Location and Animals

This study was conducted to make a compare the growth traits in two sheep breeds, Karakul and Awassi in Al-Kafeel Company / Barakat Aba Al-Fadl (peace be upon him) station, operation with laboratories in of Al-Qasim Green University. Ethical approval No. 151LBI04002. The conducted experimental procedures were mainly consisted of lambs from the two breeds, that were monitored since birth to 12 months in age. The animals were kept in similar environmental conditions in the station. All procedures performed in studies involving animals were under the ethical standards of institutional animal care and use committee at Al- Qasim Green University. Tests were performed to minimize the inconvenience and stress of lambs during measurements.

2.2. Management and Practices

In the conducted phenotypic records, 100 Karakul lambs and an equal number of Awassi lambs were used to make a total of 200 lambs. Karakul and Awassi sheep were managed on a standardized regime to make data from both groups comparable. Animals were in submitted to equivalent accommodations, and received identical diets/nutrition and veterinary care. The animals were subjected to routine veterinary examination and preventive health care based on the outcomes of growth trials provided there had been no disease outbreaks that would affect the results.

2.3. Data Collection

This study aimed to evaluate growth performance traits at birth to twelve months of age in Karakul and Awassi lamb breeds. Growth traits were evaluated included; body weight, body length wither height, rump height, chest circumference and abdominal circumference. Body weight, body length, wither height, rump height, chest circumference, and abdominal circumference. For body weight, each lamb was weighed using an electrical scale. Body length was determined using a tape from the shoulder to pin bone. The measurement of wither height

was obtained using a measuring stick to measure the distance from the ground up to their withers. With regard to rump height, the distance from the ground to the highest point of rump was recorded.

2.4. Statistical Analysis

Data were expressed as means $\pm$ standard errors using SPSS package [21]. The differences between Karakul and Awassi lambs for each growth trait were analyzed using a t-test approach. Significance levels of the conducted phenotypic records were set at $P < 0.05$ for significant and $P < 0.001$ for high significant, for comparison within the same parameter. Descriptive statistics (mean, standard error) were calculated for each measurement at each time point. The significance of differences between groups (breeds, dam ages, and sexes) was assessed using Analysis of Variance (ANOVA). Where significant differences were found, post hoc tests were applied to determine the specific differences between groups.

2.5. Correlation Matrix Analysis

The correlation matrix that depicts the relationships between various growth traits in sheep was investigated using the SRplot tool [22]. Various variables were included in the matrix, including Dam weight (kg), Dam age (days), Birth weight (kg), Birth body length (cm), Weaning weight (kg), and Weaning body length (cm). The variables are listed both horizontally and vertically, creating a grid where each cell represents the correlation between two variables. A number in the range -1 to +1 is displayed at each cell of a matrix, representing the correlation coefficient between two variables. As the value of correlation coefficient comes near 1 in positive sense, it indicates a strong and perfect linear relationship between two variables i.e. when one variable increases then other will also increase but at driving path cultures or ratio. A coefficient near -1 means there is a strong negative correlation—when one variable increases, the other often decreases. A close to 0 coefficient means that there is little or no linear correlation. Correlation strength and its direction is shown in the cells shading, ellipses. Stronger correlations (both positive and negative) are represented by darker colors and longer ellipses.

3. Results

On the basis of the data obtained, a comprehensive method for evaluating and comparing growth traits in Karakul sheep with those measured in Awassi sheep was suggested. Standardized measurement methods and maintenance practices were used in this study, which contributed to important as well as reliable conclusions about the growth performance of these two breeds.

The estimates of the breed factor effects on different growth traits in Awassi and Karakul sheep populations are shown in Table 1. At birth measurement results: The birth weight of Karakul lambs (6.62 ± 0.11 kg) was substantially superior to that of Awassi lambs (5.19 ± 0.12 kg). Karakul lambs were also longer at birth (35.75 ± 0.34 cm) than Awassi lambs (32.78 ± 0.36 cm). Both indices of the wither height (42.48 ± 0.26 cm) and rump height (42.96 ± 0.27 cm) were found to exhibit a significantly higher amounts in Karakul lambs than those in Awassi counterparts (40.88 ± 0.27 cm and 41.23 ± 0.29 cm, respectively). Karakul lambs showed a larger values of chest circumference (43.22 ± 0.30 cm) and abdominal circumference (44.80 ± 0.35 cm) compared to Awassi lambs (42.17 ± 0.32 cm and 43.74 ± 0.37 cm, respectively).

The measurements at the studied interval of 3 months of age showed that Karakul lambs continued to exert superiority over Awassi lambs with a higher body weight (25.28 ± 0.40 kg vs. 22.85 ± 0.42 kg). The measurements of body length, wither height, and rump height were all found to give significantly greater values in Karakul lambs compared to Awassi lambs. Chest and abdominal circumferences were also found to give larger diameters in Karakul lambs, with values

of 79.08 ± 0.58 cm and 87.61 ± 0.59 cm, than in Awassi lambs, with values of 77.01 ± 0.62 cm and 84.67 ± 0.63 cm, respectively.

The conducted measurements at 6 months of age showed the continuation of the trend with Karakul lambs weighing more values (33.54 ± 0.62 kg) compared to Awassi lambs (29.38 ± 0.66 kg). Karakul lambs exhibited a greater body length (72.40 ± 0.53 cm), wither height (71.44 ± 0.43 cm), and rump height (72.56 ± 0.40 cm) compared with Awassi lambs (69.30 ± 0.57 cm, 68.19 ± 0.45 cm, and 69.26 ± 0.42 cm, respectively). The chest and abdominal circumferences were also found to show significantly larger indices in Karakul lambs (94.68 ± 0.79 cm and 105.24 ± 0.76 cm) than that found in Awassi lambs (89.84 ± 0.84 cm and 100.50 ± 0.81 cm).

At 9 months of age, Karakul lambs weighed significantly more with values of 40.36 ± 0.54 kg than Awassi lambs with values of 36.05 ± 0.58 kg. As well, Karakul lambs exerted greater values of body length (76.43 ± 0.36 cm), wither height (74.83 ± 0.31 cm), and rump height (75.66 ± 0.30 cm) compared with what is shown in Awassi lambs (73.89 ± 0.39 cm, 72.06 ± 0.33 cm, and 72.91 ± 0.32 cm, respectively). The diameters of chest and abdominal circumferences were found to exhibit a significantly larger values in Karakul breed compared with the Awassi sheep.

By the age of 12 months, the Karakul lambs showed a significantly higher body weight value (46.79 ± 0.57 kg) than that found in Awassi lambs (40.33 ± 0.61 kg). The indices of body length, wither height, and rump height remained greater in Karakul lambs (78.16 ± 0.33 cm, 77.08 ± 0.29 cm, and 77.46 ± 0.27 cm) compared to Awassi lambs (75.57 ± 0.35 cm, 73.98 ± 0.31 cm, and 74.36 ± 0.29 cm). Similarly, chest and abdominal circumferences were larger in Karakul lambs (112.70 ± 0.80 cm and 122.26 ± 0.87 cm) than in Awassi lambs (107.20 ± 0.85 cm and 118.09 ± 0.92 cm).

Table 1. The effects of the breed factor (means $\pm$ standard errors) on growth traits in the studied Awassi and Karakul sheep populations.

	Measured parameter	Karakul (n=100)	Awassi (n=100)	Significance
Birth	Body weight (kg)	6.62 ± 0.11^a	5.19 ± 0.12^b	**
	Body length (cm)	35.75 ± 0.34^a	32.78 ± 0.36^b	**
	Wither height (cm)	42.48 ± 0.26^a	40.88 ± 0.27^b	**
	Rump height (cm)	42.96 ± 0.27^a	41.23 ± 0.29^b	**
	Chest circumference (cm)	43.22 ± 0.30^a	42.17 ± 0.32^b	*
	Abdominal circumference (cm)	44.80 ± 0.35^a	43.74 ± 0.37^b	*
3-month	Body weight (kg)	25.28 ± 0.40^a	22.85 ± 0.42^b	**
	Body length (cm)	61.91 ± 0.49^a	58.57 ± 0.53^b	**
	Wither height (cm)	64.72 ± 0.39^a	62.96 ± 0.41^b	**
	Rump height (cm)	65.54 ± 0.38^a	63.11 ± 0.41^b	**
	Chest circumference (cm)	79.08 ± 0.58^a	77.01 ± 0.62^b	*
	Abdominal circumference (cm)	87.61 ± 0.59^a	84.67 ± 0.63^b	*
6-month	Body weight (kg)	33.54 ± 0.62^a	29.38 ± 0.66^b	**
	Body length (cm)	72.40 ± 0.53^a	69.30 ± 0.57^b	*
	Wither height (cm)	71.44 ± 0.43^a	68.19 ± 0.45^b	**
	Rump height (cm)	72.56 ± 0.40^a	69.26 ± 0.42^b	**
	Chest circumference (cm)	94.68 ± 0.79^a	89.84 ± 0.84^b	**
	Abdominal circumference (cm)	105.24 ± 0.76^a	100.50 ± 0.81^b	**
9-month	Body weight (kg)	40.36 ± 0.54^a	36.05 ± 0.58^b	**
	Body length (cm)	76.43 ± 0.36^a	73.89 ± 0.39^b	**
	Wither height (cm)	74.83 ± 0.31^a	72.06 ± 0.33^b	**
	Rump height (cm)	75.66 ± 0.30^a	72.91 ± 0.32^b	**
	Chest circumference (cm)	104.45 ± 0.91^a	99.67 ± 0.96^b	**

12-month	Abdominal circumference (cm)	116.56±0.88 ^a	111.47±0.93 ^b	**
	Body weight (kg)	46.79±0.57 ^a	40.33±0.61 ^b	**
	Body length (cm)	78.16±0.33 ^a	75.57±0.35 ^b	**
	Wither height (cm)	77.08±0.29 ^a	73.98±0.31 ^b	**
	Rump height (cm)	77.46±0.27 ^a	74.36±0.29 ^b	**
	Chest circumference (cm)	112.70±0.80 ^a	107.20±0.85 ^b	**
	Abdominal circumference (cm)	122.26±0.87 ^a	118.09±0.92 ^b	**

Note: the data are expressed as means ± standard errors, ns, *, and ** refer to non-significant, significant at level $P < 0.05$; and significance at level $P < 0.001$, respectively. The superscript ^a refers to the higher indices than the superscript, and ^b, respectively.

The results for different growth traits of the studied sheep populations as influenced by the age of the constructed dam are presented in the Table 2. At birth, the age of the dam affected most of the growth traits in a way that was in proportion to age. Offspring born to older dams (bushes over five years) were bigger in terms of body weight, body length, wither height, rump height, chest and abdominal girth than those sired off dams of age 2-3 years. But we found that the impact of the dam's age was still apparent when the lambs were three months old. The same pattern of results was observed in the current study, whereby the lambs from older dams had significantly ($P < 0.05$) superior growth parameters that included, body weight, body length, wither height, rump height, chest girth and abdominal girth. Nonetheless, at six months of age, the effect of dam age on these traits declined, and all the recorded indexes of body weight, body length, wither height, rump height, chest circumference, and abdominal circumference were not significantly different in the different age groups of dams. This trend persisted in the nine and twelve months old lambs and the age of the dam did not influence the growth of the lambs. The growth parameters of all the age groups of dams during these later stages remained almost comparable, implying that the advantage that had been enjoyed through inheritance from the older dams up to growth at early life stages was not an advantage in the growing stages of the lambs.

Table 2. Effect of the age of dam (means ± standard errors) on growth traits of the studied sheep populations.

Age	Measured parameter	2-3 (n=53)	4-5 (n=80)	> 5 (n=67)	Significance
Birth	Body weight (kg)	5.23±0.16 ^c	5.98±0.14 ^b	6.46±0.15 ^a	**
	Body length (cm)	33.24±0.27 ^b	34.34±0.41 ^{ab}	34.90±0.44 ^a	*
	Wither height (cm)	40.43±0.38 ^c	41.94±0.33 ^b	42.59±0.35 ^a	**
	Rump height (cm)	40.89±0.39 ^c	42.34±0.34 ^b	42.89±0.37 ^a	**
	Chest circumference (cm)	41.16±0.45 ^b	43.19±0.39 ^a	43.97±0.42 ^a	**
	Abdominal circumference (cm)	42.56±0.50 ^c	44.51±0.43 ^b	45.57±0.47 ^a	**
3-month	Body weight (kg)	21.41±0.60 ^c	24.33±0.52 ^b	26.01±0.56 ^a	**
	Body length (cm)	57.94±0.72 ^b	60.91±0.62 ^a	62.08±0.68 ^a	**
	Wither height (cm)	61.89±0.58 ^c	64.24±0.50 ^b	65.41±0.54 ^a	**
	Rump height (cm)	62.30±0.57 ^c	64.79±0.49 ^b	65.87±0.53 ^a	**
	Chest circumference (cm)	75.00±0.85 ^c	77.88±0.73 ^b	80.01±0.79 ^a	**
	Abdominal circumference (cm)	83.25±0.88 ^c	85.74±0.75 ^b	88.14±0.82 ^a	*
6-month	Body weight (kg)	28.53±0.90	31.95±0.78	32.64±0.85	ns
	Body length (cm)	68.65±0.74	71.41±0.64	71.54±0.69	ns
	Wither height (cm)	68.43±0.59	70.27±0.51	71.18±0.56	ns
	Rump height (cm)	68.85±0.55	71.38±0.48	72.03±0.52	ns
	Chest circumference (cm)	88.85±1.12	92.63±0.96	94.01±1.05	ns
	Abdominal circumference (cm)	99.96±1.12	103.83±0.97	104.60±1.05	ns
9-month	Body weight (kg)	35.86±0.82	38.52±0.70	39.62±0.77	ns
	Body length (cm)	73.75±0.56	75.16±0.48	76.16±0.52	ns
	Wither height (cm)	72.84±0.42	73.82±0.36	74.06±0.39	ns

	Rump height (cm)	73.74±0.42	74.66±0.36	75.18±0.40	ns
	Chest circumference (cm)	99.15±1.32	102.55±1.14	104.60±1.24	ns
	Abdominal circumference (cm)	112.08±1.26	114.76±1.09	116.55±1.18	ns
12-month	Body weight (kg)	41.61±0.85	44.22±0.73	45.24±0.80	ns
	Body length (cm)	75.80±0.50	76.85±0.43	77.67±0.47	ns
	Wither height (cm)	75.01±0.39	76.06±0.33	76.41±0.36	ns
	Rump height (cm)	75.64±0.38	76.38±0.33	76.67±0.36	ns
	Chest circumference (cm)	107.51±1.20	110.87±1.03	111.50±1.13	ns
	Abdominal circumference (cm)	118.88±1.27	120.96±1.09	122.36±1.19	ns

Note: the data are expressed as means ± standard errors, ns, *, and ** refer to non-significant, significant at level $P < 0.05$; and significance at level $P < 0.001$, respectively. The superscript ^a refers to the higher indices than the superscripts ^b, and ^c, respectively.

Both female and male lambs exhibited similar body weight, body length, wither height, rump height, chest circumference, and abdominal circumference. This suggests that, at birth, male and female lambs begin on equal terms when it comes to these basic growth measures. At three months old, although male lambs had higher measurements, the gaps were not big enough to be meaningful. Both sexes had comparable body weights, lengths, wither heights, rump heights, chest sizes, and belly sizes. This shows that sex doesn't have a big impact on growth features in the early months after birth. But by six months clear differences between the sexes started to show up. Male lambs had much higher body weight, body length, wither height, rump height, and chest size compared to female lambs. This hints that male lambs grow faster than female lambs as they get older maybe because of hormone changes that become more noticeable as the animals age. The belly size though, didn't show a big difference between the sexes, which suggests this trait might not be as affected by sex differences at this age. At nine months, the trend kept going with male lambs growing more than females in most areas. The abdominal circumference showed no major change matching the six-month data. This suggests that as lambs grow up, males keep showing better growth features than females. At twelve months, the gap between sexes stayed the same, with males having much higher numbers in most growth areas. Males weighed more, had longer bodies, stood taller at the withers and rump, and had bigger chests than females. The belly size was about the same for both sexes hinting that this feature might not be as affected by sex-linked growth patterns in sheep.

Table 3. Effect of the sex of lambs (means ± standard errors) on growth traits of the studied sheep populations.

Age	Measured parameter	Female (n=103)	Male (n=97)	Significance
Birth	Body weight (kg)	5.77±0.12	6.05±0.12	ns
	Body length (cm)	34.04±0.35	34.33±0.37	ns
	Wither height (cm)	41.55±0.28	41.81±0.29	ns
	Rump height (cm)	41.98±0.29	42.16±0.30	ns
	Chest circumference (cm)	42.63±0.33	43.01±0.35	ns
	Abdominal circumference (cm)	44.07±0.38	44.44±0.39	ns
3-month	Body weight (kg)	23.31±0.45	24.68±0.46	ns
	Body length (cm)	60.35±0.54	60.36±0.56	ns
	Wither height (cm)	63.54±0.43	64.27±0.45	ns
	Rump height (cm)	63.97±0.42	64.79±0.44	ns
	Chest circumference (cm)	77.10±0.63	78.30±0.65	ns
	Abdominal circumference (cm)	85.54±0.65	85.95±0.68	ns
6-month	Body weight (kg)	29.75±0.67 ^b	32.60±0.70 ^a	**
	Body length (cm)	69.27±0.55 ^b	72.06±0.57 ^a	**
	Wither height (cm)	68.78±0.44 ^b	70.68±0.46 ^a	**

9-month	Rump height (cm)	69.89±0.41 ^b	71.81±0.43 ^a	**
	Chest circumference (cm)	90.02±0.83 ^b	93.99±0.87 ^a	**
	Abdominal circumference (cm)	101.09±0.84	103.94±0.87	ns
	Body weight (kg)	36.79±0.61 ^b	39.45±0.63 ^a	**
	Body length (cm)	74.19±0.41 ^b	75.99±0.43 ^a	**
	Wither height (cm)	72.51±0.31 ^b	74.32±0.33 ^a	**
12-month	Rump height (cm)	73.38±0.32 ^b	75.17±0.33 ^a	**
	Chest circumference (cm)	100.48±0.99 ^b	104.02±1.02 ^a	*
	Abdominal circumference (cm)	114.26±0.94	115.09±0.98	ns
	Body weight (kg)	42.71±0.64 ^b	44.87±0.66 ^a	*
	Body length (cm)	76.03±0.37 ^b	77.42±0.39 ^a	*
	Wither height (cm)	74.94±0.29 ^b	76.18±0.30 ^a	**
	Rump height (cm)	75.38±0.28 ^b	76.54±0.29 ^a	**
	Chest circumference (cm)	108.27±0.90 ^b	111.98±0.93 ^a	**
	Abdominal circumference (cm)	119.60±0.95	122.07±0.98	ns

Note: the data are expressed as means ± standard errors, ns, *, and ** refer to non-significant, significant at level $P < 0.05$; and significance at level $P < 0.001$, respectively. The superscript ^a refers to the higher indices than the superscripts ^b, and ^c, respectively.

The correlation coefficients between key growth traits of sheep from the Karakul and Awassi breeds are shown in Figure 1. The correlation of breed with other traits shows negative values, particularly with body weight and body length measurements at various ages. This suggests that breed has a relatively large influence, with a slight tendency for some breeds to be associated with lower values in these traits. The age of the dam has a positive impact on most growth traits, though these links aren't strong. This means that as dams get older, their offspring tend to show small gains in traits like birth weight weaning weight, and body size at various ages. Older dams might boost their offspring's growth maybe because they provide better care or have more experience with breeding. The month of birth has weak ties to early growth traits such as birth weight and body size, which suggests that the season when a lamb is born doesn't affect these early measures much. As lambs grow, the link between birth month and growth traits gets even weaker showing that birth month matters less over time. Birth weight strongly links to all later growth traits, including body size at birth weaning weight, and body size at different ages. This strong tie shows that lambs born heavier are likely to keep growing faster leading to better weight and size as they develop. This highlights the relationship between weaning weight, and therefore growth potential, and subsequent performance on heavy target weights. Body length at different ages is significantly and positively correlated with each other as well as most of the growth traits. Accordingly, body length at birth is significantly positively correlated with BW and BL during the weaning period, so it continued to be over time. The positive similarities in both trials indicate that body length of lambs at an early age gives a general idea for growth performance which does not change and hence, calves with longer bodies give the same indication. The correlation between growth traits at different ages (6, 9 and 12 months) are still strong primarily in terms of body weights and body lengths (Figure 1). Development of the growth curve in sheep was found to be relatively consistent with early measures showing some predictive ability for later growth. The strong correlations among these traits highlight the interrelatedness of each trait and hence, a whole body approach to sheep improvement where gains in one set is likely to be positively associated with improvements in some other set of traits.

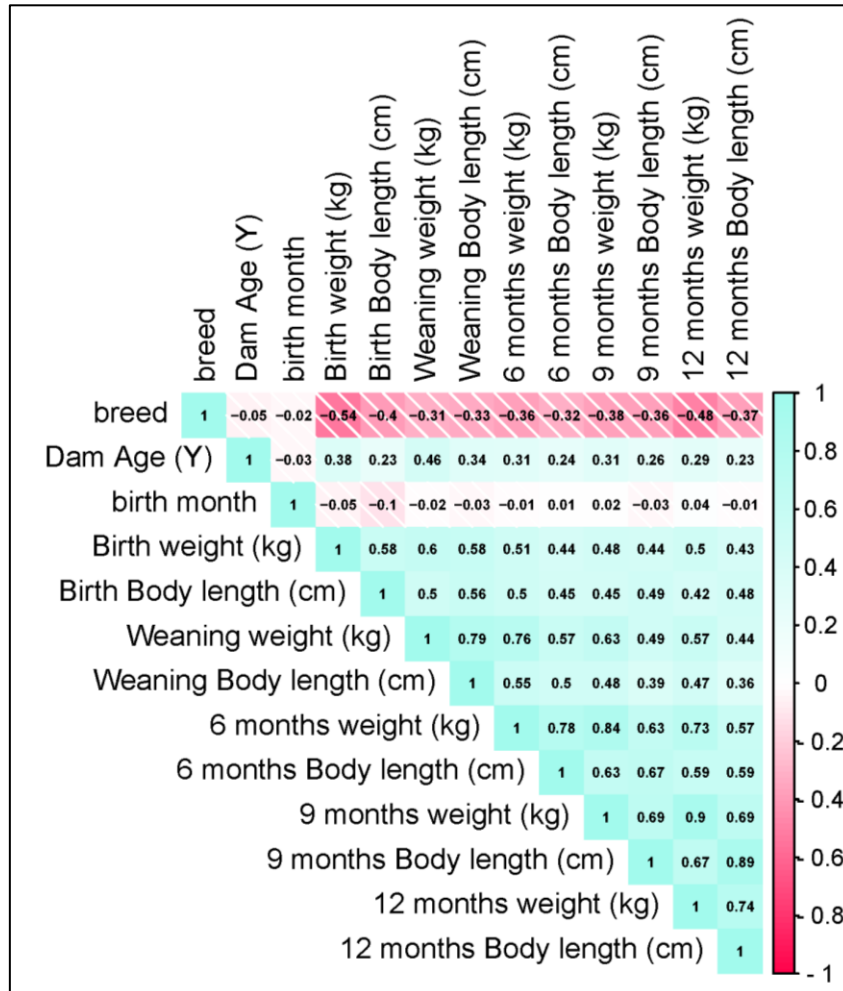


Figure 1. Correlation coefficients among the main growth traits of Karakul and Awassi sheep. Each cell contains a value from -1 to 1 representing the direction and strength of relationship between two traits. Strong positive correlations are illustrated in the heatmap, with darker colors and longer ellipses highlighting the association of these traits identified as important for sheep growth and development.

4. Discussions

This study is designed to undertake a detailed study to establish growth pattern of two different breeds of sheep, the Karakul and Awassi breeds in order to understand their potential. The results of the current study were obtained by comparison of a set of growth parameters: body weight, body length, wither height, rump height, chest circumference, and abdominal circumference at the stages of birth and three, six, nine, twelve months of age. Between the Karakul and Awassi studied ovine lineages, some similarities and differences were revealed. There are marked differences of the Karakul and Awassi sheep breeds on certain growth traits which could be due to genetic or/and environmental influence [23]. As can be seen from the above data, Karakul lambs always have a higher performance compared to Awassi lambs for all the measured parameters from birth to one year old. In birth weight, body length, wither height, rump height, chest circumference, and abdominal circumference, the Karakul breed showed the desirable

characteristics for growth . This sort of difference observed time and time again can convincingly point to the fact that the Karakul sheep enjoy an edge in terms of growth genetics over the Awassi.

Karakul sheep are well known for their high adaptability to harsh environmental conditions. They seem to carry genetic factors that favor better nutrient utilization and more rapid growth. They certainly grow much faster than Awassi lambs. And that's a big deal if you're raising sheep for their meat because more rapid growth means a quicker turnaround and a more efficient use of feed. If you've ever fed a sheep, you understand how feed costs can mount; thus, more efficient biological use of the nutrients in that feed is a top priority for any sheep raiser who hopes to make a profit [24]. The Karakul sheep's genetic endowments fitting them to produce desirable performance traits (including growth) suggest they should be chosen more often over other breeds when the goal is to improve such traits through selection. However, aficionados of improved livestock must never forget that the livestock improvement equation has two significant sides that must be balanced [25]. The first side is improving the genetics of the livestock population. The second is confronting ways to ensure that the livestock are raised in an environment that is as conducive as possible to achieving the potential their genes bestow on them [26].

The harsh environments in which they are found may lead to the Karakul's better growth performance, as these sheep are likely more efficient at converting feed to body mass. It is no accident that the Karakul sheep have managed to achieve significantly better growth rates in recent years [27]. These rates tell us something both about the sheep themselves and about the breeds with which they are compared [28]. By recognizing for growth-related genes similar to those in Karakul sheep, it may be possible to improve a wide range of phenotypic traits in Awassi sheep, making this breed more competitive in commercial utilizations [29]. Mating Awassi sheep with Karakul could inject some very useful genes that would improve growth performance while keeping the good milk and wool-coping abilities of the Awassi breed. Karakul seems to have some very useful growth traits and, as a breed, it has a very superior ability to cope with a range of environmental stressors and still perform well [30]. The superior growth traits of the Karakul breed might reflect broader genetic adaptations that confer resilience against environmental stressors [31]. Therefore, those traits that make the Karakul resilient are likely also to confer a kind of resilience to their composite breed—Karakul/Awassi. In terms of why Karakul sheep might be a good model to study and understand growth resilience, it's this idea that both breeds (recognizing that they're composite breeds) are well adapted to harsh environments and still managing to yield profit [32]. As a result, the efficiency of Karakul sheep in growth and resource utilization may lead to more sustainable livestock production [33]. Of particular note is that the differences seen between the two breeds in all growth traits show statistical significance. Karakul sheep are pretty consistently superior to the other breed (Awassi) across all growth measures, which is for the most part, pretty significant [34].

This study found that the age of the dam has a powerful influence on lamb growth traits at birth and at three months—an influence, the study says, that "could guide selection of breeding stock. However, it was found that increased dam age had a very significant effect on lamb birth weights, suggesting that older dams are better at nutrient allocation to their developing lambs during gestation [35]. The contemporary study indicates that the dam's age at lambing significantly influences lamb growth up to six months of age. After this time interval, the study found no noticeable differences in lamb growth traits related to dam age. This shift in the influence of dam age suggests that the prenatal and early postnatal growth environment might be more important to lamb growth than the genetically based, or inherited, advantages associated with the dam's age. The same study suggests, from a biological perspective, that dams that are older at lambing might confer some genetically based (inherited) advantages that positively influence lamb growth in the

early life stages, but might not have a long-lasting impact [36]. After six months, growth rates among lambs whose mothers were of different ages showed no significant differences [37]. The results of the study on the effect of sex on growth characteristics of the studied sheep population provide important insights into the influence of sex on the development of these animals over time [38].

An extensive analysis of the results are considered the consequences of the found trends, possible biological processes, and application of the obtained data to sheep production and selection. In terms of growth characteristics the sex did not differ significantly in the birth weight, birth length, withers height, pelvic height, chest girth, waist girth and abdominal girth within the first three months of lambs' age. With reference to relative growth, this is an indication that at birth, males and females are potentially similar and it is elements such as maternal care and nutrition may be more influential than sex [39]. Most importantly, few differences are recorded between the sexes at a developing infant stage perhaps because sexual hormones are not very influential during the first few months of the lives of human beings [40]. At six months of age the sex specific differences in the different growth traits such as weight; body length; height at withers; trunk height and chest girth are observed. It is supposed that these distinctions are attributed to the advanced puberty and the corresponding changes in the growth related to the hormone levels. They also observed that male lambs have got higher concentrations of testosterone as compared to the females so the muscles are more developed and the body size is larger [41]. When lambs are growing young, the hormonal factor, however, gains the upper hand, and this is where the marked disparity in size between the sexes becomes apparent. Somatic growth in male and female lambs demonstrates differences in growth rates, and these differences can be explained mainly in respect to the action of sex steroids. In males, the testosterone level increases the muscle mass and bone density and thus height and weight more than female. Females being saturated with low anabolic These hormonal differences also influence fat deposition patterns; that is why males have a greater chest circumference and other unique growth characteristics [42]. In females estrogen tends to increased fat deposition and not muscularity, thus different body compositions and such other related traits as affect abdominal girth. The results estimated in the correlation matrix may allow understanding the dependencies between different growth traits in Karakul and Awassi sheep breeds. Because the correlation coefficients between these growth indices are fairly high and have not decreased with age, the traits point to a very high level of interdependence. Livestock lambs that are born heavier and of superior body length tend to maintain the disparity in the their growth rates as they grow older. This means that early growth performance acts as a valid barometer to growth in the subsequent periods, hence using parameters such as birth weight and weaning weight as clear pointers to subsequent growth [43]. A modest positive correlation exists between dam age and several growth traits, suggesting that older dams generally produce offspring with marginally enhanced growth performance. This could stem from various elements, including increased maternal experience, superior maternal care, or better physiological condition in older dams [44]. Nevertheless, the relatively weak correlations imply that while dam age influences growth traits, it is not the primary determinant for the offspring's growth characteristics. Choosing older ewes that have demonstrated successful reproduction may enhance the growth characteristics of their lambs, particularly in settings where maternal care is crucial for the survival and development of the lambs. Although the negative correlations observed between breed and growth traits are relatively weak, they imply that certain breeds within the examined population could be linked to lower growth trait values. This may highlight the genetic distinctions between the Karakul and Awassi breeds, indicating that one breed might adapt better to varying environmental conditions or management strategies than the other [45]. Nevertheless, the weak correlation values suggest that breed alone does not significantly

influence the growth performance of the sheep, with other elements such as management practices, nutrition, and environmental factors likely having a more substantial impact [46]. The decisions as to when to mate and when to lamb can be made at other times and based on other strategies like the market requirement or the availability of workers as opposed to the effect on the growth traits. There are very high coefficients of phenotypic and partial genetic correlations between early growth traits and post-weaning growth traits at 6, 9, and 12 months of age, thus early growth traits should be given high breeding goals. If breeders can select for higher birth and weaning weights, it is likely to result in an overall improvement in the flock's performance [47]. This also suggest that any improvement of early growth, for instance through better nutrition for the pregnant ewes or careful rearing of the new born lambs would to some extent have the positive bearing on the final growth outcomes.

Conclusions

Overall the findings on this study have shown that there is a high genetic advantage of the Karakul breed over the Awassi breed in regard to the growth traits. These studies are of massive importance to both the breeding of sheep and with the production of Karakul sheep, as the results imply that these sheep are more suitable for environments that require fast growing rates and productive conversion in order to provide profitability. The finding from this study can be useful to plan future breeding programs to promote growth traits of sheep and, consequently, increase stock production profitability and sustainability. At all the assessed parameters, the Karakul lambs had higher mean values than the Awassi lambs in all the assessed growth parameters at birth, 3, 6, 9 and 12 months of age. All these differences were statistically significant; Karakul lambs had more body weight, length, height and circumferences than any of the ages. Karakul breed enjoy greater genetic merit on these growth traits hence a better overall growth performance as compared to Awassi breed.

Thus, the study reveals the level of dam age impact on some early growth traits in lambs, where the lambs originating from older dams are presented with certain benefits. That is, growth rate varied with the magnitude of dam age and the degree of variation from pen to pen increased as dam age increased. But, the ability to rebuild negative growth rates is diminished with increasing dam age, meaning that management after weaning is critical to maintaining high rates of growth across the flock. It is, therefore, possible to conclude that the results presented in this study suggest that a closer balance between breeding and management for selection with emphasis on the genetic potential as well as improving the environmental conditions for the further growth of the populations of sheep is necessary.

The study clearly demonstrates that while sex does not significantly influence growth traits at birth and during the early stages of life, it becomes a critical factor as the lambs mature. Male lambs, due to the influence of sex hormones like testosterone, exhibit significantly greater growth in body weight, length, and other parameters compared to females from six months onwards. These findings are important for breeders and producers looking to optimize growth traits in sheep populations, particularly for meat production, and highlight the need for sex-specific management strategies to maximize the potential of both male and female lambs.

The correlation matrix provides a clear picture of how growth traits in Karakul and Awassi sheep are interrelated. The strong positive correlations between early and later growth traits emphasize the importance of focusing on early growth performance in breeding and management strategies. While factors such as dam age and breed do play a role, their impact is less pronounced compared to the influence of early growth traits. These findings underscore the importance of early intervention and careful management during the initial stages of lamb development to ensure optimal growth and productivity in sheep flocks.

Acknowledgments

This study was partially funded by Al-Qasim Green University, Al-Qasim, Babil, Iraq. The author extends sincere gratitude to the entire team at Barakat Abu al Fadhl Al-Abbas Station for Raising Sheep, Al-Khafeel Co., Karbala, Iraq, for their invaluable support and assistance throughout the experimental procedures of this study.

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Gowane GR, Gadekar YP, Prakash V, Kadam V, Chopra A, Prince LLL. Climate change impact on sheep production: Growth, milk, wool, and meat. *Sheep Prod Adapt to Clim Chang* 2017;31–69.
- [2] Shinde AK, Naqvi SMK. Prospects of dairy sheep farming in India: an overview. *Indian J Small Ruminants* 2015;21:180–95.
- [3] Sejian V, Hyder I, Maurya VP, Bagath M, Krishnan G, Aleena J, et al. Adaptive mechanisms of sheep to climate change. *Sheep Prod Adapt to Clim Chang* 2017;117–47.
- [4] Aljubouri TRS, Hassan AF, Al-Shuhaib MBS. Exhibition of different hormonal regulations in Awassi and Karakul breeds at sexual maturity. *Plant Archives* 2020; 20:5937-5940.
- [5] Ombayev AM, Kuzembayuly Z, Parzhanov ZA, Yusupbayev ZS. Ecological and technological aspects of breeding Karakul sheep in the arid zone of Kazakhstan. *AIP Conf. Proc.*, vol. 3033, AIP Publishing; 2024.
- [6] Oramari RA, Alkass JE, Mahmud KI. A comparative study on growth, carcass traits and tissue distribution of Awassi and Hamdani lambs. *J Biol Agri Heal* 2014;4:36–43.
- [7] Ali W, Ceyhan A, Ali M, Dilawar S. The merits of Awassi sheep and its milk along with major factors affecting its production. *J Agric Food, Environ Anim Sci* 2020;1:50–69.
- [8] Sleiman FT, Abi Saab S. Influence of environment on respiration, heart rate and body temperature of filial crosses compared to local Awassi sheep. *Small Rumin Res* 1995;16:49–53.
- [9] Alkass JE, Hermiz HN, Baper MI. Some aspects of reproductive efficiency in awassi ewes: A review. *Iraqi J Agric Sci* 2021;52:20–7.
- [10] Mohammadi H, Edriss MA. Genetic parameters of early growth traits in Mehraban breed of sheep. *Pak J Biol Sci* 2007;10:373–7.
- [11] Şahin Ö. Evaluation of some factors on birth and weaning weights in Awassi sheep by using GLM and CART analysis. *Trop Anim Health Prod* 2022;54:400.
- [12] Bayraktar M, Durmuş M, Al-Shuhaib MBS. Identification of two novel SNPs in the myocyte enhancer factor 2B (MEF2B) gene and its association with growth traits in two breeds of Turkish sheep. *Small Rumin Res* 2022;106867.
- [13] Aljubouri TRS, Al-Shuhaib MBS, Javadmanesh A. HMGA2 gene polymorphisms and their effects on main growth traits indices in Awassi and Karakul sheep. *Agric Nat Resour* 2020;54:587–94.
- [14] Aljubouri TRS, Al-Shuhaib MBS. Genotyping of mitochondrial D-loop sequences in three breeds of sheep. *Biologia (Bratisl)* 2021;76:203–11.
- [15] Alwan IH, Aljubouri TRS, Al-Shuhaib MBS. A Novel Missense SNP in the Fatty Acid-Binding Protein 4 (FABP4) Gene is Associated with Growth Traits in Karakul and Awassi Sheep. *Biochem Genet* 2023;1–23.
- [16] Aljubouri TRS, Hassan AF, Al-Shuhaib MBS. Remarkable correlation of non-genetic factors with growth traits in two breeds of sheep. *Iraqi J Agric Sci* 2021;52:309–14.
- [17] Abdallah JM, Omar JA. Multivariate analysis of morphological characteristics of Awassi sheep in the West Bank, Palestine. *JAPS J Anim Plant Sci* 2017;27.
- [18] Ajam IK, Al-Jubouri TRS, Ghayyib QH. TGF-B super family correlation with the fertility of Iraqi Awassi Ewes. *Basrah J Agric Sci* 2019;32:26–32.
- [19] Khojastehkey M, Aslaminejad AA. Study of the environmental, genetic and phenotypic trends for pelt traits and body weight traits in Zandi sheep. *J Appl Anim Res* 2013;41:356–61.

- [20] Pettigrew E, Hickson R, Morris S, Kenyon P, Corner-Thomas R, Haslin E, et al. The Effect of Age of Dam and Birth Rank on the Reproductive Performance of Ewes as One-and Two-Year-Olds. *Animals* 2021;11:770.
- [21] Field A. *Discovering statistics using IBM SPSS statistics*. sage; 2013.
- [22] Tang D, Chen M, Huang X, Zhang G, Zeng L, Zhang G, et al. SRplot: A free online platform for data visualization and graphing. *PLoS One* 2023;18:e0294236.
- [23] Aljubouri TRS, Al-Shuhaib MBS. A missense SNP in the proopiomelanocortin (POMC) gene is associated with growth traits in Awassi and Karakul sheep. *Anim Biotechnol* 2023;1–14.
- [24] Buzu I. *Amelioration of the Karakul sheep race in different areas and countries of the world*. 2018.
- [25] Binabaj FB, Tahmoorespur M, Aslaminejad AA, Vatankhah M. The investigation of non-genetic factors affecting survival of Karakul lambs from birth to one year of age using linear and nonlinear models. *Small Rumin Res* 2013;113:34–9.
- [26] Aljubouri TRS, Hassan AF, Al-Shuhaib MBS, Mahyari SA. Association of GnRH1 Gene with Growth Traits in Two Breeds of Sheep. *Agric Res* 2020;10:285–93.
- [27] Buzu I. *The milk production variability of Moldovan Karakul ewes*. 2014.
- [28] Fadhil IA, Al-Shuhaib MBS. Phylogenetic differentiation between Awassi and Hamdani sheep using the mitochondrial 12S rRNA. *Anim Biotechnol* 2022;33:801–9.
- [29] Al-Jubouri T, Hassan A, Al-Shuhaib MB. Novel association between GnRHR gene and growth traits in Awassi and Karakul sheep. *J Microbiol Biotechnol Food Sci* 2022;11:e3241–e3241.
- [30] Al-Najjar K, Al-Momani AQ, Al-Yacoub AN, Elnahas A, Elsaid R. Estimation of Genetic Parameters and Non Genetic Factors for Milk Yield and Litter Size at Birth of Awassi Sheep in Drylands. *Egypt J Sheep Goats Sci* 2022;17:19–26.
- [31] Aljubouri TRS, Al-Khafaji FM, Al-Shuhaib MBS. Relationship of some metabolic Hormones with Increased Live Body Weight of Male and Female of Karakul and Awassi Lambs. *Basrah J Agric Sci* 2021;34:107–17.
- [32] Leite JHGM, Façanha DAE, Bermejo JVD, Guilhermino MM, Bermejo LA. Adaptive assessment of small ruminants in arid and semi-arid regions. *Small Rumin Res* 2021;203:106497.
- [33] Shiri SA, TAHMOORESPOUR M, Shariati MM. Estimates of genetic and environmental factors on growth and mortality in Karakul lambs 2015.
- [34] Aljubouri TRS, Al-Shuhaib MBS. The identification of a novel SNP in the resistin (RETN) gene associated with growth traits in Karakul and Awassi sheep. *Trop Anim Health Prod* 2023;55:1–12.
- [35] Çilek S, Gotoh T. Effects of dam age, lamb gender, and singleton or twin status on bodyweight and chest measurements of Malya lambs in Middle Anatolia, Turkey 2015.
- [36] Bell AW, Greenwood PL. Prenatal origins of postnatal variation in growth, development and productivity of ruminants. *Anim Prod Sci* 2016;56:1217–32.
- [37] Albial AM, Singh J, Singh DP, Niwas R. Environmental influences on growth traits of Nali sheep. *Indian J Anim Res* 2014;48:75–7.
- [38] Gaili ESE. Breed and sex differences in body composition of sheep in relation to maturity and growth rate. *J Agric Sci* 1992;118:121–6.
- [39] Owens FN, Dubeski P, Hanson CF. Factors that alter the growth and development of ruminants. *J Anim Sci* 1993;71:3138–50.
- [40] Smith JT. Sex steroid control of hypothalamic Kiss1 expression in sheep and rodents: comparative aspects. *Peptides* 2009;30:94–102.
- [41] Kridli RT, Abdullah AY, Shaker MM, Masa'Deh MK. Sexual activity and puberty in pure Awassi and crosses and backcrosses with Charollais and Romanov sheep breeds. *New Zeal J Agric Res* 2007;50:429–36.
- [42] Stackpole CA, Clarke IJ, Breen KM, Turner AI, Karsch FJ, Tilbrook AJ. Sex difference in the suppressive effect of cortisol on pulsatile secretion of luteinizing hormone in sheep. *Endocrinology* 2006;147:5921–31.
- [43] Mialon M-M, Nowak R, Falourd P, Marcon D, Lardy R, Boivin X. Are early-life lambs' characteristics and behavioural reactivity related to later survival and growth performance during artificial feeding? *Appl Anim Behav Sci* 2023;262:105918.



- [44] Gowane GR, Chopra A, Prakash V, Prince LLL. The role of maternal effects in sheep breeding: a review. *Indian J Small Ruminants* 2014;20:1–11.
- [45] Alwan IH, Aljubouri TRS. Effect of Breed and some Non-Genetic Factors on Productive Performance in Two Sheep Breeds. *IOP Conf. Ser. Earth Environ. Sci.*, vol. 1262, IOP Publishing; 2023, p. 72007.
- [46] Bukhari S, Ganai NA, Shanaz S, Khan HM, Rather M, Khan N, et al. Effect of breed and some non-genetic factors on growth performance of sheep under temperate conditions of Kashmir. *Small Rumin Res* 2022;215:106728.
- [47] Balasundaram B, Muralidharan J, Murali N, Cauveri D, Raja A, Okpeku M, et al. Development of selection strategies for genetic improvement in production traits of Mecheri sheep based on a Bayesian multi trait evaluation. *PLoS One* 2023;18:e0289460.

Effect of Natural Products of C3 and C4 Weeds on Yield and its Component of Some Bread Wheat Varieties (*Triticum aestivum* L.)

Ahmed Mohammed Zaki Ibrahim¹, Shaker Mahdi Saleh² and Ahmed Hawas Abdullah Anis³

¹⁻³Department of Field Crops, College of Agriculture, Tikrit University, Tikrit, Iraq.

¹E-mail: Ahmedzika@tu.edu.iq

²E-mail: shaker_nahdi@tu.edu.iq

³E-mail: Ahmed75hawas@tu.edu.iq

Abstract. The Experimental Crops Department of the College of Agriculture, Tikrit University, conducted a field experiment during the winter season (2022-2023) A.D. at the Research Station. The experiment was conducted according to a randomized complete block design (RCBD) with three sectors and a split - split-plots design, aimed to know the effect of the natural products of two weed plants, one of which is tri-carbon (C3), represented by the Malva pusilla weed. The other is quadruple carbon (C4), represented by the Wild Radish, on the components of the yield of some wheat varieties planted. The study encompassed three factors: the first factor was the impact of the natural products of weed plants on the vegetative growth and maturation stages, the second factor was the combination of the two aforementioned weed types and the comparison treatment, and the third factor was the evaluation of four wheat varieties: Fayyad, Rashid, Ibaa99 and Adna. The traits of the number of grains in the spike, the weight of 1000 grains, the grain yield, the biological yield, and the harvest index were studied. The most important results obtained are the superiority of the comparison treatment (with out weeds) in the traits of the number of grains per spike, weight of 1000 grains, grain yield, biological yield, and harvest index (52.42, 41.59, 4.65, 12.31 and 40.58) respectively. The Aba99 variety was superior in all the studied traits and gave the highest harvest index of 35.97%. The binary and triple interaction between the study factors had a significant effect on most of the traits, As the Aba99 variety's interaction with the comparison method was superior, as was its interaction in the vegetative growth period to measure the natural products. In the majority of the traits that were examined, the triple interaction between the Aba99 variety, the contrast treatment, and the vegetative growth stage was more effective in measuring the natural products and attained the highest averages.

Keywords. Natural products, Yield and its component, Wheat varieties, Weeds.

1. Introduction

Wheat (*Triticum aestivum* L.), which belongs to the Poaceae family, is a strategic crop with high nutritional value, as it contains proteins, fats, vitamins, and some mineral salts, in addition to the amino acids that humans need in their diet [1]. It ranks first in terms of cultivation area as well as

local production. Wheat production for the winter season of 2023 was estimated at 4,248 thousand tons. [2]. During its growth period, the crop suffers from competition from broad-leaved and narrow-leaved weeds, which leads to a reduction in the quantity and quality of the crop. In addition, it secretes chemicals into the environment in which it lives. Studies have shown that allelopathic substances secreted by weeds may be toxins that affect crops grown near or following them in cultivation [3]. Weed plants are traits by their ability to produce large numbers of small seeds, which helps them spread and pollute agricultural lands, which increases the difficulty of combating them [4]. Plants produce chemical compounds through secondary metabolic pathways that have negative effects that inhibit the germination process, hinder the absorption of water and nutrients, and affect the photosynthesis process. The role of these products in defending and protecting the plant is clear. [5] . The factor of competition of weed plants for wheat crops has a clear effect on the growth and development of the crop, as the survival of weeds and not combating them is a determining factor in the growth and production of the crop in the later stages [6]. The effect of weeds is not limited to competition, but they can secrete many chemicals that lead to preventing or hindering the germination and growth of neighboring plants, which are known as antimicrobial substances. In this regard, many other plants are estimated to contain more than ten thousand chemicals that are secreted from different parts of them, and they use them to protect themselves against diseases, pests, and other plants [7].

As for the competitiveness of wheat varieties, it was found that the traits of varieties with the ability to compete with weeds are the plant height and the number of branches, as these traits are considered means of reducing the appearance of weed growth and thus reducing their competition [8]. It was also found [6] that the factor of competition of weed plants for wheat crops has a clear effect on the growth and development of the crop, as the survival of weeds and not combating them is a determining factor in the growth and production of the crop in the later stages. [9] proved that the competitive genetic traits of wheat lead to a decrease in weed density of up to 50% due to the inhibition of weed growth above the soil surface. Weed plants can avoid the inhibition of crop growth because they often have higher growth rates early in the growing season when the competition between crops and weeds is not intense. Theoretically, increasing the density of crop plants can lead to an increase in the strength of the variance in competitive size and thus benefit crops at the expense of weeds [10].

The aim of the study is to evaluate the performance of wheat varieties and determine the most suitable ones for the conditions of the region, and to know the effect of the natural products of *Malva pusilla* and Wild radish plants on the components of the wheat crop yield, as well as to know the best interaction between the study factors for the studied traits to obtain the least possible growth of weeds with the studied varieties.

2. Materials and Methods

The objective of this investigation was to investigate the impact of natural products of C3 and C4 on the grain yield and growth characteristics of wheat varieties.. The land designated for planting the experiment was plowed and leveling and smoothing operations were carried out to prepare a suitable seedbed twice perpendicularly using a disc plow. Nitrogen fertilizer was added in two batches, the first half the recommended amount at planting and the second one month after planting at a rate of 90 kg ha⁻¹ nitrogen in the form of urea (40% N). Phosphate fertilizer was also added in one batch before planting at a rate of (240) kg ha⁻¹, then. Then the land was divided into three replicates with 24 experimental units in each replicate. The area of the experimental unit was 1.5 X 2 meters and each one included seven lines of wheat and the distance between one line and another was 20 cm. *Malva pusilla* seeds (C3) and also Wild radish seeds (C4) were distributed between the wheat crop lines at a rate of 25 seeds for each plant of the weeds plants in

each experimental unit and flood irrigation was used to irrigate the field. As for the varieties that were planted, they were Fayyad, which was obtained from Professor Dr. Jasim Muhammad Aziz, Rashid, Ibaa 99, and Adna. These varieties were obtained from the Seed Testing and Certification Department, Salah al-Din Branch. Wheat varieties were planted on 11/19/2022 in 72 experimental units distributed over the three sectors. The main plots included the varieties in four plots with an area of 9 x 2 m. Then the main plots were divided into 3 secondary plots, each plot included two experimental units. Weed treatments were distributed in them at two experimental units for each treatment. After that, the stages of measuring the natural outcomes of weed plants within these units were determined. The experiment was conducted according to a randomized complete block design (RCBD) with three sectors and a split - split-plots design. The combinations of treatments obtained from three factors were distributed:

- The variety factor. The experiment included four varieties of the wheat crop: Fayyad, Rashid, Ibaa99, and Adna.
- The weed type factor. The experiment contained two types of weeds: the three-carbon weeds represented by the Malva pusilla weed and the four-carbon weeds represented by the Wild radish weed, in addition to the comparison factor.
- Factor of the stages of measuring the natural products of jungle plants The experiment included two stages to measure the natural products produced by jungle plants, which are:
 - The vegetative growth stage, where the measurement of the natural products of jungle plants was taken in the vegetative growth stage.
 - The maturity stage, in which the natural products of jungle plants were measured in the maturity season.

Table 1. Some of the physical and chemical characteristics of soil.

Properties	Value
Soil texture	Silty clay
Electrical conduction (EC)	1.96 (dc/m)
Nitrogen	1.764%
Available phosphorous	0.02mg Kg ⁻¹
Available Potassium	0.04 mg Kg ⁻¹
pH	6.8
Organic matter	0.8%

2.1. The Studied Traits

2.1.1. Natural Products of Jungle Plants

Representative samples were taken for each stage of measuring the products, dried, and the natural products in them were estimated (total alkaloids, total glycosides, total phenolic content, and percentage of terpenes). The estimation process was carried out in the laboratories of the Ministry of Science and Technology, and the results obtained were as in the tables below.

Table 2. Natural vegetative products of Malva pasilla (C3).

Treatment	Vegetative growth stage / tri-carbon weed					Maturity stage / tri-carbon weed				
	Control	V1	V2	V3	V4	Control	V1	V2	V3	V4
phenolic content (mg/100 gm)	19.58	19.78	20.00	20.60	20.32	19.42	19.71	20.08	20.75	20.29
Total alkaloid content %	5.23	5.46	5.66	6.15	5.89	5.29	5.50	5.71	6.30	5.80
Total glycoside content	3.55	3.74	3.90	4.58	4.22	3.63	3.80	3.96	4.62	4.30

%										
Total terpinoid content %	1.25	1.46	1.60	2.08	1.80	1.33	1.41	1.58	2.15	1.88

Table 3. Natural vegetative products of Wild radish C4.

Treatment	Vegetative growth stage / Carbon quadruple weed					Maturity stage / quaternary -carbon weed				
	Control	V1	V2	V3	V4	Control	V1	V2	V3	V4
phenolic content (mg/100 gm)	22.58	22.98	23.69	23.20	24.00	22.50	22.90	23.73	23.27	24.09
Total alkaloid content %	6.18	6.48	7.02	6.79	7.55	6.11	6.41	7.10	6.72	7.50
Total glycoside content %	4.15	4.44	5.00	4.79	5.20	4.19	4.50	5.09	4.83	5.29
Total terpinoid content %	1.58	1.77	2.18	1.96	2.66	1.50	1.80	2.24	2.05	2.71

2.2. Traits and Components of Wheat Crop Yield

- Number of grains in the spike: 10 spikes were taken randomly from each experimental unit and were manually separated according to the total number of grains in them, then divided by ten to calculate the number of grains.Spike⁻¹.
- Weight of 1000 grains (g): 1000 grains were counted randomly in the grain counting device in the department's laboratory, and then weighed with a sensitive balance for each sample, and the process was repeated three times and the average was extracted.
- Grain yield (ton h⁻¹): It was calculated by taking the yield of the harvested plants per square meter for each experimental unit, then converting the weight based on ton h⁻¹
- Biological yield (ton h⁻¹): It was estimated by taking a square meter of plants, as the plants were weighed completely (grains + straw), then converting the weight to ton h⁻¹.
- Harvest index%: It was calculated using the following equation [11]:

$$\text{Harvest index} = (\text{grain yield} / \text{biological yield}) \times 100$$

3. Results and Discussion

The ANOVA appendix indicates that the cultivars, weeds and natural products were highly significant in all the studied traits at the probability level of 0.01. As for the two-way interaction between the cultivars and weeds, it was highly significant at the probability level of 0.01 for the traits of number of grains per spike, 1000-grain weight (g) and biological yield t h⁻¹, Although the variables of grain yield t h⁻¹ and harvest index (%) did not reach the ceiling of statistical significance. Also, the two-way interaction between the cultivars and natural products was highly significant for the traits of number of grains, 1000-grain weight (g) and biological yield tons. ha⁻¹, while it was not significant for the traits of grain yield tons. ha⁻¹ and harvest index (%), while the two-way interaction between weeds and natural products was highly significant at the probability level of 0.01 for the traits of weight of 1000 grains (g) and biological yield tons. ha⁻¹, And the attributes of the number of grains per spike, grain yield tons. ha⁻¹, and harvest index (%) did not reach the limits of statistically significant. The appendix also indicates that the triple interaction treatment shared between varieties, weeds and products was highly significant in the traits of number of grains per spike, thousand grain weight (g) and biological yield tons. ha⁻¹, while it did not reach the limits of statistical significance in the traits of grain yield ton h⁻¹ and harvest index (%).

3.1. Wheat Yield Components

3.1.1. Number of Grains in the Spike (grain spike⁻¹)

The results of Table (4) indicated that the Aba99 variety was superior to the rest of the studied varieties and gave the highest average of 51.15 grain spike⁻¹, compared to the variety below which gave the lowest average (41.88) grain spike⁻¹. This superiority in the number of grains in the spike may be due to the genetic composition of the variety and the extent of its influence by environmental conditions. This result was consistent with the results of [12] and [13]. Regarding the weed factor and its effect on this trait, the comparison treatment was superior and gave the highest average of 52.13 spike⁻¹ grains, compared to the treatment of the Wild radish weed (tetra carbon) which recorded the lowest average (40.76) spike⁻¹grains. The reason for this increase in the trait may be because the comparison treatment was free of weed plants, which allowed the crop plants to grow without competition from weeds [14].

Regarding the factor of the stages of measuring the content of natural products of the jungle plants, the vegetative growth stage was significantly superior to the maturity stage in this trait, as the average of the trait in the first stage was 47.24 grains of spike⁻¹ compared to the average of the trait in the second stage, which was 44.83 grains of spike⁻¹. The reason for this superiority may be attributed to the uprooting of the jungle plants after measuring their content of natural products, which led to the removal of the effect of those products, which chemical analyses confirmed remained until the maturity stage. The removal also led to making room for the crop plants to increase exposure to light, which increased the process of photosynthesis, Which provided a large amount of manufactured food to go to the newly growing grains to increase their set and thus increase the number of grains. These results are consistent with what was reached by [15].

Regarding the two-way interaction between the factor of varieties and weeds, the results indicated the presence of significant differences between the averages of interactions for the trait of number of grains per spike, as the interaction of the variety Aba99 with the comparison treatment recorded the highest average for these interactions (57.24) grains spike⁻¹ with a significant difference from the rest of the interactions, while the interaction between the variety Adna with the weed of the vegetation (tetra carbon) gave the lowest average for the trait (38.18) grains spike⁻¹. Similarly, the vegetative growth stage's interaction with the Aba99 variety to evaluate the results yielded the highest average (53.32) grains spike⁻¹, While the smallest average of 40.45 grains spike⁻¹ was obtained by measuring the interaction between the maturity stage and the variety Adna.

The comparison treatment's interaction with the vegetative growth stage to measure the natural results was superior, resulting in the highest average of interactions of 53.57 grains spike⁻¹, while the interaction of the sorghum weed with the maturity stage to measure the yields gave the lowest average (39.08) grains per spike⁻¹.

As for the triple interaction between the varieties, the weeds, and the stages of measuring the natural results of the weeds, The results of the variety Abaa99's interaction with the comparison treatment during the vegetative growth and maturity phases were measured, and the average interactions were the highest, with spike⁻¹ grains reaching (57.65 and 56.83), respectively, while the interaction between the variety Adna with the weeds of the Wild radish with the maturity stage to measure the results gave the lowest average (38.36) spike⁻¹ grains.

Table 4. Arithmetic averages of varieties, weeds, stages of measuring the natural products of weeds , and the overlap between them for the trait of the number of grains per spike (grain per spike⁻¹).

B / A	B1`		B2		B3		B / A	B1	B2	B3
	C1	C2	C1	C2	C1	C2				
A1	55.13 c	51.80 d	46.47 h	42.43 i	40.93 k	38.33 mn	A1	53.46 b	44.4 g	39.63 ij
A2	51.62 d	49.83 f	43.63 mn	42.33 j	39.47 j	38.37 l	A2	50.72 c	43.03 h	38.91 j
A3	58.63 a	57.67 b	51.58 d	48.31 g	50.87 de	41.60 jk	A3	58.15 a	49.94 d	46.23 f
A4	50.30 ef	44.40 i	41.23 k	39.05 lm	38.36 mn	37.90 n	A4	47.35 e	40.14 i	38.13 k
C / A	C1	C2	Averages A	C / B	C1	C2	Averages B			
A1	47.51 c	44.16 e	41.87 b	B1	53.92 a	50.93 b	52.42 a			
A2	44.91 d	43.54 f	44.22 d	B2	45.73 c	43.03 d	44.37 b			
A3	53.69 a	49.19 b	51.44 a	B3	42.41 e	39.05 f	40.72 c			
A4	43.30 f	40.45 g	45.83 c	Averages C	47.35 a	44.33 b				

3.2. Weight of 1000 Grains (g)

It is clear from Table (5) that there is a significant effect of the varieties, weeds, their natural products, and their binary and ternary interactions on the trait of weight of 1000 grains, as the variety Aba99 outperformed the rest of the varieties used in the study with an average of the trait of 41.43 g, compared to the plants of the variety Adna, which gave the lowest average of 38.76 g. The reason for the superiority in this trait may be attributed to the genetic composition of the variety, as the diversity of varieties results from differences in genetic factors and their interaction with environmental conditions. This result is consistent with many previous studies, as they indicated the existence of significant differences between wheat varieties in the trait of weight of 1000 grains, including [16], [17], and [13].

It was noted that the comparison treatment gave the highest average weight of a thousand grains, reaching 41.67 g, compared to the treatment of the grass (tetra carbon), which gave the lowest average (38.21) g. The reason for this superiority may be attributed to the fact that the comparison treatment was free of grass plants, which limited competition between them and the crop plants and allowed the crop to exploit growth factors such as water, light, and nutrients, which led to an increase in the efficiency of the photosynthesis process and an increase in the materials manufactured in the source and their transfer to the outlets (grains), and thus an accumulation of dry matter during the grain filling stage, which resulted in an increase in the grain's weight. This result agrees with [18]. For the stages of measuring natural products, the vegetative growth stage was significantly superior to the maturity stage, reaching 40.68 and 38.72 g, respectively. This increase may be due to the natural improvement of the crop's ability to grow the efficiency of the photosynthesis process and the manufacture of sugars after the absence of competition from weeds for the necessary growth requirements after their uprooting. This result agreed with what

was reached by [15]. The bilateral interaction between the varieties and the weeds had a significant effect on the average of the trait, The greatest average of 43.47 g was recorded in the interaction between the variety Aba99 and the comparison treatment, compared to the lowest average recorded by the interaction of the variety Adnah with the four-carbon weed, which gave the lowest average of the interactions of 37.32 g. The arithmetic average of 41.96 g was also recorded in the bilateral interaction among the variety Aba99 and the vegetative development stages to measure the natural products., compared to the lowest average recorded by the interaction between the variety Adnah and the maturity stage to measure the natural products (37.60) g. The comparison treatment's interaction with the vegetative growth stage to evaluate the products also surpassed expectations, resulting in the highest average of 42.01 g, while the interaction of the weed (tetra-carbon) with the maturity stage gave (36.68) g. Regarding the triple interaction between varieties, weeds, and stages of measuring the results, significant differences were recorded in the average of the trait, as the interaction of the variety Abaa99 with the comparison treatment with the first and second stages gave (43.64 and 43.30) gm respectively, while the triple interaction between the variety Adna with the weed of Wild radish with the comparison treatment recorded the lowest average interaction, reaching 35.62 gm.

Table 5. Arithmetic averages of varieties, weeds , stages of measuring the natural products of weeds , and the overlap between them for the trait of the weight of 1000 grains (g).

B / A	B1`		B2		B3		B / A	B1	B2	B3
	C1	C2	C1	C2	C1	C2				
A1	42.65 b	41.5 cd	40.63 ef	36.71 k	39.38 hi	35.5 l	A1	42.07 b	38.67 e	37.44 f g
A2	40.43 efg	39.74 gh	39.7 gh	38.52 j	39.25 hi	36.6 k	A2	40.08 d	39.11 e	37.92 f
A3	43.65 a	43.26 ab	41.76 c	39.9 fgh	40.33 efg	39.41 hi	A3	43.41 a	40.83 c	39.87 d
A4	41.00 de	40.61 efg	39.75 gh	36.2 kl	38.83 ij	35.7 l	A4	40.80 c	37.97 f	37.26 g
C / A	C1	C2	Averages A	/ B	C1	C2	Averages B			
A1	40.89 b	37.90 e	39.3 b	B1	41.91 a	41.28 e	41.59 a			
A2	39.79 d	38.28 d	39.04 bc	B2	40.46 b	37.83 d	39.14 b			
A3	41.88 a	40.86 c	41.3 a	B3	39.45 c	36.80 f	38.12 c			
A4	39.86 b	37.50 c	38.68 c	Averages C	40.60 a	38.63 b				

3.3. Grain Yield, tons. ha⁻¹

When looking at Table (6), it was noted that the Aba99 variety was significantly superior to the rest of the varieties used in the study and gave the highest average for the trait, reaching 4.4 tons. ha⁻¹, compared to the variety below, which gave the lowest average for the trait, reaching 3.14 tons. ha⁻¹. This superiority may be because the Aba99 variety was superior in the trait of the number of grains in the spike (Table 4), which was positively reflected in the grain yield. This result was consistent with what was reached by [17], [19], and [13], who showed that wheat

varieties differ among themselves in the trait of grain yield and are reduced according to the reduction of its three components

Regarding the weed factor and its effect on the grain yield trait, According to the statistical analysis, the comparison treatment was substantially superior, with an average of 4.65 tons.. ha⁻¹, compared to the treatment of the Wild radish weed (tetra carbon), which gave the lowest average for the trait (2.48) tons. ha⁻¹. The reason for this superiority may be attributed to the fact that the comparison treatment was free of weed plants, which allowed the crop plants to increase the growth of the vegetative and root system, and this led to an increase in the photosynthesis process as a result of the increase in the leaf area, which was positively reflected in the grain yield [14]. Also, the Wild radish weed is a tetra carbon plant, as chemical analyses proved that it secretes a larger amount of natural products (Tables 3 and 4), which led to a decrease in the total grain yield as a result of the crop plants being affected by these secretions.

As for the factor of the stages of measuring the natural products of jungle plants, the results of the statistical analysis confirmed the existence of significant differences in the averages of this trait, as the vegetative growth stage was significantly superior to the maturity stage and recorded an average for the trait of 3.69 tons. ha⁻¹, compared to the average for the trait in the second stage, which was 3.27 tons. ha⁻¹. The reason for this superiority may be due to the uprooting of jungle plants in the vegetative growth stage after measuring their content of natural products, which led to the lack of competition for the economic crop or the scarcity of its basic sources such as light, food, space, and water. These results are consistent with the results of [15].

In terms of the bilateral interaction between the weeds and the varieties, the combination of the Aba99 variety and the comparison treatment resulted in the maximum average of interactions, which was 5.13 tons. ha⁻¹, compared to the lowest average recorded by the interaction of the variety Rashid with the weed of the tetra carbon, which gave the lowest average for the trait, reaching 2.27 tons. ha⁻¹. Additionally, the greatest average was achieved by the interaction of the Aba99 variety with the vegetative growth stage in order to measure the results, which was 4.28 tons. ha⁻¹, with a significant variation in all interactions, while the interaction between the variety Adaana and the maturity stage to measure the results, which reached 2.96 tons.. ha⁻¹, and about the interaction between the weeds and the stages of measuring the natural results of the weed plants, the interaction between the comparison treatment and the growing vegetative stage to measure the results was preferable and resulted in the highest average of interactions, which was 4.82 tons. ha⁻¹, while it gave The interaction of the Wild radish weed (tetra carbon) with the maturity stage to measure the yields gave the lowest average for the studied trait, which was 2.46 tons.. ha⁻¹.

The triple interaction of the cultivars Aba99, Fayyad and Rashid with the comparison treatment with the first stage to measure the yields, The highest averages for the interactions were obtained by the cultivar Aba, which was subjected to the comparative treatment and the maturity stage to determine the natural yields, which were (5.21, 5.04, 4.85 and 5.06) tons. ha⁻¹, respectively, while the interaction between the cultivar Rashid with the Wild radish weed with the second stage to measure the yields gave the lowest average, which was 2.10 tons. ha⁻¹.

Table 6. Arithmetic averages of varieties, weeds, stages of measuring the natural products of weeds, and the interaction between them for the grain yield trait (ton ha⁻¹).

B / A	B1`		B2		B3		B / A	B1	B2	B3
	C1	C2	C1	C2	C1	C2				
A1	5.04 ab	4.37 cd	3.45 e	2.92 efg	2.45 ghi	2.58 ghi	A1	4.705 a	3.18 c	2.51 d e
A2	4.87	4.46	3.25	2.59	2.47	2.08	A2	4.66	2.92	2.27

B / A	B1`		B2		B3		B / A	B1	B2	B3
	C1	C2	C1	C2	C1	C2				
A3	abc 5.21	bcd 5.06	ef 4.38	ghi 3.29	ghi 3.26	i 3.08	A3	a 5.13	cd 3.83	e 3.16
A4	a 4.18	ab 4.04	cd 3.36	ef 2.72	ef 2.47	efg 2.10	A4	a 4.11	b 3.04	c 2.28
C										
/	C1	C2	Averages A		/	C1	C2	Averages B		
A					B					
A1	3.64 bc	3.29 def	3.46 b	B1	4.82 a	4.48 b	4.65 a			
A2	3.53 bcd	3.04 ef	3.28 bc	B2	3.61 c	2.88 d	3.24 b			
A3	4.28 a	3.81 b	4.04 a	B3	2.66 de	2.46 e	2.55 c			
A4	3.34 cde	2.96 f	3.14 c	Averages C	3.69 a	3.27 b				

3.4. Biological Yield, tons. ha⁻¹

When reviewing the results recorded in Table (7), a significant difference was observed between the varieties through the superiority of the variety Aba99 over the rest of the varieties used in the study and gave the highest average for the trait, reaching 12.10 tons. ha⁻¹, compared to the yield of the plants of the variety Adna, which gave the lowest average (11.02) tons. ha⁻¹. The reason for the superiority of the variety Aba99 in the biological yield trait may be due to the variation of the varieties in producing new vegetative growths and the extent of accumulation of dry materials, which in turn is related to the nature of the variety and the extent of its difference in the process of photosynthesis. This result agreed with [20] and [21].

Regarding the weed factor and its effect on the biological yield, the results of the statistical analysis indicated the superiority of the comparison treatment with an average of 12.25 tons. ha⁻¹, compared to the treatment of the weed (tetra carbon) which gave the lowest average of 10.65 tons. ha⁻¹. The reason for this superiority may be that the comparison treatment was free of weed plants, which allowed the crop plants to grow naturally as a result of the natural improvement in their ability to grow and the efficiency of the photosynthesis process and the manufacture of sugars after the absence of competition between the weeds and the crop plants, which led to an increase in growth traits which in turn was positively reflected in the biological yield trait [14].

As for the stages of measuring the natural products of the jungle plants, the first date was significantly superior to the second date and gave an average of 11.78 tons. ha⁻¹, compared to the average of the trait at the second date (11.24) tons. ha⁻¹. The reason for this superiority may be due to uprooting the jungle plants after measuring their content of natural products, which led to the removal of their inhibitory effects, which chemical analyses proved to remain until the maturity stage. This matter allowed wheat plants to exploit the growth elements and achieve an increase in the number of branches, which promoted the growth of the vegetation group and the accumulation of dried matter, as well as the creation of new growths and the opening of the way for crop plants, which was positively reflected in the trait of the biological crop. This result agreed with [22] and [23].

Regarding the bilateral interaction between the factors of varieties and weeds, the results of the statistical analysis indicated the presence of significant differences, as the interaction between the

variety Aba99 and the comparison treatment recorded the highest average of interactions, reaching 12.88 tons. ha⁻¹, compared to the lowest average recorded by the interaction of the variety Adna with the weed of the tetra carbon, which reached 10.38 tons. ha⁻¹. The highest average for the examined trait was also recorded in the interaction of the variety Aba99 with its vegetative growth stage, reaching 12.52 tons. ha⁻¹, with significant variation from the rest of the interactions, while the interaction between the variety Rashid with the second stage to measure the results recorded the lowest average (10.77) tons. ha⁻¹.

The interaction between the comparison treatment and the vegetative growth stage to measure the results achieved the highest average for the studied trait, reaching 12.32 tons. ha⁻¹, with significant variation from the rest of the interactions, while The interaction of the treatment of the weed Wild radish (tetra carbon) with the second stage of measuring the results gave the lowest average (10.34) tons. ha⁻¹. Also, the interaction between the cultivar Aba99 with the comparison treatment with the vegetative growth stage of measuring the results recorded the highest average (12.96) tons. ha⁻¹ with significant variation over the rest of the interactions, During the second phase of measuring the natural results, the lowest average of 10.11 tons was obtained when the cultivar Adnah interacted with the wild radish weed. ha⁻¹.

Table 7. Arithmetic averages of varieties, weeds, and stages of measuring the natural products of weeds and the interaction between them for the biological yield trait.

B / A	B1`		B2		B3		B / A	B1	B2	B3
	C1	C2	C1	C2	C1	C2				
A1	12.55 d	12.34 e	12.19 g	10.86 m	10.60 o	10.45 p	A1	12.45 b	11.53 f	10.53 j
A2	11.66 j	11.60 j	11.42 k	10.73 n	10.95 l	10.17 q	A2	11.63 e	11.08 h	10.56 j
A3	13.05 a	12.85 b	12.75 c	11.66 j	11.98 h	10.65 o	A3	12.95 a	12.21 c	11.32 g
A4	12.27 f	12.20 g	12.03 h	11.77 i	10.86 m	10.49 p	A4	12.23 c	11.90 d	10.68 i
C / A	C1	C2	Averages A		C1	C2	Averages B			
A1	11.78 b	11.22 e	11.49 c	B1	12.38 a	12.25 b	12.31 a			
A2	11.34 e	10.83 g	11.08 d	B2	12.10 c	11.25 d	11.67 b			
A3	12.59 a	11.72 c	12.15 a	B3	11.10 e	10.44 f	10.76 c			
A4	11.72 c	11.48 d	11.60 b	Averages C	11.85 a	11.31 b				

3.5. Harvest Index (%)

The results shown in Table (8) indicated the presence of significant effects between the study factors and their binary and ternary combinations, as the difference between the varieties used in the study led to a significant effect on the average of the harvest index trait, as the Aba99 variety outperformed the rest of the varieties significantly and gave an average of 33.36%, compared to the data recorded from the plants of the Ada variety, which gave the lowest average of 27.83%. The difference between the varieties in this trait is due to the nature of the genetic composition of the variety and its efficiency in converting dry matter into grains. As for the superiority of the

Aba99 variety in the harvest index trait, it is due to its superiority in grain yield per unit area (Table 6), and these results are consistent with what was found by [24].

The comparison treatment was significantly superior with an average of 39.07%, compared to the treatment of the weeds (tetra carbon) which gave the lowest average for the trait (24.17)%. This superiority may be because the comparison treatment was free of weed plants, which allowed the formation of a large green mass by providing more suitable conditions for the growth of crop plants without competition for growth factors, thus increasing the efficiency of the photosynthesis process and improving the performance of the crop for vital activities. This increases the conversion of manufactured dry matter from the source to the outlets (grains) and increases the weight and yield of the grains, which was reflected in the increase in the percentage of the harvest index. This result is consistent with the results Of them [25], [26] and [18].

As for the stages of measuring natural products, the results of the statistical analysis indicated that the vegetative growth stage was significantly superior to the maturity stage for the harvest index trait (31.99 and 29.37)%, respectively. The increase in the harvest index can be attributed to the inhibition of weed growth after removing it in the vegetative growth stage, which led to providing more nutrients, soil moisture, and space for the wheat crop, which gave better use of photosynthesis and improved grain formation. This result agreed with what was reached by [27] and [28].

The comparison treatment recorded the highest average of (41.99 and 40.80)% for the varieties Aba99 and Rashid, indicating a significant effect of the binary interaction within the factors of the varieties and weeds, in comparison to the lowest average observed by the interaction that occurred of the variety Adna with the contaminant of the Wild radish (tetra carbon) (21.88%). In the same treatment, the interaction between the variety Aba99 and the vegetative growth period to measure the results recorded the highest average of 34.37%, with significant variation from all other of the interactions, with the exception of the variety Rashid, The lowest average of 26.23% was obtained when the maturation stage was measured in conjunction with the variety Adna. The interaction of the comparison treatment alongside the vegetative growth phase was significantly superior to the rest of the interactions, with an arithmetic average of 40.25%. Conversely, the interaction of the treatment of a weed of the Wild radish with the maturity stage to measure the results yielded the lowest average for the studied trait of 23.21%. This trait was significantly influenced by the triple interaction among the cultivar Aba99 and the comparison treatment during the initial stage of evaluation., recording an average of 43.78% and significant variation on all interactions except the cultivar and the comparison treatment itself but with the second stage, while the interaction between the cultivar Adnah with the four-carbon shrub with the second stage of measuring the natural results gave the lowest average of 20.61%.

Table 8. Arithmetic averages of the cultivars, weeds, and stages of measuring the natural results of the weeds and the interaction between them for the harvest index trait.

B / A	B1`		B2		B3		B / A	B1	B2	B3
	C1	C2	C1	C2	C1	C2				
A1	44.7aa	42.23 ab	30.21 efg	29.65 efg	26.85 fgh	25.96 f-i	A1	39.05 ab	29.93 de	26.41 ef
A2	41.11 abc	36.99 bcd	31.47 def	26.83 fgh	25.86 f-i	22.41 hi	A2	43.96 a	29.16 def	24.14 fg
A3	45.63 a	42.29 ab	37.15 bcd	31.46 def	30.73 fgh	29.54 efg	A3	43.49 a	33.94 cd	30.50 de
A4	36.03 b-e	35.65 cde	30.26 efg	24.55 ghi	22.27 hi	20.12 i	A4	35.84 bc	27.41 ef	21.20 g

C	C1	C2	Averages A	C	C1	C2	Averages B
/				/			
A				B			
A1	32.43 bcd	31.17 cd	31.79 b	B1	41.25 a	39.92 a	40.58 a
A2	34.32 abc	30.52 d	32.41 b	B2	32.28 b	27.94 c	30.11 b
A3	36.31 a	35.65 ab	35.97 a	B3	25.91 c	25.22 c	25.56 c
A4	29.52 de	26.78 e	28.14 c	Averages C	33.14 a	31.02 b	

Table 9. Appendix Analysis of Variance for Varieties, Weeds and Outputs represented by Mean Squares (M.S) for the Traits Under Study.

S.O.V	d.f	Number of grains in the spike (grain spike ⁻¹)	Weight of 1000 grains (g)	Grain yield, tons. ha ⁻¹	Biological yield, tons. ha ⁻¹	Harvest index (%)
R	2	27.00	20.57	2.096	0.837	119.25
A	3	298.33 **	26.06 **	2.825 **	3.488 **	185.05 **
RxA	6	0.14	0.62	0.127	0.003	11.78
B	2	859.23 **	76.26 **	27.343 **	14.469 **	1424.66 **
AxB	6	5.60 **	2.16 **	0.147 N.s	0.274 **	21.28 N.s
RxB (A)	16	0.37	0.18	0.136	0.002	14.83
C	1	163.77 **	69.82 **	3.230 **	5.346 **	80.79 **
AxC	3	7.62 **	3.42 **	0.021 N.s	0.310 **	9.14 N.s
BxC	2	0.66 N.s	8.04 **	0.450 N.s	0.824 **	22.63 N.s
AxBxC	6	13.19 **	0.89 **	0.127 N.s	0.217 **	12.18 N.s
Error	24	27.000	0.165	0.110	0.001	11.709
Total	71					

** Significant at the probability level of 0.01.

Conclusion

- The natural products produced from secondary metabolism directly affected the growth characteristics of wheat.
- The effect of the C 4 weed product was higher than the effect of the C3 weed product in all characteristics, and the effect increased with the increase in the duration of the weeds remaining in the field (maturity stage).
- The Aba99 variety was superior in all the studied characteristics.

References

- [1] Costa,R; N. Pinheiro, A. Almeida, C. Gomes, J. Coco, A.Costa and B. Macas. 2013 . Effect of sowing date and seeding rate on bread wheat yield and test weight under Mediterranean conditions. J. Food Agric. 25(12): 951-961.
- [2] Directorate of Agricultural Statistics. 2023 . Wheat Crop Statistics. Planning and Follow-up Department. Ministry of Agriculture. Iraq.
- [3] Al-Sultani, Abdul Karim Haif Kazim and Baqir Abdul Khalaf Al-Jubouri. 2002 . The bactericidal effect of extracts of wild mustard grass Brassica nigra on germination and growth of two wheat varieties. Journal of Diyala University. 12: 354-365.

- [4] Al-Jubouri, Baqir Abdul Khalaf. 2002 . Jungle Science. Ministry of Higher Education and Scientific Research. Dar Al-Hikma for Printing and Publishing. Baghdad/Iraq. p. 320.
- [5] Hamadi, Rayan and Jahida Madi. 2022 . Response of durum and soft wheat cultivars to different levels of salinity. Master's thesis. University of Brothers Mentouri Constantine. Ministry of Higher Education and Scientific Research. Democratic Republic of Algeria.
- [6] Al-Jalabi, Faeq Tawfiq. 2003 . Biological response of wheat to weed control with Diclofop-methyl pesticide in succession with 2, 4-D and its effect on grain yield. Iraqi Journal of Agricultural Sciences 34 (1): 89 – 100.
- [7] Saleh, Muzaffar Abdul Mahdi. 2009 . The effect of the biotic antagonism of some winter weed species on the germination, growth and yield of bread wheat (*Triticum aestivum* L.) and yellow corn (*Zea mays*). Master's thesis. College of Agriculture, Tikrit University.
- [8] Korres, N. E and Froud-Williams, R. J. 2002 . Effects of winter wheat cultivars and seed rate on the biological traits of naturally occurring weed flora. Weed research, 42(6), 417-428.
- [9] Travlos, I.S. 2012 . Reduced herbicide rates for an effective weed control in competitive wheat cultivars. Intern. J. Plant Prod. 2012, 6, 1–14.
- [10] Lu, P; Jiang, B; Weiner, J. 2020 . Crop spatial uniformity, yield and weed suppression. Adv. Agron. 2020 . 161, 117–178.
- [11] Donald, C.M. and J. Hamblin . 1976 . The biological yield and harvest index of cereals as agronomic and plant breeding criteria. Adv. in Agron. 405-361 :28.
- [12] Al-Mohammadi, Mohammad Hani Mohammad. 2018 . Effect of spraying time with Nutrigreen amino acid compound concentrations on growth traits, yield and its components, and quality traits of wheat varieties. Master's thesis - Tikrit University - College of Agriculture - Department of Field Crops.
- [13] Al-Zankena. Dalawa R. Delshad Ali. 2023 . The role of Hydrogel in drought tolerance and its relationship to growth, yield and quality traits in bread wheat (*Triticum aestivum* L.). PhD thesis. Tikrit University. Department of Field Crops - College of Agriculture - Tikrit University.
- [14] Hassanein, Abdel Hamid Mohamed, Mohamed Al-Asmar Al-Hawari, and Sayed Farouk Saafan. 2021 . Field Crop Production. Noor Library. noor-book.com/uhrdp2.
- [15] Hamid, Nour Ali. 2023 . Response of weeds accompanying wheat (*Triticum aestivum* L.) to different spray and growth rates of Atlantis herbicide and its subsequent antagonistic effect. PhD thesis. Department of Field Crops. Faculty of Agriculture. Tikrit University.
- [16] Al-Hamdawi. Israa Rahi Saihoud. 2017 . Contribution of the flag leaf, other plant leaves and parts of the inflorescence to the growth and grain yield of three wheat and oat cultivars. Master's thesis - College of Agriculture - Al-Muthanna University.
- [17] Al-Salem, Saleh Hadi Farhoud. 2018 . Evaluation of bread wheat (*Triticum aestivum*. L) genotypes using biochemical and molecular techniques compared to morphological characterization. PhD thesis - College of Agriculture - Al-Muthanna University.
- [18] Al-Mashhadani, Ahmed Majeed Abdullah . 2020 . Effect of tillage systems, seed quantities and herbicides on the growth and yield of bread wheat (*Triticum aestivum* L.) under rainfed conditions. Master's thesis, College of Agriculture and Forestry, University of Mosul, Iraq.
- [19] Al-Nimrawi, Saad Khalaf Hammad Hussein. 2021 . Response of several bread wheat varieties to spraying with glutamic acid and the effect of seasons, locations and storage methods on growth traits, flour yield and quality. PhD thesis, Tikrit University - College of Agriculture - Department of Field Crops.
- [20] Al-Halfi, Intisar Hadi Hamidi and Mukhallad Ibrahim Falih. 2017 . Response of the yield of two varieties of bread wheat to mineral, biological and organic fertilizers. Iraqi Journal of Agricultural Sciences. 48(6): 1661-1671.
- [21] Al-Azzawi, Hussein Khadir Abbas, Mohsen Ali Ahmed Al-Janabi, and Fakhr Al-Din Abdul Qader Siddiq. 2018 . Effect of different levels of nitrogen fertilizer on grain yield and its components of eight varieties of bread wheat (*Triticum aestivum* L). Tikrit University Journal of Agricultural Sciences. 18 (1): 14- 27.

- [22] Malekian, B; Ghadiri, H; Kazemeini, S. A and Edalat, M. 2013 . Efficacy evaluation of sulfosulfuron, metsulfuron-methyl plus sulfosulfuron, mesosulfuron-methyl plus iodosulfuron-methyl and iodosulfuron plus mesosulfuron herbicides in winter wheat (*Triticum aestivum* L.). J. Biol. Environ. Sci, 7(21), 177-182.
- [23] Kadum, M. N; Mutlag, N. A; Al-Khazal, A. J; Mohamed, G. A; and Salman, K. A. 2019 . Evaluation of the performance of Bread wheat genotypes (*Triticum aestivum* L.) in central region of Iraq by using Selection technique. Research J. of Chemistry and Environment, 23(SI): 101-105.
- [24] Salman, Issam Sabti. 2016 . The effect of the interrelationship between wheat varieties and nitrogen fertilization on zinc absorption. PhD thesis, College of Agriculture, University of Baghdad.
- [25] Al-Fahdawi, Ahmed Tariq Mohammed. 2016 . The effect of Atlantis WG herbicide in controlling weeds associated with wheat and estimating its residues in plants and soil. PhD thesis. Department of Field Crops. College of Agriculture. University of Baghdad. P. 112.
- [26] Mohammad, Ramez, Ghassan Naasa and Rbab Saud . 2016 . Study of some physical and chemical properties of some Syrian durum wheat varieties. Tishreen University Journal for Scientific Research and Studies. Science and Biology Series 38(1) 155-165.
- [27] Amare T, Raghavaiah CV, Zeki T.(2016). Productivity, yield attributes and weed control in wheat (*Triticum aestivum* L.) as influenced by integrated weed management in Central High Lands of Ethiopia, East Africa. Advances of Crop Science and Technology. 2016; 4(1):1-4.
- [28] Al-Taie, Saja Issam Abdul-Wahab. 2023 . The effect of chemical pesticides on the growth and yield of two varieties of soft wheat (*Triticum aestivum* L.) and on accompanying weeds. Master's thesis. Department of Field Crops. College of Agriculture and Forestry. University of Mosul.

Some Factors Limiting Rural Women's Participation in the Development and Sustainability of the Family Farm

Nadia Kazem Anoon

Department of Agricultural Extension and Technology Transfer, College of Agricultural Engineering Science, University of Baghdad, Baghdad, Iraq.

E-mail: nadia_annon@coagri.uobagdad.edu.iq

Abstract. The study aimed to understand the administrative and economic empowerment of rural women, with a focus on sustainable family farming. The research encompassed various aspects, including increasing living standards, achieving economic independence, economic awareness, special projects for income generation, administrative capabilities, and the integration of rural women into agricultural activities. The study surveyed 750 rural women in different agricultural regions, specifically in the province of Baghdad. The findings revealed that a majority of the surveyed women had low levels of living standards improvement, with only 45% experiencing an increase. However, 54% achieved economic independence, while 63.3% demonstrated economic awareness. Impressively, 87% of the women possessed financial literacy. Despite these strengths, many of them lacked the ability to secure project proposals for income generation. The study recommended the necessity of governmental involvement in providing opportunities for rural women to enhance their economic situations, particularly through development projects. This would ultimately contribute to improving the economic status of rural women and their families.

Keywords. Administrative factors, Rural development, Economic awareness.

1. Introduction

The issue of rural women and their role in Arab societies presents significant challenges and opportunities. This issue is central to various social and field studies and naturally extends to the empowerment of women and their vital contributions to rural development [1].

Rural women play a crucial role in agricultural activities, contributing to both productive and agricultural sectors. They manage agricultural enterprises independently, whether individually or collectively, to meet the needs of their families or for economic purposes [2].

Furthermore, sustainable development initiatives emphasize the importance of involving diverse segments of society, particularly women, in economic, social, environmental, and political dimensions. Balancing these aspects is crucial for rural development [3].

Agriculture is a vital sector for the economy of any nation, providing the foundation for trade, industry, and community development [4]. The indicators of sustainable development are also divided into four main categories based on the definition of sustainable development: economic, social, environmental indicators, as well as institutional indicators that provide an assessment of the progress of governance in any society [5]. Sustainable rural development necessitates investing in women's education, training, leadership, research, and knowledge dissemination,

recognizing their pivotal role in rural development [6]. The agricultural sector remains one of the most important sectors in developing countries, including Iraq [7].

The development of this sector and the improvement of its production methods have become an urgent necessity for the success of agricultural development in general [8].

Scientific progress is one of the main pillars for achieving sustainable economic and social development. With proper utilization of agricultural resources, women can contribute significantly to agricultural productivity and economic growth [9].

The multifaceted roles and diverse activities of rural women should be acknowledged. Rural women represent an essential part of the productive workforce and should be empowered with education, training, leadership, research, and access to knowledge to excel in their roles in rural development [10].

Understanding the conditions of women, both in general and rural women in particular, is vital. Women's development should be viewed differently from men's, as gender integration is essential for comprehensive development [11].

"Investment in knowledge, information, and rural women's empowerment is essential through the use of modern technology and agricultural programs [12]. Enhancing women's access to rural areas and providing specialized development programs supported by agricultural research results are crucial [13].

It is well-established that when strategic plans align with political will and decision-makers' support for women's roles and responsibilities, successful rural development becomes attainable [14]. The effectiveness of rural women's development relies on various internal and external factors and the nature of the tasks assigned [15].

It is evident that diverse development initiatives in rural regions still fall short of achieving their intended outcomes. This implies the presence of developmental obstacles without harnessing the full potential of rural women, resulting in missed opportunities for economic growth [16].

Based on the above, the research problem can be framed through several research questions:

- What are the administrative factors hindering rural women's participation in family farm development and sustainability?
- What are the economic factors restricting rural women's involvement in family farm development?

1.1. Research Objectives

Based on the presentation of the research problem, the study's objectives are as follows:

- Firstly: to identify the social and economic characteristics of the research subjects.
- Secondly: to understand the administrative factors that hinder rural women's participation in family farm development and sustainability.
- Thirdly: to comprehend the economic factors limiting rural women's engagement in family farm development.

1.2. Operational Definitions

1.2.1. Administrative Factors

These are factors that hinder rural women's participation in the development and sustainability of family farms. They arise from low and ineffective administrative levels, introversion, stagnation, procedural complexity, routine-based behavior, slow decision-making, mutual interference, non-adherence to declared slogans, indifference, and dominance.

"Personal Factors on Official Work Relationships, Difficulty in Coordinating with New Administrative Units, and the Shortage of Qualified and Trained Administrative Competencies Capable of Bearing Responsibility.

1.2.2. Economic Factors

These are the factors that limit the participation of rural women in the development and sustainability of family farms, stemming from low living standards, a lack of savings, minimal investments, and technological underdevelopment.

1.3. The Ability to Improve Living Standards

This refers to rural women's ability to engage in a specific profession, start their own projects, or expand income-generating activities to enhance living standards and family well-being.

1.4. Economic Independence and Self-Reliance

This points to the neglect of women's roles in public work due to increased family responsibilities towards household, spouse, and children, along with a lack of interest in employment, especially after marriage. Additionally, it results from women being placed in a lower status compared to men, a lack of belief in manual labor, and a loss of women's control over their own decisions related to their productive and economic activities, including income.

1.5. Economic Awareness

This denotes the hidden knowledge possessed by rural women regarding economic aspects arising from their proficiency in their work and their ability to make decisions confidently.

1.6. Special Loans for Establishing Projects

This refers to rural women's capability to access credit facilities with the aim of obtaining loans for income-generating projects that secure economic independence and provide sustainable livelihood resources.

1.7. Availability of Administrative Competencies

This indicates the shortage of qualified and trained administrative competencies capable of shouldering development responsibilities and enhancing legislative development. It also reflects the development of communication channels between higher and lower administrative levels and vice versa.

1.8. Coordination Among Administrative Units

This entails the lack of balance among different sectors and development areas, as well as the ambiguity of planning objectives and the absence of community participation in planning and implementation.

2. Material and Methods

2.1. Research Methodology

The field survey method was employed to achieve the objectives of the current research because it is suitable for obtaining data in this field [17]. It branches from the descriptive approach in social research [18].

2.2. Research Community

The Abi Ghraib district in Baghdad province was chosen for conducting the research for two reasons:

The presence of a large number of rural women in this district.

A significant percentage of women in this district work in agriculture.

The research community includes all rural women in Baghdad province (Abi Ghraib district). Their number is approximately 750 women distributed over three agricultural regions of the province (Textile, Akerkof, Alhaswa) [19].

2.3. Research Sample

I selected a random sample from Abi Ghraib district, representing 20% of the total rural women in the district, whose number is 750 research participants. They are distributed across three agricultural regions within the district (Textile, Akerkof, Alhaswa) to constitute a sample size of 150 research participants, as shown in the following table:

Table 1. Distribution of Respondents Among Agricultural Sectors in the Abu Ghraib District.

The conclusive	The judiciary	Agricultural sector	The number of women in the society	The number of women in the sample
Abu Ghraib	The judicial center of Abu Ghraib	Alnasr Alsalam	275	55
		Akarkouf	300	60
		Alhasoa	175	35
		The sum	750	150

2.4. Data Collection Tool

The questionnaire was used to obtain information from the respondents, as it is suitable for the nature of the research and the required data. The questionnaire is considered an appropriate tool for data collection because it is more objective than other data collection methods to achieve the research goals. The preparation relied on [20]:

- Scientific literature related to the research topic.
- Annual reports on extension activities directed at rural women issued by the Ministry of Agriculture.
- Interviews with rural women who worked on the family farm within the research community.
- Review of extension studies and research related to rural women.

Based on the above, the researcher prepared a preliminary questionnaire consisting of two parts: the first part includes a description of the respondents and their agricultural systems, which includes eight items, and the second part includes 29 items to identify the administrative and economic factors that limit rural women's participation in the sustainability of the family farm in the Abu Ghraib district, as mentioned in some studies.

2.5. Scale Validity

The face validity of the scale was examined by presenting the measurement tool, after its completion, to a group of experts in agricultural extension to assess both face and content validity. Face validity refers to how well the tool appears to measure what it is supposed to measure based on the type of items, their clarity, and formulation (the scale's format). It doesn't indicate what the tool measures but rather its superficial or apparent appearance [21]. Content validity, on the other hand, pertains to the scientific material contained within the items and reflects the degree to which the scale measures what it was designed to measure within a specific

content area, meaning the items are representative and appropriate for the behavior being measured [22].

A preliminary test (pre-test) was conducted on a sample of 15 respondents from the Abu Ghraib district, Baghdad Governorate. The reliability of the questionnaire was measured using Cronbach's alpha, and the reliability coefficient was found to be 0.98, indicating that all statements had high reliability. Data was collected from October 2022 to December 2022 through personal interviews. The following statistical tools were used: frequencies, percentages, and the arithmetic mean.

2.6. Scale Reliability

The researcher employed a four-point Likert scale, ranging from (Strongly Agree - Agree - Disagree - Strongly Disagree), assigning weights (3, 2, 1, 0) to the responses. This scale was administered to a total of 29 female participants to assess administrative and economic barriers faced by rural women in sustainable family farming in Abu Ghraib. The scale's reliability was confirmed by calculating Cronbach's alpha, which yielded a value of 0.98, indicating the internal consistency of the items. The reliability test was conducted on 17 respondents for economic barriers and 12 respondents for administrative barriers, with the scale scores ranging from (0 to 87).

3. Results and Discussion

3.1. To Identify the Characteristics of the Social and Economic Aspects of the Respondents

3.1.1. Income

The study results showed that the majority of respondents have a low-income percentage (65%), while women with high income reached (20%), as shown in the following table:

Table 2. Distribution of respondents according to monthly income variable.

The monthly income of the respondent	Number	%
one thousand dinars (550-150)low income	65	43.3
one thousand dinars (950-551) medium income	55	36.3
Or more one thousand dinars-951) high income	30	20

From the above table, we conclude that the majority of respondents have a low income, which necessitates relevant authorities to undertake projects that contribute to enhancing the economic status of rural women.

3.1.2. Openness to the Outside World

The results indicate that (46.6%) of the respondents fall within the medium category, while those in the low category constituted (36.6%), and the high category comprised (16.6%), as shown in the following table:

Table 3. Distribution of respondents according to the variable of openness to the outside world.

Openness to the outside world	Number	%
level(8-5) low	55	36.6
level(12-9) medium	70	46.6
more 13)high	25	16.6

We can conclude from the above table that the majority of respondents have a moderate level of openness to the outside world due to the lack of necessary facilities for rural women, which contribute to enhancing this openness, such as specialized educational platforms for rural women.

3.1.3. Educational Level

The results show that the majority of rural women fall within the categories (read and write, elementary, preparatory), with percentages of (13.3%, 26.6%, 33.4%) respectively. Meanwhile, the percentage of those holding a degree (preschool, university, higher degree) is (10%, 6.7%, 3.3%) respectively.

As shown in the following table:

Table 4. Distribution of respondents according to the variable of educational level.

Educational level	Number	%
Illiterate	10	6.7
Read and write	20	13.3
Elementary	40	26.6
preparatory	50	33.4
Institute	15	10
University	10	6.7
Higher degree	5	3.3

We can conclude from the above table that the majority of respondents are educated, and this is a positive factor that helps rural women to work in various fields, whether agricultural or non-agricultural, to improve their living standards.

3.1.4. Marital Status

The results show that the vast majority of rural women fall within the category of married, constituting 53.3%, while the percentage of unmarried women is 16.7%, widows are 23.3%, and divorced women are 6.7%, as shown in the following table:

Table 5. Distribution of respondents according to the variable of marital status.

Marital status	Number	%
Unmarried	50	33.3
Married	60	40
widowed	30	20
Divorced	10	6.7

We can conclude from the above table that regardless of the social status, rural women require the provision of all means and the removal of all obstacles that help them participate in income-generating activities.

The results show that the majority of respondents fall within the (farmer) category with a percentage of 40%, while the percentage of respondents in the (employee) category is 33.3%, and those in the (housewife) category is 20%. As for the (self-employed) category, their percentage reached 6.7%.

3.1.5. Occupational Status

As shown in the following table:

Table 6. Distribution of respondents according to the variable of occupational status.

Occupational status	Number	%
Employee	50	33.3
Farmer	60	40
Housewife	30	20
Self-employed	10	6.7

We can conclude from the above table that rural women, whether employed, working in the field, or housewives, are capable of working and contributing, performing various roles that contribute to increasing their income.

3.1.6. Cultural Openness

The results showed that (40%) of the respondents fall within the medium category, (30%) in the low category, and (30%) in the high category, as shown in the following table:

Table 7. Distribution of respondents according to the variable of cultural openness.

Cultural openness	Number	%
Low (14-7)	45	30
medium (22-15)	60	40
High 22&more	45	30

3.1.7. Women's Perception of Their Status in Society

The results showed that (50%) of the respondents fall within the medium category, while the percentage of respondents in the high category is (33.4%), and the percentage in the low category is (16.6%), as shown in the following table:

Table 8. Distribution of respondents according to the variable of Women's perception.

Women's perception of their status in society	Number	%
low(8-5)	25	16.6
medium(12-9)	75	50
High 13&more	50	33.4

3.1.8. Renewability

The results showed that (46.6%) of the respondents fall within the medium category, while the percentage of respondents in the high category is (30%), and the low category comprises (23.3%), as shown in the following table:

Table 9. Distribution of respondents according to the variable of Renewability.

Renewability	Number	%
low(8-5)	35	23.3
Medium (16-12)	70	46.6
High 17&more	45	30

3.2. Identifying the Economic Factors that Hinder the Participation of Rural Women in the Development and Sustainability of the Family Farm in Various Fields

3.2.1. Ability to Change the Standard of Living

The results from Table (10) show that (45%) of the respondents have the ability to change the standard of living in the low category, and (41%) of the respondents fall within the medium category, while the percentage of respondents in the high category is (14%).

Table 10. Distribution of respondents according to the ability to change the standard of living.

The ability to change the standard of living	Number	%	The average	Standard deviation
(9 – 4)High	21	14	11.8	5.2
(15 – 10)medium	63	41		
&more (21 – 16)	66	45		

low		
sum	150	100

We can conclude from the above table that the majority of respondents have a low ability to change their standard of living. Therefore, relevant authorities need to take necessary measures to assist rural women in improving their living standards.

3.2.2. *Economic Independence and Self-Reliance*

The results from Table (11) show that (54%) of the respondents have the ability for economic independence and self-reliance, while the percentage of respondents in the medium category is (40.7%), and in the low category is (5.3%).

Table 11. Distribution of respondents according to the ability for economic independence and self-reliance.

The ability for economic independence and self-reliance	Number	%	The average	Standard deviation
(12-8)low	8	5.3	14.2	6.8
(17 – 13)medium	61	40.7		
high18 &more	81	54		
sum	150	100		

We can conclude from the above table that the economic independence and self-reliance of rural women are low and significantly so. Therefore, providing financial assistance and loans to support income-generating projects is crucial.

3.2.3. *Economic Awareness*

The results from Table (12) show that (63.3%) of the respondents are in the high category, while (5.3%) are in the medium category, and the percentage of respondents in the low category is (31.3%).

Table 12. Distribution of respondents according to economic awareness.

Economic awareness	Number	%	The average	Standard deviation
(7-3)low	47	31.4	10.9	2.9
(12 – 8)medium	8	5.3		
High 16 &more	95	63.3		

We can conclude from the above table that most respondents have a high economic awareness. This aspect can be harnessed for comprehensive development by optimally investing in the potential of rural women for their self-development, reflecting positively on various aspects, whether agricultural, social, or economic

3.2.4. *Loans for Establishing Projects*

The results from Table (13) show that (87.3%) of the respondents do not have the ability to take private loans for establishing projects. Respondents in the medium category are (4.7%), while those in the high category are (8%).

Table 13. Distribution of respondents according to the ability to take private loans for establishing projects.

Loans for establishing projects	Number	%	The average	Standard deviation
(8 - 6)high	12	8	10.2	1.9
(11 – 9)medium	7	4.7		

low 12&more	131	87.3

Based on the findings, it is essential to provide administrative facilitations and reduce the conditions imposed by relevant authorities to ensure the necessary loans are made available.

3.3. Identifying the Administrative Factors that Hinder the Participation of Rural Women in the Development and Sustainability of the Family Farm

3.3.1. Lack of Administrative Competencies

The results from Table (14) show that (81.3%) of the respondents are in the high category, while the percentage of respondents in the medium category is (9.3%), and the low category comprises (9.3%).

Table 14. Distribution of respondents according to the availability of administrative competencies.

Lack of administrative competencies	Number	%	The average	Standard deviation
(17-15)low	14	9.3	17.3	2.1
(20 – 18)medium	14	9.3		
&more21 high	122	81.4		

Based on the presented findings, it is crucial to provide administrative competencies and expertise in various fields, whether in rural or urban areas, to achieve sustainable development.

3.3.2. Weak Coordination Between Administrative Units

The results from Table (15) show that (94%) of the respondents are in the high category, while the percentage of respondents in the medium category is (2%), and in the low category is (4%).

Table 15. Distribution of respondents according to the coordination between administrative units.

Weak coordination between administrative units	Number	%	The average	Standard deviation
(18 -16)low	6	4	19.9	2.1
(21 – 189)medium	3	2		
22& more high	141	94		
sum	15	100		

Based on the provided information, it is essential to work on enhancing relationships between official entities and strengthen them to contribute to achieving comprehensive coordination among relevant departments, thereby overcoming challenges faced by rural women.

Important Findings

- A large percentage of rural women are unable to improve their living standards due to a lack of sufficient job opportunities that contribute to this change.
- Most rural women have a high level of economic awareness and sufficient knowledge that can help establish income-generating projects.
- The necessary facilities for rural women to obtain loans for establishing their own projects, which could increase their income and improve their economic situation, are not available.
- Administrative routines and the lack of sufficient administrative competencies have led to a decrease in the economic level of rural women and hindered many economic projects.
- Most of the respondents have educational qualifications that qualify them for various types of work, even if these jobs require a high level of education.

- A significant percentage of the respondents, possibly up to 60% or more, do not have a provider due to reasons such as not being married, divorced, or widowed. This specific situation necessitates the provision of job opportunities for them.

Recommendations

Based on the aforementioned results, the study recommends the following:

- Empower rural women economically by involving them in sustainable and income-generating projects, in collaboration with relevant government agencies, such as the Ministry of Women's Affairs and the Ministry of Agriculture.
- Eliminate administrative obstacles and institutional hindrances through streamlining administrative procedures and enhancing coordination between various sectors to increase women's capacities for production and development.
- Provide loans for rural women, along with the necessary support and reasonable interest rates, to alleviate financial pressures.
- Focus on training rural women in various skills, such as sewing, embroidery, and home-based businesses like livestock breeding and beekeeping, to help them achieve independent income and elevate their family's standard of living.
- Encourage rural women to engage in small-scale projects like handicrafts, dairy farming, and poultry farming to secure their own income.
- Promote educational programs for rural women to acquire skills that enable them to have their own source of income.

References

- [1] Al-Heithawi, Najwa Salim (2012). The Role of Rural Women in Household Food Security in Some Districts of Baghdad Governorate, PhD thesis, College of Agriculture, University of Baghdad.
- [2] Al-Badri, Ashwaq Abdul Razzaq Najee (2007). Proposed Strategies for Developing the Role of Rural Women in Agricultural Extension in the Governorates of Iraq, Iraqi Journal of Agricultural Sciences, Volume 2, Number 38.
- [3] Yasseri, Abdul Mawla (2005). The Role of Rural Women in Political Participation in the Governorates of Menoufia and Beni Suef, Center for Agricultural Research and Rural Development - Arab Republic of Egypt.
- [4] Ahmed daher Shahyed and Al-Badri, Ashwaq Abdul Razzaq Najee (2018). Agricultural Information and Communication Technologies Used Before Farming to Reduce the Gap for Wheat and Barley Production in the Governorates of the Middle Euphrates Region of Iraq, Iraqi Journal of Agricultural Sciences, Volume 49, Number 1, Page 84
- [5] Al-Azzawi, Kadhim (2012). Empowering Rural Women in Sustainable Development in the Rural Areas of Baghdad Governorate, PhD thesis, College of Agriculture, University of Baghdad.
- [6] Abdul Razzaq, Abdullah Mohammed, and Ashwaq Abdul Latif Salman (2018). Modern Communication Technologies and Their Role in Agricultural Extension Work in the Middle Euphrates Governorates of Iraq, Iraqi Journal of Agricultural Sciences, Volume 49, Number 5, Page 827.
- [7] Al-Shibawi, Ahmed Raheem Hamza, and Al-Wasati, Raja'a Tu'ma (2022). "An Economic Study to Measure the Impact of Major Variables on Rural Poverty in Iraq from 1990-2019," Journal of Agricultural Sciences, Vol. 53, No. 6, p. 1447.
- [8] Alsahli, ahmed thoini hadom (2016). The Role of Information and Communication Technologies for Extension Workers in Agricultural Extension and Their Relationship to Modern Agriculture Systems in Kirkuk Governorate, Iraqi Journal of Agricultural Sciences, Volume 47, Number 1, Page 35.



- [9] Mohammad Omar Al-Toubi (2001). Rural Arab Women, Technical Radiance Library, First Edition.
- [10] Al-Hout, Mohamed Sabri - Nahid Abdel Shaheen (2007). Education and Development, Faculty of Arts, Cairo University.
- [11] Aabdeen, Mahmoud Abdel Aziz (2000). Sustainable Development from the Human Perspective: A Study of the Environmental Impact Assessment, Faculty of Education, Zagazig University.
- [12] Jihad, Afeen Safaa, and Bayan Abdul Jabbar (2017). "The Reality of Some Specialized Tasks for Human Resources Management in the Agricultural Extension System in the Central Provinces of Iraq," Iraqi Journal of Agricultural Sciences, Vol. 48, No. 1, p. 311.
- [13] Al-Mashhadani, Muthal Abed (2015). Awareness of Rural Women's Role in Agricultural Projects in Plastic Greenhouses within the Framework of Agricultural Extension and Training in Babylon Governorate, Agricultural Sciences Journal, Volume 46, Number 5, Pages 870-875.
- [14] Raheem, Ibrahim; Eid, Saad; Barkat, Mohammad Mahmoud (2005). Rural Development, Ain Shams University, Faculty of Agriculture.
- [15] Tahe, Samir Mohyi and Ashwaq Abdul Latif Salman (2020). Appropriate Communication Methods for the Smart Agriculture Systems in Iraqi Agricultural Systems, Iraqi Journal of Agricultural Sciences, Volume 51, Special Issue.
- [16] Human Development Report (2003). Assessing Human Development: The Role of Women in Development, UNDP Regional Office for Arab States, National Women's Council Library.
- [17] Saleh, Mohammed Faleh (2004). *Human Resource Management: Presentation and Analysis*, 1st edition, Dar Hamed for Publishing and Distribution, Amman, p. 45.
- [18] Melhem, Sami Mohammed (2000). *Research Methods in Education and Psychology*, 1st edition, Dar Al-Maysarah for Publishing and Distribution.
- [19] Republic of Iraq - Council of Ministers - Central Statistical Organization - Baghdad Governorate (2011).
- [20] Khamaqani, Mubarka (2017). "Methods and Tools for Data Collection," *Journal of Memory*, No. 9, pp. 42-50.
- [21] Al-Zuhairi, Haider Abdul Karim Muhsin (2017). *Methods of Educational Research*, 1st edition, De Bono Center for Teaching Thinking, Dubai, UAE.
- [22] Al-Dhamin, Mundher (2009). *Fundamentals of Scientific Research*, 1st edition, Dar Al-Maseerah for Publishing, Distribution, and Printing, Amman, Jordan.

Effect of Nano and Organic Fertilizers on the Vegetative Traits of Local Orange Seedlings Grafted on Two Different Rootstocks

Nebras Ghanem Mohammed Al-Ameri¹ and Raad Taha Mohammed Ali Blackett²

^{1,2}Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University, Najaf, Iraq.

¹E-mail: nebrasalamree@gmail.com

²E-mail: Raadbalaket87@gmail.com

Abstract. The experiment was conducted in lathhouse of the Plant Production Department at Al-Musaib Technical College during the fall and spring growing seasons of 2023 and 2024. To study the effect of nano-fertilizers and seaweed extract on the growth of local orange seedlings grafted on two different rootstocks (sour orange and Volkameriana) at the age of (one year) which were obtained from the certified citrus seedling production nursery belonging to the Iraqi Ministry of Agriculture / General Directorate of Horticulture and Forestry in Al-Hindiyah District / Karbala province, homogeneous in size. The experiment included three factors, the first factor is the grafted varieties, the variety grafted on sour orange and the variety grafted on Volkameriana and symbolized by the symbol (A1 and A2), while the second factor is the use of seaweed extract, which was added to the soil after planting at three levels (0, 10, 20 g. seedling⁻¹) and symbolized by the symbol (B1, B2, B3). The third factor is spraying with nano-fertilizer (NPK). And at three levels (0, 1.5, 2 g/L) and symbolized by the symbol (C3, C2, C1). A factorial experiment was conducted according to the Randomized Complete Blocks Design (RCBD), with three replicates. The results were analyzed using the Genstat program and compared with the least significant difference (L.S.D) test at a probability level of 0.05. The results were summarized as follows: The results showed that the rootstock had a significant effect on the vegetative growth and chemical characteristics of orange seedlings, as the rootstock of sour orange (A1) was significantly excelled to the rootstock of volkameriana (A2) and gave the highest average for traits of seedling height 60.05 cm, number of leaves 94.80 leaves.plant⁻¹, leaf area 6.22 cm², stem diameter 4.80 mm, chlorophyll pigment intensity 103.46 SPAD, fresh and dry weight of leaves (24.52 and 10.24 g) respectively. The treatment of adding seaweed extract at a concentration of 20 gm.pot⁻¹ (B3) was significantly excelled and gave the highest rate of seedling height 58.19 cm, number of leaves 100.90 leaves.plant⁻¹, leaf area 8.79 cm², stem diameter 4.84 mm, chlorophyll pigment intensity 117.58 SPAD, fresh and dry weight of leaves (26.10 and 10.59 gm) respectively. The treatment of spraying nano NPK at a concentration of 2 mg.L⁻¹ (C3) recorded the highest rate of seedling height 59.47 cm, number of leaves 93.93 leaves.plant⁻¹, leaf area 7.76 cm², stem diameter 4.95 mm, chlorophyll pigment intensity 112.94 SPAD, fresh and dry weight of leaves (25.20 and 10.90 gm) respectively. The triple interaction treatment consisting of (stem sour orange root and seaweed extract at a concentration of 20 gm. and spraying with nano fertilizer NPK at a concentration of 2 gm. L⁻¹) was significantly excelled to the rest of the interaction treatments and recorded the highest values for the traits of seedling height

65.73 cm, number of leaves 130.27 leaves. plant⁻¹, leaf area 9.13 cm², stem diameter 5.48 mm, chlorophyll pigment intensity 160.93 SPAD, fresh and dry weight of leaves (30.88 and 11.95 gm) respectively.

Keywords. Organic fertilizers, Spraying, Rootstock.

1. Introduction

Citrus fruits are evergreen fruit plants that belong to the Rutaceae family and include many genera, the most important of which is the Citrus genus, which includes four groups: the lemon group, the acid group, the orange group, and the hybrid group [1]. The orange tree is an evergreen tree, but its leaves are renewed, producing annually a number of leaves exceeding those that fell in the same season. Its flowers are white in color and have a pleasant smell. Citrus fruits are of a special type, berries (Hesperidium) [2]. Orange fruits are rich in a group of vitamins, including vitamins (A and B) and vitamin C. And a group of acids, including citric acid. Orange fruits have many benefits, including stimulating blood circulation, increasing the body's immunity, and resisting colds. Orange fruits contain polyphenols that protect the body from exposure to viral infections and also protect the digestive system from severe constipation. It also plays a role in protecting the eye from atrophy Al-Buqai in addition to many other health benefits [3]. Commercial varieties of the genus Citrus are propagated on appropriate rootstocks. Given that the tree depends in its basic structure on two main parts, which are the graft represented by the green group and the rootstock such as the seedling orange rootstock and the volcameriana which represents the root group and is part of the stem and the physiological relationship that links them, it is known that the rootstock has an effect on several characteristics of the grafted variety, including the shape, size and nature of tree growth, the beginning of pregnancy and production, and tolerance to environmental factors such as soil and climate, as well as widespread diseases. Therefore, it is considered one of the most important requirements for its use as a rootstock. The slow growth of different citrus rootstocks and the long period of time for them to reach the stage suitable for grafting are among the main problems that lead to an increase in their production costs, which calls for the use of means to accelerate the seedling's arrival to the appropriate size for grafting, including the use of nano and biofertilizers [4]. The field of nanotechnology As a result of various factors like washing, adsorption, and sedimentation, the majority of added fertiliser elements degrade during fertilisation. To combat this, new applications involving nanotechnology and nanomaterials have led to the development of nanofertilizers or nanonutrient encapsulations. These products have the ability to regulate plant growth and enhance their targeted activity, as well as speed up seedling growth and release nutrients when needed [5]. Foliar (non-root) nutrition is one of the best fertilization technologies for nutrients in the nano form, as it helps in high utilization of nutrients and reducing environmental pollution [6]. As for seaweed extract, it is considered an essential element in achieving healthy and sustainable growth for orange trees, as it provides the necessary nutrients such as nitrogen, phosphorus and potassium in a sustainable manner. These fertilizers contribute to improving soil properties and enhancing soil structure to facilitate the absorption of nutrients and water by the roots, as the modern scientific trend has begun to use natural compounds and move away from chemical compounds [7]. In view of the above, the research aims to:

- Determine the optimal level of nano-fertilizer spraying to obtain strong-growing seedlings.
- Determine the optimal rootstock to increase the vegetative growth of orange seedlings

- Determine the optimal concentration of seaweed extract in influencing the growth of orange seedlings grafted on two different rootstocks
- Know the joint effects and the best interaction between the levels of the study factors in order to obtain vegetative and root growth and obtain seedlings with a strong structure.

2. Materials and Methods

The experiment was conducted in the wooden shade of the Plant Production Department at the Technical College, Al-Musayyab during autumn and spring growing seasons of 2023 and 2024. To study the effect of nano-fertilizers and seaweed extract on the growth of local orange seedlings grafted on two different rootstocks (sour orange and Volkameriana) at the age of (one year) which were obtained from the certified citrus seedling production nursery belonging to the Iraqi Ministry of Agriculture / General Directorate of Horticulture and Forestry in Al-Hindiyah District / Karbala Governorate. At the age of one year, orange seedlings from the local area were chosen for grafting onto two distinct rootstocks: sour orange and sour lemon (Volkameriana). The seedlings were planted in black plastic bags and then transferred to an 8-kilogram plastic pot filled with a mixture of river sand and peat moss in a ratio of 1:3. Under a green agricultural net (Saran) shaded area, the seedlings were treated in the same way. In the morning, they were sprayed with water until they were completely wet. A day before spraying, the field was irrigated to improve the plants' ability to absorb the sprayed material. Humidity plays a role in the process of swelling.

Table 1. Some physical and chemical properties of the soil used in the experiment.

Traits	Clay	Silt	Sand	Soil texture	pH	Electrical conductivity Ece	Organic matter O.M	Total nitrogen	Potassium
Values	24.8	24.6	50.6	Sandy loam soil	7.5	2.32 DS.m ⁻¹	% 3.3	%3.18	44.07 mg kg ⁻¹ soil

2.1. Study Factors

The factors were as follows:

2.1.1. The First Factor (A)

Where the rootstock includes two

A1 = the rootstock of the sour orange

A2 = the rootstock of the Volkameriana

2.1.2. The Second Factor (B)

Using seaweed extract (soil helpmate produced by the American company Eco safe consisting of (NPK at a rate of 30%, seaweed 10%, trace elements 1%, organic materials 45%, moisture 10%) was added to the soil after planting and at three levels:

- 1 - Control treatment (0) without addition symbolized by the symbol B1.
- 2 - Adding (10) grams/seedling symbolized by the symbol B2.
- 3 - Adding (20) grams/seedling symbolized by the symbol B3.

2.1.3. The Third Factor (C)

Spraying with nano fertilizer (NPK) produced by the Iranian company Khazra, the proportions of nano elements (NPK) in it (20-20-20 %) respectively and at three levels (0, 1.5, 2 g. L⁻¹) (C3, C2,, C1) and the date of the first spray was 10/7/2023.

- Control treatment (0) without adding water spray only, symbolized by the symbol C1
- Spraying with a concentration of (1.5 g. L⁻¹) and symbolized by the symbol C2
- Spraying with a concentration of (2 g. L⁻¹) and symbolized by the symbol C2

2.2. Experimental Design and Analysis

A factorial experiment (2*3*3) was implemented according to the Randomized Complete Blocks Design (RCBD), on (270) seedlings with three replicates, as each replicate contains (18) treatments with (5) seedlings for each experimental unit, and the results were analyzed using the Genstat program and compared with the least significant difference (L.S.D) test at a probability level of 0.05 and according to the method mentioned In [9].

2.3. Periods of Spraying and Ground Fertilization

Orange seedlings grafted on sour orange and Volkameriana were sprayed and ground fertilized at a rate of 3 sprays every (15) days between one spray and another for the fall season. In November 10/8/2023, 10/23/2023, 11/7/2023 and the spring season on (3/1/2024, 3/16/2024, 4/1/2024).

Table 2. Components of seaweed extract.

Nutritional elements	% Percentage
N	%4
P ₂ O ₅	%4
k ₂ O	% 4
Mg	32ppm
Fe	30ppm
Mn	31 ppm
Zn	17.5ppm
Cu	12.6ppm

2.4. Studied Traits

The samples were taken for the purpose of measuring the studied characteristics on 4/27/2024.

2.4.1. Seedling Height Rate (cm)

The seedling height rate was measured using a metric measuring tape starting from the location of the stem's contact with the soil surface until the highest growing peak on the main stem of the seedling, and the seedling height rate was taken.

2.4.2. Main Stem Diameter Rate (mm)

The diameter of the main stem of the seedlings was measured using a Vernier caliper at a height of (5 cm) above the soil surface and according to the average for each experimental unit.

2.4.3. Total Number of Leaves Seedling⁻¹

The number of leaves was calculated for each seedling and according to the average for each experimental unit.

2.4.4. Average Leaf Area cm² Seedling⁻¹

At the conclusion of the experiment, we determined the seedling's leaf area by adding the area of each leaf to the total number of leaves. First, we used the computer program Image J to measure three full-width leaves from various areas of each experimental unit and then we averaged their areas to get the leaf area. To get the leaf area, we ran the procedure three times and averaged the results. The following equation was used to determine the area of one seedling's leaves after

determining the average leaf area and the number of leaves on each seedling at the end of the experiment:

$$\text{Leaf area of seedlings} = \text{Number of leaves per seedling} \times \text{Average area of one leaf (cm)}$$

2.4.5. Relative Content of Chlorophyll in Leaves (SPAD)

The intensity of chlorophyll pigment in the leaves was estimated using a (Chlorophyll Meter) device of type (SPAD 502: Soil Plant Analysis Device) equipped by the Japanese company Minolta Co. LTD. The reading was taken for three leaves from different places for each seedling and the average was calculated [10].

2.4.6. Leaf Fresh Weight (g)

The fresh weight of the leaves was calculated after the leaves were separated from the stem and root system, then they were washed and cleaned from any remaining dust attached to them, then they were weighed using a sensitive electric balance to fix the weight.

2.4.7. Leaf Dry Weight (g)

The leaves for which the fresh weight was calculated were placed in perforated paper bags and dried in an electric oven ((oven for the purpose of getting rid of moisture at a temperature of 70 degrees Celsius for 48 hours until the weight stabilized, and the dry weight was taken using a sensitive electric balance [8].

3. Results and Discussion

3.1. Seedlings Height (cm)

The rootstock significantly affected the plant's height (in cm), according to the data in Table (3). The sour orange seedling rootstock (A1) produced the tallest seedlings, measuring 60.05 cm, while the volcameriana rootstock (A2) produced the shortest, measuring 54.29 cm. Also, the results demonstrated that seaweed significantly affected seedling height; at 20 g.pot⁻¹ (B3), the seaweed concentration significantly outperformed all others, producing a seedling height rate of 58.19 cm; at 10 g.pot⁻¹ (B2), the seedling height rate was 57.26 cm; at 55.56 cm, the plant height rate was recorded in the treatment without addition (B1); and at 2 mg, the nano-fertilizer spraying treatment was the lowest. In terms of seedling height rate, L⁻¹ (3C) was the most successful, reaching 59.47 cm, while the 1.5 mg spraying treatment came in second. In the 2C treatment, the seedling height rate was 56.77 cm, whereas the lowest plant height rate was 54.85 cm in the 1C treatment without spraying. The results also demonstrated that the seedlings' height was significantly affected by bi-interaction. The most effective treatments involved combining seedling sour orange with seaweed at a concentration of 20 g.pot⁻¹. These treatments achieved the highest seedling height rate of 61.98 cm, while the rootstock of the volcameriana treatment without seaweed resulted in the lowest rate of 52.99 cm. While the interaction treatment between the rootstock of the sour orange (A1) and spraying nano-fertilizer at a concentration of 2 mg. L⁻¹ (3C) gave the highest rate of 61.98 cm, while the treatment of the rootstock of the volcameriana (A2) and without spraying nano-fertilizer (1C) gave 51.43 cm. The interaction treatment between adding seaweed at a concentration of 20 g.pot⁻¹ (B3) and spraying nano-fertilizer at a concentration of 2 mg. L⁻¹ (3C) also recorded the highest rate of height of The plant reached 62.27 cm, while the treatment without adding seaweed (B1) and without spraying nano fertilizer (1C) recorded the lowest rate of plant height of 54.78 cm. The results of Table (3) also showed that the triple interaction between the experimental factors had a significant effect on the height

of the seedlings, as the interaction factor between the rootstock of the orange seed and seaweed at a concentration of 20 g. pot⁻¹ and spraying nano fertilizer at a concentration of 1.5 mg. L⁻¹ was significantly excelled and gave an average of 65.73 cm, while the interaction treatment between the rootstock of the volkameriana and adding seaweed (B1) and without spraying nano fertilizer (1C) recorded the lowest rate of 48.63 cm.

Table 3. Effect of rootstock, seaweed, spraying nano fertilizer and their interaction on the height of orange seedlings (cm).

(A) Rootstock	Seaweed (B) extract	Nano Fertilizer Spraying (C)			A × B
		Without spraying (C1)	1.0 mg.L ⁻¹ (C2)	2mg.L ⁻¹ (C3)	
sour orange (A1)	without				
	adding(B1)	56.20	59.27	60.93	58.80
	10 g.pot ⁻¹ (B2)	57.27	59.67	61.13	59.36
	20g.pot-1(B3)	59.60	60.60	65.73	61.98
Volkameriana (A2)	without	48.63	52.40	57.93	52.99
	adding(B1)				
	10 g.pot ⁻¹ (B2)	52.27	53.33	53.53	53.04
	20g.pot ⁻¹ (B3)	53.40	55.33	58.80	55.84
L.S.D 0.05			6.91		3.32
Interaction between rootstock and nanofertilizer spraying					(A)rootstock
(A1) sour orange		57.58	60.58	61.98	60.05
(A2) Volkameriana		51.43	55.22	56.22	54.29
L.S.D 0.05			3.32		1.99
Interaction between seaweed extract and nanofertilizer spraying					seaweed (B)extract
without adding(B1)		54.78	54.83	57.07	55.56
10 g.pot ⁻¹ (B2)		56.47	56.7	58.6	57.26
20g.pot ⁻¹ (B3)		55.77	56.53	62.27	58.19
L.S.D 0.05			4.31		2.12
Nano Fertilizer Spraying (C)		54.85	56.77	59.47	
L.S.D 0.05			2.12		

3.2. Number of Leaves (leaf.plant⁻¹)

The results in Table (4) showed that the rootstock had a significant effect on the number of leaves (leaf.plant⁻¹), as the seedling rootstock of sour orange (A1) was significantly excelled and gave the highest number of leaves, reaching 94.80 leaves.plant⁻¹, while the rootstock of volkameriana (A2) gave a number of leaves, reaching 84.31 leaves.plant⁻¹. The results also demonstrated that the amount of seaweed had a notable impact on the total number of leaves; at a concentration of 20 g.pot⁻¹ (B3), the leaves averaged out to 100.90, which was the highest value. After that, I measured the amount of leaves (86.62 total) and applied a concentration of 10 g.pot-1 (B2).plant⁻¹, but the addition-free treatment (B1) produced the fewest leaves on average, amounting to 81.39 leaves. The treatment with a concentration of 2 mg.L⁻¹ (3C) of nano fertiliser spray outperformed all others and produced the maximum rate of leaf number, reaching 98.93 leaves per plant. The average number of leaves reached 86.61 after plant⁻¹ was treated with a spray solution containing 1.5 mg.L⁻¹ (2C).plant-1, with the lowest rate of leaf numbering at 83.19 leaves in the treatment that did not involve spraying (1C).plant one. Additionally, the results demonstrated that the quantity of leaves was significantly affected by the bilateral interaction, as seen by the interaction treatment using 20 g of seaweed and seed oranges. At 107.40 leaves per cent, pot⁻¹ was the most

productive treatment without seaweed and the treatment with Volcameriana rootstock had the lowest rate of leaf number, reaching 74.60 leaves per plant⁻¹ cm.plant one. As the 2 mg nano-fertilizer was sprayed onto the sour orange rootstock (A1), an interaction treatment was carried out. The rate of 103.62 leaves was highest for L⁻¹ (3C).Plant 1 received the treatment, but Volcameriana rootstock received treatment A2 and treatment 1C did not include spraying the nano-fertilizer. At its lowest point, there were 77.45 leaves on average.plant one. A 20 g.pot⁻¹ (B3) seaweed addition and a 2 mg nano-fertilizer spray constitute the interaction treatment. With an average of 117.83 leaves, L⁻¹ (3C) produced the most foliage. The treatment that did not include seaweed (B1) and did not spray nano-fertilizer (1C) had the fewest leaves on average, reaching 78.98 leaves, compared to the other treatments.plant one. The results of Table (4) also showed that the triple interaction between the experimental factors had a significant effect on the number of leaves. The interaction factor between the rootstock of the sour orange seed and seaweed at a concentration of 20 g.pot⁻¹ and spraying nano-fertilizer at a concentration of 2 mg. L⁻¹ was significantly excelled, giving an average of 130.27 leaves.plant⁻¹, while the interaction treatment between the rootstock of the volcameriana was recorded Adding seaweed (B1) without spraying nano-fertilizer (1C) gave the lowest rate of leaf number, which reached 70.00 leaves.plant⁻¹.

Table 4. Effect of rootstock, seaweed, spraying nano-fertilizer and their interaction on the number of leaves (leaf/plant⁻¹).

(A) Rootstock	Seaweed (B) extract	(Nano Fertilizer Spraying (C)			A × B
		Without spraying (C1)	Spray 1.5 (C2) mg.L ⁻¹	Spray 2 mg.L ⁻¹ (C3)	
sour orange (A1)	without adding(B1)	70.67	84.93	87.27	80.96
	(B2) g.pot ⁻¹ 10	72.35	93.73	93.33	86.47
	(B3) g.pot ⁻¹ 20	89.33	102.60	130.27	107.40
Volkameriana (A2)	without adding(B1)	70.00	68.20	85.60	74.60
	10gm / pot (B2)	85.67	82.13	99.93	89.24
	20gm / pot(B3)	105.40	85.27	105.33	98.67
	L.S.D 0.05		6.32		3.54
Interaction between rootstock and nanofertilizer spraying					(A)rootstock
(A1) sour orange		87.02	93.76	103.62	94.80
(A2) Volkameriana		77.45	78.53	96.96	84.31
	L.S.D 0.05		3.54		1.83
Interaction between seaweed extract and nanofertilizer spraying					seaweed (B)extract
without adding(B1)		78.98	83.53	81.67	81.39
10g.pot ⁻¹ (B2)		88.00	85.40	86.47	86.62
20g.pot ⁻¹ (B3)		90.25	94.63	117.83	100.90
	L.S.D 0.05		4.14		2.72
Nano Fertilizer Spraying (C)		83.19	86.61	98.93	
	L.S.D 0.05		2.72		

3.3. Leaf Area (cm²)

Leaf area (cm²) was significantly affected by the rootstock, according to Table (5). A2 of the Volkameriana rootstock produced leaves with a surface area of 5.91 cm², whereas A1, the seed orange rootstock, produced the largest leaf area at 6.22 cm². Seaweed had a notable impact on leaf area, according to the results. The concentration of 20 g.pot⁻¹ (B3) produced the highest average leaf area of 8.79 cm², followed by 10 g.pot⁻¹ (B2), which produced a leaf area of 7.34 cm². The treatment without addition (B1) produced the lowest average leaf area of 6.20 cm², and the nano-fertilizer spraying treatment with a concentration of 2 mg yielded the lowest average leaf area. After the spraying treatment with a concentration of 1.5 mg, L⁻¹ (3C) achieved the maximum average leaf area of 7.76 cm². Compared to the treatment without spraying (1C), which yielded the lowest average leaf area of 5.16 cm², L⁻¹ (2C) resulted in an average leaf area of 5.97 cm². Another important finding was the substantial impact of bi-interaction on leaf area, as it At a dosage of 20 g.pot⁻¹, the interaction factor between seeded sour orange and seaweed achieved an outstanding rate of 7.76 cm², in contrast to the 4.67 cm² rate obtained by the sour orange treatment and the control group without seaweed. However, a 2 mg concentration of spray-on nano-fertilizer was used in an interaction treatment with rooted sour orange (A1). The root-sour orange (A1) and non-spraying nano-fertilizer (1C) treatments yielded 5.10 cm², whereas L⁻¹ (3C) achieved the maximum rate of 7.30 cm². A 20 g.pot⁻¹ (B3) seaweed addition and a 2 mg nano-fertilizer spray constitute the interaction treatment. L⁻¹ (3C) had the largest rate of leaf area at 10.69 cm², whereas treatments B1 and 1C, which did not include seaweed, had the smallest rates at 4.59 cm². The interaction coefficient between the rootstock of the orange seed and seaweed at a concentration of 20 g.pot⁻¹ and spraying nano fertiliser at a concentration of 2 mg had a significant effect on leaf area, as shown in Table (5) along with the results of the other two experimental factors. The interaction treatment including the orange rootstock A1 and the absence of seaweed (B1) and nanofertilizer spray (1C) had the lowest rate (4.18 cm²), whereas L⁻¹ substantially excelled with a rate of 9.13 cm².

Table 5. Effect of rootstock, seaweed, spraying nano fertilizer and their interaction on leaf area (cm²).

(A) Rootstock	Seaweed (B) extract	Nano Fertilizer Spraying (C)			A × B
		Without spraying (1C)	Spray 1.5 (C2) mg.L ⁻¹	Spray 2 mg.L ⁻¹ (C3)	
sour orange (A1)	without adding(B1)	4.18	4.51	5.33	4.67
	10g.pot ⁻¹ (B2)	4.50	5.05	8.93	6.16
	20g.pot ⁻¹ (B3)	6.78	7.36	9.13	7.76
Volkameriana (A2)	without adding (B1)	4.27	4.85	5.32	4.81
	gm / pot 10 (B2)	4.30	5.03	6.80	5.38
	20gm / pot(B3)	4.69	6.04	7.22	5.98
L.S.D 0.05		1.20			0.57
Interaction between rootstock and nanofertilizer spraying					(A)rootstock
sour orange A1		5.10	6.25	7.30	6.22
A2 Volkameriana		5.35	5.93	6.45	5.91
L.S.D 0.05		0.57			0.18
Interaction between seaweed extract and nanofertilizer spraying					seaweed (B)extract
without adding (B1)		4.59	5.40	8.60	6.20

(A) Rootstock	Seaweed (B) extract	Nano Fertilizer Spraying (C)			A × B
		Without spraying (1C)	Spray 1.5 (C2) mg.L ⁻¹	Spray 2 mg.L ⁻¹ (C3)	
10g.pot ⁻¹ (B2)		5.97	6.52	9.54	7.34
20g.pot ⁻¹ (B3)		6.99	8.70	10.69	8.79
L.S.D 0.05			0.65		0.37
Nano Fertilizer Spraying (C)		5.16	5.97	7.76	
L.S.D 0.05			0.37		

3.4. Stem Diameter (mm)

The results in Table (6) showed that the rootstock had a significant effect on the stem diameter (mm), as the seeded sour orange rootstock (A1) was significantly excelled and gave the highest stem diameter of 4.80 mm, while the volcamerian rootstock (A2) gave a stem diameter of 4.58 mm. The results also demonstrated that seaweed had a notable impact on stem diameter. At a concentration of 20 g.pot⁻¹ (B3), the stem diameter was 4.84 mm, which was significantly higher than the next highest concentration of 10 g.pot⁻¹ (B2), which produced a stem diameter of 4.76 mm. The treatment without addition (B1) resulted in the lowest stem diameter at 4.46 mm, and the treatment using 2 mg of nano-fertilizer was the least effective. With a stem diameter of 4.95 mm, L⁻¹ (3C) outperformed all other treatments, including the 1.5 mg spray. In the 2C group, the average stem diameter was 4.64 mm, but in the 1C group, the lowest average stem diameter was 4.49 mm, due to the absence of spraying. In addition, the results demonstrated that bi-interaction significantly impacted the stem diameter; for example, the rootstock of the volcameriana treatment without seaweed had the smallest average stem diameter of 4.51 mm, whereas the seeded sour orange and seaweed at a concentration of 20 g.pot⁻¹ had the highest average of 5.17 mm. While the interaction treatment between the rootstock of the sour orange (A1) and spraying nano-fertilizer at a concentration of 2 mg.L⁻¹ (3C) recorded the highest average of 4.94 mm, while the treatment of the rootstock of the volcameriana (A2) and without spraying nano-fertilizer (1C) gave.

The lowest stem diameter rate was 4.44 mm. The interaction treatment between adding seaweed at a concentration of 20 g.pot⁻¹ (B3) and spraying nano fertilizer at a concentration of 2 mg. L⁻¹ (3C) gave the highest stem diameter rate of 4.95 mm, while the treatment without adding seaweed (B1) and without spraying nano fertilizer (1C) recorded the lowest stem diameter rate of 4.24 mm. The results of Table (6) also showed that the triple interaction between the experimental factors had a significant effect on stem diameter. The interaction factor between the rootstock of the sour orange seed and seaweed at a concentration of 20 g.pot⁻¹ and spraying nano fertilizer at a concentration of 2 mg. L⁻¹ was significantly excelled and gave a rate of 5.48 mm, while the interaction treatment between the rootstock of the volcameriana and adding seaweed (B1) and without spraying nano fertilizer (1C) gave the lowest rate. The stem diameter was 4.23 mm.

Table 6. Effect of rootstock, seaweed, nano-fertilizer spraying and their interaction on stem diameter (mm)

(A) rootstock	Seaweed (B) extract	Nano Fertilizer Spraying (C)			A x B
		Without spraying (1C)	Spray 1.5 (C2) mg.L ⁻¹	Spray 2 mg.L ⁻¹ (C3)	
sour orange (A1)	without adding(B1)	4.26	4.57	4.78	4.54
	10g.pot ⁻¹) B2(	4.25	4.67	4.96	4.63
	20g.pot ⁻¹) B3(	5.01	5.02	5.48	5.17

Volkameriana A2	without adding(B1)	4.23	4.39	4.90	4.51
	10gm / pot (B2)	4.35	4.44	4.92	4.57
	20gm / pot(B3)	4.81	4.49	5.01	4.77
L.S.D 0.05		1.49			0.86
Interaction between rootstock and nanofertilizer spraying					(A)rootstock
(A1) sour orange	4.61	4.86	4.94	4.80	
(A2) Volkameriana	4.44	4.47	4.82	4.58	
L.S.D 0.05		0.86			0.49
Interaction between seaweed extract and nanofertilizer spraying					seaweed (B)extract
without adding(B1)	4.24	4.34	4.80	4.46	
10g.pot ⁻¹ (B2)	4.53	4.87	4.89	4.76	
20g.pot ⁻¹ (B3)	4.70	4.88	4.95	4.84	
L.S.D 0.05		1.05			0.61
Nano Fertilizer Spraying (C)	4.49	4.64	4.95		
L.S.D 0.05		0.61			

3.5. Chlorophyll Intensity in Leaves (spad)

The results in Table (7) showed that the rootstock had a significant effect on the chlorophyll intensity in leaves (spad), as the seed orange rootstock (A1) was significantly excelled and gave the highest chlorophyll intensity in leaves, reaching 103.46 spad, while the volcamerian rootstock (A2) gave the chlorophyll intensity in leaves, reaching 102.42 spad.

Also, the results demonstrated that seaweed significantly affected the intensity of chlorophyll in the leaves. At 20 g.pot⁻¹ (B3), the concentration was the highest, with a rate of 117.58 spad of chlorophyll intensity in the leaves. At 10 g.pot⁻¹ (B2), the rate was recorded at 102.00 spad, and at no addition (B1), the rate was recorded at 0 spad. After being treated with a 2 mg dosage of nano-fertilizer spray, the leaves' chlorophyll intensity dropped to 97.10 spad. Following the 1.5 mg spray treatment, which was excelled by L⁻¹ (3C), the leaves' chlorophyll intensity increased at the fastest pace, reaching 112.94 spad. In the presence of L⁻¹ (2C), the leaves of 98.80 spad showed an increase in chlorophyll intensity, whereas the leaves of 98.43 spad showed the lowest increase in chlorophyll intensity in the treatment without spraying (1C). Furthermore, the results demonstrated that the intensity of chlorophyll in the leaves was significantly affected by the bilateral interaction. The interaction treatment involving seeded sour orange and seaweed at a concentration of 20 gm. pot⁻¹ achieved the highest rate of 133.03 spad, while the treatment involving only the sour orange rootstock and no seaweed resulted in the lowest rate of 86.72 spad. The sour orange rootstock (A1) was treated with an interaction treatment that involved spraying a 2 mg dose of nano-fertilizer. The rootstock of the sour orange ((A1)) and without spraying the nano-fertilizer (1C) were treated, and the maximum rate of 114.46 spad was obtained with L⁻¹ (3C). At its lowest point, the leaves' chlorophyll intensity was 93.19 spad. The interaction treatment between adding seaweed at a concentration of 20 g.pot⁻¹ (B3) and spraying nano fertilizer at a concentration of 2 mg. L⁻¹ (3C) gave the highest rate of chlorophyll intensity in the leaves, reaching 128.41 spad, while the treatment without adding seaweed (B1) and without spraying nano fertilizer (1C) recorded the lowest rate of chlorophyll intensity in the leaves, reaching 89.68 spad. The results of Table (7) also showed that the triple interaction between the experimental factors had a significant effect on the intensity of chlorophyll in the leaves. The

interaction factor between the rootstock of the orange seed and seaweed at a concentration of 20 g.pot⁻¹ and spraying nano fertilizer at a concentration of 2 mg. L⁻¹ was significantly excelled, giving a rate of 160.93 spad, while the interaction treatment between the origin of the volcameriana and the addition of seaweed (B1) and without spraying nano-fertilizer (1C) and gave the lowest rate of chlorophyll intensity in the leaves, reaching 84.74 spad.

Table 7. The effect of rootstock, seaweed, spraying nano-fertilizer and the interaction between them on the intensity of chlorophyll in the leaves (spad) of the orange plant.

(A) rootstock	seaweed (B) extract	Nano Fertilizer Spraying (C)\			A x B
		Without spraying (1C)	Spray 1.5 (C2) mg.L ⁻¹	Spray 2 mg.L ⁻¹ (C3)	
sour orange (A1)	without adding(B1)	96.17	56.17	107.81	86.72
	10g.pot ⁻¹ (B2)	108.83	82.49	113.64	101.65
	20g.pot ⁻¹ (B3)	126.27	111.90	160.93	133.03
Volkameriana (A2)	without adding(B1)	84.74	95.89	87.19	89.27
	10gm / pot (B2)	91.78	101.65	104.45	99.29
	20gm / pot(B3)	93.19	111.92	118.87	107.99
L.S.D 0.05		56.48			32.61
Interaction between rootstock and nanofertilizer spraying					rootstock)A(
(A1) sour orange		93.19	102.72	114.46	103.46
(A2) Volkameriana		99.86	100.65	106.75	102.42
L.S.D 0.05		32.61			18.82
Interaction between seaweed extract and nanofertilizer spraying					seaweed (B)extract
without adding(B1)		89.68	99.78	101.84	97.10
10g.pot ⁻¹ (B2)		96.28	108.68	109.05	102.00
20g.pot ⁻¹ (B3)		110.38	113.96	128.41	117.58
L.S.D 0.05		39.94			23.06
Nano Fertilizer Spraying (C)		98.34	98.80	112.94	
L.S.D 0.05		23.06			

3.6. Fresh Weight of Leaves (g)

The results in Table (8) showed that the rootstock had a significant effect on the green weight of leaves (g), as the seed orange rootstock (A1) was significantly excelled and gave the highest green weight of leaves, reaching 24.52 g, while the rootstock of Volkameriana (A2) gave the green weight of leaves, reaching 22.56 g. Additionally, the results demonstrated that seaweed had a notable impact on the green weight of leaves. Concentration 20 g.pot⁻¹ (B3) yielded the highest average green weight of leaves at 26.10 gm, followed by concentration 10 g.pot⁻¹ (B2) at 25.49 gm. The treatment without addition (B1) resulted in the lowest average green weight of leaves at 19.91 g, and the nano fertiliser spraying treatment at 2 mg was the least effective. The spraying treatment with a concentration of 1.5 mg was followed by L⁻¹ (3C), which excelled and recorded the greatest rate of green leaf weight at 25.20 g. In the 2C treatment, the green leaf weight rate was 22.82 g, whereas the lowest rate of green leaf weight was 19.91 g in the 1C treatment, which did not involve spraying. The results also demonstrated that the green leaf weight was significantly affected by the bilateral interaction. Specifically, the interaction factor between the

seeded sour orange rootstock and seaweed at a concentration of 20 g.pot⁻¹ yielded the highest rate of 28.46 g, while the treatment without seaweed yielded the lowest rate of 16.64 g. Nano fertiliser at a concentration of 2 mg was detected in the interaction treatment between spraying and the sour orange rootstock (A1). The treatment yielded the root of Volcameriana (A2) without spraying nano fertiliser (1C), but L⁻¹ (3C) produced the greatest rate at 27.85 g. At its lowest point, the leaf weight was 19.34 g. The combination of 20 g.pot⁻¹ seaweed (B3) and 2 mg. L⁻¹ nano fertiliser (3C) resulted in the greatest average green weight of leaves (29.86 g) of any treatment. At 18.25 g, the average green weight of the leaves was lowest in the treatment that did not include seaweed (B1) and did not spray nano fertiliser (1C). The results of Table (8) also showed that the triple interaction between the experimental factors had a significant effect on the green weight of leaves. The interaction factor between the rootstock of the sour orange seed and seaweed at a concentration of 20 g.pot⁻¹ and spraying nano fertilizer at a concentration of 2 mg. L⁻¹ was significantly excelled and gave an average of 30.88 g. While the interaction treatment between the rootstock of the volcameriana and adding Seaweed (B1) and without spraying nano fertilizer (1C) gave the lowest rate weight of 13.25 g.

Table 8. Effect of rootstock, seaweed, spraying nano fertilizer and their interaction on the fresh weight of leaves (g) of orange plant.

(A) rootstock	Seaweed (B) extract	Nano Fertilizer Spraying (C)			A x B
		Without spraying (1C)	Spray 1.5 (C2) mg.L ⁻¹	Spray 2 mg.L ⁻¹ (C3)	
sour orange (A1)	without adding(B1)	14.40	16.13	19.39	16.64
	10g.pot ⁻¹ (B2)	19.32	20.31	22.58	20.74
	20g.pot ⁻¹ (B3)	25.97	28.53	30.88	28.46
Volkameriana (A2)	without adding(B1)	13.25	17.75	19.76	16.92
	10gm / pot (B2)	19.87	22.30	23.96	22.04
	20gm / pot(B3)	23.20	25.37	28.55	25.71
L.S.D 0.05		2.96			1.71
Interaction between rootstock and nanofertilizer spraying					(A)rootstock
(A1) sour orange		21.02	24.70	27.85	24.52
(A2) Volkameriana		19.34	23.13	25.20	22.56
L.S.D 0.05		1.71			0.98
Interaction between seaweed extract and nanofertilizer spraying					seaweed extract)B(
without adding(B1)		18.25	19.94	21.54	19.91
10g.pot ⁻¹ (B2)		23.39	25.41	27.66	25.49
20g.pot ⁻¹ (B3)		20.97	27.48	29.86	26.10
L.S.D 0.05		2.09			1.21
Nano Fertilizer Spraying (C)		19.91	22.82	25.20	
L.S.D 0.05		1.21			

3.7. Dry Weight of Leaves (g)

Based on the data in Table (9), it was observed that the rootstock had a notable impact on the dry weight of leaves (g). Specifically, the seed orange rootstock (A1) produced the highest dry weight of leaves (10.24 g), while the volcamerian rootstock (A2) contributed the least amount (9.58 g).

The results also showed that seaweed had a significant effect on the dry weight of leaves, as the concentration of 20 g.pot⁻¹ (B3) was significantly excelled and gave the highest average dry weight of leaves, reaching 10.59 g, followed by the concentration of 10 g.pot⁻¹ (B2) and recorded the dry weight of leaves, reaching 10.09 g, while the treatment without addition (B1) gave the lowest average dry weight of leaves, reaching 9.44 g, while the treatment of spraying nano fertiliser with a concentration of 2 mg.L⁻¹ (3C) and recorded the highest rate of dry weight of leaves amounting to 10.90 g, followed by the spray treatment at a concentration of 1.5 mg.L⁻¹ (2C) and gave a dry weight rate of leaves amounting to 9.81 g, while the treatment without spraying (1C) recorded the lowest rate of d Additionally, the results demonstrated that bi-interaction significantly impacted the dry weight of leaves. The interaction coefficients involving seed orange and seaweed at a concentration of 20 g.pot⁻¹ performed the best, with a rate of 11.95 g. In contrast, the rootstock of Volcameriana treatment and the control group recorded the lowest rate of dry weight of leaves, at 8.97 g. However, a 2 mg concentration of spray-on nanofertilizer was used in an interaction treatment with orange seed stock (A1). Among the treatments that included the root of Volcameriana (A2) but did not include spraying nano fertiliser (1C), L⁻¹ (3C) had the highest rate at 11.19 gm. At their lightest, the leaves weighed only 9.14 g. A 20 g.pot⁻¹ (B3) seaweed addition and a 2 mg. spray of nano fertiliser constitute the interaction treatment. The leaves with the maximum dry weight, 11.01 g, were obtained from L⁻¹ (3C). The treatment that did not include seaweed (B1) or nano fertiliser spray (1C) had the lightest leaf dry weight, at 9.00 g. The dry weight of leaves was significantly affected by the triple interaction of the experimental parameters, as shown in Table (9). Incorporating 20 g.pot⁻¹ of seaweed into the sour orange seed stock and spraying 2 mg of nano fertiliser create an interaction factor. The rate of 11.95 g was provided by L⁻¹, which was greatly surpassed. With a dry weight rate of 8.55 g, the interaction treatment using the volcameriana rootstock and seaweed (B1) but without spraying Nano fertiliser (1C) had the lowest yield.

Table 9. Effect of rootstock, seaweed, nano fertilizer spraying and their interaction on the dry weight of leaves (g) of orange plants.

(A) rootstock	Seaweed (B) extract	Nano Fertilizer Spraying (C)			A x B
		Without spraying (1C)	Spray 1.5 (C2) mg.L ⁻¹	Spray 2 mg.L ⁻¹ (C3)	
sour orange (A1)	without adding(B1)	9.05	9.07	9.47	9.20
	10g.pot ⁻¹ (B2)	9.76	10.06	11.97	10.60
	20g.pot ⁻¹ (B3)	10.98	12.03	12.83	11.95
Volkameriana (A2)	without adding(B1)	8.55	8.62	9.74	8.97
	10gm / pot (B2)	8.9	9.29	10.92	9.70
	20gm / pot(B3)	9.1	9.51	11.96	10.19
L.S.D 0.05			1.20		0.42
Interaction between rootstock and nanofertilizer spraying					(A)rootstock
(A1) sour orange		9.61	9.93	11.19	10.24
(A2) Volkameriana		9.14	9.21	10.39	9.58
L.S.D 0.05			0.42		0.40
Interaction between seaweed extract and nanofertilizer spraying					seaweed extract)B(
without adding(B1)		9	9.43	9.9	9.44

10g.pot ⁻¹ (B2)	9.52	10.26	10.48	10.09
20g.pot ⁻¹ (B3)	10.25	10.52	11.01	10.59
L.S.D 0.05		0.97		0.71
Nano Fertilizer Spraying (C)	9.44	9.81	10.90	
L.S.D 0.05		0.71		

It is clear from Tables (3-9) that the rootstock has a significant effect on the vegetative characteristics and that the reason for the difference and variation between rootstocks in the vegetative growth characteristics and the variation between rootstocks, this variation is due to the genetic difference between rootstocks resulting from the variation of genetic factors responsible for the vegetative growth characteristics which were positively reflected in the physiological processes necessary for vegetative and root growth [11], and agrees with [12] that rootstocks differ with each other according to their genetic characteristics. The results of Tables (3-9) showed that nano-NPK fertilization had a significant effect on vegetative traits, as the treatment of spraying 2 mg.L⁻¹ (3C) significantly outperformed the other treatments and gave the highest rate of the studied traits, as the reason for the increase in seedling height Table (3) is due to the treatment with nano-NPK fertilizer because it has distinct properties resulting from the high surface area and small particles, which leads to an increase in the rate of absorption and spread and then their availability to the plant, accompanied by stimulating plant growth [13]. A longer main stem is a direct result of nano-NPK fertilization's ability to supply the plant with the essential nutrients it needs to carry out photosynthesis, respiration, and other metabolic processes that are crucial to the process of cell division and elongation [14]. Nitrogen enhances the activity of meristematic tips, which in turn increases cell division and elongation. This is due to an increase in auxin concentrations or the preparedness of the building blocks the plant needs, like amino acids and enzyme companions like NAD and NADP, which contain nitrogen. Phosphorus also plays a fundamental role in vegetative growth, and the major nano elements contribute to the plant's ability to carry out photosynthesis, which in turn increases the length of its bran Furthermore, potassium promotes an increase in the number of leaves (4) by modulating the osmotic potential of plant cells, which in turn forms leaf principles [15]. The major nutrients contained in nano fertilizer also play an important role in meeting the plant's need for nutrients to carry out vital processes, including photosynthesis, respiration and various metabolic processes due to the small and major elements it contains, which It helps in increasing meristematic activity and thus increasing the process of cell division and elongation [16] and thus increasing the leaf area and stem diameter, Table (5 and 6). These results are consistent with what was mentioned by [17] as spraying with major nutrients has a positive effect on the stem diameter of citrus fruits. The increase in the chlorophyll content of the leaves (Table 7) is due to the role of nitrogen and phosphorus in the process of manufacturing chlorophyll, as nitrogen enters into the construction of the porphyrins ring, which is essential in the formation of chlorophyll or improving vital activities and increasing enzymatic activity leading to stimulating chlorophyll, in addition to its entry into the composition of cytochromes, which are essential in the process of photosynthesis and the role of phosphorus in the formation of energy compounds ATP, which leads to an increase in its content in the leaves and the formation of large leaves with a high chlorophyll content [18]. The increase in chlorophyll may also be due to the role of nano-nitrogen in improving vital activities and increasing enzymatic activity leading to stimulating chlorophyll and thus raising the efficiency of the photosynthesis process The increase in the fresh and dry weight of leaves (Tables 8 and 9) may be due to the levels of added fertilizer used, which helped in forming new vegetative growth for the plant, in addition to absorbing large amounts of water, thus increasing the water content in the different plant parts. Or to the vital role of each of the

elements included in the nano fertilizer, which includes nitrogen, phosphorus and potassium, in forming chlorophyll and activating the enzymes specific to the photosynthesis process [15]. In light of these functions and the solidarity between the above elements, they led to improving the specifications of vegetative growth of seedlings, especially increasing the number of leaves, leaf area and the rate of manufactured carbohydrates, which ultimately led to an increase in the fresh and dry weight of the leaves. These results agreed with [19,20] on figs in terms of increasing the leaf area of seedlings treated with nano-NPK fertilizer.

The reason for the increase in vegetative growth characteristics (Tables (3-9)) may be due to the role of the components of the seaweed extract and the organic acids it contains, which directly affect various enzymatic reactions and various vital processes of the plant, such as photosynthesis, respiration, and protein synthesis, as their effect is similar to the effect of plant hormones, causing an increase in the growth rate of the plant and creating the best conditions for cell division and elongation [21] thus increasing the height of the plant (Table (3)). The increase in the number of leaves and leaf area (Tables (4 and 5)) can also be attributed to the role of the extract in providing plants with the necessary nutrients, including nitrogen, which is of great importance in increasing the formation of leaf primordia, in addition to the role of the element in increasing the tissue content of nucleic acids (DNA and RNA) and the synthesis of proteins that are important in stimulating cell division and increasing their size. The important role played by spraying the food extract in preparing and improving the absorption of major and minor nutrients, which contribute to improving the permeability of cell membranes, which results in various biochemical processes that enhance plant growth and increase its resistance or perhaps due to the content of the seaweed extract of nitrogen and zinc, which is important in building the amino acid Tryptophan, which is the basic substance in the manufacture of auxin IAA, which is important in increasing the expansion and elongation of cells and increasing the flexibility and plasticity of the cell wall, which contributes to increasing growth, in addition to the fact that some elements enter directly into the composition of some important compounds such as cytokines, which are necessary in the process of photosynthesis and activating cell growth, and thus leads to an increase in the number of leaves and leaf area. The seaweed extract also contains substances similar to plant hormones such as auxins, cytokinins, gibberellins, and growth-stimulating substances such as amino acids, organic acids, and vitamins that play a role in stimulating division and thus increasing the diameter of the stem, Table (6). Increasing the absorption of mineral elements into the leaves such as nitrogen and zinc stimulates various vital processes of the plant by activating various enzymes necessary for various metabolic processes [22] The reason for the increase in chlorophyll pigment Table (7) is attributed to the role of the elements present in the seaweed extract, especially nitrogen, iron and zinc, which are necessary for building protein and forming chloroplasts, and thus increasing the formation of chlorophyll pigment [23]. Increasing the concentration of the used algae extract leads to increased growth by stimulating cell division and expansion, leading to the growth of young leaves, which are a source or center for the manufacture of cytokinin, which works to activate the process of growth of lateral buds, thus increasing the number of branches and improving vegetative growth, by providing a good leaf area and amount of chlorophyll, which led to improving the process of photosynthesis and thus increasing nutrients and thus increasing the fresh and dry weight Tables (8 and 9).

Conclusions

- All the studied traits showed the same behavior in both rootstocks (sour orange and Volkameriana) under the influence of the study factors, as the response was similar but

- different in magnitude and sour orange rootstock was distinguished from fulcameriana in most of the studied traits
- The treatment of seaweed extract at a concentration of 20 g. Sandana⁻¹ achieved significant superiority and gave the highest values for most vegetative growth traits
 - Foliar spraying with acid with nano fertilizer NPK at a concentration of 2 g.L⁻¹ showed a significant effect and achieved the highest rates for most vegetative growth traits
 - The triple interaction treatment (stem of sour orange and marine waste at a concentration of 20 g. pot⁻¹ and spraying nano fertilizer at a concentration of 1.5 mg.L⁻¹) was significantly superior and gave the highest rates for most vegetative growth traits

References

- [1] Mahawesh, H. H. (2023). Response of Citrus aurantium L. orange seedlings to the addition of NPK complex nanofertilizer and humic and their effect on vegetative traits and nutrients. University of Thi-Qar Journal of agricultural research, 12(2), 195-205.
- [2] Ennab, H. (2016). Effect of Organic Manures, Biofertilizers and NPK on Vegetative Growth, Yield, Fruit Quality and Soil Fertility of Eureka Lemon Trees (Citrus limon (L.) Burm). Journal of Soil Sciences and Agricultural Engineering, 7(10), 767-774.
- [3] Talibi, I., Boubaker, H., Boudyach, E. H., & Ait Ben Aoumar, A. (2014). Alternative methods for the control of postharvest citrus diseases. Journal of Applied Microbiology, 117.
- [4] Al-Douri, A.H. and Al-Rawi, A.K.S. (2000). Production of fruits / first edition, Dar al-Kutub for printing and publishing / University of Mosul/Ministry of Higher Education and Scientific Research.
- [5] Kamali-Andani, N., Fallah, S., Peralta-Videa, J. R., & Golkar, P. (2022). A comprehensive study of selenium and cerium oxide nanoparticles on mung bean: Individual and synergistic effect on photosynthesis pigments, antioxidants, and dry matter accumulation. Science of the Total Environment, 830, 154837.
- [6] Al-Jalihawi, Iqbal Abdul-Ridha. (2019). Response of bitter orange seedlings to foliar spraying with growth stimulant and NPK fertilizer produced by nanotechnology. Ministry of Higher Education and Higher Research. University of Kufa. Faculty of Agriculture. Master's Thesis. Al-Areq.
- [7] Morales-Payan, J. P., & Norrie, J. (2010). Accelerating the growth of Avocado (Persea americana) in the nursery using a soil applied commercial extract of the brown alga Ascophyllum nodosum. In International Seaweeds Symposium (p. 189).
- [8] Al-Sahaf, Fadhel Hussein Reda. 1989. Applied Plant Nutrition. Ministry of Higher Education and Scientific Research. University of Baghdad. Bayt Al-Hikma for Publishing, Translation and Distribution. Iraq. p. 259.
- [9] Al-Rawi, K.M. & Khalaf Allah A.M. (2000). Design and Analysis of Agricultural Experiments. Dar Al Kutub Prin. & Pub. Univ. Mosul. Min. High. Educ. Sci. Res.. Rep. Iraq: 213pp.
- [10] Jemison, J., & Williams, M. (2006). Potato–Grain Study Project Report. Water Quality Office. University of Maine, Cooperation Extension.
- [11] Hartmann, H., Kester, D. E., Davies, F. T., & Geneva, R. (2002). Plant propagation: Principles and practices (6th ed.). Prentice Hall.
- [12] Ibrahim, Alaa Suhail, Ishraq Suleiman Ali, Raed Nazih Subaih, Rafiq Ali Abboud, and Fadel Suleiman Al-Qaim. 2014. The effect of seven citrus rootstocks on the growth and production of Abu Navel orange cultivar (Washington Navel 141). Syrian Journal of Agricultural Research. 1(1): 59- 69.
- [13] Kole, C. ; D. S. Kumar and Khodakovskaya, M. V. 2016. Plant Nanotechnology: Principles and Practices. Springer International Publishing .Switzerland.
- [14] Awad, S. M. and A. R. Atawia .1995. Effect of foliar sprays with some micronutrients on "Le-Conte" pear trees. Tree growth, flowering & leaf mineral contents. Annals of Agricultural Science, Ain Shams Univ. 40(1): 359-367.



- [15] Yassin, Bassam Taha. 2001. Fundamentals of Plant Physiology. College of Science - Qatar University.
- [16] Abdul, Karim Saleh. (1988). Physiology of Nutrients in Plants. University of Mosul. Ministry of Higher Education and Scientific Research. Iraq.
- [17] AL- Falahy, H.R. T. 2014. Effect of foliar application with urea and CO₂ enrichment on some growth characteristics and mineral content of sour orange seedlings. Euphrates Journal of Agriculture Science-6 (3): 30- 49.
- [18] Hagagg, L. F., Mustafa, N. S., Genaidy, E. A. E., & El-Hady, E. S. (2018). Impact of nanotechnology application on decreasing used rate of mineral fertilizers and improving vegetative growth of Aggizi olive seedlings. Bioscience Research, 15(2), 1304-1311.
- [19] Al-Taie, Muhammad Hussein Hamza. 2020. The effect of biofertilization and foliar spraying with nano-fertilizer on the vegetative and root growth of orange seedlings. Ministry of Higher Education and Scientific Research. Al-Furat Al-Awsat Technical University. Technical College / Al-Musayyab. Master's thesis. Iraq.
- [20] Abdul Zaid, Mushtaq Jabbar. 2016. Response of lemon seedlings grafted on different rootstocks to foliar spray of NPK-TE fertilizer and G-GANA growth stimulant. Master's thesis. College of Agriculture. University of Kufa. Republic of Iraq.
- [21] Kulikova, N.A.; Dashitsyrenova, A.D.; Perminova, I.V. and Lebedeva, G.F. 2003. Auxin-like activity of different fractions of coal humic acids. Bulgarian J. Ecol. Sci., 2(3-4): 55-56 .
- [22] Osman, S.M.; M.A. Khamis and A.M. Thorya. 2010. Effect of mineral and Bio-NPK soil application on vegetative growth, flowering, fruiting and leaf chemical composition of young olive trees. Res. J. Agric. & Biol. Sci. 6 (1)54-63.
- [23] Prajapati, H. B., Shah, J. P., & Dabhi, V. K. (2017). Detection and classification of rice plant diseases. Intelligent Decision Technologies, 11(3), 357-373.

Effect of Organic and Bio Fertilizer and Spraying with Nano Zinc on the Vegetative Growth of Aswd Diyala Fig Seedlings

Lara Qaisar Abdel Khader¹ and Akram Abd Alkadem Hadi²

^{1,2}Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Najaf, Iraq.

¹E-mail: lara.qayssar.tcm.79@student.atu.edu.iq

²E-mail: com.akr@atu.edu.iq

Abstract. Between April 2023 and July 2024, researchers from Al-Musaib Technical College, which is a part of Al-Furat Al-Awsat Technical University, carried out the experiment at their lab. The experiment included three factors to study the effect of organic and bio fertilization, spraying with nano zinc, and the overall growth of Aswd Diyala fig seedlings. The seedlings were one year old, obtained from the Horticulture Department in Al-Hindiya / Karbala province, and planted in uniformly sized plastic bags measuring 30×25 cm. The first factor: - Organic fertilization Different types of organic fertilizer were used (control treatment, vermicompost at an average of 20 g/pot, cow and sheep waste at an average of 20 g/pot + palm frond waste, wheat and rice at an average of 20 g/pot) and symbolized by the symbol (A0, A1, A2) The second factor is spraying EM1, Sefo Alexi at three levels (without spraying (control treatment), spraying EM1 at a concentration of 5 ml/L, spraying Sefo Alexi 5 ml.L⁻¹) and symbolized by (B0, B1, B2) The third work: Spraying nano zinc. Nano zinc is sprayed at three concentrations: (2, 1, 0 g. L⁻¹) and its symbol is (N0, N1, N1). A factorial experiment was conducted according to the Randomized Complete Blocks Design (RCBD), with three replicates. The results were analyzed using the Genstat program and compared with the least significant difference (L.S.D) test at a probability level of 0.05. The results were summarized as follows: Animal and plant wastes (A2) significantly increased the seedling height to 108.98 cm, stem diameter to 20.23 cm, chlorophyll intensity in leaves to 43.20 Spad, fresh weight of leaves to 55.33 g and dry weight of leaves to 13.98 g. The results also revealed that the treatment with 5 ml of EM1 (B1) was the most effective compared to the other treatments. It resulted in the highest seedling height of 105.21 cm, stem diameter of 20.24 cm, chlorophyll intensity in leaves of 43.43 Sppad, fresh weight of 54.74g, and dry weight of 13.38g for the leaves. The treatment with 2g of nano zinc spraying was the least effective. With a seedling height of 103.52 cm, a stem diameter of 21.46 cm, a chlorophyll intensity of 41.84 Spad, an average fresh weight of 54.08 g, and a dry weight of 13.06 g. L⁻¹ (N2) greatly outperformed the other groups. Additionally, the results demonstrated that the examined attributes were significantly impacted by the triple interaction. With a seedling height of 129.21 cm, stem diameter of 24.08 cm, chlorophyll intensity in the leaves of 48.47 Spad, fresh leaf weight of 62.75 g, and dried leaf weight of 17.10 g, the triple interaction treatment A2B1N2 considerably outperformed the other interaction treatments.

Keyword. Vermicompost, EM1, Sefo Alexi, Nano zinc.

1. Introduction

The genus *Ficus* is one of 40 genera of the Moraceae family. The original home of figs is Western Asia and its cultivation has spread to the Mediterranean basin and the fertile part of the Arabian Peninsula. It still grows in its wild state and from there it spread to southern Syria and then to the shores of the Mediterranean. At present, its cultivation has spread to different regions of the world, including Turkey, Egypt, Spain, Greece, America, Italy, Brazil and other places in the world [1]. Figs are one of the medicinal plants known since ancient times because they have multiple medical and therapeutic benefits, as they contain vitamins in reasonable quantities such as vitamin B1, B2, B5, citric acid, ascorbic acid, vitamin D2, D3, and vitamin E, in addition to minerals such as phosphorus, iron, sodium, and potassium, in addition to small amounts of proteins, fats, and carbohydrates [2]. Therefore, it has multiple uses, as its fruits are used to treat abscesses, while fruit juice is given to treat constipation, indigestion, relieve headaches, and treat intestinal infections resulting from bacterial and parasitic infections [3]. While the soft pulp of the fruit is useful for treating tumors and gum abscesses, treating dry and irritated coughs, and bronchitis [4]. The world has recently turned to using organic fertilizers to reduce pollution to plants, humans and the environment by using natural materials [5]. Organic waste, which includes both plant and animal matter, improves the soil's physical and chemical characteristics when added to it. In addition to enhancing soil porosity, controlling water and air movement, gas distribution, increasing water retention, lowering pH, increasing cation exchange capacity (C.E.C.), and providing plants with growth-supporting nutrients, organic matter is a rich source of these elements. Chelation by organic compounds increases the availability of nutrients, particularly trace elements, by preventing their deposition in soil. Plants will have enough nutrients for a long enough amount of time thanks to organic matter's gradual breakdown [6]. When opposed to mineral fertilizers, which are costly and harmful to the environment, biofertilizers are more cost-effective and have a smaller impact on plant development and growth [7]. EM1 is an abbreviation for (Effective Micro-organism) and is a natural preparation produced by the Japanese company Emro and contains a compatible group of beneficial microorganisms that have an active and effective role in improving the fertility of agricultural soil [8] and it is a safe preparation from a health perspective as the microorganisms in it are not genetically modified and do not contain harmful substances. It was prepared by the scientist Higa in the early eighties and called it EM1 and that this biological solution EM1 consists of a large group of microorganisms, which are [9] As for nano-fertilizers, they are characterized by unique properties due to their small size and large surface area that lead to an increase in the absorption surface and thus increase the process of photosynthesis and thus increase production in the plant [10]. The research aims to know Effect of Organic and bio fertilizer and spraying with nano zinc on the vegetative growth of Aswd Diyala fig seedlings

2. Materials and Methods

Between April 2023 and July 2024, researchers from Al-Mussaib Technical College, which is a part of Al-Furat Al-Awsat Technical University, carried out the experiment at their lab. In order to investigate how Aswd Diyala fig seedlings responded to organic and biofertilization treatments as well as nanozinc spraying during the vegetative growth stage.

2.1. Preparing the Seedlings

One-year-old fig seedlings (Aswd Diyala cultivar) were brought and obtained from the Horticulture Department in Al-Hindiya / Karbala provainc. They were planted in plastic bags measuring (30×25) cm. 324 seedlings were selected that were as homogeneous in growth and size as possible. The service operations were conducted equally for all treatments. The soil will be

analyzed to determine some of its physical and chemical properties according to the methods mentioned in [11,12].

2.2. The Experiment Included Three Factors

- The First Factor: Organic fertilization Different types of organic fertilizer were used (control treatment, vermicompost at an average of 20 g/pot, cow and sheep waste at an average of 20 g/pot + palm frond waste, wheat and rice at an average of 20 g/pot) and symbolized by the symbol (A0, A1, A2).
- The second factor: Spraying EM1, Sefo Alexi at three levels (without spraying (control treatment), spraying EM1 at a concentration of 5 ml.L⁻¹, spraying Sefo Alexi 5 ml.L⁻¹) and symbolized by (B0, B1, B2)
- The third factor: - Spraying nano zinc Nano zinc was sprayed at three concentrations (2, 1, 0 g.L⁻¹) and symbolized by (N0, N1, N1).

Table 1. Some chemical and physical properties of the soil.

Soil separators	Units	Values
sand	g.kg ⁻¹ soil	608
Silt	g.kg ⁻¹ soil	287
clay	g.kg ⁻¹ soil	105
texture	-----	Sand loamy
traits	units	values
Electrical conductivity EC	DS.m ⁻²	2.35
pH	-----	7.60
available nitrogen	g.kg ⁻¹	3.89
available phosphorus	mg.kg ⁻¹	4.10
available potassium	mg.kg ⁻¹	3.15

2.3. Experimental Design and Treatments Experiment

Every one of the three replications of the research will consist of 81 seedlings, with three seedlings assigned to each of the three experimental units and factors. This design is known as a (3*3*3) completely randomized experiment. We used the Least Significant Difference (LSD) test to compare the averages.

2.4. Vegetative Traits

- Plant height (cm): The seedling height was measured using a measuring tape.
- Main stem diameter (mm): The seedling diameters were measured using a Vernia scale and the average was used for each experimental unit.
- Total number of leaves (1 seedling leaf): The number of leaves for each seedling was calculated.
- Leaf area (cm²): The leaf area was measured using a (PLANIMETER) device and three complete leaves were taken from different places of the same plant and the leaf area was measured and the average was calculated for each seedling and then the average was calculated for each experimental unit.
- Fresh weight of leaves (g): The fresh weight of leaves was calculated after the leaves were separated from the stem and root system, then they were washed and cleaned from any remaining dust stuck to them, then they were weighed using a sensitive electric balance to fix the weight.

- Dry weight of leaves (g): The leaves for which the fresh weight was calculated were placed in perforated paper bags and dried in an electric oven ((oven for the purpose of getting rid of moisture at a temperature of 70 degrees Celsius for 48 hours until the weight stabilized, and the dry weight was taken using a sensitive electric balance [13].

3. Results and Discussion

3.1. Seedling Height (cm)

Table (2) shows that there was a significant influence of organic fertilizer on seedling height (cm). With a seedling height of 108.98 cm, animal and plant residues (A2) produced the best results, followed by vermicompost (101.17 cm), and the control treatment (A0) produced the lowest result (85.20 cm). Adding EM1 at a rate of 5 ml per plant was one of the biofertilizers that significantly affected seedling height, according to the data. In comparison to the other treatments, L⁻¹ (B1) produced the tallest seedlings at 105.21 cm, and the next best thing was adding 5 ml of Sifo Alexi. L⁻¹ (B2) and measured 102.34 cm for seedling height, whereas the control group recorded the lowest seedling height rate of 87.81 cm (B0). The results also demonstrated that the application of nano zinc spray, at a dosage of 2 g, significantly affected the height of the seedlings. The spray treatment at a dosage of 1 g was followed by L⁻¹ (N2), which showed a considerably higher seedling height rate of 103.52 cm. The treatment without spraying (0N) resulted in the lowest rate of plant height, at 93.23 cm, whereas L⁻¹ (1N) resulted in a seedling height rate of 98.60 cm. The findings also demonstrated that the seedling height was significantly impacted by the bi-interaction. While the interaction treatment A0B0 produced the lowest seedling height rate of 79.91 cm, the A2B1 interaction treatment recorded the highest rate of seedling height at 119.40 cm. Also, the results demonstrated that the interaction treatment A2N2 was the most effective, with a seedling height rate of 115.37 cm, whereas the interaction treatment A0N0 yielded the lowest, at 81.49 cm. At 111.72 cm, the interaction treatment B1N2 produced the tallest seedlings, whereas at 82.89 cm, the interaction treatment B0N0 produced the shortest. The triple interaction of the experimental parameters significantly affected the seedling height, as shown in Table (2) as well. With a seedling height of 129.21 cm, the A2B1N2 interaction treatment was far and away the best of the bunch. The seedling height rate was lowest in the interaction treatment A0B0N0, which reached 77.41 cm.

Table 2. Effect of Organic and bio fertilizer, nano zinc spraying and their interaction on the height of fig seedlings (cm).

Organic fertilizer (A)	Biofertilizers (B)	Nano Zinc (g.L ⁻¹) (N)			A × B
		Without adding(N0)	1 g.L ⁻¹ (N1)	2 g.L ⁻¹ (N2)	
control (A0)	Without adding (B0)	77.41	80.79	81.53	79.91
	EM1 (B1)	84.17	89.01	92.17	88.45
	Sifo Alexi (B2)	82.88	87.88	91.00	87.25
Vermicompost (A1)	Without adding (B0)	85.17	92.95	95.21	91.11
	EM1 (B1)	102.21	107.30	113.79	107.77
	Sifo Alexi (B2)	99.85	102.21	111.88	104.65
Animal and plant waste (A2)	Without adding (B0)	86.10	93.90	97.21	92.40
	EM1 (B1)	105.12	123.88	129.21	119.40
	Sifo Alexi (B2)	116.21	109.49	119.68	115.13

L.S.D 0.05	0.60	0.40
Interaction between Organic fertilizer and zinc spraying (A×N)		Organic fertilizer (A)
A0	81.49	85.89
A1	95.74	100.82
A2	102.48	109.09
L.S.D 0.05	0.40	0.20
Interaction between Organic fertilizer and Biofertilizers (A×B)		Biofertilizers (B)
Without adding (B0)	82.89	89.21
EM1(B1)	97.17	106.73
Sifo Alexi (B2)	99.65	99.86
L.S.D 0.05	0.40	0.20
Nano Zinc (g.L ⁻¹) (N)	93.23	98.60
L.S.D 0.05	0.20	

3.2. Stem Diameter (cm)

The organic fertilizer significantly affected the stem diameter (cm), as shown in Table (3). With an average stem diameter of 20.23 cm, the Vermicompost medium produced the greatest quality stems, followed by animal and plant waste (A2), and finally, the control treatment (A0) yielded the lowest quality stems, with an average diameter of 17.25 cm. Adding EM1 at an average of 5 ml demonstrated that biofertilizers significantly affected stem diameter. When compared to the other treatments, L⁻¹ (B1) produced the most impressive stem diameter rate of 20.24 cm, with an average of 5 ml of Sifo Alexi following. The treatment without adding (B0) resulted in the lowest stem diameter rate of 17.83 cm, whereas L⁻¹ (B2) recorded a stem diameter of 18.94 cm. Additionally, the results demonstrated that the diameter of the stem was significantly affected by nano zinc spraying, at a concentration of 2 g. With a stem diameter rate of 19.43 cm, L⁻¹ (N2) was noticeably superior, and a concentration of 1 cm came in second. Using L⁻¹ (1N), a stem diameter rate of 19.39 cm was obtained.

The stem diameter rate was 18.18 cm lowest in the treatment that did not involve spraying (0N). A notable impact of the bi-interaction on the stem diameter was also demonstrated by the data. While the A0B0 interaction treatment produced the smallest stem diameter rate of 16.16 cm, the A2B1 and A0B0 interaction treatment produced the greatest rate of 21.46 cm. Furthermore, the results demonstrated that the interaction treatment A2N2 was the most effective, with a stem diameter rate of 21.27 cm, whereas the interaction treatment A0N0 had the lowest rate of 16.49 cm. A stem diameter rate of 20.03 cm was obtained with the B1N2 interaction therapy, whereas a rate of 16.93 cm was obtained with the B0N0 interaction treatment. Table (3) further demonstrated that the experimental components' triple interaction significantly affected the stem diameter. The interaction treatment A2B1N1 produced the largest stem diameter (24.08 cm), surpassing all of the others by a wide margin. At 15.72 cm, the interaction treatment A0B0N0 had the smallest stem diameter rate.

Table 3. Effect of Organic and bio fertilizer, nano zinc spraying and their interaction on stem diameter (cm).

Organic fertilizer (A)	Biofertilizers (B)	Nano Zinc (g.L ⁻¹) (N)			A × B
		Without adding(N0)	1 g.L ⁻¹ (N1)	2 g.L ⁻¹ (N2)	
control (A0)	Without adding	15.72	16.33	16.43	16.16

		(B0)			
Vermicompost (A1)	EM1 (B1)	17.11	18.18	18.45	17.92
	Sifo Alexi (B2)	16.63	18.03	18.33	17.67
	Without adding	17.33	18.83	19.30	18.49
	(B0)				
Animal and plant waste (A2)	EM1 (B1)	20.23	21.33	22.43	21.33
	Sifo Alexi (B2)	20.07	20.43	22.08	20.86
	Without adding	17.73	19.06	19.73	18.84
	(B0)				
	EM1 (B1)	21.08	24.08	19.21	21.46
	Sifo Alexi (B2)	17.71	18.20	18.93	18.28
L.S.D 0.05			2.23		1.29
Interaction between Organic fertilizer and zinc spraying (A×N)					Organic fertilizer (A)
A0		16.49	17.52	17.74	17.25
A1		18.84	20.45	19.29	19.53
A2		19.21	20.20	21.27	20.23
L.S.D 0.05			1.29		0.74
Interaction between Organic fertilizer and Biofertilizers (A×B)					Biofertilizers (B)
Without adding (B0)		16.93	18.08	18.49	17.83
EM1(B1)		19.48	21.20	20.03	20.24
Sifo Alexi (B2)		18.14	18.89	19.78	18.94
L.S.D 0.05			1.29		0.74
Nano Zinc (g.L ⁻¹) (N)		18.18	19.39	19.43	
L.S.D 0.05			0.74		

3.3. Chlorophyll Intensity in Leaves (Spad)

The results in Table (4) showed that the Organic fertilizer had a significant effect on the intensity of chlorophyll in leaves (Spad). Animal and plant residues (A2) were significantly excelled and gave the highest rate of chlorophyll intensity in leaves, reaching 43.20 Spad, followed by the Vermicompost medium, which gave a rate of chlorophyll intensity in leaves, reaching 41.78 Spad, while the control treatment (A0) gave the lowest intensity of chlorophyll in leaves, reaching 38.17 Spad.

Additionally, the results demonstrated that the concentration of chlorophyll in the leaves was significantly influenced by biofertilizers. The other treatments were greatly outperformed by the addition of 5 ml of EM1 L⁻¹ (B1), which resulted in the greatest rate of chlorophyll intensity in the leaves (43.43 Spad). Then, 5 ml of Sifo Alexi was added. L⁻¹ (B2) and measured a chlorophyll intensity in the leaves of 40.75 Spad, whereas the lack of addition (B0) resulted in the lowest rate of chlorophyll intensity in the leaves, measuring 38.97 Spad. Nano zinc spray significantly affected leaf chlorophyll intensity, according to the data. The spray treatment with a concentration of 1 g of nano zinc was the second most effective, with a rate of chlorophyll intensity in the leaves reaching 41.84 Spad, after the 2 g.L⁻¹ (N2) nano zinc treatment. The rate was given as L⁻¹ (1N). The treatment without spraying (0N) resulted in the lowest rate of chlorophyll intensity in leaves, reaching 39.72 Spad, whereas the treatment with spraying reached 41.59 Spad.

The findings also demonstrated that the binary interaction significantly impacted the leaf chlorophyll intensity. While A0B0 recorded and gave the lowest rate of chlorophyll intensity in

the leaves (36.66 Spad), the interaction treatments A2B1 and A1B1 achieved the highest rates of chlorophyll intensity in the leaves (45.59 Spad, respectively). In addition, the results demonstrated that the interaction treatment A2N2 achieved the best rate of chlorophyll intensity in the leaves (45.92 Spad), whereas the interaction treatment A0N0 yielded the lowest rate of chlorophyll intensity (37.35 Spad). In terms of leaf chlorophyll intensity rates, the B1N2 interaction treatment achieved the maximum rate at 43.11 Spad, whereas the B0N0 interaction treatment recorded the lowest rate at 37.94 Spad. The results of Table (4) also showed that the triple interaction between the experimental factors had a significant effect on the intensity of chlorophyll in the leaves. The interaction treatment A1B1N2 significantly excelled the other interaction treatments and gave the highest intensity of chlorophyll in the leaves, reaching 48.47 Spad. While the interaction treatment A0B0N0 recorded the lowest rate of chlorophyll intensity in the leaves, reaching 36.24 Spad.

Table 4. Effect of Organic and bio fertilizer, nano zinc spraying and their interaction on the intensity of chlorophyll in the leaves (Spad).

Organic fertilizer (A)	Biofertilizers (B)	Nano Zinc (g.L ⁻¹) (N)			A × B
		Without adding (N0)	1 g.L ⁻¹ (N1)	2 g.L ⁻¹ (N2)	
control (A0)	Without adding (B0)	36.24	36.51	37.24	36.66
	EM1 (B1)	38.21	39.37	39.77	39.11
	Sifo Alexi (B2)	37.62	39.13	39.49	38.74
Virmicompost (A1)	Without adding (B0)	39.17	40.24	40.94	40.11
	EM1 (B1)	41.49	46.82	48.47	45.59
	Sifo Alexi (B2)	41.45	41.85	48.37	43.89
Animal and plant waste (A2)	Without adding (B0)	38.42	40.75	41.26	40.14
	EM1 (B1)	46.52	49.15	41.11	45.59
	Sifo Alexi (B2)	38.40	40.52	39.93	39.62
L.S.D 0.05		3.34			1.93
Interaction between Organic fertilizer and zinc spraying (A×N)					Organic (A) fertilizer
A0		37.35	38.33	38.83	38.17
A1		41.11	43.47	40.77	41.78
A2		40.70	42.97	45.92	43.20
L.S.D 0.05		1.93			1.11
Interaction between Organic fertilizer and Biofertilizers (A×B)					Biofertilizers (B)
Without adding (B0)		37.94	39.16	39.81	38.97
EM1(B1)		42.07	45.11	43.11	43.43
Sifo Alexi (B2)		39.16	40.50	42.59	40.75
L.S.D 0.05		1.93			1.11
Nano Zinc (g.L ⁻¹) (N)		39.72	41.59	41.84	
L.S.D 0.05		1.11			

3.4. Fresh Weight of Leaves (g)

Table (5) shows that the fresh weight of leaves (g) was significantly affected by the organic fertilizer. The average fresh weight of the leaves was 55.33 g in the Vermicompost medium, 53.15 g in the treatment involving animal and plant residues (A2), and 48.93 g in the control treatment (A0), thus indicating a significant difference between the two. Biofertilizers also significantly affected the fresh weight of leaves, according to the results. On average, 5 milliliters of EM1 is added. The average fresh weight of leaves was 54.74 g.L⁻¹ (B1), which was significantly higher than the other treatments. The next best treatment was adding 5 ml of Sifo Alexi. The treatment without adding (B0) resulted in the lowest average fresh weight of leaves, reaching 49.36 g.L⁻¹ (B2) and recorded a fresh weight of 53.30 g achieved the most. The findings also demonstrated that the fresh weight of leaves was significantly impacted by the nano zinc spray. Two grams of nano zinc sprayed on as a therapy. After the spray treatment at a concentration of 1 g.L⁻¹ (N2) achieved the highest average fresh weight of leaves, reaching 54.08 g, which was greatly exceeded. The average fresh weight of the leaves was 52.70 g.L⁻¹ (1N). On the other hand, the rate of fresh weight of leaves reached 50.63 g in the treatment that did not involve spraying (0N). Furthermore, the results demonstrated that the bi-interaction significantly impacted the fresh weight of leaves. Specifically, the interaction treatments involving A2B1 and B1 achieved the highest rate of fresh weight of leaves, measuring 58.99 g, while the interaction treatment A0B0 recorded the lowest rate, reaching 47.05 g. The results also demonstrated that the interaction treatment A2N2 achieved the highest rate of fresh weight of leaves (57.55 g), the lowest rate (47.55 g) was recorded by the interaction treatment A0N0, the highest rate (56.95 g) was recorded by the interaction treatment B1N2, and the lowest rate (48.02 g) was recorded by the interaction treatment formed of B0N0.

Table 5's findings further demonstrated that the experimental factors' triple interaction significantly impacted the fresh weight of leaves. A1B1N2 was the most effective interaction treatment, producing leaves with the highest fresh weight (62.75 g), and it outperformed all of the others. However, with a fresh weight of 46.27 g, the interaction treatment A0B0N0 had the lowest weight of leaves.

Table 5. Effect of Organic and bio fertilizer, nano zinc spraying and their interaction on the fresh weight of leaves (g).

Organic fertilizer (A)	Biofertilizers (B)	Nano Zinc (g.L ⁻¹) (N)			A × B
		Without adding(N0)	1 g.L ⁻¹ (N1)	2 g.L ⁻¹ (N2)	
control (A0)	Without adding (B0)	46.27	47.27	47.60	47.05
	EM1 (B1)	48.40	50.50	51.29	50.06
	Sifo Alexi (B2)	47.98	50.27	50.81	49.69
Virmicompost (A1)	Without adding (B0)	48.60	50.50	51.40	50.17
	EM1 (B1)	53.14	55.50	56.82	55.15
	Sifo Alexi (B2)	52.40	53.81	56.14	54.12
Animal and plant waste (A2)	Without adding (B0)	49.18	51.18	52.26	50.87
	EM1 (B1)	55.18	59.05	62.75	58.99
	Sifo Alexi (B2)	54.50	56.18	57.65	56.11
L.S.D 0.05					
Interaction between Organic fertilizer and zinc spraying (A×N)					Organic fertilizer (A)

Organic fertilizer (A)	Biofertilizers (B)	Nano Zinc (g.L ⁻¹) (N)			A × B
		Without adding(N0)	1 g.L ⁻¹ (N1)	2 g.L ⁻¹ (N2)	
A0		47.55	49.35	49.90	48.93
A1		51.38	53.27	54.79	53.15
A2		52.95	55.47	57.55	55.33
L.S.D 0.05					
Interaction between Organic fertilizer and Biofertilizers (A×B)					Biofertilizers (B)
Without adding (B0)		48.02	49.65	50.42	49.36
EM1(B1)		52.24	55.02	56.95	54.74
Sifo Alexi (B2)		51.63	53.42	54.87	53.30
L.S.D 0.05					
Nano Zinc (g.L ⁻¹) (N)		50.63	52.70	54.08	
L.S.D 0.05					

3.5. Dry Weight of Leaves (g)

The organic fertilizer significantly affected the dry weight of leaves (g), as shown in Table (6). The vermicompost medium produced leaves with an average dry weight of 12.95 g, animal and plant residues (A2) with a significantly higher average dry weight of 13.98 g, and the control treatment (A0) with the lowest average dry weight of 9.38 g.

Biofertilizers significantly affected the dry weight of leaves, according to the data. The treatment with EM1 added at a concentration of 5 ml.L⁻¹ (B1) produced the highest average dry weight of leaves at 13.38 g, followed by the addition of Sifo Alexi at a concentration of 5 ml.L⁻¹ (B2), which produced leaves at 12.67 g, and the treatment without any additions (B0) produced the lowest leaves at 10.26 g. Nano zinc spray significantly affected the leaves' dry weight, according to the data. The spray treatment with a concentration of 1 g of nano zinc was the second most effective, with an average dry weight of 13.06 g of leaves, while the treatment with a concentration of 2 g L⁻¹ (N2) was the most outstanding. The average dry weight of the leaves was 12.20 g.L⁻¹ (1N), while it reached 11.06 g when treated without spraying (0N), the lowest rate of dry weight of the leaves. Additionally, the results demonstrated that the bi-interaction significantly impacted the dry weight of leaves. Specifically, the interaction treatments involving A2B1 and B1 achieved the highest rates of dry weight of leaves, measuring 15.72 g and 7.91 g, respectively, while the interaction treatment A0B0 yielded the lowest rates. Furthermore, the results demonstrated that the interaction treatment A2N2 was the most effective, with a rate of dry leaf weight of 15.04 g, whereas the interaction treatment A0N0 yielded the lowest rate of dry leaf weight, at 8.55 g. With a rate of 14.47 g, the interaction treatment B1N2 produced the heaviest leaves, while the interaction treatment B0N0 produced the lightest, with a rate of 9.28 g. The results of Table (6) also showed that the triple interaction between the experimental factors had a significant effect on the dry weight of leaves. Among the interaction treatments, A1B1N2 produced the most impressive results, with a maximum dry weight of 17.10 g of leaves. However, with a dry weight of only 7.33 g, the interaction treatment A0B0N0 achieved the lowest leaf weight.

Table 6. Effect of Organic and bio fertilizer, nano zinc spraying and their interaction on the dry weight of leaves (g).

Organic fertilizer (A)	Biofertilizers (B)	Nano Zinc (g.L ⁻¹) (N)			A × B
		Without adding(N0)	1 g.L ⁻¹ (N1)	2 g.L ⁻¹ (N2)	

Organic fertilizer (A)	Biofertilizers (B)	Nano Zinc (g.L ⁻¹) (N)			A × B
		Without adding(N0)	1 g.L ⁻¹ (N1)	2 g.L ⁻¹ (N2)	
control (A0)	Without adding (B0)	7.33	7.92	8.48	7.91
	EM1 (B1)	9.33	10.38	11.28	10.33
	Sifo Alexi (B2)	8.98	10.01	10.68	9.89
Virmicompost (A1)	Without adding (B0)	9.98	11.43	12.23	11.22
	EM1 (B1)	12.99	14.23	15.03	14.09
	Sifo Alexi (B2)	12.63	13.33	14.68	13.55
Animal and plant waste (A2)	Without adding (B0)	10.53	12.10	12.33	11.66
	EM1 (B1)	14.05	16.01	17.10	15.72
	Sifo Alexi (B2)	13.68	14.33	15.68	14.57
L.S.D 0.05			0.06		0.03
Interaction between Organic fertilizer and zinc spraying (A×N)					Organic fertilizer (A)
A0		8.55	9.44	10.15	9.38
A1		11.87	13.00	13.98	12.95
A2		12.76	14.15	15.04	13.98
L.S.D 0.05			0.03		0.018
Interaction between Organic fertilizer and Biofertilizers (A×B)					Biofertilizers (B)
Without adding (B0)		9.28	10.49	11.02	10.26
EM1(B1)		12.13	13.54	14.47	13.38
Sifo Alexi (B2)		11.77	12.56	13.68	12.67
L.S.D 0.05			0.03		0.018
Nano Zinc (g.L ⁻¹) (N)		11.06	12.20	13.06	
L.S.D 0.05			0.018		

Reviewing the previous results showed a clear increase in the vegetative traits as a result of adding organic, nano and bio-fertilizers and the interaction between them. The results were shown in the tables (2-6). An increase was found in the vegetative traits of the Aswd Diyala fig seedlings, represented by (plant height, stem diameter, chlorophyll pigment intensity, fresh and dry weight in leaves). The reason for this increase in these traits may be attributed to the role of these fertilizers in improving the physical and chemical traits of the soil, as bio-fertilizers work to increase the surface area of the roots and stimulate root hairs to absorb nutrients present in the soil as a result of their secretion of plant hormones in the growth medium, including auxins and gibberellins, which in turn work to increase cell division and increase their numbers [14]. The reason for the increase in the above traits may be attributed to the fact that EM1 contains a mixture of microorganisms that work to produce growth stimulants and activators and form plant defense mechanisms, which contribute significantly to increasing the readiness of nutrients in the root zone and thus facilitate their absorption by the plant [15] and its content of bacteria and fungi extends several centimeters from the surface of the roots and works to extract nutrients from the outer area of the infected roots [16]. The reason may be due to its important role in producing enzymes and organic compounds important for the growth and development of the plant in the root area [17], as well as stimulating root hairs, which is positively reflected in the process of nutrient absorption and thus an increase in vegetative traits represented by plant height, stem

diameter, leaf area, fresh weight of the vegetative group and dry weight of the vegetative group in improving vegetative traits, or it is attributed to the role of organic matter added to the soil has a great effect in improving the physical, chemical and biological traits of the soil, in order to make sandy soil more compact and clay soil less compact, thereby supplying the soil with the oxygen needed for root respiration and microbial activity; when air circulation improves, carbon is converted into CO₂ gas, which can Carbonic acid is formed when it reacts with oxygen in the air or dissolves in water; nitrogen is transformed into ammonium ions (NH₄), which are subsequently changed to nitrate (NO₃), and sulfur is transformed into sulfate ions. As a source of these essential nutrients for plant and microbial growth, organic matter also improves soil porosity, controls water and air movement, spreads gases, increases water holding capacity, raises cation exchange capacity, lowers pH, and contains various minor and major nutrients and other rare elements in organic media. Fertilizers, particularly trace elements, are more readily available to plants when bound to organic compounds, and plants can draw on organic matter's nutrients for an extended length of time due to the slow decomposition process [18,19]. One possible explanation is that nano-fertilizers play a role in enhancing plant growth. This is because the molecules that make up nano-fertilizers are very small, which means that their surface area is very large. This, in turn, increases the effective area for interaction, which in turn increases the efficiency of enzymes and biochemical reactions. As a result, there are more cell divisions. Another possible explanation is that nano-fertilizers reduce or inhibit the formation of free radicals, which in turn reduces their activity and oxidation. This, in turn, delays the aging process, which encourages vegetative growth [20]. The effective role of the zinc element, which performs vital processes such as food production and enzyme work, regulates plant growth and cell elongation through the hormone indole acetic acid, which is tryptophan (which zinc is included in its composition) and is considered the main component of the hormone, which increases vegetative traits [21,22].

Conclusions

It is concluded that the Animal and plant wastes (A2) and the treatment of adding EM1 at 5 mL⁻¹ (B1) and nano zinc spraying at a concentration of 2g.L⁻¹ (N2) are superior and the highest values were recorded for most of the studied traits.

References

- [1] Singh, D., Singh, B., & Goel, R. K. (2011). Traditional uses, phytochemistry and pharmacology of *Ficus religiosa*: A review. *Journal of ethnopharmacology*, 134(3), 565-583.
- [2] Jacobo-Salcedo, M., Valdez-Cepeda, R. D., Sanchez-Cohen, I., Arreola-Ávila, J. G., González-Espíndola, L. A., & Trejo-Calzada, R. (2024). Ability of fig tree (*Ficus carica* L.) accessions to thrive under limited and unlimited soil water conditions. *Acta Agrobotanica*, 77.
- [3] Falistocco, E. (2020). The millenary history of the fig tree (*Ficus carica* L.). *Adv Agric Horticult Entomol*, 5, 130.
- [4] Hussain, S. Z., Naseer, B., Qadri, T., Fatima, T., & Bhat, T. A. (2021). Fig (*Ficus Carica*)—Morphology, taxonomy, composition and health benefits. In *Fruits Grown in Highland Regions of the Himalayas: Nutritional and Health Benefits* (pp. 77-90). Cham: Springer International Publishing.
- [5] Al-Hamidawi, Abbas Mohsen Salman. (2010). The effect of pruning and spraying BA on the vegetative growth characteristics of fig trees (*Ficus carica* L.) Black Diyala variety. *Kufa Journal for Agricultural Sciences*, 2(2).
- [6] Al-Zuhawi, Samir Muhammad Ahmad. 2007. The effect of different organic fertilizers and soil cover on the growth, production and quality of potatoes (*Solanum tuberosum* L.). Master's thesis - Department of Horticulture - College of Agriculture - University of Baghdad - Iraq.

- [7] Mishra, S., Bhuyan, S., Mallick, S. N., Biswal, S., & Singh, V. B. (2022). Role of Biofertilizer in Agriculture. *Biotica Research Today*, 4(6), 461-463.
- [8] Osman, S. O., Moustafa, F. M. A., Abd El-Galil, H. A., & Ahmed, A. Y. M. (2011). Effect of yeast and effective microorganisms (EM1) application on the yield and fruit characteristics of Bartamuda date palm under Aswan conditions. *Assiut J. of Agric. Sci*, 42(5), 332-349.
- [9] Higa, T. (1998). Effective Microorganisms, concept and recent advances in technology. In *Proceedings of the Conference on Effective Microorganisms for a sustainable agriculture and environment. 4th International Conference on Kyusei Nature Farming, Bellingham-Washington USA* (pp. 247-248).
- [10] El-Nwehy, S. S., El-Naggar, A. A. M., & El-Nasharty, A. B. (2023). Impact of biofertilizer EM1 combined with NPK on growth, flowering, corm production, chemical composition and reduction of mineral fertilization of gladiolus hybrida cv. rose supreme. *Pakistan Journal of Agricultural Research*, 36(3), 217-229.
- [11] Black, C.A. .1965. Methods of soil analysis . Part1 . Physical and mineralogical properties. Part2. Chemical and microbiological properties. Am. Soc. Agron. , Inc. Madison , Wisconsin U.S.A
- [12] Page, A.L., Miller, R.H. and Keeney, D.R. (1982) Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy. In Soil Science Society of America, Vol. 1159.
- [13] Al-Sahaf, Fadhel Hussein. 1989. Applied Plant Nutrition. University of Baghdad - Ministry of Higher Education and Scientific Research - Bayt Al-Hikma for Publishing, Translation and Distribution - Higher Education Press in Mosul - Iraq.
- [14] Fahad, S., Nie, L., Chen, Y., Wu, C., Xiong, D., Saud, S., ... & Huang, J. (2015). Crop plant hormones and environmental stress. *Sustainable Agriculture Reviews: Volume 15*, 371-400.
- [15] Ezeagu, G. G., Omotosho, A. O., & Suleiman, K. O. (2023). Effective Microorganisms, Their Products and Uses-A Review.
- [16] Demir, S. (2004). Influence of arbuscular mycorrhiza on some physiological growth parameters of pepper. *Turkish Journal of Biology*, 28(2), 85-90.
- [17] Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., & Kopriva, S. (2017). The role of soil microorganisms in plant mineral nutrition—current knowledge and future directions. *Frontiers in plant science*, 8, 1617.
- [18] Tisdale, S. L. W. L. Nelson, J.D. Beaton and J. Havlin.1993. soil fertility and fertilizers. 5th .E. d. The Macmillan pub. Co. New York. USA.
- [19] Granatstein, D. (2004). Center for sustaining Agriculture and Natural Resources. Washington State University, Wenatchee, Wa.USA.
- [20] Laware, S.L, and S.V.Raskar SV .2014. Influence of Zinc Oxide Nanoparticles on Growth, Flowering and Seed Productivity in Onion. *Int J Curr Microbiol AppSci* 3:874-881.
- [21] Mahajan, P., S. Dhole, and A. Khanna, .2011. Effect of Nano – ZnO particle suspension on growth of mung (*Vigna radiata* L.) and gram (*Cicer arietinum* L.) seedling using plant agar method. *J. Nanotech.*, 1(1): 1-7.
- [22] Saleh, A. L.; A. A. Abd EL- Kader and S. A. M. Hegab. 2003. Responses of onion to organic fertilizer under irrigation with saline water. *Egypt J. Appl. Sci.* 18 (12): 707 – 716.

Effect of Adding Gum Arabic to the Dietary on the Productive Characteristics of Quail Birds

Yasin Jasim Mohammed¹ and Mokhalad Oraibi Hasan²

^{1,2} Department of Animal Production, Faculty of Agriculture, University of Tikrit, Tikrit, Iraq.

¹E-mail: yasin.j.mohammed001@st.tu.edu.iq

²E-mail: Mokhalad082@tu.edu.iq

Abstract. This study was conducted in the quail house of the Department of Animal Production, Faculty of Agriculture, Tikrit University to knowing the effect of adding gum Arabic to the dietary on the productive performance of quail birds. 80 birds were randomly distributed to four treatments, where the first treatment was the control, while gum Arabic was added to the second, third, and fourth treatments at 0.3, 0.6 and 0.9% of the feed, respectively. The result of the study showed that the third treatment significant improved ($P \leq 0.05$) in final cumulative egg production, HD egg production and feed consumption, while in egg mass, the third and fourth treatments were significant improved ($P \leq 0.05$) compared to other treatments, while in feed efficiency, the third and fourth treatments improved significant compared with other treatments, while in egg weight, there were no significant differences between treatments. It concludes from the current study that the addition of gum Arabic to the feed led to an improvement in most of the production traits of quail birds.

Keywords. Gum Arabic, Quail eggs, Production characteristics.

1. Introduction

One of the main issues facing the 21st century is securing the human need for protein sources, as many countries, especially developing countries, suffer from a decline in nutrition levels due to an increase in population and a decrease in food sources, and despite the increase in global production of table eggs have not met the requirements of the food gap caused by the growing demand, as the volume of egg production worldwide reached 76.7 million tons of eggs in 2018, up from 73.9 million tons of eggs in 2016. The volume of global egg production increased by more than 95 percent [1-3]. Domestic bird eggs are products of high nutritional value, as they contain protein of high biological value, so one egg per day sufficient about 14.4% of the protein needs of an adult human. The quail bird is characterized by high activity despite its low weight, which is estimated at 110-140 g for males and 120-160 g for females, and the short generation period, as it can produce four generations during one year. The average egg production rate for quail birds reached 280 eggs/year with an average weight of 10 g per egg [4]. The reality of raising laying hens imposes costs on production, the most important of these costs are the components of the feed and its manufacture, where the cost of feed constitutes about 65-75% of the total costs of eggs due to the nature of the feed materials involved in the composition of laying hens, represented by

protein and energy sources [5]. Therefore, researchers and producers have resorted to the use of many feed alternatives and additives that would reduce these costs, provided that these additives are cheap and do not compete with the main human food, and do not have negative effects on the health of chickens and eggs produced and the health of the consumer. Gum Arabic is the secretions of the acacia tree, it comes mainly from the African Sahel countries, and it is considered an additive [6], Gum Arabic is a natural food supplement that plays an important role in intestinal and hepatic metabolism and has other potential health benefits. It is also considered a natural biomaterial that supports overall health and growth performance in poultry. Ismail [7], observed that the use of Gum Arabic powder (0.5%, 1%, 1.5%) in laying hen diets resulted in a weight gain in the 1.5% addition treatment and a significant increase in the relative rate of egg production by 55.89%. In addition, a significant improvement ($p < 0.05$) was observed in the feed conversion factor for all addition treatments (0.5%, 1%, 1.5%) but no significant improvement was observed in feed consumption rate, egg weight, and mortality rate at the end of the experiment. [8], found that the addition of Gum Arabic powder (1, 3, 5, and 7%) to the Lohmann laying hen treatments resulted in a decrease in the level of cholesterol and triglycerides for the addition treatment containing 7% Gum Arabic powder. In contrast, no significant improvement was observed in the level of total fat, phospholipids, and total protein at the end of the experiment.

This study aimed to evaluate the effect of gum Arabic addition to laying quail dietary on production characteristics.

2. Materials and Methods

This study was conducted in the poultry field of the Department of Animal Production, Faculty of Agriculture, University of Tikrit operation with laboratories in Mosul university Ethical approval No. um.VET.2021.5, and the birds raised up to (56) days and distributed the experimental treatments as follows:

The first treatment without addition

In the second treatment added 0.3% gum Arabic.

The third treatment added 0.6% gum Arabic.

The fourth treatment added 0.9% gum Arabic.

The birds were randomly distributed inside the breeding cages into four treatments, each treatment has four replicates with five birds per replicate. Appropriate conditions were provided for the birds inside the breeding hall from nutrition and environmental conditions (temperature, humidity, and lighting) and the lighting program was 8 hours of darkness and 16 hours of light. Feed and water were provided freely to the birds, where the birds were fed on a special ready-made feed for egg production that was purchased from a reliable feed factory, Gum Arabic was purchased from local markets in Tikrit in the form of powder. Feed consumption was recorded weekly for each replicate and eggs were collected daily and weighed with a sensitive electronic scale.

3. Results and Discussion

The results of Table (1) showed the effect of adding gum arabic powder in the quail diets on cumulative egg production, where significant differences were found at ($p \leq 0.05$) during the production period from (1-14) days, where the third treatment increased significant compared with the control and the fourth treatment, but there were no significant differences between the second and third treatment, while during the production period from (15-28) days, the third treatment increased significant compared with the control and the fourth treatment, but there were no significant differences between the second and third treatment. The results of the table also

showed that during the production period from (29-42) days, the third treatment increased significant compared with other treatments of the study, and the results of the table for the production period from (43-56) days showed that the second and third treatments increased significant compared with the control and the fourth treatment.

Table (2) shows the effect of using gum Arabic in the diet on daily egg production HD (1 - 14) days, the third treatment was superior to the fourth treatment, but there were no significant differences between the third treatment and the first and second treatments, while the range between (15 - 28) days, the third treatment was significantly superior to the first treatment, but the second treatment was superior to the first treatment, but there were no significant differences between the third treatment and the first and second treatments.

In the range between (29-42) days, the third treatment outperformed all the studied treatments, but the second treatment outperformed the first treatment, but there are no significant differences between the first treatment and the fourth treatment, while the range between (43-56) days, the third increased significant compared with other treatments, as well as the second treatment increased significant compared with the first and fourth treatment, but there are no significant differences between the first and fourth treatment.

Table 1. Effect of gum Arabic on eggs Cumulative egg production.

Treatments	The days				
	1-14	15-28	29-42	43-56	Total
First	56.33±2.18b	51.33±2.90b	48.66±1.76c	52.66±1.20b	209.00±2.64c
Second	53.00±2.51ab	55.66±1.20ab	56.66±3.38b	62.33±1.66a	227.66±1.45b
Third	58.33±1.20a	61.00±1.00a	64.33±0.33a	59.66±0.33a	243.33±1.66a
Fourth	50.33±2.72b	54.00±1.73b	54.00±2.30bc	50.00±2.64b	208.33±6.56c

-Different letters within a column indicate significant differences at $p \leq 0.05$,

T1: Control treatment without addition, T2: Addition of 0.3 % gum Arabic powder to the diet, T3: adding 0.6% gum Arabic to the diet, T4: Addition 0.9% of gum Arabic to the diet.

Table 2. The effect of adding gum Arabic to the diet on HD egg production%.

Treatments	The days				
	1-14	15-28	29-42	43-56	Total
First	80.48±3.12ab	73.33±4.15b	69.52±2.52c	75.24±1.71b	74.64±0.94c
Second	75.71±3.59ab	79.52±1.71ab	80.95±4.83b	89.05±2.38a	81.31±0.51b
Third	83.33±1.71a	87.14±1.42a	91.90±0.47a	85.23±0.47a	86.90±0.59a
Fourth	71.90±3.89b	77.14±2.47b	77.14±3.29bc	71.42±3.78b	74.40±2.34c

-Different letters within a column indicate significant differences at $p \leq 0.05$,

T1: Control treatment without addition, T2: Addition of 0.3 % gum Arabic powder to the diet, T3: adding 0.6% gum Arabic to the diet, T4: Addition 0.9% of gum Arabic to the diet.

The results of Table (3) showed the effect of using gum Arabic in the diet on egg mass (1-14), there are no significant differences between the study treatments, with the range between (15-28) as well as there are no significant differences between the studied treatments. And the range between (29-42) days, there are also no significant differences between the studied coefficients, as for the range between (43-56) days, where the second and third treatment increased significant compared with the first and fourth treatment.

Table 3. The effect of adding gum Arabic to the diet on egg mass grams.

Treatments	The days				
	1-14	15-28	29-42	43-56	Total
First	8.31±0.42a	8.15±0.81a	8.02±0.3b	8.64±0.16b	8.28±0.11b

Second	8.73±0.52a	9.04±0.22a	9.59±0.49a	10.45±0.16a	9.45±0.19a
Third	8.51±0.58a	9.70±0.29a	10.27±0.12a	9.38±0.09a	9.58±0.17a
Fourth	7.86±0.21a	8.90±0.44a	8.97±0.57ab	8.65±0.38b	8.59±0.21b

-Different letters within a column indicate significant differences at $p \leq 0.05$,

T1: Control treatment without addition, T2: Addition of 0.3 % gum Arabic powder to the diet, T3: adding 0.6% gum Arabic to the diet, T4: Addition 0.9% of gum Arabic to the diet.

Table (4) shows the effect of using gum Arabic in the diet on feed consumption. The results of the statistical analysis showed that during the productive period from (1-14) days, there are no significant differences between all treatments, while the period between (15-28) days. Likewise, there are no significant differences between all treatment and found during the productive period from (29-42) days there are no significant differences between all treatments, and in the period between (43-56) days, there are also no significant differences between treatments.

Table 4. The effect of adding gum Arabic to the diet on feed consumption grams.

Treatments	The days				
	1-14	15-28	29-42	43-56	Total
First	26.73±1.19a	27.54±4.46a	32.14±1.11b	34.90±0.96a	121. 31± 7.72 b
Second	27.26±0.55a	29.14±1.20a	32.24±0.35b	37.14±1.22a	125.78± 3.32ab
Third	26.88±1.27a	32.57±0.96 a	35.88±0.38a	36.45±1.01a	131.78±3.62a
Fourth	26.78±0.18a	31.02±0.34a	33.42±0.74b	36.28±0.35a	127.5 ±1.61 ab

-Different letters within a column indicate significant differences at $p \leq 0.05$,

T1: Control treatment without addition, T2: Addition of 0.3 % gum Arabic powder to the diet, T3: adding 0.6% gum Arabic to the diet, T4: Addition 0.9% of gum Arabic to the diet.

Table (5) shows the effect of the use of gum Arabic in the diet on the efficiency of nutritional conversion (1-14 days), there were no significant differences between all studied treatments, as well as the period between (15-28 days), there were no significant differences between all studied treatments, as well as found during the period between (29-42 days), there were no significant differences between all studied treatments, as well as found during the period between (29-42 days), there were no significant differences. As for the period between (43-56), the fourth treatment increased significant compared with the third and second treatment, but there are no significant differences between the fourth treatment and the first treatment, as well as no significant differences between the first treatment and the third treatment.

Table 5. The effect of adding gum Arabic on the feed efficiency.

Treatments	The days				
	1-14	15-28	29-42	43-56	Total
First	3.23±0.25a	3.33±0.24a	4.01±0.08a	4.03±0.10ab	3.65±0.13ab
Second	3.15±0.25a	3.23±0.18a	3.38±0.20a	3.55±0.07c	3.32±0.05b
Third	3.20±0.33a	3.35±0.05a	3.49±0.02a	3.71±0.06bc	3.44±0.06b
Fourth	3.41±0.11a	3.50±0.15a	3.76±0.28a	4.21±0.18a	3.72±0.10a

-Different letters within a column indicate significant differences at $p \leq 0.05$,

T1: Control treatment without addition, T2: Addition of 0.3 % gum Arabic powder to the diet, T3: adding 0.6% gum Arabic to the diet, T4: Addition 0.9% of gum Arabic to the diet.

Table (6) showed the effect of using gum arabic in the bush on the average weight of eggs, where it was found during the period between (1-14) days that there were no significant differences between all studied treatments and also during the period between (15-28) days that there were no significant differences It was also found during the period between (29-42) days that there were

no significant differences between all treatments, and it was found during the period between (43-56) days that there were no significant differences between all the studied treatments.

Table 6. The effect of adding gum Arabic to the diet on the average weight of eggs grams.

Treatments	The days				
	1-14	15-28	29-42	43-56	Total
First	10.32±0.19a	11.06±0.55a	11.53±0.24a	11.50±0.22a	11.10±0.08a
Second	11.54±0.47a	11.37±0.11a	11.87±0.29a	11.76±0.41a	11.63±0.28a
Third	10.23±0.81a	11.13±0.17a	11.18±0.1a	11.53±0.05a	11.02±0.19a
Fourth	10.98±0.46a	11.54±0.51a	11.62±0.42a	12.13±0.43a	11.56±0.38a

-Different letters within a column indicate significant differences at $p \leq 0.05$,

T1: Control treatment without addition, T2: Addition of 0.3 % gum Arabic powder to the diet, T3: adding 0.6% gum Arabic to the diet, T4: Addition 0.9% of gum Arabic to the diet.

When reviewing the results obtained from our study, we may notice that there are arithmetic differences between the addition and control treatments. The reason may be due to the fermentation of gum Arabic inside the digestive tract by microorganisms, i.e. creating an environment similar to the action of bor and ionic within the body by changing the balance ratios between harmful and beneficial bacteria, which reflects positively on bird health, thus increasing production performance rates [9,10,11,12]. A number of studies conducted on Gum Arabic (Senegal Acacia) have confirmed that it is rich in soluble sugars such as galactose, rhamnose, rapinose, essential amino acids, and minerals [13,14], thus leading to increased growth and improved nutritional conversion factor (NCF). The maximum improvement in growth performance in the first weeks of the experiment was consistent with the results obtained [14] who found that broilers showed a higher growth rate compared to the feed conversion ratio which was lower, The total amount of feed consumed was lower in the addition treatments when compared to the control treatment, this may be attributed to the relative stability in the variables that affect the rate of feed consumption with respect to the experimental conditions such as temperature [15].

The content of gum arabic in active compounds, including lignin, at a rate of 25%. 58 had an effective and obvious effect on increasing the appetite of the birds in the addition treatments and although the feed consumption rates did not record significant differences between the addition and control treatments, they did record arithmetic differences between the experimental treatments and this was explained in the results of our study and our results obtained agreed with the researchers Leite *et al.* [16] and Phillips *et al.* [17]; Yang *et al.* [18] Gum Arabic is a fiber-rich biomaterial that improves the nutrient conversion factor by coating the intestinal lining with carbohydrate components of gum Arabic, which enhances the behavior of beneficial bacteria, increases the height of the villi and the depth of the crypts, improves the internal environment of the intestine, and promotes growth [19]. When Gum Arabic is analyzed, it contains many effective and active compounds that have a distinctive effect in creating an ideal environment for the digestive system, including the terpene compound, which is found at 12. 55%, as this compound is very important to create an ideal environment with an acidic pH suitable for the work and activity of lactobacilli bacteria, which in turn stimulates the digestion and absorption of nutrients by increasing the surface area of the small intestine and increasing the height of the villi and the depth of the crypts and then increasing growth and productive performance [20-22]. Roberfroid [23] found that the best results obtained for eggshell quality parameters are partly because prebiotics affect the metabolic activity of the colony of beneficial bacteria within the gut of laying hens, which positively affects the absorption rate of minerals, especially those of Ca^{2+} and Mg^{2+} .

Conclusions

From the current study, we conclude that the addition of gum Arabic to the quail dietary at 0.6 percent resulted in a significant improvement in the productive qualities of quail birds.

References

- [1] Shahbandeh, M., 2020. Global egg production from 1990 to 2018. online: <https://www.statista.com/statistics/263972/egg-productionworldwide-since-1990/>. Accessed.
- [2] Hasan, M. O., Al-Kaisi, H. R. M., Al-Samrai, M. K., & Aljumaily, T. K. H. (2024, April). Study the qualities and characteristics of stored quail eggs in the refrigerator which is fed with Aloe vera. In AIP Conference Proceedings (Vol. 3079, No. 1). AIP Publishing.
- [3] Al-Saada, Q., Al-Nasseri, A. N., Hasan, M. O., & Taha, M. W. (2024, February). Effect of Rhus coriaria L. on performance productive, physiological and carcass characteristics of broiler chicks. In IOP Conference Series: Earth and Environmental Science (Vol. 1302, No. 1, p. 012053). IOP Publishing.
- [4] AL-Hadeedy, I. Y., Mohammed, A. K., & AL-Tikriti, S. S. (2023). Heritability and Genetic Correlation Between Productive and Biochemical Traits of White Japanese Quail. Journal of Kirkuk University for Agricultural Sciences, 14(3).
- [5] Hasan, M. O., Hasan, T. K., & Naji, S. A. H. (2020, August). Effect of adding pumpkin and flax oil to diets on the meats physical and chemical traits of broilers. In IOP Conference Series: Earth and Environmental Science (Vol. 553, No. 1, p. 012009). IOP Publishing.
- [6] Al-Baadani, H. H., Al-Mufarrej, S. I., Al-Garadi, M. A., Alhidary, I. A., Al-Sagan, A. A., & Azzam, M. M. (2021). The use of gum Arabi as a natural prebiotic in animals: A review. Animal feed science and technology, 274, 114894.
- [7] Ismail, F. S. A., Hassan, R. A., & Abu El-Hassan, E. A. (2016). Effect of gum arabic on egg production and some blood constituents of laying hens under hot summer conditions in Egypt. Journal of Animal and Poultry Production, 7(3), 77-83.
- [8] NM, A. R., MK, S., & Idris, O. F. (2010). Effect of Gum Arabic (Acacia senegal, L. Willd) on lipid profile and performance of Laying Hens. Journal of Applied biosciences, 32, 2002-2007.
- [9] Kishimoto, A., Ushida, K., Phillips, G. O., Ogasawara, T., & Sasaki, Y. (2006). Identification of intestinal bacteria responsible for fermentation of gum arabic in pig model. Current microbiology, 53, 173-177.
- [10] Calame, W., Weseler, A. R., Viebke, C., Flynn, C., & Siemensma, A. D. (2008). Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. British journal of nutrition, 100(6), 1269-1275.
- [11] Adil, S., Banday, T., Bhat, G. A., Mir, M. S., & Rehman, M. (2010). Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Veterinary medicine international, 2010(1), 479485.
- [12] Gultemirian, M. D. L., Corti, H. R., Chaia, A. P., & Apella, M. C. (2014). Fermentation in vitro of a mixture of dietary fibers and cane molasses by the cecal microbiota: Application on mineral absorption through the laying hen's colonic epithelium. Animal Feed Science and Technology, 191, 76-82.
- [13] Khojah, E. Y. (2017). Biological effects of low protein diet with gum Arabic on rat's chronic kidney disease. Adv. Environ. Biol, 11(4), 60-69.
- [14] El-Ratel, I. T., Ismail, R. F., & Fouda, S. F. (2019). Productive performance, carcass traits, lipid profile, antioxidants and immunity of growing rabbits treated with gum Arabic under Egyptian summer condition. Egyptian Journal of Nutrition and Feeds, 22(2), 383-394.
- [15] Al-Yasin, Ali Abdul Khaliq. (1990). Poultry - Food and Nutrition. By J.P Patr.H Schaible.
- [16] Leite, A. M., Lima, E. D. O., Souza, E. L. D., Diniz, M. D. F. F. M., Trajano, V. N., & Medeiros, I. A. D. (2007). Inhibitory effect of beta-pinene, alpha-pinene and eugenol on the growth of potential infectious endocarditis causing Gram-positive bacteria. Revista Brasileira de Ciências Farmacêuticas, 43, 121-126.

- [17] Phillips, M. A., Wildung, M. R., Williams, D. C., Hyatt, D. C., & Croteau, R. (2003). cDNA isolation, functional expression, and characterization of (+)- α -pinene synthase and (-)- α -pinene synthase from loblolly pine (*Pinus taeda*): Stereocontrol in pinene biosynthesis. *Archives of Biochemistry and Biophysics*, 411(2), 267-276.
- [18] Yang, H., Woo, J., Pae, A. N., Um, M. Y., Cho, N. C., Park, K. D., ... & Cho, S. (2016). α -Pinene, a major constituent of pine tree oils, enhances non-rapid eye movement sleep in mice through GABAA-benzodiazepine receptors. *Molecular pharmacology*, 90(5), 530-539.
- [19] Spring, P., Wenk, C., Dawson, K. A., & Newman, K. E. (2000). The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. *Poultry science*, 79(2), 205-211.
- [20] Hart, P. H., Brand, C., Carson, C. F., Riley, T. V., Prager, R. H., & Finlay-Jones, I. J. (2000). Terpinen-4-ol, the main component of the essential oil of *Melaleuca alternifolia* (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. *Inflammation Research*, 49, 619-626.
- [21] Mondello, F., De Bernardis, F., Girolamo, A., Cassone, A., & Salvatore, G. (2006). In vivo activity of terpinen-4-ol, the main bioactive component of *Melaleuca alternifolia* Cheel (tea tree) oil against azole-susceptible and-resistant human pathogenic *Candida* species. *BMC infectious diseases*, 6, 1-8.
- [22] Tighe, S., Gao, Y. Y., & Tseng, S. C. (2013). Terpinen-4-ol is the most active ingredient of tea tree oil to kill *Demodex* mites. *Translational Vision Science & Technology*, 2(7), 2-2.
- [23] Roberfroid, M. B. (2000). Prebiotics and probiotics: are they functional foods? *The American journal of clinical nutrition*, 71(6), 1682S-1687S.

Effect of Adding Gum Arabic to the Quails Diet on the Qualitative Characteristics of Stored Eggs

Yasin J. Mohamm¹ and Mokhalad Oraibi Hasan²

^{1,2}Department of Animal Production, Faculty of Agriculture, University of Tikrit, Tikrit, Iraq.

¹E-mail: yasin.j.mohammed001@st.tu.edu.iq

²E-mail: Mokhalad082@tu.edu.iq

Abstract. This study was conducted in the quail breeding hall of the Department of Animal Production, Faculty of Agriculture, University of Tikrit to determine the effect of adding gum Arabic to the quail larva on the qualitative characteristics of eggs stored for three weeks at room temperature. The result of the study showed that there were no significant differences in all qualitative characteristics of the stored eggs in the first week except for the significant superiority of the fourth treatment over the rest of the treatments in the yolk index characteristic. In the second week of the storage period, the third treatment significantly outperformed the control treatment and the rest of the treatments in the whiteness index, and the third and fourth treatments recorded significant superiority over the first and second treatments in the Hue unit, as well as the second and fourth treatments outperformed the first and third treatments in the yolk index, and also the fourth treatment outperformed the first treatment in pH, while in the third week of the storage period, the fourth treatment significantly outperformed the third treatment in relative shell weight, and the fourth treatment significantly outperformed the whiteness index and Hue unit over the rest of the treatments, the third and fourth treatments were significantly superior to the other treatments. The treatments did not differ significantly in the average weight of eggs and the relative weight of whites and yolks, as well as the pH of yolks and whites. We conclude from the present study that the addition of gum Arabic to the quail perch resulted in significant improvement in most qualitative traits and decreased oxidation indices of eggs stored at room temperature.

Keywords. Gum Arabic, Quails, Egg quality traits, Oxidative indicators.

1. Introduction

As a result of the change in the level of human lifestyle in many countries, as well as the economic and living development of those countries, he resorted to searching for good food elements, including bird products from meat and eggs these sources are important sources of animal protein, as developed countries tended to develop this source by raising different types of birds, such as the Japanese quail to produce eggs mainly [1,2] Which is considered of high nutritional value due to the high biological value of protein and its content of mineral elements and vitamins needed by the human body [3,4]. Egg yolk is one of the most important substances rich in cholesterol, and researchers and specialists all over the world have realized the necessity of using natural additives in human consumption products, due to the severe adverse effects

caused by the use of synthetic additives. This shift led to the search for natural sources that are safer for the consumer and the animal itself [5] and one of these natural additives is Gum Arabic, which is the secretions of the acacia tree, which comes mainly from the countries of the African Sahel, and is considered a natural supplement that plays an important role in intestinal and hepatic metabolism and has other potential health benefits. This review evaluates the effects and mechanisms of action of GA as a natural biomaterial on overall health and growth performance in livestock and poultry.

Arabic Gum is a natural food supplement, recognized by the US Food and Drug Administration as one of the safest food additives for humans and is a dried gum exudate obtained from the stems and branches of Senegal Acacia trees or other related African species, These trees are abundant in West Africa and the State of Sudan [6] Gum Arabic is a natural biomaterial containing high molecular weight glycoproteins, which are carbohydrate groups with peptide chains, if we obtain sugar These carbohydrates are complex and simple sugars and when hydrolyzed arabinose, galactose, rhamnose, and glucuronic acid. Gum Arabic stimulates the growth and activity of beneficial bacteria in the intestinal tubule as well as in the fermentations that occur in the cecal region, and it contains organic substances, amino acids, and minerals [7] found that adding gum Arabic to the diet of laying hens led to a significant increase in total egg weight ($P < 0.05$) compared to the control treatment. Egg length, egg diameter, shape index, and shell percentage were not affected. A decrease was observed in shell weight, shell thickness, albumin weight, albumin diameter, and yolk weight for birds fed a control diet. Albumin height was significantly lower ($P < 0.05$) for birds fed 5% gum arabic. Moreover, albumin percentage, Hugh unit, yolk diameter, yolk index, yolk percentage, and yolk color did not differ significantly ($P > 0.05$) between treatments[8]. The results of the study conducted by [9] showed that adding different levels of gum Arabic to the diets led to a significant increase ($P \leq 0.05$) in egg weight, eggshell percentage, and shell thickness compared to the control group.

The study aimed to evaluate the effect of adding gum Arabic gum to the basal diet of laying quail on the qualitative characteristics of stored eggs and lipid oxidation indices of stored eggs.

2. Materials and Methods

This study was conducted in the field of poultry birds belonging to the Department of Animal Production - Faculty of Agriculture - University of Tikrit operation with laboratories in The Ministry of Science and Technology Ethical approval No. ISO 17025 and lasted for (56) days.

The birds were randomly distributed inside the breeding cages into four treatments, each treatment has four replicates with four birds in each replicate. The first treatment was without addition, while the second, third, and fourth treatments added 0.3, 0.6, and 0.9% gum Arabic to the feed, respectively, and the lighting program was 8 hours of darkness and 16 hours of light Feed and water were provided freely to the birds throughout the study period, Gum Arabic was purchased from local markets in the city of Tikrit in the form of powder. Eggs from the first experiment were stored at room temperature for three weeks and the qualitative characteristics of the eggs were taken for each week of the storage period.

2.1. Measuring the Qualitative Characteristics of the Eggs

The total weight of the egg was taken with a sensitive scale and the length and width of the egg were taken by (Vernia) then the egg was broken on a flat surface to take measurements of the inner egg length and diameter of the white, white height, white diameter, yolk diameter, yolk color, yolk weight, yolk weight, shell weight with membranes, shell thickness and the yolk and white index was calculated according to the following equations:

$$\text{Whiteness index} = \frac{\text{Whiteness height (mm)}}{\text{Whiteness Diameter (mm)}} \left\{ \frac{\text{White Length} + \text{Whiteness Width}}{2} \right\} \times 100\%$$

$$\text{Yolk index} = \frac{\text{Yolk height (mm)}}{\text{Yolk Diameter (mm)}}$$

2.2. Estimation of Oxidation Markers for Yolk

2.2.1. Determination of Peroxide Value

According to [10] 2 g of extracted fat was weighed using a succulent apparatus and estimated according to the following equation:

$$\text{Peroxide number (m Eq)} = \frac{(\text{Number of millimeters of sodium thiosulfate} \times 0.01 \times 1000)}{\text{Weight of the sample}}$$

2.2.2. Estimation of Thiobarbituric Value

Lipid oxidation in the model was measured by estimating thiobarbituric acid according to the method [11] and according to the following equation:

$$\text{TBA value mg / MDA kg} = A530 \times 5.2$$

2.2.3. Estimation of Fatty Acid Concentration

Free Fatty Acids were estimated according to the method [10] and the method is summarized by cold extraction of fat.

2.2.4. Statistical Analysis

The data of this study was analysis complet randomized design by SAS program and the different between means of treatment according Duncan [11].

3. Results and Discussion

The results of Table (1) show the effect of adding gum Arabic to the quail bird perch on the qualitative characteristics of the stored eggs, as it was observed in the first week that there was no significant difference in all studied traits except for the yolk index trait, where the fourth treatment was significantly superior to the rest of the experimental treatments with a value of 41.39, [12] found that albumin weight was significantly lower for birds fed a control diet, albumin weight was higher for birds fed 2.5% Arabic gum, and albumin height was significantly lower for birds fed 5% Arabic gum compared to other birds. Albumin diameter was significantly lower for birds fed the control diet compared to other birds. The ratio of albumin and HAU did not differ between all dietary groups. Yolk weight was significantly lower in the control group, while albumin weight was higher in the group fed with 7.5% Arabic gum, and yolk weight was significantly lower in the group fed with 5% Arabic gum compared to the other treatment, and yolk diameter, yolk index, yolk ratio, and yolk color did not differ between all dietary groups.

Table 1. Impact using gum Arabic on quail's diet on egg qualities stored for 1 week.

Treatments Characteristics	First	Second	Third	Forth
Egg weight	10.43 + 0.32	10.81+ 0.36	10.50+ 0.59	11.28+ 0.93
Shell weight%	12.42 + 0.44	12.49 + 0.73	12.84 + 0.74	12.11 + 0.77
Albumin weight%	53.61 + 1.39	51.94 + 0.68	52.55 + 2.92	53.80 + 1.94
Albumin index	7.40 + 0.39	7.56 + 0.11	7.91 + 0.73	8.55 + 0.74
pH Albumin	8.00 + 0.20	8.00 + 0.20	8.00 + 0.20	8.50 + 0.28
Haugh unit	80.91 + 0.65	80.22 + 0.21	80.20 + 1.16	82.08 + 2.01

Yolk weight%	33.97 + 0.99	35.56 + 0.71	34.60 + 2.88	34.09 + 1.17
Yolk index	39.94 + 1.05 b	35.57 + 0.65 b	38.96 + 2.20 b	41.93 + 0.18 a
pH Yolk	7.62 + 0.12	7.62 + 0.12	7.75 + 0.14	7.75 + 0.14

Table (2) shows the effect of adding gum Arabic to quail bird perch on some qualitative characteristics of the eggs after two weeks of storage period, as it shows that there are no significant differences in the average weight of the eggs and the relative weight of the shell, white and yolk, in addition to the pH of the yolk and white, the third treatment was significantly superior to the control and the rest of the treatments, the third treatment was superior to the control and the rest of the treatments The third and fourth treatments outperformed the control and the second treatment, the second and fourth treatments outperformed the control and the third treatment in the whiteness index, and the fourth treatment outperformed the first treatment in pH and did not differ significantly with the second and third treatments.

[13] their study indicated the effect of adding different levels of gum Arabic powder to the treatments of laying hens at the age of 20 weeks, in proportions (0, 5, 10, and 15%). The results showed a significant decrease ($p < 0.05$) in serum cholesterol level for the 15% gum Arabic powder supplementation treatment (80 mg/100 ml) compared to the control treatment (180 mg/100 ml). This decrease also led to a reduction in egg yolk cholesterol (60 mg/100 ml). The results also indicated a significant ($p < 0.05$) gradual increase in egg yolk calcium and phosphorus content, which may be attributed to the role of gum Arabic in improving the efficiency of absorption of calcium, phosphorus, and other minerals in the blood, which is reflected in the observed increase in egg yolk.

Table 2. Impact using gum Arabic on quail's diet on egg qualities stored for 2 weeks.

Treatments Characteristics	First	Second	Third	Forth
Egg weight	10.42 + 0.30	11.73 + 0.16	11.20 + 0.58	11.32 + 0.92
Shell weight%	11.92 + 0.33	10.94 + 0.34	11.84 + 0.30	12.05 + 1.18
Albumin weight%	54.13 + 0.78	51.96 + 3.48	51.38 + 2.98	55.59 + 2.96
Albumin index	8.62 + 0.25 b	8.55 + 0.30 b	10.80 + 0.41 a	8.41 + 0.21 b
pH Albumin	8.87 + 0.12	8.87 + 0.12	8.87 + 0.12	8.87 + 0.12
Haugh unit	83.12 + 0.04 b	81.64 + 0.85 b	86.80 + 0.79 a	83.29 + 1.37 a
Yolk weight%	33.93 + 0.64	37.09 + 3.61	36.77 + 2.84	32.35 + 1.99
Yolk index	36.16 + 1.10 b	42.44 + 0.90 a	37.20 + 2.78 b	40.80 + 0.61 a
pH Yolk	8.25 + 0.14 b	8.62 + 0.23 ab	8.75 + 0.14 ab	8.87 + 0.12 a

Table (3) shows the effect of adding gum Arabic to the quail bird perch on some qualitative characteristics of the eggs after three weeks of the storage period, as it shows that there are no significant differences in the average weight of eggs and the relative weight of whites and yolks in addition to the pH of the yolks and whites, the fourth treatment was significantly superior to the third treatment in the relative weight of the shell and did not differ significantly with the first and second treatment, the fourth treatment was superior in the whiteness index and Hue unit to the control treatment and the second and third treatments. The third treatment in the relative weight of the shell did not differ significantly from the first and second treatments, the fourth treatment in the whiteness index and Hue unit was superior to the control treatment, and the second and third treatments, the third and fourth treatments were superior to the control treatment and the second treatment in the yolk index characteristic. The results of the study agreed with [14] who studied the effect of gum Arabic as a supplemental food on serum lipids, egg yolk, meat, and the performance of laying hens. Egg weight, egg weight, and daily egg production increased significantly ($P \leq 0.05$) in egg weight and there was no significant difference in daily

egg production. The results indicate that the addition of gum Arabic as a dietary supplement to laying hens can lead to a significant decrease in triglycerides, total lipids, and phospholipids, and a significant increase in egg and body weight. This was attributed to the high soluble fiber content of gum Arabic.

Table 3. Impact of using gam Arabic on quail's diet on egg qualities stored for 3 weeks.

Treatments Characteristics	First	Second	Third	Forth
Egg weight	10.91 + 0.03	10.56 + 0.16	10.73 + 0.40	10.20 + 0.09
Shell weight%	12.04+0.63ab	11.14 + 0.17ab	10.72 + 0.49b	12.26 + 0.43 a
Albumin weight%	51.89 + 1.21	53.60 + 0.45	52.48 + 2.34	50.19 + 1.20
Albumin index	8.90 + 0.23 b	9.76 +0.34 b	9.90 +0.37 b	11.73 + 0.44 a
pH Albumin	8.87 + 0.12	8.87 + 0.12	8.87 + 0.12	8.87 + 0.12
Haugh unit	80.01 +0.16 c	81.32 +0.73 bc	82.81 +0.62 b	86.22 + 0.78 a
Yolk weight%	36.05 + 0.58	35.24 + 0.45	36.79 + 2.13	37.54 + 0.85
Yolk index	30.22 +0.10 c	33.21 + 0.40 b	34.79 +0.41 a	35.76 + 0.82 a
pH Yolk	8.87 + 0.12	8.87 + 0.12	8.87 + 0.12	8.87 + 0.12

The results of Table (4) showed the effect of gum Arabic on lipid oxidation indices of stored quail eggs showed a decrease in the oxidation indices of peroxide value (PV), thiobarbituric acid (TBA), and free fatty acids (FFA) for all the addition treatments compared with the control treatment. This may be due to the limonene compound present in Gum Arabic at 10.25%, which plays an important role in oxidative processes that serve the preservation of meat for a period of time [15,16].

Table 4. Impact of using gam Arabic on quail's diet on oxidation indexes of stored eggs.

Treatments Characteristics	First	Second	Third	Forth
PV	6.255 +0.027 a	5.047 +0.022 b	4.742 + 0.026c	4.265 +0.018 d
TBA	0.055 + 0.001 a	0.037 +0.0004 b	0.033+0.0004c	0.025 +0.0008 d
FFA	0.615 + 0.006 a	0.435 +0.009 b	0.362 +0.010 c	0.305 +0.002 d

Conclusions

We conclude from the current study that the addition of gum Arabic to the quail birds at 0.9% resulted in a significant improvement in most qualitative traits and reduced oxidation indices for eggs stored at room temperature, and this preserved the eggs for a longer period of time.

References

- [1] Al-Saada, Q., Al-Nasseri, A. N., Hasan, M. O., & Taha, M. W. (2024, February). Effect of *Rhus coriaria* L. on performance productive, physiological and carcass characteristics of broiler chicks. In IOP Conference Series: Earth and Environmental Science (Vol. 1302, No. 1, p. 012053). IOP Publishing.
- [2] Abbasi, I. A., Shamim, A., Shad, M. K., Ashari, H., & Yusuf, I. (2024). Circular economy-based integrated farming system for indigenous chicken: Fostering food security and sustainability. *Journal of Cleaner Production*, 436, 140368.
- [3] Nayyef, S. H., Wahab, M. H. A., Ahmed, N. M., & Hasan, M. O. (2023, July). The Effect of *Cupressus arizonica* Fruit Ethyl Alcoholic Extract Toward some Bacterial Species that Isolated from Different Infections of Human Body's Sites. In IOP Conference Series: Earth and Environmental Science (Vol. 1213, No. 1, p. 012049). IOP Publishing.

- [4] Hasan, M. O., Al-Kaisi, H. R. M., Al-Samrai, M. K., & Aljumaily, T. K. H. (2024, April). Study the qualities characteristics of stored quail eggs in the refrigerator which it's fed with Aloe vera. In AIP Conference Proceedings (Vol. 3079, No. 1). AIP Publishing.
- [5] Al-Baadani, H. H., Alhotan, R. A., & Azzam, M. M. (2023). Evaluation of the interaction between gum Arabic addition and stocking density on growth performance, carcass characteristics, and general health parameters of broiler chickens. *Animals*, 13(19), 3024.
- [6] Hay, T. O., Kontogiorgos, V., Thompson, S., Nastasi, J. R., & Fitzgerald, M. (2024). A new hydrocolloid to rival gum Arabic: Characterisation of a traditional food gum from Australian *Acacia cambagei*. *Food Hydrocolloids*, 153, 110003.
- [7] Sulieman, A. M. E. H. (2018). Gum arabic as thickener and stabilizing agents in dairy products. *Gum arabic*, 151-165.
- [8] Abdalla, S. A., Abdelatti, K. A., Malik, H. E., Elamin, K. M., & Dousa, B. M. (2015). Effect of dietary inclusion of gum arabic (*Acacia senegal*) on layer hen performance, egg quality and egg cholesterol.
- [9] Ismail, F. S. A., Hassan, R. A., & Abu El-Hassan, E. A. (2016). Effect of gum arabic on egg production and some blood constituents of laying hens under hot summer conditions in Egypt. *Journal of Animal and Poultry Production*, 7(3), 77-83.
- [10] Egan, H., Kirk, R. S., & Sawyer, R. (1981). *Pearson's Chemical Analysis of Foods*. Eight Edition. Churchill Livingstone, New York. L (Fabaceae).
- [11] Duncan, D.B. 1955. Multiple range and multiple F test. *Biometrics*.11:1 -42.
- [12] Witte, V. C., Krause, G. F., & Bailey, M. E. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of food Science*, 35(5), 582-585.
- [13] Abdalla, S.A., Abdel-Atti, K.A., Malik, H.E.E., Elamin, K.M., and Dousa B.M. (2015). Effect of Dietary Inclusion of Gum Arabic (*Acacia Senegal*) On Layer Hen Performance Egg Quality and Egg Cholesterol. *Global Journal of Animal Scientific Research*, 3(3), 636-640.
- [14] Elkhier, M.K., Ishag, K.E., Yagoub, A.A., and Abu Baker, A.A. (2009). Supplementation Laying Hen Diet with Gum Arabic (*Acacia Senegal*): Effect on Egg Production, shell thickness and Yolk content of cholesterol, calcium and phosphorus. *Asian J. Poult. Sci.*, 8, 1 -3.
- [15] Abd-Razig, N. M., Sabahelkhier, M. K., & Idris, O. F. (2010). Effect of gum Arabic (*Acacia senegal*, L. Willd) on lipid profile and performance of laying hens. *Journal of Applied Biosciences*, 2002. Published online at www.biosciences.elewa.org on August 9, 2010
- [16] Soulimani, R. ; J. Bouayed and R. K. Joshi (2019). Limonene: natural monoterpene volatile compounds of potential therapeutic interest . *American j. of essential oils and natural products* , 7(4), 1-10.

Effect of Using Algae Oil (Sources of Omega-3) on the Productive Performance of Broiler

Mustafa Saleh Hadi¹, Maad A. K. Albaddy² and Mokhalad Oraibi Hasan³

¹⁻³Department of Animal Production, College of Agriculture, Tikrit University, Tikrit, Iraq.

²E-mail: maadalbaddy@tu.edu.iq

Abstract. The experiment aimed to study the effect of using algae oil (plant sources of omega-3) and its effect on the productive performance of broiler. This experiment was conducted in the field of Anwar Al-Faris Company - Research Hall - in Babylon province, for the period from 1/3/2024 to 5/4/2024. The experiment included 360 unsexed broiler from the commercial strain ROS-308. The chicks were raised on the litter and randomly distributed into 6 treatments, each treatment had 3 replicates, and each replicate included 20 chicks. Which were fed on three productive rations, the first ration was a starter without adding algae oil (1-10) days prepared by Anwar Fares Company, the second ration was growth, and the third was final, algae oil was added to it as follows: T1 (control treatment) using 100% sunflower oil, T2 replacing 20% of sunflower oil with algae oil, T3 replacing 40% of sunflower oil with algae oil, T4 replacing 60% of sunflower oil with algae oil, T5 replacing 80% of sunflower oil with algae oil, T6 replacing 100% of sunflower oil with algae oil, and these rations were similar in energy and protein levels. The results showed no significant differences in body weight, weight gain, feed conversion ratio and feed consumption at 35 days of age. Also, no significant difference was observed for total weight gain, total feed conversion efficiency and cumulative feed consumption for the algae oil supplementation treatment compared to the control treatment.

Keywords. Omega-3, Algae Oil, Broiler feeds.

1. Introduction

The poultry industry is considered one of the basic and important pillars in meeting part of the human nutritional needs, and thus it has achieved great progress in terms of nutrition and economy in recent years, as the productivity of poultry has increased significantly and with high efficiency, as a result of applied research in various fields of science in this industry, most notably nutrition science [1]. Therefore, recent studies and research have tended towards finding alternatives without side effects, including adding antibiotics and stimulants to poultry feed for many years in order to control diseases, improve production performance, and avoid the risks of antibiotic residues and their impact on the health of birds and consumers [2,3]. In addition to the exposure of protein sources that enter into the composition of feed, including soybeans, yellow corn, and fish meal, which are traditional components of poultry feed, to negative environmental effects, including the deterioration of agricultural lands, drought, water scarcity, and gas emissions [4,5]. Also in light of the upcoming increase in population, which is expected to reach

seven billion by 2050 [6]. As a result of the increasing consumer demand for these resources, the ongoing environmental degradation and climate change, the need has become necessary to shift to more sustainable feeds in the animal production diet [7]. There has been a trend towards using marine plants and natural resources to improve animal health and production due to their anti-inflammatory, immune, antioxidant and antibacterial functions, as they are considered active and effective substances [8]. Including seaweed, which represents a large and new source as one of the types of natural food additives that can be added to poultry feed due to its good content of nutrients, nutritional compositions, richness in minerals, fatty and amino acids, and other health benefits [9]. Algae oils are also characterized by containing long-chain unsaturated fatty acids such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and have various benefits for human health, as they reduce the risk of heart disease, promote eye health, and combat inflammation [10]. [11] indicated that the use of algae rich in Docosahexaenoic acid (DHA) at a rate of 1% and 2% (1.7 and 3.4 g DHA / kg feed) No significant difference was observed while adding it at a rate of 4% (6.8 g DHA / kg feed) led to a decrease in productive characteristics by 19% compared to the control treatment. In general, food additives containing omega-3 sources have a better effect on the productive characteristics of poultry birds, and no harmful effect was observed when consuming these feeds in all studies conducted so far [12]. Therefore, this study aimed to add these important elements, which are an important source of omega-3, to broiler feed, including algae oil rich in Docosahexaenoic acid, and to know its effect on productive performance.

2. Materials and Methods

This experiment was conducted in the field of Anwar Al-Faris Company - Research Hall - in Babylon province, Operation with laboratories in of Al-Furat Al-Awsat Technical University. Ethical approval No. 151LBI04002. for the period from 1/3/2024 to 5/4/2024 using 360 unsexed broiler of the Ross308 strain from the Anwar hatchery in Babylon province. The average initial weight of the chick was 40 g/bird, as they were randomly distributed into 6 treatments, each treatment had 3 replicates of 20 birds/replication. The birds were raised for 35 days in the ground rearing hall. Modern methods were followed in preparing the hall and equipping it with all the requirements for rearing, including feeders and watering places, as well as the appropriate temperature. The chicks were fed from the age of one day until the end of the experiment (35 days) on three diets, the first without adding algae oil, and the second and third with algae oil added to them, as shown in Table 1, T1 (control treatment) using 100% sunflower oil, T2 replacing 20% of oil Sunflower with algae oil, T3 40% replacement of sunflower oil with algae oil, T4 60% replacement of sunflower oil with algae oil, T5 80% replacement of sunflower oil with algae oil, T6 100% replacement of sunflower oil with algae oil. According to the chemical composition according to the recommendations of NRC (1994), the feed materials for the second and third feed were prepared from local markets, and the feed was crushed in one of the feed factories in Babylon province. As for the first feed, it was prepared by Anwar Al-Faris Company. The following production characteristics were studied: live body weight, weight gain (g/bird), amount of feed consumed, feed conversion ratio, and total mortality rate. The statistical program Statistical Analysis System -SAS (2018) was used to analyze the data to study the effect of different treatments on the studied traits according to a completely random design (CRD), and the significant differences between the averages were compared using the [13] multinomial test.

Table 1. Components of broiler feeds with the calculated chemical composition.

Feed materials	Diets	
	Grower	Finisher
Corn (%)	45	46.3
Soybean Meal (48%)	31.1	26
Wheat	19.35	23.35
*Premix (%)	2.5	2.5
Sunflower Oil (%)	1	1
Limestone (%)	0.8	0.6
Table Salt (%)	0.25	0.25
Total	100	100
**Calculated chemical composition		
Metabolic energy (kcal/kg feed)	3075	3119
Crude protein (%)	21.51	19.63
Crude fiber (%)	2.38	2.36
Lysine (%)	1.27	1.16
Methionine (%)	0.67	0.65
Methionine + Cysteine (%)	1.01	0.97
Calcium (%)	0.79	0.71
Phosphorus (%)	0.48	0.48

*The premix used is of Turkish origin and consists of Crude Protein 21.46%, Sodium 5.53%, AV Phosphorus 15.75%, Crude FAT 1.04%, Crude Fiber 0.21%, Crude Ash 60.84%, Lysine 11.74%, Methionine 14.48%, Meth + CYS 15.22, Linoleic Acid 0.50%, Potassium 0.03%, Chloride 9.78 %, Arginine 0.80%, Threonine 2.06, Leucine 0.80%, Isoleucine 1.36%, Valine 0.80%, Tryptophan 0.90%, Phenylalanine 0.80% ,3a672 Vitamin-A520.000,00IU/kg , 3a671Vitamin -D3 200.000,00 IU/kg , 3a700 Vitamin-E 2.800,00mg/kg ,3a710Vitamin -K3 120,00mg/kg ,3a821Vitamin -B1 120,00 mg/kg

** According to the chemical composition of the materials included in the feed composition according to [14].

3. Results and Discussion

3.1. Body Weight and Weight Gain

It is noted from Tables (2 and 3) the effect of different treatments in some breeding periods on live body weight and weight gain. Table (2) shows a significant difference in live body weight during the rearing period (11-24) days, where the birds in the 60% algae oil treatment (T4) outperformed, as the average weight of the birds reached (1032.50 g) compared to the weight of the birds in the 100% algae oil treatment (T6), while it did not differ from the birds in the T1, T2, T3, T5 treatments, while the birds in these treatments did not differ with the T6 treatment. While significant differences ($P < 0.05$) did not appear between the birds in the adding treatments and the control treatment in live body weight during the end of the experiment period (25-35). As for Table (3), it is noted that there is a significant difference in the rate of weight gain for the algae oil addition treatments during the rearing period (11-24) days of rearing, as a significant superiority ($P < 0.05$) was observed for the birds in the 60% algae oil treatment (T4) compared to the 100% algae oil treatment. (T6) While it did not differ significantly with birds of treatments T1, T2, T3, T5, as these treatments did not show a significant difference with birds of treatment T6. There were no significant differences for the period (25-35) days of rearing with regard to the rate of weight gain as well as the total.

Table 2. Effect of the studied treatments on the rate of body weight for different ages.

Treatments	Mean $\pm$ standard error (g/bird)		
	10-1day	24-11day	35-25day
T1	1.92 $\pm$ 291.40	23.01 $\pm$ 982.75ab	10.88 $\pm$ 1896.50
T2	3.38 $\pm$ 299.83	14.11 $\pm$ 1002.67ab	45.14 $\pm$ 1956.17
T3	1.87 $\pm$ 303.75	36.12 $\pm$ 978.75ab	56.22 $\pm$ 1937.25
T4	9.01 $\pm$ 302.25	13.44 $\pm$ 1032.50a	55.26 $\pm$ 1959.42
T5	1.50 $\pm$ 305.75	8.89 $\pm$ 1001.17ab	24.72 $\pm$ 1969.92
T6	1.46 $\pm$ 295.00	15.31 $\pm$ 946.25b	21.05 $\pm$ 1881.00
Level of Significance	N.S	*	N.S

Means with different lowercase letters within a column and different uppercase letters within a row are significantly different from each other. * (P<0.05), N.S.: not significant.

Table 3. Effect of the studied treatments on the rate of weight gain for different ages Feed consumption.

Treatments	Mean $\pm$ standard error (g/bird)			
	10-1day	24-11day	35-25day	Total
T1	251.56 2.64 $\pm$	21.25 $\pm$ 691.68 ab	913.91 12.92 $\pm$	10.89 $\pm$ 1856.50
T2	259.58 3.31 $\pm$	11.40 $\pm$ 702.83 ab	953.50 31.20 $\pm$	45.25 $\pm$ 1916.33
T3	265.75 3.59 $\pm$	34.49 $\pm$ 674.67 ab	958.50 20.11 $\pm$	56.21 $\pm$ 1897.25
T4	262.58 9.16 $\pm$	7.22 $\pm$ 730.46a	6.31 $\pm$ 965.03	55.25 $\pm$ 1919.42
T5	265.33 1.42 $\pm$	9.26 $\pm$ 695.25 ab	973.25 14.67 $\pm$	24.72 $\pm$ 1929.92
T6	254.75 1.28 $\pm$	14.73 $\pm$ 651.00 b	934.83 29.49 $\pm$	$\pm$ 1840.92 Docosaheaxaenoic acid 20.97
Level of Significance	N.S	*	N.S	N.S

Means with different lowercase letters within a column and different uppercase letters within a row are significantly different from each other. * (P<0.05), N.S.: not significant.

Table (4) shows the effect of different algae oil addition treatments on the amount of feed during the rearing periods (11-24, 25-35) days. The table shows no significant differences (P<0.05) between birds treated with algae oil and birds treated with the control treatment during the rearing periods (11-35) days.

Table 4. Effect of the studied treatments on the rate of feed consumed for different ages.

Treatments	Mean $\pm$ standard error (g/bird)			
	10-1day	24-11day	35-25day	Total
T1	1.01 $\pm$ 314.25	6.62 $\pm$ 1111.00	10.25 $\pm$ 1552.33	326.63 $\pm$ 2644.25
T2	5.64 $\pm$ 317.50	24.17 $\pm$ 1138.00	52.92 $\pm$ 1563.67	82.22 $\pm$ 3019.17
T3	2.75 $\pm$ 316.67	34.57 $\pm$ 1134.83	4.75 $\pm$ 1632.00	39.13 $\pm$ 3083.50
T4	6.33 $\pm$ 312.17	41.95 $\pm$ 1172.58	52.02 $\pm$ 1566.58	100.30 $\pm$ 3051.42
T5	4.71 $\pm$ 320.83	20.22 $\pm$ 1106.53	26.82 $\pm$ 1580.50	28.85 $\pm$ 2992.83
T6	7.48 $\pm$ 315.67	28.75 $\pm$ 1085.33	42.01 $\pm$ 1572.33	7.14 $\pm$ 2973.33
Level of Significance	N.S	N.S	N.S	N.S

N.S .

3.2. Feed Conversion Ratio

Table (5) shows the effect of different treatments to which algae oil was added on the feed conversion ratio for the rearing periods (11-24, 25-35) days. No significant differences were observed between birds treated with algae oil during the rearing period (11-35) days, compared with birds treated with control regarding the feed conversion factor for the same period of time as well as the cumulative feed conversion factor. These results are consistent with Long et al. (2018) where (indicating that adding algae and its oil rich in Docosahexaenoic acid (0.55 and 2.55 g DHA / kg feed) in broiler feed did not affect the productive traits. While these results differed from [15] who stated that birds fed a basic diet with algae rich in Docosahexaenoic acid (DHA) added at a rate of 1.2 g DHA / g feed and a diet with algae oil rich in the same acid added at a rate of 1.5 and 3.0 g ADH / g, a highly significant increase ($P>0.05$) was observed in the traits Productivity.

Table 5. Effect of the studied treatments on the feed conversion efficiency rate for different ages.

Treatments	Mean $\pm$ standard error (kg meat/kg feed)			
	10day	24day ¹	35day ¹	Total
T1	0.008 $\pm$ 1.246a	0.051 $\pm$ 1.603	0.018 $\pm$ 1.693	0.173 $\pm$ 1.416
T2	0.003 $\pm$ 1.216ab	0.012 $\pm$ 1.613	0.007 $\pm$ 1.636	0.006 $\pm$ 1.570
T3	0.006 $\pm$ 1.190b	0.034 $\pm$ 1.676	0.030 $\pm$ 1.700	0.025 $\pm$ 1.620
T4	0.017 $\pm$ 1.186b	0.050 $\pm$ 1.600	0.048 $\pm$ 1.617	0.008 $\pm$ 1.586
T5	0.023 $\pm$ 1.203ab	0.028 $\pm$ 1.586	0.037 $\pm$ 1.620	0.018 $\pm$ 1.546
T6	0.024 $\pm$ 1.233ab	0.016 $\pm$ 1.663	0.021 $\pm$ 1.680	0.020 $\pm$ 1.610
Level of Significance	*	N.S	N.S	N.S

Means with different lowercase letters within a column and different uppercase letters within a row are significantly different from each other. * ($P<0.05$), N.S.: not significant.

Conclusions

The results showed no significant differences in body weight, weight gain, feed conversion ratio and feed consumption at 35 days of age. Also, no significant difference was observed for total weight gain, total feed conversion efficiency and cumulative feed consumption for the algae oil supplementation treatment compared to the control treatment.

References

- [1] Xu, J., R. Sangthong, E. McNeil, R. Tang, and V. Chongsuvivatwong .2020. Antibiotic use in chicken farms in northwest China. *Antimicrobial Resistance & Infection Control*, 9(1), 1-9.
- [2] Kareem, K.Y.; Abdulla, N.R.; Foo, H.L.; Zamri, A.N.M.; Shazali, N.; Loh, T.C.; Alshelmani, M.I. Effect of feeding larvae meal in the diets on growth performance, nutrient digestibility and meat quality in broiler chicken. *Indian J. Anim. Res.* 2018, 88, 1180–1185. [Google Scholar]
- [3] Veldkamp, T.; van Duinkerken, G.; van Huis, A.; Lakemond, C.M.M.; Bosch, G.; van Boekel, M.A.J.S. *Insects as a Sustainable Feed Ingredient in Pig and Poultry Diets—A Feasibility Study*; Wageningen UR Livestock Research: Lelystad, The Netherlands, 2012.
- [4] OECD/FAO. *OECD-FAO Agricultural Outlook 2020–2029*; OECD: Paris, France, 2020.
- [5] Finley, J.W. Evolution and future needs of food chemistry in a changing world. *J. Agric. Food Chem.* 2020, in press.
- [6] Abdel Aziz, E.A.; El-Nabtity, S.M.; El Barawy, A.A.M.; Saleh, M.A.M. Residues of Ceftiofur Sodium in Rabbit Tissues. *Zagazig Vet. J.* 2017, 45, 104–111
- [7] Lagha, A.B.; Haas, B.; Gottschalk, M.; Grenier, D. Antimicrobial potential of bacteriocins in poultry and swine production. *Vet. Res.* 2017, 48, 22.

- [8] Long SF, Kang S, Wang QQ, Xu YT, Pan L, Hu JX, et al. Dietary supplementation with DHA-rich microalgae improves performance, serum composition, carcass trait, antioxidant status, and fatty acid profile of broilers. *Poult Sci.* 2018;97:1881–90.
- [9] Khan IA, Parker NB, Lohr CV, Cherian G. Docosahexaenoic acid (22:6 n-3)-rich microalgae along with methionine supplementation in broiler chickens: effects on production performance, breast muscle quality attributes, lipid profile, and incidence of white striping and myopathy. *Poult Sci.* 2021;11:865–74.
- [10] Sun T, Tolba SA, Magnuson AD, Lei XG. Excessive *Aurantiochytrium acetophilum* docosahexaenoic acid supplementation decreases growth performance and breast muscle mass of broiler chickens. *Algal Res.* 2022;63:102648.
- [11] Coudert E, E. Elisabeth & C. Berri. 2020. Use of algae in poultry production: a review. *World's Poultry Science Journal*. Volume 76, Issue 4
- [12] Mahmoud Alagawany, Shaaban S. Elnesr, Mayada R. Farag, Mohamed E. Abd EL-Hack, Asmaa F. Khafaga, Ayman E. Taha, Ruchi Tiwari, Moha. Iqbal Yattoo, Prakash Bhatt, Sandip Kumar Khurana, and Kuldeep Dhama. 2019. Omega-3 and Omega-6 fatty acids in poultry nutrition: Effect on production performance and health. *Animals (Basel)*. 2019 Aug; 9(8): 57.
- [13] Sahil Kalia, Andrew D. Magnuson, Tao Sun, Guanchen Liu, Woo Kyun Kim, Zackary Johnson and Xin Gen Lei. 2023. Supranutrition of microalgal docosahexaenoic acid and calcidiol improved growth performance, tissue lipid profiles, and tibia characteristics of broiler chickens. *Journal of Animal Science and Biotechnology*.
- [14] Duncan, D.E. 1955. Multiple Range and Multiple F Tests. *Biometrics*. 11:4-42.
- [15] W.S. Harris, N.L. Tintle, F. Imamura, F. Qian, A.V.A. Korat, M. Marklund, L. Djoussé, J.K. Bassett, P.H. Carmichael, Y.Y. Chen, Y. Hirakawa, L.K. Küpers, F. Laguzzi, M. Lankinen, R.A. Murphy, C. Samieri, M.K. Senn, P. Shi, J.K. Virtanen, I.A. Brouwer, K.L. Chien, G. Eiriksdottir, N.G. Forouhi, J.M. Geleijnse, G.G. Giles, V. Gudnason, C. Helmer, A. Hodge, R. Jackson, K.T. Khaw, M. Laakso, H. Lai, D. Laurin, K. Leander, J. Lindsay, R. Micha, J. Mursu, T. Ninomiya, W. Post, B.M. Psaty, U. Risérus, J.G. Robinson, A.H. Shadyab, L. Snetselaar, A. Sala-Vila, Y. Sun, L.M. Steffen, M.Y. Tsai, N.J. Wareham, A.C. Wood, J.H.Y. Wu, F. Hu, Q. Sun, D.S. Siscovick, R.N. Lemaitre, D. Mozaffarian. 2021. Blood n-3 fatty acid levels and total and cause-specific mortality from 17 prospective studies. *Nat. Commun.*, 12 (2021), Article 2329.

Effect of Different Concentrations of Floratone Growth Regulator and Spray Dates on the Growth Traits of Two Types of Wheat *Triticum aestivum* L.

Sana Qasim Hussein¹ and Hawraa Ali Abbas²

^{1,2}Al-Furat Al-Awsat Technical University, Al-Mussaib Technical College, Najaf, Iraq.

¹E-mail: sanaqasim977@gmail.com

²E-mail: Hawraa.abbas@uokerbala.edu.iq

Abstract. A field experiment was carried out for the wheat crop *Triticum aestivum* L. for the winter season 2021-2020 in Babil Governorate / project area to see (the effect of different concentrations of floratone growth regulator and spray dates on the growth traits of two cultivars of wheat. The experiment was carried out according to the arrangement of split split plot and according to Randomized Complete Blok Design with three replications. The growth regulator (Floratone) was used with a concentration of (4.2.0) g.l.lt1- and the symbols for the coefficients (T3.T2.T1) were given and the sub-plates were filled by Plot sub sub. It was sprayed in three stages (branching stage, elongation stage, endothelial stage) and the following bananas were given (F1.F2.F3) and the secondary plot was occupied by sub plot. Two cultivars of wheat (Sham 6 and Latifia 2) were given the symbols (V1 and V2) and the main plot was filled. The results showed: The variety Sham 6 (V2) excels in all studied traits, as it exceeds the height of the plant, number of tillers.m², flag leaf area.cm², spikes number. m², grains number. Spike⁻¹, weight of 1000 tablets.gm and grain yield.ton.ha⁻¹ 103.12 cm, 439.76m², 34.52cm², 339.25m², 59.64, 36.74gm, 7.43ton.ha⁻¹. The results showed that F3 the date(stage of underlay) excelled in flag leaf area, number grain.Spike⁻¹, Weight of 1000 grain.gm and grain yield.ton.ha⁻¹, 35.43cm² 60.82 grain.Spike⁻¹, 36.44gm, 7.48 ton.ha⁻¹. As for the concentrations of the growth regulator (Floratone), the concentration T3 (4gm. L⁻¹) excelled in all studied traits and gave a grain yield of 6.96 ton.ha⁻¹. The results showed that there was a significant effect of bi interaction between the cultivars and the dates of spraying, as the combination F3 + V2 excelled in a weight of 1000 grain.gm and the grain yield.ton.ha⁻¹. 37.22 gm, 7.66 ton.ha⁻¹. While the combination F1 + V2 excels in the number of spikes (m²) and the number of grains.Spike⁻¹ only 341.08 spike.Spike.m², 60.91 grain.Spike⁻¹. The results also showed a significant effect of the interaction between the cultivars and the spray concentrations in some traits, The combination T3 + V2 excelled at a plant height.Cm, number of spikes. m² and grains number Spike⁻¹ only, 106.69 cm, 339.53 Spike.m², 61.48 grain.Spike⁻¹. Also, the bi-interaction between spray dates and concentrations showed a significant effect on some traits, as the combination T3 + F3 excelled on the weight of 1000 grain.gm, and grain yields ton.ha⁻¹. 36.83 gm, 7.82 ton.ha⁻¹. The results showed a significant effect of triple interaction in some traits. The combination excelled V2 + F2 + T3 in trait height of the plant only, 109.77 cm. Whereas, the combination V2 + F3 + T3 excels in the weight of 1000 grain Only 38.00 gm.

Keywords. Wheat, Floratone, Spray dates, Growth regulator.

1. Introduction

The wheat crop wheat *Triticum aestivum* L. is one of the most important cereal food crops globally as it is an important source of carbohydrates, starch, protein and vitamins. Because of its importance, this prompted researchers and scientists to think of new ways that improve quality and increase the amount of grains yield and One of the common methods in modern agriculture is the technique of using plant growth regulators as they are used in very low concentrations. It encourages plants to take advantage of their physiological and genetic potentials to use nutrients with high efficiency that may not be obtained through fertilization processes. And the growing interest in wheat crop has led to the development of new cultivars with high productivity by many institutions that are interested in growing cereal crops, including wheat, whose cultivars differ in their genetic nature and productive capacity and Many researchers have indicated[1,2,3] through their studies on different cultivars of wheat that the cultivars differ from each other in most of the growth and yield traits, and from these traits are the number of spikes.m², grains number.Spike⁻¹, weight of 1,000 grains.gm, and the grain yield tons.ha⁻¹. Iraq annually consumes 4.400 million tons of wheat grains from which it imports 2.600 million tons, and thus comes in the sixth place among the countries that import wheat[4]. The researchers found [5,6]. To the presence of a significant increase in some of the studied traits, including the tillers number. m², flag leaf area.cm², The grains number. spike⁻¹ and the total yield of grains ton.ha⁻¹. This is when spraying with the kinetin growth regulator at the beginning of the branching stage. Kinetin is one of the most common circulating synthetic cytokines that have an effect on cell division and its specificity in combination with auxin [7], Which leads to morphological change of the plant and stimulates growth and lateral buds through a process of preventing or reducing apical dominance, which plays an important role in the expansion of the leaves through the cells grow and increase and thus the leafy area of the plant increase too.

The study aims to:

- The effect of foliar feeding on growth regulator on growth and yield of wheat cultivars.
- Knowing the response of the two cultivars of wheat to different concentrations of the growth regulator (Floratone).

2. Materials and Methods

A field experiment was conducted for wheat *Triticum aestivum* L. for the winter season 2020-2021 in Babil Governorate / project area to see (the effect of different concentrations of floratone growth regulator and spray dates on the growth traits of two cultivars of wheat.

The experiment was carried out according of split split plot according to design Randomized Complete Blok Design(RCBD) And by three reduplicate and the growth regulator (Floratone) was used in it at a concentration of (4.2.0) gm.L⁻¹ and codes were given for the coefficients (T3.T2.T1) and occupied Plot sub sub. It was sprayed in three stages (branching stage, elongation stage, liner stage) and gave symbols (F1.F2.F3) and occupied sub plot and two cultivars of wheat (Sham 6 and Latifia 2) were given the symbols (V1 and V2) and occupied main plot.

The experiment land has been prepared after plowing, smoothing, leveling and dividing it into experimental units. There are (54) experimental units with dimensions (2×2 m). Each board contains 10 lines, the distance between one line and another 20 cm and separated the plot buttress and the wide 1 m. The field soil was analyzed before planting by taking samples at a depth of (0 - 30 cm) (Table 1). Use fertilizer super calcium phosphate P₂O₅ 45% at a rate of 100 kg.ha⁻¹, add at once when preparing the soil after tillage and before smoothing and urea fertilizer (N% 46) at a rate of 200 kg.ha⁻¹.

Add in four equal batches(when planting and when three whole leaves appear and And when the second node appears on the stem and at liner). It used a sprinkler capacity(20 L)to spray the crop

manually , it used seeding rate 120 Kg.h⁻¹ and after that All soil and crop service operations were performed such as weeding and fertilize the soil manually and irrigate according needed and plants were harvested on 13/5/2019 after taking all necessary measurements in the research before harvest.

Table 1. Some chemical and physical properties of the soil sample which used before planting.

Properties	Value
Degree of soil reaction (pH)	7.5
Ready nitrogen (mg.kg ⁻¹ soil)	38
Electrical conductivity (DC.S.m ⁻¹)	2.3
Ready potassium (mg.kg ⁻¹ soil)	281
Ready Phosphorus (mg.kg ⁻¹ soil)	14.28
Organic matter (g.kg ⁻¹ soil)	3.2
Soil components	
Sand (g.Kg ⁻¹ soil)	135
Clay (g. Kg ⁻¹ soil)	315
Silt (g.Kg ⁻¹ soil)	550
soil texture	Sandy clay salty

3. Results and Discussion

3.1. Plant Height

Table (2) data indicated a significant effect of the growth regulator (fluraton) on the plant height trait of the cultivars. There were significant differences between the two cultivars, as the cultivar V2 excelled and gave the highest average of 103.12 cm while cultivar V1 gave the lowest average capacity of 97.06 cm. The reason is due to the variation of cultivars among them in the height of the plant due to their genetically different length in internodes , which is one of the important traits that distinguish the cultivars from each other in height [8]. These results are in agreement with reached [9], as they found significant differences between 30 genotypes of wheat species in the height of the plant.

Also, from the data of the same table, there were significant differences between the dates in this traits, as the F2 appointment exceeded the rest of the dates, which gave the highest average of 101.32 cm, while the F3 appointment gave the lowest average of 98.66 cm, while the growth regulator concentrations, the of concentration T3 showed superiority to the other concentrations by giving it the highest average capacity of 103.37 cm, while the control treatment gave T1 the lowest average of 97.53 cm. This result is consistent with what was reached [10] they stated that a significant increase occurred with increasing concentrations of growth regulator compared to the control treatment. Responses vary a lot depending on the sensitivity of the plant organ, and the stem responds to a wide range of oxin concentrations [11]. There was no significant effect of the interaction between (F and V). As for the interaction between the cultivars and the growth regulator concentrations, a significant effect of the interaction is observed. The combination (V2 and T3) excelled the rest of the combinations and gave the highest average of 106.69 cm while giving the variety V1 with the control treatment T3 The lowest average was 94.30 cm. It is also noted that there was no significant effect of the interaction between spray dates and growth regulator concentrations. The results of the same table showed a significant effect of triple interaction, as the combination V2 F2 T3 gave the highest average plant height reached 109.77 cm, while the combination V1 F3 T3 gave the lowest average of 91.27 cm.

Table 2. The effect of foliar fertilization on spray dates and concentrations for the cultivars (plant height.cm).

Cultivars	Spray dates	Growth regulator concentrations (g. L ⁻¹)			Average F × V
		T1	T2	T2	
V1	F1	95.87	97.43	99.63	97.64
	F2	95.77	97.13	100.77	97.89
	F3	91.27	95.90	99.77	95.64
V2	F1	100.37	101.40	107.03	102.93
	F2	101.33	103.13	109.77	104.74
	F3	100.57	101.20	103.27	101.68
L. S. D _(0.05)			1.60		N.S
Growth regulator concentrations		T1	T2	T2	Average
Cultivars					V
V1		94.30	96.82	100.06	97.06
V2		100.76	101.91	106.69	103.12
L. S. D _(0.05)			0.93		0.89
Growth regulator concentrations		T1	T2	T2	Average
/					F
Spray dates					
F1		98.12	99.42	103.33	100.29
F2		98.55	100.13	105.27	101.32
F3		95.92	98.55	101.52	98.66
L. S. D _(0.05)			N.S		1.00
Average T		97.53	99.37	103.37	
L. S. D _(0.05)			0.66		

3.2. Flag Leaf Area.cm²

The results of Table (3) showed the presence of a significant effect only on the effect of the cultivars, spray dates and spray concentrations. While we did not find a significant effect of bi and triple interaction in this variety. V2 excelled V1 and gave the highest average reach of 34.52 cm², The reason may be attributed to the fact that variety V2 exploited its genetic and physiological capabilities very efficiently to obtain better growth than variety V1, as this was reflected in the increase in the flag leaf area, This result is consistent with them findings [12,13] who found significant differences between wheat cultivars in the flag leaf area,

While the addition dates, from the data of the same schedule, the F3 appointment appeared superior to the rest of the dates and gave the highest rate of 35.43 cm², while the F2 appointment gave the lowest rate of the paper area reached 32.04 cm². The data of the same table showed an increase in flag leaf area by increasing the concentrations of auxin (fluraton), as T3 gave the highest average flag leaf area reaching 36.18 cm², while the control treatment gave the lowest rate of 30.19 cm². These results were in agreement with what was reported by [10] as they found an increase in the leafy area of wheat when increasing concentrations of IAA, while we did not find a significant effect of bi-interactions between the cultivars and the spraying dates, the cultivars and spray concentrations, spray dates and spray concentrations. We also did not find significant differences for the triple interaction of the study factors in this trait.

Table 3. The effect of foliar fertilization on spray dates and concentrations for the recipe (flag leaf area.cm²).

Cultivars	Spray dates	Growth regulator concentrations (g. L ⁻¹)			Average F × V
		T1	T2	T2	
V1	F1	29.60	32.55	34.76	32.30

V2	F2	27.76	32.01	33.71	31.16
	F3	31.09	34.34	36.36	33.93
	F1	30.64	34.28	36.22	33.71
	F2	30.85	33.07	34.86	32.92
	F3	31.22	38.41	41.18	36.94
L. S. D _(0.05)			N.S		N.S
Growth regulator concentrations					
/		T1	T2	T2	Average V
Cultivars					
V1		29.48	32.96	34.94	32.46
V2		30.90	35.25	37.42	34.52
L. S. D _(0.05)			N.S		1.79
Growth regulator concentrations					
/		T1	T2	T2	Average F
Spray dates					
F1		30.12	33.41	35.49	33.01
F2		29.31	32.54	34.29	32.04
F3		31.16	36.37	38.77	35.43
L. S. D _(0.05)			N.S		0.66
Average T		30.19	34.11	36.18	
L. S. D _(0.05)			1.02		

3.3. The Tillers Number.m²

A number of factors significantly affected the number of tillers, as shown in Table (4). According to the data in the table, variety V2 had the highest average of 439.76 branches.m², which is a measure of stoppers, while variety V1 had the lowest average of 417.28 branches.m². The variation in tiller numbers among the cultivars is likely due to genetic differences and growth characteristics. The trait of early maturity and branching is associated with genetics and is influenced to varying degrees by environmental factors, which supports previous research [14,15]. Date F1 had the greatest number of tillers per square meter, reaching 430.83 branches per square foot, while date F3 had the lowest number of tillers, at 426.72 beaches per square foot, according to the same table's data, indicating that spraying dates had a major impact. This might be because spraying cytokinein at the start of the branching stage causes the number of tillers to increase [5,6]. Another reason why spraying salicylic acid at the start of the branching stage increased tiller count by 13.5 percent compared to not spraying was because the hormone, when added often and in high concentrations, improves the plant's vegetative development. However, these findings corroborated those of the mechanism, which indicated that adding cytokinin to wheat at the start of the branching stage increased the number of tillers [5].

Table (4) shows that the concentrations of the growth regulator had a significant effect, with the maximum mean number of tillers obtained at concentration T3, indicating that the number of tillers rose as the concentration of the growth regulator increased.m², with the lowest average of 426.95 tiller. m² produced by comparative treatment T1. In terms of the interactions between the bi and triple variables, this feature did not show any significant differences.

Table 4. The effect of foliar fertilization on spray dates and concentrations for trait (tillers number. m²).

Cultivars	Spray dates	Growth regulator concentrations (g. L ⁻¹)			Average F× V
		T1	T2	T3	
V1	F1	418.93	420.13	420.83	419.97
	F2	415.63	417.53	417.83	417.00

	F3	412.27	415.20	417.13	414.87
	F1	440.60	441.07	443.40	441.69
V2	F2	438.07	438.30	440.63	439.00
	F3	436.20	439.30	440.23	438.58
L. S. D _(0.05)			N.S		N.S
Growth regulator concentrations					
/	T1		T2	T3	Average V
Cultivars					
	V1	415.61	417.62	418.60	417.28
	V2	438.29	439.56	441.42	439.76
L. S. D _(0.05)			N.S		3.57
Growth regulator concentrations					
/	T1		T2	T3	Average F
Spray dates					
	F1	429.77	430.60	432.12	430.83
	F2	426.85	427.92	429.23	428.00
	F3	424.23	427.25	428.68	426.72
L. S. D _(0.05)			N.S		2.36
	Average T	426.95	428.59	430.01	
L. S. D _(0.05)			0.98		

3.4. Spikes Number.m²

Table (5) data indicated that there were significant differences between the cultivars, spray dates and spray concentrations in this trait, as well as a significant effect of interaction between cultivars, spray dates, cultivars and growth regulator concentrations in this trait. We did not find a significant effect of bi interaction between spray dates, spray concentrations, and triple interaction of the three factors in this trait.

According to Table (5), variety V2 has more spikes per square meter than variety V1, and it also produced the highest average number of spikes (339.25 spikes per square meter). This could be because variety V2 was more productive in terms of tillers, a trait that is influenced by genetic variation among cultivars. These outcomes corroborate the mechanism's findings [16,17] that the number of spikes per square meter is different. In terms of the impact of spray dates, Table (5) shows that F1's date had the greatest average of 339.20 spike. m², which was higher than the other days. On the other hand, the average number of spikes per square meter was 337.54 on the F2 appointment and 337.30 on the F3 spraying date, which were statistically indistinguishable. This might be because the number of spikes per square meter increased as a result of the plant's elongation and branching brought about by the early-season spraying. Results from the same table demonstrated that spike.m² increased as spray concentrations rose. With an average of 338.23 spikes/m², T3 was the most effective. In terms of spikes per square meter, though, it was statistically indistinguishable from the T1 + T2 concentration. The data from the same table show that the cultivars and spraying dates interacted significantly; for example, the combination V2 + F1 produced the best average, with 341.08 spike m² compared to the other combinations, and the combination F2 + V1 produced the lowest average, with 336.32 spike m². According to the same table, bi-interaction between cultivars and spray concentrations had a significant impact. For example, the combination V2 + T3 resulted in the highest average capacity of 339.53 spike m², which was not significantly different from the combination V2 + T2 (339.41 spike m²), and the lowest average reached 336.56 spike m² with the combination V1 + T2. The results from the same table did not indicate that the bi interaction between the spray dates and concentrations had

any discernible impact. We also failed to detect any statistically significant interaction between the three components of this attribute.

Table 5. Effect of foliar fertilization on spray dates and concentrations for the adjective (Spikes number.m²).

Cultivars	Spray dates	Growth regulator concentrations (g. L ⁻¹)			Average F × V
		T1	T2	T3	
V1	F1	337.07	337.17	337.73	337.32
	F2	336.13	336.13	336.70	336.32
	F3	337.33	336.37	336.37	336.69
V2	F1	340.83	341.13	341.27	341.08
	F2	338.03	339.00	339.23	338.76
	F3	337.53	338.10	338.10	337.91
L. S. D _(0.05)			N.S		0.39
Growth regulator concentrations					
/		T1	T2	T3	Average V
Cultivars					
V1		336.84	336.56	336.93	336.78
V2		338.80	339.41	339.53	339.25
L. S. D _(0.05)			0.41		0.23
Growth regulator concentrations					
/		T1	T2	T3	Average F
Spray dates					
F1		338.95	339.15	339.50	339.20
F2		337.08	337.57	337.97	337.54
F3		337.43	337.23	337.23	337.30
L. S. D _(0.05)			N.S		0.27
Average T		337.82	337.98	338.23	
L. S. D _(0.05)			0.29		

3.5. Grains Number.Spike⁻¹

The data from Table (6) demonstrated that the amount of grains varied significantly across cultivars and spray dates. A significant bi-interaction between (cultivars + spray dates, concentrations, and cultivars) was also found to exist in this trait, but no such effect was found for (spray dates and concentrations), and no such effect was found for triple-interaction either.

According to Table (6), the cultivars had a notable impact on this characteristic; specifically, the V2 variety produced the best results, with an average of 59 grains. The Spike⁻¹ There are an average number of grains. There was a spike of 55.92 grains in variety V1. The first spike, The different cultivars may have differed in this trait because, as shown in Table 5, the V2 variety had a larger flag leaf area. This meant that more photosynthesis-processed food could reach the emerging seeds, which meant that they suffered less from miscarriage and had more contractile strength. [11] In line with previous research, this result confirms that there were notable variations in this feature across the various spraying dates (F3 date yielded the highest average of 60.82 grains) using the same schedule data. Spike⁻¹, with F1 receiving the lowest average of 55.38 grains based on the date. A Spike⁻¹.

The results from Table (6) shows that the spray concentrations employed had a substantial effect, with T3 receiving the highest average of 59.25 grain. The T1 therapy produced the lowest mean of 56.02grain, while Spike⁻¹ performed better than the other concentrations. The Spike⁻¹ This shows that the floratone organizer contributes to a higher grain number. Based on the data in the table, it appears that there is a strong relationship between the cultivars and the spraying dates; for

example, the V2 + F1 combination outperformed the others and produced the best average grain yield of 60.91. The combinations of V2 and F3 and V1 and F3 did not differ significantly from Spike⁻¹, but V1 and F1 produced the lowest average of 49.86 grains. The first spike, V2+T3 outperformed all other combinations and produced the highest average of 61.48 grain, among the data from the same table showing significant differences for bi interaction between the cultivars and spray concentrations. V1+T1 produced the lowest average of 53.99 grain, while Spike⁻¹ was the result of the combo. There was no significant effect of bi interaction between spray dates and spray concentrations on this characteristic, according to Spike⁻¹ and table 7 data. The triple interaction including cultivars, spray dates, and concentrations was likewise not found to have a substantial impact on this characteristic.

Table 6. The effect of foliar fertilization on spray dates and concentrations for the trait (grains number. Spike⁻¹).

Cultivars	Spray dates	Growth regulator concentrations (g. L ⁻¹)			Average F×V
		T1	T2	T3	
V1	F1	47.50	51.60	50.47	49.86
	F2	56.30	57.37	57.63	57.10
	F3	58.17	61.27	62.97	60.80
V2	F1	60.33	59.90	62.50	60.91
	F2	56.30	56.53	58.63	57.16
	F3	57.50	61.73	63.30	60.84
L. S. D _(0.05)			2.76		3.33
Growth regulator concentrations /		T1	T2	T3	Average V
Cultivars					
V1		53.99	56.74	57.02	55.92
V2		58.04	59.39	61.48	59.64
L. S. D _(0.05)			1.59		2.05
Growth regulator concentrations /		T1	T2	T3	Average F
Spray dates					
F1		53.92	55.75	56.48	55.38
F2		56.30	56.95	58.13	57.13
F3		57.83	61.50	63.13	60.82
L. S. D _(0.05)			1.95		2.36
Average T		56.02	58.07	59.25	
L. S. D _(0.05)			1.13		

3.6. Weight 1000 Grain.gm

According to the results in Table 7, the cultivars had a considerable impact on this feature. Specifically, the V2 variety outperformed the V1 variety, yielding an average weight of 36.74 gm, while the V1 variety only managed 30.67 gm. The V2 variety's high efficiency in turning photosynthesis products into seeds and its superior flag leaf area (Table 5) greatly aided in processing growing grains with the processed food they needed for fullness and weight, which is a major reason for this. [13] his conclusions are supported by this outcome [18]. In terms of spraying dates, the F3 appointment stood out from the rest of the dates on the same schedule, with an average of 36.44 gm, while the F1 appointment had the lowest average of 29.78 gm.

According to Table (7), the concentrations of the sprays had a significant impact. The T3 concentration produced the highest average of 34.39 gm, which was higher than all the other concentrations. However, there was no statistical difference between T2 and T3, which each

produced an average of 33.89 gm. In contrast, the control treatment yielded the lowest rate of 32.83 gm from T1. The explanation behind this is that as oxyin levels increased, the leafy area also increased, but at a faster rate than T3, leading to an increase in photosynthesis and providing the spike with food supplies straight from the flag leaf. This information is presented in Table 5. Although there was no statistically significant difference between the interactions of the same variety at concentrations F1 and F2, the combination V2 + F3 performed the best and produced the highest average of 37.22 gm, indicating a significant effect of the interaction between the cultivars and dates in the bi interaction. Table (7) shows that there was no significant effect of the interaction between cultivars and concentrations on the 1000-grain weight trait, even though the combination V1 + F1 produced the lowest mean of 23.44 grams. Cloud The results showed a significant interaction between spraying dates and concentrations. The F3 + T3 combination had the highest average of 36.83 gm, while the F1 + T1 combination had the lowest. The same table also showed that there was a significant effect of the triple interaction (cultivars, dates, and concentrations) on this trait. The V2 + F3 + T3 combination had the highest average of 38.0 gm, but it was not statistically different from the V2 + F3 + T2 and V2 + F2 + T3. The lowest average weight was 23.0 grams when V1 + F1 was combined with T1 + T2.

Table 7. The effect of foliar fertilization on spray dates and concentrations for a trait (weight 1000 grain.gm).

Cultivars	Spray dates	Growth regulator concentrations (g. L ⁻¹)			Average F × V
		T1	T2	T3	
V1	F1	23.00	24.33	23.00	23.44
	F2	30.33	32.67	35.67	32.89
	F3	35.00	36.33	35.67	35.67
V2	F1	35.67	36.33	36.33	36.11
	F2	36.33	36.67	37.67	36.89
	F3	36.67	37.00	38.00	37.22
L. S. D _(0.05)			1.73		1.39
Growth regulator concentrations / cultivars		T1	T2	T3	Average V
V1		29.44	31.11	31.44	30.67
V2		36.22	36.67	37.33	36.74
L. S. D _(0.05)			N.S		3.47
Growth regulator concentrations / Spray dates		T1	T2	T3	Average F
F1		29.33	30.33	29.67	29.78
F2		33.33	34.67	36.67	34.89
F3		35.83	36.67	36.83	36.44
L. S. D _(0.05)			1.22		0.98
Average T		32.83	33.89	34.39	
L. S. D _(0.05)			0.71		

3.7. Grain Yield ton.ha⁻¹

With an average of 7.43 tons per hectare, the V2 variety outperformed all others, proving that cultivars significantly affect the grains yield attribute (Table 8). variety V1 produced an average of 5.86 tons per hectare. Photosynthesis and storage (metabolic compounds) in the mature seed determine the grain yield. Since the V2 variety produced the most average number of spikes per square meter and the most average number of grains, it offered the highest rate of grain yield per

unit area, making it the superior variety in terms of grain yield. An increase (Tables 6 and 7). These findings are in line with his previous research [19], which also identified notable variations among five wheat cultivars. The data from Table (8) indicates that the addition dates had a substantial impact, with the F3 date providing the greatest mean average, surpassing all other appointments with a total of 7.48 ton.ha⁻¹. From the same table, we can see that when spray concentrations climbed, yields did as well; for example, at 6.96 tons per hectare, the T3 concentration was much higher than the others. Compared to the control treatment, T1 achieved the lowest average yield of 6.27 tons per hectare. This average rise occurred because When a high dose of auxin was sprayed, the characteristic manifested as an increase in flag area, leaf area, spikes per square meter, and grains per plant. The spike and 1000 grain weight are listed in Tables 8, 7, 6, and 5.

The data shows that this feature is significantly affected by the bi-interaction between cultivars and dates; for example, the V2 + F3 combination outperformed all other combinations and yielded the highest average of 7.66 ton.ha⁻¹. In contrast to the V1 + F1 combination, which was administered, this one did not vary much from the V2 + F1 combination. The average lowest was 3.94 tons per hectare. The bi-interaction between the cultivars and the spray concentrations did not yield any meaningful results (Table 8).

According to Table (8), the average of 7.82 tons per hectare was achieved by combining F3 and T3, which was significantly higher than the other combinations. This was due to the fact that there was a bi-interaction between the spray dates and concentrations. There was no statistically significant influence of the triple interaction on this feature, even though the combination yielded the lowest average of 5.50 ton.ha⁻¹.

Table 8. The effect of foliar fertilization on spraying dates and concentrations for the trait (grain yield ton.ha⁻¹).

Cultivars	Spray dates	Growth regulator concentrations (g. L ⁻¹)			Average F × V
		T1	T2	T3	
V1	F1	3.68	4.23	3.92	3.94
	F2	5.74	6.31	6.92	6.32
	F3	6.86	7.49	7.56	7.30
V2	F1	7.33	7.42	7.75	7.50
	F2	6.91	7.03	7.49	7.14
	F3	7.11	7.72	8.13	7.66
L. S. D _(0.05)			N.S		0.35
Growth regulator concentrations / cultivars		T1	T2	T3	Average V
V1		5.43	6.01	6.13	5.86
V2		7.12	7.39	7.79	7.43
L. S. D _(0.05)			N.S		0.91
Growth regulator concentrations / Spray dates		T1	T2	T3	Average F
F1		5.50	5.83	5.84	5.72
F2		6.32	6.67	7.21	6.73
F3		6.99	7.61	7.84	7.48
L. S. D _(0.05)			0.30		0.24
Average T		6.27	6.70	6.96	
L. S. D _(0.05)			0.17		

Conclusions

We conclude from the experiment that the treatment is superior. The variety Sham 6 (V2), F3 the date (stage of underlay) treatment and the combination F1 + V2 excels in all studied traits.

References

- [1] Al-Jumaili, Ismail Ahmad Farhan (2011). The effect of spraying with microelements on the growth and yield of three cultivars of wheat bread. *Anbar Journal of Agricultural Sciences*. 9 (2): 90-80.
- [2] Al-Shabib, Emad Abdel-Hussein Badr (2013). Evaluation of wheat cultivars *Triticum aestivum* L. cultivated at different levels of nitrogen fertilizer and the determination of electrolar evidence using the path factor, Master Thesis, College of Agriculture - University of Basra. Iraq.
- [3] Samadiyan, F.; A. Soleymani and H. R. Javanmard (2013). Under the Influence of light Absorption of Nitrogen Fertilizer Treatments on Yield and Yield Components of Wheat Different Cultivars in Isfahan Region *International J. of Agronomy and Plant Production*, 4(8):1884-1889.
- [4] Food and Agriculture Organization. 2011. Statistical Data of the Organization for International Food and Agriculture. Rome.
- [5] Ibrahim, M. E., Bekheta, M. A., A. ElMoursi and N. A. Gaafar. (2009). Improvement of growth and seed yield quality of (*Triticum aestivum* L.) plants as Affected by application plant growth regulators. *Journal of Basic and applied sciences*. 1 (4) : 657 – 666.
- [6] Sakri, F. A., Shireen, A. A. (2009). The response of two cultivars *Triticum* spp. to cytokinin and water stress treatments and their interaction. *Journal of Zankoy Sulaimani*. 12 (1) part A : (51-58).
- [7] Sakakibara, A. R.; Fatkhutdinova, D. R.; Bezrukova, M. V. and Shakirova F. M. (2006). Kinetin prevents the damaging action of stress factors on wheat plants. *Bulg. J. Plant physiol., Special Issue*:314- 319.
- [8] Mohammad, Hanaa Hassan. 2000. Traits of growth, yield, and quality of wheat cultivars due to the effect of planting dates. Faculty of Agriculture. Baghdad University. Iraq.
- [9] Gulanz, S.; M. Sajjad; I. Khaliq; A. S. Khan and S. H. Khan. 2011. Relationship among coleoptile length, plant height and tilering capacity for developing improved wheat cultivars. *Int. J. Agric. Biol.* 13:130-133.
- [10] Faisal, Mohamed Saeed and Shaimaa Mohamed Abdel-Tayeb. 2013. Response of wheat cultivars to growth regulator (IAA) under drought conditions. *Agricultural Sciences Journal. Tikrit University*. Volume 13. Issue 1, pp. 120-111.
- [11] Essa, Talib Ahmed (1990). *Crop Plant Physiology*, Ministry of Higher Education and Scientific Research. University of Baghdad, p. 496 (translated).
- [12] Amer, Sarhan Anam Abdo (2004). Response of some *Triticum aestivum* L. wheat cultivars to water stress under field conditions. PhD thesis. Department of Field Crops. College of Agriculture, University of Baghdad. P. 142.
- [13] Al-Hayani, Ahmad Abdel-Wahid Ali Merhi (2009). Cultivars Seed rates and bush pesticide spray rates as integrated management agents for bush control in the wheat crop *Triticum aestivum* L. Master Thesis, College of Agriculture_ Anbar University, Iraq, p: 100.
- [14] Al-Rafeeqi, Zina Thamer Abdul-Hussein (2012). Diagnosis of phenotypic and genetic variations in wheat cultivars *Triticum aestivum* L. .. and estimation of the parameters of genetic and phenotypic correlation under different levels of added nitrogen fertilizer. Master Thesis - Faculty of Education for Pure Sciences - University of Karbala - page 80.
- [15] Al-Taie, Ali Karim Hussein. 2019. The effect of foliar fertilization and spraying stage on growth, yield and quality of two cultivars of wheat bread. Al-Musayyib College of Technology. Middle Euphrates Technical University. Iraq.
- [16] Al-Taie, Ali Karim Hussein. 2019. The effect of foliar fertilization and spraying stage on growth, yield and quality of two cultivars of wheat bread. Al-Musayyib College of Technology. Middle Euphrates Technical University. Iraq.



- [17] Al-Yasiri, Ayat Shansul Musa Muhammad and Qais Hussain Al-Sammak (2015). The effect of potassium leaf feeding on the growth and yield of five cultivars of wheat *Triticum aestivum* L. Journal of Karbala University Scientific Journal, Volume (13) No. (3) pp. 114-105.
- [18] Kilic, H., and Gürsoy, S. .2010. Effect of seeding rate on yield and yield components of durum wheat cultivars in cotton-wheat cropping system. Scientific Research and Essays 5(15): 2078-2084.
- [19] Eskandari, H. and K. Kazemi . 2010. Response of different bread wheat (*Triticum aestivum* L.) genotypes to post-anthesis water deficit. J. Sci. Biol., 2 (4) : 49-52 .

Importance of the Azotobacter and Mineral Fertilizers in Plant Growth

Haider A. Al-Maamori

Department of Biology, College of Education for Pure Sciences, University of Wasit, Wasit, Iraq.

E-mail: haidera.f@uowasit.edu.iq

Abstract. Sustainable agricultural development is about protecting natural and environmental resources from pollutants. In line with the goal of sustainable development, this study aimed to use nitrogen-fixing bacteria as a natural bio fertilizer. A biological experiment was conducted in the Department of Biology/College of Education for Pure Sciences/University of Wasit To study the effect of bacteria and Mineral fertilizers on soil properties and wheat growth and yield. The experiment was conducted in a randomized complete block design (RCBD). The results showed that A mixture of *Azotobacter vinelandii* and *Azotobacter chroococcum* was superior in terms of preformed nitrogen , phosphorus available and Total bacteria, 34.20 mg kg⁻¹ and 22.74 mg kg⁻¹ and 8.10*10⁻⁶ Cfu g⁻¹ dry soil respectively, in the soil. The bacterial mix treatment also showed superior results in plant growth indicators, including: plant height, number of branches, leaf area, 1000 grain weight, and grain yield, 66.50 cm , 6.81 branch plant⁻¹ 19.46 cm² plant⁻¹, 43.23gm, 2.97 Mg ha⁻¹). The Mineral fertilizer-bacteria interaction treatment yielded higher values than the no addition treatment for all measured traits (nitrogen, preformed phosphorus and Total bacterial count in soil after planting, plant height,number of branches, leaf area, 1000 grain weight and grain yield (36.30 mg kg⁻¹, 24.70 mg kg⁻¹,8.30*10⁻⁶ Cfu g⁻¹ dry soil ,69.82 cm,7.35 branch plant⁻¹ , 20.65 cm² plant⁻¹, 45.05gm and 3.07 Mg ha⁻¹).

Keywords. *A.vinelandii*, *A.chroococcum*, Nutrient availability, Sustainable agricultural, Wheat plant.

1. Introduction

Agricultural development that contributes to improving resource efficiency, increasing resilience to climate change, ensuring equity and social responsibility at the agricultural sector and food system level, achieving food security and nutrition for all, now and in the future. Sustainable agricultural development allows for sustainable production and environmental protection, bringing tangible benefits to water and soil, and reducing pollutants such as the use of mineral fertilizers that cause soil pollution and increase carbon emissions. There is therefore a trend to move away from mineral fertilizers to bacterial and fungal biofertilizers in order to maintain soil vitality and fertility and produce highly efficient and healthy plants. With the increase in world population, and it has become a major challenge to provide an adequate amount of food, feed, and agricultural products majorly in developing countries. The use of chemical fertilizers causes the plant to grow efficiently and rapidly to meet the food demand. The drawbacks of using a higher quantity of chemical or synthetic fertilizers are environmental pollution, persistent changes in the soil ecology, physiochemical composition, decreasing agricultural productivity and cause several health hazards .[1]. Plant growth-promoting rhizobacteria (PGPR) are the best-known bacterial species among all other microorganisms that have a greater impact on the physiological and

structural properties of soil. PGPR help to replace chemical fertilizers for sustainable agricultural production by fixing atmospheric nitrogen and producing growth-promoting substances. Among the PGPR group, *Azotobacter* is a ubiquitous, aerobic, free-living, and N₂-fixing bacteria that commonly inhabit rhizosphere soil. *Azotobacter*, a major group of soil-borne bacteria, plays various beneficial roles by producing various types of vitamins, amino acids, plant growth hormones, antifungal substances, hydrogen cyanide, and siderophores. Growth-promoting substances such as indole acetic acid, gibberellic acid, and arginine produced by *Azotobacter* species directly affect shoot length, root length, and seed germination of several agricultural crops (soil rhizosphere). Some species of the genus *Azotobacter*, namely *A. vinelandii*, *A. chroococcum*, *A. salinestris*, *A. tropicalis*, and *A. nigricans*, are capable of producing antibacterial compounds that inhibit the growth of plant pathogens.[2] Also, the problem of nutrient deficiency and lack of availability is currently one of the major issues, and the use of environmentally less harmful and highly efficient sources such as *Azotobacter* is being considered. Studies of *Azotobacter* in crop production have revealed its importance in plant nutrition and its contribution to soil fertility. [3] The problem of reduced quality and plant yield, as well as the deterioration of some physiological characteristics, is one of the problems experienced by professionals in the field of agriculture and field crop production. To solve the problem of low availability of nutrients such as nitrogen and phosphorus, the addition of substances that improve soil properties and increase the availability of nutrients, such as biofertilizers, is necessary. The addition of growth-promoting substances increases physiological and biological processes in plants, increasing cell division and proper growth.[4] To increase productivity and improve the quantitative and physiological characteristics of plants, studies were conducted to examine the effect of microorganisms and *Azotobacter* bacteria on wheat growth and yield, as well as on increasing soil nitrogen fixation compared to chemical fertilizers. The results showed that plant growth-promoting bacteria are superior in growth criteria and physiological characteristics [5]. Experiments were conducted to examine the effect of inoculation with *Azotobacter* and *Phosphobacterium* on wheat growth and productivity. The experiments consisted of different treatments. The bacteria were added to liquids, carriers, seeds, or sprayed directly on the soil. The results showed an increase in plant height, panicle number, panicle length, and an increase in yield per kg of plant [6]. The aim of study is to determine the role of different isolates of *Azotobacter* in enhancing the availability of some nutrients in soil and their effects on wheat growth and yield under mineral fertilization levels.

2. Materials and Methods

Biological experiments were carried out in the field of the Faculty of Pure Science Education, Wasit University, and laboratory analyses were carried out in the laboratory of the Faculty of Life Sciences. Pots with holes in the bottom were prepared, the surface was sterilized with alcohol from the inside, and the soil sieved through a 4 mm sieve was packed into the pots. Then, 9 kg of sieved soil was placed in each anvil according to the different experimental treatments (individual treatments of bacterial isolates, and interactions of bacteria with mineral fertilizers). The soil was moistened and the pot soil was fertilized according to [7] at the levels of 150 kg/ha of nitrogen (46% urea), 100 kg/ha of phosphorus (triple superphosphate, 21% phosphorus), and 100 kg/ha (potassium sulfate, 50% K₂O). Then, the treatments were randomly distributed in the field according to the design (RCBD).

2.1. Isolation Azotobacter

To isolate *Azotobacter chroococcum*, a dilution series of soil samples taken from different agricultural regions and fields was prepared. 10 g of selected soil sample was added to 90 ml of

sterile distilled water in a 100 ml plastic bottle and shook well to prepare the dilution series. For each soil sample, 1 ml of soil suspension was transferred to a test tube containing 9 ml of sterile distilled water to prepare a 10^{-1} to 10^{-7} fold dilution series. Using a sterile pipette, 1 ml of dilution (10^{-5} , 10^{-6} , 10^{-7}) was transferred to a test tube containing 9 ml of sucrose Chemical salts liquid medium described by [8]. This medium was prepared and autoclaved at a temperature of 121 °C and a pressure of 15 pounds Ang2. With two replicates for each dilution, the test tubes were incubated at a temperature of 28 °C , 5 days the test tubes were examined by observing the ring-shaped film formed on the surface. This film is a positive indicator of *Azotobacter chroococcum* [11].

2.1.1. Purification and Identification Azotobacter vinelandii and Azotobacter chroococcum

0.1ml of the growth that showed positive indications of growth in the tube was taken and spread on a Petri dish containing solid medium (SMSA) [11]. The dish was incubated for 72 hours at 28°C until colonies appeared. plot then repeated three times in succession to obtain pure isolates of bacteria. This method resulted in 10 pure isolates with general characteristics of *Azotobacter vinelandii* and *Azotobacter chroococcum* . Slant agar tubes were then prepared from the dedicated medium in order to store the isolates in a refrigerator for microscopic and biochemical examination. The bacterial isolates were diagnosed by studying some cultural and microscopic characteristics, as well as biochemical tests.

2.1.2. Agronomic Characteristics

To study the cultural characteristics of the bacterial isolates, a special solid culture medium (S M S A) was prepared. Dishes containing special culture medium for the isolates were inoculated by planning method and incubated at 29 ° C for 48 h, growth was examined and phenotypic characteristics such as colony color, texture, and growth density were recorded according [21].

2.1.3. Microscopic Characteristics

2.1.3.1. Gram stain

Gram stain, examined under a light microscope according to Bergey's manual [9].

2.1.3.2. Mobility

Mobility tests were performed. According [10], the spread of growth outside the stabbing area after 24 hours of incubation at 28°C is an indicator of the ability of the isolate to migrate.

2.1.4. Biochemical Tests

2.1.4.1 .Growth Test in 1% Sodium Chloride

Bacterial isolates were grown on a special solid sucrose Chemical salts medium after the addition of 1% sodium chloride 1 g of substance per 100 ml of medium and incubation at a temperature of 28°C for 72 hours. According to [11], the growth of colonies on solid medium is a positive indicator.

2.1.4.2 .Growth Test with Glycerol as the Sole Carbon Source

Bacterial isolates were grown on solid medium (sucrose Chemical salts) with 2% glycerol added instead of sucrose and incubated at a temperature of 28°C for 72 hours. According to [12], the appearance of colonies is an indication of growth.

2.1.4.3. Growth at a Temperature of 37°C

Bacteria were grown on solid medium (sucrose Chemical salts) at a temperature of 37°C for 4. According to [12], the appearance of colonies is considered a positive indication.

2.2. Field Experiment Factors Included

2.2.1. First Factor: *Azotobacter* Bacteria

- Without *Azotobacter* : AZ0
- With *Azotobacter vinelandii* : AZ1
- *Azotobacter chroococcum* AZ2
- A mixture of *Azotobacter vinelandii* and *Azotobacter chroococcum* AZ3

2.2.2. Second Factor: Mineral Fertilizer

- No added : MF0
- 50% fertilizer recommended MF1
- 100% complete fertilizer recommended: MF2

Number of experimental units = 4X3X3 = 36 experimental units

2.3. Statistical Analysis

The results were statistically analyzed using the analysis of variance method and using the Genstat program, and the means were compared according to the least significant difference (LSD) test at a probability level of 0.05 .

Soil Characteristics were estimated before planting according to [22].

Table 1. Chemical, Physical and Biological Characteristics of Soil Pre – planting.

Character		Value
pH		7.03
EC 1:1	dsm ⁻¹	3.11
nutrients Soluble	Ca ⁺²	7.36
	Mg ⁺²	4.19
	Na ⁺	5.87
	K ⁺	0.85
	CL ⁻	11.64
Avialable	SO4 ⁻²	9.45
	N	22.75
	P	9.14
Nutrients	K	201.31
Texture		Clay Loam
Total bacteria		10 ⁶ *0.5 Cf _u g ⁻¹ dry soil
Total fungus		10 ⁵ *0.32 Cf _u g ⁻¹ dry soil

3. Results and Discussion

3.1. Isolation and Diagnosis of *Azotobacter*

The results showed that 10 bacterial isolates were obtained from rhizosphere soil and the microscopic and biochemical characteristics of these isolates when grown on sucrose Chemical salts medium. As shown in Table 2, they are gram-negative motile rods with the ability to grow in

1% sodium chloride and 2% glycerol and can grow at a temperature of 37°C . Based on the study of these characteristics of the isolates and what was reported in previous studies, these characteristics are consistent with the cultural and microscopic characteristics of the genus *Azotobacter* described in the Bergeys Manual [21].

Table 2. Diagnostic microscopy and biochemical tests for *Azotobacter* isolates.

Microscopic characteristics of cells				Biochemical characteristics of the isolates		
Isolation number	Gram stain	Cell Mobility	Colony color	%1 NaCl	2% Glycerol	37 c'
1	-	+	light brown	++	+	++
2	-	+	Brown	++	++	++
3	-	+	dark brown	+	+	++
4	-	+	dark brown	++	+	+
5	-	+	Brown	+	+	+
6	-	+	light brown	+	+	++
7	-	+	Brown	+	+	+
8	-	+	light brown	++	+	++
9	-	+	light brown	+	++	++
10	-	+	light brown	++	++	++

3.2. Nitrogen Availability mg N kg^{-1}

Table (3) significant increase in the average nitrogen concentration when adding *Azotobacter vinelandii* and *Azotobacter chroococcum* AZ3, which reached $34.20 \text{ mg kg soil}^{-1}$ compared to the AZ0 treatment, which gave the lowest average, which amounted to $31.60 \text{ mg kg soil}^{-1}$. Mineral fertilizer treatment MF2 showed superiority in soil available nitrogen reaching $34.80 \text{ mg kg soil}^{-1}$ compared to $31.80 \text{ mg kg soil}^{-1}$ for comparison treatment MF0. The table indicates that the interaction between bacteria and Mineral fertilizer significantly affected the average nitrogen, as the AZ3MF3 treatment gave the best average of $36.30 \text{ mg kg soil}^{-1}$ compared to the AZ0MF0 treatment, which gave the lowest average $27.90 \text{ mg kg soil}^{-1}$.

Table 3. Effect of *Azotobacter* isolates and Mineral fertilizer on the available nitrogen mg N kg^{-1} .

Azotobacter isolates	Mineral fertilizer			Mean (AZ)
	MF0	MF1	MF2	
AZ 0	27.90	32.90	34.10	31.60
AZ 1	27.90	32.90	34.10	31.60
AZ 2	36.80	30.80	34.70	34.10
AZ 3	34.60	31.80	36.30	34.20
LSD _{AZ MF}		2.10		L.S.D _{AZ} = 1.20
Mean (MF)	31.80	32.10	34.80	L.S.D _{MF} = 1.00

3.3. Phosphorus Availability mg P kg⁻¹

Table (4) showed a significant increase in the average concentration of phosphorus when adding the *Azotobacter vinelandii* and *Azotobacter chroococcum* AZ3, if it reached 22.74 mg P kg⁻¹ soil of soil compared to the AZ0, which gave the lowest average of 8.05 mg P kg⁻¹ soil of soil Mineral fertilizer treatment MF2 showed superiority in soil available phosphorus reaching 17.10 compared to 13.21 for comparison treatment MF0. The table indicates that the interaction between the *Azotobacter* and Mineral fertilizer had a significant effect on the average phosphorus concentration if AZ3MF2 gave the best average of 24.70. mg P kg⁻¹ soil with AZ0CF0. Which gave the average of 6.87 mg P kg⁻¹ soil.

Table 4. Effect of *Azotobacter* isolates and Mineral fertilizer on available phosphorus mg P kg⁻¹.

Azotobacter isolates	Mineral fertilizer			Mean (AZ)
	MF0	MF1	MF2	
AZ 0	6.87	8.10	9.17	8.05
AZ 1	8.20	9.96	12.11	10.09
AZ 2	17.00	19.66	22.44	19.70
AZ 3	20.77	22.77	24.70	22.74
LSD _{AZ MF}		0.68		L.S.D _{AZ} = 0.39
Mean (MF)	13.21	15.12	17.10	0.34L.S.D _{MF} =

3.4. Total bacterial count in soil after planting Cfu g⁻¹ dry soil

The results in table (5) show that when *Azotobacter vinelandii* and *Azotobacter chroococcum* AZ3 were added, the total number of bacteria in the soil increased significantly, reaching 8.10*10⁻⁶ Cfu g⁻¹ dry soil, while the average without the addition of bacteria AZ0 was 0.82*10⁻⁶ Cfu g⁻¹ dry soil. The interaction treatments also showed a significant effect on the total number of bacteria in the soil after planting, with the interaction treatment of *Azotobacter vinelandii* and *Azotobacter chroococcum* and the interaction treatment with full fertilization recommended showing the best average value of 8.30*10⁻⁶ Cfu g⁻¹ dry soil. Compared to the AZ0MF0 treatment 0.71*10⁻⁶ Cfu g⁻¹, which had the lowest number of bacteria,

Table 5. Effect of *Azotobacter* isolates and Mineral fertilizer Total bacterial count in soil after planting Cfu g⁻¹ dry soil.

Azotobacter isolates	Mineral fertilizer			Mean (AZ)
	MF0	MF1	MF2	
AZ 0	0.71	0.84	0.91	0.82
AZ 1	5.53	5.98	6.21	5.90
AZ 2	7.89	8.01	8.14	8.01
AZ 3	7.92	8.10	8.30	8.10
LSD _{AZ MF}		0.14		0.08L.S.D _{AZ} =
Mean (MF)	5.51	5.73	5.89	0.07L.S.D _{MF} =

The increase in the total number of bacteria in the soil is due to the added biofertilizers and the role of bacteria in increasing the secretion of organic acids and hormones that promote plant

growth, root elongation and density of the root system, promoting the secretion of bacteria-stimulating substances by the roots, increasing the growth and density of bacteria in the rhizosphere, which is reflected in an increase in the availability of nutrients in the soil. [26]. increase in the concentration of ready-to-use nitrogen in the bacterial treatments could be due to the availability of nitrogen in a form that can be absorbed by plants as a result of the presence of bacteria that fix nitrogen freely. The presence of *Azotobacter chroococcum* + *Azotobacter vinelandii* contributes to an increase in the rate of nitrogen fixation, as they fix nitrogen freely. Thus, we see an increase in the value of ready-to-use nitrogen in the experimental soils that were amended with bio fertilizers. They also secrete substances important for plant growth, such as the production of the plant hormones auxin, gibberellin, and cytokinin. These properties have made *Azotobacter chroococcum* the most successful and widespread group, and they have significantly increased (IAA), K, Mg, N. [15] They also play a role in improving soil properties and increasing the absorption of nutrients. These results indicate that *Azotobacter chroococcum* + *Azotobacter vinelandii* play a positive role in increasing the amount of ready-to-use nitrogen in the soil with increasing amounts of addition, as this is one of the factors with the addition of bacteria [16]. Also, the increase in the amount of preformed P in the soil may be due to the increase in the amount of preformed P as a result of the presence of *Azotobacter chroococcum*+*Azotobacter vinelandii* .

which increases the solubility of nutrients and increases the concentration of preformed phosphorus in the soil. As the results show, the increase in the concentration of preformed phosphorus in the soil may be due to the role of phosphate fertilizers added to the soil. The presence of nitrogen fixing bacteria *Azotobacter* has the ability to increase nutrient availability by fixing atmospheric nitrogen and secreting hormones, growth promoters, gibberellins and organic acids, which increase the availability of nitrogen and phosphorus in the soil. [24].

3.5.Plant Height

The statistical analysis results in table (6) show that when *Azotobacter chroococcum* + *Azotobacter vinelandii* AZ3 was added, the average plant height reached 66.50 cm, which increased significantly compared to the AZ0 treatment, which showed the lowest average 59.43cm.Mineral fertilizer treatment MF2 showed superiority in soil available nitrogen reaching 65.15 cm compared to 60.09 cm for comparison treatment MF0 The table shows that the interaction of bacteria and Mineral fertilizers had a significant effect on plant height when treatments were given. AZ3MF2 showed the best average at 69.82 cm, compared to 57.73 cm for the AZ0CF0 treatment, which showed the lowest average.

Table 6. Effect of *Azotobacter* isolates and Mineral fertilizer on wheat plant height (cm).

Azotobacter isolates	Mineral fertilizer			Mean (AZ)
	MF0	MF1	MF2	
AZ 0	57.73	59.06	61.48	59.43
AZ 1	57.33	62.33	62.82	60.82
AZ 2	62.33	64.33	66.48	64.38
AZ 3	63.00	66.67	69.82	66.50
LSD _{AZ MF}		3.87		L.S.D _{AZ} = 3.64
Mean (MF)	60.09	63.09	65.15	L.S.D _{MF} = 3.29

3.6. Number of Branch (branch plant⁻¹)

The results of the statistical analysis in table (7) show that the average number of branches of wheat increased significantly when *Azotobacter chroococcum* + *Azotobacter vinelandii* bacteria was added, reaching 6.81 branch plant⁻¹, compared to the lowest of 4.26 branch plant⁻¹ in the AZ0 treatment. The table shows that the interaction of biological and Mineral fertilizers had a significant effect on the average number of branches of wheat crops. In the AZ3MF2 treatment, the average was the highest, 7.35 branch plant⁻¹ compared to the lowest of 3.68 branch plant⁻¹ in the AZ0MF0 treatment.

Table 7. Effect of Azotobacter isolates and Mineral fertilizer on Number of branch (branch plant⁻¹).

Azotobacter isolates	Mineral fertilizer			Mean (AZ)
	MF0	MF1	MF2	
AZ 0	3.68	4.5	4.59	4.26
AZ 1	5.9	4.74	5.58	5.40
AZ 2	5.1	6.25	6.53	5.96
AZ 3	5.82	7.26	7.35	6.81
LSD _{AZ MF}		0.20		L.S.D _{AZ} = 0.10
Mean (MF)	5.12	5.68	6.01	L.S.D _{MF} = 0.09

3.7. Leaf Area

The results of statistical analysis in table (8) show that when AZ3 was added, the average leaf area of the plants increased significantly with 19.46 cm² plant⁻¹, whereas AZ0 treatment showed the lowest average value with 9.77 cm² plant⁻¹. compared to 11.80 cm² plant⁻¹ for comparison treatment MF0. This table shows that the interaction of bio fertilizer and Mineral fertilizer had a significant effect. AZ3MF2 20.65 cm² plant⁻¹ treatment showed the best average value of, whereas AZ0MF0 treatment showed the lowest average value with 8.35 cm² plant⁻¹.

Table 8. Effect of Azotobacter isolates and Mineral fertilizer on the leaf area of wheat plants (cm² plant⁻¹).

Azotobacter isolates	Mineral fertilizer			Mean (AZ)
	MF0	MF1	MF2	
AZ 0	8.35	9.60	11.36	9.77
AZ 1	8.85	12.15	13.65	11.55
AZ 2	12.50	17.87	18.85	16.40
AZ 3	17.82	19.92	20.65	19.46
LSD _{AZ MF}		0.61		L.S.D _{AZ} = 0.35
Mean (MF)	11.80	14.88	16.12	L.S.D _{MF} = 0.32

Adding bacteria to plants provides the plants with the necessary nutrients, increasing the average plant height, leaf area, number of branches and therefore the yield. The presence of bacteria improves the growth criteria of plants, which is reflected in an increase in the photosynthetic process, an increase in carbohydrate production and dry matter accumulation in the trophic

system. The interaction of *Azotobacter vinelandii* and *Azotobacter chroococcum* also increases growth parameters such as number of branches and leaf area compared to their absence. This is because bacteria secrete organic acids, which increase root elongation and the density of the root system. This increases the absorption of nutrients and Chemicals by the plant, which increases the growth indicators. Plant growth [23].

3.8. 1000 Grain Weight (g)

The results of the statistical analysis of table (9) showed that when *Azotobacter vinelandii* and *Azotobacter chroococcum* was added, the weight per 1000 seeds reached 43.23 gm, which was significantly increased compared to the AZ0 treatment 30.35 gm which had the lowest average. Mineral fertilizer treatment MF2 showed superiority in 1000 grain weight reaching 39.41gm compared to 32.94 gm for comparison treatment MF0. The table shows that the interaction of bacteria and Mineral fertilizers had a significant effect on the weight per 1000 seeds when the AZ3MF2 treatment was used. The highest average was 45.05gm, which was higher compared to the AZ0MF0 treatment 27.52gm.

Table 9. Effect of *Azotobacter* isolates and Mineral fertilizer on 1000 grain weight gm.

Azotobacter isolates	Mineral fertilizer			Mean (AZ)
	MF0	MF1	MF2	
AZ 0	27.52	30.4	33.15	30.35
AZ 1	28.80	35.35	36.95	33.70
AZ 2	35.75	41.23	42.51	39.83
AZ 3	39.71	44.95	45.05	43.23
LSD _{AZ MF}		0.44		L.S.D _{AZ} = 0.26
Mean (MF)	32.94	37.98	39.41	L.S.D _{MF} = 0.21

3.9. Grain yield (Mg ha⁻¹)

The results of statistical analysis in table (10) show that the grain yield increased significantly when AZ3 was added, reaching 2.97 Mg ha⁻¹ compared to AZ1 treatment. AZ0 reached the lowest average of 2.61 Mg ha⁻¹ and 2.49. Mg ha⁻¹. Also, the interaction of *Azotobacter vinelandii* and *Azotobacter chroococcum* and Mineral fertilizer had a significant effect on the grain yield of wheat, with AZ3MF2 treatment showing the highest average of 3.07 Mg ha⁻¹ and AZ0MF0 treatment showing 2.42 Mg ha⁻¹.

Table 10. Effect of *Azotobacter* isolates and Mineral fertilizer on wheat grain yield (Mg ha⁻¹).

Azotobacter isolates	Mineral fertilizer			Mean (AZ)
	MF0	MF1	MF2	
AZ 0	2.42	2.5	2.55	2.49
AZ 1	2.42	2.68	2.75	2.61
AZ 2	2.67	2.91	2.91	2.83
AZ 3	2.78	3.07	3.07	2.97

LSD _{AZ MF}	0.05	L.S.D _{AZ} = 0.031
Mean (MF)	2.57 2.79 2.82	L.S.D _{MF} = 0.028

Azotobacter chroococcum play a role in increasing wheat grain yield and grain number due to bacterial enzyme activity and secretion of organic acids. Organic acids play an important role in plant vital activities, such as root hair growth, increased plant activity, and increased photosynthesis and food production processes in plants. *Azotobacter vinelandii* and *Azotobacter chroococcum* also play a role. The improvement of soil properties and increased nutrient preparation, as well as biological nitrogen fixation, provide plants with nutrients such as nitrogen that they need, and these results are consistent with [17].

As for Chemical fertilizer, the results show that it has a significant effect on increasing plant yield, and the reason for this may be due to the role of Chemical fertilizer in plant growth, plants acquiring the nutrients they need, and performing its important activities, including the process of photosynthesis, which is reflected in plant growth and yield [18].

Also, the role of Chemical fertilizer in providing good nutrients and increasing biological activity increases the value of these properties. Similar results were obtained with the addition of Chemical fertilizers to the soil [19].

This may be due to the increased level of ready-made Chemical elements in the soil solution, allowing the plant to absorb the amount. Suitable for both, phosphorus plays an important role in many physiological processes in plants. It is involved in the formation of nucleic acids, which leads to an increase in the root system, improving nutrient absorption and increasing productivity [20]. The reason for this is also due to the increased concentration of phosphorus in the plant. Thus, the soil increases the amount absorbed by the plant, which has a positive effect on cell division and increased plant growth. However, the addition of bio fertilizers increases the availability of nutrients, which increases plant growth and yield. From the results so far, the increase that occurred in these plants due to the effect of treating urea with *Azotobacter chroococcum* has been noted. Urea contains nitrogen, which promotes the growth of the vegetative parts of the plant and also plays a role in the process of photosynthesis since it contains 46% nitrogen. Also, the phosphate in the fertilizer is a direct source of the mineral phosphorus. Therefore, these two elements have various effects on plant growth since they are involved in many important activities. Nitrogen is involved in the increase of cell division, which leads to an increase in the height of the plant. Also, nitrogen is involved in the construction of chlorophyll molecules, so relatively, growth indicators such as leaf area and number of branches increase. The increase in chlorophyll content leads to an increase in the photosynthetic process, which leads to the conversion of manufactured carbohydrate compounds into storage areas, and the *Azotobacter vinelandii* and *Azotobacter chroococcum* bacteria lead to the fixation of atmospheric nitrogen by the bacteria, which leads to an increase in all the characteristics of vegetative growth and yield of the plant, and through good nutrition with nitrogen, the plant growth is improved, which leads to an improvement in the photosynthetic process, which was reflected in the yield characteristics [25].

Conclusion

- The interaction of *Azotobacter vinelandii* and *Azotobacter chroococcum* with mineral fertilizers is one-way, increasing wheat growth parameters and yield, increasing nutrient absorption and enhancing soil nutrient availability.
- Expand research and investigation into the use of bacterial biofertilizers and encourage inoculation with bacterial isolates tested for their ability to increase soil nutrient availability.

References

- [1] Chaudhary, P., Singh, S., Chaudhary, A., Sharma, A., & Kumar, G. (2022). Overview of biofertilizers in crop production and stress management for sustainable agriculture. *Frontiers in Plant Science*, 13, 930340.
- [2] Chennappa, G., Naik, M. K., Amaresh, Y. S., Nagaraja, H., & Sreenivasa, M. Y. (2017). *Azotobacter*: a potential biofertilizer and bioinoculants for sustainable agriculture. *Microorganisms for Green Revolution: Volume 1: Microbes for Sustainable Crop Production*, 87-106.
- [3] Wani, S. A., Chand, S., Wani, M. A., Ramzan, M., & Hakeem, K. R. (2016). *Azotobacter chroococcum*—a potential biofertilizer in agriculture: an overview. *Soil science: agricultural and environmental perspectives*, 333-348.
- [4] Pathak, J., Jaiswal, J., Shukla, R. K., Singh, D. K., and R. P. Sinha .2024. Cyanobacterial/algal biofertilizers as plant growth stimulants for green sustainable agriculture. *crops*, 42, 43.
- [5] Alotaibi, M. M., Aljuaid, A., Alsudays, I. M., Aloufi, A. S., AlBalawi, A. N., Alasmari, A. and M. M. Awad-Allah . 2024. Effect of Bio-Fertilizer Application on Agronomic Traits, Yield, and Nutrient Uptake of Barley (*Hordeum vulgare*) in Saline Soil. *Plants*, 13(7), 951 .
- [6] McCarthy-Neumann, S., and R. K. Kobe.2019. Site soil-fertility and light availability influence plant-soil feedback. *Frontiers in Ecology and Evolution*, 7, 383.
- [7] Ali, N. S, H. S. Rahi, and A-W..Shaker. 2014. *Soil Fertility*, Dar Al-Kutub Al-Ilmiyya for Printing, Publishing and Distribution.
- [8] Becking,J.H.(1981). The family Azotobacteraceae . In: Starr, M.P. (Ed): "The prokaryotes" Vol 1 . Springer-Verlag. Berlin. Heidelberg. New York. P. 795-817.
- [9] Garrity, G. M., Bell, J. A., and T. G. Lilburn .2004. Taxonomic outline of the prokaryotes. *Bergey's manual of systematic bacteriology*.
- [10] Collee, J. G , Miles, R. S. and B. Watt,. 1996. Tests for the Identification of Bacteria. In : *Practical Medical Microbiology* 14th ed. Ed. by J. Gerald Collee, Barrie, P, Marmion, Andrew, G. ; Fraser and Anthony Simmons. Churchill Livingstone. New York. P. 132–149.
- [11] Tchan,Y.T. and N.B. Peter. 1984. Genus *Azotobacter*. In: Sneath,P.H., Mair,N.S. Sharpe,M.E. and Holt,J.G.(ed.s.):"Bergey's manual of systematic Bacteriology" 1. William and Wilkins,219-229.
- [12] Thompson,J.P. and V.B.D. Skerman 1979.*Azotobacteraceae*. The Taxonomy and Ecology of Aerobic Nitrogen-Fixing Bacteria. Academic Press, London.
- [13] Richards,L.A. 1954. Diagnosis and improvement of saline and alkali soils. United. States, Department of Agriculture , Washington,D.C. Handbook No. (60): 4-18.
- [14] Page, A. L.; R. H. Miller and D. R. Kenney. 1982. *Method of soil analysis part 2*. 2nd ed. Agronomy 9. Am. Sco. Agron. Madison Wisconsin.
- [15] Song, Y., Li, Z., Liu, J., Zou, Y., Lv, C., and F.Chen . 2021. Evaluating the impacts of *Azotobacter chroococcum* inoculation on soil stability and plant property of maize crop. *Journal of Soil Science and Plant Nutrition*, 21, 824-831.
- [16] Al-juthery, H. W., and Q. M. Al-Shami, 2019. Impact of Irrigation Fertilization with Nano NPK Fertilizers on Some Parameters of Growth and Yield of Potato (*Solanum tuberosum* L.). *AL-Qadisiyah Journal of Agricultural Sciences*, 9(2), 225-232.
- [17] Kaur,S. A. and S. Sharma.2024. "Azotobacter and Streptomyces Bioformulation for Improving Growth and Yield of Wheat (*Triticum aestivum* L.): A Field Study." *Journal of Plant Growth Regulation* (2024): 1-17
- [18] Cohan, J.P., C. Hannon, B. Houilliez,; J.M. Gravouelle, A. Geille, E. Lampaert and F. Laurent 2018. Influence of potato cultivars on components of nitrogen use efficiency. *Potato Research*, 61(3): 231-246
- [19] Yang, X., Zhang, C., Ma, X., Liu, Q., An, J., Xu, S and J. Geng. 2021. Combining organic fertilizers with slow-release urea to reduce nitrogen leaching and increase wheat yields. *Frontiers in Plant Science*, 12, 802137
- [20] Saquee, F. S., Diakite, S., Kavhiza, N. J., Pakina, E and M. Zargar . 2023. Effect of micronutrient fertilizers on wheat grain yield composition and quality. *Agronomy*, 13(2), 566



- [21] Black , C.A. 1965a. Methods of soil analysis. Part2. chemical and microbiological properties. Am. Soc. Agron , Inc. Madison , Wisconsin , USA.
- [22] Salem, S. J. and N. S. Ali. 2017. Directory of Chemical Analyzes of Soil, Water, Plants and Fertilizers. Ministry of Higher Education and Scientific Research. College of Agriculture - University of Baghdad. pp: 407. (In Arabic).
- [23] El-zawawy, H. A. H., El-aziz, A., and A. I. A. El-Ghomary.2023. Efficiency of *Azotobacter* on wheat plant growth and its anatomical characteristics. *Journal of Agricultural Chemistry and Biotechnology*, 14(6), 65-72
- [24] Azzawi, S. S., & Kamal, J. A. K. (2023, April). Effective of Bacterial Inoculation *Azotobacter Chroococcum* and *Bacillus Subtillis* in Inoculation Density and Available Phosphorus in Soil and Plant. In IOP Conference Series: Earth and Environmental Science (Vol. 1158, No. 2, p. 022002). IOP Publishing.
- [25] brahim, G. H., & Al-Alawy, H. H. M. (2024, July). Effect of Enriching Vermicompost with Chemical and Biological Fertilizers on the Growth and Yield of Sunflower. In IOP Conference Series: Earth and Environmental Science (Vol. 1371, No. 8, p. 082034). IOP.
- [26] Arbi, H. N., Dahham, A. A., & Kamal, J. A. K. (2021, April). Effect of Chemical and Organic Fertilization and *Aztobacter Chorocoocum* inoculation in IAA Acid and Growth plant (*Lycopersicon esculentum* Mill). In IOP Conference Series: Earth and Environmental Science (Vol. 735, No. 1, p. 012059). IOP Publishing.

Production of Lettuce Under Low Levels of Mineral Fertilizers in the Presence of Mycorrhizal Fungi and Azospirillum Bacteria and Spraying Organic Nutrients and Glutamic Acid

Ayad Ali Hunaish Al-Samah¹ and Ridha Mustafa Abed Al-Hussain Al-Ubaidy²

^{1,2}Department of Horticulture and Landscape, College of Agriculture Engineering Sciences, University of Baghdad, Baghdad, Iraq.

¹E-mail: ayad.ali2105p@coagri.uobaghdad.edu.iq

²E-mail: ridha.mustfa@coagri.uobaghdad.edu.iq

Abstract. The experiment was conducted in one of the fields of the organic fertilizer project in Al-Shatra District, Bani Zaid Sub-district, affiliated to the Directorate of Agriculture in Dhi Qar for the two agricultural seasons 2022-2023 and 2023-2024 with the aim of studying the effect of reducing mineral fertilizers using inoculation with mycorrhizal fungi and azospirillum and spraying organic nutrients and glutamic acid on lettuce production. The factorial experiment (4×3×3) was implemented according to randomized complete block design (RCBD) so that the number of treatments reached 36 treatments and three replicates, so the number became 108 experimental units with 15 plants per experimental unit. The experiment included three factors: the first factor was four levels of biofertilization (without inoculation, symbolized by A0, Mycorrhizae 15 g. plant⁻¹, symbolized by A1, Azospirillum 5 g. plant⁻¹, symbolized by A2, Mycorrhizae + Azospirillum, symbolized by A3). The second factor was spraying the organic nutrient Forteorgan at three levels (without addition, symbolized by B0, 2 ml L⁻¹, symbolized by B1, 4 ml L⁻¹, symbolized by B2). The third factor was spraying glutamic acid at three levels (without addition, symbolized by C0, 250, symbolized by C1, 500 mg L⁻¹, symbolized by C2). The averages of the results of the treatments were compared according to the least significant difference (LSD) test at a probability level of 5%. The most important results reached can be summarized as follows: Biofertilization treatments were significantly excelled at the level of treatment A3 in total plant weight (925 and 954.8 g), total yield per hectare (64.24 and 66.31 tons) and percentage of twisted heads for both seasons. Treatment A3 for the first season and A2 for the second season were excelled in lettuce stem weight. Most organic nutrient spray treatments were not significantly excelled. Glutamic acid spray treatments were also superior at the level of treatment C1 in head weight (620.5 and 645.5 g), total plant weight (864.3 and 876.4 g) and total yield per hectare (59.86 and 60.64 tons) for both seasons. As for bi-interaction between biofertilization and organic nutrient spraying, treatment A3B1 was excelled in the percentage of twisted heads for both seasons, and treatment A3B2 was excelled in head weight (691.3 and 760.2 g), total plant weight (980 and 1028.8 g), and total plant yield per hectare (68.05 and 71.45 tons) for both seasons. Treatment A3B2 was excelled for the first season and A2B1 for the second season in stem weight, and treatment A3B1 for the first season and A3B2 for the second season were excelled in the percentage of twisted heads. The interaction of biofertilization and glutamic acid spraying resulted in a

significantly excelled of treatment A3C1 in head weight (748.3 and 768.4 g), total plant weight (1043.4 and 1046.9 g), and total plant yield per hectare (72.46 and 72.7 tons) for both seasons. Treatment A3C1 for the first season and A2C1 for the second season were excelled in stem weight, and treatment A3C2 for the first season and A3C1 for the second season were excelled in the percentage of twisted heads. As for the interaction of organic nutrient spraying and glutamic acid, treatment B2C1 was excelled in stem weight for both season, and treatment B1C1 was excelled in head weight (658.8 and 685.1 g) for both season respectively, and treatment B1C2 was excelled in the percentage of twisted heads for both season, and treatment B1C1 for the first season and B2C2 for the second season were excelled in total plant weight (897.4 and 924 g) and plant yield per hectare (62.32 and 64.17 tons) for both season respectively. As for the triple interaction between biofertilization, organic nutrient spraying and glutamic acid, treatment A3B2C1 was excelled in stem weight, head weight (905.9 and 907.2 g), total plant weight (1288.2 and 1267 g) and total plant yield per hectare (89.46 and 88.02 tons) for both season respectively, and treatment A3B2C2 was excelled for the first season and A3B2C1 for the second season in the percentage of twisted heads.

Keywords. Organic Nutrients, Spraying, Lettuce.

1. Introduction

Lettuce (*Lactuca sativa* L.) belongs to the Asteraceae family, which is one of the largest plant families [1]. It is one of the most widely consumed leafy vegetable crops worldwide [2]. It is considered one of the important winter vegetable crops that is grown in the world and Iraq alike due to its high nutritional value as it contains water 94.61 g, protein 1.23 g, fat 0.30 g, carbohydrates 3.29 g, fiber 2.1 g, calcium 33 mg, phosphorus 30 mg, potassium 247 mg, zinc 0.23 mg, iron 0.97 mg, vitamins A 8710 IU, C 4.0 mg, B6 0.074 mg, thiamine 0.072 mg, and niacin 0.313 mg [3]. It is grown for its leaves, which are eaten fresh, due to its high nutritional value, as it is a crop rich in niacin and calcium, and has a medium content of iron, riboflavin and vitamin A. It also contains some organic acids such as citric acid [4]. Lettuce has shallow roots and needs high fertilization, especially nitrogen. Nitrogen plays an important role in the synthesis of amino acids and proteins, plant growth, chlorophyll formation, and the development of lettuce production [5]. Lettuce is a leafy vegetable that needs a lot of nitrogen fertilization. Since the irrational use of mineral fertilizers in agricultural areas causes pollution of groundwater and surface water, the need to use other techniques to reduce the use of mineral fertilizers without affecting the crop has emerged [6]. Lettuce and spinach are among the leafy vegetables richest in nitrate content accumulated in their leaves due to high amounts of mineral fertilizers [7]. Using more than the recommended amount of nitrate may cause some health problems [8]. The risk of exposure of the body to high nitrite is the conversion of blood hemoglobin to methemoglobin, which leads to a deficiency in oxygen transport in the blood [9]. Biofertilization is one of the modern techniques to reduce the excessive use of mineral fertilizers and reduce sources of pollution. This technique includes the use of beneficial microorganisms to improve soil properties. The use of fungal and bacterial biofertilizers has spread recently because they are inexpensive and environmentally safe [10,11]. Mycorrhizal fungi (*Glomus mosseae*) form symbiotic relationships with plant roots, where both organisms benefit from this symbiotic relationship. These fungi take carbohydrates (sugars) and growth factors from the plant and provide many benefits to the plant, such as increased nutrient absorption. It is a symbiotic relationship, formed between the roots of most vascular plant species. These symbioses are characterized by a two-way movement of nutrients, whereby fungi take up carbon while nutrients

are transferred to the plant. The growth of mycorrhizae in plant roots greatly increases the surface area of the root for external absorption. The absorption of nutrients by mycorrhizal fungi improves plant growth and production, and improves the mass of vegetative growth [12].

Azotobacter and Azospirillum are among the most efficient free-living microorganisms in terms of their ability to fix atmospheric nitrogen [13]. Azospirillum has the ability to fix atmospheric nitrogen, produce plant hormones, and secrete compounds that improve soil structure and absorption of nutrients [14]. Mineral fertilizers increase the production of vegetable crops, however, the use of mineral fertilizers at high levels leads to increased levels of pollutants and increased pollution of soil and groundwater and increased concentration of nitrates in the leaves of vegetable crops [15]. However, with the use of organic fertilizers, the severity of these problems can be alleviated [16]. Amino acids, including glutamic acid, work to homogenize the growth of lettuce crops [17]. Glutamic acid is considered a stimulator of increased vegetative growth and early production [18]. Spraying amino acids contributes to increasing the growth and productivity of many agricultural crops [19]. aims of the study:

- Rationalizing the use of mineral fertilizers and knowing the effect of biofertilizers on the growth and productivity of lettuce.
- Knowing the effect of spraying the organic nutrient Forteorgan and the amino acid glutamic on lettuce productivity. 3- Studying the effect of the interaction between mycorrhizal fungi and Azospirillum bacteria and spraying the organic nutrient and glutamic acid on the growth and productivity of lettuce.

2. Materials and Methods

An experiment was conducted in autumn seasons 2022-2023 and 2023-2024 in one of the fields affiliated with the Organic Fertilizers Project - Dhi Qar Agriculture Directorate in Bani Zaid District, affiliated with Shatrah District. Random samples were taken from the field soil at a depth of 0-30 cm before planting, and laboratory analyses of the physical and chemical properties were conducted in the laboratories affiliated with the Agricultural Research Unit - Ministry of Agriculture.

The land allocated for the study was prepared by ploughing it perpendicularly with a rototiller, then it was leveled with a leveling machine after smoothing it well with disc harrows. The area allocated for the experiment was divided into three sectors (in the form of terraces) and the sectors were divided into 36 experimental units (36 experimental units $\times$ 3 replicates). The area of the experimental unit was 2.16 m² with dimensions of (2.4 $\times$ 0.9) m, the distance between one experimental unit and another was 0.8 m, between one sector and another was 1.5 m, the distance between one plant and another was 0.30 m, and between lines was 0.40 m. Each experimental unit contained 15 plants. A total of 108 experimental units. The drip system was installed, then the drip irrigation pipes were connected at a rate of 15 pipes, for each sector 5 pipes, the distance between the pipes in the sector is 0.40 m, and the distance between the drippers in the pipe is 0.30 m, where the seedlings were planted at this distance (0.30 m between plants). All the above land preparation steps were implemented for both autumn agricultural seasons. Lettuce seeds (local) were planted for both autumn seasons on 10/1/2022 and 10/1/2023 in the panels that were well prepared to produce lettuce seedlings, then the seeds were scattered and covered with a light layer of soil and lightly irrigated to prevent them from drifting, after which they were irrigated when needed. The seedlings were transferred to the permanent field at the age of 30 days from the date of planting after forming 4-5 true leaves. Holes were dug 5-10 cm deep for the seedlings, the seedlings were irrigated immediately after transplanting, weeding was carried out to get rid of weeds as needed and manually only, and drip irrigation was carried out when the plants needed water.

The factorial experiment included three factors within the Randomized Complete Block Design (R.C.B.D) and three replicates with 36 treatments for each replicate, so that the total number of treatments in the experiment was 108 treatments.

Urea fertilizer was used as a source of nitrogen and diammonium phosphate (P₂O₅) DAP (46% P₂O₅, 18% N) was used as a source of phosphorus, where half of the fertilizer recommendation was added to all experimental units (110 kg ha⁻¹ urea was added and 60 kg ha⁻¹ DAP was added before the transplanting process and for all treatments) [20,21] For both agricultural seasons

2.1. Experimental Treatments for Both Seasons

2.1.1. The First Factor is Four Levels of Biofertilization

Adding 15 g.pit⁻¹ of peat moss and plant roots loaded with mycorrhizal fungal inoculum with an inoculum density of 50 spores g. moss⁻¹ at a depth of 5 cm below the soil surface under the roots of lettuce seedlings, and adding 5 g.pit⁻¹ of Azospirillum lipoferum bacterial inoculum at an inoculum density (1.5×10⁹ cells ml⁻¹) under the roots of lettuce seedlings [22] was added when planting plants in the permanent field as shown in the following steps:

- Without addition and symbolized by A0.
- Inoculation with mycorrhizal fungus alone. (Adding 15 g.pit⁻¹) at a depth of 5 cm below the roots of lettuce seedlings and symbolized by A1.
- Inoculation with Azospirillum bacteria alone. Add 5 g.pit⁻¹ and symbolize it A2.
- Inoculation with Mycorrhizal fungus + Azospirillum bacteria and symbolize it A3.

2.1.2. The Second Factor (Fertiorgan Organic Fertilizer)

It consists of (organic matter 42%, calcium 1.68%, magnesium 1.2%, boron 0.012%, iron 0.024%, manganese 0.012%, zinc 0.0024%) where the lettuce crop was sprayed with three concentrations of organic fertilizer:

- Without adding and symbolized by B0.
- Spraying the recommended concentration 2 ml L⁻¹ according to the recommendation of the producing company and symbolized by B1.
- Spraying 4 ml L⁻¹ and symbolized by B2.

Spraying organic fertilizer:

- The first spray after 15 days from the planting date.
- The second spray after 30 days from the planting date.
- The third spray after 45 days from the planting date.

2.1.3. The Third Factor (Amino Acid)

Spraying the lettuce crop with three concentrations of glutamic acid (0, symbolized by C0, 250, symbolized by C1, 500 mg L⁻¹, symbolized by C2) [23].

Glutamic acid was sprayed:

- The first spray after 18 days from the planting date.
- The second spray after 33 days from the planting date.
- The third spray after 48 days from the planting date.

The results were analyzed after completing the study indicators using the statistical analysis program GenStat 12. The averages were tested at the least significant difference (LSD) at the probability level of 0.05 for the purpose of comparing the averages [24].

2.2. Study Indicators

2.2.1. Stem Weight ($g\ plant^{-1}$)

The plant was cut from the area where it was in contact with the soil, then all the leaves were removed and the stem was weighed (which is from the area where the stem was in contact with the soil to the top of the stem without the leaves).

2.2.2. Marketing Head Weight ($g.plant^{-1}$)

Five heads were weighed and the average weight of the marketing head was calculated after removing the uncured and damaged leaves, where the weights of the marketing heads in the experimental unit were divided by their number according to the following equation:

$$\text{Average weight of marketing head (g)} = \frac{\text{Total weights of marketing heads (g)}}{\text{Number marketing heads}}$$

2.2.3. Total Plant Weight ($g. plant^{-1}$)

According to the average plant after removing it from the place of contact with the soil by dividing the total weights of the plants in the experimental unit by their number and according to the equation below:

$$\text{Average weight of the plant (g)} = \frac{\text{Total weights of plants in the experimental unit (g)}}{\text{Number of plants in the experimental unit}}$$

2.2.4. Total Yield of The Plant ($ton .ha^{-1}$)

According to the yield of the experimental unit for the plant according to the following equation:

$$\text{Experimental unit yield} = \text{Average plant weight} \times \text{Number of plants in the experimental unit}$$

Then the total yield was calculated For the plant according to the following equation:

$$\text{Total yield (ton ha}^{-1}\text{)} = \frac{\text{Yield of the experimental unit (ton plant}^{-1}\text{)} \times \text{area of the hectare (10,000 m}^2\text{)}}{\text{(Area of the experimental unit m}^2\text{)}}$$

2.2.5. Percentage of Twisted Heads %

The number of twisted heads in the experimental unit was calculated divided by the total number of plants in the same experimental unit multiplied by 100 according to the following equation:

$$\text{Percentage of twisted heads} = \frac{\text{Number of twisted heads in the experimental unit}}{\text{(Total number of plants in the experimental unit)}} \times 100$$

3. Results and Discussion

3.1. Stem Weight ($g\ plant^{-1}$)

The results of Table 1 indicate that the biofertilization treatment A3 and A2 was significantly excelled to the control treatment only, as it gave a weight of (145.4 and 139.4) $g\ plant^{-1}$ for both growth season, respectively, compared to the control treatment with the lowest weight of (115.3 and 116.6) $g\ plant^{-1}$ for both growth season. The results of spraying organic nutrients showed that treatment B2 was excelled without significant differences, with a weight of (138.5 and 131.5) $g\ plant^{-1}$ for both growth season, while the control treatment B0 gave the lowest weight of (128.8 and 125.3) $g\ plant^{-1}$ for both growth season. The glutamic acid treatments indicated the superiority of treatment C1 without significant differences and with a weight of (138.7 and 130.8)

g plant⁻¹ for both growth season, as the control treatment had the lowest weight of (129.5 and 126.1) g plant⁻¹ for both growth season. In the same table, the results of the dual interaction between biofertilization and organic nutrient spraying showed that treatment A3B2 and A2B1 were excelled with a significant difference compared to the control treatment with a weight of (157.7 and 156.5) g plant⁻¹ for both growth season respectively, while the control treatment A0B0 had the lowest weight of (112.3 and 111.8) g plant⁻¹ for both growth season.

The interactions of biofertilizer and glutamic acid showed that treatment A3C1 and A2BC1 were significantly excelled compared to the control treatment with a weight of (162.2 and 145.8) g plant⁻¹ for both growth season respectively, while the lower weight was for the control treatment which was (111.8 and 112.4) g plant⁻¹ for both growth season.

The interactions of organic nutrient spraying and glutamic acid showed a significantly excelled for treatment B2C1 compared to the control treatment for the first season with a weight of 151.6 g plant⁻¹ for the first growth season, while the control treatment had the lowest weight of 121.7 g plant⁻¹ for the first growth season. No significant differences were shown for the organic nutrient spraying and glutamic acid treatments during the second season, but treatment B2C1 was significantly excelled and gave a weight of 138.1 g plant⁻¹ for the second growth season. The results of the triple interaction indicated the superiority of treatment A3B2C1 for the first and second seasons and A2B2C0 for the second season compared to the control treatment with values of (235.9, 183.8 and 184.6) g plant⁻¹ for both growth season respectively, and the lowest values were in the control treatment A0B0C0 with a weight of (95.9 and 98.9) g plant⁻¹ for both growth season.

Table 1. Effect of inoculation with *Glomus mosseae* and *Azospirillum lipoferum*, organic nutrient spraying, glutamic acid and their interactions on lettuce stem weight (g plant⁻¹).

Organic nutrient spray (B)	Glutamic acid spray (C)	First autumn agricultural season 2022-2023					Second autumn agricultural season 2022-2023				
		Biofertilization (A)				Average B ×C	Biofertilization (A)				Average B ×C
		0	1	2	3		0	1	2	3	
0	0	95.9	103.3	137.8	149.8	121.7	98.9	102.1	133	144.5	119.6
	1	125	151.6	113.3	115.4	126.3	112.2	147.8	116.7	111.6	122.1
	2	117.1	120	167.1	149.8	138.5	124.3	106.7	161.4	144.2	134.1
1	0	141.2	108.5	187.7	121.7	139.8	137.8	113.1	184.6	105.4	135.2
	1	122.3	121.6	173.9	135.2	138.2	118.7	118.7	168.1	123.2	132.1
	2	96.7	117.1	113.3	163.6	122.7	112.1	113	116.7	157.6	124.8
2	0	98.3	177.4	125	107.1	127	100.6	171.2	108.3	113.3	123.4
	1	100	111.9	158.4	235.9	151.6	107.4	108.5	152.6	183.8	138.1
	2	141.2	160.2	116.7	130	137	137.1	154.8	113.3	126.7	133
L.S.D interaction		42.12				21.06	44.14				22.07N.S
average bio A		115.3	130.2	143.7	145.4	average B	116.6	126.2	139.4	134.5	average B
L.S.D		14.04					14.71				
A× B	0	112.7	125	139.4	138.4	128.8	111.8	118.8	137	133.4	125.3
	1	120.1	115.7	158.3	140.1	133.6	122.8	114.9	156.5	128.7	130.7

	2	113.2	149.8	133.4	157.7	138.5	115	144.8	124.8	141.3	131.5
L.S.D			24.32			12.16 N.S		25.48			12.74N.S
						average C					average C
	0	111.8	129.7	150.2	126.2	129.5	112.4	128.8	142	121.1	126.1
A × C	1	115.8	128.4	148.6	162.2	138.7	112.8	125	145.8	139.5	130.8
	2	118.3	132.4	132.4	147.8	132.7	124.5	124.8	130.5	142.8	130.6
L.S.D			24.32			12.16 N.S		25.48			12.74 N.S

Biofertilization A: A0 Without addition. A1 Mycorrhizae 15g hole⁻¹. A2 Azospirillum 5g hole⁻¹. A3 Mycorrhizae + Azospirillum interaction.

Organic fertilization B: B0 Spray water only. B1 Spray 2 ml L⁻¹. B2 Spray 4 ml L⁻¹.

Glutamic acid C: C0 Spray water only. C1 Spray 250 mg L⁻¹. C2 Spray 500 mg L⁻¹.

3.2. Weight of the Marketing Head (g plant⁻¹)

Table 2 shows the effect of biofertilization, organic nutrient spraying and glutamic acid and their interactions on the weight of the marketing head (g plant⁻¹) for both growth season. The biofertilization treatments showed that treatment A3 was significantly excelled to all treatments, reaching (661.1 and 704.9) g plant⁻¹ for both growth season, while the control treatment was the lowest value, reaching (524.5 and 540.7) g plant⁻¹ for both growth season. The organic nutrient B1 treatment was significantly excelled to the control treatment only, reaching 609.1 g plant⁻¹ for the first growth season, while the control treatment B0 had the lowest weight, reaching 568.3 g plant⁻¹ for the first growth season. In the second season, the organic nutrient B2 treatment was significantly excelled, reaching 622.5 g plant⁻¹ for the second growth season, while the control treatment B0 had the lowest weight, reaching 608.2 g plant⁻¹ for the second growth season. The glutamic acid treatments showed a significantly excelled in the C1 treatment over the control treatment only, as it gave a weight of (620.5 and 645.5) g plant⁻¹ for both growth season, compared to the control treatment, which had the lowest weight, reaching (544.8 and 569.8) g plant⁻¹ for both growth season. The same table indicates the effect of bi-interaction between biofertilization and organic nutrient spraying, indicating that treatment A3B2 was significantly excelled compared to the control treatment, giving a weight of (691.3 and 760.2) g plant⁻¹ for both growth season, while treatment A0B0 and A0B2 recorded the lowest value of (491.1 and 513.7) g plant⁻¹ for both growth season, respectively. When biofertilization and glutamic acid were interacted, treatment A3B1 significantly excelled the control treatment only, reaching (748.3 and 768.4) g plant⁻¹ for both growth season, while treatment A0C0 recorded the lowest value of (460.1 and 493.2) g plant⁻¹ for both growth season. When the organic nutrient spray and glutamic acid were combined, treatment B1C1 was significantly excelled compared to the control treatment, with a value of (658.8 and 685.1) g plant⁻¹ for both growth season, while the lowest treatment was B2C0, which was (512.2 and 551.2) g plant⁻¹ for both growth season, respectively. As for the triple interaction between the study factors, treatment A3B2C1 was significantly excelled to all treatments, and gave a weight of (905.9 and 907.2) g plant⁻¹ for both growth season, while the control treatment A0B0C0 was the lowest, with a weight of (365.4 and 406.8) g plant⁻¹ for both growth season.

Table 2. Effect of inoculation with *Glomus mosseae*, *Azospirillum lipoferum*, organic nutrient spray, and glutamic acid and their interactions on head weight (g head⁻¹).

Organic nutrient spray (B)	Glutamic acid spray (C)	First autumn agricultural season 2022-2023					Second autumn agricultural season 2022-2023				
		Biofertilization (A)				Average B × C	Biofertilization (A)				Average B × C
		0	1	2	3		0	1	2	3	
0	0	365.4	504.6	623.4	660.5	538.5	406.8	525.7	655.1	711.4	574.8
	1	553.3	601.1	467	625.2	561.6	593	623.4	540.3	652.4	602.3
	2	554.6	510.9	699.2	654.8	604.9	595.2	525.9	727	741.9	647.5
1	0	606.2	563.2	627.7	538.3	583.9	627.9	594.9	610.8	500.1	583.4
	1	628.6	576.3	716.4	713.8	658.8	653.6	597.4	743.7	745.6	685.1
	2	510	582.1	562.4	683.7	584.6	448.6	613.8	531.4	712.5	576.6
2	0	408.5	683.7	438.4	518.1	512.2	444.8	706.1	443.8	610	551.2
	1	477.1	564.9	616.6	905.9	641.1	452.3	593.1	643.8	907.2	649.1
	2	616.6	700.9	507.6	650	618.8	644.1	733.5	528	763.3	667.2
L.S.D interaction		124.54				62.27	137				68.5
average bio A		524.5	587.5	584.3	661.1	average B	540.7	612.7	602.7	704.9	average B
L.S.D		41.51				B	45.67				
A × B	0	491.1	538.9	596.6	646.8	568.3	531.7	558.3	640.8	701.9	608.2
	1	581.6	573.8	635.5	645.3	609.1	576.7	602.1	628.6	652.7	615
	2	500.7	649.9	520.9	691.3	590.7	513.7	677.6	538.5	760.2	622.5
L.S.D		71.9				35.95	79.1				39.55 N.S
						average C					average C
A × C	0	460	583.8	563.2	572.3	544.8	493.2	608.9	569.9	607.2	569.8
	1	553	580.7	600	748.3	620.5	566.3	604.7	642.6	768.4	645.5
	2	560.4	598	589.8	662.9	602.7	562.6	624.4	595.5	739.2	630.4
L.S.D		71.9				35.95	79.1				39.55

Biofertilization A: A0 Without addition. A1 Mycorrhizae 15g hole⁻¹. A2 Azospirillum 5g hole⁻¹. A3 Mycorrhizae + Azospirillum interaction.

Organic fertilization B: B0 Spray water only. B1 Spray 2 ml L⁻¹. B2 Spray 4 ml L⁻¹.

Glutamic acid C: C0 Spray water only. C1 Spray 250 mg L⁻¹. C2 Spray 500 mg L⁻¹.

3.3. Total Plant Weight (g plant⁻¹)

Table 3 shows the effect of biofertilization, organic nutrient spraying, and glutamic acid and their interactions on the total plant weight (g plant⁻¹) for both growth season, where biofertilization A3 recorded a significantly excelled over all treatments and reached (925 and 954.8) mg 100 g⁻¹ fresh weight for both growth season, while control treatment had the lowest weight, reaching (777.1

and 774.6) g⁻¹ fresh weight for both growth season. The result of organic nutrient spraying for treatment B1 (851.2) g⁻¹ fresh weight for the first growth season was significantly excelled to the control treatment only. And B2 with a result of (856.6) g⁻¹ fresh weight for both growth season without a significant difference, and the control treatment had the lowest value, reaching (828.8) g⁻¹ fresh weight for the second growth season. As for the effect of glutamic acid, treatment C1 showed a significantly excelled over the control treatment only, with a value of (864.3 and 876.4) g⁻¹ fresh weight for both growth season, and the lowest value for the control treatment C0, which reached (767.3 and 788) g⁻¹ fresh weight for both growth season, respectively. As for the results of bi-interaction between biofertilization and organic nutrient spraying, treatment A3B2 was significantly excelled with a weight of (980 and 1028.8) gm⁻¹ fresh weight for both growing seasons compared to the control treatment. The interaction of biofertilization and glutamic acid indicated that treatment A3C1 was significantly excelled to all treatments in the first season and most treatments in the second season, as it gave a weight of (1043.4 and 1046.9) gm⁻¹ fresh weight for both growing seasons, and the control treatment A0C0 appeared with the lowest values, amounting to (681 and 706.6) gm⁻¹ fresh weight for both growing seasons. As for the interaction of organic nutrient spraying and glutamic acid, treatment B2C1 and B2C2 were significantly excelled and gave a result of (897.6 and 924) gm⁻¹ fresh weight for both growing seasons compared to the control treatment. The results of the same table on the triple interaction indicate that treatment A3B2C1 was significantly excelled, with values of (1288.2 and 1267.5) g⁻¹ fresh weight for both growing seasons, compared to the standard treatment, which gave the lowest values, reaching (567.1 and 630.1) gm⁻¹ fresh weight for both growing seasons, respectively.

Table 3. Effect of inoculation with *Glomus mosseae*, *Azospirillum lipoferum*, organic nutrient spray, glutamic acid and their interactions on total plant weight (g plant⁻¹).

Organic nutrient spray (B)	Glutamic acid spray (C)	First autumn agricultural season 2022-2023					Second autumn agricultural season 2022-2023				
		Biofertilization (A)				Average B × C	Biofertilization (A)				Average B × C
		0	1	2	3		0	1	2	3	
0	0	567.1	656.2	861.1	853.4	734.4	630.1	707.4	879.1	893.8	777.6
	1	806.5	842.2	659.9	883.5	798	831.5	851.5	737.1	896.4	829.1
	2	859.4	713.1	948.9	925.2	861.7	883.1	674.1	962.9	998.6	879.6
1	0	856.7	787.1	918.8	836.5	849.8	856.4	807.3	885.9	797.8	836.8
	1	880.3	820.8	930	958.4	897.4	865.7	829	944.1	976.8	903.9
	2	720.4	818.1	758.8	928.3	806.4	618.1	835.5	715.9	943.5	778.3
2	0	619.2	916.2	600.3	734.7	717.6	633.2	926.6	635.5	803.3	749.6
	1	706.1	748.8	847.3	1288.2	897.6	668.8	788.1	860.3	1267.5	896.2
	2	978.2	912.8	755.6	916.9	890.9	984.9	933.4	762.1	1015.7	924
L.S.D interaction		151.42				75.71	160.6				80.3
average bio A		777.1	801.7	809	925	average B	774.6	817	820.3	954.8	average B
L.S.D		50.47				B	53.53				

Organic nutrient spray (B)	Glutamic acid spray (C)	First autumn agricultural season 2022-2023					Second autumn agricultural season 2022-2023						
		Biofertilization (A)				Average B ×C	Biofertilization (A)				Average B ×C		
		0	1	2	3		0	1	2	3			
A× B	0	744.3	737.1	823.3	887.4	798	781.6	744.3	859.7	929.6	828.8		
	1	819.1	808.6	869.2	907.7	851.2	780.1	823.9	848.7	906	839.7		
	2	767.8	859.3	734.4	980	835.4	762.3	882.7	752.6	1028.8	856.6		
L.S.D		87.42				43.71	92.72				46.36 N.S		
						average C							average C
A× C	0	681	786.5	793.4	808.2	767.3	706.6	813.7	800.2	831.6	788		
	1	797.6	803.9	812.4	1043.4	864.3	788.7	822.9	847.2	1046.9	876.4		
	2	852.7	814.6	821.1	923.5	853	828.7	814.3	813.6	985.9	860.6		
L.S.D		87.42				43.71	92.72				46.36		
Biofertilization A: A0 Without addition. A1 Mycorrhizae 15g hole ⁻¹ . A2 Azospirillum 5g hole ⁻¹ . A3 Mycorrhizae + Azospirillum interaction.													
Organic fertilization B: B0 Spray water only. B1 Spray 2 ml L ⁻¹ . B2 Spray 4 ml L ⁻¹ .													
Glutamic acid C: C0 Spray water only. C1 Spray 250 mg L ⁻¹ . C2 Spray 500 mg L ⁻¹ .													

3.4. Total Yield of Lettuce (ton ha⁻¹)

Table 4 shows the effect of biofertilization, organic nutrient spraying and glutamic acid on the total yield of lettuce, ton ha⁻¹ for both growing seasons, as treatment A3 showed significantly excelled over all treatments, which gave a weight of (64.24 and 66.31) tons ha⁻¹ for both growing seasons, while the control treatment A0 had the lowest weight (53.96 and 53.79) tons ha⁻¹ for both growing seasons. As for the organic nutrient spray treatments, treatment B11 significantly excelled the control treatment only and gave a weight of (59.11) tons ha⁻¹ for the first growing season compared to the control treatment with the lowest value of (55.42) tons ha⁻¹ for the first growing season.

As for treatment B2, it excelled on the rest of the treatments with a non-significant difference, as it reached (59.27) tons ha⁻¹ for the second growing season, and the control treatment B0 had the lowest value of (57.56) tons ha⁻¹ for the second growing season. As for the glutamic acid treatments, treatment C1 significantly excelled the control treatment with a result of (59.86 and 60.64) tons ha⁻¹ for both growing seasons compared to the control treatment C0 with the lowest value of (53.28 and 54.72) tons ha⁻¹ for both growing seasons. From the same table, the results of the dual interaction, biofertilization and organic nutrient spraying, indicate that treatment A3B2 significantly excelled all treatments except treatment A3B1 with a weight of (68.05) tons ha⁻¹ for the first growing season, and treatment A3B2 significantly excelled all treatments with a weight of (71.45) tons ha⁻¹ for the second growing season, and the lowest weight was in treatment A2B0 and A1B0, which amounted to (51 and 51.69) tons ha⁻¹ for both growing seasons in succession.

As for biofertilization and glutamic acid, treatment A3C1 excelled on all treatments in the first season except treatment A3C2 in the second season, giving them a weight of (72.46 and 72.7)

tons ha⁻¹ for both growing seasons in succession, and the lowest weight was in control treatment A0C0, which amounted to (47.29 and 49.07) tons ha⁻¹ for both growing seasons in succession. The results of the interaction of organic nutrient spraying and glutamic acid indicated the superiority of treatment B1C1 and B2C2 with a weight of (62.32 and 64.17) tons ha⁻¹ for the two successive growth seasons with a significant difference with the control treatment, and the lowest weight was for treatment B2C0 and B0C0 with a weight of (49.83 and 54) tons ha⁻¹ for the two successive growth seasons. The results of the same table indicate that the triple interaction had a significant effect as treatment A3B2C1 excelled on all treatments with a significant difference by giving it the highest average weight of (89.46 and 88.02) tons ha⁻¹ for both growth season, and the lowest weight was in control treatment A0B0C0 which reached (39.38 and 43.76) tons ha⁻¹ for both seasons.

Table 4. Effect of inoculation with *Glomus mosseae*, *Azospirillum lipoferum*, organic nutrient spray, glutamic acid and their interactions on total yield (ton ha⁻¹).

Organic nutrient spray (B)	Glutamic acid spray (C)	First autumn agricultural season 2022-2023					Second autumn agricultural season 2022-2023				
		Biofertilization (A)				Average B ×C	Biofertilization (A)				Average B ×C
		0	1	2	3		0	1	2	3	
0	0	39.38	45.57	59.8	59.26	51	43.76	49.12	61.05	62.07	54
	1	56.01	58.48	45.83	61.35	55.42	57.74	59.13	51.19	62.25	57.58
	2	59.68	49.52	65.9	64.25	59.84	61.32	46.81	66.87	69.35	61.09
1	0	59.49	54.66	63.8	58.09	59.01	59.47	56.06	61.52	55.4	58.11
	1	61.13	57	64.58	66.55	62.32	60.12	57.57	65.57	67.83	62.77
	2	50.03	56.81	52.69	64.46	56	42.92	58.02	49.71	65.52	54.05
2	0	43	63.63	41.69	51.02	49.83	43.97	64.34	44.13	55.79	52.06
	1	49.04	50.03	58.84	89.46	61.84	46.45	52.07	59.75	88.02	61.57
	2	67.93	63.39	52.47	63.68	61.87	68.4	64.82	52.92	70.54	64.17
L.S.D interaction		10.592				5.296	11.074				5.537
average bio A		53.96	55.45	56.18	64.24	average B	53.79	56.44	56.97	66.31	average B
L.S.D		3.531					3.691				
A× B	0	51.69	51.19	57.17	61.62	55.42	54.28	51.69	59.7	64.56	57.56
	1	56.88	56.16	60.36	63.04	59.11	54.17	57.22	58.93	62.92	58.31
	2	53.32	59.01	51	68.05	57.85	52.94	60.41	52.27	71.45	59.27
L.S.D		6.115				3.058	6.394				3.197 N.S
						average C					average C
A× C	0	47.29	54.62	55.1	56.13	53.28	49.07	56.51	55.57	57.75	54.72
	1	55.39	55.17	56.42	72.46	59.86	54.77	56.26	58.83	72.7	60.64
	2	59.21	56.57	57.02	64.13	59.23	57.55	56.55	56.5	68.47	59.77

L.S.D	6.115	3.058	6.394	3.197
Biofertilization A: A0 Without addition. A1 Mycorrhizae 15g hole ⁻¹ . A2 Azospirillum 5g hole ⁻¹ . A3 Mycorrhizae + Azospirillum interaction.				
Organic fertilization B: B0 Spray water only. B1 Spray 2 ml L ⁻¹ . B2 Spray 4 ml L ⁻¹ .				
Glutamic acid C: C0 Spray water only. C1 Spray 250 mg L ⁻¹ . C2 Spray 500 mg L ⁻¹ .				

3.5. Percentage of Twisted Heads %

The results of Table 5 showed the effect of biofertilization treatments on the percentage of twisted heads, as treatment A3 significantly excelled the control treatment only by giving a percentage of 86.56% for the first growing season compared to the control treatment which gave the lowest percentage of 83.23% for the first growing season. As for treatment A3 for the second season, it significantly excelled the rest of the treatments and gave a percentage of twisted heads of 95.51% for the second growing season compared to the control treatment which gave the lowest value of 91.78% for the second growing season. The organic nutrient spray treatments did not show any superiority in the percentage of twisted heads, but treatment B1 excelled on by a non-significant difference and gave a percentage of twisted heads of (85.23 and 93.84)%. Also, glutamic acid treatments did not show any significantly excelled, but treatment C2 and C1 were excelled by a non-significant difference and their percentage reached (85.26 and 93.9)% for both growth season respectively. As for the interaction of biofertilization and organic nutrient spraying, treatment A3B1 was excelled by a non-significant difference and gave a percentage of 86.56% for the first growth season. Treatment A3B2 was excelled significantly and gave a percentage of twisted heads that reached 95.69% for the second growth season compared to the control treatment that gave the lowest percentages, reaching 91.35% for the second growth season. The interaction coefficients between biofertilization and glutamic acid showed that treatment A3C2 and A3C1 were significantly excelled and gave percentages of (86.92 and 95.69)% for both growth season respectively compared to the control treatment with the lowest percentage which was (81.45 and 89.93)% for both growth season. The organic nutrient spraying and glutamic acid treatments did not show any significant difference, but treatment B1C2 was excelled by a non-significant difference and the percentage of twisted heads was (86.23 and 94.85)% for both growth season. The same table indicated the effect of the triple interaction coefficients between the study factors, as treatment A3B2C2 was excelled by a non-significant difference in the first season and gave a percentage of 86.96% for the first growth season. In the second season, treatment A3B2C1 was significantly excelled, reaching 96.11% for the second growth season, compared to the control treatment, which gave the lowest value, reaching 90.61% for the second growth season.

Table 5. Effect of inoculation with *Glomus mosseae*, *Azospirillum lipoferum*, organic nutrient spray, glutamic acid and their interactions on the percentage of twisted heads %.

Organic nutrient spray (B)	Glutamic acid spray (C)	First autumn agricultural season 2022-2023					Second autumn agricultural season 2022-2023				
		Biofertilization (A)				Average B × C	Biofertilization (A)				Average B × C
		0	1	2	3		0	1	2	3	
0	0	81.51	84.45	82.76	86.72	83.86	90.61	95.29	90.89	95.4	93.04
	1	84.13	83.86	84.33	86.82	84.78	92.44	93.17	92.67	95.4	93.42
	2	82.79	84.46	84.47	86.91	84.66	91	92.83	92.83	95.4	93.02
1	0	81.19	84.46	84.47	86.72	84.21	89.84	92.83	92.83	95.4	92.73

Organic nutrient spray (B)	Glutamic acid spray (C)	First autumn agricultural season 2022-2023					Second autumn agricultural season 2022-2023				
		Biofertilization (A)				Average B × C	Biofertilization (A)				Average B × C
		0	1	2	3		0	1	2	3	
2	1	86.55	84.37	83.15	86.96	85.26	96.11	92.73	91.41	95.56	93.95
	2	86.82	86.72	84.47	86.91	86.23	95.78	95.4	92.83	95.4	94.85
	0	81.66	86.82	82.98	86.8	84.57	89.33	95.4	91.19	95.4	92.83
	1	82.23	86.72	86.8	84.24	85	90.44	95.4	95.4	96.11	94.34
	2	82.23	83.62	86.82	86.96	84.91	90.44	91.88	95.4	95.56	93.32
	L.S.D interaction	6.943 N.S				3.471 N.S	4.308				2.154 N.S
average bio A		83.23	85.05	84.47	86.56	average B	91.78	93.88	92.83	95.51	average B
L.S.D		2.314				average C	1.436				average C
A × B	0	82.81	84.26	83.85	86.81		91.35	93.76	92.13	95.4	
	1	84.85	85.18	84.03	86.86		93.91	93.65	92.36	95.45	
	2	82.04	85.72	85.54	86		90.07	94.22	94	95.69	
L.S.D		4.008 N.S				2.004 N.S	2.487				1.244 N.S
A × C	0	81.45	85.24	83.4	86.75	84.21	89.93	94.51	91.64	95.4	92.87
	1	84.3	84.98	84.76	86	85.01	93	93.77	93.16	95.69	93.9
	2	83.95	84.93	85.25	86.92	85.26	92.41	93.37	93.69	95.45	93.73
	L.S.D	4.008				2.004 N.S	2.487				1.244 N.S

Biofertilization A: A0 Without addition. A1 Mycorrhizae 15g hole⁻¹. A2 Azospirillum 5g hole⁻¹. A3 Mycorrhizae + Azospirillum interaction.

Organic fertilization B: B0 Spray water only. B1 Spray 2 ml L⁻¹. B2 Spray 4 ml L⁻¹.

Glutamic acid C: C0 Spray water only. C1 Spray 250 mg L⁻¹. C2 Spray 500 mg L⁻¹.

The use of biofertilizer showed a significant effect in increasing the growth indicators and yield of lettuce plants as shown in Tables (1, 2, 3, 4, 5). This may be due to improving the ability of roots inoculated with mycorrhizal fungi to absorb water and nutrients, as mycorrhizal fungi increase the ability of roots to transport water and solutes to the plant by expanding the root hair network as well as fungal hyphae [25]. Mycorrhizal fungi can be used as an environmentally friendly alternative and complement to chemical fertilizers in agriculture, as the results of inoculation indicated an increase in lettuce yield [26] This may be due to the role of mycorrhizae in increasing the absorption of nutrients that the plant needs for growth and development processes [27]. One study has proven the importance of mycorrhizal fungi in increasing the production of the biological yield of a group of legumes and non-legumes, as the size and weight of the root system in these plants increased, and this increase benefits the overall growth and

development processes as a result of its activity and the increase in the surface area of the root system and the expansion of the spread area, thus benefiting from the water and nutrients that the roots reach, as the weight of the green system of all non-legume plants increased [28]. Lettuce inoculated with mycorrhizae had good growth, quality and high chlorophyll content even when using half the recommended fertilizer for phosphorus. This may be attributed to the ability of these fungi to increase the efficiency and stimulate the roots of the plant to absorb nutrients, as the content of the vegetative group of nitrogen, phosphorus, potassium and magnesium increased compared to plants not inoculated with mycorrhizae, which increased the weight of the vegetative group of the plant [29]. Phosphorus is one of the major elements in plant nutrition. The symbiosis of the plant with mycorrhizae increases plant growth and enhances the absorption of phosphorus from the soil, which is not readily available in abundant quantities in the root zone by dissolving phosphate by the enzyme phosphatase to provide mineral phosphorus for the plant [30]. These results are consistent with the results of experiments on lettuce plants, where the effect of mycorrhizae was significant on the yield of lettuce plants [31]. Azospirillum bacteria also provide the associated plant with nitrogen, which is necessary for the life, growth and development of the plant [32]. Climate changes have affected the production of agricultural crops using traditional methods, so researchers have turned to some alternatives, including the use of beneficial microorganisms such as (bacteria and fungi) that help plants adapt to these conditions and are also capable of fixing atmospheric nitrogen, which is one of the most important nutrients for plants and increases the size and weight of their green group [33]. These results are consistent with what was obtained by [34] when using Azospirillum bacteria on lettuce and watercress plants. These results are also consistent with a study on lettuce by the researcher [35] The use of microorganisms as plant inoculants is a growing practice in agricultural operations, and the reason is that the combination of two or more beneficial microorganisms can produce synergistic effects that are beneficial to plants. This procedure is not correct in all cases, as sometimes unfavorable antagonistic interactions may arise [36]. Through the results obtained in Table (2, 3, 4), it was found that spraying organic nutrients created significant differences in the weight of the marketing head, the total plant weight, and the total yield of lettuce per hectare, respectively. The use of spraying Moringa extract as an organic nutrient with feeding with mineral solutions contributed well to reducing mineral fertilization and gave positive results in the values of vegetative growth, chemical properties, plant productivity, and decreased nitrate content [37]. In a comparative study between the protected and exposed cultivation system on lettuce plants to compare the effect of organic nutrients, the results obtained were positive as a result of feeding with organic nutrients in most of the studied traits. It was found that the use of organic nutrients led to a significant improvement in vegetative growth traits and the total yield of lettuce heads [38].

The increase in plant weight and total yield may be attributed to the organic nutrient containing a group of major and minor nutrients that enter into the photosynthesis processes and the general growth of the plant, as some of these elements contribute to the construction of amino acids and proteins such as nitrogen, and some of them are deposited in the cell walls such as calcium. Magnesium also enters into the composition of the chlorophyll molecule and has a vital role in activating enzymes. Sulfur also enters into the compounds of some amino acids. Iron is also necessary for the formation of the chlorophyll molecule, and manganese is necessary for some enzymes. Zinc and boron participate in protein synthesis. As a result of the work of these elements and their availability to the plant, an increase in the total yield of the plant is usually observed, which is the result of growth processes and is the final result of the biochemical activities carried out by the plant during its life cycle [39]. The results of adding organic nutrients to the lettuce plant also indicate superiority in some yield indicators, as the total yield was

significantly excelled [40]. The lettuce yield also increased when using biofertilizer and spraying organic nutrients. These results are consistent with what was reached by [41]. It may be attributed to the fact that spraying organic nutrients increases tolerance to adverse conditions and stresses and maintains the cell content of chlorophyll and the water balance of the cells, which enhances the growth of lettuce. Foliar nutrients also reduce the percentage of nitrates in the vegetative group in addition to a significant increase in the total yield of the lettuce crop, as the plant needs water and fertilizer to grow. Water plays a role in maintaining cell cohesion and the process of photosynthesis, and organic fertilizers provide nutrients to the plant and thus increase plant growth [42]. These results agreed with what was reached by [43] on the cabbage plant. Spraying glutamic acid also affected the achievement of an increase in quantitative yield characteristics such as plant circumference, head weight, plant weight and total plant yield per hectare as in Tables (2, 3, 4). Spraying glutamic acid has many positive effects on the growth of lettuce plants, as one study indicates that spraying glutamic acid plays an important role in the synthesis of chlorophyll and proline acid, which contributes to raising the efficiency of the photosynthesis process as well as resistance to water stress in lettuce plants [44]. Proline helps in building plant cells as it enters into the formation of the plant cell wall due to the enzyme Prolyl hydroxylase, and proline acid also provides a nitrogen and carbon source for the plant cell under normal conditions [45]. Proline increases the ability to absorb water and nutrients from the soil, thus increasing the efficiency of the plant for photosynthesis after the availability of water, nutrients and chlorophyll pigment [46]. This may be attributed to the easy absorption of amino acids by plants, which are considered a source of nitrogen and contribute in some way to the transfer of nitrogen. It has been proven that amino acids play an important role in metabolic processes, growth and plant physiology, as they are linked to many metabolic pathways such as their role in the Krebs cycle (citric acid cycle) and the response of acids to biotic stresses and their contribution to protein formation. Through these important roles, the results usually appear on the plant yield through an increase in growth indicators and total weight [47,48,49,50].

Conclusions

We conclude that the triple interaction between biofertilization, organic nutrient spraying and glutamic acid has a significant effect. Treatment A3B2C1 was superior in stem weight, head weight (905.9 and 907.2 g), total plant weight (1288.2 and 1267 g) and total plant yield per hectare (89.46 and 88.02 tons) for the two seasons respectively. Treatment A3B2C2 was superior for the first season and A3B2C1 for the second season in the percentage of twisted heads.

References

- [1] Shi, M.; J. Gu; H Wu et al. 2022. Phytochemicals, Nutrition, Metabolism, Bioavailability, and Health Benefits in Lettuce-A Comprehensive Review. *Antioxidants* (Basel). 11(6):1158. doi: 10.3390/antiox11061158.
- [2] Peiris, P. U. S. and W. A. P. Weerakkody. 2015. Effect of organic based liquid fertilizers on growth performance of leaf lettuce (*Lactuca Sativa* L.). In international conference on agricultural, ecological and medical sciences (AEMS-2015) April (pp. 7-8).
- [3] United States Department of Agriculture (USDA) (2018). National Nutrient Database for Standard Reference Release. Available Online at: <https://ndb.nal.usda.gov/ndb/foods/show/3001>.
- [4] Khalil, Mahmoud Abdel Aziz Ibrahim. 2004. Vegetable plants. Al-Maaref for Publishing and Distribution. Alexandria. Arab Republic of Egypt.
- [5] Sukor, A.; .2013. Effects of cyanobacterial fertilizers compared to commonly-used organic fertilizers on nitrogen availability, lettuce growth, and nitrogen use efficiency on different soil textures. M.Sc. Thesis, Colorado State Univ., USA.

- [6] Pandorf, M.; L. Pourzahedi; L. Gilbertson et al . 2020. Graphite nanoparticle addition to fertilizers reduces nitrate leaching in growth of lettuce (*Lactuca sativa*). Environmental Science: Nano. Issue 1.
- [7] Qiu, W.; Z. Wang ; C. Huang; B. Chen and R. Yang . 2014 . Nitrate accumulation in leafy vegetables and its relationship with water. Journal of Soil Science and Plant Nutrition, 14 (4), 761-768. Research Article.
- [8] Brkić, D.; J. Bošnjir; M. Bevardi; A. G. Bošković et al. 2017. Nitrate In Leafy Green Vegetables And Estimated Intake. African Journal of Traditional, Complementary and Alternative Medicines, 14(3), 31–41. <https://doi.org/10.21010/ajtcam.v14i3.4>.
- [9] Ördögh, M.; Z. Beregi and A. T. Mándy . 2019 . The effect of different biostimulators on morphological and biochemical parameters of micropropagated Hosta 'Gold Drop'. Int J Hortic Sci. 25(1-2):22–9.
- [10] Al-Kartani, Abdul Karim Arabi Sabaa and Salah Al-Din Hammadi Mahdi Al-Taie. 2011. The effect of biofertilization with mycorrhizal fungi (*Glomus mosseae*) and organic fertilization with humic acid and chemical fertilization on some growth characteristics of yellow corn plants grown in gypsum soil. The Fifth Scientific Conference of the College of Agriculture. Tikrit University. For the period April 26-27.
- [11] Taha, Hiba Hadi. 2021. Mycorrhizal fungi. Journal of Rafidain Sciences. Volume 33, Issue 3. Pp. 52-64.
- [12] Ali, Nour El-Din Shawqi and Muhammad Mustafa Allawi and Nada Hamid Majeed. 2022. Rhizosphere management and agricultural sustainability. University of Baghdad. College of Agricultural Engineering Sciences. p. 325. Iraq.
- [13] Foriani, G., Pastorli, R., Branzoni, M. and Favilli, F. 1995. Root colonization efficiency and potentially related properties in plant associated bacteria. J.Gent plant Breeding.49(4):343-431.
- [14] Helman, Y.; S. Burdman and Y. Okon; .2011. Plant growth promotion by rhizosphere bacteria through direct effects. In: Rosenberg E, Gophna U (eds) Beneficial microorganisms in multicellular life forms. Springer, Berlin, pp 89–103.
- [15] Saleh, S.S.; A.A. Glala; M.I. Ezzo; and A.A. Ghoname; . 2010. An attempt for reducing mineral fertilization in lettuce production by using bio-organic farming system. Acta Horticulture, doi: 10.17660/ActaHortic.2010.852.39.
- [16] Ekinci, M.; E. Yildirim; M. Turan and R. Kul; .2020. Organic Fertilizers Improved Plant Growth and Mineral Content of Lettuce (*Lactuca sativa* L.). Acta Horticulture, doi:10.17660/ActaHortic.2020.852.39.
- [17] Tsouvaltzis, Pavlos. Koukounaras, Athanasios and Siomos, A. S. .2014. Application of Amino Acids Improves Lettuce Crop Uniformity and Inhibits Nitrate Accumulation Induced by the Supplemental Inorganic Nitrogen Fertilization. International Journal Of Agriculture & Biology. 16 (5) 951–955. <http://www.fspublishers.org>.
- [18] Baqir, H. A. and N.H. Zeboon .2019. Response Of Some Wheat Growth Traits For Foliar Spraying With Humic And Glutamic Acid. Iraqi Journal of Agricultural Sciences. 50 (6) pp: 1455-1464.
- [19] Saaseea, K. G. and N. J. K. Al-a'mry .2024. Effects of mineral fertilization and spraying with salicylic acid and amino acids on the growth and productivity of industrial potatoes. Iraqi J. Agri. Sci. :55(Special Issue):162-174.
- [20] Al-Zaidi, Raed Jumaa Abdul. 2021. The effect of fish farm wastewater and spraying with Shamblan extract on the growth and yield of lettuce. Master's thesis. College of Agricultural Engineering Sciences. University of Baghdad. Iraq.
- [21] AL-Mharib, M. .Z.K.; A. M. Attalah and A. B. Ali .2019. Effect of Adding Humic Acid and Phosphate Fertilizer Levels on Growth and Yield of Lettuce. IOSR J. of Agri. and Veterinary Sci. Vol. 12 (4) pp: 12-15. DOI: 10.9790/2380-1204011215.
- [22] Issa, Falah Hassan and Nasser Habib Muhaibis. 2020. The effect of mycorrhizal fungus and licorice extract on the growth and productivity of okra crop (*Abelmoschus esculentus* L.)) Husaynawiah variety. Diyala Journal of Agricultural Sciences. Special issue of the proceedings of

- the Fourth Scientific Conference and the First International Conference on Agricultural Research. July 15-16, 2020. ISSN: 2073-9524.
- [23] Turfan, N. and B. Turan .2023. Effects of glutamic acid applications on the yield and growth parameters in garlic (*Allium sativum* L.) cultivation. Harran tarim ve gıda bilimleri dergisi, Harran Journal of Agricultural and Food Sciences, 27(1), 1-14.
- [24] Al-Rawi, Khasha Mahmoud and Abdul Aziz Muhammad Khalaf Allah. 1980. Design and analysis of agricultural experiments. Ministry of Higher Education and Scientific Research. Dar Al-Kutub for Printing and Publishing, University of Mosul. Mosul. Iraq.. p. 488.
- [25] Fiorilli, V.; C. Vannini; F. Ortolani et al. 2018. Omics approaches revealed how arbuscular mycorrhizal symbiosis enhances yield and resistance to leaf pathogen in wheat. Sci Rep 8, 9625. <https://doi.org/10.1038/s41598-018-27622-8>.
- [26] Epelde, L.; J. Urrea; M. Anza; J. Gamboa and C. Garbisu .2020. Inoculation of arbuscular mycorrhizal fungi increases lettuce yield without altering natural soil communities. Archives of Agronomy and Soil Science, 68(3), 413–430. <https://doi.org/10.1080/03650340.2020.1839056>.
- [27] AL-Samarai, M.M. A.; R.M. A. Al-Ubaidy and H. H.A. Al-Umrani .2020. Response Of *Abelmoschus Esculentus* L. Forinoculation With Mycorrhizae And Folair Application With Biostimulators And Effect On Vegetative Growth Characters. Iraqi J. of Agri. Sci. 2020:51(Special Issue):61-71.
- [28] Wu, S.; Z. Shi; X. Chen; J. Gao and X. Wang .2022. Arbuscular mycorrhizal fungi increase crop yields by improving biomass under rainfed condition: a meta-analysis. Plant Biology. PeerJ 10:e12861 <https://doi.org/10.7717/peerj.12861>.
- [29] Cela, F.; L. Avio; T. Giordani; et al .2022. Arbuscular Mycorrhizal Fungi Increase Nutritional Quality of Soilless Grown Lettuce while Overcoming Low Phosphorus Supply. Foods (Basel, Switzerland), 11(22), 3612. <https://doi.org/10.3390/foods11223612>.
- [30] De Souza Buzo, F.; L.M. Garé; N.F.S. Garcia et al. 2023. Effect of mycorrhizae on phosphate fertilization efficiency and maize growth under field conditions. Sci Rep 13, 3527. <https://doi.org/10.1038/s41598-023-30128-7>.
- [31] Goshki, M. G.; F. Abdollahi and A. S. Lari .2021. Effect of Mycorrhiza Fertilizer on Physiological Traits and Economical Yield of Lettuce (*Lactuca sativa* L.) under Water Stress Conditions. Journal of Vegetables Sciences. Vol. 9, No 5(1) Pp:157-173.
- [32] Xiong, C.; K. B. Singh; Y.-G. Zhu et al. 2024. Microbial species pool-mediated diazotrophic community assembly in crop microbiomes during plant development. mSystems, 9(4), e0105523. <https://doi.org/10.1128/msystems.01055-23>.
- [33] Novello, G.; E. Bona; M. Nasuelli; et al .2024. The Impact of Nitrogen-Fixing Bacteria-Based Biostimulant Alone or in Combination with Commercial Inoculum on Tomato Native Rhizosphere Microbiota and Production: An Open-Field Trial. Biology, 13(6), 400. <https://doi.org/10.3390/biology13060400>.
- [34] Oliveira, C. E. da S.; I. M. B. Gato; V. de A. Moreira et al .2023. Inoculation methods of *Azospirillum brasilense* in lettuce and arugula in the hydroponic system. Revista Brasileira de Engenharia Agrícola e Ambiental. vol.27 (9), p.653-662.
- [35] Díaz; P. R.; F. Merlo; L. Carrozzi; C. Valverde; C. M. Creus and G. A. Maroniche .2023. Lettuce growth improvement by *Azospirillum argentinense* and fluorescent *Pseudomonas* co-inoculation depends on strain compatibility. Vol. 189. 104969.
- [36] Negi, R., Sharma, B., Jan, T. et al. 2024. Microbial Consortia: Promising Tool as Plant Bioinoculants for Agricultural Sustainability. Curr Microbiol 81, 222. <https://doi.org/10.1007/s00284-024-03755-0>.
- [37] Elbagory, M. 2018. Effectiveness of Organic Fertigation and Moringa Leaf Extract Spray as An Alternative to Chemical Fertigation for Improving Yield and Quality of Lettuce under Soilless Condition. Env., Biodiv. Soil Security. Vol. 2. Pp 175-182.
- [38] Al-Chalabi, A. T. M.; F. F. R. Ibraheem; A. M. Hussein and A. Alsawaf .2023. Comparison the Effect of an Organic Nutrient (Nektafol) Under Production and Open Field Condition on the Growth and Yield of Lettuce. IOP Conf. Ser.: Earth Environ. Sci. 1262 042003.

- [39] Al-Hlfie, R. G. and W. A. Hussin .2023. Response of three beetroot cultivars to organic fertilization and foliar application. Iraqi Journal of Market Research and Consumer Protection. 15 (2): 221-234.
- [40] Hadid, R.A.; R.M. Abdel Hussein .2023. Effect of spraying with organic nutrients and adding levels of potassium fertilizer on the growth and yield of red cabbage. Revis Bionatura J. Vol. 8 (2) 69. <http://dx.doi.org/10.21931/RB/CSS/2023.08.02.69>.
- [41] Chau, M. H. and V. V. Hung .2023. Effect of inoculation methods of *Azospirillum brasilense* on growth and yield of lettuce in the hydroponic system. Journal of Forestry Science and Technology 8(2):3-9.
- [42] Shalaby, O. A. 2024. Moringa leaf extract increases tolerance to salt stress, promotes growth, increases yield, and reduces nitrate concentration in lettuce plants. Scientia Horticulturae Vol. 325,112654. <https://doi.org/10.1016/j.scienta.2023.112654>.
- [43] Al-Zaidi, Ali Karim Naheer and Reda Mustafa Abdul-Hussein Al-Obaidi. 2017. Effect of adding wheat peat and spraying with its extract and the organic nutrient Vegeamino on the growth and yield of red hannah. Iraqi Journal of Agricultural Sciences. 48 (2) 429-438.
- [44] Franzoni, G.; G. Cocetta; A. Trivellini; C. Garabello; V. Contartese and A. Ferrante .2022. Effect of exogenous application of salt stress and glutamic acid on lettuce (*Lactuca sativa* L.). Scientia Horticulturae. Vol. 299, 111027. <https://doi.org/10.1016/j.scienta.2022.111027>.
- [45] Taiz, L. and E. Zeiger .2010. Plant Physiology. Fifth Edition. Sinauer Associates Inc., Publishers Sunderland, Massachusetts U.S.A. p. 623.
- [46] Salman, Abeer Dawood and Sadiq Qasim Sadiq. 2017. Effect of spraying with solutions containing CO₂ and amino acids on the plant proline content and total yield of cherry tomato plants under open and protected cultivation conditions. Zagazig Journal of Agricultural Research. Vol. 44 (4) pp. 1217-1236.
- [47] Rahman, S.; S. Mehta and A. Husen .2024. Use of amino acids in plant growth, photosynthetic assimilation, and nutrient availability. Biostimulants in Plant Protection and Performance. Chapter 8. Pp: 117-127. <https://doi.org/10.1016/B978-0-443-15884-1.00016-6>.
- [48] Ibrahim, Waldan Ali Abdul-Amir. 2022. The effect of *Stenotrophomonas maltophilia* and *Bacillus megaterium* bacteria and methods of adding vermicompost tea on phosphorus availability, calcareous soil, growth and yield of zucchini (*Cucurbita pepo* L.). PhD thesis, College of Agricultural Engineering Sciences, University of Baghdad.
- [49] Mohammed, A. A.; J. M. Abbas and M. H. K. Al-Baldawi .2020. Effect Of Plant Source Organic Fertilizers On Yield and It's Components Of Linseed Cultivars. Iraqi Journal of Agricultural Sciences. Vol. 51(Special Issue):86-95. <https://doi.org/10.36103/ijas.v51iSpecial.885>.
- [50] Abduraseool, I. J. and Z. R. J. Al-Malikshah .2022. Effect of Adding Fulzyme plus and Spraying with Green Tea Extract on Vegetative Growth and Yield of Pepper cv. California Wonder. Basrah J. Agric. Sci., 35(2), 302-312.

Effect of Reducing Mineral Fertilizers, Using Biofertilizers and Spraying Organic Nutrients and Glutamic Acid on Some Vegetative Traits of Lettuce

Ayad Ali Hunaish Al-Samah¹ and Ridha Mustafa Abed Al-Hussain Al-Ubaidy²

^{1,2}Department of Horticulture and Landscape, College of Agriculture Engineering Sciences, University of Baghdad, Baghdad, Iraq.

¹E-mail: ayad.ali2105p@coagri.uobaghdad.edu.iq

²E-mail: ridha.mustafa@coagri.uobaghdad.edu.iq

Abstract. The organic fertilizer project in Al-Shatra District, Bani Zaid Sub-district, affiliated to the Directorate of Agriculture in Dhi Qar, conducted the experiment in one of its fields from 2022-2023 to 2023-2024 to study the effects of reducing mineral fertilizers with mycorrhizae and azospirillum and spraying organic nutrients and glutamic acid on lettuce growth and production. The factorial experiment (4x3x3) used a randomized complete block design (RCBD) with 36 treatments and three repetitions, resulting in 108 experimental units with 15 plants each. The experiment had three factors: four levels of biofertilization (0 without inoculation, A0, Mycorrhizae 15 g. plant⁻¹, A1, Azospirillum 5 g. plant⁻¹, A2, Mycorrhizae + Azospirillum, A3), three levels of Forteorgan spraying (0, B0, 2 ml L⁻¹, B1, 4 ml L⁻¹, B2), and three levels of glutamic acid spraying. The LSD test at 5% was used to compare treatment averages. Plant height, stem diameter, total number of leaves, number of outer leaves, and leaf area were considerably higher in biofertilization treatment A3 for both seasons. At treatment B1, organic nutrition spray treatments excelled in lettuce plant height, total leaves, and outer leaves for both seasons. Both seasons, Treatment B2 had superior stem diameter and leaf area. Glutamic acid spray treatments excelled at treatment C1 in plant height, stem diameter, total number of leaves, and leaf area for both seasons, and treatment C2 in outer leaf number. Regarding the interaction of spraying organic fertilizer and glutamic acid, treatment B2C1 excelled in stem diameter for both seasons, whereas treatment B1C1 excelled in total leaves and outer leaves. Leaf area was outstanding in B2C2 for both seasons, while plant height was excelled in B2C1 and B1C2. Regarding the triple interaction between biofertilization, organic nutrient spraying, and glutamic acid, treatment A3B2C2 excelled in plant height and leaf area for both seasons, stem diameter for both seasons, outer leaf number for both seasons, and total leaf number for both seasons.

Keywords. Bio-fertilizer, Organic Nutrient, *Lactuca sativa* L.

1. Introduction

Lettuce (*Lactuca sativa* L.) belongs to the Asteraceae family, also known as the Sunflower Family, and is considered one of the largest plant families [1,2]. Its presumed origin is the Mediterranean basin. It is a cold-weather vegetable, with seeds capable of germinating in

temperatures ranging from 4 to 26 °C, while the optimal temperature is 21 °C. It withstands cold to a degree; nevertheless, temperatures over 26 °C result in a decline in qualitative attributes, as the leaves get disintegrated and bitter, turn yellow, and the plants are prone to flowering [3]. It is one of the most consumed leafy green crops in the world [4]. It is considered one of the important winter vegetable crops that is grown in the world and Iraq alike due to its high nutritional value as it contains water 94.61 g, protein 1.23 g, fat 0.30 g, carbohydrates 3.29 g, fiber 2.1 g, calcium 33 mg, phosphorus 30 mg, potassium 247 mg, zinc 0.23 mg, iron 0.97 mg, vitamins A 8710 IU, C 4.0 mg, B6 0.074 mg, thiamine 0.072 mg, and niacin 0.313 mg [5]. It is grown for its leaves, which are eaten fresh. It is one of the most popular vegetable crops among salad crops, due to its high nutritional value, as it is rich in niacin and calcium, and has a medium content of iron, riboflavin, and vitamin A. It also contains some organic acids such as citric acid [6]. Lettuce has shallow roots and needs high fertilization, especially nitrogen. Nitrogen plays an important role in the synthesis of amino acids and proteins, plant growth, chlorophyll formation, leaf photosynthesis, and the development of lettuce production [7]. Lettuce is a leafy vegetable that needs a lot of nitrogen fertilization. Since the irrational use of mineral fertilizers in agricultural areas causes pollution of groundwater and surface water, the need to use other techniques to reduce the use of mineral fertilizers without affecting the crop has emerged [8]. Lettuce and spinach are among the leafy vegetables richest in nitrate content accumulated in their leaves due to high amounts of mineral fertilizers [9]. Using more than the recommended amount of nitrate may cause some health problems [10]. The risk of exposure of the body to high nitrite is the conversion of blood hemoglobin to methemoglobin, which leads to a deficiency in oxygen transport in the blood [11]. Biofertilization is one of the modern techniques to reduce the excessive use of mineral fertilizers and reduce sources of pollution. This technique includes the use of beneficial microorganisms to improve the physical, chemical and biological properties of the soil. The use of fungal and bacterial biofertilizers has spread recently because they are inexpensive and environmentally safe [12,13]. Mycorrhizal fungi (*Glomus mosseae*) form symbiotic relationships with plant roots, where both organisms benefit from this symbiotic relationship, as these fungi take carbohydrates (sugars) and growth factors from the plant and provide many benefits to the plant, such as increased nutrient absorption [14,15]. It is a symbiotic relationship, formed between the roots of most vascular plant species. These symbioses are characterized by a two-way movement of nutrients, whereby the fungi take up carbon while the nutrients are transferred to the plant. The growth of mycorrhizae in plant roots greatly increases the surface area of the root for external absorption. The absorption of nutrients by mycorrhizal fungi improves plant growth and production, and increases the plant's immunity against pathogens and harmful organisms (as this fungus is effective against nematodes and some types of fungi, it can suppress up to 60 types of pathogens that cause rot and scab) and improves the mass of vegetative growth [16]. It works to transfer water and mineral nutrients from the soil to the plant [17,18]. It provides improvements to the plant's ability to deal with drought. *Azotobacter* and *Azospirillum* are among the most efficient free-living nitrogen-fixing microorganisms in terms of their ability to fix atmospheric nitrogen [19]. *Azospirillum* has the ability to fix atmospheric nitrogen, produce plant hormones, and secrete compounds that improve soil structure, absorb nutrients, and protect against some pathogens [20]. Lettuce is a leaf-eating plant, so it needs high fertilization. Since mineral fertilizers at high concentrations cause problems for human health and also pollute the environment, it is necessary to use safer and cleaner methods for human health and the environment, leading to the production of environmentally sound plant foods [21]. Fertilization plays an important role in providing nutrients to the plant. One of the methods of adding fertilizers is spraying on the green group, as it was found that foliar feeding is about 8-20 times more efficient than ground fertilization, in addition to being an effective method

for better transfer of nutrients within the plant. It is a complementary method to ground additions [22]. Mineral fertilizers increase the production of vegetable crops. However, the use of mineral fertilizers at high levels leads to increased levels of pollutants, increased pollution of soil and groundwater, and increased concentration of nitrates in the leaves of vegetable crops [23]. However, with the use of organic fertilizers, the severity of these problems can be alleviated [24]. Amino acids, including glutamic acid, work to homogenize the growth of lettuce crops [25]. Glutamic acid is considered a stimulator of increasing vegetative growth and early production [26]. Spraying amino acids also contributes to increasing the growth and productivity of many agricultural crops [27].

1.1. Study Aim

- Knowing the response of lettuce crop to biofertilizers and reducing the use of mineral fertilizers and their effect on growth and productivity.
- Studying the effect of spraying the organic nutrient Forte organ and glutamic amino acid on the growth and productivity of lettuce.
- Studying the effect of the interaction between biofertilizers and spraying the organic nutrient and glutamic amino acid on the growth, productivity and quality of lettuce heads.

2. Materials and Methods

The experiment was conducted in the two autumn agricultural seasons 2022-2023 and 2023-2024 in one of the fields affiliated with the Dhi Qar Agriculture Directorate - Organic Fertilizers Project in Bani Zaid District, affiliated with Al-Shatrah District. Random samples were taken from the field soil at a depth of 0-30 cm before planting, and laboratory analyses of the physical and chemical properties were conducted in the laboratories affiliated with the Agricultural Research Unit - Ministry of Agriculture.

The land designated for the research was prepared by ploughing it perpendicularly with a rototiller, then it was leveled with a leveling machine after smoothing it well with disc harrows. The area designated for the experiment was divided into three sectors (in the form of terraces) and the sectors were divided into 36 experimental units (36 experimental units $\times$ 3 replicates). The area of the experimental unit was 2.16 m² with dimensions of (2.4 $\times$ 0.9) m, the distance between one experimental unit and another was 0.8 m, between one sector and another was 1.5 m, the distance between one plant and another was 0.30 m, and between lines was 0.40 m. Each experimental unit contained 15 plants. A total of 108 experimental units. The drip system was installed, then the drip irrigation pipes were extended at a rate of 15 pipes, for each sector 5 pipes, the distance between the pipes in the sector is 0.40 m, and the distance between the drippers in the pipe is 0.30 m, where the seedlings were cultivated at this distance (0.30 m) between the plants. All the above land preparation steps were implemented for the two autumn agricultural seasons. Lettuce seeds (local) were cultivated on 10/1/2022 - 2023 for both seasons in the panels that were well prepared to produce lettuce seedlings, then the seeds were scattered and covered with a light layer of soil and lightly irrigated to prevent them from drifting, after which irrigation was done when needed until the seedlings were transferred to the field. The seedlings were transferred to the permanent field at the age of 30 days from the date of planting after forming 4-5 true leaves. Where pits were dug 5-10 cm deep for the seedlings and the seedlings were irrigated immediately after transplanting, the weeding process was conducted to get rid of weeds as needed and manually only, and drip irrigation was conducted when the plants needed water.

The factorial experiment included three factors within the Randomized Complete Block Design (R.C.B.D) and three replicates with 36 treatments for each replicate, so that the total number of treatments in the experiment was 108 treatments. Urea fertilizer was used as a source of nitrogen

and diammonium phosphate fertilizer (P_2O_5) DAP (46% P_2O_5 , 18% N) was used as a source of phosphorus, where half of the fertilizer recommendation was added to all experimental units (110 kg ha^{-1} urea was added and 60 kg ha^{-1} DAP was added before the transplanting process and for all treatments). For both agricultural seasons

2.1. Experimental Treatments for Both Seasons

2.1.1. The First Factor is Four Levels of Biofertilization

Adding 15 g.pit⁻¹ of peat moss and plant roots loaded with mycorrhizal fungal inoculum with an inoculum density of 50 spores per g.peat moss⁻¹ at a depth of 5 cm below the soil surface under the roots of lettuce seedlings, and adding 5 g.pit⁻¹ of Azospirillum lipoferum bacterial inoculum at an inoculum density (1.5×10⁹ cells ml⁻¹ per 2 kg peat moss) under the roots of lettuce seedlings (Essa and Muhaibis, 2020) was added when planting plants in the permanent field as shown in the following steps:

- Without addition and symbolized by A0.
- Inoculation with mycorrhizal fungus alone. (Adding 15 g.pit⁻¹) at a depth of 5 cm below the roots of lettuce seedlings and symbolized by A1.
- Inoculation with Azospirillum bacteria alone. Add 5 g.pit⁻¹ and symbolize it A2.
- Inoculation with mycorrhizal fungus + Azospirillum bacteria and symbolize it A3.

2.1.2. The Second Factor (Organic Fertilizer Fertiorgan)

It consists of (organic matter 42%, calcium 1.68%, magnesium 1.2%, boron 0.012%, iron 0.024%, manganese 0.012%, zinc 0.0024%) where the lettuce crop was sprayed with three concentrations of organic fertilizer

- Without addition and symbolize it B0.
- Spraying the recommended concentration 2 ml L⁻¹ according to the recommendation of the producing company and symbolize it B1.
- Spraying 4 ml L⁻¹ and symbolize it B2.

- Organic Fertilizer Spraying

- The first spray after 15 days from the planting date.
- The second spray after 30 days from the planting date.
- The third spray after 45 days from the planting date.

2.1.3. The Third Factor (Amino Acid)

Spraying the lettuce crop with three concentrations of glutamic amino acid (0 and its symbol is C0, 250 and its symbol is C1, 500 mg L⁻¹ and its symbol is C2) [28].

- Glutamic Acid was Sprayed
 - The first spray after 18 days from the planting date.
 - The second spray after 33 days from the planting date.
 - The third spray after 48 days from the planting date.

The results were analyzed after completing the study indicators using the statistical analysis program GenStat 12. The averages were tested at the least significant difference (LSD) at the probability level of 0.05 for the purpose of comparing the averages[29].

2.2. Vegetative Growth Indicators

2.2.1. Plant Height (cm plant^{-1})

The plant height (when harvested) was measured from the point of contact with the soil to the end of the leaves using a metric measuring tape for the experimental unit plants, then the average was taken for them.

2.2.2. Stem Diameter (cm plant^{-1})

The stem diameter was measured using a foot (Vernia) when harvesting at a distance of 1 cm from the point of contact of the stem with the soil for the experimental unit plants, then the average was calculated for them.

2.2.3. Total Number of Leaves (leaf plant^{-1})

All leaves were counted for each of the selected plants except for the very small leaves at the growing tip, then the average was calculated for them[30].

2.2.4. Number of Outer Leaves of the Plant (leaf.plant^{-1})

The number of outer leaves (uncurled) was calculated for each of the selected plants after that according to the average.

2.2.5. Leaf Area of the Plant ($\text{dm}^2 \text{ leaf}$)

The method of [31] was used. The leaf area was measured by the weight method using a cork borer with a known diameter and according to the dry weight of the leaves, where ten leaves (the fourth - fifth leaf from each plant) were taken from a group of selected plants from the experimental unit, 30 discs with a known diameter were taken from them using the cork borer. The discs and leaves were dried in an electric oven (Oven) at a temperature of 70 C until the weight stabilized.

$$\text{Leaf area} = (\text{Leaf area of discs} \times \text{total dry weight of plant leaves}) / (\text{Dry weight of discs})$$

3. Results and Discussion

3.1. Plant Height (cm plant^{-1})

The results of Table 1 indicate that the treatment of inoculation interaction Mycorrhizae + Azospirillum A3 significantly excelled on the plant height, which reached (35.37 and 35) cm plant^{-1} for both seasons, respectively, over the rest of the treatments, while the lowest height treatment was A2 and A0, which reached (34.56 and 33.23) cm plant^{-1} for both seasons, respectively. The organic nutrient spraying treatment at level B1 also showed a significantly excelled for both seasons for the average plant height, which reached (35.08 and 34.49) cm plant^{-1} , respectively, compared to the control treatment, which had the lowest height (33.83 and 33.15) cm plant^{-1} for both seasons. While the treatment of spraying glutamic acid at concentration C1 gave the highest value of (35.08 and 34.49) cm plant^{-1} for both seasons, significantly excelled compared to the treatment of the lowest height (33.83 and 33.15) cm plant^{-1} for both seasons. Bi-interaction treatments showed significantly excelled in the average plant height, as treatment A3B1 gave the highest average height of (36.56 and 35.92) cm plant^{-1} for both seasons, respectively, compared to treatment A2B0 (31.11 and 30.56) cm plant^{-1} for both seasons. The results of the interaction of biofertilizer and glutamic acid in treatment A3C2 showed significantly excelled, as the average height reached (37.22 and 36.5) cm plant^{-1} for both seasons, respectively, compared to treatment A2C0, which gave the lowest average height of (33.44 and

32.88) cm plant⁻¹ for both seasons. The interaction treatment between spraying organic nutrients and glutamic acid B2C1 and B1C2 showed significantly excelled, as the average height value reached (35.83 and 35.73) cm plant⁻¹ for both seasons, respectively, compared to the control treatment, which gave the lowest average height of (33 and 32.44) cm plant⁻¹ for both seasons. The triple interaction treatment of the study factors A3B2C2 was significantly excelled and gave the highest value for plant height, reaching (38 and 37.03) cm plant⁻¹ for both seasons, respectively, while the combination A2B0C0 and A2B0C2 gave the lowest values of (29.67 and 31.1) cm plant⁻¹ for both seasons, respectively.

Table 1. Effect of inoculation with *Glomus mosseae* and *Azospirillum lipoferum*, organic nutrient spraying and glutamic acid and their interactions on lettuce height (plant cm⁻¹).

Spray organic nutrient (B)	Glutamic acid spray (C)	First autumn season 2022-2023					Second Autumn Season 2023-2024				
		Biofertilization (A)				Average B × C	Biofertilization (A)				Average B × C
		0	1	2	3		0	1	2	3	
0	0	33.3 ₃	34	29.6 ₇	35	33	32.77	33.4	32.1 ₅	34.4 ₃	32.44
	1	35.6 ₇	34.6 ₇	32	34.6 ₇	34.25	35.05	34.0 ₂	31.4 ₃	34.1 ₃	33.66
	2	34	35.6 ₇	31.6 ₇	35.6 ₇	34.25	32.12	35.0 ₈	31.1	35.1 ₂	33.35
1	0	32.6 ₇	33	33.6 ₇	37	34.08	32.05	32.4 ₈	33.0 ₇	36.3 ₃	33.48
	1	36.6 ₇	32	36	34.6 ₇	34.83	36.08	31.4 ₇	35.4	34.0 ₇	34.25
	2	35	35.6 ₇	36.6 ₇	38	36.33	34.45	35.0 ₈	36.0 ₃	37.3 ₅	35.73
2	0	35.6 ₇	35.6 ₇	37	32	35.08	35.07	35.0 ₈	36.4 ₃	31.4 ₈	34.52
	1	35	36.6 ₇	35.6 ₇	36	35.83	34.42	36.0 ₈	35.0 ₇	35.0 ₇	35.16
	2	33	32	32.6 ₇	38	33.92	32.47	31.4 ₃	32.1 ₇	37.0 ₃	33.27
L.S.D (0.05)		3.127				1.563	3.044				1.522
average biofertilizer A		34.5 ₆	34.3 ₇	33.8 ₉	35.6 ₇	average B	33.2 ₃	33.7 ₉	33.3 ₂	35	average B
L.S.D (0.05)		1.042					1.015				
A × B	0	34.3 ₃	34.7 ₈	31.1 ₁	35.1 ₁	33.83	33.31	34.1 ₇	30.5 ₆	34.5 ₆	33.15
	1	34.7 ₈	33.5 ₆	35.4 ₄	36.5 ₆	35.08	34.19	33.0 ₁	34.8 ₃	35.9 ₂	34.49
	2	34.5 ₆	34.7 ₈	35.1 ₁	35.3 ₃	34.94	33.98	34.2	34.5 ₆	34.5 ₃	34.32

Spray organic nutrient (B)	Glutamic acid spray (C)	First autumn season 2022-2023					Second Autumn Season 2023-2024				
		Biofertilization (A)				Average B × C	Biofertilization (A)				Average B × C
		0	1	2	3		0	1	2	3	
L.S.D (0.05)		1.805				0.903	1.758				0.879
						average C					average C
A × C	0	33.89	34.22	33.44	34.67	34.06	33.29	33.66	32.88	34.08	33.48
	1	35.78	34.44	34.56	35.11	34.97	35.18	33.86	33.97	34.42	34.36
	2	34	34.44	33.67	37.22	34.83	33.01	33.87	33.1	36.5	34.12
L.S.D (0.05)		1.805				0.903 N.S	1.758				0.879
Biofertilization A: A0 without addition. A1 Mycorrhizae 15g hole ⁻¹ . A2 Azospirillum 5g hole ⁻¹ . A3 Mycorrhizae + Azospirillum interaction.											
Organic fertilization B: B0 Spray water only. B1 Spray 2 ml L ⁻¹ . B2 Spray 4 ml L ⁻¹ .											
Glutamic acid C: C0 Spray water only. C1 Spray 250 mg L ⁻¹ . C2 Spray 500 mg L ⁻¹ .											

3.2. Stem Diameter (mm plant⁻¹)

Table 2 shows that the biofertilization treatment A3 was significantly excelled in the average stem diameter by recording the highest value of (38.11 and 36.79) mm plant⁻¹ for both seasons, while the control treatment gave the lowest value of (34.15 and 33.40) mm plant⁻¹ for both seasons. The organic nutrient spraying treatment B2 was also significantly excelled in stem diameter and gave the highest value of (37.11 and 35.85) mm plant⁻¹ for both seasons, while the control treatment B0 had the lowest value of (35.42 and 34.19) mm plant⁻¹ for both seasons. The glutamic acid C1 treatment showed a significantly excelled in stem diameter, as its value reached (37.28 and 36.04) mm plant⁻¹ for both seasons, while the C0 treatment gave the lowest value, reaching (34.92 and 33.69) mm plant⁻¹ for both seasons. Bi-interaction between biofertilization and spraying organic nutrient A3B2 showed the highest value, reaching (39.22 and 37.84) mm plant⁻¹ for both seasons, significantly excelled to the control treatment, while the A0B2 and A0B0 treatments had the lowest values, reaching (33.33 and 32.17) mm plant⁻¹ for both seasons, respectively. The interaction of the biofertilizer and glutamic acid A3C1 treatments gave the highest values (39.67 and 38.29) mm plant⁻¹ for both seasons, significantly excelled to the control treatment, which gave the lowest value (31.89 and 30.7) mm plant⁻¹ for both seasons. The organic nutrient spraying treatment and glutamic acid B2C1 were significantly excelled, giving a value of (38.5 and 37.21) mm plant⁻¹ for both seasons. As for the treatments with the lowest value, they were from the B2C0 and B1C0 treatments, where their values reached (34.58 and 33.74) mm plant⁻¹ for both seasons, respectively. The results of the triple interaction between biofertilization, organic nutrient spraying and glutamic acid show a significantly excelled in treatment A3B2C1, where the average stem diameter value reached (42.67 and 41.17) mm plant⁻¹ for both seasons compared to control treatment, which gave the lowest value (31.67 and 30.37) mm plant⁻¹ for both seasons.

Table 2. Effect of inoculation with *Glomus mosseae* and *Azospirillum lipoferum* mycorrhizae, organic nutrient spraying, and glutamic acid and their interactions on stem diameter.

Spray organic nutrient (B)	Glutamic acid spray (C)	First autumn season 2022-2023					Second Autumn Season 2023-2024				
		Biofertilization (A)				Average B × C	Biofertilization (A)				Average B × C
		0	1	2	3		0	1	2	3	
0	0	31.6 ₇	35	37.6 ₇	36.6 ₇	35.25	30.37	33.8 ₃	36.4	35.3	33.98
	1	34.3 ₃	40	31.3 ₃	38	35.92	32.97	38.7	30.4 ₇	36.7 ₃	34.72
	2	34.3 ₃	33.3 ₃	34.6 ₇	38	35.08	33.17	32.2	33.4 ₇	36.7	33.88
1	0	31.6 ₇	37.3 ₃	34	33.3 ₃	34.92	33.8	36.1 ₇	32.7 ₇	32.2 ₃	33.74
	1	37.3 ₃	35	39	38.3 ₃	37.42	36.1	33.9 ₇	37.7 ₇	36.9 ₇	36.2
	2	34.6 ₇	39.6 ₇	37.6 ₇	41	38.25	33.6	38.3 ₃	36.5 ₃	39.6 ₃	37.03
2	0	31.6 ₇	39.3 ₃	34.3 ₃	35.6 ₇	34.58	31.9 ₃	38.0 ₃	33.1 ₃	34.3 ₃	34.17
	1	33.3 ₃	36.3 ₃	41.6 ₇	42.6 ₇	38.5	32.2	35.1 ₇	40.3	41.1 ₇	37.21
	2	37.6 ₇	39.3 ₃	36.6 ₇	39.3 ₃	38.25	36.5	38	35.4 ₃	38.0 ₃	36.99
L.S.D FOR INTERACTION		5.264				2.632	5.131				2.565
average biofertilizer A		34.1 ₅	37.2 ₆	36.3 ₃	38.1 ₁	average B	33.40	36.0 ₄	35.1 ₄	36.7 ₉	average B
L.S.D		1.755					1.71				
A × B	0	33.4 ₄	36.1 ₁	34.5 ₆	37.5 ₆	35.42	32.17	34.9 ₁	33.4 ₄	36.2 ₄	34.19
	1	35.6 ₇	37.3 ₃	36.8 ₉	37.5 ₆	36.86	34.5	36.1 ₆	35.6 ₉	36.2 ₈	35.66
	2	33.3 ₃	38.3 ₃	37.5 ₆	39.2 ₂	37.11	32.21	37.0 ₇	36.2 ₉	37.8 ₄	35.85
L.S.D		3.039				1.52	2.962				1.481
						average C					average C
A × C	0	31.8 ₉	37.2 ₂	35.3 ₃	35.2 ₂	34.92	30.7	36.0 ₁	34.1	33.9 ₆	33.69
	1	35	37.1	37.3	39.6	37.28	33.76	35.9	36.1	38.2	36.04

Spray organic nutrient (B)	Glutamic acid spray (C)	First autumn season 2022-2023					Second Autumn Season 2023-2024				
		Biofertilization (A)				Average B × C	Biofertilization (A)				Average B × C
		0	1	2	3		0	1	2	3	
			1	3	7			4	8	9	
	2	35.56	37.44	36.33	39.44	37.19	34.42	36.18	35.14	38.12	35.97
L.S.D			3.039			1.52		2.962			1.481

Biofertilization A: A0 without addition. A1 Mycorrhizae 15g hole⁻¹. A2 Azospirillum 5g hole⁻¹. A3 Mycorrhizae + Azospirillum interaction.
Organic fertilization B: B0 Spray water only. B1 Spray 2 ml L⁻¹. B2 Spray 4 ml L⁻¹.
Glutamic acid C: C0 Spray water only. C1 Spray 250 mg L⁻¹. C2 Spray 500 mg L⁻¹.

3.3. Total Number of Leaves (leaf⁻¹)

The results of Table 3 indicate the significantly excelled of the biofertilization treatment A3, which reached (56.7 and 55.73) leaves⁻¹ for both seasons compared to control treatment A0, which gave the lowest average of leaves, which reached (53.09 and 54.57) leaves⁻¹ for both seasons. As for the organic nutrient spray treatments, treatment B1 was significantly excelled by giving the highest value of 56.07 leaves.plant⁻¹ for the first growth season compared to control treatment with the lowest value of 54.01 leaves.plant⁻¹ for the first growth season, while the same treatment was for the second growth season without significant differences, as treatment B1 gave the highest values of 57.85 leaves.plant⁻¹ for the second growth season and the lowest value for control treatment B0 was leaves.plant⁻¹ for the second growth season without significant differences. As for the glutamic acid treatments, treatment C1 was significantly excelled by giving the highest rate of (57.01 and 58.52) leaves.plant⁻¹ for both seasons, and treatment C0 gave the lowest value of (52.69 and 54.53) leaves.plant⁻¹ for both seasons. In the same table, bi-interaction between biofertilization and organic nutrient spraying were observed, as treatment A1B2 significantly excelled on most of the treatments by giving the value (61 and 63.47) leaf plant⁻¹ for both seasons, while the treatment with the lowest value was A0B2 (49.11 and 50.64) leaf plant⁻¹ for both seasons. As for the results of the interaction between biofertilization and glutamic acid, treatment A3C1 significantly excelled on all treatments for the first season, while it excelled on most of the treatments in the second season, giving values (64.22 and 64.65) leaf plant⁻¹ for both seasons, while the measurement treatment had the lowest value (50.44 and 51.84) leaf plant⁻¹ for both seasons. The interaction treatment of organic nutrient spraying and glutamic acid B1C1 significantly excelled on all treatments of the first season and most treatments of the second season, as it gave values of (61.67 and 63.82) leaf plant⁻¹ for both seasons. The lowest results were for treatments B2C0 and B0C1, which reached values of (51.21 and 53.13) leaf plant⁻¹ for both seasons in succession. The triple interaction treatment between the study factors also appeared, as treatments A3B2C1 and A3B1C1 significantly excelled on most treatments, and their values reached (70.17 and 69.33) leaf plant⁻¹ for both seasons in succession. The lowest value treatments were A0B2C0 and A2B0C1, which reached (41.83 and 43) leaf plant⁻¹ for both seasons.

Table 3. Effect of inoculation with *Glomus mosseae* and *Azospirillum lipoferum*, organic nutrient spraying, glutamic acid and their interactions on the total number of leaves of lettuce (plant cm⁻¹).

Spray organic nutrient (B)	Glutamic acid spray (C)	First autumn season 2022-2023					Second Autumn Season 2023-2024				
		Biofertilization (A)				Average B × C	Biofertilization (A)				Average B × C
		0	1	2	3		0	1	2	3	
0	0	49.5	45.6 ₇	57.5	61	53.42	51.2	47.3 ₄	59.8 ₈	63.4 ₉	55.48
	1	53.8 ₃	55	42	55.5	51.58	55.9 ₇	56.5 ₃	43	57.0 ₃	53.13
	2	53.8 ₃	53.3 ₃	59.3 ₃	61.6 ₇	57.04	54.0 ₃	54.6 ₂	61.4 ₇	63.9 ₁	58.51
1	0	60	52	50.5	51.3 ₃	53.46	61.3 ₁	54.1 ₆	51.3 ₉	53	54.96
	1	66	54.5	59.1 ₇	67	61.67	68.0 ₄	56.3 ₇	61.5 ₂	69.3 ₃	63.82
	2	47.3 ₃	51.5	54.5	59	53.08	48.6 ₇	53.1 ₂	55.9 ₆	61.3 ₈	54.78
2	0	41.8 ₃	59.8 ₃	53.8 ₃	49.3 ₃	51.21	43.0 ₁	62.1 ₉	55.9 ₅	51.4 ₅	53.15
	1	48	57.5	55.5	70.1 ₇	57.79	49.4 ₂	59.8 ₈	57.5 ₂	67.5 ₈	58.6
	2	57.5	65.6 ₇	53.3 ₃	54.1 ₇	57.67	59.5	68.3 ₃	55.1 ₃	55.7 ₃	59.67
L.S.D FOR INTERACTION		6.884				3.442	8.4				4.2
average biofertilizer A		53.0 ₉	55	53.9 ₆	58.8	average B	54.5 ₇	56.9 ₅	55.7 ₆	60.3 ₂	average B
L.S.D		2.295					2.8				
A × B	0	52.3 ₉	51.3 ₃	52.9 ₄	59.3 ₉	54.01	53.7 ₃	52.8 ₃	54.7 ₈	61.4 ₈	55.71
	1	57.7 ₈	52.6 ₇	54.7 ₂	59.1 ₁	56.07	59.3 ₄	54.5 ₅	56.2 ₉	61.2 ₄	57.85
	2	49.1 ₁	61	54.2 ₂	57.8 ₉	55.56	50.6 ₄	63.4 ₇	56.2	58.2 ₆	57.14
L.S.D		3.974				1.987	4.85				2.425 N.S
						average C					average C
A × C	0	50.4 ₄	52.5	53.9 ₄	53.8 ₉	52.69	51.8 ₄	54.5 ₆	55.7 ₄	55.9 ₈	54.53
	1	55.9 ₄	55.6 ₇	52.2 ₂	64.2 ₂	57.01	57.8 ₁	57.6	54.0 ₁	64.6 ₅	58.52

Spray organic nutrien t (B)	Glutami c acid spray (C)	First autumn season 2022-2023					Second Autumn Season 2023-2024				
		Biofertilization (A)				Averag e B × C	Biofertilization (A)				Averag e B × C
		0	1	2	3		0	1	2	3	
	2	52.8 9	56.8 3	55.7 2	58.2 8	55.93	54.0 7	58.6 9	57.5 2	60.3 4	57.66
L.S.D		3.974				1.987	4.85				2.425

Biofertilization A: A0 without addition. A1 Mycorrhizae 15g hole⁻¹. A2 Azospirillum 5g hole⁻¹. A3 Mycorrhizae + Azospirillum interaction.

Organic fertilization B: B0 Spray water only. B1 Spray 2 ml L⁻¹. B2 Spray 4 ml L⁻¹.

Glutamic acid C: C0 Spray water only. C1 Spray 250 mg L⁻¹. C2 Spray 500 mg L⁻¹.

3.4. Number of Outer Leaves of the Plant (leaf.plant⁻¹)

Table 4 shows that the biofertilization treatment A3 was significantly excelled, reaching 20.17 leaves⁻¹ plant for the first growing season, over the less valuable treatment A1, which reached 18.59 leaves⁻¹ plant for the first growing season, without any significant difference with control treatment A0 for the first season. As for the second season, there were no significant differences, but the treatment A3 gave the highest value of 19.69 leaves⁻¹ plant for the second growing season, without any significant difference. The organic nutrient spraying treatment B1 was significantly excelled to the rest of the treatments, reaching values of (20.51 and 20.05) leaves plant⁻¹ for both season, compared to the less valuable standard treatment (18.62 and 18.17) leaves plant⁻¹ for both season. As for the glutamic acid treatments, treatment C2 gave the highest values reaching (20.12 and 19.65) leaf plant⁻¹ for both seasons, significantly excelled to the control treatment which gave the lowest values (18.06 and 17.65) leaf plant⁻¹ for both seasons respectively. The results of bi-interaction between biofertilization and organic nutrient spraying showed significantly excelled in treatment A0B1 with values reaching (21.67 and 21.23) leaf plant⁻¹ for both seasons compared to the control treatment with the lowest value (17.06 and 16.62) leaf plant⁻¹ for both seasons. As for the interaction of biofertilization and glutamic acid, treatment A3C1 was significantly excelled to most treatments and gave a result of (23 and 22.42) leaf plant⁻¹ for both seasons, and the lowest values were for treatment A1C0 (17.06 and 16.7) leaf plant⁻¹ for both seasons. The interaction of spraying organic nutrients and glutamic acid showed that treatment B1C1 was significantly excelled with values reaching (21.42 and 20.93) leaf plant⁻¹ for both seasons compared to the control treatment, which gave the lowest values (17 and 16.58) leaf plant⁻¹ for both seasons in succession. The triple interaction between the study factors showed a significantly excelled of treatment A3B1C1 with values of (25 and 24.46) leaf plant⁻¹ for both seasons, while the lowest values were for treatment A3B2C0, which were (14 and 13.72) leaf plant⁻¹ for both seasons, respectively.

Table 4. Effect of inoculation with *Glomus mosseae* and *Azospirillum lipoferum*, organic nutrient spraying and glutamic acid and their interactions on the number of outer leaves of the plant (leaf per plant⁻¹).

Spray	Glutami	First autumn season 2022-2023					Second Autumn Season 2023-2024				

organic nutrien t (B)	c acid spray (C)	Biofertilization (A)				Averag e B × C	Biofertilization (A)				Averag e B × C
		0	1	2	3		0	1	2	3	
0	0	16.1 7	15	18.1 7	18.6 7	17	15.7 4	14.6 7	17.6 8	18.2 2	16.58
	1	14.5	22.3 3	17.6 7	21.5	19	14.1	21.8 7	17.2	20.8 9	18.52
	2	20.5	20.6 7	18.3 3	20	19.88	20.0 3	20.1 5	17.8 7	19.5 8	19.41
1	0	20.6 7	18	22.1 7	19.6 7	20.12	20.2 5	17.6 3	21.6 5	19.2	19.68
	1	24.3 3	18.1 7	18.1 7	25	21.42	23.8 4	17.7 7	17.6 5	24.4 6	20.93
	2	20	20.8 3	20.5	18.6 7	20	19.6	20.3 2	19.9 6	18.2 5	19.53
2	0	16.5	18.1 7	19.5	14	17.04	16.0 8	17.7 9	19.1 5	13.7 2	16.69
	1	22.3 3	14.8 3	18.5	22.5	19.54	21.8 2	14.4 8	17.9 9	21.9 2	19.05
	2	22.5	19.3 3	18.6 7	21.5	20.5	21.9 6	18.8 7	18.2	21	20.01
L.S.D FOR INTERACTION		4.149				2.074	4.068				2.034
average biofertilizer A		19.7 2	18.5 9	19.0 7	20.1 7	average B	19.2 7	18.1 7	18.5 9	19.6 9	average B
L.S.D		1.383					1.356 N.S				
A × B	0	17.0 6	19.3 3	18.0 6	20.0 6	18.62	16.6 2	18.9	17.5 8	19.5 7	18.17
	1	21.6 7	19	20.2 8	21.1 1	20.51	21.2 3	18.5 7	19.7 6	20.6 4	20.05
	2	20.4 4	17.4 4	18.8 9	19.3 3	19.03	19.9 5	17.0 5	18.4 5	18.8 8	18.58
L.S.D		2.395				1.198	2.349				1.174
						average C					average C
A × C	0	17.7 8	17.0 6	19.9 4	17.4 4	18.06	17.3 5	16.7	19.4 9	17.0 5	17.65
	1	20.3 9	18.4 4	18.1 1	23	19.99	19.9 2	18.0 4	17.6 1	22.4 2	19.5
	2	21	20.2 8	19.1 7	20.0 6	20.12	20.5 3	19.7 8	18.6 8	19.6 1	19.65

Spray organic nutrien t (B)	Glutami c acid spray (C)	First autumn season 2022-2023					Second Autumn Season 2023-2024				
		Biofertilization (A)				Averag e B × C	Biofertilization (A)				Averag e B × C
		0	1	2	3		0	1	2	3	
L.S.D		2.395				1.198	2.349				1.174

Biofertilization A: A0 without addition. A1 Mycorrhizae 15g hole⁻¹. A2 Azospirillum 5g hole⁻¹. A3 Mycorrhizae + Azospirillum interaction.

Organic fertilization B: B0 Spray water only. B1 Spray 2 ml L⁻¹. B2 Spray 4 ml L⁻¹.

Glutamic acid C: C0 Spray water only. C1 Spray 250 mg L⁻¹. C2 Spray 500 mg L⁻¹.

3.5. Leaf Area Per Plant (dm² plant⁻¹)

The table 5 shows the effect of biofertilization on leaf area, where treatment A3 was significantly excelled and recorded a value of 127.7 dm² plant⁻¹ for the first growth season compared to control treatment, which had a value of 114.5 dm² plant⁻¹ for the first growth season, while no treatment was excelled for the second growth season, but the highest values were for treatment A3, which had a value of 129.6 dm² plant⁻¹ for the second growth season, and the lowest value was for control treatment, which had a value of 120.7 dm² plant⁻¹ for the second growth season. As for the organic nutrient spray factor, treatment B2 was significantly excelled to all treatments, reaching (133.1 and 142.5) dm/plant⁻¹ for both seasons, while control treatment B0 had the lowest values, reaching (10.8 and 113) dm/plant⁻¹ for both seasons, respectively. Spraying glutamic acid had a significantly excelled for the first season only, as treatment C1 recorded a result of 125 dm/plant⁻¹ for both seasons, and without a significantly excelled for the second season, compared to control treatment, which gave the lowest value, reaching (113.2 and 121.3) dm/plant⁻¹ for both seasons, respectively. The same table shows bi-interaction between the effect of biofertilization and organic nutrient spraying, which led to a significantly excelled of treatment A3B2, which gave the highest leaf area rate of (144 and 155.7) dm/plant⁻¹ for both seasons, while the lowest values were for treatment A1B1 (102.4 and 108.6) dm/plant⁻¹ for both seasons. As for the interaction of biofertilization and glutamic acid, treatments A3C1 and A3C2 were significantly excelled, and their values reached (139.6 and 140.3) dm/plant⁻¹ for both seasons, respectively, compared to the control treatment, which had the lowest values, reaching (97.6 and 109.6) dm/plant⁻¹ for both seasons. The interaction of organic nutrient spraying and glutamic acid showed significantly excelled in treatment B2C2, as its result was (145.3 and 156.6) dm-plant⁻¹ for both seasons, while the lowest treatment was for B0C2 and B1C2, with values of (102.6 and 107.1) dm-plant⁻¹ for both seasons, respectively. The results of the triple interaction treatment between the study factors, treatment A3B2C2 showed significantly excelled, as it reached (186 and 210) dm-plant⁻¹ for both seasons, compared to control treatment A0B0C0. Which appeared with the lowest values in the first season, reaching (87.4) dm-plant⁻¹ for the first growth season, while in the second season, the lowest values were for treatment A2B0C1, reaching (92.8) dm-plant⁻¹ for the second growth season.

Table 5. Effect of inoculation with *Glomus mosseae* and *Azospirillum lipoferum*, organic nutrient spraying and glutamic acid and their interactions on leaf area (dm² plant⁻¹).

Spray organic	Glutami c acid	First autumn season 2022-2023		Second Autumn Season 2023-2024	
		Biofertilization (A)	Averag	Biofertilization (A)	Averag

nutrien t (B)	spray (C)	0	1	2	3	^e B × C	0	1	2	3	^e B × C
0	0	87.4	155. 5	95.3	107. 9	111.6	93	127. 2	120. 7	116. 1	114.3
	1	155. 6	91	94.8	106. 1	111.9	132. 1	105. 2	92.8	112	110.5
	2	99.7	99.3	111. 5	100	102.6	126. 3	106	118. 1	106. 6	114.3
1	0	105. 8	98.7	143. 7	107. 5	113.9	111. 2	107. 5	165	111. 9	123.9
	1	120. 2	101. 9	109. 3	164. 9	124.1	127. 1	109. 9	116	148	125.2
	2	104. 7	106. 8	101	130. 9	110.8	110. 7	108. 5	105	104. 2	107.1
2	0	99.6	157. 6	104. 9	98.3	115.1	124. 7	166. 5	111. 6	100. 2	125.8
	1	126. 7	137. 7	143. 6	147. 8	138.9	124	147. 5	152. 5	156. 9	145.2
	2	131	106. 1	158. 3	186	145.3	137. 1	113. 4	166	210	156.6
L.S.D FOR INTERACTION			37.02			18.51		30.66			15.33
average biofertilizer A		114. 5	117. 2	118	127. 7	average B	120. 7	121. 3	127. 5	129. 6	average B
L.S.D			12.34					10.22 N.S			
A × B	0	114. 2	115. 3	100. 6	104. 7	108.7	117. 2	112. 8	110. 5	111. 6	113
	1	110. 2	102. 4	118	134. 5	116.3	116. 3	108. 6	128. 6	121. 4	118.7
	2	119. 1	133. 8	135. 6	144	133.1	128. 6	142. 5	143. 4	155. 7	142.5
L.S.D			21.37			10.69		17.7			8.85
						average C					average C
A × C	0	97.6	137. 3	114. 6	104. 6	113.5	109. 6	133. 7	132. 4	109. 4	121.3
	1	134. 2	110. 2	115. 9	139. 6	125	127. 7	120. 9	120. 4	139	127
	2	111. 8	104. 1	123. 6	139	119.6	124. 7	109. 3	129. 7	140. 3	126
L.S.D			21.37			10.69		17.7			8.85

N.S

Biofertilization A: A0 without addition. A1 Mycorrhizae 15g hole⁻¹. A2 Azospirillum 5g hole⁻¹. A3 Mycorrhizae + Azospirillum interaction.

Organic fertilization B: B0 Spray water only. B1 Spray 2 ml L⁻¹. B2 Spray 4 ml L⁻¹.

Glutamic acid C: C0 Spray water only. C1 Spray 250 mg L⁻¹. C2 Spray 500 mg L⁻¹.

From the results of Tables 1, 2, 3, 4 and 5, it is clear that the biofertilization treatments (inoculation with mycorrhizae and Azospirillum) are excelled compared to the measurement treatments in the vegetative growth traits under study (plant height, stem diameter, total number of leaves, number of outer leaves, leaf area). It was noted that treatment A3 was excelled to the rest of the biofertilization treatments in plant height, stem diameter, stem length, total number of leaves, number of outer leaves and leaf area for both seasons). The interaction between mycorrhizal fungi and Azospirillum bacteria increased the values of vegetative growth indicators, as the interaction between the microorganisms under study as a biofertilizer has a positive effect on stimulating plant growth. Therefore, this is a beneficial relationship between mycorrhizal fungi and Azospirillum bacteria in encouraging plant growth and increasing vegetative growth indicators[32].

There are also synergistic effects between mycorrhizal fungi and root bacteria that stimulate plant growth to enhance the absorption of elements and retain water in the rhizosphere (the root zone of the plant). These effects are conducted by organisms individually or cooperatively, and in both cases they provide growth requirements in the area affected by root secretions. Biofertilizers also provide some stimulating growth regulators, and as a result, an increase in plant growth and an increase in their biomass is observed[33]. There is a close relationship between plant roots and mycorrhizal fungi, as these fungi work to increase the efficiency of the soil in retaining water and absorbing nutrients. Fungal threads (hyphae) also work to increase the surface area of the roots. These hyphae benefit from root secretions to obtain sugars from the roots of the host plant. In return, the roots of the host plant improve by improving their absorption of water and nutrients from the soil. Mycorrhizal fungi also dissolve phosphate using the phosphatase enzyme to provide the phosphorus element necessary for plant growth and development [34]. This may be attributed to the role of mycorrhizae in increasing the availability of elements such as nitrogen, phosphorus and potassium in a ready-made form in the soil solution, thus increasing the plant's absorption of these elements. What helps in this absorption is biofertilization and in various ways such as the secretion of enzymes and hormones such as auxin and others, and thus its results appear in increasing vegetative growth indicators [35,36].

These plant hormones resulting from the secretion of biofertilizers have the ability to divide and elongate cells and grow and develop the plant, thus increasing the root system and the surface area of the absorption area, then increasing the rate of absorption of water and dissolved nutrients, which is reflected in an increase in vegetative growth. This is consistent with [37], the increase in vegetative growth indicators of lettuce plants is due to the use of bacteria that stimulate plant growth and mycorrhizal fungi, as biofertilizers increase the enrichment of the soil with nutrients such as nitrogen, phosphorus and potassium and improve soil aeration, thus increasing soil fertility and plant absorption of nutrients and photosynthesis rates for lettuce plants. One of the most important elements that encourage plant growth and its continuation in life is nitrogen, as it is considered one of the basic nutrients for plant growth, and without it the plant does not complete its life cycle. Azospirillum bacteria carry out the biological fixation of atmospheric nitrogen in the root zone of the plant, and thus these bacteria provide an essential food source that is easy and close to the roots of the plant. Azospirillum bacteria also provide a group of growth

regulators that stimulate plant growth, such as auxin (indole 3 acetic acid) IAA, cytokinin, and gibberellin. All of these regulators are organic materials that encourage plant growth and increase its vegetative growth indicators [38].

The results of the same tables above also show that spraying organic nutrients had a significant effect on vegetative growth indicators (plant height, stem diameter, total number of leaves, number of outer leaves, leaf area), as the values of vegetative growth indicators increased with a significant difference, as some studies indicate the existence of an interaction between the addition method such as spraying and the concentration of organic nutrients on growth indicators, as the spraying method had the greatest effectiveness than other addition methods on lettuce plants [39]. This is consistent with what [40] reached, as spraying organic nutrients increases the results of growth indicators under open field conditions for lettuce plants. These results are consistent with the results of [41] on garlic plants, and also with [42] and with [43] on lettuce plants. The addition of biofertilizers and spraying organic fertilizers affected vegetative growth indicators such as stem length, number of leaves per plant, and lettuce plant productivity. Organic fertilizers also positively affected the plant's nitrogen, phosphorus, and potassium content [44,45]. This may be attributed to the fact that organic nutrients increase the nutrient content in the plant, as nutrients such as nitrogen, phosphorus, potassium, and other elements that the plant needs to build cells and develop the growth of plant organs with the chlorophyll molecule, which is the main pigment in the plant's building process, and thus the growth indicator values increase [46]. These results are consistent with a study on lettuce, which indicated that the use of organic nutrient spraying increased the root system and nutrient absorption [47]. It is consistent with the results of [48]. The effect of spraying glutamic acid was studied as an alternative to reducing traditional nitrogen fertilizers, and the results of this study indicated significant values in wet and dry weight and the efficiency of using traditional fertilizers on spinach plants[49]. Nitrogen fertilizer was associated with the growth and development of lettuce in a study to determine the effect of compensating for the deficiency of nitrogen fertilizers with a group of amino acids, including glutamic acid, on the growth and development of lettuce plants, as the results of these acids and glutamic acid indicated positive results regarding vegetative growth such as wet and dry weight and leaf area[50]. Glutamic acid is considered a vital growth stimulant, easy to decompose and absorb, and a good contributor to improving plant growth without going to plant breeding and improvement programs[51]. In a study aimed at knowing the effect of spraying glutamic acid on lavender *Lavandula angustifolia* Mill on some vegetative growth traits and plant mineral content, the results indicated excelled in plant height, chlorophyll content, leaf area and wet weight [52]. Glutamic acid participates in various vital processes, including chlorophyll and proline synthesis processes. Chlorophyll is the pigment responsible for photosynthesis, and proline acts as a stimulating and stress-resistant substance. Glutamic acid has also been used to improve nutrient efficiency, reduce environmental impacts, as a chelating agent for microelements and as a nitrogen source. In one of the experiments, the results were positive when glutamic acid was used to stimulate the growth of lettuce in aquatic and traditional plant environments [53-57].

Conclusions

The triple interaction among biofertilization, organic nutrient application, and glutamic acid demonstrated significant superiority at treatment A3B2C2 in terms of plant height and leaf area across both seasons. Treatment A3B2C1 excelled in stem diameter for both seasons, while treatment A3B1C1 was superior in the number of outer leaves for both seasons. Additionally, treatment A3B2C1 was superior in total leaf count for the first season, whereas A3B1C1 excelled in the second season.

References

- [1] Shi M, Gu J, Wu H, et al. 2022. Phytochemicals, Nutrition, Metabolism, Bioavailability, and Health Benefits in Lettuce-A Comprehensive Review. *Antioxidants* (Basel). 11(6):1158. doi: 10.3390/antiox11061158.
- [2] AL-Taey, D. K. A., S. S. M. AL-Azawi, M. J. H. AL-Shareefi, and A. R. AL-Tawaha. (2018). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (*Lactuca sativa* var. Parris Island) *Research on Crops*.19 (3) : 441-449. DOI : 10.31830/2348-7542.2018.0001.14
- [3] Hassan, Ahmed Abdel-Moneim and Mohammed Abdul-Majeed Badawi and Ahmed Hassan Khreibia and Mohammed Ramadan Ajwa and Sayed Fathi Al-Sayed. 2003. Vegetable production. Cairo University Center for Open Education. Egypt.
- [4] Peiris, P. U. S. and W. A. P. Weerakkody. 2015. Effect of organic based liquid fertilizers on growth performance of leaf lettuce (*Lactuca Sativa* L.). In international conference on agricultural, ecological and medical sciences (AEMS-2015) April (pp. 7-8).
- [5] United States Department of Agriculture (USDA) (2018). National Nutrient Database for Standard Reference Release. Available Online at: <https://ndb.nal.usda.gov/ndb/foods/show/3001>.
- [6] Khalil, Mahmoud Abdel Aziz Ibrahim. 2004. Vegetable plants. Al-Maaref for Publishing and Distribution. Alexandria. Arab Republic of Egypt.
- [7] Sukor, A.; .2013. Effects of cyanobacterial fertilizers compared to commonly-used organic fertilizers on nitrogen availability, lettuce growth, and nitrogen use efficiency on different soil textures. M.Sc. Thesis, Colorado State Univ., USA.
- [8] Pandorf, M.; L. Pourzahedi, L. Gilbertson; G. V. Lowry; P. Herckes and P. Westerhoff . 2020. Graphite nanoparticle addition to fertilizers reduces nitrate leaching in growth of lettuce (*Lactuca sativa*). *Environmental Science: Nano*. Issue 1.
- [9] Qiu, W.; Z. Wang ; C. Huang; B. Chen and R. Yang . 2014 . Nitrate accumulation in leafy vegetables and its relationship with water. *Journal of Soil Science and Plant Nutrition*, 14 (4), 761-768. Research Article.
- [10] Brkić, D.; J. Bošnjir; M. Bevardi; A. G. Bošković; S. Miloš; D. Lasić; A. Krivohlavek; A. Racz; Ana Mojsović – Čuić and N. U. Trstenjak . 2017 . Nitrate In Leafy Green Vegetables And Estimated Intake. *Afr J Tradit Complement Altern Med*. 14 (3): 31-41.
- [11] Ördögh, M.; Z. Beregi and A. T. Mándy . 2019 . The effect of different biostimulators on morphological and biochemical parameters of micropropagated Hosta 'Gold Drop'. *Int J Horti Sci*. 25(1-2):22–9.
- [12] Ali, Nour El-Din Shawqi and Mohammed Mustafa Allawi and Nada Hamid Majeed. 2022. Rhizosphere Management and Agricultural Sustainability. University of Baghdad. College of Agricultural Engineering Sciences. Pp. 325. Iraq.
- [13] Al-Taey, D.K.A., M.J.H. Al-Shareefi , A.K. MijweL, A. RZ. Al-Tawaha, A. RM. Al-Tawaha.(2019). The beneficial effects of bio-fertilizers combinations and humic acid on growth, yield parameters and nitrogen content of broccoli grown under drip irrigation system. *Bulgarian Journal of Agricultural Science*, 25 (5), 959–966.
- [14] Khanday, M.; Bhat, R.A.; Haq, S.; Dervash, M.A.; Bhatti, A.A.; Nissa, M.; Mir, M.R. .2016. "Arbuscular Mycorrhizal Fungi Boon for Plant Nutrition and Soil Health". In: Hakeem KR et al., (eds) *Soil science: agricultural and environmental prospective*. Springer International Publishing, Switzerland, pp. 317–332.
- [15] Al-Dulaimy, A.F.Z., B. M. Abed , G.I. Abd and D. K. A. ALTaey.2024. Response of *Gazania* (*Gazania splendens* L.) Plants to Foliar Application with Bio Health and Salicylic Acid on some Growth and Flowering Traits. *IOP Conf. Series: Earth and Environmental Science* 1371 (2024) 042007. doi:10.1088/1755-1315/1371/4/042007
- [16] Taha, Hiba Hadi. 2021. Mycorrhizal fungi. *Journal of Rafidain Sciences*. Volume 33, Issue 3. Pp. 52-64.

- [17] Turk, M.A.; Assaf, T.A.; Hameed, K.M.; Tawaha, A.M. (2006). Significance of mycorrhizal. J. Agricultural Sci., 2(1),16-20.
- [18] Al-Maliki S., A.Al-Amery., M. Sallal, A. Radhi and D. K.A. Al-Taey,2021. Effects of Arbuscular Mycorrhiza and Organic Wastes on Soil Carbon Mineralization, Actinomycete and Nutrient Content in Maize Plants (*Zea Mays* L.). Malaysian Journal of Soil Science Vol. 25: 107-124
- [19] Conrad, R.; .2009. "Mycorrhiza". 3th ed. Springer. pp.1-200.
- [20] Helman, Y.; S. Burdman and Y. Okon; .2011. Plant growth promotion by rhizosphere bacteria through direct effects. In: Rosenberg E, Gophna U (eds) Beneficial microorganisms in multicellular life forms. Springer, Berlin, pp 89–103.
- [21] Mahdi, Hanin Saleh and Wafaa Ali Hussein. 2017. Effect of Biohealth and mineral fertilizers on yield and quality of four eggplant varieties. Al-Furat Journal of Agricultural Sciences. Volume 9, Issue 4: 603-612.
- [22] Al-Jawari, Abdul-Rahman Khamas Suhail. 2002. Effect of spraying with different nutrients on the growth and yield of sweet pepper *Capsicum annuum* L.. Master Thesis. College of Agriculture. University of Baghdad. Iraq.
- [23] Saleh, S.S.; A.A. Glala; M.I. Ezzo; and A.A. Ghoname; . 2010. An attempt for reducing mineral fertilization in lettuce production by using bio-organic farming system. Acta Horticulture, doi: 10.17660/ActaHortic.2010.852.39.
- [24] Ekinci, M.; E. Yildirim; M. Turan and R. Kul; .2020. Organic Fertilizers Improved Plant Growth and Mineral Content of Lettuce (*Lactuca sativa* L.). Acta Horticulture, doi:10.17660/ActaHortic.2020.852.39.
- [25] Tsouvaltzis, Pavlos. Koukounaras, Athanasios and Siomos, A. S. .2014. Application of Amino Acids Improves Lettuce Crop Uniformity and Inhibits Nitrate Accumulation Induced by the Supplemental Inorganic Nitrogen Fertilization. International Journal Of Agriculture & Biology. 16–5–951–955. <http://www.fspublishers.org>.
- [26] Baqir, H.A.; N. H. Zeboon and A.A. J. Al-behadili; .2019. The role and importance of amino acids within plants : A review. Plant Archives Vol. 19, Supplement 2, pp. 1402-1410.
- [27] Saaseea, K. G. and N. J. K. Al-a'mry .2024. Effects of mineral fertilization and spraying with salicylic acid and amino acids on the growth and productivity of industrial potatoes. Iraqi J. Agri. Sci. :55(Special Issue):162-174.
- [28] Turfan, N. and B. Turan .2023. Effects of glutamic acid applications on the yield and growth parameters in garlic (*Allium sativum* L.) cultivation. Harran tarim ve gıda bilimleri dergisi, Harran Journal of Agricultural and Food Sciences, 27(1), 1-14.
- [29] Al-Rawi, Khasha Mahmoud and Abdel Aziz Muhammad Khalaf Allah. 1980. Design and analysis of agricultural experiments. Ministry of Higher Education and Scientific Research.
- [30] Dar Al-Kutub for Printing and Publishing, University of Mosul. Mosul. Iraq.. P. 488.
Al-Khazraji, Safwan Nawaf Fayyad. 2020. The effect of spraying iron and zinc on the accumulation of nitrates and nitrites in plants resulting from nitrogen fertilization and the growth and yield of lettuce. Master's thesis. University of Anbar. College of Agriculture. Iraq.
- [31] Watson ,D.J. and M .A.Watson .1953. Comparative Physiological Studies on the growth of yield crops . 111. Effect of infection with beet yellow . Annals of Applied Biology .40(1) :1– 37 .
- [32] Ammar, G. A. G.; .2024. Plant Beneficial Symbionts: Charming Members in the Phytomicrobiome Community. Future Perspectives of Medical, Pharmaceutical and Environmental Biotechnology. Vol. 1 (1) pp: 19-30.
- [33] Alshegaihi, R. M.; A. Alatawi and M. A. Alenezi .2024. Ameliorative Effects of Plant Growth Promoting Rhizobacteria and Arbuscular Mycorrhizal Fungi on Cu Stress in Maize (*Zea mays* L.) with a Focus on Oxidative Damage, Antioxidant Responses, and Gene Expression. J. of Soil Sci. and Plant Nutrition. Vol. 24. Pp: 2437-2455. <https://doi.org/10.1007/s42729-024-01662-8>.
- [34] Bhandana, P.; M.S. Rana; Xc. Sun et al. .2021. Arbuscular mycorrhizal fungi and its major role in plant growth, zinc nutrition, phosphorous regulation and phytoremediation. Symbiosis. Vol. 84 (1) pp: 19–37. <https://doi.org/10.1007/s13199-021-00756-6>.

- [35] Balci, G.; .2023. The Effect of the Application of Mycorrhiza on Vegetative Growth, Mineral Element Intake, and Some Biochemical Characteristics of Strawberry Seedlings under Lime Stress. *Horticultural Studies*, 40(2), 62-71. <https://doi.org/10.16882/hortis.1330523>.
- [36] Al-Taey, D.K.A., Z.N. A. Kamaluddin and H. H. S. Al-Kazemy .(2022). Impact of Zytonic-M and organic fertilizer on growth and yield of hybrid tomato Awahr (F1) under saline conditions, *International Journal of Vegetable Science*, 28(4) :1-6 DOI: 10.1080/19315260.2022.2070569
- [37] Chatzistathis, T.; V. Kavvadias; T. Sotiropoulos and I. E. Papadakis .2021. Organic Fertilization and Tree Orchards. *J. Agric. Vol. 11 (8)*. 692-. doi:10.3390/agriculture11080692.
- [38] Joe, M. M. and A. Benson .2024. *Azospirillum*-based aggregated cells as inoculant delivery for sustaining plant growth under challenging conditions. *Beneficial Microbes for Sustainable Agriculture Under Stress Conditions*. Chapter 15. Pp: 281-311.
- [39] Frasetya, B.; A. Taofik and L. Nasrulloh .2021. The effectiveness of the application method of liquid organic fertilizer in different concentration on lettuce. *IOP Conf. Ser.: Earth Environ. Sci.* 694 012024.
- [40] Al-Chalabi, A. T. M.; F. F. R. Ibraheem; A. M. Hussein and A. Alsawaf .2023. Comparison the Effect of an Organic Nutrient (Nektafol) Under Production and Open Field Condition on the Growth and Yield of Lettuce. *IOP Conf. Ser.: Earth Environ. Sci.* 1262 042003.
- [41] Al-Bayati, Nazik Haqi Khalil and Redha Mustafa Abdul-Hussein Al-Obaidi. 2011. Effect of foliar nutrient spraying on vegetative characteristics and yield components of garlic *Allium sativum*. *Diyala Journal of Agricultural Sciences*. 3 (2): 656-668.
- [42] Al-Zaidi, Raed Jumaa Abdel. 2021. The effect of fish farm wastewater and spraying with Shamblan extract on the growth and yield of lettuce. Master Thesis. College of Agricultural Engineering Sciences. University of Baghdad. Iraq.
- [43] Hadid, R.A.; R.M. Abdel Hussein .2023. Effect of spraying with organic nutrients and adding levels of potassium fertilizer on the growth and yield of red cabbage. *Revis Bionatura J. Vol. 8 (2)* 69. <http://dx.doi.org/10.21931/RB/CSS/2023.08.02.69>.
- [44] Mohammed, A. A.; J. M. Abbas and M. H. K. Al-Baldawi .2020. Effect Of Plant Source Organic Fertilizers On Yield and It's Components Of Linseed Cultivars. *Iraqi Journal of Agricultural Sciences*. Vol. 51(Special Issue):86-95. <https://doi.org/10.36103/ijas.v51iSpecial.885>.
- [45] Al-Hlfie, R. G. and W. A. Hussin .2023. Response of three beetroot cultivars to organic fertilization and foliar application. *Iraqi Journal of Market Research and Consumer Protection*. 15 (2): 221-234.
- [46] Ahmed, Z. F. R.; A. K. H. Alnuaimi; A. Askri and N. Tzortzakis .2021. Evaluation of Lettuce (*Lactuca sativa* L.) Production under Hydroponic System: Nutrient Solution Derived from Fish Waste vs. Inorganic Nutrient Solution. *Horticulturae* 7, no. 9: 292. <https://doi.org/10.3390/horticulturae7090292>.
- [47] Tunio, M. H.; J. Gao; I. A. Lakhiar; K. A. Solangi; W. A. Qureshi; S. A. Shaikh and J. Chen . 2021. Influence of Atomization Nozzles and Spraying Intervals on Growth, Biomass Yield, and Nutrient Uptake of Butter-Head Lettuce under Aeroponics System. *Agronomy* 11, no. 1: 97. <https://doi.org/10.3390/agronomy11010097>.
- [48] Abdurassool, I. J. and Z. R. J. Al-Malikshah .2022. Effect of Adding Fulzyme plus and Spraying with Green Tea Extract on Vegetative Growth and Yield of Pepper cv. California Wonder. *Basrah J. Agric. Sci.*, 35(2), 302-312.
- [49] Chen, L.; L. Fei; Z. Wang; M. K. Salahou; L. Liu; Y. Zhong and Z. Dai .2018. The effects of ploy (γ -glutamic acid) on spinach productivity and nitrogen use efficiency in North-West China. *Plant Soil Environ.* 64, no. 11: 517-522.
- [50] Salinas , M.; E. Gandolfo; G. Hakim; E. Giardina and A. Di Benedetto .2019. Foliar Amino Acids Sprays on Lettuce (*Lactuca sativa* L.) Biomass Accumulation. *Int.J.Curr.Microbiol.App.Sci.* 8 (1) pp: 2183-2196. <https://doi.org/10.20546/ijcmas.2019.801.228>.
- [51] Alfosea-Simón, M.; S. Simón-Grao, E. A. Zavala-Gonzalez; I. Navarro-Morillo; J. J. Martínez-Nicolás; F. J. Alfosea-Simón; I. Simón and F. García-Sánchez .2022. Ionomic, metabolic and

- hormonal characterization of the phenological phases of different tomato genotypes using omics tools. *Scientia Horticulturae*. Vol. 293 (5). 110697. <https://doi.org/10.1016/j.scienta.2021.110697>.
- [52] Hamada, J. and M. A. Ahmed .2022. Effect of Glutamic Acid Spraying and Nano Zinc Oxide in the Growth and Content Qualities of *Lavendula Angustifolia* Mill. *International Journal of Health Sciences*. 6 (S8):884-896. <https://doi.org/10.53730/ijhs.v6nS8.9852>.
- [53] Galieva, G.; P. Kuryntseva; S. Selivanovskaya; V. Brusko; B. Garifullin; A. Dimiev and P. Galitskaya .2024. Glutamic-N,N-Diacetic Acid as an Innovative Chelating Agent in Microfertilizer Development: Biodegradability, Lettuce Growth Promotion, and Impact on Endospheric Bacterial Communities. *Soil Systems* 8, no. 2: 67. <https://doi.org/10.3390/soilsystems8020067>.
- [54] AL-Mharib, M. .Z.K.; A. M. Attalah and A. B. Ali .2019. Effect of Adding Humic Acid and Phosphate Fertilizer Levels on Growth and Yield of Lettuce. *IOSR J. of Agri. and Veterinary Sci*. Vol. 12 (4) pp: 12-15.
- [55] AL-Samarai, M.M. A.; R.M. A. Al-Ubaidy and H. H.A. Al-Umrani .2020. Response Of *Abelmoschus Esculentus* L. Forinoculation With Mycorrhizae And Folair Application With Biostimulators And Effect On Vegetative Growth Characters. *Iraqi J. of Agri. Sci*. 2020:51(Special Issue):61-71.
- [56] Al-Zaidi, Ali Karim Nahir and Redha Mustafa Abdul-Hussein Al-Obaidi. 2017. Effect of adding wheat peat and spraying with its extract and the organic nutrient Vegeamino on the growth and yield of red hannah. *Iraqi Journal of Agricultural Sciences*. 48 (2) 429-438.
- [57] Al-Omrani, Hussein Hamid Aboud. 2018. Effect of inoculation with mycorrhizae and spraying with ferrous sulphate and Armurox solution on the growth and yield of okra. Master Thesis. College of Agricultural Engineering Sciences. University of Baghdad. Iraq.

The Impact of Potassium Fertilizer Levels and the Method of Addition on the Growth and Yield of Cauliflower Plants *Brassica Oleracea* var. *Botrytis*

Omar A. Ibrahim¹, Amin H. Jabal² and Abdullah A. Abdullah³

^{1,3}Horticulture Department, College of Agriculture, University of Basrah, Basrah, Iraq.

²Soil Science Water Resources Department, College of Agriculture, University of Basrah, Basrah, Iraq.

¹E-mail: Omar.ibrahim@uobasrah.edu.iq

²E-mail: amin.hussain@uobasrah.edu.iq

³E-mail: Abdulla.Abdulaziz@uobasrah.edu.iq

Abstract. The research was conducted at the Agricultural Research Station of the College of Agriculture, University of Basrah, in Karma Ali during the winter agricultural season 2022-2023. The objective of the study was to investigate the impact of the addition of potassium fertilizer at varying levels (0, 100, 200.300) kg ha⁻¹, as well as two methods of sprinkler and ground application on the plant growth and yield of hybrid cauliflower. Flake of white color. The results indicated that the dry weight of vegetative growth, the weight of the flower disc, and the total yield were significantly surpassing the fertilizer level of 200 kg ha⁻¹. Additionally, the percentage of total soluble minerals in the discs was significantly surpassing all levels. The foliar addition resulted in a significant increase in the number of leaves, leaf area, dry weight of vegetative growth, the diameter of the flower disc, its weight, and the total yield. Similarly, the ground addition increased the percentage of dry matter in the flower discs. The interaction between the two factors had a significant impact on the majority of the studied traits. The plants that were sprayed with potassium fertilizer at a level of 200 kg ha⁻¹ achieved the highest weight of the disc and the total yield, which were 2.180 kg and 59.95 tons ha⁻¹, respectively.

Keywords. Cauliflower, Potassium Fertilizer, Soil.

1. Introduction

Cauliflower *Brassica oleracea* var. botrytis is an important winter vegetable crop belonging to the Brassicaceae family. It is grown for its curd, which is the edible part of the plant. It is the flower buds before they open, with the fleshy, enlarged flower stalks [1]. It is considered to have a high nutritional and medical value due to its content of vitamins (B6, B5, K, C), fiber, folic acid, and minerals (P, Mg, Fe, Zn, K) [2]. It also contains polyphenolic antioxidant compounds [3]. The area planted with cauliflower in Iraq for the year 2020 is estimated at about 4,500 dunums with a total productivity of 12,400 tons and a production rate of 2.724 tons. dunum⁻¹ [4]. In order to help increase production per unit area, attention must be paid to agricultural service operations, including the fertilization process, due to the nutrients it provides to the plant by determining its type, quantity, and method of addition. One of the most important nutrients that the cauliflower plant needs, which ranks second to the plant, is potassium K [5] for its important role in many vital activities such as photosynthesis, protein metabolism, enzyme activity, regulation of cell osmotic potential, expansion, and thickening of its walls [6,7]. It is a carrier and activator of many enzymatic activities and is responsible for the process of opening and closing the stomata through its effect on the guard cells located around the stomata, so it controls the level of gas and water exit from During the stomata [8]. [9] concluded that when *Brassica napus* rape plants were

sprayed With potassium sulphate (K 42%) at concentrations (0, 1500, 300, 4500) mg K L⁻¹, the concentration of 4500 mg K L⁻¹ caused a significantly excelled in the number of fruits, seed weight and seed yield for both seasons of the experiment.[10]explained that spraying cauliflower plants, White cloud cultivar, with potassium nitrate KNO₃ at different concentrations (2, 1, 0)%, the concentration of 2% was significantly excelled in increasing the number of leaves, weight of the inflorescence without leaves and the total yield compared to the control treatment for both seasons of the experiment [11]. noted that when adding potassium fertilizer at different levels to Paraiso hybrid broccoli plants (300, 200, 0) kg ha⁻¹, the level of 300 kg ha⁻¹ was significantly higher in increasing plant height, leaf area, total yield, and percentage of dry matter in the flower disc compared to the control treatment. [12] indicated that adding potassium sulfate fertilizer to cowpea plants at levels (0, 43, 86, 129) kg ha⁻¹ led to an increase in plant height, leaf area, number of pods, number of seeds per pod, plant yield, and total yield. [13]. showed that spraying Nahar hybrid cauliflower plants with high potassium fertilizer at different concentrations (3, 1.5, 0) ml L⁻¹ caused both concentrations to significantly increase the number of leaves, leaf area, flower disc weight, total yield and percentage of dry matter in the flower disc compared to the control treatment. [14] noted that when spraying Galaxy and Durra hybrid cauliflowers with different concentrations of potassium humate (5, 2.5, 0) g L⁻¹, both concentrations caused a significant increase in the number of leaves, leaf area, flower disc weight and total yield compared to the control treatment. [15] indicated that spraying Organza hybrid cauliflower plants with different concentrations of potassium (4, 2, 0) g L⁻¹, the concentration of 4 g L⁻¹ caused a significant increase in the number of leaves, fresh and dry weight of plant leaves, flower disc diameter and yield. Total compared to control treatment. The study aims to find the best method and the best level of addition of potassium fertilizer added either by irrigation in the soil or spraying on the green group and the interaction between them in the growth and yield of the White Flake cauliflower hybrid grown under the conditions of Basra city.

2. Materials and Methods

The experiment was conducted at the Agricultural Research Station - College of Agriculture - University of Basra located in Karmat Ali area during the agricultural season 2022-2023 AD to study the effect of different levels of potassium fertilizer in the form of N:P: K (0:0:40) on the growth and yield of cauliflower, White flake cultivar. The experiment included two factors: the first factor was adding potassium fertilizer at levels of 0, 100, 200 and 300 kg K ha⁻¹, and the second factor was the method of adding potassium fertilizer, as the concentrations were added in two ways: the first was spraying on the plant and the other was a ground addition. The experiment was designed as a factorial experiment with a randomized block design (R.C.B.D) and with three replicates to make the number of experimental units 24 units. The land was prepared by plowing it twice perpendicularly to a depth of 0.4 m and was well smoothed after carrying out the adjustment and leveling operations. The cultivation was carried out on two rows, the distance between each row was 1 m and the width of the row was 0.5 m. The soil was fertilized with animal manure (cow waste) at a level of 5 tons ha⁻¹ at a depth of 0.2 m. The rows were divided into three sectors, each containing one replicate of the experimental treatments (24) experimental units, so that the length of the experimental unit was 2 m, and each experimental unit contained 5 plants, and the distance between one plant and the next was 40 cm. Broccoli seedlings (White flake) were grown in plastic plates and then transferred to the ground after reaching the stage of three true leaves. The experimental units were fertilized with nitrogen fertilizer (urea 46% N) at a rate of 250 kg N ha⁻¹ and phosphate fertilizer (superphosphate) at a rate of 65 kg P ha⁻¹. Some vegetative growth indicators were estimated such as plant height, number of and leaf area was calculated according to [16]. The fresh weight of the vegetative mass (kg), the dry weight of the

vegetative mass (g), the yield and its components were estimated such as the weight of the flower disc (g), the diameter of the flower disc (cm), and the total productivity of the flower discs (tons.ha⁻¹). The total yield was calculated as follows: yield per plant (kg) × plant density/plant. ha⁻¹. Some qualitative and chemical traits were also estimated such as the content of the flower discs of total chlorophyll and carotene (mg/100g⁻¹ fresh weight) according to the method described by [17] and the percentage of total soluble solids in the flower discs. The percentage of dry matter in the flower discs was also estimated.

Table 1. Some chemical and physical properties of the study soil.

Traits		Values	Units
pH		7.7	
Electrical conductivity EC		5.22	dS m ⁻¹
Available phosphorus		38.3	mgkg ⁻¹
Total nitrogen		0.23	gkg ⁻¹
Available potassium		101.20	mgkg ⁻¹
dissolved positive ions	Calcium	16.5	
	Magnesium	11	
	Sodium	21.3	
	Carbonates	0.00	mmol L ⁻¹
dissolved negative ions	Bicarbonates	13.6	
	Sulphates	18.5	
	Chlorides	28.0	
	Sand	593	
Soil separators	silt	271.5	g kg ⁻¹
	Clay	135.5	
Texture		Sandy loam	

leaves.plant⁻¹, total leaf area/plant (dm²). The results were analyzed statistically using the statistical program GenStat according to the adopted design, and the results were compared to the arithmetic averages according to the least significant difference test at a significance level of 0.05.

3. Results and Discussion

It is clear from Table (2) that the levels of potassium fertilizer have significantly affected the number of leaves and the fresh and dry weights of the plant, but had no significant effect on the plant height and leaf area, as the level of 300 kg ha⁻¹ was significantly excelled in increasing the number of leaves compared to the levels of 100, 200 kg ha⁻¹, with an increase rate of (14.70, 23.18), respectively, and did not differ significantly in control treatment, while the level of 100 kg ha⁻¹ was significantly excelled in increasing the fresh weight of the vegetative group compared to the level of 300 kg ha⁻¹, with an increase rate of 57.34% It did not differ significantly with control treatment and the level of 200 kg ha⁻¹, while the dry weight of the vegetative group was significantly excelled to the level of 300 kg ha⁻¹ compared to control treatment and the level of 100 kg ha⁻¹, with an increase rate of (99.05 ,102.49)% respectively and did not differ significantly with the level of 200 kg ha⁻¹. The significantly excelled of potassium fertilizer levels may be due to the role of potassium in growth and control of important physiological activities for plant growth, including controlling the opening and closing of stomata, carbohydrate formation and regulating water balance in leaves [18]. These results are consistent with what was obtained by [15]. The same table shows that foliar addition caused a significant increase in the number of leaves, leaf area and dry weight of the vegetative group compared to ground addition, with an increase rate of (45.25, 13.12, 7.24)% respectively, and the method of addition did not have a significant effect on plant height and fresh weight of the vegetative group. The significant

increase may be due to adding fertilizer as a spray on vegetative growth, as it makes its absorption by the leaves easier and faster, and thus the direct benefit from the potassium element, which works to stimulate enzymes specific to vital activities such as photosynthesis, and also helps in the movement and transfer of food manufactured in the leaves to other plant tissues [8] and increasing the readiness and absorption of necessary nutrients such as nitrogen, phosphorus and potassium from the soil to the plant, which leads to an increase in vital activities in the plant, which is positively reflected in growth [19,20]. The interaction between the two factors showed a significant effect on the traits under study, with the exception of plant height, as plants sprayed with potassium at a level of 300 kg ha⁻¹ gave the highest values for the number of leaves, leaf area and dry weight of the vegetative group, which reached (23.0 leaves, 74.3 dm², 147.7 g), respectively, while plants sprayed at a level of 100 kg ha⁻¹ gave the highest fresh weight of the group. The vegetative mass reached 1.776 kg. While the lowest number of leaves per plant was at the level of 100 kg ha⁻¹, reaching 15.67 leaves. The lowest leaf area and fresh weight of the vegetative mass when adding potassium fertilizer to the ground at the level of 300 kg ha⁻¹, reached (20.7 dm², 0.668 kg), respectively, and the lowest dry weight of the vegetative growth in plants sprayed at the level of 100 kg ha⁻¹ reached 35.9 g.

Table 2. Effect of potassium addition level, addition method and interaction between them on some vegetative growth indicators of cauliflower plant.

Treatments		Plant height (cm)	Number of leaves	Leaf area dm ²	Fresh weight of vegetative growth (g)	Dry weight of vegetative growth (g)	
Average effect of potassium fertilizer levels kg ha ⁻¹	0	63.5	19.19	46.2	1.282	52.1	
	100	57.5	15.83	51.8	1.361	53.0	
	200	64.3	17.00	44.6	1.104	99.5	
	300	61.3	19.50	47.5	0.865	105.5	
LSD 0.05		NS	1.65	NS	0.363	10.5	
Average effect of adding method	Foliar	62.1	18.5	55.1	1.224	91.8	
	Ground	61.2	17.25	39.9	1.082	63.2	
LSD 0.05		NS	1.17	6.9	NS	7.4	
The effect of the interaction between the adding levels and the addition method	0	Foliar	60.7	17.33	38.5	0.840	45.7
		Ground	66.3	21.00	53.9	1.725	58.5
	100	Foliar	59.0	15.67	57.4	1.770	35.9
		Ground	56.0	16.00	46.3	1.011	70.1
	200	Foliar	71.0	18.00	50.3	1.282	138.0
		Ground	57.7	16.00	38.6	0.925	61.0
	300	Foliar	57.7	23.00	74.3	1.062	147.7
		Ground	65.0	16.00	20.7	0.668	63.4
LSD 0.05		NS	2.34	13.9	0.514	14.9	

Table (3) shows that the levels of potassium fertilizer significantly affected the weight of the flower disc, the total yield, and the percentage of total soluble solids in the disc, where the level of 200 kg ha⁻¹ significantly excelled the increase in the weight of the flower disc and the total yield compared to the control treatment and the levels of 100 and 300 kg ha⁻¹, with an increase rate of (29.45, 26.50, 84.28)% for each, respectively. The levels did not significantly affect the diameter of the flower disc and the percentage of dry matter in it. The significantly excelled of the level of 200 kg ha⁻¹ may be due to the level of 200 kg ha⁻¹, as it is the best level that achieved a significant increase in the dry weight of the vegetative group (Table 2) as a result of the accumulation of manufactured nutrients as a result of adding potassium at the appropriate level and its important role in the process of photosynthesis through controlling the mechanism of opening and closing the stomata, which is directly related to the collection of potassium and

sugars in the guard cells [18,21] and its work in improving the process of transporting nutrients. It is manufactured in the leaves to other storage sites in the plant [22] and stimulates enzymes associated with the process of transferring energy necessary to metabolize CO₂ and in building sugars, starch and proteins, as ATP is the main energy carrier [7]. These results are consistent with [10,11,13,14,15]. It appears from the same table that the addition method has a significant effect on all the traits under study, except for the percentage of total soluble solids in the flower disc, as the foliar addition achieved a significant increase in the diameter of the flower disc, its weight, and the total yield, with an increase rate of (35.84, 35.84, 48.32)%, respectively, while the ground addition was significantly excelled in the percentage of dry matter in the disc, with an increase rate of 12.09%. The significantly excelled of the foliar addition may be due to its positive role in increasing the vegetative growth indicators represented by the number of leaves, leaf area, and dry weight of the vegetative group (Table 2), which was positively reflected in increasing the diameter of the flower disc, its weight, and the total yield. As for the increase in the percentage of dry matter in the flower disc when adding potassium fertilizer to the ground, which stimulated the plant to build a root system with high efficiency in absorbing nutrients from the soil, which encouraged the process of photosynthesis and thus increased its nutritional products in the leaves such as carbohydrates and proteins and their transfer to the discs, which leads to an increase in the percentage of dry matter in them. The interaction between the two study factors showed a significant effect in all the studied traits except for the percentage of dry matter in the disc, as the plants sprayed at the level of 300 kg ha⁻¹ gave the largest diameter of inflorescence disc and the highest percentage of total soluble solids, which amounted to (23.66 cm, 5.8%), respectively. The plants sprayed at the level of 200 kg ha⁻¹ gave the highest weight of inflorescence disc and the total yield, which amounted to (2.180 kg, 59.95 tons ha⁻¹), respectively. While the lowest diameter of inflorescence disc and the percentage of total soluble solids when adding soil at the level of 200 kg ha⁻¹ amounted to (12.34 cm, 4.50%), respectively. The lowest weight of inflorescence disc and the total yield in the plants not added potassium fertilizer amounted to (0.823 kg, 22.69 tons ha⁻¹), respectively.

Table 3. Effect of potassium addition level, addition method and interaction between them on some yield traits of cauliflower plant.

Treatments		Inflorescence diameter (cm)	Inflorescence weight kg	Total yield T.ha ⁻¹	Dry matter per disc %	Percentage of total soluble solids %	
Average effect of potassium fertilizer levels kg ha-1	0	15.15	0.961	26.44	9.56	4.65	
	100	15.62	1.400	38.51	10.42	5.35	
	200	15.04	1.771	48.70	10.38	5.06	
	300	15.13	1.368	37.62	10.64	5.63	
	LSD 0.05	NS	0.215	3.93	NS	0.34	
Average effect of adding method	Foliar	18.20	1.584	43.56	9.67	5.29	
	Ground	12.27	1.166	32.07	10.84	5.06	
	LSD 0.05	1.79	0.152	4.19	0.88	NS	
The effect of the interaction between the adding levels and the addition method	0	Foliar	13.15	1.097	30.18	9.12	4.53
		Ground	17.15	0.823	22.69	10.01	4.76
	100	Foliar	18.26	1.651	48.41	9.75	5.20
		Ground	12.99	1.150	31.62	11.09	5.50
	200	Foliar	17.74	2.180	59.95	9.73	5.63
		Ground	12.34	1.362	37.45	11.04	4.50
	300	Foliar	23.66	1.405	38.72	10.07	5.80
		Ground	6.60	1.326	36.52	11.21	5.50

Treatments	Inflorescence diameter (cm)	Inflorescence weight kg	Total yield T.ha ⁻¹	Dry matter per disc %	Percentage of total soluble solids %
LSD 0.05	3.58	0.305	18.38	NS	0.48

Conclusion

Based on the research, it is determined that in order to enhance the development and productivity of the White Flake hybrid cauliflower plant cultivated in Basra city, it is necessary to apply foliar potassium fertilizer at a rate of 200 kg ha⁻¹.

References

- [1] Matlub, Adnan Nasser and Izz Al-Din Sultan Muhammad and Karim Saleh Abdul (1989). Vegetable production, Part One, Ministry of Higher Education and Scientific Research, University of Mosul. Republic of Iraq.
- [2] Nonnecke, I.L. (1989). Vegetable production .Avi, Newyork.p:657.
- [3] Ramarathram, N.; H. Ochi and M. Takeucki .(1997). Anti oxidative defense system in vegetable extracts. In: shahidi, F., eds. Natural antioxidants. chapters:5;76-87 AOCS press, Illinois, USA.
- [4] Central Statistical Organization (2021). Crop and Vegetable Production, Directorate of Agricultural Statistics, Ministry of Planning, Republic of Iraq.
- [5] Takeishi, J.; AB. Ceciliofitho and PR. Oliveire . (2009). Growth and accumulation of food in cauliflower Verona hybrid. Bio Science Journal ,25:1-10.
- [6] Taiz, L. and E. Zeiger (2003). Plant physiology 3rd ed. Sinauer Associates, Inc. publishes Sunderland, Massac huts, U.S.A.
- [7] AL-Taey D.K.A. and , A.K. Burhan.(2021). The effect of water quality, cultivar, and organic fertilizer on growth and yield of volatile oil carvone and limonene in Dill. International Journal of Vegetable Science., 27(5)1-7: DOI: 10.1080/19315260.2021.1970075.
- [8] IPI International Potash Institute.(2001). Potassium dynamics and its availability in the soil. international fertilizer correspondent .p,1-5.
- [9] Muhsen, B.M. ; A. A. Al-Guburi and Y. M. Abu-Dahi (2009). Effect of Phosphorus fertilization and foliar Feeding of Potassium on yield, yield components and oil, yield of rapeseed (*Brassica napus* L.) Basrah J. Agric. Sci., 22(2): 143-154.
- [10] Al-Hakim, Mumtaz Sahib Muhammad (2010). Effect of spraying with gibberellic acid, cyclosil and potassium nitrate on some vegetative and floral growth traits and production of cauliflower *Brassica oleracea* Var. botrytis. Al-Furat Journal of Agricultural Sciences 2(4):43-54.
- [11] Hashem, A.I.; N.M. Al-Bayaty and M.J. Mohamed.(2018). Effect of cultivation distances and potassium fertilization on the growth and yield of broccoli (*Brassica oleracea* Var. italica plemok). Euphrates Journal of Agricultural Science. 10(3):163-174.
- [12] AL-Furtuse, A.K.; K. A. Aldoghachi and W. A. Jabail (2019). Response of three varieties of Cowpea (*Vigna sinensis* L.) to different levels of Potassium fertilizer under southern region conditions of Iraq. Basrah J. Agric. Sci., 32(2): 25-34.
- [13] Obaid, A.K.; J.H. Hiji and Z. Abd .Alamer .(2020). The effect of Humic acid and high potassium on some growth traits and yield of cauliflower plant cell Biotechnology and Molecular Biology, 21(67868):14-20.
- [14] Maleh, B.A.H. and A.A.K. Obaid (2022) .Effect of salicylic acid and potassium humate on the growth and yield of two hybrids of cabbage (*Brassica Oleracea* L. Capitata) in southern Iraq. Euphrates Journal of Agriculture Science, 13(4)250-266.
- [15] Saleem, J.S.M and A.J.I. Saeid. (2023) .Effect of foliar application of Licorice root extract and potassium and their interaction on Vegetative growth and yield of cauliflower (*Brassica Oleracea* L. Var. botrytis). hyperbird. Journal of University of Duhok, 26 (1): 87-95.

- [16] Watson, D. J. and M. Watson (1953). Comparative physiological studies on the growth of filed crops. III-Effect of in fraction with Beet yellow. Ann. Appl. Bio, 40:1-18.
- [17] Abbas, Mu'ayyad Fadhel and Mohsen Jalab Abbas (1992). Practical care and storage of fruits and vegetables, Dar Al-Hikma Press, University of Basra. Iraq.
- [18] Krauss, A. (1995). Potassium, the forgotten nutrient in west Asia and North Africa. I.P.I. Basel Switzer.
- [19] Abdul Karim, Muhammad Abdullah (1994). Effect of adding nitrogen, phosphorus and potassium by spraying or to the soil on the growth and productivity of tomato plants. Master's thesis, College of Agriculture, University of Basra.
- [20] AL-Taey, D. K. A., S. S. M. AL-Azawi, M. J. H. AL-Shareefi, and A. R. AL-Tawaha. (2018). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (*Lactuca sativa* var. Parris Island) Research on Crops. 19 (3) : 441-449. DOI : 10.31830/2348-7542.2018.0001.14
- [21] Al-Falahi, M. N. A., K. H. Al-Dulaimi, E. T. A. Ghani, D.K. A. Al-Taey and K. J. Farhan. 2022. EFFECT OF HUMIC ACIDS AND THE AMOUNT OF MINERAL FERTILIZER ON SOME CHARACTERISTICS OF SALINE SOIL, GROWTH AND YIELD OF BROCCOLI PLANT UNDER SALT STRESS CONDITIONS. Agraarteadus. 32 (1):11-20. <https://doi.org/10.15159/jas.22.24>
- [22] Mengle, K. and H.E. Haeder. (1977). Effect of potassium supply on the rate of phloem sap exudation and the composition of phloem sap *Ricinus communis*. plant physiol, Sq: 282-284.

Detection the Active Compounds of the Ethanol Extract of *Cordia myxa* L. Leaves Using GC Mass Technology

**Duaa Ahmed Hassan Al-Kailani¹, Sarab Waleed Hassan Al-Samarrai² and
Aseel Salah Mahmoud Akuola³**

¹⁻³Department of Biotechnology, Faculty of Applied Science, Samarra University,
Samarra, Iraq.

¹E-mail: duaa.ahmed@uosamarra.edu.iq

²E-mail: sarab.waleed@uosamarra.edu.iq

³E-mail: aseelsalah@uosamarra.edu.iq

Abstract. The study's objectives were to identify secondary metabolites in the ethanolic extract of *Cordia myxa* L. tree leaves and identify the various types of chemical compounds that compose the extract. The leaves were harvested from a farm in the northern region of the Baghdad Governorate, and it underwent a process of cleaning, drying, washing, and grinding. A Soxhlet apparatus was utilized to create the ethanolic alcoholic extract, and GC-MS equipment was employed to identify the chemical components. Among the substances that showed up were: (ether 4-Boroxin, tris(4-bromophenyl) 4-(1-Gala-1-ido-octose), Lup-20(29)-ene-3,21,28-triol, 28-acetate, Ethyl tetracosanoate, 1-Heptatriacotanol, Squalene, (E)-9-Octadecenoic acid ethyl ester 4-Oleic acid, 3-(octadecyloxy)propyl ester 4-Ethyl iso-allocholate 4-Tetratetracontane 4-beta-Sitosterol 4-Spiro[androst- 5-ene-17,1'-cyclobutan]-2'-one).

Keywords. *Cordia myxa* L., GC-MS, Soxhlet extractor, Secondary metabolite, Chemical detection.

1. Introduction

Cordia myxa L. One of the semi-tropical plants grown in the country's center and southern Iraq, particularly Basra, is the pampier plant, which is a member of the Boraginaceae family [1]. Its distribution range can be found worldwide in the area that stretches from eastern India to the east of the Mediterranean Sea. It was first used a long time ago. It is ancient and found in Australia, tropical Asia, and Africa. It was just discovered in the Americas.

1.1. Plant Taxonomy [2]

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Lamiales

Family: Boraginaceae

Genus: *Cordia* L.

Species: *Cordia myxa* L.

It is a medium-sized tree with deciduous leaves, reaching a height of about 12 meters. The leaves are wide, smooth, oval, with wavy edges. The flowers are small white in terminal inflorescences and axillary clusters. The fruits are edible, about 2 cm in diameter and bright brown in color. A laboratory study published in the Journal of Herbs showed, Spices & Medicinal Plants in 2019 AD 2023), (Taylor and Francis, mentioned that the fruits of this plant are rich in protein and low in fat. As for minerals, they consist mainly of nitrogen, potassium, and calcium. They also contain a large amount of phenols and flavonoids and thus possess It has a strong antioxidant

effect [4] and the presence of small amounts of antioxidants such as phytic acid and tannins makes it a fruit suitable for human consumption [5].

The fruit of the tree, which bears seeds and cuttings for reproduction, contains mucus, a gelatinous substance that is used to treat a variety of ailments. Certain industries also make use of its wood. Mistletoe, a mucous substance, is frequently employed in bird trapping. In addition, it is planted for shade and adornment. It is a little, tropical tree that grows to a height of 12 to 15 meters. It is native to tropical Asia. The lifespan of this perennial tree is sixty to seventy years. When the fruit ages, its yellow hue turns darker. It contains a viscous substance inside that resembles clear, sweet glue. Due to its high vitamin content and favorable effects on hair follicles, it is regarded as one of the best fruits. These healing herbs are widely cultivated in many different parts of the world, which are extensively employed in numerous medical applications, both directly and indirectly. These plants were primarily used to make a variety of therapeutic remedies, including anti-inflammatory, antibacterial, antifungal, antioxidant, and antihypertensive medications. This plant is used not only for medical applications but also for the production of drinks, oil compounds, and aromatic oils. The gummy, slimy flesh of *Cordia myxa* L. plant's fruit is used in traditional medicine.

The alcoholic extract of the fruits contains flavonoids, alkaloids, gum, and tanning materials. The fruits have a sweet taste and play a major role in stimulating the immune system, in addition to the fact that the fruit contains water, antioxidants, and vitamin C, which protect cells from damage and cancer. In addition to the presence of chemicals such as alkaloids, polyphenols, saponins, and flavonoids in addition to essential vitamins, which are important compounds in reducing the risk of cancer and help strengthen the body's immunity and prevent the weakness of the immune system associated with aging, as it activates genes and Enzymes are concerned with antioxidants in immune cells and reducing free radicals, in addition to their importance in treating cancer diseases and their causes and preventing some liver infections [5] Chinese medicine has been using *Cordia myxa* L. to cure insomnia and promote better sleep since ancient times. This is because it has the relaxing property of saponin, which makes it perfect for naturally inducing, sleep It functions to lower blood levels of dangerous cholesterol, stop atherosclerosis, and also maintain especially if consumed frequently, it keeps the body in shape and helps avoid fat since fruits can be ingested in the form of juice that is combined with other fruits. *Cordia myxa* L. fruits also help in treating chest and respiratory diseases, including treating allergic asthma. They soothe coughs, expel phlegm, help clean the esophagus, and treat throat ulcers that occur due to severe inflammation. It is possible to consume at least 40 milligrams of pamper fruit every day because its fruits combat toxic substances and so enhance the overall environment of the digestive system, which is highly useful for stomach health. Because it reduces indigestion and improves the health of the stomach walls, it also cures stomach ulcers.

Because it improves the health of the stomach walls and prevents indigestion, Bamper fruit is widely used in medicine to treat stomach ulcers, eradicate intestinal worms, treat *Helicobacter pylori* in the stomach, and maintain the overall health of the digestive system.

1.2. Phytochemistry

[5] explained that the word Phyto is of Greek origin, meaning "Plant." Therefore, Phytochemistry means plant chemistry, and thus Phytochemicals means defensive molecules against various diseases. The chemical compounds produced by the plant are by-products of metabolic processes that it uses for the purpose of sustaining its life or for protection and defense against organisms, [6] These natural products are found in fruits and vegetables and show the potential to modify or modify human metabolism in a beneficial way to prevent chronic and degenerative diseases [7].

According to [8] phytochemicals are distributed non-uniformly (asymmetrically) in plants, with variations in kind and quantity between individual plants and sections. For example, alkaloids are typically present in larger concentrations in roots, seeds, and fruits than in leaves, and in lower amounts in vegetative tissues when compared to phenolic compounds. According to [9] plants are sometimes referred to as "green factories" because many of these biological compounds are high-level pharmaceuticals. Due to the large number of chemical compounds that are increasingly being discovered in plants, especially after conducting multiple chemical analysis methods, it is necessary to detect these compounds because of their importance in human life as well as animals, by relying on chromatographic analysis devices to classify the various chemical compounds and the possibility of proving their presence in plants Without the other and basically the classification of flowering plants [10].

1.3. Phytochemical Contents Can be Divided Into Two Parts [11]

Primary metabolic compounds: These are non-medically effective substances or compounds that participate directly in the growth, reproduction, and energy requirements of the plant, such as carbohydrates and amino acids.

Secondary metabolic compounds: These are organic compounds produced by the plant and do not participate directly in the natural growth, development, or reproduction of the plant. Secondary metabolic compounds often play an important role in defending the plant against living organisms such as agricultural pests and microorganisms, and these compounds are often used. In drugs, dyes, and flavours, these compounds are divided according to their chemical nature into volatile oils, alkaloids, Glycosides, tannins, phenolics, and terpenes. [12] stated that different parts of the plant can be used in treatment.

1.3.1. Gas Chromatography-Mass Spectrometry (GC-MS)

This technic is coupled analytical technique that combines the separation properties of gas and liquid chromatography with the detection and mass spectrometry properties to identify many substances within the test. GC is used to separate volatile and refractory substances as stable alternatives in the sample, while GC-MS fractionates the sample to be determined based on its mass [13].

It is widely used in pure and applied sciences like chemistry, polymers, nanotechnology, biotechnology, etc. and is thought to be a potent and unique technology that offers a unique opportunity to perform the analysis of new compounds as well as identify and characterize the total number of derived compounds. Acquiring information that can be applied to the global dissemination of research is beneficial. In 1940, it was first discovered [14].

1.4. The Importance of of GC-MS Technology

This is Advance technology that cannot be compared with other modern analytical devices. It has a wide range of applications that meet the needs of academic research and quality control as well as industrial applications. Its concise and efficient automated system gives fast, reproducible and efficient results playing a major role in the advancement of science and technology[15] also confirmed that GC coupled with MS is a powerful technique and a preferred method for analyzing small and volatile molecules.

The technique is well-known for its capacity to examine multi-component compounds with unknown and quantitative constituents. Only the analysis of esters, amino acids, alcohols, aldehydes, terpenes, and other chemicals has been done using this GC-MS method. Food, oil, and other materials can also have pollutants found in them and measured using GC-MS technology. The method has also been used to bioanalyze blood and urine for the presence of drugs, alcohol,

and residual solvents. The medications that have been detected include opioids, foodstuffs, anesthetics, anticonvulsants, antihistamines, and antiepileptic pharmaceuticals. This technique was used to determine the properties of fatty acids in microbes, the presence of free steroids, blood pollutants, metabolites in blood serum, chlorinated organic pesticides in river water, drinking water, soft drinks, pesticides in sunflower oil, and others [13].

1.5. The Study's Objectives

- Determine, examine, and classify the chemical components that make up a *Cordia myxa* L. plant leaf's makeup.
- Being informed about the significance of and operation of the gas chromatography-mass spectrometry technology.
- Learn how to prepare an alcoholic extract of *Cordia myxa* L. , as well as the basics of utilizing a Soxhlet extractor for extraction and how it differs from other extraction techniques.

2. Materials and Methods

2.1. Methodology

2.1.1. Devices Used

- Sensitive electronic balance.
- soxhlet extractor .
- Gas chromatography - mass spectrometry device.
- Electric grinder.

2.1.2. The Materials

- 250 ml of ethanol alcohol was used.
- *Cordia myxa* L plant leaves: In December 2023, the leaves of the plant under study were gathered from one of the Tarmiyah farms in the Baghdad Governorate. In order to avoid influencing the study's findings, the plant leaves were thoroughly cleaned with distilled water to remove any dust and contaminants that had adhered to them. Then they were dried in places away from influences. External factors (dust, humidity, sunlight) to preserve the compounds they contain and this is to avoid the effect of the various enzymes present in the plant. After grinding using an electric grinder, it was stored in paper bags until use.

2.1.3. The Process of Creating an Alcoholic Ethanol Extract with a soxhlet Extractor

After weighing 25 grams of powdered plant leaves with a sensitive weighing instrument, the alcoholic extract (ethanol 70%) was made using a soxhlet device that was available in the central laboratories of the Samarra University Faculty of Applied Sciences.

The plant powder placing in the round flask of the device, that is attached to the condenser of the soxhlet, and then adding 250 milliliters of ethanol alcohol to it. Following a 16-hour process that involves heating the solvent to a temperature that may reach 78 C°, *Cordia myxa* L. plant powder is thoroughly mixed with the ethanol alcohol. Throughout this time, the soxhlet device needs to be periodically checked to ensure that the plant's alcoholic extract is ready.

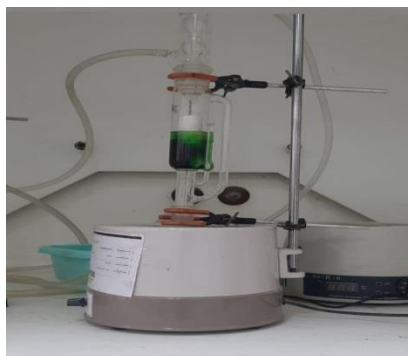


Figure 1. The soxhlet extractor device.

2.1.4. Examining a Plant Sample Using a GC-MS Device

10 microliters of the sample was taken and diluted in 5 ml of ethanol, and the device injector was set to 2 microliters of the diluted sample using a capillary column of the type (InertCap 1 is a non-polar column bonded 100% dimethylpolysiloxane) with a length of 30 meters and the carrier gas is helium at a flow rate (30 mL/min), The oven temperature program was set to begin at 50°C with a split ratio of 2:1. This temperature was held for three minutes, and then it was increased by 25°C per minute to 200°C, which was held for three more minutes for a total holding period of twenty minutes. [18] the procedure is developed rather than established and differs depending on the components of the sample.

3. Results and Discussion

As shown in Table (1) and Figure (2), the chemical compounds present in the alcoholic extract of the leaves of the Cordia myxa plant, which were detected using gas chromatography technology equipped with mass spectrometry. The detection showed the presence of 14 chemical compounds. The mass spectra were recorded with a range of m/z 900-50 and EV72 card. The chemical compounds extracted from the studied samples were identified by comparing the resulting mass spectra with the mass spectra present in the libraries available in the device's software.

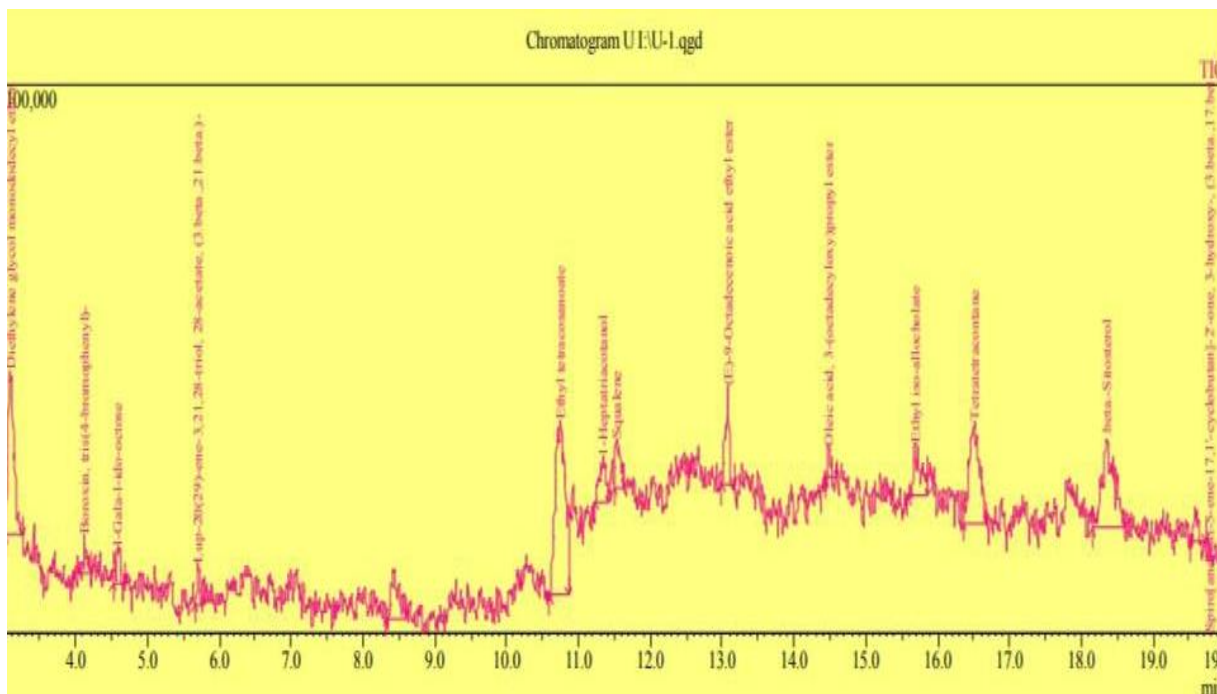
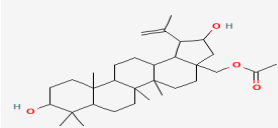
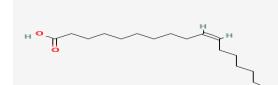
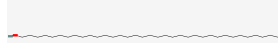
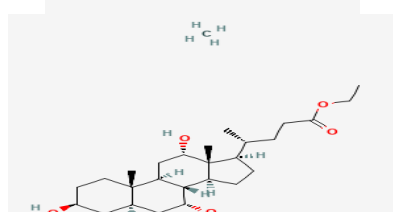


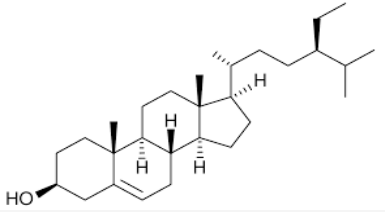
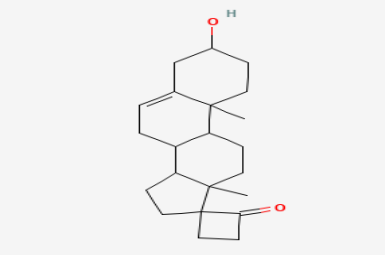
Figure 2. Curves for each chemical constituent that surfaced during the plant extract testing.

The compounds showed differences in their distance from one another, their appearance and reading in the device at different times, and the height of the peak of the curve that represents each compound. Table (1) is displayed below and shows the graph and its findings.(1). Findings and conversation:

Table 1. GC-MS analysis of the alcoholic extract of *Cordia myxa* leaves.

The peak	Retention time	The expansion	Name	Chemical formula	Structural formula
1	3.083	14.10	Diethylene glycol monododecyl ether	C16H34O3	
2	4.125	1.46	Boroxin, tris(4-bromophenyl)-	18H12B3Br3O ₃	
3	4.585	1.89	l-Gala-l-ido-octose	C8H16O8	

The peak	Retention time	The expansion	Name	Chemical formula	Structural formula
4	5.704	2.21	Lup-20(29)-ene-3,21,28-triol, 28-acetate	C ₃₂ H ₅₂ O ₄	
5	8.438	5.08	cis-10-Heptadecenoic acid	C ₁₇ H ₃₂ O ₂	
6	10.752	24.56	Ethyl tetracosanoate	C ₂₆ H ₅₂ O ₂	
7	11.346	4.14	1-Heptatriacotanol	C ₃₇ H ₇₆ O	
8	11.535	3.63	Squalene	C ₂₀ H ₃₈ O ₂	
9	13.085	4.83	(E)-9-Octadecenoic acid ethyl ester	C ₂₀ H ₃₈ O ₂	
10	14.487	0.66	Oleic acid, 3-(octadecyloxy)propyl ester	C ₃₉ H ₇₆ O ₃	
11	15.690	5.83	Ethyl iso-allocholate	C ₂₇ H ₄₈ O ₅	

The peak	Retention time	The expansion	Name	Chemical formula	Structural formula
12	16.516	14.57	Tetratetracontane	C ₄₄ H ₉₀	
13	18.355	15.72	.beta.-Sitosterol	C ₂₉ H ₅₀ O	
14	19.590	1.32	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one	C ₂₂ H ₃₂ O ₂	

According to the results shown in the table above, the highest peak area appeared for the chemical compound Ethyl tetracosanoate with a retention time of 10.752 and the smallest peak area that appeared for the chemical compound Oleic acid, 3-(octadecyloxy)propyl ester, with a retention time of 14.487. The compounds varied in their appearance times, and there were compounds that appeared repeatedly over different periods of time.

From the results height peak of the compound Diethylene glycol monododecyl ether that appeared in the GC-MS analysis is equal to 16.43, while the peak height of the compound (Boroxin, tris(4-bromophenyl)-) is equal to 3.74, the peak height of the compound l-Gala-l-idooctose is equal to 3.63, The mother of the compound Lup-20(29)-ene-3,21,28-triol, 28-acetate, 3.beta is equal to 3.98, while the compound cis-10-Heptadecenoic acid has a peak height of 4.91. The peak height of the compound Ethyl tetracosanoate is equal to 17.44, the compound Heptatriacotanol 1- the peak height is equal to 4.59, while the peak height of the compound Squalene is equal to 4.71, the compound E)-9-Octadecenoic acid ethyl ester, the peak height is equal to 9.91, the compound Oleic acid, 3-(octadecyloxy). (For propyl ester, the peak height is equal to 3.14, the peak height for the compound Ethyl iso-allocholate is equal to 5.31, the peak height for the compound Tetratetracontane is equal to 9.98, the peak height for the compound beta.-Sitosterol is equal to 8.95, and finally the peak height for the compound Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, equals 3.47.

The types of chemical compounds that emerged in this study were different from those in the study [19] This difference could be attributed to variations in the extraction technique, the solvent type utilized, and the experimental setup. The type of the thermal program, the variation in the amount of time spent in custody, which may be due to the difference in the plant environment and surrounding conditions in terms of temperatures, irrigation method, etc., led to the difference in the quality of the chemical compounds that appeared.

Compounds identified by preliminary qualitative analysis GC-MS this analysis has many uses in the medical field. Each of the identified compounds has its own unique character for treating different diseases. There is a need for more studies to reveal its importance in a specific field of treatment, especially since many studies have proven the effectiveness of the extract of the leaves of the bamber plant, according to [20]. In a research published in the Journal of the Babylon University of Pure and Applied Sciences, the effect of the extract of alkaloid compounds was demonstrate. by leaves of the *Cordia myxa* plant in the biological performance of *Culex pipienes* mosquitoes.

A study showed [21] the antibacterial activity of the alcoholic extract of the *Cordia myxa* L fruit, as the extract showed promising antimicrobial activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Salmonella enterica*, *Escherichia coli*, and *Pseudomonas aeruginosa*).

Gallic acid, ferulic acid, chlorogenic acid, caffeic acid, and pumaric acid were all found in the *Cordia myxa* L fruit extract, according to HPLC research. The findings suggest that these compounds' existence, together with those of other physiologically active compounds, may be connected to the notable antibacterial activity of the *C. myxa* ethanol extract.

Conclusion

Using a soxhlet device to prepare the ethanol extract and gas chromatography-mass spectrometry technology to detect the compounds, fourteen chemical compounds from the secondary metabolism of the *Cordia myxa* plant's extract were identified through this study. These compounds have medicinal significance and may be used to develop specific treatments, such as antifungal , certain types of pathogenic bacteria.

Acknowledgments

The researchers are grateful to the Dean of the Faculty of Applied Sciences at the University of Samarra, and to the professors of applied chemistry present in the central laboratories of the Faculty of Applied Sciences - Samarra University.

References

- [1] <https://apiv3.iucnredlist.org/api/v3/taxonredirect/137733199> Al-Snafi, A. E., (2016). The Pharmacological and therapeutic importance of *Cordia myxa*- A review .IOSR Journal of Pharmacy, 6, 4757.
- [2] ITIS (2021). Integrated Taxonomic Information System – Report. *Cordia myxa* L., accessed 26 July 2021, https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=565086#null.
- [3] Murthy, Hosakatte Niranjana; Joseph, Kodanthottu Sebastian; Gaonkar, Akshata Arun; Payamalle, Shivanand (2019). "Evaluation of Chemical Composition and Antioxidant Activity of *Cordia myxa* Fruit Pulp". Journal of herbs, spices & medicinal plants the original on 2021-06-29. ISSN:1540-3580.
- [4] Payamalle, Shivanand, Gaonkar, Akshata Arun , Joseph, Kodanthottu Sebastian, Murthy, Hosakatte Niranjana, .(2019). "Evaluation of Chemical Composition and Antioxidant Activity of *Cordia myxa* Fruit Pulp". Journal of Herbs, Spices & Medicinal Plants.DOI:10.1080/10496475.2019.1585399. ISSN:1049-6475.
- [5] Kumar, A.; Ilavarasan, R. ; Jayachandran, T. ; Decaraman, M. ; Aravindhnan, P.N.; Padmanabhan ; Krishanan, M.R. V. (2009). Pak.J.Nut. , 8(1) : 83-85. In : In: Parvez, M. (2014). Biochemical , Enviromental and Medicinal Exploration of *Euphorbia prostrate* Aiton and *Euphorbia granulate* Forssk. Ph.D. Thesis, Univ. of Peshawar, Pakistan.

- [6] Carolus Linnaeus. (1753). <https://biodiversitylibrary.org/page/358209>.
- [7] Sandhar, H.K.; Kumar, B.; Prasher, S.; Tiwari, P.; Salhan, M.; Sharma, P.; (2011). Internationale Pharmaceutica Science , 1(1): 25-41. In: Parvez, M. (2014). Biochemical , Enviromental and Medicinal Exploration of Euphorbia prostrate Aiton and Euphorbia granulate Forssk. Ph.D. Thesis, Univ. of Peshawar, Pakistan.
- [8] Khan , A.M.; Qureshi, R.A.; Ullah, F.; Gilani, S.A.; Nosheen, A.; Sahreen, S.; Laghari, M.K.; Laghari, M.Y. ; Shafiq-Ur-Rehman.; Hussain, I. (2011). ;Murad. Jour. Med. Plants Res. 5(25): 6017-6023. In: Parvez, M. (2014). Biochemical , Enviromental and Medicinal Exploration of Euphorbia prostrate Aiton and Euphorbia granulate Forssk. Ph.D. Thesis, Univ. of Peshawar, Pakistan.
- [9] Vaghasiya, Y.; Dave, R.; Chanda, S. (2011). Res.J.Med. Plant , 5(5):567-576. In : Parvez, M. (2014). Biochemical , Enviromental and Medicinal Exploration of Euphorbia prostrate Aiton and Euphorbia granulate Forssk. Ph.D. Thesis, Univ. of Peshawar, Pakistan. vol. 1, p. 190, QID:Q21856106 .
- [10] Harborne, J. B. and Turner, B.L. (1984). Plant Chemosystematics. Academic Press, London, New York.
- [11] Seigler, D.S. (1994). Phytochemical and systematics of Euphorbiaceae . Ann. Mo.Bot. Gard. ; 81: 380-401.
- [12] Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. Nutrients, 2(12), 1231-1246.
- [13] Chauhan , A. ; Goyal, M.K. and Chauhan,P. (2014). GC-MS Technique and its Analytical Applications in Science and Technology . Journal of uoJ Analytical & Bioanalytical Techniques . Volume 5 • Issue 6 • ISSN: 2155-9872.
- [14] Sahil K, Prashant B, Akanksha M, Premjeet S, Devashish R (2011) . GC-MS: Applications. International Journal Pharma & Biological Archives 2: 1544-1560.
- [15] Jackie ; Cindy Lee and Toyama , A. (2020). Gas Chromatography Mass Spectrometry (GCMS) . First Edition.
- [16] Meyer, Markus. (2016). Hans-Joachim Hübschmann: Handbook of GC-MS: fundamentals and applications, 3rd ed. Analytical and Bioanalytical Chemistry. 408. 10.1007/s00216-015-9282-1.

Estimating the Effectiveness of some Types of Sugars on Oxidative Stress Indicators and Kidney Function in Animals with Induced Diabetes and Obesity

Omar I. Ammar¹, Faryal F. Hussein² and Mohammed J. Mohammed³

¹⁻³Department of Food Sciences, College of Agriculture, Tikrit University, Tikrit, Iraq.

¹E-mail: omer.e2017@gmail.com

²E-mail: dr.feryalalazawi@tu.edu.iq

³E-mail: m_jamel68@yahoo.com

Abstract. During the period from March 6th to April 28th, 2022, researchers from the Biotechnology Research Center / Al-Nahrain University / Baghdad studied the effects of various sugars on kidney function and oxidation markers in rats. The rats were given normal diets with glucose replaced with sugar (Stevia, Inulin, xylitol), and the rats were then tested for the treatment of type 2 diabetes and induced obesity. We isolated 6 rats to serve as controls and gave them a regular diet. The other 24 rats were each given an injection of alloxan at a dosage of 150 mg/kg; one group got diabetes, and the other became infected. The effects of a 30% caloric increase on the weight of 24 rats fed a regular diet. There were a total of forty-six rats distributed across the four groups. Here are the treatments that were administered: a healthy control group, a diabetic control group, and a diabetic group that was given a diet with 12 mg of stevia per kilogram, 160 mg/kg of inulin, and 100 mg/kg of xylitol. All four groups of obese people were given the identical regimens of a regular diet and the same amounts of sugar replacements as described above. After confirming the incidence of induced diseases for two weeks, a nutritional trial was conducted for a period of (50 days) and oxidative stress indicators were estimated, which included the concentration of malondialdehyde (MDA), the effectiveness of glutathione peroxidase (GPX), and kidney function, which included an estimation of the concentration of creatinine and urea in the blood serum. Inducing diabetes with Alloxan in laboratory animals and causing obesity with a 30% increase in fat in another group led to a significant increase at the level ($0.05 \geq P$) in the concentration of MDA in the two groups and a significant decrease at the level ($0.05 \geq P$) in the concentration of GPX compared to the control group. Uninfected. It also turns out that feeding diabetic and obese laboratory animals standard diets modified by replacing glucose with sugar (stevia, inulin), respectively, led to a significant decrease at the level of ($P \geq 0.05$) in the level of malonic aldehyde (MDA) and a significant increase at the level of ($0.05 \geq P$) in (GPX) level compared to the positive control group. As for the groups that were fed a diet containing the sugar xylitol, the results of the increase in the concentration of (GPX) and the decrease in the concentration of (MDA) were similar to the same results in the two positive control groups that were fed the standard diet throughout the experimental period. The results of the statistical analysis showed a significant increase in the concentration of urea and creatinine at the probability level ($P < 0.05$) for the group of control animals with diabetes and obesity compared to the healthy, unaffected control group. It was also shown that feeding animals in the diabetic and obese group with a standard diet and replacing the glucose in it with sugar (stevia,

inulin), respectively, led to a significant decrease at the level ($P < 0.05$) in the concentration of urea and creatinine in the blood serum for all groups compared to the control groups. positive subjects were fed a standard diet. While xylitol in the standard diet fed by diabetic and obese women did not have any positive effect. On the contrary, there was a significant increase in the concentration of urea and creatine in blood serum compared to the two control groups that were fed a standard diet.

Keywords. Diabetes, Obesity, Oxidative stress indicators, Kidney function.

1. Introduction

The first paragraph after a heading is not indented (Bodytext style). Other paragraphs are indented (BodytextIndented style). Use the following reference style [1] and [2, 3].

The obesity and diabetes epidemic are increasing in industrialized countries, resulting in a number of harmful health conditions, including type 2 diabetes, heart disease, cancer, accelerated aging, and increased risk of death [1] Researchers have identified the relationship between insulin resistance, which is a major factor. In metabolic disorders and many non-communicable diseases such as hyperglycemia and hyperinsulinemia, which are promoted by obesity and may subsequently lead to type 2 diabetes [2] the key here is insulin resistance, which can be exacerbated by lifestyle changes and intake. Unhealthy food [3].

To address current concerns, diabetics, obese and overweight individuals often use zero-calorie artificial sweeteners as a tool for blood sugar control, weight loss, and metabolic risk reduction [4]. Stevia leaves, extracts, and have been used as a low-calorie sweetener. Thermal content has been used for several years in South America, Asia, Japan, China, Brazil, Korea, and various countries of the European Union [5] because it enhances insulin secretion and glucose absorption in the human body in the long term [6] and also inulin, as it is considered a low-calorie carbohydrate. Because it gives less than 50% of the energy that other digestible carbohydrates give, it is not digested in the stomach, as it does not cause an increase in blood glucose levels [7], as well as natural alcoholic sugars such as sorbitol, mannitol, erythritol, maltitol, and lactitol, the most important of which is xylitol. It is more effective because it easily crosses cell membranes by easy diffusion to provide nutrients and energy to cells without causing high blood sugar and regulates inflammatory reactions [8].

2. Materials and Methods

2.1. Preparing the Standard Diet for Feeding Experimental Animals

The standard ration for feeding experimental animals was prepared according to the source [9] and several modifications were made to it, operation with laboratories in The Ministry of Science and Technology Ethical approval No. ISO 17025. The first was to add 30% fat to the standard ration itself to feed the animals in which obesity was intended. The second was to prepare the ration itself while replacing the glucose with proportions of stevia, xylitol, and inulin, so as to provide 12 And 160 and 100 gm/kg of body weight, respectively, for all groups except the control groups, which continued to be fed the standard diet without any change in it.

2.2. Biochemical Tests of Blood

2.2.1. Blood Samples

The animals were fasted for 12 hours after the end of the experiment period, then anesthetized with chloroform, and blood samples in quantities of 3-5 ml were drawn by cardiac puncture using 5 ml syringes. The blood samples were placed directly in test tubes designated for isolating blood serum using centrifugation at a speed of 3000. revolutions/minute for 15 minutes, the serum was withdrawn using a micropipette and kept frozen at (-20°C) until the necessary blood tests were performed.

2.2.2. Estimation of Malondialdehyde Concentration in Blood Serum

Some text. The concentration of MDA in blood serum was examined using a solution of (TBA) Thiobarbituric acid with a concentration of 0.04 molar and two solutions of (TCA) Trichloro Acetic Acid, the first with a concentration of 17.5% and the second with a concentration of 70% and following the method of work mentioned by the researchers Guidet and Shah [10], as the estimation method is based on the reaction of peroxides. Fats with acid (TBA) in an acidic medium. The absorbance is measured with a spectrophotometer at the wavelength (532nm), as it reflects its concentration in the sample, as in the following equation.

$$\text{Dilution factor} \times (\text{At the 532 nm level Absorbency}) / (E_0 \times L) = \text{Concentration MDA}$$

whereas:

L = light path = 1cm

E0 = 1.56×10⁵ / molar. Poison

$$21 = (1+1+1+0.15) / (0.15) = (\text{The volumes used are ml}) / (0.15) = \text{Dilution factor}$$

Table 1. Spectrophotometer readings at wavelength (532nm) for concentrations.

Standard curve	
ng/ml	OD
0	0.105
25	0.457
50	0.599
100	0.801
200	1.108
300	1.742

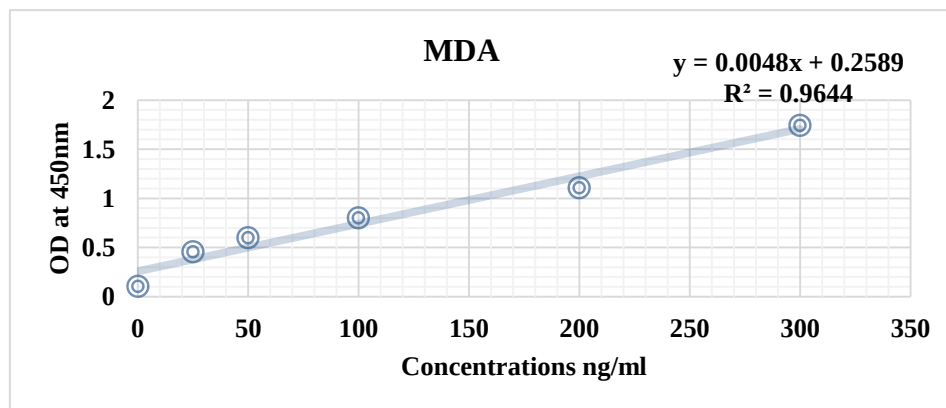


Figure 1. Standard curve of malondialdehyde (MDA) concentration in blood serum.

2.2.3. Estimation of the Effectiveness of Glutathione Peroxidase in Blood Serum

Glutathione in serum was determined according to the method mentioned by [11] using a solution of hydrogen peroxide H₂O₂, 4-Amino antipyrine, and phenol at molar concentrations (0.0017, 0.0025, and 0.17), respectively, after which 1.4 ml of (phenol and 4-Amino antipyrine) were incubated. And 1.5 ml of (H₂O₂) at a temperature of 25°C for 3 to 4 minutes, after which 0.1 ml of blood serum was added and the absorbance of the sample was recorded with a spectrophotometer immediately after addition at a wavelength of 510 nm - and the absorbance was repeated after 5 minutes at The same wavelength, where the reagent reacts quickly with glutathione and is reduced by the sulfhydryl group of glutathione to produce a yellow compound. The concentration of the resulting product depends on the concentration of glutathione present in the serum. Then the following equation was applied for calculation:

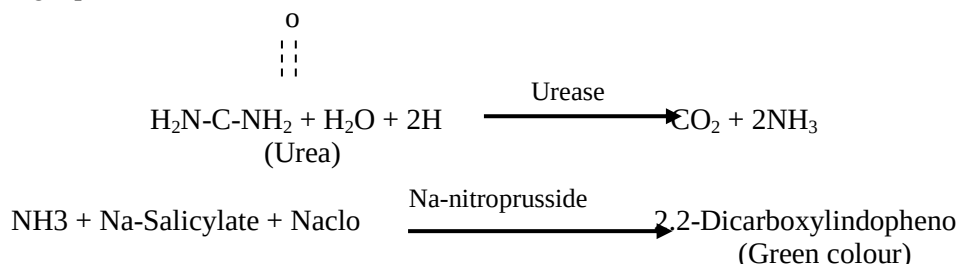
$$(\text{Absorbance per minute zero} - \text{Absorbance per minute is five}) / 5 = \Delta A / \text{min}$$

$$100000 \times Tv/Vs \times (\Delta A / \text{min}) / E = \text{Efficiency of (GPX) enzyme per liter}$$

2.3. Estimation of Kidney Function

2.3.1. Determination of Urea Concentration

Serum urea level was calculated according to the source [12]. Using a ready-made test kit from a French company Biolabo, the basis of this method depends on the decomposition of urea in the presence of water into ammonia (NH₃) and carbon dioxide (CO₂), and then the ammonia reacts with salicylate and hypochlorite, resulting in Green colored compound (Grenindophenol). As in the following equation:



The intensity of the resulting complex color above is proportional to the urea concentration in the blood, and the urea concentration is calculated after reading the absorbance of the sample with a spectrophotometer according to this equation:

$$\text{Urea concentration} = (\text{Sample absorbance}) / (\text{Concentration of the standard solution}) \times \text{Concentration of the (mg/100ml)}$$

The intensity of the resulting complex color above is proportional to the urea concentration in the blood, and the urea concentration is calculated after reading the absorbance of the sample with a spectrophotometer according to this equation:

$$\text{Urea concentration} = (\text{Sample absorbance}) / (\text{Concentration of the standard solution}) \times \text{Concentration of the (mg/100ml standard solution(50mg/100ml)}$$

2.3.2. Creatinine Concentration

Blood creatine was estimated by using an AFLO-prepared pen kit with the settings that [13] only then reported for calculation:

$$(\text{Creatinine concentration}) = (\text{Sample absorbance}) / (\text{Concentration of the standard solution}) \times \text{Concentration of the Mg/dl standard solution (2mg/100ml)}$$

2.4. statistical Analysis

The Statistical Analysis System [14] program was used to analyze the results of some experiments to study the effect of different parameters on the traits under study. The significant differences between the means were compared with the Least Significant Difference Test (LSD) at the probability level > 0.05 P.

2.5. Approval

Operating with laboratories at Fallujah University Ethical Approval No.9-2022.

3. Results and Discussion

3.1. Estimation of Oxidative Stress Indicators in Blood Serum

Table (1) shows the effect of treatments on the levels of malondialdehyde (MDA) and glutathione peroxidase (GPX) on infected animals:

Table 1. Concentrations of malon aldehyde and glutathione peroxidase.

Disease condition	Type of transaction	MDA	GPX
Not infected	Co	8.5	2.88
	D	17.7	0.97
Diabetes	DS	11.6	2.43
	DI	13.4	2.05
	DX	16	1.4
	O	12.3	1.29
Obesity	OS	9.2	2.78
	OI	10.4	2.56
	OX	7.9	1.48

The results showed that the induction of diabetes in groups of laboratory animals (D, DS, DI, DX) and the induction of obesity in groups of laboratory animals (O, OS, OI, OX) led to a significant increase at the level ($0.05 \geq P$) in the concentration of MDA compared to the group of healthy control animals (Co), which recorded the lowest values, because diabetic patients are characterized by increased oxidative stress resulting from an increase in free radicals when infection is caused [15] and increased oxidative stress resulting from an increase in the proportion of fats in the diet, which results in It has a decrease in the G-Px enzyme as evidence that the body uses it to get rid of free radicals and some types of oxygen formed in the body and an increase in the percentage of malondehyde (MDA) as evidence of an increase in the level of oxidation (a direct relationship). This is due to an increase in the level of free radicals and thus an increase in the occurrence of Damage to cells in the body [16, 17, 18].

This has been explained by the fact that oxidative stress leads to an imbalance between oxidant factors and their antioxidants, thus generating effective free radicals that increase oxidant substances, especially the concentration of malonial aldehyde (MDA), and thus they are a stimulating factor for the occurrence of the disease compared to those who are not infected [19].

The results in the same aforementioned table also showed a significant decrease in the concentration of GPX at the level ($0.05 \geq P$) for groups of laboratory animals with diabetes (D, DS, DI, DX) and those with obesity (O, OS, OI, OX) compared to a group of healthy control animals. (Co), which recorded the highest values, is due to the increase in free radicals and increased oxidative stress in diabetic and obese patients, as we mentioned previously, which weakens the internal defense system, as glutathione peroxidase works as an antioxidant to protect cells from free radical damage [20, 21]

It was also clear from the results studied that feeding laboratory animals with diabetes (DS, DI, DX) and those with obesity (OS, OI, OX) on standard diets modified by replacing the glucose sugar in them with sugar (stevia, inulin, xylitol), respectively, resulted in obtaining A significant decrease at ($0.05 \geq P$) in the level of malonial aldehyde (MDA) compared to the two positive control groups (D) and (O), which recorded the highest value (17.7, 12.3), respectively, and a significant increase at the level of ($0.05 \geq P$) in the level Glutathione peroxidase (GPX) compared to the positive control group (D) and (O), which recorded the lowest value (0.97, 1.9), respectively.

The reason for the increase in the concentration of (GPX) and the decrease in the concentration of (MDA) in the groups (DS, OS) that were fed a diet containing Stevia sugar is due to the therapeutic properties that this herb has that reduce the risk of oxidative stress [22], The antioxidant activity of the Stevia plant is often due to its high percentage of polyphenols, represented by phenols, phenolic acids, and flavonoids [23, 24] as phenols have an effective role in reducing oxidative stress by activating cellular antioxidant pathways, which It enhances its role in combating free radicals, the most important of which are rosmarinic acid and rosmarinic acid, which is one of the powerful antioxidants that contribute to protecting phosphorylated lipids from oxidative stress in cellular membranes [25]. These results were consistent with the results of [26], as the extracts showed Organic Stevia leaf antioxidant properties and a high restorative response in diabetic rats.

The reason for the increase in the concentration of (GPX) and the decrease in the concentration of (MDA) in the groups (DI, OI) that were fed a diet containing the sugar inulin is attributed to enhancing antioxidants in the blood by raising the level of alpha-tocopherol, as the results indicate that The use of inulin in nutrition stimulates local and systemic antioxidant defense systems in mice [27]. He confirmed this [28], stating that inulin can prevent oxidative stress and lipid peroxidation and reduce the effects of endotoxemia. As a prebiotic, the results were consistent with what he found. [29] When inulin was used to treat diabetic mice as a result of increased lipid levels, an improvement in the activity of cardiac antioxidant factors was observed, including total antioxidant capacity (TAC) and glutathione peroxidase (GPx) ($P < 0.001$, $P < 0.01$.) respectively, and the level of cardiac malondialdehyde (MDA) decreased ($P < 0.05$).

As for the groups (DX, OX) that were fed a diet containing xylitol sugar, the results of the increase in the concentration of (GPX) and the decrease in the concentration of (MDA) were similar to what was found [30]. In comparison to the controls, animals that were administered xylitol showed a notable rise ($P < 0.05$) in GSH levels and antioxidant enzyme activity in both their serum and examined tissues. The results show that xylitol can effectively combat the oxidative stress caused by type 2 diabetes. This leads us to xylitol's possible use as a diabetic dietary supplement. [31] treated rats with type 2 diabetes with 10% xylitol. Glutathione (GSH) levels dropped and malondialdehyde (MDA) levels rose as a consequence of this treatment. In addition to reshaping the liver, it changes fat metabolism. Hepatic dysfunction due to type 2 diabetes was more effectively treated with 10% xylitol, according to the data. The fact that modest oral administration of xylitol protects intestinal mucosal inflammatory lesions produced by diabetes and alterations in intestinal permeability was corroborated by the statement in [32].

Xylitol reduces intestinal inflammation in diabetic rats by enhancing tight junction protein expression and blocking the secretion of pro-inflammatory cytokines, which in turn reduces damage to the intestinal structure. More clinical studies are still needed to confirm these results because there are potential side effects associated with its normal or higher doses. Its use has been cautioned according to the relevant Food and Drug Administration guidelines. For the same reason, future studies should also focus on the toxicity and potential side effects associated with the consumption of sugar alcohols in order to determine their safety [33].

3.2. Kidney Functions

3.2.1. Determination of Urea and Creatinine in Blood Serum

The results of the statistical analysis shown in Table (2) showed a significant increase in the concentration of urea and creatinine at the probability level ($P < 0.05$) for the control group of animals with diabetes (D), reaching (41.2) and (0.74) mg/dl, respectively, for the group of animals with diabetes (D). Control animals with obesity (O), which amounted to (54.2) and (0.77) mg/dl, respectively, compared to the healthy control group (Co), which amounted to (27.6) and (0.57) mg/dl, respectively.

As for the group of diabetic animals, the urea results agreed with both the results of [34] and the results of [35] in the high percentage of urea in the blood serum of diabetic rats. The reason is due to a defect in kidney function and a lack of urea excretion due to hyperglycemia. Which is one of the main causes of progressive kidney damage, and as a result, urea excretion decreases, accumulates and its level rises in the blood and the creatinine results agreed with what Anjaneyulu and [36] and [37] found in high creatinine levels. In the blood serum of diabetic rats. The reason for this is that low insulin levels lead to severe muscle proteolysis, and thus loss of muscle mass, which greatly interferes with creatinine levels and raises them in the blood serum [37,38] or the reason may be due to the possibility of gradual kidney damage. [39, 40] Previous studies indicate that hyperglycemia is one of the main causes of progressive kidney damage, and this is an important clinical sign for detecting kidney failure [36,41]. Chronic exposure to high concentrations of glucose also causes oxidative stress, which is responsible for abnormalities in mesangial cells (MCs) that regulate blood flow in the kidneys, which is associated with most kidney diseases associated with diabetes [42].

The results of high urea and creatinine for the obese group agreed with the results of many studies and research that showed the relationship between high levels of cholesterol in the blood serum and chronic kidney disease and the accompanying high levels of urea and creatinine resulting from a defect in kidney function. The results were completely consistent with as stated in [43] this is due to feeding the rats a high-fat diet, which leads to an increase in insulin hyperemia, as we mentioned previously, causing relaxation of the arteries and increased renal glomerular filtration, in addition to changes in body weight and the size of the kidneys, cortex, glomeruli, and tubules. spirochete resulting in kidney dysfunction.

Table 2. Shows the effect of different treatments on urea level.

Disease condition	Type of transaction	Urea mg/dl	Creatinine mg/dl
Not infected	Co	27.6	0.57
	D	41.2	0.74
Diabetes	DS	28.9	0.61
	DI	32.1	0.65
	DX	42.3	0.89
	O	54.2	0.77
Obesity	OS	34.4	0.58

OI	36.2	0.62
OX	53.4	0.87

The results show that feeding the animals of the diabetic group (DS) and those affected by obesity (OS) a standard diet with the glucose replaced with Stevia sugar led to a significant decrease at the level ($P<0.05$) in the concentration of urea and creatinine in the blood serum, as their concentrations reached the same level in the blood of the affected group. In diabetes (28.9) and (0.61) mg/dl, respectively, and in the blood of the obese group (34.4) and (0.58) mg/dl compared to the two affected control groups (D) and (O) that were fed a standard diet. These results are consistent with what It was mentioned by [44] that Stevia works to lower blood sugar and thus improves kidney function and the levels of creatine and urea in the blood. Stevia is also antioxidant and anti-inflammatory because it contains phenolic compounds, organic acids and vitamins.

The reason for these decreases may be due to the role of stevia in improving kidney function [45]. It has been found that stevia extract improves some biochemical parameters for patients suffering from chronic kidney disease, in particular, improvement in the level of creatinine and urea [46] and among [47] This is achieved through systemic and renal vasodilation, decreased blood pressure, and diuresis, which leads to an increase in renal plasma flow and stabilization of the glomerular filtration rate after administration of stevioside to experimental rats for 60 days.

Feeding the diabetic group (DI) and the obese group (OI) a standard diet with glucose replaced by inulin led to a significant decrease at the level ($P<0.05$) in the concentration of urea and creatinine, as their concentration in the blood of the diabetic group reached (32.1). (0.65) mg/dl, respectively, and in the blood of the obese group there were (36.2) and (0.62) mg/dl compared to the two affected control groups (D) and (O) that were fed a standard diet. These results were consistent with what was mentioned [48] showed that consuming fructans (in the synthesis of inulin) led to a reduction in the levels of glucose, total cholesterol, uric acid, and creatinine in the blood serum of experimental animals, which indicates that fructans of the inulin type contribute to reducing the risk of kidney disease. Therefore, it has been indicated [49] that since inulin showed a general benefit or neutral effect, inulin is considered a safe and reliable strategy to improve the microtoxicological and inflammatory status in patients with CKD. The reason may be due to the role of inulin in increasing the number of cells responsible for secreting the glucan-like peptide, which may be a cause of the displacement of amino acids in the intestine, leading to a decrease in the formation of the essential protein in the production of urea and an increase in creatinine, thus reducing its presence in the blood serum of animals [50].

While xylitol in the standard diet fed to the diabetic group (DX) and the obese group (OX) did not have any positive effect. On the contrary, there was a significant increase in the concentration of urea and creatine in the blood serum of the rats, as their concentration in the group's blood reached In the blood of the diabetic group (42.3) and (0.89) mg/dl, respectively, and in the blood of the obese group (53.4) and (0.87) mg/dl, respectively, compared to the two positive control groups (D) and (O) that were fed a standard diet.

Findings [51] of a higher-than-normal increase in creatinine in humans (the normal range is up to 7.2 mg/dL) and a partial decrease in the urea level after ingesting a drink containing xylitol by 7% corroborated the results of the creatinine rise and the modest decrease in urea. Reduced blood flow to the kidneys is indicated by these two numbers. This is because xylitol improves metabolism and decreases kidney excretion. For example, one study indicated that after four weeks of using xylitol as a sugar substitute, the Uric acid levels in the blood of diabetic children increased by 1 mg/dL.

Despite xylitol's widespread use as a sweetener, recent research on its short-term effects on metabolic rate and renal function in people with obesity is lacking.

Conclusion

Feeding diabetic and obese laboratory animals standard diets modified by replacing glucose with sugar (stevia, inulin) at a ratio of (12 and 160) mg/kg, respectively, led to a decrease in the level of malonal dialdehyde (MDA) and an increase in the level of (GPX). There was also a lower level of urea and creatinine concentrations compared to the positive control group.

While xylitol in the standard diet fed by diabetic and obese women did not have any positive effect on the concentration of (GPX) and (MDA) and led to an increase in the concentration of urea and creatinine in blood serum compared to the two control groups that were fed the standard diet.

References

- [1] Roth, J., Qiang, X., Marbán, S. L., Redelt, H., & Lowell, B. C. (2004). The obesity pandemic: where have we been and where are we going? *Obesity research*, 12(S11), 88S-101S.
- [2] Watal, G., Dhar, P., Srivastava, S. K., & Sharma, B. (2014). Herbal medicine as an alternative medicine for treating diabetes: the global burden. *Evidence-based Complementary and Alternative Medicine*, 2014.
- [3] Goodrich, K. M., Crowley, S. K., Lee, D. C., Sui, X. S., Hooker, S. P., & Blair, S. N. (2012). Associations of cardiorespiratory fitness and parental history of diabetes with risk of type 2 diabetes. *Diabetes Research and Clinical Practice*, 95(3), 425-431.
- [4] Dunne, A. (2021). Low-calorie Sweetener Exposure: Associations with Weight Status, Glycaemic Control, and Food-related Cognition (Doctoral dissertation, Ulster University).
- [5] Peteliuk, V., Rybchuk, L., Bayliak, M., Storey, K. B., & Lushchak, O. (2021). Natural sweetener *Stevia rebaudiana*: Functionalities, health benefits and potential risks. *EXCLI journal*, 20, 1412.
- [6] Jeppesen, P. B., Gregersen, S., Poulsen, C. R., & Hermansen, K. (2000). Stevioside acts directly on pancreatic β cells to secrete insulin: Actions independent of cyclic adenosine monophosphate and adenosine triphosphate—sensitive K^+ -channel activity. *Metabolism*, 49(2), 208-214.
- [7] Shoaib, M., Shehzad, A., Omar, M., Rakha, A., Raza, H., Sharif, H. R., ... & Niazi, S. (2016). Inulin: Properties, health benefits and food applications. *Carbohydrate polymers*, 147, 444-454.
- [8] Mäkinen, K. K. (2016). Gastrointestinal disturbances associated with the consumption of sugar alcohols with special consideration of xylitol: scientific review and instructions for dentists and other health-care professionals. *International journal of dentistry*, 2016.
- [9] NRC (National Research Council Recommended) (2002). *Dietary Allowance*. 15th ed. Washington. D.C National Academy. Press.
- [10] Guidet, B. E. R. T. R. A. N. D., & Shah, S. V. (1989). Enhanced in vivo H_2O_2 generation by rat kidney. in glycerol-induced renal failure. *American Journal of Physiology-Renal Physiology*, 257(3), F440-F445.
- [11] Cunningham-Rundles, S. (1996). Zinc modulation of immune function: specificity and mechanism of interaction. *The Journal of laboratory and clinical medicine*, 128(1), 9-11.
- [12] Kaplan, A. (1984). *Clinical Chemistry*. 1st ed. C.V. Mosby Co. St Louis, Toronto. Princeton. Pp: 1257-1260 and 437+418.
- [13] Tietz, Y., (2005). *Clinical Biochemistry* 6th ed; Mc Graw – Hill, New York, 825PP.
- [14] SAS. (2012). *Statistical Analysis System, User's Guide*. Statistical. Version 9.1th ed. SAS. Inst. Inc.Cary. N.C. USA.
- [15] Rozenberg, O., Shiner, M., Aviram, M., and Hayek, T. (2008). Paraoxonase 1 (PON1) attenuates diabetes development in mice through its antioxidative properties. *Free Radical Biology and Medicine*, 44(11), 1951-1959.

- [16] Wahda A. Khrofa, Ramzia H. Abdul Rahman , Maha A.AL-Samak (2008). Estimation of Glutathione in Three Organs of Experimentally Infected Mice by *Trichomonas vaginalis* Parasite. *Journal of Al-Rafidain* .Vol 19. Issue 1 . P65-71.2008
- [17] Dafer Al-Kufy A. Faysel (2009). Measuring MDA & GSH as Biomarkers for Oxidative Stress in Bladder Cancer Patients. *Journal of Kerbala University*, Vol. 7 No.3 Scientific 2009
- [18] Michitaka Naito, Xiaohong Wu, Hideki Nomura, Michiteru Kodama, Yuriko Kato, Yoji Kato, Toshihiko Osawa (2002). The Protective Effects of Tetrahydrocurcumin on Oxidative Stress in Cholesterol-fed Rabbits. *Journal of Atherosclerosis and Thrombosis*. ISSN-L: 1340-3478. p. 243-250.
- [19] Murri, M.; Luque-Ramirez, M.; Insenser, M.; Ojeda-Ojeda, M.; Escobar Morreale, H.F.(2013). Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis, *Human Reproduction Update*, Volume 19, Issue 3, May/June 2013, Pages 268–288.
- [20] Kolarovic, J., Popovic, M., Zlinská, J., Trivic, S., and Vojnovic, M. (2010). Antioxidant activities of celery and parsley juices in rats treated with doxorubicin. *Molecules*, 15(9), 6193-6204.
- [21] Shehata, M. M. S. M., and Soltan, S. S. (2012). The effects of purslane and celery on hypercholesterolemic mice. *World J Diary Food Sci*, 7, 212-221.
- [22] Shivanna, N., Naika, M., Khanum, F., & Kaul, V. K. (2013). Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana*. *Journal of Diabetes and its Complications*, 27(2), 103-113
- [23] Pacifico, S., Piccolella, S., Nocera, P., Tranquillo, E., Dal Poggetto, F., & Catauro, M. (2019). New insights into phenol and polyphenol composition of *Stevia rebaudiana* leaves. *Journal of pharmaceutical and biomedical analysis*, 163, 45-57.
- [24] Karakose, H., Jaiswal, R., & Kuhnert, N. (2011). Characterization and quantification of hydroxycinnamate derivatives in *Stevia rebaudiana* leaves by LC-MS n. *Journal of agricultural and food chemistry*, 59(18), 10143-10150.
- [25] Gu, J., Wang, H., Zhou, L., Fan, D., Shi, L., Ji, G., & Gu, A. (2020). Oxidative stress in bisphenol AF induced cardiotoxicity in zebrafish and the protective role of N-acetyl N-cysteine. *Science of the Total Environment*, 731, 139190.
- [26] Papaefthimiou, M., Kontou, P. I., Bagos, P. G., & Braliou, G. G. (2023). Antioxidant activity of leaf extracts from *Stevia rebaudiana* Bertoni exerts attenuating effect on diseased experimental rats: A systematic review and meta-analysis. *Nutrients*, 15(15), 3325.
- [27] Wu, W. T., & Chen, H. L. (2011). Konjac glucomannan and inulin systematically modulate antioxidant defense in rats fed a high-fat fiber-free diet. *Journal of agricultural and food chemistry*, 59(17), 9194-9200.
- [28] Kandil, A. (2022). Effects of pre-treatment with inulin on the kidney in lipopolysaccharide-induced endotoxemia in rats. *Polish Journal of Veterinary Sciences*, 25(1), 103-108.
- [29] Sefidgari-Abrasi, S., Karimi, P., Roshangar, L., Morshedi, M., Bavafa-Valenlia, K., Saghafi-Asl, M., ... & Rahimiyan-Heravan, M. (2020). *Lactobacillus plantarum* and inulin: therapeutic agents to enhance cardiac ob receptor expression and suppress cardiac apoptosis in type 2 diabetic rats. *Journal of Diabetes Research*, 2020.
- [30] Chukwuma, C. I., & Islam, S. (2017). Xylitol improves anti-oxidative defense system in serum, liver, heart, kidney and pancreas of normal and type 2 diabetes model of rats. *Acta Pol Pharm*, 74(3), 817.
- [31] Msomi, N. Z., Erukainure, O. L., Salau, V. F., Olofinson, K. A., & Islam, M. S. (2022). Xylitol improves antioxidant, purinergic and cholinergic dysfunction, and lipid metabolic homeostasis in hepatic injury in type 2 diabetic rats. *Journal of Food Biochemistry*, 46(4), e14040.
- [32] Han, F., Li, X., Jin, Y., Zuo, Q., Liu, W., & Han, J. (2024). Xylitol attenuates diabetes induced intestinal permeability changes and inflammatory injury by improving intestinal tight junction protein expression and mucus secretion in rats. *CyTA-Journal of Food*, 22(1), 2303447.
- [33] Msomi, N. Z., Erukainure, O. L., & Islam, M. S. (2021). Suitability of sugar alcohols as antidiabetic supplements: A review. *Journal of food and drug analysis*, 29(1), 1.

- [34] Giribabu, N., Karim, K., Kilari, E. K., and Salleh, N. (2017). Phyllanthus niruri leaves aqueous extract improves kidney functions, ameliorates kidney oxidative stress, inflammation, fibrosis and apoptosis and enhances kidney cell proliferation in adult male rats with diabetes mellitus. *Journal of ethnopharmacology*, 205, 123-137.
- [35] Matboli, M., Eissa, S., Ibrahim, D., Hegazy, M. G., Imam, S. S., and Habib, E. K. (2017). Caffeic acid attenuates diabetic kidney disease via modulation of autophagy in a high-fat diet/streptozotocin-induced diabetic rat. *Scientific reports*, 7(1), 1-12
- [36] Anjaneyulu, M., and Chopra, K. (2004). Quercetin, an antioxidant bioflavonoid, attenuates diabetic nephropathy in rats. *Clinical and Experimental pharmacology and physiology*, 31(4), 244-248.
- [37] Rocha, D. S., Casagrande, L., Model, J. F. A., dos Santos, J. T., Hoefel, A. L., and Kucharski, L. C. (2018). Effect of yerba mate (*Ilex paraguariensis*) extract on the metabolism of diabetic rats. *Biomedicine and Pharmacotherapy*, 105, 370-376.
- [38] Thomson, S. C., Vallon, V., and Blantz, R. C. (2004). Kidney function in early diabetes: the tubular hypothesis of glomerular filtration. *American Journal of Physiology-Renal Physiology*, 286(1), F8-F15.
- [39] Barski, L., Nevzorov, R., Jotkowitz, A., Rabaev, E., Zektser, M., Zeller, L., ... and Almog, Y. (2013). Comparison of diabetic ketoacidosis in patients with type-1 and type-2 diabetes mellitus. *The American journal of the medical sciences*, 345(4), 326-330.
- [40] Shrestha, S., Gyawali, P., Shrestha, R., Poudel, B., and Sigdel, M. (2008). Serum urea and creatinine in diabetic and non-diabetic subjects. *Journal of Nepal Association for Medical Laboratory Sciences P*, 11, 12.
- [41] Bamanikar, S. A., Bamanikar, A. A., and Arora, A. (2016). Study of Serum urea and Creatinine in Diabetic and nondiabetic patients in a tertiary teaching hospital. *The Journal of Medical Research*, 2(1), 12-15.
- [42] Du, S., Neiman, A., Batis, C., Wang, H., Zhang, B., Zhang, J., and Popkin, B. M. (2013). Understanding the patterns and trends of sodium intake, potassium intake, and sodium to potassium ratio and their effect on hypertension in China. *The American journal of clinical nutrition*, 99(2), 334-343.
- [43] Alper Sonmez; Mahmut Ilker Yilmaz; Mutlu Saglam; Hilmi Umut Unal; Mahmut Gok; Hakki Cetinkaya; Murat Karaman2, Cem Haymana; Tayfun Eyileten; Yusuf Oguz; Abdulgaffar Vural; Manfredi Rizzo; and Peter P Toth. (2015). The role of plasma triglyceride/high-density lipoprotein cholesterol ratio to predict cardiovascular outcomes in chronic kidney disease. *Lipids in Health and Disease* 14:29 DOI 10.1186/s12944-015-0031-4.
- [44] Ozbayer, C., Kurt, H., Kalender, S., Ozden, H., Gunes, H. V., Basaran, A., ... & Degirmenci, I. (2011). Effects of *Stevia rebaudiana* (Bertoni) extract and N-nitro-L-arginine on renal function and ultrastructure of kidney cells in experimental type 2 Diabetes. *Journal of medicinal food*, 14(10), 1215-1222.
- [45] Peteliuk, V., Rybchuk, L., Bayliak, M., Storey, K. B., & Lushchak, O. (2021). Natural sweetener *Stevia rebaudiana*: Functionalities, health benefits and potential risks. *EXCLI journal*, 20, 1412.
- [46] Rizwan, F., Rashid, H. U., Yesmine, S., Monjur, F., & Chatterjee, T. K. (2018). Preliminary analysis of the effect of *Stevia* (*Stevia rebaudiana*) in patients with chronic kidney disease (stage I to stage III). *Contemporary clinical trials communications*, 12, 17-25.
- [47] Melis, M. S. (1995). Chronic administration of aqueous extract of *Stevia rebaudiana* in rats: renaleffects. *Journal of Ethnopharmacology*, 47(3), 129-134.
- [48] Liu, C., Yuan, P., Wang, Y., Yang, X., Xu, Y., Zhang, W., ... & Shao, T. (2023). Effects of burdock inulin-type fructans exposure on the physiological function of healthy mice and their filial generation. *Journal of Veterinary Medical Science*, 85(4), 425-433.
- [49] Mitrović, M., Stanković-Popović, V., Tolinački, M., Golić, N., Bajić, S. S., Veljović, K., ... & Dimković, N. (2023). The Impact of Synbiotic Treatment on the Levels of Gut-Derived Uremic Toxins, Inflammation, and Gut Microbiome of Chronic Kidney Disease Patients—A Randomized Trial. *Journal of Renal Nutrition*, 33(2), 278-288.



- [50] Zou, J., Chassaing, B., Singh, V., Pellizzon, M., Ricci, M., Fythe, M. D., ... & Gewirtz, A. T.(2018). Fiber-mediated nourishment of gut microbiota protects against diet-induced obesity by restoring IL-22-mediated colonic health. *Cell host & microbe*, 23(1), 41-53.

Extension Needs for Sheep Breeders in Nimrud District / Nineveh Governorate

Ali Tariq Hameed¹, Talal Saeed Hameed², Zahraa Meiser Sultan³ and Esam M. I. El-Baaly⁴

¹⁻³Agriculture Extension and Technology Transfer Department, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq.

⁴Department of Agricultural Economics, Faculty of Agriculture, Tanta University, Egypt.

¹E-mail: ali_tariq@uomosul.edu.iq

²E-mail: stalal1982@uomosul.edu.iq

³E-mail: zahraa.albasso@uomosul.edu.iq

Abstract. The research targeted all sheep breeders in Nimrud district in Nineveh Governorate and the research aimed to identify the extension needs of sheep breeders in Nimrud district / Nineveh Governorate Identify the extension needs of sheep breeders in each field of sheep breeding. and Identify the correlation between the extension needs of sheep breeders according to some independent variables (age, educational level, number of years of work in sheep breeding, Number of sheep owned by the breeder, agricultural information sources). The research community included all sheep breeders in the Nimrud hand, as the total size of the community (100) breeders, and the research sample was comprehensive, after excluding 20 forms for the purpose of ensuring the stability of the scale and thus the research sample became (80) sheep breeders. For the purpose of achieving the objectives of the research, a questionnaire was prepared consisting of two parts, the first included the independent variables and the second included a scale of the extension needs of respondents and consisted of 40 items distributed over 5 areas: (Reproduction and selection of rams - nutrition and knowledge of nutritional requirements - care of pregnant ewes - breeding and improvement of sheep - sheep production). The results showed that the extension needs in the field of sheep breeding among the respondents are moderate . The results also did not show significant relationships in the extension needs of the respondents and the following variables (educational level, number of sheep owned by the breeder, agricultural information sources, number of years of work in sheep breeding), while there was a significant relationship between the extension needs of the respondents in the field of sheep breeding according to (age).

Keywords. Extension needs, Agricultural extension, Sheep breeder.

1. Introduction

Livestock is one of the most important tributaries of the agricultural economy because of the resources it provides that have a close relationship with foreign currency, where the revenues of this wealth are concentrated in the provision of animal products (red meat, milk, poultry, fish, and eggs) that man cannot give up in his food for one of these materials, and there is no suitable

alternative to replace them and that their absence in human food causes nutritional problems and is the basic building block in malnutrition diseases [1]. Sheep constitute a large sector in livestock that supplies the country with food products and products included as raw materials in many industries, and sheep are distributed in Iraq heterogeneously, as their numbers increase in areas where pastures are available, especially in the Jazira region, and decrease in desert areas with little water and pasture [2]. Where sheep contribute to filling a large part of the human needs of animal protein, whether meat or milk, and that consumers prefer these products to a large extent, and research indicates a decrease in the number of these animals for many reasons, the most important of which are: high feed prices, lack of pastures, reliance on nomads in breeding and failure to follow scientific methods and proper management. One of the main requirements for sheep herds is to be productive of milk and meat, and for the birth rate to be high, especially since sheep do not remain productive for a long time and half of the births are males, so it is preferable to keep most of the females resulting from the herd and take care of them so that the breeder can improve his herd or increase its size [3]. At the present time, the Arab countries suffer from the problem of low domestic production in red meat, which led to a reduction in the per capita share of animal protein from the standard rates necessary to cover the needs of the individual per day, which is reflected in public health, which negatively affects the rate of individual performance of his work properly. The progress of peoples is measured by several criteria, including the rate of per capita animal protein, and therefore the government imports red meat from abroad to fill part of this food gap, which strains the state budget in procuring financial resources from hard currency necessary to cover the expenses and costs of importing from abroad [4]. The Asserts [5] Success in increasing agricultural production, both plant and animal, depends on the development of knowledge and skill in the use of agricultural techniques, which indicates the need for farmers to agricultural extension to obtain information and train them on various agricultural practices, so the need for agricultural extension appears to help farmers manage their farms in an effective scientific way that increases their production and improves its quality. Despite this importance for the animal sector, but in general it did not receive its luck from the care as is the case in the field of plant production, where the production rates of most plant crops have increased while the rates of animal production increase significantly, and there is no doubt that the lack of good knowledge of breeders methods of care and good nutrition in the field of sheep breeding had a negative impact on the production of meat, milk and wool, which led to the inability of livestock to participate more strongly in Facing the steady increase in demand for their products This growing gap between the population and the availability of sheep production has resulted in a decrease in the average per capita share of animal products [6]. One of the important issues that attracted the attention of the researcher is the existence of a decrease in the number of sheep significantly in recent years as a result of the retention of rain or its absence, which led to the lack of herbs and pasture or for other reasons, as the number of sheep owned by breeders decreased to less than half the size of the herd than it was before the drought in the district of Nimrud / Nineveh Governorate, and all this is due to the lack of awareness of breeders in the issue of storing quantities of hay in good agricultural seasons (many rains) To maintain their herds in drought years, because each breeder can store enough hay for at least three years so that it remains palatable to the sheep, using rooms built of clay roofed with reeds and papyrus covered with straw and mud, all available locally and at a low price. Moreover, the state did not lift a finger about what happened to this productive segment of the people, as it was able, at least after the first drought year, to build large warehouses to store hay and fodder in areas where there is an abundance of production of field crops to fill their shortage in the dry seasons, as is the case in many Arab countries. And for lack of tech research Reveal the extension needs of sheep breeders in Nimrud district This research was conducted.

1.1. Research Objectives

- Identifying the guiding needs of sheep breeders in Nimrud district / Nineveh Governorate. In general.
- Identify the extension needs of sheep breeders in each field of sheep breeding.
- Identifying the correlation between the extension needs of sheep breeders according to some independent variables (age, educational level, number of years of work in sheep breeding, number of sheep owned by the breeder, agricultural information sources).

2. Materials and Methods

2.1. Research Methodology

This research is one of the researches that fall within the descriptive approach, as this approach is suitable for accessing detailed data and facts about the target at a certain time [7], as this approach describes what is currently being and interprets it by collecting data on a level, opinions, attitudes or reactions on a particular topic or phenomenon [8], and then classifying, processing and analyzing those data and facts accurately, extracting their implications, and reaching conclusions and generalizations about the phenomenon or subject of research in an adequate and accurate manner [9].

2.2. The Research Community and Sampled

The research was conducted in the area of Nimrud / Nineveh Governorate, as the research community included all sheep breeders in the district of Nimrod, as the size of the total community (100) breeders, and the research sample was comprehensive, after excluding 20 forms for the purpose of ensuring the stability of the scale, and thus the research sample became (80) sheep breeders.

2.3. Research Tool

For the purpose of achieving the objectives of the research, a questionnaire has been prepared, being one of the means of collecting information from the respondents through a set of questions or phrases required to be answered by the respondents, and the questions are directed about the guiding needs followed [10], as it was determined after reviewing the literature, studies and research related to the subject of the research, the questionnaire form consisted of three parts as follows:

- Part I: The first part included the independent variables related to sheep breeders, namely (age - educational level - number of years of work in sheep breeding - the number of sheep owned by the breeder - agricultural information sources).
- Part II: This part included a scale to measure the indicative needs of sheep breeders, and the scale consisted of 75 items. In front of each item (3) alternatives were put to measure the indicative needs of sheep breeders, which are (large need, medium need, do not need) and were given numerical values (3, 2, 1) respectively.

2.4. Measuring Research Variables

- Age: Measured by the number of years of the researcher's life at the time of data collection.
- Educational level: It was measured according to the following levels (illiterates. reads and writes, primary, intermediate, preparatory, institute, college and higher certificate) and has been given the following codes: (7,6,5,4,3,2,1,8) respectively.

- Number of years of work in sheep breeding: measured by the number of years of work of the researcher in sheep breeding
- Number of sheep owned by the breeder: Measured by the number of sheep owned by the respondent.
- Agricultural information sources: This variable was measured by a scale consisting of (10) paragraphs related to agricultural information sources, and three alternatives were developed for them: (always, sometimes and I do not get) and the following code were given to them: (3, 2, 1) respectively, and the total scores on the scale represent the total degree of the respondent .

2.5. Measuring the Dependent Variable

2.5.1. The Indicative Needs of Sheep Breeders in the Field of Sheep Breeding

The dependent variable was measured through a three-paragraph scale consisting of 40 items distributed over the following areas, namely) reproduction and selection of rams - nutrition and knowledge of nutritional requirements - care of pregnant ewes - breeding and improvement of sheep - sheep production) each area has 8 items. Three alternatives (large, medium and non-need) were developed and given a numerical value (3, 2, 1) respectively. By collecting the answers of each researcher on the paragraphs, we will get the final score for each researcher, and this result represents the guiding needs of sheep breeders in the field of sheep breeding.

2.6. The Validity of the Tool (scale)

It means that the scale measures what was set to measure, that is, the extent to which the scale achieves the goal for which it was set, and this is known as face validity [11] The sincerity of the content means the extent to which the components of the scale represent the aspects of the measured side, i.e. the extent to which the objectives set are covered [12], and this was achieved through the presentation of the questionnaire in its initial form to a group of experts specialized in the field of agricultural extension to measure the virtual honesty The questionnaire was distributed in its initial form to Experts of the Department of Extension and Transfer of Agricultural Techniques at the Faculty of Agriculture and Forestry, as for the content validity, the form was presented to the teachers of the Department of Animal Production at the Faculty of Agriculture and Forestry.

2.7. Reliability

Reliability means the stability of the results if the scale is repeated after a while on the same group of individuals [13]. To find stability, a random survey sample of (20) sheep breeders was selected from the community sample, and the preliminary test data was collected through the questionnaire form and by personal interview with sheep breeders in Nimrud district. The initial test was used for the following purposes: to ensure the clarity of the items contained in the questionnaire for the subject subjects, to find the reliability coefficient of the research tool. Through the initial test, the reliability of the instrument was calculated by the Alpha-Cronbach method by the SPSS program[14], and the reliability value was (0.98), as most sources consider the scale to be constant if the value of the stability coefficient exceeds (0.70) [15].

2.8. Statistical Means

After collecting the data, it was unloaded and analyzed using the spss program, and the following statistical methods were used: range - percentage frequencies - arithmetic mean - weighted

arithmetic mean - standard deviation - Cronbach alpha coefficient - Pearson correlation coefficient - rank correlation coefficient.

3. Results and Discussion

3.1. The First Objective: to Identify the Extension Needs of Sheep Breeders in Nimrud District / Nineveh Governorate in General

The level of indicative needs was extracted by relying on the range between the highest value (65) and the lowest value (24), where it reached 41, and after dividing it by the number of categories, it was found that it was (14), where the percentage of the small category reached (24-37) (36%), while the medium needs category reached (38-51) (44%), while the percentage of the large category (52-65) (20%).

Table 1. Shows distribution of sheep breeders according to their extension needs.

Categories of needs	No. of farmers	%
Low needs (24-37)	29	25.00
Medium needs (39-51)	35	65.28
High needs (52-65)	16	9.72
Total	80	100%

We Can see Table (1), it was found that the categories of indicative needs are average, and this may be due to the large number of experiences that he may have experienced or heard about in the field of sheep breeding, which is the most numerous if it reaches (80%), and the arithmetic mean reached 41.06 and the standard deviation was 10.23. This may be due to the possession of the majority of respondents of knowledge related to sheep breeding, to attach this activity to their living life and their source of livelihood, which may be the only one in most cases, and their interest in improving their herds by choosing good rams and ewes that have the ability to give birth to twins that are strong and safe from diseases and parasites and fast-growing, especially males that are used in the production of meat, as well as attention to knowledge related to nutrition, barns and others.

3.2. The Second Objective is to Identify the Extension Needs of Sheep Breeders in Each Field of Sheep Breeding

3.2.1. The First field is Reproduction and the Selection of Rams

The results showed that the highest numerical value obtained by the respondents on the paragraphs of the field (23.44), and the lowest value (5.73) with an average of (14.88) numerical value, and the respondents were distributed into three categories for indicative needs, as shown in Table 2.

Table 2. Distribution of respondents according to their extension needs in the field of reproduction and selection of rams.

Categories	Number	%	Average of need
Low needs (5.73-11.63)	21	26.25	10.39
Medium needs (11.64-17.54)	50	62.5	14.32
High needs (17.55-23.45)	9	11.25	18.23
Total	80	100%	

X=14.88 s.d=2.99.

Table (2) indicates that (26.25%) of the respondents fall within the category of low needs with an average of (10.39) numerical values. The percentage of (62.5%) of the respondents falls within the category of medium needs with an average of (14.32) numerical value. The percentage of (11.25%) of the respondents falls within the category of large needs with an average of (18.23) numerical value. It is clear from this that the respondents had their extension needs in the field of reproduction and the selection of rams average. This may be due to the respondents' knowledge regarding the selection of appropriate ages for rams and ewes used in mating, as well as information related to preparing the herd for the insemination season and the appropriate time for insemination.

3.2.2. The Second Area is Nutrition and Knowledge of Nutritional Requirements

The results showed that the highest numerical value obtained by the respondents on the items of the field (22.6), and the lowest value (8) with an average of (16.19) numerical value. The respondents were distributed into three categories of extension needs in Table 3.

Table 3. Distribution of respondents according to their needs in the field of nutrition and knowledge of nutritional requirements.

Categories	Number	%	Average of need
Low needs (8-12.86)	12	15	11.51
Medium needs (12.87-17.73)	18	22.5	16.43
High needs (17.55-23.45)	50	62.5	20.64
Total	80	100%	

X=16.19 s.d=3.22

Table (3) shows that (15%) of the respondents fall within the category of low needs with an average of (11.51) numerical value. The percentage of (22.5%) of the respondents falls within the category of medium needs with an average of (16.43) numerical value. The percentage of (62.5%) of the respondents falls within the category of large needs with an average of (20.64) numerical value. It is clear from this that the respondents had their needs in the field of nutrition and knowledge of nutritional requirements high. This may be due to the fact that the respondents do not have the knowledge and information related to the symptoms of protein deficiency and salts that may appear on the herd, or they may be able to know how to prepare and prepare the diet for sheep, which reflected on their needs.

3.2.3. The Third Area is Care for Pregnant Ewes

The results showed that the highest numerical value obtained by the respondents on the Items of the field (14.3), and the lowest value (4.2) with an average of (10.18) numeric value. The respondents were divided into three categories of extension needs, as in Table 4.

Table 4. Distribution of respondents according to their needs in the field of care method of pregnant ewes.

Categories	Number	%	Average of need
Low needs (4.2-7.56)	10	12.5	6.53
Medium needs (7.57-10.93)	20	25	9.12
High needs (10.94-14.3)	50	62.5	12.25
Total	80	100%	

X=10.18 s.d=2.33

Table (4) shows that (12.5%) of the respondents fall within the category of low needs with an average of (6.53) numerical value. The percentage of (25%) of the respondents falls within the

category of medium needs with an average of (9.12) numerical value. The percentage of (62.5%) of the respondents falls within the category of large needs with an average of (12.25) numerical value. It is clear from this that the need for respondents in the field of caring for pregnant ewes is high. This may be due to the lack of knowledge of the respondents on how to care and care for newborns from the first moment after birth and so that he can rely on himself, and the way to breastfeed newborns whose mothers die, and the way they feed on the colostrum and grass.

3.2.4. The Fourth Area is Sheep Breeding and Improvement

The results showed that the highest numerical value obtained by the respondents on the items of the field (11.5), and the lowest value (1.62) with an average of (7.18) numerical value, and the respondents were distributed into three categories for indicative needs, as in Table 5.

Table 5. Distribution of respondents according to their needs in the field of sheep breeding and improvement.

Categories	Number	%	Average of need
Low needs (1.62-4.91)	10	12.5	1.98
Medium needs (4.92-8.21)	35	43.75	5.68
High needs (8.22-11.51)	35	43.75	9.16
Total	80	100%	

X=7.18 s.d=2.29

Table (5) shows that (12.5%) of the respondents fall within the category of low needs with an average of (1.98) numerical value. The percentage of (43.75%) of the respondents falls within the category of medium needs with an average of (5.68) numerical value. The percentage of (43.75%) of the respondents falls within the category of large needs with an average of (9.16) numerical value. It is clear from this that the respondents have a high guidance needs in the field of sheep breeding and improvement, and this may be due to the respondents do not have knowledge of how to elect good ewes when buying them from the market or the methods used to reduce the effort expended in raising and improving sheep.

3.2.5. The Fifth Field is Sheep Production

The results showed that the highest numerical value obtained by the respondents on the items of the field (6), and the lowest value (1.6) with an average of (6.23) numerical value. The respondents were divided into three categories of indicative needs, as shown in Table 6.

Table 6. Distribution of respondents according to their needs in the field of sheep production.

Categories	Number	%	Average of need
Low needs (1.6-3.73)	10	12.5	2.88
Medium needs (3.74-5.87)	10	12.5	4.8
High needs (5.88-8.01)	60	75	7.10
Total	80	100%	

X=6.23 s.d=1.60

Table (6) shows that (12.5%) of the respondents fall within the category of low needs with an average of (2.88) numerical values. The percentage of (12.5%) of the respondents falls within the category of medium needs with an average of (4.8) numerical value. The percentage of (75%) of the respondents falls within the category of large needs with an average of (7.10) numerical value. It is clear from this that the respondents have a high extension needs in the field of sheep production. This may be due to the lack of knowledge of the respondents in this field, especially

with regard to their accumulated experience in the field of wool production and its relationship to the age of the ewe and the weight of the carrot produced by local sheep.

3.3. Third Objective: Identify the Correlation Between the Extension Needs of Sheep Breeders According to Some Independent Variables

Academic achievement: When distributing educators according to the categories of academic achievement, it was found that the percentage of illiterates was high, as their percentage represented (26.5%) and those who could read and write represented (26.5%), and the percentage of primary graduates was (17.5%), and (12.5%) The percentage of intermediate graduates, and preparatory graduates reached (17.5%), and the percentage of graduates of institutes was (5%), while the percentage of college graduates or higher was (2.5%) of the total respondents, and to find the correlation between the extension needs of sheep breeders and academic achievement, the correlation coefficient was used Spearman Brown, which amounted to Its value (0.135) is a non-significant value, this disagree with [16].

3.3.1. Age

It has been shown that the lowest age of the respondents is 25 years and the highest age is 60 years and when the respondents are distributed according to age groups, it was found that the age group (25-36) years increased, where their percentage reached (55%), while the age group (37-48) years reached (16.25%) while the age group (49-60) years reached (28.75%), and this indicates the high percentage of young elements of educators and the decrease in the elderly. To find the relationship between the indicative needs of sheep breeders and age, Pearson's simple correlation coefficient was used, which was (0.190) and at a probability level (0.05), which is a positive significant value. This agree with [17]. This result may be due to the enthusiasm, enthusiasm and activity of young and middle-aged breeders that may be the reason for their application of modern and new methods of sheep breeding.

3.3.2. The Number of Years of Work in Sheep Breeding

The results showed that the lowest number of years of work in sheep breeding was 3 years and the highest number of years of work in sheep breeding was 38 years, and when distributing the respondents according to the number of years of work in sheep breeding, it was found that the percentage of the category (3-14) years was high, where their percentage reached (58.75%), and the percentage of the category (15-26) years (20%) while the percentage of the category (27-38) years (21.25%) of the total farmers surveyed, and to find the relationship between the extension needs of sheep breeders and the number of Years of work in sheep breeding used Pearson's simple correlation coefficient, which amounted to (0.020), which is a non-significant value, and this result may be due to the lack of differentiation between the respondents with regard to this variable.

Table 7. Shows the correlation between the extension needs of sheep breeders according to some independent variables.

The variables	Frequency	%	person	Spearman
Age				
Low (25-36)	44	55	0.190*	-----
Medium (37-48)	13	16.25		
High (49-60)	23	28.75		
Level of education				
Illiterate	21	26.25	-----	0.135 n.s
Read and write	21	26.25		

Primary	14	17.5		
Secondary	10	12.5		
High school	14	17.5		
Institute	4	5		
Collage	2	2.5		
number of years of work in sheep breeding				
Low (3-14)	47	58.75	0.020 n.s	-----
Moderate (15-26)	16	20		
High (27-38)	17	21.25		
Number of sheep owned by the breeder				
(50-290) animal	47	58.75		
(300-540) animal	19	23.75	0.124 n.s	
(550-790) animal	14	17.5		
Sources of agricultural information				
Low (less than 15)	45	56.25	0.338*	-----
Moderate (16-22)	19	23.75		
High (more than 23)	16	20		

3.3.3. Number of Sheep Owned by the Breeder

It appeared that the smallest animal possession among breeders is 50 heads and that the largest animal possession among breeders is 790 head, and when distributing the respondents according to animal possession, it was found that the percentage of the category that owns (50-290) animals was high, reaching (58.75%), while the category that owns (300-540) animals reached (23.75%), while the percentage of the category (550-790) animals reached (17.5%) of the total respondents. To find the relationship between the indicative needs of sheep breeders and the number of sheep owned by the breeder in sheep breeding, Pearson's simple correlation coefficient was used, which amounted to (0.124), which is a non-significant value, and this result may be due to the fact that most breeders own a small animal possession and that there is no need to apply modern methods and recommendations in animal husbandry due to small tenure.

3.3.4. Agricultural Information Sources

The results showed that the highest numerical value of agricultural information sources is 30 and the lowest value is 10, and when distributing the respondents according to the categories of agricultural information sources, it was found that the percentage of respondents who fall into the low communication category was high, as their percentage reached (56.25%), while the percentage of farmers in the medium communication category reached (23.75%), while the percentage of the many communication category reached (20%). Its value is (0.021), which is a non-significant value, this disagree with [18] and this result may be due to the fact that the sources of information to which farmers were exposed did not affect their application of modern methods and recommendations or that they were not in the field of applying modern agricultural methods in the field of animal husbandry.

Conclusions

Based on the results mentioned in the research, we conclude the following:

- The results showed that the extension needs in the field of sheep breeding among the respondents are average. This makes us conclude from this that the respondents have general information on the subject of sheep breeding .
- The results did not show significant relationships in the extension needs of the respondents according to the following research variables (educational level, number of

- sheep owned by the breeder, agricultural information sources, number of years of work in breeding cattle). We conclude from this that these variables do not play a major role in the development of the guidance needs of the respondents in the fields of sheep breeding .
- Due to the existence of a significant relationship between the guidance needs of the respondents in the field of sheep breeding different (age), it can be concluded that the elderly breeders may have weak guidance needs, especially those related to sheep breeding, and they do not want to renew their knowledge and try to maintain their knowledge in their practices of this activity, and believe that what they possess of information and knowledge is sufficient in the field of sheep breeding.

Recommendations

- Attention to activating the role of agricultural extension in rural areas, which is very limited and almost non-existent in some areas, as breeders do not hear about agricultural extension except through television programs, whose programs often focus on the agricultural aspect of the plant and neglect the animal side.
- The need to increase the support of the Ministry of Agriculture, represented by the agricultural people in rural areas, for breeders, providing fodder to them, and sparing them the loss of their herds due to the drought years that have been repeated in the last decade, by establishing warehouses to store hay in good years and distributing it to breeders in drought years, as well as educating the breeder about the importance of this issue and pushing him towards storing hay, which is sufficient for more than two years.
- The need to pay attention to agricultural information sources with the necessary recommendations and instructions that must be delivered to breeders and try to benefit from the mobile phone in the delivery of modern information through opinion leaders in the rural community, as well as activating the role of television represented by local satellite channels (Mosulia satellite channel), as the majority of breeders prefer to watch their programs, in the delivery of agricultural knowledge related to sheep breeding through extension programs broadcast regularly.
- Attention must be paid to the remaining sheep herds after the drought years and promote them healthily through the veterinary divisions in the region and the fight against diseases and pests that kill sheep, lambs and embryos and the introduction of breeds and improved types with high productivity of meat, milk and wool because the sheep of our regions are poorly produced in quantity and quality.

Acknowledgements

We extend our thanks and appreciation to the presidency of Agricultural Extension and Technical Transfer Department, Deanship of College of Agriculture and Forestry / University of Mosul for providing research requirements and continuous support for us that helped us to complete this research.

References

- [1] Hassan, Mohammed Radi (2005), The Role of Agricultural Extension in Rural Development, Iraqi Agriculture Magazine, Volume (1), Issue (3).
- [2] Al-Haboby, A.H.,K.h.shedeed and G.K.katab . (1997).sheep fertility survey in Iraq (APreliminary report).Socioeconomic group meeting for the mashreq countries in the mashreq /Maghreb project ,24-37,Amman ,Jordan.

- [3] Mohammed, Abdel Moneim Mahdi Saleh (2008), The effect of different protein ratios in formaldehyde diets on production performance and some biochemical characteristics of Awassi sheep, PhD thesis, College of Agriculture and Forestry, University of Mosul.
- [4] Al-Barbary, Adel Sayyid Ahmed (2006), Breeder's Guide to Sheep Breeding and Care in the Arab World, Dar Al-Kutub Foundation for Printing and Publishing, University of Mosul, Iraq.
- [5] Emadi ,H.Mohammad.(2006).Application Of In Indigenous Knowledge In The Process Of Dry Land Mongment ICARDA.1th Ed .
- [6] Al-Kadi, Mohamed El-Sayed (1998), An Agricultural Extension Analytical Study of the Knowledge and Skill Levels of Sheep Breeders in Kafr El-Zayat Center in Gharbia Governorate, Alexandria Journal for Agricultural Research, Faculty of Agriculture, Alexandria University, Vol. (3), Issue (1).
- [7] Al-Asadi, Saeed Jassim (2008), Ethics of Scientific Research in the Humanities, Educational and Social Sciences, 2nd Edition, Warith Cultural Foundation, Department of Studies and Research, Iraq.
- [8] Melhem, Sami Mohammed, 2010, Research Methods in Education and Psychology, 6th Edition, Dar Al-Masirah for Publishing and Distribution, Amman, Jordan.
- [9] Keshta, Abdel Halim Abbas, (2012), Agricultural Extension: A New Vision, Dar Al-Nada Printing,
- [10] Al-Jubouri, Maher Ibrahim Dawood (2010), The level of knowledge of sheep breeders in the district of Tal Afar, Nineveh Governorate and its relationship to some variables, Master's thesis, College of Agriculture, University of Mosul, Iraq.
- [11] Kawafha, Tayseer Mufleh (2010), Measurement, Evaluation and Methods of Measurement and Diagnosis in Special Education, 3rd Edition, Al-Masirah, Jordan.
- [12] Abdul Hafeez, Ikhlas Muhammad and Mustafa Hussein (2000). Methods of scientific research and statistical analysis in the fields of educational, psychological and sports, Book Center, Egypt.
- [13] Abbasi, Amel Fadel Khalil, 2018. Methods of Scientific Research and Statistical Analysis in the Behavioral Sciences - Dar Noon for Printing, Publishing and Distribution - 1st Edition
- [14] Al-Nabhan, Musa (2004), Fundamentals of Measurement in Behavioral Sciences, Dar Al-Shorouk, Amman.
- [15] Allam, Salah El-Din Mahmoud, (2009).Diagnostic and Educational Standards, Dar Al-Masirah for Distribution and Publishing, Jordan.
- [16] Farmers in Zammar region\Nineveh Governorate\Iraq.2019. Journal of Physics: Conference Series - (9)1294.
- [17] Hameed, T.S., Al Faraje, D.M.A. Cows breeder's awareness of the transitional diseases that affect the farm animals in Rabiaa district, Nineveh Governorate, Iraq.2020. Plant Archives ‘20pp. 3455-3460.
- [18] Hameed, T.S., Abd Al Faraje, D.M.2021. Importance of problems farmers facing of tomato hybrid varieties cultivated in AL-Koba and AL-Shreikhan of Nineveh. International Journal of Agricultural and Statistical Sciences (1)17pp. 255-260-2021.

Molecular Diagnosis of Armyworm in *Zea mays* L. Fields in Iraq

Majid Mahmood Esa Slubi¹, Majeed Hameed Nawar² and Hasan Moamin Lilo Al-Saedi³

^{1,2}Plant Protection Department, College of Agricultural Engineering, University of Baghdad, Baghdad, Iraq.

³Ministry of Agriculture, Department of Crop Protection, Baghdad, Iraq.

¹E-mail: Majid.eesa2204p@coagri.uobaghdad.edu.iq

²E-mail: majeed.hameed@coagri.uobaghdad.edu.iq

³E-mail: hasaanmoomin@gmail.com

Abstract. The results of the molecular diagnosis of the DNA extracted from Armyworm in *Zea mays* fields infected with Armyworm and multiplexed DNA products (PCR-amplified products) by polymerase chain reaction, each of size 617 nitrogen base pairs (bp) and in the presence of the forward and reverse primer. The results of analyzing the nitrogenous bases sequences of the nucleic acid products using the BLAST program showed that all the isolated isolates belonged to the *Spodoptera frugiperda*. The results of the analysis showed that all isolates which were collected from Four provinces in Iraq, were 100% similar to each other in sequences of nitrogenous bases, as well as to some isolates diagnosed and registered in the National Center for Biotechnology Information (NCBI), including Those diagnosed in USA (AY714298). The isolates diagnosed in this study had a similarity rate of 99% with some other isolates diagnosed in the mentioned center (NCBI), including those diagnosed in USA (AY714304 and AY714303 and AY714302), Korea (MN803322), China (PP230662, MT732476) and through Armyworm it was found that all *Zea mays* fields. And by polymerase chain reaction using primers for *Spodoptera frugiperda* it was found that all Armyworm were positive for *Spodoptera frugiperda* and the sequences was registered in Genbank under accession numbers: PQ097295.

Keywords. Armyworm, *Zea mays* fields, Sequences.

1. Introduction

Noctuid moths, Armyworm (*Spodoptera frugiperda*), are well-known agricultural pests of economical importance. Armyworms are known to attack at least 60 varieties of crops, most notably corn, peanuts alfalfa and forage grasses, cotton, soybeans [1-3]. Recent studies on *S. frugiperda* genetic variation have revealed that populations can be divided two morphologically identical, but genetically distinct strains using mtDNA cytochrome oxidase sequences [4-6]. Pheromone traps are a very easy and popular method for collecting adult malenoctuid moths in areas where it is prudent to monitor for destructive insects. the moths trapped inside may become difficult to identify to species [7,8]. A molecular diagnostics protocol may be very useful in distinguishing the armyworm from other noctuids commonly found in northwest Iraq [9-11]. The polymerase chain reaction, technique offers a very affordable and accurate method for the identification of insect species, and it can be used on the insect during any developmental stage

from egg to adult [12-14]. The objective of this study was to distinguish of Armyworm in *Zea mays* L. fields in Iraq from other noctuid moths.

2. Materials and Methods

2.1. Collect Armyworm Samples

A survey was conducted on the presence of the insect in four governorates, and three areas were selected from each governorate, which are abundant in yellow corn cultivation, by setting up specialized pheromone traps for the type. The traps were set up a week after planting at a height of 1.5 m, and the appearance of the insect in the trap was monitored periodically. The appearance of the insect was recorded, as shown in the Table 1. The appearance of the insect was recorded in Nineveh and Kirkuk governorates and in some areas of Baghdad governorate, and the appearance of the insect was not recorded in Wasit governorate. Samples were taken during the spring season of 2024 for planting the yellow corn crop, and the appearance of the insect was recorded 10 days after planting in most areas where the insect was present. Samples were taken at the first appearance of the insect, 5 days after it, and 10 days after it. The traps were set on 4/26/2024 and the first appearance of the insect was recorded on 5/12/2024.

Four governorates in Iraq were sampled for Armyworm infected *Zea mays* fields, as indicated Baghdad, Wasit, Kirkuk and Nineveh. Microscopic diagnosis was the primary approach employed to confirm the presence of Armyworm[15,16].

Table 1. Areas to record insect occurrence.

Governorates		First survey	Second survey	Third survey
1	Baghdad	Abu Ghraib	2	17
		Mahmoudiya	-	-
		Al-Mada'in	-	-
		Suwayrah	-	-
2	Wasit	Aziziya	-	-
		Al-Numaniyah	-	-
		Hawija	7	35
3	Kirkuk	Daquq	5	22
		Laylan	3	13
		Hamam Al-Alil	7	8
		Qayyarah	7	16
4	Nineveh	Tel Keif	2	16
				39

2.2. Molecular Diagnosis of Armyworm

In order to confirm the results of the microscopic diagnosis of Armyworm species diagnosed in this study, which were collected from some governorates of Iraq, DNA was extracted from them, following the following steps:

- Taking one ml of the spore suspension and subjecting it to the osmosis process at a speed of 13000 revolutions / minute for two minutes, after which the liquid was discarded and what was accumulated at the end of the tube was kept (Pellet).
- Resuspend the spores by adding 200 microliters of buffer solution CL and 20 microliters of protein-digesting enzyme (Proteinase K) and mix well using a mixing device (Vortex mixer) and incubate for 30 minutes at a temperature of 46 °C.
- After the end of the incubation process, add 200 microliters of BL buffer solution and mix well with the mixing device and incubate the sample at a temperature of 70 °C for 30

- minutes, then add 200 microliters of absolute ethanol to the sample and mix well with the mixing device.
- The mixture was transferred to the (Mini-column) separation tube, and the osmosis process was performed at a speed of 13000 revolutions per minute for one minute, after which the separation tube (Mini-column) was transferred to a new collection tube (Collection tube).
 - 700 microliters of TW buffer solution was added and the osmosis process was performed for one minute at a speed of 13000 rpm/min to get rid of the filtrate, return the mini-column to the collection tube, and repeat the osmosis process at the same speed and time mentioned. To get rid of leftover wash solution (TW).
 - Transferring the separation tube (Mini-column) to a test structure (1.5ml Eppendorf tube) to add 100 microliters of buffer solution AE and incubate for one minute vertically at room temperature, then the ectopia process was performed for five minutes at a speed of 5000 rpm .

The concentration and purity of DNA extracted from Armyworm samples were measured and the extracted DNA was kept at -20°C until use. Carry out the polymerase chain reaction (PCR) using the fixed primers in Table2 [17,18]. An agarose gel layer was prepared and the PCR-amplified products were transferred, where the sequences of the nitrogenous bases of the duplicated DNA products were analyzed, and a phylogenetic tree was drawn. The use of polymerase chain reaction (PCR) in diagnosis.

Table 2. Forward and reverse primers used in the polymerase chain reaction (PCR) to diagnose Armyworm (*Spodoptera frugiperda*).

Primer name	Primer sequence (5'-3')	Primer annealing °C	PCR product size
C1-J-2797	CCTCGACGTTATTCAGATTACC	46	617
C2-N3400	TCAATATCATTGATGACCAAT		

3. Results and Discussion

The results of the molecular diagnosis of the DNA extracted from Armyworm and multiplexed DNA products (PCR-amplified products) by polymerase chain reaction, each of size 617 nitrogen base pairs (bp), figure (1), and in the presence of the forward primer and The results shown in Table (1) showed that the results of analyzing the sequences of the nitrogenous bases of the DNA products, using the BLAST program, proved that all the isolated isolates belonged to *Spodoptera frugiperda*. The results of the analysis showed that all isolates were 100% similar to each other in the sequences of nitrogenous bases, as well as to some isolates diagnosed and registered in the US National Center for Biotechnology Information (NCBI), including those diagnosed in in USA (AY714298). The isolates diagnosed in this study had a similarity rate of 99% with some other isolates diagnosed in the mentioned center (NCBI), including those diagnosed in USA (AY714304 and AY714303 and AY714302), Korea (MN803322), China (PP230662, MT732476) (Fig. 2).

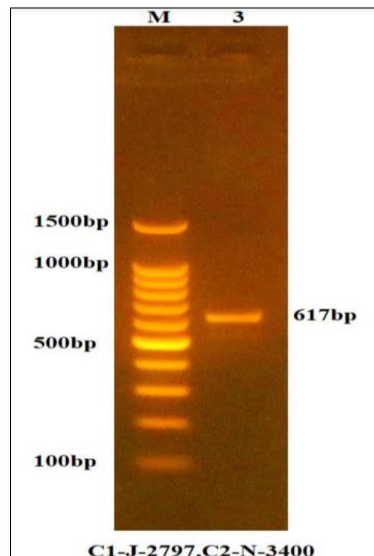


Figure 1. Nucleic acid products (PCR products) multiplexed using polymerase chain reaction (PCR) from *Spodoptera frugiperda* isolated in this study from in *Zea mays* fields With the exception of M: the nucleic acid scale (Molecular-weight size marker) with the number of base pairs (bp) and the sizes of each of them fixed on the left side of the figure.



Figure 2. Phylogenetic tree shows the relationship between the sequences of the nitrogenous bases of the doubled DNA products of *Spodoptera frugiperda* isolated in this study.

Conclusion

By PCR using primers specific for *Spodoptera frugiperda* it was found that all *Zea mays* fields are infected with *Spodoptera frugiperda*. And by polymerase chain reaction using primer for *Spodoptera frugiperda* and the sequence was registered in Genbank under accession numbers: PQ097295.

References

- [1] Abrahams, P.,T.;Beale, M.;Cock, N.;Corniani, R.;Day, J.;Godwin, S. Murphy,;G.Richards and J. Vos, 2017. Fall Armyworm: Status Impacts and control options in Africa CABI-Preliminary Evidence Note (April 2017).
- [2] Abbas, A.; Ullah, F.; Hafeez, M.; Han, X.; Dara, M.Z.N.; Gul, H. and Zhao,C.R. 2022 . Biological Control of Fall Armyworm, *Spodoptera frugiperda*. *Agronomy*,12:2704.
- [3] Alba, M.C.1990.Use of natural enemies for controlling sugar cane pests in the Philippine .*Sugar Quarterly*, 1(1):4-15.
- [4] Assefa, F. and Ayalew,D.2019. Status and control measures of fall armyworm (*Spodoptera frugiperda*) infestations in maize fields in Ethiopia: A review. *Cogent Food Agric*. 5:(16)41-90
- [5] Baker, R.; Gilioli,G.; Behring,C.; Candiani,D.; Gogin,A.; Kaluski,T.; Kinkar,M.; Mosbach-Schulz,O.; Neri,F.M.; Preti,S.; Rosace,MC.; Siligato,R.; Stancanelli,G. and Tramontini,S. .2018.*Spodoptera frugiperda* Pest Report to support ranking of EU candidate priority pests,European Commission. EFSA-Q-2018-00401.
- [6] Balla, A.; Bhaskar, M.; Bagade, P.; and Rawal,N.(2019).Yield losses in maize (*Zea mays*) due to fall armyworm infestation and potential IoT-based interventions for its control. *Journal of Entomology and Zoology Studies*,7:920–927.
- [7] Genetic Variation of the Southern Corn Rootworm, (Coleoptera: Chrysomelidae).Allen L. Szalanski, Carrie B. Owens.The Florida Entomologist, Vol. 86, No. 3 (Sep.2003), pp. 329-333.
- [8] J A Altre, J D Vandenberg Comparison of blastospores of two *Paecilomyces fumosoroseus* isolates: in vitro traits and virulence when injected into fall armyworm, *Spodoptera frugiperda* *Journal of Invertebrate Pathology*, 78 (2001), pp. 170-175.
- [9] R Andrade, C Rodriguez, A C Oehlschlager Optimization of a pheromone lure for *Spodoptera frugiperda* (Smith) in Central America *Journal of the Brazilian Chemical Society*, 11 (2000), pp. 609-613.
- [10] Y Assefa Molecular identification of the invasive strain of *Spodoptera frugiperda* (JE smith) (Lepidoptera: Noctuidae) in Swaziland *International Journal of Tropical Insect Science*, 39 (2019), pp. 73-78.
- [11] C Ayra-Pardo, O Borrás-Hidalgo. Fall armyworm (FAW; Lepidoptera: Noctuidae): Moth oviposition and crop protection J F Picimbon (Ed.), *Olfactory Concepts of Insect Control-Alternative to Insecticides*, Springer, Switzerland (2019), pp. 93-116.
- [12] R Banerjee, J Hasler, R Meagher, R Nagoshi, L Hietala, F Huang, K Narva, J L Jurat-Fuentes Mechanism and DNA-based detection of field-evolved resistance to transgenic Bt corn in fall armyworm (*Spodoptera frugiperda*) *Scientific Reports*, 7 (2017), Article 10877.
- [13] G P Barrera, M N Belaich, M A Patarroyo, L F Villamizar, P D Ghiringhelli Evidence of recent interspecies horizontal gene transfer regarding nucleopolyhedrovirus infection of *Spodoptera frugiperda* *BMC Genomics*, 16 (2015), Article 1008.
- [14] M L Bateman, R K Day, B Luke, S Edgington, U Kuhlmann, M J W Cock, Assessment of potential biopesticide options for managing fall armyworm (*Spodoptera frugiperda*) in Africa *Journal of Applied Entomology*, 142 (2018), pp. 805-819.
- [15] L G Batista-Pereira, K Stein, A F de Paula, J A Moreira, I Cruz, C de Lourdes, M Figueiredo, J Perri Jr, A G Correa Isolation, identification, synthesis, and field evaluation of the sex pheromone of the Brazilian population of *Spodoptera frugiperda*.*Journal of Chemical Ecology*, 32 (2006), pp. 1085-1099.



- [16] R W Behle, H J R Popham. Laboratory and field evaluations of the efficacy of a fast-killing baculovirus isolate from *Spodoptera frugiperda*. *Journal of Invertebrate Pathology*, 109 (2012), pp. 194-200.
- [17] D Boaventura, A Bolzan, F E O Padovez, D M Okuma, C Omoto, R Nauen Detection of a ryanodine receptor target-site mutation in diamide insecticide resistant fall armyworm, *Spodoptera frugiperda* *Pest Management Science*, 76 (2020), pp. 47-54.
- [18] S Gimenez, H Abdelgaffar, G L Goff, F Hilliou, C A Blanco, S Hanniger, A Bretaudeau, F Legeai, N Negre, J L Jurat-Fuentes, E d'Alencon, K Nam .Adaptation by copy number variation increases insecticide resistance in the fall armyworm, *Communications Biology*, 3 (2020), p. 664.

Antioxidants and Their Important Role in Resisting Stresses in Plants: A Review

**Ahmed F. Z. Al-Dulaimy¹, Gaith Ibrahim Abd², Basim Mohammed Abed³,
Y. S. Sekhi⁴, Omar H. M. Almohammed⁵ and D. K. A. Al-Taey⁶**

¹⁻⁵Department of Horticulture and Landscape Gardening, College of Agriculture,
University of Anbar, Anbar, Iraq.

⁶Department of Horticulture and Landscape, College of Agriculture, University
of Al-Qasim Green, Babylon, Iraq.

¹E-mail: ag.ahmed.fatkhan@uoanbar.edu.iq

²E-mail: gaith882020@uoanbar.edu.iq

³E-mail: bassim19866@uoanbar.edu.iq

⁴E-mail: yassirsayel@uoanbar.edu.iq

⁵E-mail: ohmosleh@uoanbar.edu.iq

⁶E-mail: duraidaltaey@gmail.com

Abstract. Antioxidants can be classified into primary, secondary, and cooperative antioxidants based on their mode of action. Primary antioxidants donate a hydrogen atom to lipid radicals, forming stable compounds and thus breaking the reaction chain. Secondary antioxidants decompose lipid peroxides into more stable end products. Additionally, cooperative antioxidants work in a number of ways, such as by removing reactive oxygen species (deoxygenation) and chelating metals to stop autoxidation. They chelate metal ionThey chelate metal ions, which catalyze oxidation, decompose peroxides (breaking the oxidation reaction chain), and regenerate antioxidants, such as the production of tocopherol from ascorbic acid. ts delay or prevent the oxidation of lipids and proteins by inhibiting or preventing oxidative reactions induced by oxygen. Moreover, they slow down and alter the kinetics of oxidation during the autoxidation of lipid membranes by reducing membrane fluidity, thereby slowing down the oxidation process.

Keywords. Antioxidants, Plant, Growth, Yield, Stresses.

1. Introduction

Antioxidants are substances or compounds that counteract oxidative damage and delay or prevent the action of free radicals. They protect plants through various mechanisms, including direct inhibition of reactive oxygen species (ROS) production, preventing their propagation, or scavenging them [1,2]. Cells employ diverse antioxidant systems, whose nature varies across tissues, cell types, and intracellular and extracellular environments [3-5].

1.1. Non-Enzymatic Antioxidants

Non-enzymatic antioxidants are positively charged molecules with an extra electron in their outer orbital. They react or merge with negatively charged atoms or molecules (free radicals) to inhibit

their activity or capacity to damage plant tissues [6,7]. These compounds are readily oxidized by ROS, thus neutralizing free radicals in cells and plant tissues [8,9]. Cellular antioxidant content decreases with increasing stress levels, leading to a significant increase in the activity of oxidative enzymes. These enzymes produce more free radicals, accelerating cellular and tissue degradation and inducing senescence [10,11].

Antioxidants exist naturally in plants and can take various forms, including enzymes, minerals, vitamins, and organic acids. Their activity is stimulated under stress conditions [12,13]. The enzyme whose activity increases in response to a specific type of stress can be used to combat that stress and plays a crucial role in resistance. Important non-enzymatic antioxidants include ascorbate (Vit. C), glutathione, tocopherol (Vit. E), carotene, and flavonoids [14,15].

Ascorbate and glutathione must be present at adequate concentrations during stress to remove reactive oxygen species. Additionally, alpha-tocopherol plays a vital role in scavenging ROS [16]. Alpha-tocopherol prevents the formation of hydroxyl and singlet oxygen and can remove lipid peroxyl radicals. It is essential for protecting cellular membranes, especially in chloroplasts, and thus accumulates in plants under water stress. Carotenoids also play a role in removal singlet oxygen [17]. Figure 1 illustrates the interaction of ascorbic acid with glutathione (GSH), thioredoxin (Trx), coenzyme Q, α -tocopherol, and retinol [18-20].

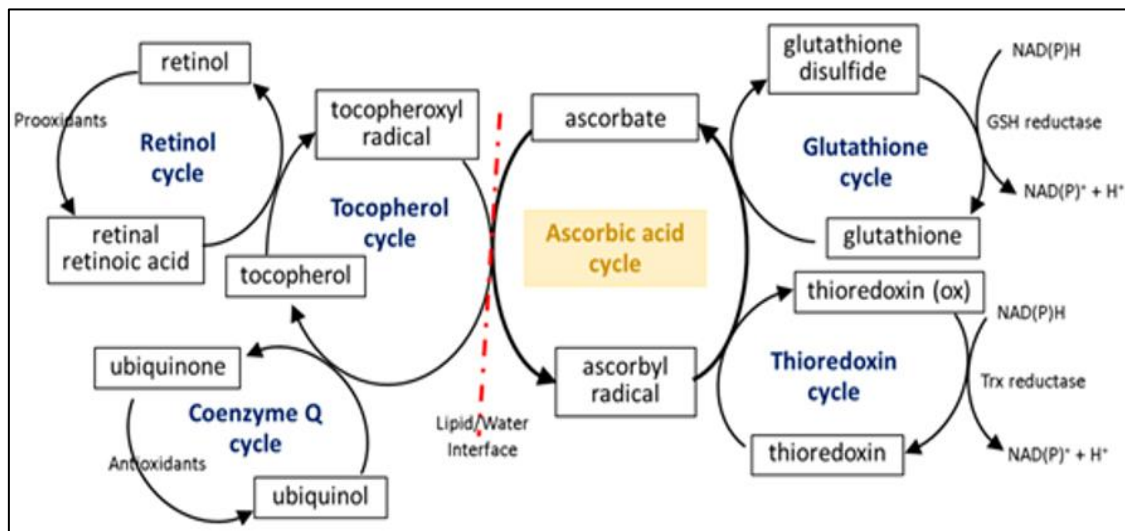


Figure 1. Scheme of the interaction of ascorbic acid with glutathione (GSH), thioredoxin (Trx), coenzyme Q, α -tocopherol, and retinol.

1.2. Non-Enzymatic Antioxidants

The following compounds represent some of the most significant non-enzymatic antioxidants:

- Phenolic Compounds: This category includes flavonoids, which exhibit potent antimutagenic and anticancer properties. Examples include myricetin, glabridine, quercetin, and hesperetin [21-23]. Anthocyanidins are particularly effective, surpassing other flavonoids in potency by approximately 150 times. Examples include delphinidin, nasunin, pelargonidin, cyanidin, and glucoside [24,25]. These compounds are prevalent in various plant families, including legumes, composites, umbellifers, and crucifers [26,27].

- Tannins: These compounds contain ellagic acid, which plays a crucial role in protecting nucleic acids. Tannins are found in a wide range of plants, such as tea, berries, grapes, apples, and persimmons [28,29].
- Carotenoids: Characterized by long hydrocarbon chains with multiple double bonds, carotenoids include zeaxanthin and lycopene [30,31].
- Diterpenes: These compounds act as antioxidants due to the presence of erythroxydiol [32,33].
- Organosulfur Compounds: Found in garlic, these compounds possess antioxidant, antimutagenic properties. Examples include ajoene and allicin derivatives [34,35].
- Vitamins: Tocopherol (Vitamin E), present in various vegetable oils like soybean, pistachio, corn, and avocado, is a potent antioxidant [36,37]. Ascorbic acid (Vitamin C), abundant in fruits and vegetables, especially citrus fruits, also exhibits antioxidant properties [38,39]. Vitamin E, comprising beta-carotene, is found in numerous plants, including carrots, apricots, broccoli, and spinach [40,41].
- Mineral Salts: Selenium, present in broccoli, lentils, onions, celery, cucumber, and watermelon, is a significant mineral salt with antioxidant properties [42,43].
- Minerals: Zinc, a cofactor in the synthesis of antioxidants, is found in legumes and grains [44,45].

1.3. Antioxidative Enzymes

- Catalase: Ubiquitous in plant tissues and intracellularly, particularly within peroxisomes, catalase catalyzes intracellular oxidation and detoxification. Its primary function is the dismutation of hydrogen peroxide into water and oxygen. Remarkably, a single catalase molecule can convert 83,000 hydrogen peroxide molecules per second [46,47].
- Superoxide Dismutase (SOD): Located in mitochondria and cytosol, SOD is associated with metal cofactors such as iron, copper, zinc, and manganese. Functioning as both an oxidant and reductant, SOD scavenges superoxide radicals (O_2^-) by converting them to hydrogen peroxide (H_2O_2) and oxygen (O_2). The rate of this reaction is accelerated by the presence of metals like iron, copper, zinc, and manganese [48,49].
- Ascorbate Peroxidase (APX): Found in chloroplasts, cytosol, and leaf cells, APX is a crucial antioxidative enzyme, especially against hydrogen peroxide. APX catalyzes the transfer of electrons from ascorbate to hydrogen peroxide, yielding dehydroascorbate and water. APX is a central component of the glutathione-ascorbate cycle [50,51].
- Glutathione Peroxidase (GP): Localized in the cytosol, GP is a primary antioxidative enzyme that protects lipid membranes and fats from peroxidation. GP transfers electrons from glutathione to hydrogen peroxide or organic compounds containing peroxide groups. Subsequently, glutathione reduces the GP enzyme, halting the reaction [52-54].
- Dehydro Ascorbate Reductase (DAR): DAR utilizes the sulfur group as an electron donor. This enzyme operates within three systems: ascorbate and aldarate metabolism, glutamate metabolism, and glutathione metabolism [55,56].
- Mono-Dehydro Ascorbate Reductase (MDAR): MDAR is present in peroxisomes, mitochondria, cytosol, and chloroplasts. As an enzyme in the glutathione-ascorbate cycle, MDAR employs NADH and NADPH as electron donors [57,58].

2. How Antioxidants Reduce the Harmful Effects of Stresses

Antioxidants combat the harmful effects of salt stress by inducing the accumulation of metabolic products such as proline, amines, sugars, and ions. This increases the osmotic pressure of the cell

sap to resist external salt stress [59,60]. One of the most important biochemical indicators of antioxidants is the amino acid proline, which is closely related to the mechanism of stress resistance. It performs several functions, including regulating the osmolarity of plant tissue cells, removing the toxic effects of ammonia in cells [61,62], and maintaining the vitality and stability of proteins and cytoplasmic membranes under stress conditions [63,64]. Additionally, it plays a role in eliminating reactive oxygen and hydroxyl radicals from plant tissues [65-67]. (Figure 2) illustrates the mechanism of eliminating the O_2^- radical by antioxidant enzymes [68].

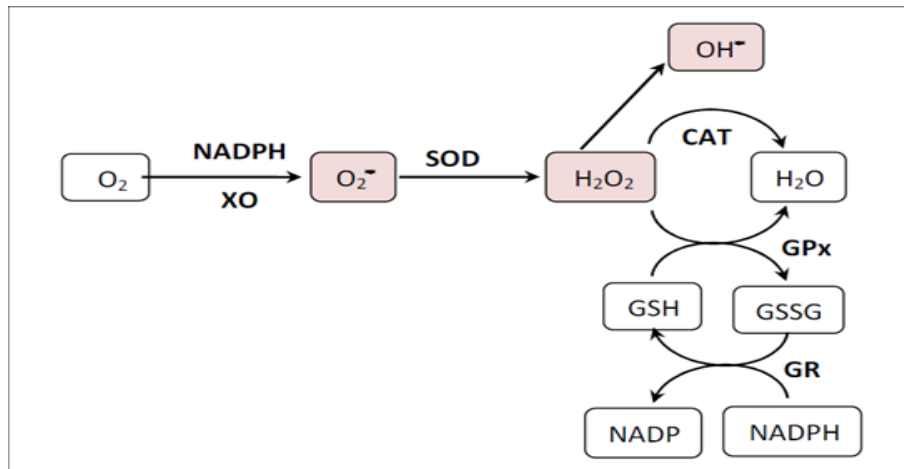


Figure 2. Mechanism of the elimination of O_2^- radical by antioxidant enzymes.

Proline plays a crucial role in protecting plant tissues from absorbed toxic elements and in safeguarding and stabilizing mitochondrial enzymes under stress conditions [69,70]. It contributes to increased soluble sugar content, as a strong correlation has been observed between elevated proline accumulation and increased soluble sugar content, which aids in regulating the osmolality of plant cells and tissues [71,72]. Under stress conditions, sucrose synthesis is inhibited, while mannitol synthesis is activated, leading to increased cell osmolality [73,74]. Certain legumes and conifers accumulate pinitol in chloroplasts under stress conditions, which raises cell osmolality [75,76].

Additionally, proline is involved in maintaining protein viability and stability, as well as the synthesis of proteins like osmosing [77,78]. It protects cytoplasmic membranes from oxidative damage and safeguards mitochondrial enzyme stability [79,80]. Proline also regulates cytoplasmic pH, increases cytochrome synthesis, which protects photosynthetic membranes and the accumulation of photosynthetic products by increasing CO_2 fixation and net photosynthesis [81,82]. Furthermore, proline activates the formation of chlorophyll-synthesizing enzymes and inhibits the degradation of photosynthetic pigments [83,84]. It also plays a significant role in increasing the synthesis and stability of nucleic acids and inducing intracellular hormonal balance [85,86]. Additionally, proline stimulates cell division, delays senescence, and maintains the viability and integrity of plasma membranes, protecting them from oxidation [87,88]. Figure 3 shows the effect of natural antioxidants on stimulating and accelerating growth and promoting bud development [89].

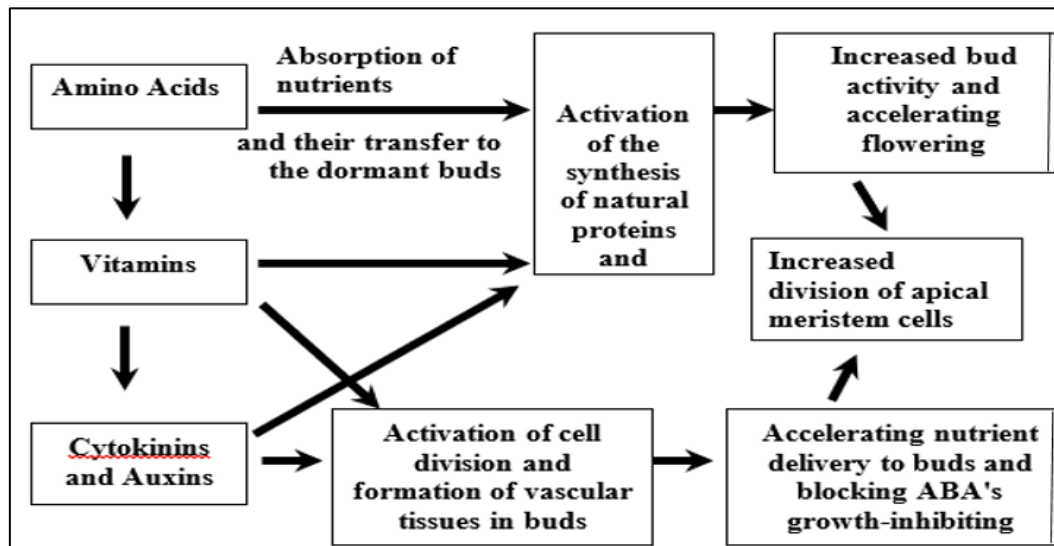


Figure 3. The effect of natural antioxidants on stimulating, accelerating, and promoting the growth of buds.

3. Mechanism of Antioxidants

Figure 4 illustrates the mechanism of action of antioxidants in counteracting the harmful effects of various stresses, which occurs through the donation of an electron or hydrogen atom to the free radical, transforming it into a stable, inactive compound [90].

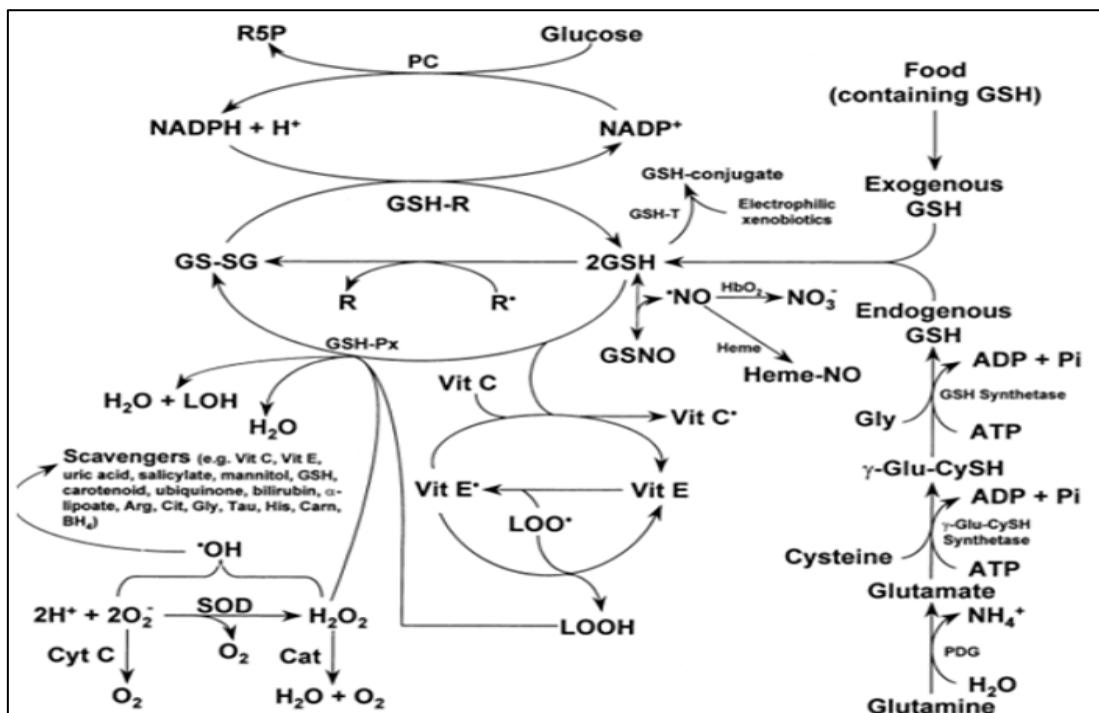


Figure 4. Elimination of Reactive Oxygen Species by Antioxidant Systems.

4. Glutathione-Ascorbate Cycle

This cycle is the metabolic pathway responsible for eliminating hydrogen peroxide (H_2O_2), a reactive oxygen species that is a harmful byproduct of photosynthesis. This cycle involves the metabolic reactions of ascorbate, glutathione, enzymes, and NADPH [89].

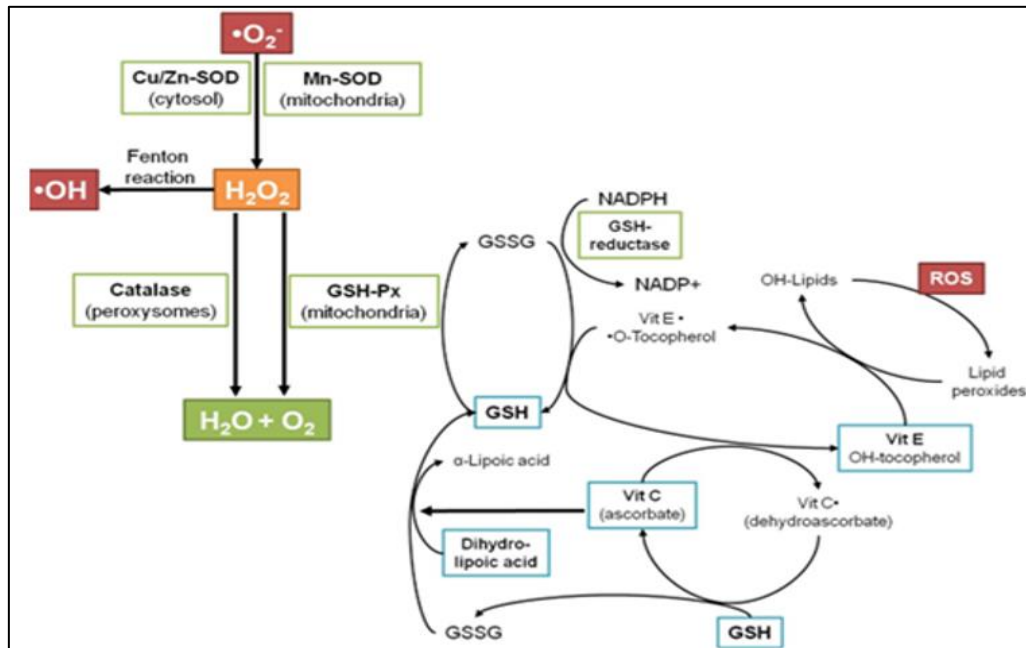


Figure 5. Enzymes in the Glutathione-Ascorbate Cycle.

Conclusions

As demonstrated in the presented review, antioxidants exhibit antioxidant and anti-inflammatory properties at various levels of cellular function. These properties range from directly scavenging free radicals and suppressing pro-inflammatory pathways to activating intracellular antioxidant systems and supporting the action of other exogenous antioxidants. In this context, DNA, proteins, and lipids are protected from oxidation, which can lead to inflammation and even cell death (apoptosis). However, the role of antioxidants in preventing DNA mutations and cell apoptosis, particularly in relation to cancer cells, is still debated.

References

- [1] Sies, H 1997. Oxidative stress: oxidants and antioxidants. *Exp Physiol.* 82, 291–295.
- [2] Apel, K, and Hirt, H 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol.* 55, 373–399.
- [3] Al-Dulaimy, AF and Jumaa, FF 2020. Effect of foliar spray with yeast suspension, licorice roots extract and amino Quelant-K compound on chemical content of Black Hamburg grape cultivar berries. *Diyala Agricultural Sciences Journal.*, 12(Special Issue), 546–557.
- [4] Inzé, D, and Van Montagu, M 1995. Oxidative stress in plants. *Curr Opin Biotechnol* 6:153–158.
- [5] SekhI, Y, Kadhim, Z, and Hamad, A 2022. Effect of Sodium Azide on some vegetative and biochemical properties of Strawberry under Polyethylene Glycol of Albion variety in vitro. *Annals of Agri-bio Research.* 28(1), 121-128.
- [6] Al-Taey,D. K. A., Z. Z. Majid.(2018). The activity of antioxidant enzymes and NPK contents as affected by water quality, Kinetin, Bio and organic fertilization lettuce (*Lactuca sativa* L). *The Iraqi Journal of Agricultural Science:* 49(3):506- 518. file:///C:/Users/aw/Downloads/123-Article%20Text-257-1-10-20181201.pdf

- [7] Alani, MHI, Al-Dulaimy, AFZ, Sekhi, YS, Almehemdi, AF 2021. Chemical analysis of Fig transplants cv Wazeri under with cutting type and humic acid. IOP Conference Series: Earth and Environmental Science. 761(1), 012032.
- [8] Ahmed, ZR, and Almohammed, OHM 2021. Response of Cherry tomato to foliar spray of amino acid, bio-catalyst and dotted type in qualities and evaluation of drip irrigation system. IOP Conference Series: Earth and Environmental Science., 910, 012100.
- [9] Abbas A.K. , H. A. Mohammed., D.K. A. Al-Taey., R. A. Al-Khafajy., S. S. Mahmood and M. F. Alsaffar.2024. The Effect of NaCl Treatment on the Nutritional Balance of Wheat under Salt Stress Conditions. IOP Conf. Series: Earth and Environmental Science 1371 (2024) 052090. doi:10.1088/1755-1315/1371/5/052090.
- [10] Mittler, R, Vanderauwera, S, Gollery, M and Van Breusegem, F 2004. Reactive oxygen gene network of plants. Trends Plant Sci. 9, 490–498.
- [11] Al-Dulaimy, AFZ, Alalaf, AH, Al-Hayali, REY and ALTaey, DKA 2023. Flowers and fruits abortion in fruit trees ... Causes and solutions: A review. IOP Conference Series: Earth and Environmental Science. 1158(4), 042010.
- [12] Al-Issawi, AK and Al-Mohammed, OHM 2023. A study of three planting dates and spraying with zinc, boron and polyamine (Putrescine) on the growth and yield of broccoli. IOP Conference Series: Earth and Environmental Science., 1225, 012029.
- [13] Al-Taey, D.K.A. and Z.J. M. Al-Musawi.(2022). THE IMPACT OF NANO FERTILIZATION AND SALICYLIC ACID ON GROWTH, YIELD AND ANTI-OXIDANT CONTENTS IN ROCKET PLANT UNDER SALT STRESS. *Agraarteadus*. 32 (1) :43-47. <https://dx.doi.org/10.15159/jas.21.30>
- [14] Murad, HJ and Al-Dulaimy, AFZ 2021. Response of date palms cv. Zahdi to foliar spray with urea and seaweed extract. IOP Conference Series: Earth and Environmental Science. 761(1): 012052.
- [15] Navrot, N, Rouhier, N, Gelhay, E and Jacquot, J 2007. Reactive oxygen species generation and antioxidant systems in plant mitochondria. *Physiol Plant*. 129,185–195.
- [16] Sekhi, YS, Hamad, RM and Neamah, SI 2021. Effect of acridine orange in promoting growth and physiological characteristics of fragaria ananassa duch under salinity stress in vitro. IOP Conference Series: Earth and Environmental Science. 761, 012048.
- [17] Dixon, DP, Skipsey, M and Edwards, R 2010. Roles for glutathione transferases in plant secondary metabolism. *Phytochemistry*. 71, 338–350.
- [18] Packer, L, Weber, SU and Rimbach, G 2001. Molecular aspects of α -tocotrienol antioxidant action and cell signalling. *J. Nutr*. 131, 369S–373S.
- [19] Li, X, Qu, ZC and May, JM 2001. GSH is required to recycle ascorbic acid in cultured liver cell Lines. *Antioxid. Redox Signal*. 3, 1089-1097.
- [20] Young, AM and Gregoriadis, G. 1996. Photolysis of Retinol in Liposomes and Its Protection with Tocopherol and Oxybenzone. *Photochem. Photobiol*. 63, 344-352.
- [21] Mohammed, AB and Almohammed, OHM 2023. Effect of soil mulching and spraying with Naphthalene acetic acid on growth and yield of Broccoli. IOP Conference Series: Earth and Environmental Science., 1252, 012086.
- [22] Winkel-Shirley, B 2002. Biosynthesis of flavonoids and effects of stress. *Curr Opin Plant Biol*,223.
- [23] Panche, AN, Diwan, AD and Chandra, SR 2016. Flavonoids: an overview. *J Nutr Sci* 5, 47.
- [24] Al-Dulaimy, AFZ and Jubair, N A 2023. Improving chemical content of date palm cv. Khastawi under different levels of spraying with micro-elements, gibberellic acid and salicylic acid. *Anbar Journal of Agricultural Sciences*., 22(1), 147-159.
- [25] Ayaprakasam, B, Vareed, SK and Olson, LK 2005. Insulin secretion by bioactive anthocyanins and anthocyanidins present in fruits. *J Agric Food Chem*. 53(1), 28–31.
- [26] Brown, JE and Kelly, MF 2007. Inhibition of lipid peroxidation by anthocyanins, anthocyanidins and their phenolic degradation products. *Eur J Lipid Sci Technol*. 109(1), 66–71.

- [27] Murad, HJ and Al-Dulaimy, AF 2021. Effects of spraying with urea and seaweed extract (Tecamin Algae) on growth and chemical content of date palm tree cv. Zahdi. IOP Conference Series: Earth and Environmental Science. 904(1), 012065.
- [28] Dai, J, and Mumper, RJ 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 15,7313–7352.
- [29] Almohammadi, OH, and Kokaz, NM 2021. Effect of adding the biological fertilizer, Fulzyme Plus, and spraying CaCl_2 on efficiency of the rhizoctonia fungus, and the qualitative of the potato (*Solanum tuberosum* L). IOP Conference Series: Earth and Environmental Science., 790, 012060.
- [30] Maoka, T 2020. Carotenoids as natural functional pigments. J. Nat. Med., 74, 1-16.
- [31] Mahmood, MM and Al-Dulaimy, AF 2022. Effect of media culture, nano and normal micronutrients on some flowers and yield traits of Strawberry. AIP Conference Proceedings., 2394(1), 1-13.
- [32] Sergi, M, Maren, M, Karin, S and Leonor, A. 2001. Diterpenes and antioxidative protection in drought-stressed *Salvia officinalis* plants. Journal of Plant Physiology. 158(11), 1431-1437.
- [33] Almohammadi, O, Sekhi, Y, and Ismail, M 2023. A Review of Nano fertilization and its role on growth, yield and quality characteristics of fruit trees. Tikrit Journal for Agricultural Sciences, 23(1), 158-167.
- [34] Al-Dulaimy, AFZ, Al-Janabi, AMI, Abd, NT and ALTaey, DKA 2024. Fruit pome cracking, causes and solutions: A review. IOP Conf. Series: Earth and Environmental Science., 1371(042025), 1-11.
- [35] Nakamoto, M, Kunimura, K, Suzuki, JI and Kodera, Y 2020. Antimicrobial properties of hydrophobic compounds in garlic: Allicin, vinylidithiin, ajoene and diallyl polysulfides. Experimental and Therapeutic Medicine. 19(2), 1550–1553.
- [36] Al-Dulaimy, Alani, AFZ, MHI, Awad, IM, Khudair, RIA, Salim, HA, and Jasem, SM, 2021. Response of *Gazania* plants (*Gazania Splendens* L.) to seaweed extract (Tecamin Algae) and nano stimulator (Proteck CalBor). IOP Conference Series: Earth and Environmental Science., 910(1), 012052.
- [37] Munne-Bosch, S 2005. The role of alpha-tocopherol in plant stress tolerance. J Plant Physiol. 162, 743–748.
- [38] Al-Janabia, AMI, Al-Dulaimy, AFZ, Sekhi, YS, Almohammadi, OHM and Al-Taey, DKA 2024. Effect of salt stress on growth and yield of plants: A Review. IOP Conf. Series: Earth and Environmental Science., 1371(042028), 1-16.
- [39] Farhan, TF, and Sekhi, YS 2024. Effect of plant growth regulators and explants on the micropropagation of *Capparis spinosa* L. Anbar Journal of Agricultural Sciences, 22(1),
- [40] Mahmood, MM and Al-Dulaimy, AF 2021. Response of Strawberry cv. Festival to culture media and foliar application of nano and normal micronutrients. IOP Conference Series: Earth and Environmental Science. 904(1), 012067.
- [41] Kobayashi, N, and DellaPenna, D 2008. Tocopherol metabolism, oxidation and recycling under high light stress in *Arabidopsis*. Plant J. 55, 607–618.
- [42] Liu, H, Xiao, C, Qiu, T, Deng, J, Cheng, H, Cong, X, Cheng, S, Rao, S and Zhang Y. 2023. Selenium regulates antioxidant, photosynthesis, and cell permeability in plants under various abiotic stresses: A Review. Plants. 12(1), 32-44.
- [43] Al-Issawi, AK and Al-Mohammadi, OHM 2023. A study of three planting dates and spraying with zinc, boron and polyamine (Putrescine) on the qualitative characteristics of Broccoli. IOP Conference Series: Earth and Environmental Science., 1213, 012055.
- [44] Dilina, DM, Kyria, JCC, Jennifer, BSM, Jéssica, BB, Juliana, SS and Ana, RSD 2017. Zinc and oxidative stress: current mechanisms. Antioxidants. 6(2), 13-24.
- [45] Al-Ealayawi, ZA and Al-Dulaimy AFZ 2023. The impact of spraying Marine Algae Extract, Arginine, and Brassinolide on vegetative growth traits of Albion Strawberry saplings. IOP Conference Series: Earth and Environmental Science. 1262(4), 042061.
- [46] Dat, J, Van Breusegerm, F, Vandenabeele, S, Vranova, E, Van Montagu, M and Inze, D 2000. Active oxygen species and catalase during plant stress response. Cell Mol. Life Sci. 57, 779–786.

- [47] Abd, GI, Omer, IK, Basim, MA, Idrees, HMA, Harith, SMA, Mohammed, IKA and Amer, HA 2024. Effect of spraying with salicylic acid (sa) on the yield of several cultivars of cucumber, *Cucumis sativus* L., under protected cultivation conditions. IOP Conf. Series: Earth and Environmental Science., 1371(042002), 1-8.
- [48] Al-Taey, D.K.A., Z.N. A. Kamaluddin and H. H. S. Al-Kazemy .(2022). Impact of Zytonic-M and organic fertilizer on growth and yield of hybrid tomato Awahr (F1) under saline conditions, International Journal of Vegetable Science, 28(4) :1-6 DOI: 10.1080/19315260.2022.2070569
- [49] Al-Dulaimy, AFZ, Abbood, AA and Alian, AT 2022. Effect of spraying with normal and nano Iron on the growth and flowering of Petunia plant Petunia hybrid L. IOP Conference Series: Earth and Environmental Science., 1060 (1), 012033.
- [50] Shigeoka, S, Ishikawa, T, Tamoi, M, Miyagawa, Y, Takeda, T, Yabuta, Y and Yoshimura, K 2002. Regulation and function of ascorbate peroxidase isoenzymes. J Exp Bot. 53, 1305-1319.
- [51] Ziydan, B, Alrawi, M, Almeahmedi, AF, and Sekhi, Y 2024. A Growth and seed yield of seven fennel cultivars with two types of ploughing systems. Plant Science Today. 11(2),
- [52] Jubair, NA and Al-Dulaimy, AFZ 2023. Response of date palm cv. Khastawi to foliar spray with micro-elements, gibberellic acid and salicylic acid. IOP Conference Series: Earth and Environmental Science., 1158(4), 042063.
- [53] Noctor, G, Mhamdi, A, Chaouch, S, Han, Y and Neukermans, J 2012. Glutathione in plants: an integrated overview. Plant Cell Environ. 35, 454–484.
- [54] AL-Taey D.K.A. , K.M.N.AL-Dirbil and S.S.M.AL-Azawi.(2021). Study The Effect of Water Quality, Bio Booster (Zytonic F) and Nano NPK fertilizer on Tomato Growth and Yield. IOP Conf. Series: Earth and Environmental Science 910 (2021) 012063. doi:10.1088/1755-1315/910/1/012063
- [55] Al-Mohammed, AN, Al-mehemdi, AF and Al-mehemdi, OH 2016. Some physical properties of essential oil of barakaseed *Nigella sativa* L. impacted by bat guano otonycteris hemprichii camd and seaweed extract. Iraqi Journal of Agricultural Sciences., 47(4), 12-36.
- [56] Zhong, C and Daniel, RG 2006. Dehydroascorbate reductase affects leaf growth, development, and Function. Plant Physiol. 142(2), 775–787.
- [57] Al-Ealayawi, ZA and Al-Dulaimy, AFZ 2023. Marine algae and applications to plant nutrition: A review. IOP Conference Series: Earth and Environmental Science. 1158(4), 042004.
- [58] Yeh, HL, Lin, TH, Chen, CC, Cheng, TX, Chang, HY and Lee, TM, 2019. Monodehydroascorbate reductase plays a role in the tolerance of chlamydomonas reinhardtii to photooxidative stress. Plant Cell Physiol. 60, 2167–2179.
- [59] Hasan A.A. , Aubied I.A. and D.K. A. Al-Taey. (2023). The Effect of Organic Amendments and Minerals on Increasing Soil Fertility (A review). IOP Conf. Series: Earth and Environmental Science. 1262 (2023) 042012. doi:10.1088/1755-1315/1262/4/042012
- [60] Odo, AO 2016. Abiotic stress, antioxidants and crop productivity: the mitigating role of exogenous substances. Greener J Agric Sci. 6, 79–86.
- [61] Al-Hayali, REY, AL-Jaf, IHM, AL Miahhy, FHR and Al-Dulaimy, AFZ 2023. Evaluation of orange and biofertilizer on some yield traits of Cucumber (*Cucumis sativus* L.). Bionatura., 8(1), 1-5.
- [62] Kram, S, Ayyub, C, Shahzad, M and Shahzad, A 2021. Role of proline in mitigating the deleterious effects of heat stress in chillies. Contemporary Agriculture, 70, 28–35.
- [63] Al-Dulime, MLA, and Al-Mohammed, OHM 2023. Effect of spraying with growth regulators Brassinolide and Thidiazuron on the chemical content of leaves and pods of three Okra hybrids under protected cultivation conditions. Conference Series: Earth and Environmental Science., 1252, 012089.
- [64] Al-Taey, D.K.A., Z. J. M. Al-Musawi ., S. M. AbdulKadium. , A. K. Abbas4., M. F. Alsaffar and S. S. Mahmood. 2024. Brassinolides' Function and Involvement in Salt Stress Response: A Review. IOP Conf. Series: Earth and Environmental Science 1371 (2024) 042032. doi:10.1088/1755-1315/1371/4/042032.

- [65] Trovato, M, Mattioli, R and Costantino, P 2008. Multiple roles of proline in plant stress tolerance and development. *Rend Lincei*. 19, 325–346.
- [66] Hardan, ME and Al-Dulaimy, AF 2022. Effect of humic acid addition and spraying with ginger rhizome extract on the growth and some chemical contents of apricot seedlings *Prunus armeniaca* L . cv. Bionatura. 7(4), 1-6.
- [67] Hayat, S, Hayat, Q, Alyemeni, MN, Wani, AS, Pichtel, J and Ahmad, A 2012. Role of proline under changing environments: a review. *Plant Signal Behav*. 7, 1456–1466.
- [68] Shilina, NM 2009. Mechanisms of the antioxidant defense in children. *Vopr Pitan*. 78, 11-17.
- [69] Liang, X, Zhang, L, Natarajan, SK and Becker, DF 2013. Proline mechanisms of stress survival. *Antioxid Redox Signal*. 19, 998–1011.
- [70] Jubair, NA and Al-Dulaimy, AFZ 2023. Effect of spraying with micro-elements, gibberellic acid and salicylic acid on some yield traits of date palm cv. Khastawi. *Bionatura*. 8(1), 1-14.
- [71] Hoque, MA, Okuma, E, Banu, MNA, Nakamura, Y, Shimoishi, Y and Murata, Y 2007. Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. *J Plant Physiol*. 164, 553–561.
- [72] AL-Jaf, IHM, Al-Hayali, REY, Al-Dulaimy, AFZ and AL Miah, FHR 2023. Response of Cucumber plants to the addition of animal waste and dry yeast suspension., *Bionatura*. 8(1), 1-5.
- [73] Shraida, AS and Almohammadi, OHM 2021. Effect of salicylic acid and arginine spraying on growth and some of its active compounds of basil *Ocimum Basilicum* L. *IOP Conference Series: Earth and Environmental Science*. 761:(0120611).
- [74] Thakur, P and Nayyar, H 2013. Facing the cold stress by plants in the changing environment: sensing, signaling, and defending mechanisms, In: N. Tuteja and S. Singh Gill (Eds.) *Plant Acclimation to Environmental Stress*. New York, NY: Springer, pp. 29–69.
- [75] Al-Ealayawi, ZA and Al-Dulaimy, AFZ 2023. Response of Strawberry saplings Cv. Albion to spraying with Seaweed Extract, Arginine and Brassinolide. *IOP Conference Series: Earth and Environmental Science*., 1262(4), 042065.
- [76] Almohammadi, OHM, Al-Abdaly, MM, and Mahmood, SA 2019. Study of genotype and environment interaction on yield analysis of tuber of potato (*Solanum tuberosum* L.) using AMMI in Iraq. *Plant Archives*. 19(1), 978-982.
- [77] AL-Taey, D. K. A., S. S. M. AL-Azawi, M. J. H. AL-Shareefi, and A. R. AL-Tawaha. (2018). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (*Lactuca sativa* var. Parris Island) *Research on Crops*. 19 (3) : 441-449. DOI : 10.31830/2348-7542.2018.0001.14
- [78] Al-Jaf, IHM, Mubarak, AK, Al-Dulaimy, AFZ and ALTaey, DKA 2024. Variance estimation and genotypic, phenotypic correlation and broad heritability percentage in cucumber (*Cucumis sativus* L.). *IOP Conf. Series: Earth and Environmental Science*., 1371(042020), 1-8.
- [79] Szabados, L and Savoure, A 2010. Proline: a multifunctional amino acid. *Trends Plant Sci.*, 89.
- [80] Musleh, SH and Omar Almohammadi, HM 2023. Response of potato to organic fertilizer and zinc sulfate spraying on some growth characteristics and yield. *Revis Bionatura*. 8(2), 1-7.
- [81] Ghosh, U, Islam, M, Siddiqui, M, Cao, X and Khan, M 2022. Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms. *Plant Biology*. 24, 227–239.
- [82] Al-Dulaimy, AFZ, Abed, BM, Abd, GL and ALTaey, DKA 2024. Response of gazania (*Gazania splendens* L.) plants to foliar application with bio health and salicylic acid on some growth and flowering traits. *IOP Conf. Series: Earth and Environmental Science*., 1371(042007), 1-12.
- [83] Iqbal, N, Fatma, M, Khan, NA and Umar, S 2019. Regulatory role of proline in heat stress tolerance: Modulation by salicylic acid. In: M.I.R. Khan, Reddy P.S., Ferrante A. and Khan N.A. (Eds.) *Plant Signaling Molecules*. Amsterdam, The Netherlands: Elsevier. pp. 437–448.
- [84] Al-Enzi, AH Almohammadi, OH 2024. Effect of CPPU and Cobalt on the growth and productivity of two potato cultivars under field conditions. *Conference Series: Earth and Environmental Science*. 1371, 042009.



- [85] Alani, OMT and Al-Dulaimy, AFZ 2024. Effect of packaging type and calcium spraying on some chemical traits of seedless pomegranate variety Rawa. IOP Conf. Series: Earth and Environmental Science., 1371(042027), 1-10.
- [86] Giordano, M, Petropoulos, SA and Rouphael, Y 2021. Response and defence mechanisms of vegetable crops against drought, heat and salinity stress. Agriculture. 11, 463.
- [87] Almohammed, OHM, and Musleh, SH 2024. Response of potato to organic fertilizer and zinc spraying on some growth characteristics and yield. Anbar Journal of Agricultural Sciences. 22(1), 383-397.
- [88] Jonytiene, V, Burbulis, N, Kupriene, R and Blinstrubiene, A 2012. Effect of exogenous proline and de-acclimation treatment on cold tolerance in Brassica napus shoots cultured in vitro. Journal of Food, Agriculture & Environment. 10, 327–330.
- [89] Zalmeh, MT 2015. Stress Physiology. Agricultural Research Center, Seed Technology Research Division, Egypt.
- [90] Fang, YZ, Yang, S and Wu, G 2002. Free radicals, antioxidants, and nutrition. Nutrition.18(10),872-879.

Influence of Calcium Spraying and Packaging Type on Some Anatomical Characteristics of Seedless Pomegranate Fruits Rawa cv.

Omar M. T. Alani¹ and Ahmed F. Z. Al-Dulaimy²

¹Anbar Education Directorate, Ministry of Education, Anbar , Iraq.

²Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Anbar, Iraq.

¹E-mail: oma22g5004@uoanbar.edu.iq

²E-mail: ag.ahmed.fatkhan@uoanbar.edu.iq

Abstract. A study was conducted for research looked in to the effect of calcium spraying and fruit packaging on some anatomical characteristics of seedless Pomegranate fruit Rawa cv. The experiment comprised two factors: the first involved calcium spraying at concentrations of 0, 1, and 2 g L⁻¹. The second factor included covering the fruits with several kinds of bags, including (white paper bags, Saran, muslin clothes and untreated control). The results showed that spraying by calcium at the high concentration (2 g L⁻¹) attained the optimal values for traits (thickness of the epidermal and vascular bundle layers), at (21.4 µm, and 80.7 µm), respectively. Conversely, the level of 1 g L⁻¹ produced the best result for the thickness of the cuticle layers at 4.2 µm, on the other side the treatment (0 g L⁻¹) showed the highest value for the thickness of the fruit peel at 273.0 µm. Regarding fruit covering treatments significantly influenced all studied anatomical properties except for the thickness of the fruit peel, bagging with saran resulted in the best outcomes for the traits thickness of the epidermal and peel layers, the values recorded were 21.9 µm and 289.5 µm, respectively. Conversely, the treatment with muslin clothes resulted in the best outcomes for the cuticle and vascular bundle layers, with values of 4.7 µm and 63.7 µm, respectively.

Keywords. Pomegranate, Calcium Spraying, Packaging Type, Anatomical Characteristics.

1. Introduction

The pomegranate, *Punica granatum* L., is a sub-tropical (tropical) tree that grows to a height of five meters [1]. It is a shrub or deciduous tree with dense branches that give rise to numerous tumors that appear close to the earth's surface [2]. The East is thought to have been the pomegranate's original habitat [3], however some sources place Iran as its birthplace. From there, it expanded throughout the Arab world and the Mediterranean region. It traveled, and cultivation expanded throughout Afghanistan and India [4,5]. The high amount of sugar in pomegranate fruits-up to 16%, mostly in the form of monosaccharides-and protein-up to 1%-make them an excellent source of nutrients [6]. Citric acid, one of the organic acids, makes up between 0.5% and 2.0% of its weight, whereas water makes up about 75% of the fruit's weight [7,8].

Fruit covering with various materials, is a physical technique that enhances fruit quality, protects it from adverse weather, particularly in hot and dry regions, and establishes optimal conditions for successful pollination [9,10]. Additionally, it shields fruit from mechanical harm, birds, and

insects that could lower fruit quality and tree yield [11,12]. Numerous studies have demonstrated the potential benefits of employing various cover materials, such as paper, saran, polyethylene, and muslin fabric [13]. Moreover, based on the intended use of the cover, multiple colors are offered [14,15].

Calcium is one of the vital components that is crucial to the development of cell walls[16,17]. Since the calcium salt of the fatty substance lecithin contributes to the building of the membrane [18,19], calcium also has an impact on the formation of cellular membranes [20-22]. Since sugars and starches build up in plants growing in calcium-deficient settings, making them unable to take nitrate nitrogen, it appears that calcium is important for the absorption of nitrate nitrogen, albeit this is a constantly changing scenario[23-25]. In order to neutralize the organic acids generated in plant cells and prevent damage from an increase in the concentration of these acids, plants absorb it as calcium ions (Ca^{++}) [26-29]. This study looked into the effects of calcium applied topically and various fruit packaging methods on a few anatomical characteristics of the seedless pomegranate Rawa cv.

2. Materials and Methods

A study on seedless pomegranate trees of the Rawa cv. was carried out in the area of Rawa. The study examined the impact of calcium applied topically and fruit packing on a few anatomical characteristics of the peel fruit..

2.1. Treatments

- Spraying with calcium (D) as three concentrations (D0 0 g L⁻¹ as a control, D1 1 g L⁻¹ and D2 2 g L⁻¹.
- Fruit packaging (H) as four types, H0 as a control (non-packaged), H1 *Muslin Cloths*, H2 *Saran* and H3 *White paper bags*. Three times, calcium was sprayed: once at the onset of flower bud opening, once a month after the first spray, and once again a month after the second spray.

2.2. Experimental Design

A randomized complete block design (RCBD) was used to conduct a factorial experiment with three components (4 x 3). With one tree for each of the twelve treatments and three replications that made up the experimental units. The Genstat statistical program was used to statistically analyze the data and compare the averages using the least significant difference (L.S.D.) test at a probability threshold of 5% [30]

2.3. Studied Traits

The following components of the fruit peels were measured during the experiment:
thickness of the cuticle layers, thickness of the epidermal layers, thickness of the cortex layers and thickness of the vascular bundle layers [31] .

3. Results and Discussion

3.1. Thickness of the Cuticle Layers

The findings displayed in Figure (1) suggest that the thickness of the peel fruit's cuticle layers was significantly impacted by fruit covering procedures, the treatment (H2) recorded the lowest value at 3.3 μm , while treatments (H3) produced the greatest value at 4.7 μm . Conversely, Table (1) showed that applying a calcium spray to fruits had a noteworthy impact, with treatment (D1) registering the maximum value at 4.2 μm , Treatment (D2) at 4.0 μm comes next, in contrast, the untreated control (D0) had the lowest value, measuring 3.6 μm . The interaction between the

research parameters resulted in significant changes in these qualities, as evidenced by the results, In particular, treatment (D1H3) produced the greatest value at 5.1 μm . Conversely, the control treatment (D0H0) had the lowest value at 2.6 μm .

3.2. Thickness of the Epidermal Layers

The results displayed in Figure (1) showed that the thickness of the peel fruit's epidermal layers was significantly impacted by fruit covering procedures, the treatment (H2) recorded the highest value at 21.9 μm , while treatment (H0) produced the lowest value at 17.6 μm . Conversely, applying a calcium spray to trees had a noteworthy impact, with treatment (D2) registering the maximum value at 21.4 μm , Treatment (D0) at 20.0 μm comes next, Conversely, Table (1) showed that the treatment (D1) had the lowest value at 16.6 μm . In particular, treatment (D2H2) produced the greatest value at 26.6 μm . on the other hand the treatment (D1H1) had the lowest value at 15.3 μm .

3.3. Thickness of the Cortex Layers

The Figure (1) suggest that the thickness of the peel fruit's cortex layers was significantly impacted by fruit packing procedures, the treatment (H2) recorded the highest value at 289.5 μm , while treatments (H1) produced the lowest value at 245.4 μm . On the other side, Table (1) showed that applying a calcium spray to fruits had a significant impact, with treatment (D0) registering the maximum value at 273.0 μm , followed by treatment (D2) at 266.7 μm , in contrast, the treatment (D1) had the lowest value, measuring 252.4 μm . The interaction between the research parameters resulted in significant changes in these qualities, as evidenced by the results, In particular, treatment (D2H2) produced the greatest value at 315.7 μm . Conversely, the control treatment (D2H1) had the lowest value at 233.4 μm .

3.4. Thickness of the Vascular Bundle Layers

The findings displayed in Figure (1) suggest that the thickness of the peel fruit's vascular bundle layers was significantly impacted by fruit covering procedures, the treatment (H3) recorded the highest value at 63.7 μm , while treatments (H2) produced the lowest value at 50.8 μm . Conversely, Table (1) showed that spraying with calcium to fruits had a significant effect, with treatment (D2) registering the maximum value at 80.7 μm , Treatment (D0) at 49.5 μm comes next, Conversely, the untreated control (D1) had the lowest value, measuring 42.9 μm . The interaction between the two factors resulted in significant changes in these trait, as evidenced by the results, In particular, treatment (D2H1) produced the greatest value at 98.4 μm . On the other side, the control treatment (D1H1) had the lowest value at 28.0 μm .

Table 1. Effect of packaging type and calcium spraying interaction on some anatomical characteristics of seedless Pomegranate fruit peel Rawa cv.

(D) Spraying with calcium (g L ⁻¹)	(H) packaging type	Thickness of the cuticle layers (μm)	Thickness of the epidermal layers (μm)	Thickness of the cortex layers (μm)	Thickness of the vascular bundle layers (μm)
D0 (0 g L ⁻¹)	H0 Control (Non-packaged)	2.6	19.3	255.6	49.0
	H1 (Muslin Cloths)	3.3	22.6	263.9	51.9
	H2 (Saran)	3.5	21.3	303.3	34.3
	H3 (White paper bags)	4.8	16.8	269.0	62.9

(D) Spraying with calcium (g L ⁻¹)	(H) packaging type	Thickness of the cuticle layers (μm)	Thickness of the epidermal layers (μm)	Thickness of the cortex layers (μm)	Thickness of the vascular bundle layers (μm)
D1 (1 g L ⁻¹)	H0 Control (Non-packaged)	4.5	17.7	252.9	58.9
	H1 (<i>Muslin Cloths</i>)	3.9	15.3	239.0	28.0
	H2 (Saran)	3.3	17.9	249.4	44.4
	H3 (White paper bags)	5.1	15.5	268.1	40.2
D2 (2 g L ⁻¹)	H0 Control (Non-packaged)	4.6	16.0	252.2	62.6
	H1 (<i>Muslin Cloths</i>)	4.2	16.8	233.4	98.4
	H2 (Saran)	3.2	26.6	315.7	73.8
	H3 (White paper bags)	4.1	26.3	265.6	88.1
LSD _{5%}		0.35	3.64	3.76	6.31

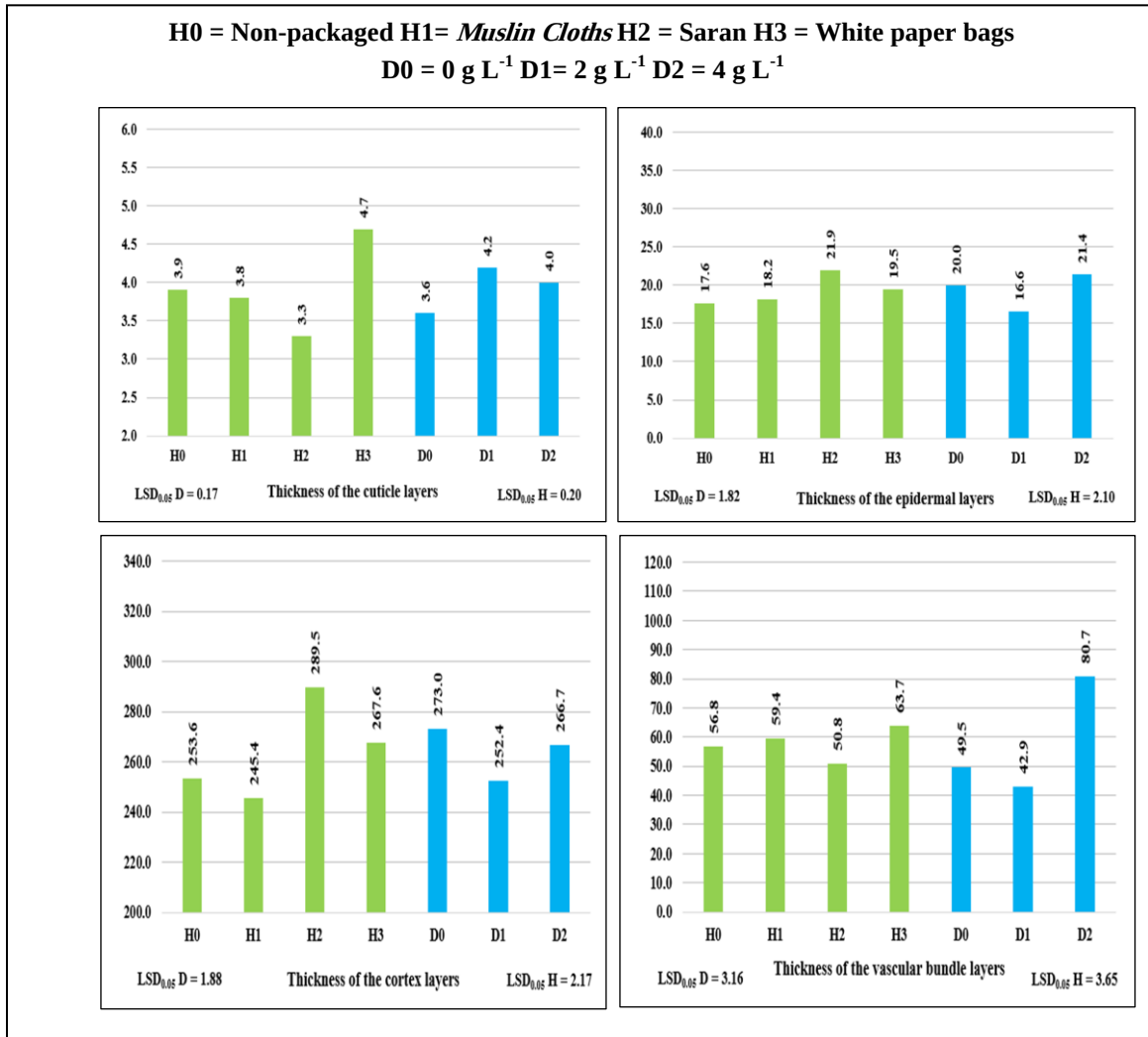


Figure 1. Effect of calcium spraying and packaging type on some anatomical characteristics of seedless Pomegranate fruit peel Rawa cv.

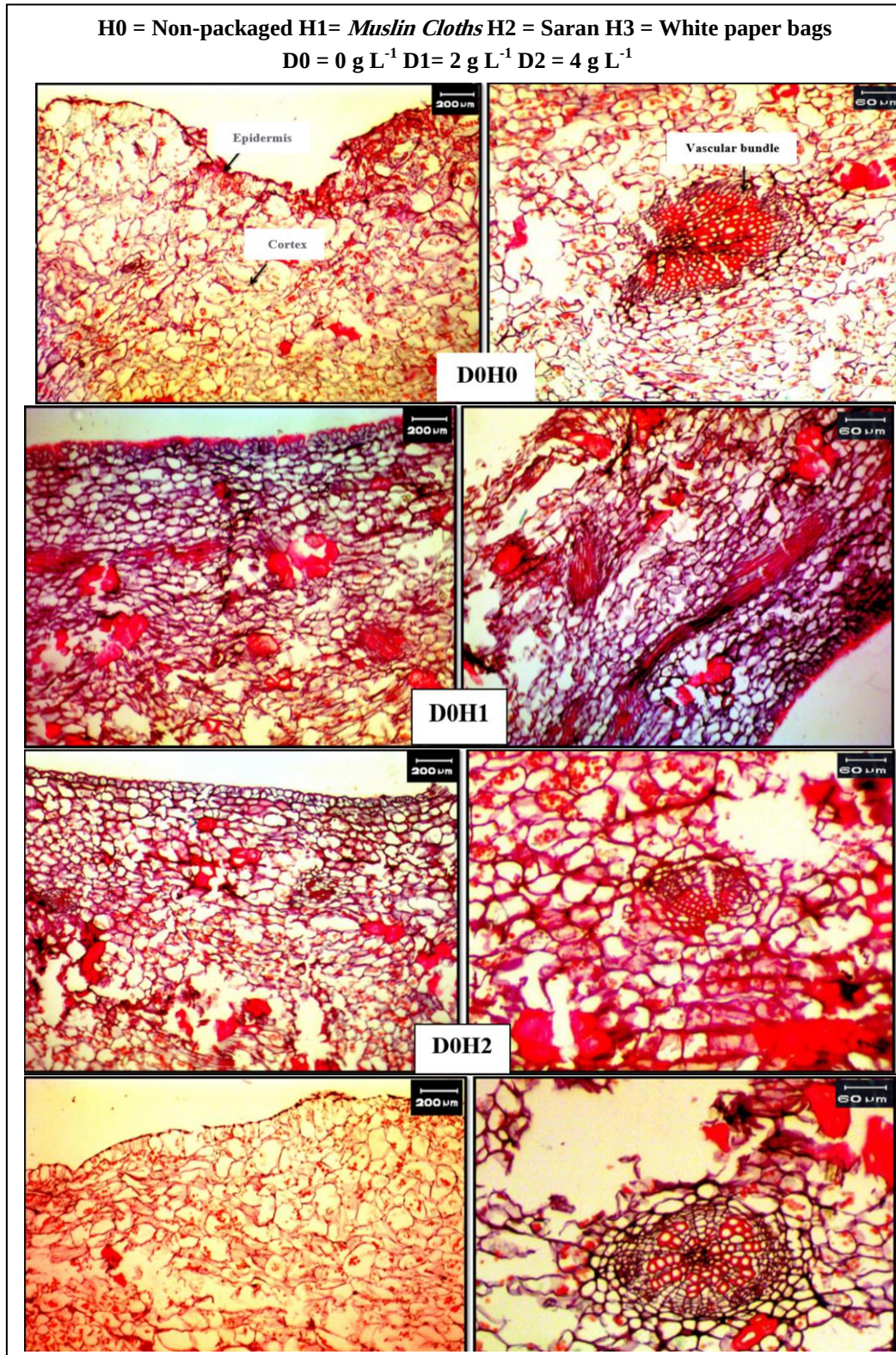


Figure 2. Effect of packaging type and calcium spraying on some anatomical traits of fruit peel.

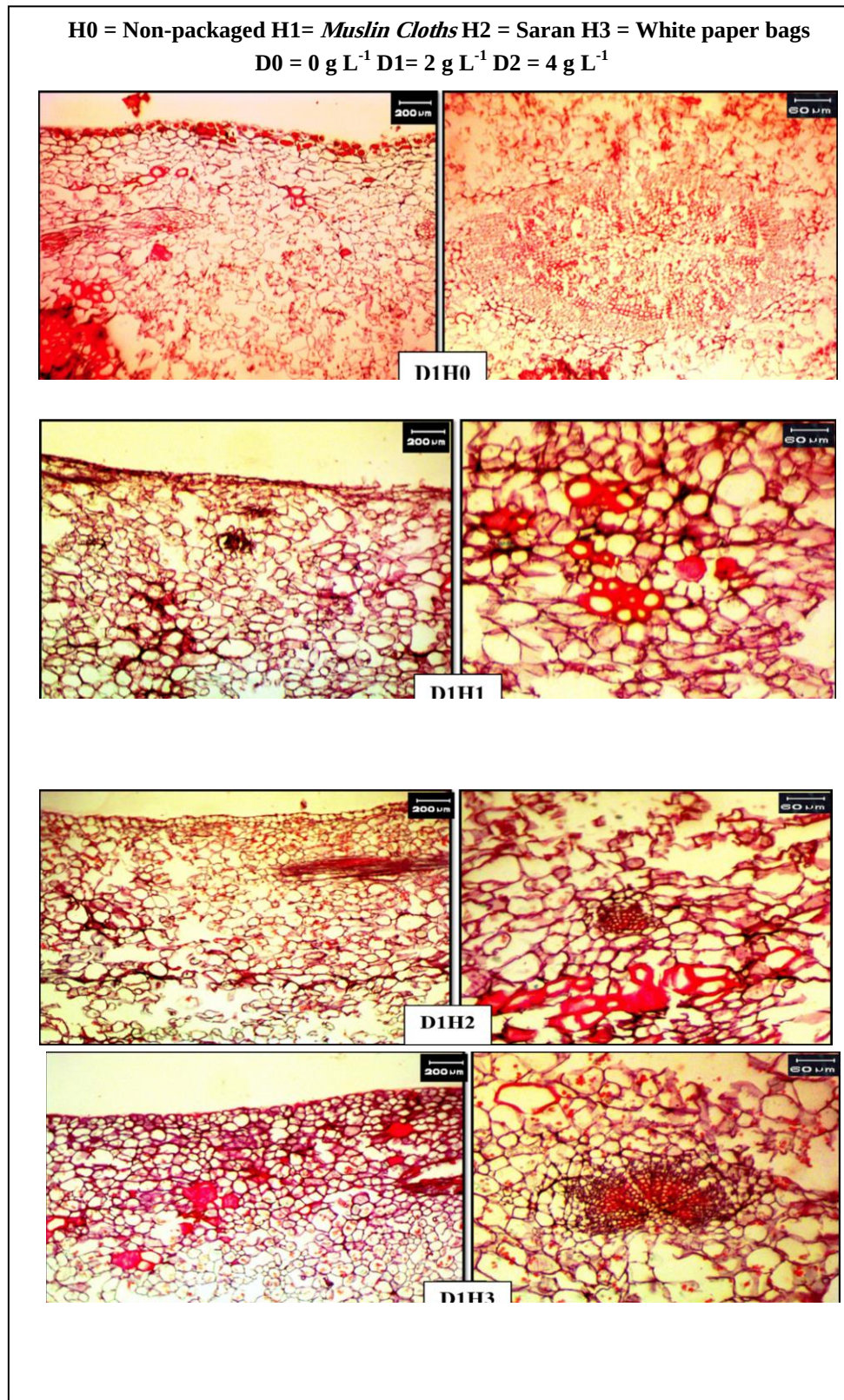


Figure 3. Effect of packaging type and calcium spraying on some anatomical traits of fruit peel.

H0 = Non-packaged H1= *Muslin Cloths* H2 = Saran H3 = White paper bags
D0 = 0 g L⁻¹ D1= 2 g L⁻¹ D2 = 4 g L⁻¹

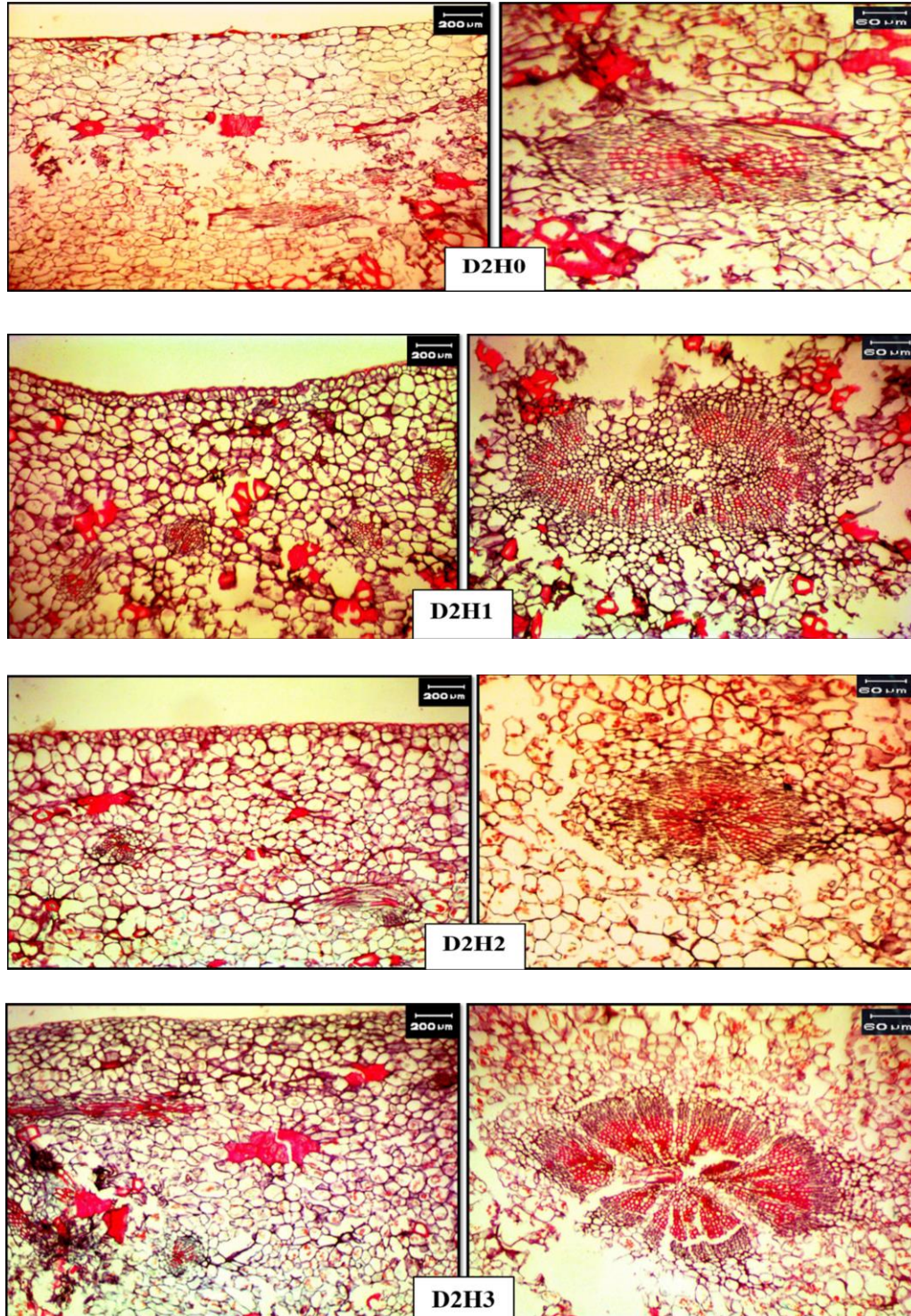


Figure 4. Effect of packaging type and calcium spraying on some anatomical traits of fruit peel.

Fruit packaging treatments contributed improving the anatomical characteristics of the fruit peel, the fruits were largely shielded from outside environmental forces by the covering [32-34], which decreased the rate at which the fruits lost water [35,36]. It also stopped photooxidation from happening, which led to higher levels of internal hormones that speed up growth and improve the absorption of a lot of nutrients-some of which are transmitted to the fruits [37].

In the middle lamella, where it exists as calcium pectate, calcium is an essential component and plays a critical role in the creation of cell structures [38,39]. Furthermore, calcium activates a wide range of enzyme systems that support multiple metabolic processes and carry out essential biochemical tasks that promote the healthy growth of plants [40,41]. Additionally, it has been shown that plant tissue cells with severe calcium deficiencies leak more low-molecular-weight solutes, which contributes to the collapse of cell membranes and the reduction of cell division activity [42,43]. By increasing the amount of chlorophyll, leaf area, and water absorption, calcium also plays a critical role in boosting tree nutrition. This increases the amount of major and minor nutrients that are present in the fruit [44,45]. Furthermore, calcium helps prevent damage from water stress by promoting the transfer of carbohydrates, creating calcium pectate in cell walls, and increasing the permeability of cellular walls, which permits nutrients and water-including potassium-to pass from leaves to fruits [46,47].

Conclusions

One significant method for successfully improving the fruit's quality and look is to cover the fruit while it grows and develops on the tree. This technique is important since it allows you to manage the fruit's environment, including humidity, temperature, and light intensity. As a result, it is essential for both biotic and abiotic resistance, boosting root and vegetative development characteristics and facilitating the absorption of greater amounts of nutrients produced by photosynthesis in the leaves. Fruits and other plant components store the excess of these nutrients. Furthermore, applying calcium spray on pomegranate trees has helped to raise the fruit's nutritional value.

References

- [1] Hassan, TA 1998. Fruit trees in Arab countries. Its cultivation, its types, its service, and its benefits. Dar Aladdin for publishing, distribution and translation. First edition. Damascus. Syria: 237.
- [2] Alani, OMT and Al-Dulaimy, AFZ 2024. Effect of packaging type and calcium spraying on some chemical traits of seedless pomegranate variety Rawa. IOP Conf. Series: Earth and Environmental Science., 1371(042027), 1-10.
- [3] Al-Issa, I, Hamed, F, and Bathha, M 2012. Fruit production. Damascus University Press. Syria: 325.
- [4] Al-Dulaimy, AF and Jumaa, FF 2020. Effect of foliar spray with yeast suspension, licorice roots extract and amino Quelant-K compound on chemical content of Black Hamburg grape cultivar berries. Diyala Agricultural Sciences Journal., 12(Special Issue), 546–557.
- [5] Gratiou, RR, Selvasubramanian, S and Jayasaundar, S 2001. Immunomodulatory activity of *Punica granatum* in rabbits, a preliminary study. J. Ethnopharmacology., 78(1), 85-87.
- [6] Afaq, F, Saleem, M and Mukhtat, H 2003. Pomegranate fruit extract is evolve agent for cancer chemoprevention . Studies in mouse skin 2nd Annual AACR . Int. Conf. on frontiers in cancer prevention Res., Biomar., 12, 1351s. part 2. Suppl. S.
- [7] Al-Dulaimy, AFZ, Al-Issawi, MHE, Alani, MHI, and Jubair, NA 2020. Effect of pulp extract and peels powder of Banana in growth and yield of Cucumber cv. Rola. Biochem. Cell. Arch., 20(1), 2221-2227.

- [8] Glozer, K and Ferguson, L 2008. Pomegranate Production in Afghanistan.(UCDAVIS). Department of Plant Sciences. College of Agriculture Environmental Sciences.
- [9] Al-Janabia, AMI, Al-Dulaimy, AFZ, Sekhi, YS, Almohammed, OHM and Al-Taey, DKA 2024. Effect of salt stress on growth and yield of plants: A Review. *IOP Conf. Series: Earth and Environmental Science.*, 1371(042028), 1-16.
- [10] Sharma, R, Reddy, SVR, Jhalegar, MJ 2014. Pre-harvest fruit bagging: A useful approach for plant protection and improved post-harvest fruit quality-A review. *J. Hortic. Sci. Biotechnol.*, 89, 101-113.
- [11] Al-Dulaimy, AFZ, Alalaf, AH, Al-Hayali, REY and ALTaey, DKA 2023. Flowers and fruits abortion in fruit trees ... Causes and solutions: A review. *IOP Conference Series: Earth and Environmental Science.* 1158(4), 042010.
- [12] Sharma, RR, Nagaraja, A, Goswami, AK, Thakre, M, Kumar, R, Varghese, E 2020. Influence of on-the-tree fruit bagging on biotic stresses and postharvest quality of rainy-season crop of 'Allahabad Safeda' guava (*Psidium guajava* L.). *Crop Prot.*, 135, 105216.
- [13] Al-Jaf, IHM, Mubarak, AK, Al-Dulaimy, AFZ and ALTaey, DKA 2024. Variance estimation and genotypic, phenotypic correlation and broad heritability percentage in cucumber (*Cucumis sativus* L.). *IOP Conf. Series: Earth and Environmental Science.*, 1371(042020), 1-8.
- [14] Issarakraisila, M 2018. Effect of types of bagging materials on growth, quality and disease-insect damages in pummelo fruit in tropical humid conditions. *Acta Hortic.*, 1208, 319-324.
- [15] Murad, HJ and Al-Dulaimy, AFZ 2021. Response of date palms cv. Zahdi to foliar spray with urea and seaweed extract. *IOP Conference Series: Earth and Environmental Science.* 761(1): 012052.
- [16] White, P.J. 2000. Calcium channels in higher plants. *BBA- Bio membranes*, 1465(1-2):171-189.
- [17] Alani, MHI, Al-Dulaimy, AFZ, Sekhi, YS, Almehemdi, AF 2021. Chemical analysis of Fig transplants cv Wazeri under with cutting type and humic acid. *IOP Conference Series: Earth and Environmental Science.* 761(1), 012032.
- [18] Fageria, VD 2001. Nutrient interactions in crop plants. *J. Plant Nutr.*, 24(8),1269-1290
- [19] Al-Dulaimy, Alani, AFZ, MHI, Awad, IM, Khudair, RIA, Salim, HA, and Jasem, SM, 2021. Response of *Gazania* plants (*Gazania Splendens* L.) to seaweed extract (Tecamin Algae) and nano stimulator (Proteck CalBor). *IOP Conference Series: Earth and Environmental Science.*, 910(1), 012052.
- [20] Drake, RS 2008. Calcium sprays: better fruit. Healthier trees. <http://www.tfrec.wsu.edu>.
- [21] Salisbury, F and Ross, C 2000. Plant physiology 1. Cells: water, solutions and surfaces. Trad. por J. M. Alonso. Thomson Internat. Madrid, Spain.
- [22] Mahmood, MM and Al-Dulaimy, AF 2022. Effect of media culture, nano and normal micronutrients on some flowers and yield traits of Strawberry. *AIP Conference Proceedings.*, 2394(1), 1-13.
- [23] Mengel, K and Kirkby, EA 2000. Principles of plant nutrition. BaselSwitzerland: International PotashInstitute.
- [24] White, PJ and Broadley, MR 2003. Calcium in Plants- Review. *Annals of Botany* 92, 487-511.
- [25] Murad, HJ and Al-Dulaimy, AF 2021. Effects of spraying with urea and seaweed extract (Tecamin Algae) on growth and chemical content of date palm tree cv. Zahdi. *IOP Conference Series: Earth and Environmental Science.* 904(1), 012065.
- [26] Azcón-Bieto, J and Talón, M 2000. Fundamentals of plant physiology. McGraw-Hill American. Barcelona, Spain.
- [27] Hepler, PK 2005. Calcium: a central regulator of plant growth and development. *Plant Cell.*, 17, 2142-2155.
- [28] El Habbasha, SF and Ibrahim, FM 2015. Calcium: Physiological Function, Deficiency and Absorption. *International Journal of ChemTech Research.*, 8(12), 196-202.
- [29] Mahmood, MM and Al-Dulaimy, AF 2021. Response of Strawberry cv. Festival to culture media and foliar application of nano and normal micronutrients. *IOP Conference Series: Earth and Environmental Science.* 904(1), 012067.

- [30] Al-Mehmedi, S, and Al-Mehmedi MFM (2012). "Statistics and Experimental Design". Dar Usama for publishing and distributing. Amman- Jordan., 376 pp.
- [31] Johanson, DA 1968. Plant Microtechnique. McGraw-Hill Book company.
- [32] Rajametov, S, and Nurbekov, A 2020. Effects of different types of paper bags on 'Golden Delicious' apple fruits. *Acta Hort.*, 1275, 415-418.
- [33] Al-Ealayawi, ZA and Al-Dulaimy AFZ 2023. The impact of spraying Marine Algae Extract, Arginine, and Brassinolide on vegetative growth traits of Albion Strawberry saplings. *IOP Conference Series: Earth and Environmental Science*. 1262(4), 042061.
- [34] Al-Dulaimy, AFZ, Abbood, AA and Alian, AT 2022. Effect of spraying with normal and nano Iron on the growth and flowering of Petunia plant Petunia hybrid L. *IOP Conference Series: Earth and Environmental Science*., 1060 (1), 012033.
- [35] Reddy, VS and Reddy ASN 2004. Proteomics of calcium-signaling components in plants. *Phytochemistry*., 65, 1745-1776.
- [36] Bothwell, JHF and Ng, CKY 2005. The evolution of Ca^{2+} signalling in photosynthetic eukaryotes. *New Phytol.*, 166, 21-38.
- [37] Jubair, NA and Al-Dulaimy, AFZ 2023. Response of date palm cv. Khastawi to foliar spray with micro-elements, gibberellic acid and salicylic acid. *IOP Conference Series: Earth and Environmental Science*., 1158(4), 042063.
- [38] Al-Ealayawi, ZA and Al-Dulaimy, AFZ 2023. Marine algae and applications to plant nutrition: A review. *IOP Conference Series: Earth and Environmental Science*. 1158(4), 042004.
- [39] Hardan, ME and Al-Dulaimy, AF 2022. Effect of humic acid addition and spraying with ginger rhizome extract on the growth and some chemical contents of apricot seedlings *Prunus armeniaca* L. cv. Bionatura. 7(4), 1-6.
- [40] Al-Hayali, REY, AL-Jaf, IHM, AL Miah, FHR and Al-Dulaimy, AFZ 2023. Evaluation of orange and biofertilizer on some yield traits of Cucumber (*Cucumis sativus* L.). *Bionatura*., 8(1), 1-5.
- [41] Jubair, NA and Al-Dulaimy, AFZ 2023. Effect of spraying with micro-elements, gibberellic acid and salicylic acid on some yield traits of date palm cv. Khastawi. *Bionatura*. 8(1), 1-14.
- [42] Lara, I, Garcia, P, Vendrell, M 2004. Modifications in cell wall composition after cold storage of calcium-treatment strawberry (*Fragaria × ananassa* Duch.) fruit. *Postharvest Biology and Technology*., 34, 331-339.
- [43] Al-Dulaimy, AFZ, Abed, BM, Abd, GL and ALTaey, DKA 2024. Response of gazania (*Gazania splendens* L.) plants to foliar application with bio health and salicylic acid on some growth and flowering traits. *IOP Conf. Series: Earth and Environmental Science*., 1371(042007), 1-12.
- [44] Taiz, L, and Zeiger, E 2006. *Plant physiology*. 4th ed. Sinauer Associates, Inc., publishers Sunderland, Massachusetts.
- [45] AL-Jaf, IHM, Al-Hayali, REY, Al-Dulaimy, AFZ and AL Miah, FHR 2023. Response of Cucumber plants to the addition of animal waste and dry yeast suspension., *Bionatura*. 8(1), 1-5.
- [46] Al-Dulaimy, AFZ, Al-Janabi, AMI, Abd, NT and ALTaey, DKA 2024. Fruit pome cracking, causes and solutions: A review. *IOP Conf. Series: Earth and Environmental Science*., 1371(042025), 1-11.
- [47] Al-Ealayawi, ZA and Al-Dulaimy, AFZ 2023. Response of Strawberry saplings Cv. Albion to spraying with Seaweed Extract, Arginine and Brassinolide. *IOP Conference Series: Earth and Environmental Science*., 1262(4), 042065.

Effect of Spraying with Arginine and Indole Acetic Acid on Growth and Flowering of Marigold *Tagetes erecta* L.

Anmar K. Alalwani¹, Thamer M. Badawi², Ahmad Kamis³, Ahmed F. Z. Al-Dulaimy⁴, Idrees H. M. Al-Jaf⁵ and D. K. A. Al-Taey⁶

¹Department of Biotechnology, College of Science, University of Anbar, Iraq

²Directorate of Education in Anbar, Ministry of Education, Iraq

³Department of Biology, College of Science, University of Anbar, Iraq.

^{4,5}Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Anbar, Iraq.

⁶Department of Horticulture and Landscape, College of Agriculture, University of Al-Qasim Green, Babylon, Iraq.

¹E-mail: anmar_kamil@uoanbar.edu.iq

²E-mail: Thamer_ma1980@yahoo.com

³E-mail: yyazen780@uoanbar.edu.iq

⁴E-mail: ag.ahmed.fatkhan@uoanbar.edu.iq

⁵E-mail: idresshussein@uoanbar.edu.iq

⁶E-mail: duraidaltaey@gmail.com

Abstract. This research looked at what happened to marigold plants grown in a protected area during the 2023 growing season when amino acid (arginine) and indole acetic acid (IAA) were sprayed on them and how they affected growth, flowering, and chemical parameters. We studied two factors during the experiment: spraying arginine at concentrations of 100 and 200 mg L⁻¹ and spraying IAA at concentrations of 50, 100, and 150 mg L⁻¹. Additionally, we sprayed distilled water as the control treatment for both factors. Amino acid (arginine) spraying significantly impacted all of the examined characteristics, except flower weight. At a concentration of 200 mg L⁻¹, we obtained the greatest results for plant height, branch number, flower number, flower diameter, total chlorophyll, total carbohydrates, nitrogen, phosphorous, and potassium. The values were (43.08 cm, The values were 43.08 cm, 5.73 branch plant⁻¹, 6.97 flower plant⁻¹, 6.16 cm, 44.50 mg 100 gm⁻¹, 5.10%, 1.96%, 0.41%, and 1.78%, respectively. (IAA) spraying treatments significantly impacted all the attributes examined, except for branch number. The 100 mg L⁻¹ treatment yielded the highest values for the traits, including plant height, flower number, flower diameter, total chlorophyll, total carbohydrates, and potassium. The values were (42.17 cm, 7.29 flower plant⁻¹, 6.50 cm, 43.74 mg 100 gm⁻¹, 5.03%, and 1.72%), respectively. In contrast in contrast, the treatment of 150 mg L⁻¹ yielded the best results for the traits of nitrogen and phosphorous, with values of 1.93% and 0.38%, while the treatment of 50 mg L⁻¹ achieved the highest value for the trait of flower weight, reaching Additionally, the results showed significant differences in all the studied traits, except for branches number and flower weight. This was attributed to the interaction between the research components, particularly N2M2, which produced the highest values, despite the fact that the control group, N0M0, obtained the lowest values for most traits.

Keywords. Marigold, *Tagetes erecta*, Arginine, IAA, Growth, Flowering.

1. Introduction

The Marigold plant is a member of the Asteraceae family and is native to Mexico, which is located in the southern United States west of Arizona. It is grown in most nations across the world. Additionally, it is a medium-height herbaceous plant that grows from early October to January across the majority of Iraq's governorates [1,2]. It has strong stems and lanceolate leaves with a serrated edge. Propagated by seeds, these flowers are feathery or rectangular, with colors ranging from yellow to orange. planted successfully in locations with sunlight [3,4].

One of the most significant and important commercial flower crops, African marigold is extensively cultivated for the production of loose flowers [5,6]. Because the flower petals of this ornamental plant species contain a high concentration of carotenoids and phenolics, it is recognized to have medicinal uses [7,8]. Due to its wide range of appealing colors, shape, and good keeping quality, it is well-liked all across the world. Marigold's year-round production, wide range of adaptability, and ease of cultivation have contributed to its growing popularity [9,10]. Its flower petals are employed for more than just aesthetics; they also produce xanthophyll, a significant carotenoid portion that makes about 80–90% of lutein [11,12]. Due to the flowers' extensive adaptability to a wide range of soil and climatic conditions and their lengthy blossoming period, they are often employed in religious ceremonies and social gatherings [13,14].

Particularly when sprayed on plants as nutritional solutions, amino acids are naturally occurring chelating substances with low molecular weights that are highly soluble in water and readily pass through cell membranes [15,16,17]. As a result, they may have an impact on physiological processes that result in the synthesis of fundamental substances including proteins, carbohydrates, lipids, vitamins, and others, either directly or indirectly [18,19,20]. Additionally, greater pollen germination and pollen tube elongation are facilitated by amino acids, increasing the proportion of fruit set [21,22]. Additionally, they are crucial for enhancing plant health and vegetative growth, which strengthens the plants' defenses against illness, pests, and stress [23,24].

Plant growth regulators function to either stimulate or inhibit vegetative growth, flowering, fruiting, or any other physiological process in plants, hence contributing to the regulation of physiological processes in plants [25,26]. When administered in small amounts to certain plant tissues in biological experiments, auxins-plant hormones-promote the growth of cell elongation. In addition to IAA, the most common auxin, plants have been discovered to synthesize many other natural auxins. Naturally occurring in plants are both conjugated auxins and free acids [27,28]. Cell elongation and organ creation or development are caused by auxins [29,30], in addition to the way it helps plants new roots [31,32]. Research on using environmentally benign compounds, such as indole acetic acid, to treat ornamental plants to promote their flowering growth has been carried out in several countries, with some degree of success attained [33]. In small quantities, plant growth regulators (PGRs) either stimulate, inhibit, or quantitatively alter growth and development, and they are crucial to the creation of flowers. For the reasons mentioned above, this experiment was carried out to study the effect of spraying arginine, and IAA on growth, flowering and chemical parameters of Marigold plant.

2. Materials and Methods

2.1. Experiment Site

This study was conducted in the lath house of the Hort. Depart. at the Coll. of Agric., Univ. of Anbar, to study the impact of spraying with amino acid (arginine), and Indole acetic acid (IAA) and their interaction on growth, flowering and chemical parameters of Marigold. Table 1 shows some of properties of the soil.

Table 1. Some chemical and physical properties of agricultural soil.

pH	EC ds m ⁻¹	O.M %	Bulk density g cm ⁻³	CaCO ₃ g Kg ⁻¹	Total N %	P Av. mg Kg ⁻¹
7.57	3.94	1.68	1.35	147.62	0.38	1.21
K Av. mg Kg ⁻¹	Ca ⁺⁺ Mq L ⁻¹	Mg ⁺⁺ Mq L ⁻¹	Na ⁺ Mq L ⁻¹	CO ₃ ⁼ Mq L ⁻¹	HCO ₃ ⁼ Mq L ⁻¹	Cl ⁻ Mq L ⁻¹
126.75	28.84	43.17	0.70	Nil	4.31	36.82
SO ₄ ⁼ Mq L ⁻¹	Sand g Kg ⁻¹	Silt g Kg ⁻¹	Clay g Kg ⁻¹	Texture		
29.13	438.5	291.0	270.5	Sandy Clay Loam		

2.2. Treatments

- Spraying with the amino acid arginine (N) at three concentrations: N0 (spraying with distilled water), N1 (100 mg L⁻¹), and N2 (200 mg L⁻¹).
- Spraying with the growth regulator Indole acetic acid IAA (M) at four concentrations: M0 (spraying with distilled water), M1 (50 mg L⁻¹), M2 (100 mg L⁻¹) and M3 (150 mg L⁻¹).

The plants were sprayed with a 2-liter sprayer until they were thoroughly covered. In order to minimize the surface tension of the spray solution molecules and enable the plants to absorb the most of the sprayed solutions, liquid soap was added as a dispersant at a rate of 0.1 ml L⁻¹. The research components were sprayed on the plants twice: once after the appearance of four to five pairs of true leaves, and again a month later, with a two-day gap between each spraying. In order to stop the spray solutions from transferring between the treatments, a mobile barrier was erected before the spraying operations began in the early morning.

2.3. Experimental Design

A randomized complete block design was used to conduct a two-factors. Twelve treatments total, spread across three blocks, with five plants in each experimental unit, made up the experiment. After statistical analysis of the data, the means were compared at the 5% level of significance using the least significant difference (LSD) test [34]. The Genstat software was used to evaluate the data. The following traits were studied: Plant height (cm), Branches number (branch plant⁻¹), Flowers number (flowers plant⁻¹), Flower diameter (cm), Flower weight (g), Total chlorophyll content in leaves (mg 100 gm⁻¹), [35] Total carbohydrates content in leaves (%) [36] and macrolelements content in leaves including nitrogen [37], phosphorous [38], potassium [39].

3. Results and Discussion

3.1. Growth Traits

3.1.1. Plant Height (cm)

The findings show that arginine spraying was a significant factor in the variations in plant height (Figure 1). The no-spray treatments (N0) had the lowest value, measuring 35.10 cm, while the treatments (N2) had the greatest values, measuring 43.08 cm. However, foliar spraying with the growth regulator IAA had a notable impact, particularly in treatment (M2), which had the greatest value at 42.17 cm, and treatment (M0), which had the lowest value at 37.67 cm. Table 2 shows that there was a significant two-way interaction between arginine and IAA. The treatment (M2N2) resulted in the highest plant height at 49.12 cm, whereas the control treatment (M0N0) produced the lowest value at 34.09 cm.

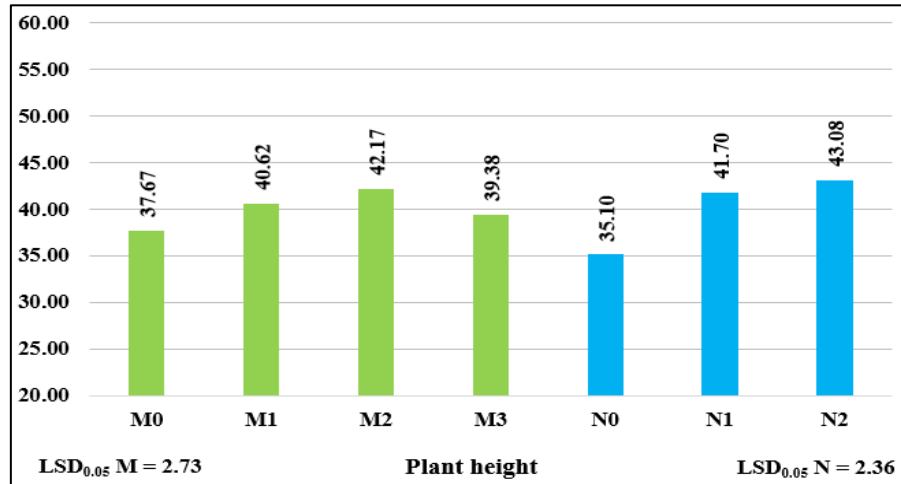


Figure 1. Effect of spray with Arginine and Indole acetic acid (IAA) on plant height (cm) of Marigold.

3.1.2. Branches Number (*branch plant⁻¹*)

Figure's 2 findings demonstrate that arginine spraying had a noteworthy impact on the number of branches, treatment (N2) had the highest values, reaching 5.73 branch plant⁻¹, while treatment (N0) had the lowest value, 4.87 branch plant⁻¹. In contrast, the foliar spraying with the growth regulator (IAA) and two-way interaction did not reach a significant level of influence on the studied trait (Table 2).

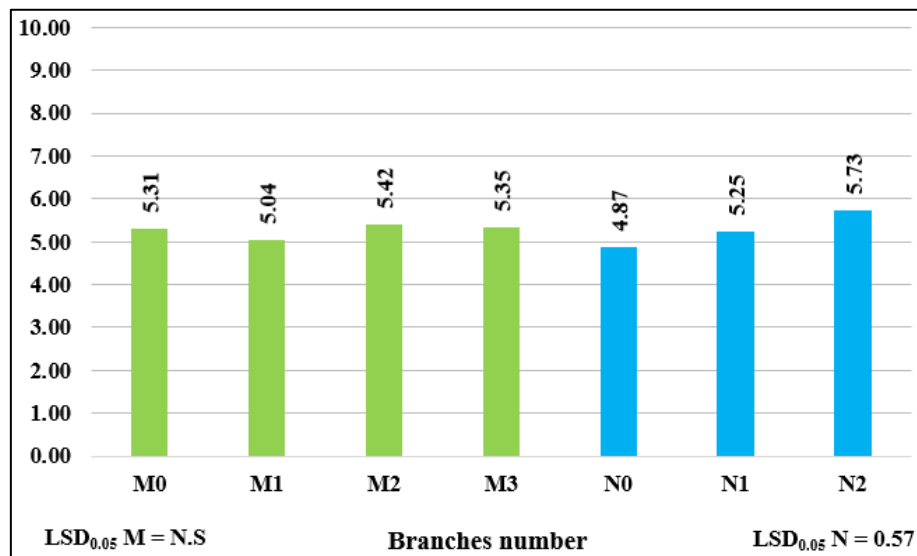


Figure 2. Effect of spray with Arginine and Indole acetic acid (IAA) on branches number (branch plant⁻¹) of Marigold.

3.2. Flowering Traits

3.2.1. Flowers Number (flowers plant⁻¹)

As shown in Figure 3, the results show that foliar spraying with arginine significantly affected the number of flowers, treatment (N2) had the maximum content, recording 6.97 flower plant⁻¹, while treatment (N0) had the lowest value, 5.47 flower plant⁻¹. Reaching 7.29 flower plant⁻¹, treatment (M2) had the highest value for spraying growth regulator (IAA), treatment (M0) had the lowest value, with 5.92 flower plant⁻¹. The results indicate that there was a two-way interaction between the growth regulator and amino acid at treatments (M2N2) of 8.19 flower plant⁻¹, whereas treatment (M0N0) produced the fewest flowers at 5.08 flower plant⁻¹ (Table 2).

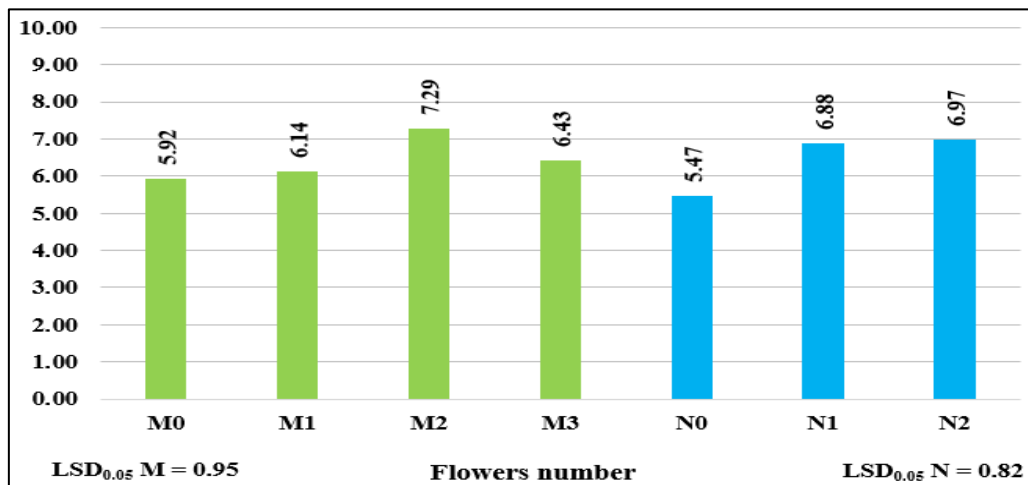


Figure 3. Effect of spray with Arginine and Indole acetic acid (IAA) on flowers number (flower plant⁻¹) of Marigold.

3.2.2. Flower Diameter (cm)

Figure 4 illustrates the considerable impact of arginine spraying treatments on flower diameter. Treatment (N2) exhibited the maximum value of 6.16 cm, whereas treatment (N0) displayed the lowest value of 5.62 cm. The IAA spray demonstrated a noteworthy impact, with treatment (M2) exhibiting the maximum value of 6.50 cm and treatment (M0) displaying the lowest value of 5.07 cm. A significant two-way interaction was found between arginine and IAA; treatment (M2N3) produced the highest plant height, measuring 49.12 cm, whereas treatment (M0N0) produced the lowest value, measuring 34.09 cm (Table 2).

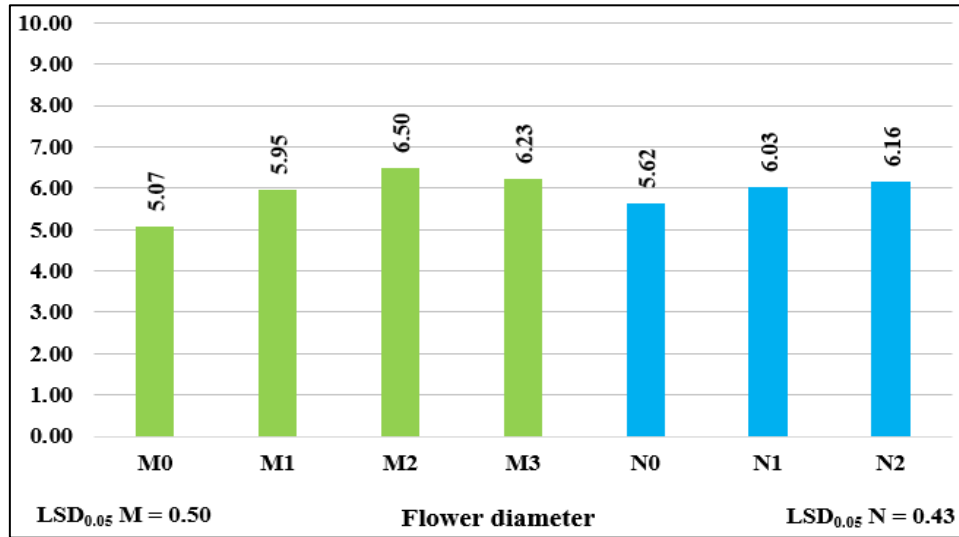


Figure 4. Effect of spray with Arginine and Indole acetic acid (IAA) on Flower diameter (cm) of Marigold.

3.2.3. Flower Weight (gm)

The foliar spraying with the amino acid (arginine) did not reach a significant level of influence on the flower weight (Figure 5). The studied trait was significantly affected by IAA spraying, according to the data, treatment (M1) had the highest levels of influence, reaching 0.86 gm, while treatment (M0) had the lowest value, 0.79 gm. On the other hand, the results indicated no significant differences in the flower weight due to the interaction between the two factors of the study (Table 2).

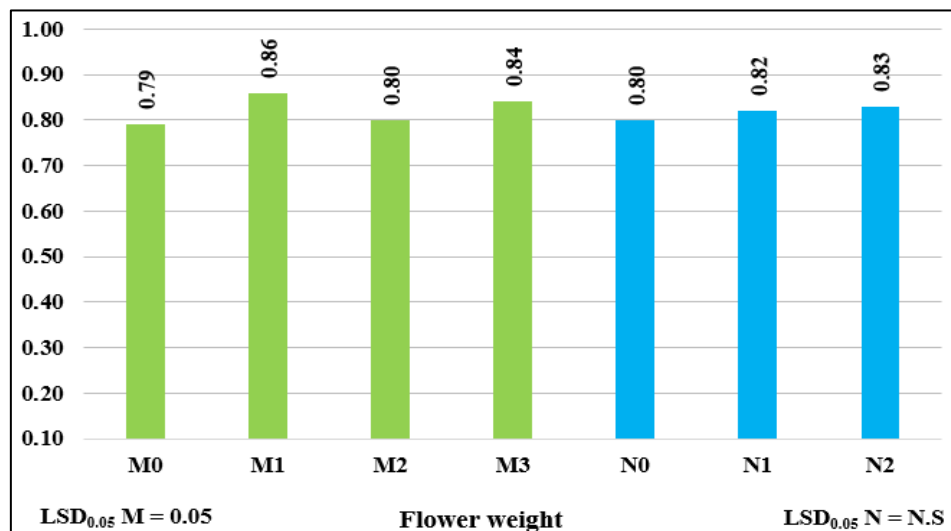


Figure 5. Effect of spray with Arginine and Indole acetic acid (IAA) on Flower weight (gm) of Marigold.

Table 2. Effect of spray with Arginine and Indole acetic acid (IAA) on growth and flowering traits of Marigold.

Arginine (N)	IAA (M)	Plant height (cm)	Branches number (branch plant ⁻¹)	Flowers number (flowers plant ⁻¹)	Flower diameter (cm)	Flower weight (gm)
N0 (0 mg L ⁻¹)	M0 (0 mg L ⁻¹)	34.09	4.64	5.08	4.76	0.82
	M1 (50 mg L ⁻¹)	34.93	4.80	5.52	5.94	0.80
	M2 (100 mg L ⁻¹)	35.62	5.04	6.11	6.56	0.78
	M3 (150 mg L ⁻¹)	35.74	4.98	5.18	5.20	0.83
N1 (100 mg L ⁻¹)	M0 (0 mg L ⁻¹)	38.36	5.49	6.77	5.41	0.78
	M1 (50 mg L ⁻¹)	42.69	4.78	7.16	6.18	0.87
	M2 (100 mg L ⁻¹)	41.77	5.32	7.57	6.52	0.81
	M3 (150 mg L ⁻¹)	43.95	5.40	6.03	6.04	0.84
N2 (200 mg L ⁻¹)	M0 (0 mg L ⁻¹)	40.54	5.81	5.91	5.06	0.79
	M1 (50 mg L ⁻¹)	44.25	5.54	5.73	5.73	0.90
	M2 (100 mg L ⁻¹)	49.12	5.92	8.19	6.41	0.82
	M3 (150 mg L ⁻¹)	38.43	5.67	8.07	7.45	0.83
LSD _{5%}		4.71	N.S	1.64	0.87	N.S

3.3. Chemical Traits

3.3.1. Total Chlorophyll (mg100 gm⁻¹)

According to Figure 6, the results show that arginine spraying had a substantial impact on total chlorophyll. Treatment (N2) had the highest levels, reaching 44.50 mg100 gm⁻¹, while treatment (N0) had the lowest values, 40.48 mg100 gm⁻¹. Conversely, foliar spraying with IAA significantly impacted this trait; treatment (M2) had the highest value, whereas, at 43.74 mg100 gm⁻¹; treatment (M0) had the lowest value, 41.80 mg100 gm⁻¹. The two study components' interaction had a substantial impact, with treatment (N2M3) showing the highest values at 46.37 mg 100 gm⁻¹ and treatment (N0M0) showing the lowest values at 38.87 mg 100 gm⁻¹ (Table 3).

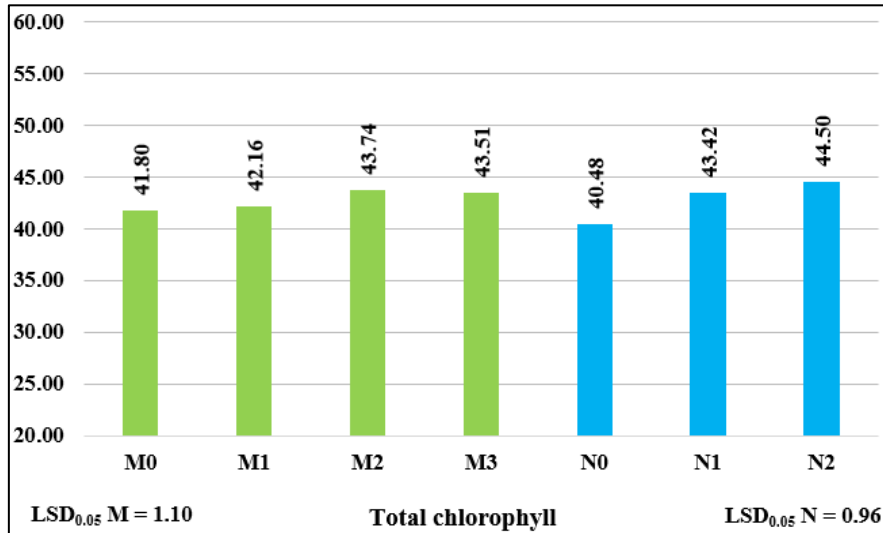


Figure 6. Effect of Spray with Arginine and Indole acetic acid (IAA) on Total chlorophyll (mg100 gm⁻¹) of Marigold.

3.3.2. Total Carbohydrates (%)

According to the findings, foliar spraying with the arginine significantly increased the amount of total carbohydrates in the leaves, with a maximum content of 5.10% (Figure 7). This was seen in the treatment (N2), which was significantly different from the treatment (N0), which produced the lowest percentage of 4.31%. However, foliar spraying with (IAA) had a substantial impact on the characteristic under study, treatment (M2) had the highest percentage, 5.03%, while treatment (M0) had the lowest percentage, 4.56%. The two study components exhibited a similar pattern of substantial effect in their interaction, with the treatment (N2M2) achieving the maximum percentage of 5.74%, the control therapy (N0M0) had the lowest percentage of 20% (Table 3).

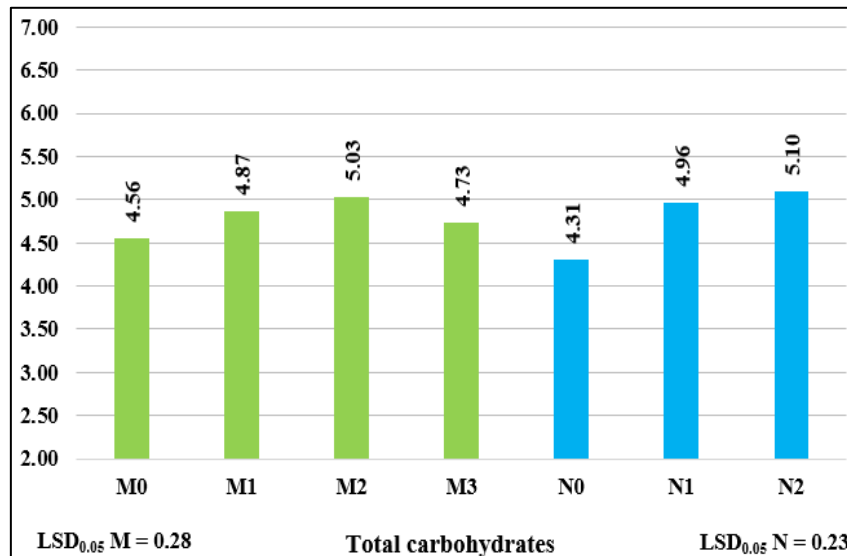


Figure 7. Effect of spray with Arginine and Indole acetic acid (IAA) on total carbohydrates (%) of Marigold.

3.3.3. Nitrogen Content in Leaves (%)

The nitrogen content of the leaves exhibited a notable advantage with the foliar spraying of arginine (N2), reaching a maximum percentage of 1.96%, while the non-sprayed treatment (N0) saw a fall in percentage to 1.82% (Figure 8). However, the growth regulator (IAA) foliar spraying had a considerable impact on the characteristic under study. Treatments M3 and M1 yielded the highest percentages of 1.93 and 1.92%, respectively. Treatment (M0) showed the lowest rate, at 1.84%. The treatment (N2M3) stood out with the highest percentage of 2.09%, while the control treatment (N0M0) had the lowest value at 1.79%, indicating that the interaction between the two study parameters had a substantial impact (Table 3).

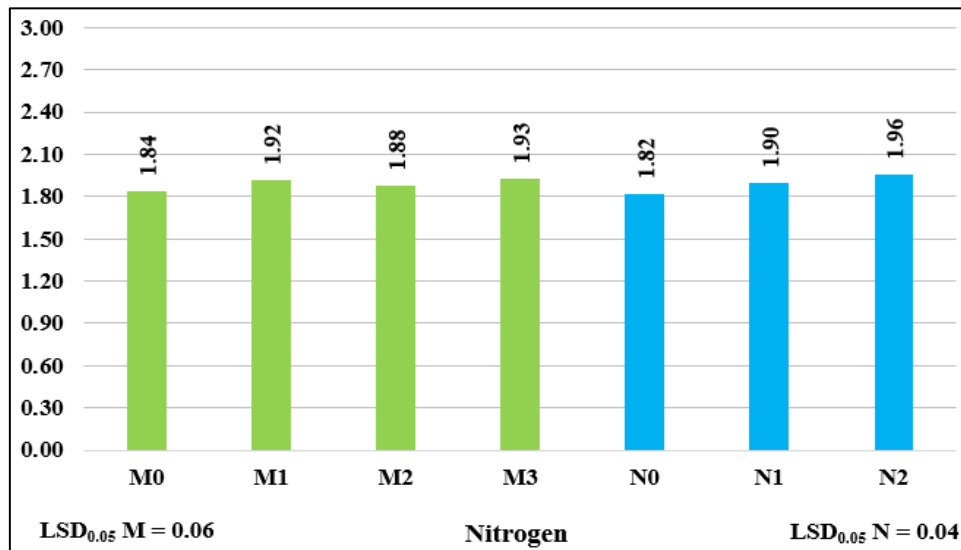


Figure 8. Effect of spray with Arginine and Indole acetic acid (IAA) on Nitrogen (%) of Marigold.

3.3.4. Phosphorous Content in Leaves (%)

According to the results shown in (Figure 9), foliar spraying with the amino acid arginine significantly increased the phosphorus content of the leaves, reaching a maximum of 0.41% in treatment (N2), while treatment (N0) showed the lowest percentage at 0.27%. However, foliar spraying with the growth regulator had a notable impact, particularly in treatment (M3), which had the greatest percentage at 0.38%. At 0.31%, the therapy (M0) had the lowest value. But the relationship between the two research components had a significant path of effect, particularly in treatment (N2M3), which attained the maximum phosphorus content in the leaves at 0.47%, while treatment (N0M1) obtained the lowest percentage at 0.23% (Table 3).

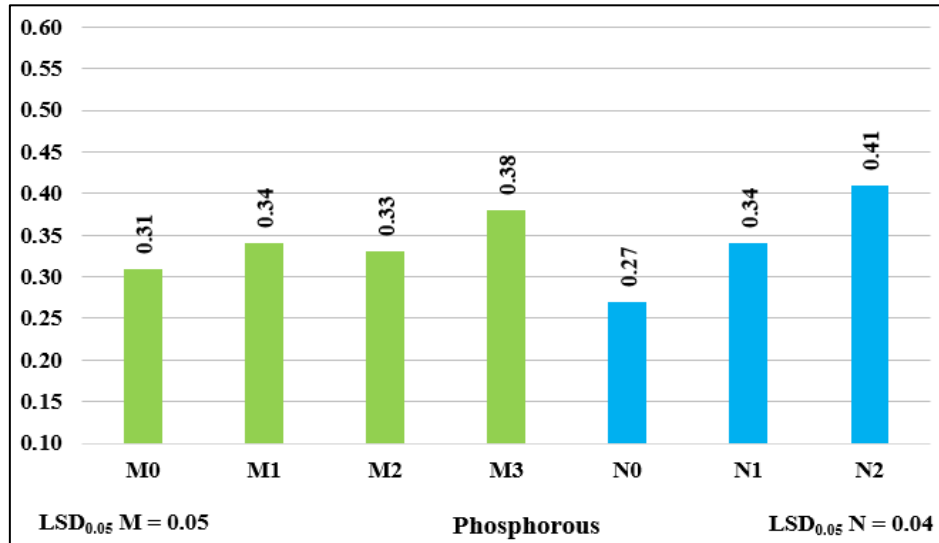


Figure 9. Effect of spray with Arginine and Indole acetic acid (IAA) on phosphorous (%) of Marigold.

3.3.5. Potassium Content in Leaves (%)

Based on the data presented in Figure 10, it is evident that the amino acid treatment (N2) had a significant impact and the highest potassium percentage in the leaves 1.78%, whereas the non-sprayed treatment (N0) had the lowest percentage 1.61%. The growth regulator (IAA) foliar spraying had a significant impact on the trait under study, particularly in treatment (M2 and M3), which had the greatest percentages of 1.72 and 1.70%, respectively. The therapy (M0), on the other hand, had the lowest proportion, 1.62%. The study's two components interacted significantly, with treatment (N2M2) exhibiting the highest percentage of potassium in the leaves 1.91% and control treatment (N0M0) exhibiting the lowest percentage 1.54% (Table 3).

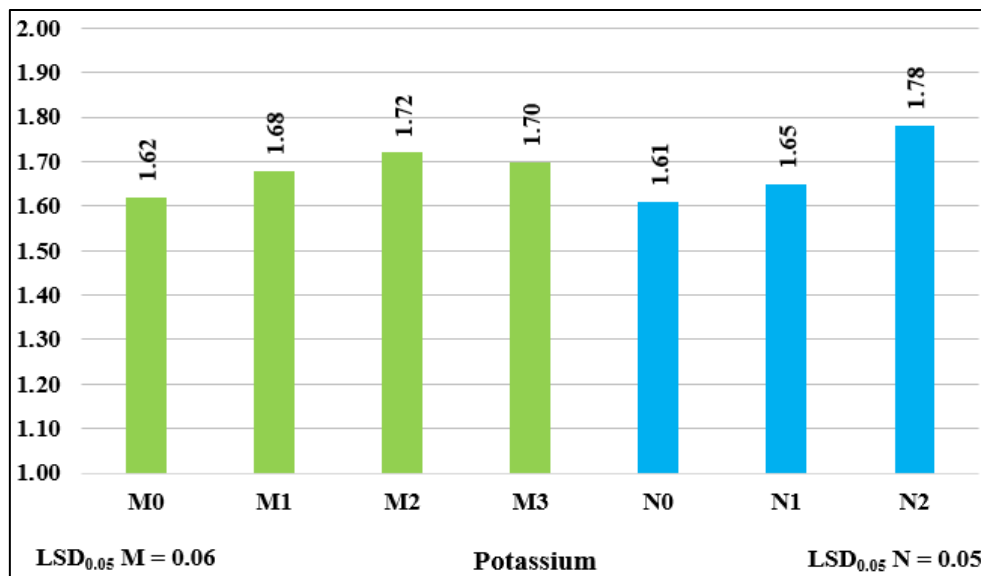


Figure 10. Effect of Spray with Arginine and Indole acetic acid (IAA) on Potassium (%) of Marigold.

Table 3. Effect of Spray with Arginine and Indole acetic acid (IAA) on chemical content of of Marigold leaves.

Arginine (N)	IAA (M)	Total chlorophyll (mg100 gm ⁻¹)	Total carbohydrates (%)	Nitrogen (%)	Phosphorous (%)	Potassium (%)
N0 (0 mg L ⁻¹)	M0 (0 mg L ⁻¹)	38.87	4.20	1.79	0.26	1.54
	M1 (50 mg L ⁻¹)	39.56	4.32	1.83	0.23	1.68
	M2 (100 mg L ⁻¹)	41.00	4.36	1.85	0.28	1.56
	M3 (150 mg L ⁻¹)	42.48	4.38	1.82	0.30	1.65
N1 (100 mg L ⁻¹)	M0 (0 mg L ⁻¹)	42.58	4.63	1.86	0.27	1.60
	M1 (50 mg L ⁻¹)	42.49	5.06	1.94	0.34	1.66
	M2 (100 mg L ⁻¹)	43.85	4.97	1.91	0.38	1.69
	M3 (150 mg L ⁻¹)	44.87	5.19	1.87	0.36	1.63
N2 (200 mg L ⁻¹)	M0 (0 mg L ⁻¹)	43.95	4.85	1.87	0.40	1.71
	M1 (50 mg L ⁻¹)	44.43	5.22	1.98	0.45	1.68
	M2 (100 mg L ⁻¹)	46.37	5.74	1.88	0.32	1.91
	M3 (150 mg L ⁻¹)	43.26	4.61	2.09	0.47	1.83
LSD _{5%}		1.91	0.48	0.10	0.08	1.11

The increase in growth, flowering and chemical parameters of Marigold can be explained by the amino acid (arginine) role as a stimulant of plant growth and a major source of nitrogen, which is required for the synthesis of proteins and enzymes essential for physiological processes, particularly the processes of growth, division, and expansion of plant cells [40,41]. Because arginine absorbed by guard cells through the stomata right away, this is the cause [42,43]. It also has a high nitrogen content, which helps the plant produce auxins and synthesise proteins. These mechanisms encourage cells to develop both longitudinally and transversely, which lengthens and dilates vegetative growths [44,45]. Arginine lowers the water potential of cells while improving their capacity to absorb water and dissolved nutrients from the growth media [46,47]. As a result, trees' vegetative development is increased [48,49]. Because of this, there are more vegetative Arginine increases the cell's capacity to absorb water and dissolved nutrients from the growth

media while lowering the cell's water potential, which encourages trees to grow more vegetatively [50,51]. Since amino acids are the building blocks of proteins, the plant produces more of them, which could explain why arginine spraying causes an increase in total chlorophyll, carbohydrates, and dry matter in the vegetative and root sections of the plant [52,53]. Among the many functions that proteins perform are regulating metabolic processes and transporting and storing nitrogen. Moreover, arginine increases the amount of chlorophyll pigments, which boosts the pace of photosynthesis and increases the amount of carbohydrates produced. It also aids in delaying the senescence of leaves [54,55].

The majority of vegetative and flowering development characteristics were positively impacted by spraying florets with indole acetic acid; the best results were obtained with a concentration of 150 mg L⁻¹ for the percentage of total carbohydrates in the leaves, as well as the percentage of nitrogen, phosphorus, and potassium in the leaves, the date of the first basal floret opening, and the number of inflorescences. This rise might be brought on by indole acetic acid's ability to promote cell elongation and division [56,57]. Since the cell wall is made up of pectin and cellulose materials, auxin is thought to cause the removal of ions that bind to carboxyl groups responsible for determining growth [58,59]. This results in the decomposition of the cell wall's components and their softening, increasing the amounts of cellulosic and pectin substances. Auxin stimulates growth (elongation and cellular expansion) through changes that occur in the plasticity of the cell wall [60,61].

Auxin's function in promoting and developing gene cloning and translation-which in turn promotes the synthesis of RNA and protein-could potentially be responsible for this. It might occur by turning on the hydrogen ion pump (protons), reducing the cell's pH, increasing the acidity of the cell wall, and altering the bonds, making the cell wall softer [62]. The findings demonstrated that plants treated with 150 mg L⁻¹ of indole acetic acid displayed significantly more flowering growth features. This could be explained by the rise in vegetative growth indicators, which raises the efficiency of the plant's metabolic processes and photosynthesis, improving the plant's ability to process nutrients produced in the leaves and transmit them. This is corroborated by [63] on carnation, and the physiological functions of auxin in the plant's growth and development by promoting cell division, elongation, and lateral bud growth may be the cause. Because of this, it promotes growth and results in more buds forming in the leaf axils [64].

Conclusion

The proportion of carbohydrates, nitrogen, phosphorous, and potassium in leaves, as well as the quantity of inflorescences, were among the qualities that the data indicated the red strain outperformed the white strain in. When compared to untreated plants, the interaction between Indole acetic acid and the red strain resulted in a considerable rise in the percentage of nitrogen, phosphorous, and potassium as well as the number of inflorescences.

References

- [1] Sultan, SM, Talal, MC and Mohammed, DA 1992. Ornamental plants. University Mosul/ Ministry of Higher Education and Scientific Research. Iraq. P. 407.
- [2] Al-Dulaimy, AFZ, Abed, BM, Abd, GL and ALTaey, DKA 2024. Response of gazania (*Gazania splendens* L.) plants to foliar application with bio health and salicylic acid on some growth and flowering traits. IOP Conf. Series: Earth and Environmental Science., 1371(042007), 1-12.
- [3] Bussmann, RW, Batsatsashvili, K, Kikvidze, Z, Paniagua-Zambrana, NY, Khutsishvili, M, Maisaia, I, Sikharulidze, S and Tchelidze, D 2020. *Tagetes erecta* L. Asteraceae. in Ethnobotany of the Mountain Regions of Far Eastern Europe; Springer: Berlin/Heidelberg, Germany, pp. 1–5.

- [4] Al-Dulaimy, AFZ, Abbood, AA and Alian, AT 2022. Effect of spraying with normal and nano Iron on the growth and flowering of *Petunia* plant *Petunia hybrid* L. IOP Conference Series: Earth and Environmental Science., 1060 (1), 012033.
- [5] Paradiković, N, Teklić, T, Zeljković, S, Lisjak, M and Špoljarević, M 2019. Biostimulants research in some horticultural plant species-A review. Food Energy Secur. 8, e00162.
- [6] AL-Jaf, IHM, Al-Hayali, REY, Al-Dulaimy, AFZ and AL Miahay, FHR 2023. Response of Cucumber plants to the addition of animal waste and dry yeast suspension., Bionatura. 8(1), 1-5.
- [7] Choudhary, M, Beniwal, BS and Kumari, A 2014. Characterization of marigold genotypes using morphological characters. Res. Crop. 15, 839.
- [8] Al-Janabia, AMI, Al-Dulaimy, AFZ, Sekhi, YS, Almohammed, OHM and Al-Taey, DKA 2024. Effect of salt stress on growth and yield of plants: A Review. IOP Conf. Series: Earth and Environmental Science., 1371(042028), 1-16.
- [9] Afzal, I, Rahim, A, Qasim, M, Younis, A, Nawaz, A and Bakhtavar, MA 2017. inducing salt tolerance in french marigold (*Tagetes patula*) through seed priming. Acta Sci. Pol. Hortorum Cultus, 16, 109-118.
- [10] Al-Ealayawi, ZA and Al-Dulaimy, AFZ 2023. Marine algae and applications to plant nutrition: A review. IOP Conference Series: Earth and Environmental Science. 1158(4), 042004.
- [11] Singh, R, Meena, ML, Verma, S, Vilas, R, Saurabh, V, Mauriya, SK, Maurya, R, and Kumar, M 2019. A review on performance of gibberellic acid on African marigold. J. Pharmacogn. Phytochem, 8, 43-46.
- [12] Al-Hayali, REY, AL-Jaf, IHM, AL Miahay, FHR and Al-Dulaimy, AFZ 2023. Evaluation of orange and biofertilizer on some yield traits of Cucumber (*Cucumis sativus* L.). Bionatura., 8(1), 1-5.
- [13] Riaz, A, Younis, A, Taj, AR, Karim, A, Tariq, U, Munir, S and Riaz, S 2013. Effect of drought stress on growth and flowering of marigold (*Tagetes erecta* L.). Pak. J. Bot., 45, 123-131.
- [14] Al-Ealayawi, ZA and Al-Dulaimy AFZ 2023. The impact of spraying Marine Algae Extract, Arginine, and Brassinolide on vegetative growth traits of Albion Strawberry saplings. IOP Conference Series: Earth and Environmental Science. 1262(4), 042061.
- [15] Hassan, HAS, Sarrwy, SMA and Mostafa, EAM 2010. Effect of foliar spraying with liquid arginine fertilizer, some micronutrients and gibberellins on leaf mineral content, fruit set, yield and fruit quality of Hollywood Plum trees. *Agriculture and Biology Journal of North America*, 1(4), 638-643.
- [16] Al-Dulaimy, AF and Jumaa, FF 2020. Effect of foliar spray with yeast suspension, licorice roots extract and amino Quelant-K compound on chemical content of Black Hamburg grape cultivar berries. Diyala Agricultural Sciences Journal., 12(Special Issue), 546-557.
- [17] AL-Mohammad MH and AL-Taey DKA. 2019. Effect of tyrosine and sulfur on growth, yield and antioxidant compounds in arugula leaves and seeds. Res. on Crops 20 (1) : 116-120
10.31830/2348-7542.2019.016. DOI : 10.31830/2348-7542.2019.016
- [18] Baqir, HA, Zeboon, NH and Al-behadili, AAJ 2019. The role and importance of amino acids within plants : A review. *Plant Archives*, 19(2), 1402-1410.
- [19] Al-Jaf, IHM, Mubarak, AK, Abed, BM and Al-Dulaimy AFZ 2023. Artificial seeds technology: A review. Anbar Journal of Agricultural Sciences., 21(2), 396-407.
- [20] Rai, VK 2012. Role of amino acids in plant responses to stresses. *Biologia plantarum*, 45(4), 481-487.
- [21] Al- Duraid MH, Al-Taey KA, and Al-Kikhani AHJ. 2019. Effect of Phenylalanine and Naphthalene Acetic Acid on Growth, Yield and Antioxidant Activity of Fenugreek *Trigonella foenum-graecum*. IOP Conf. Series: Earth and Environmental Science; 388 (2019) 012073
doi:10.1088/1755-1315/388/1/012073.
- [22] Al-Ealayawi, ZA and Al-Dulaimy, AFZ 2023. Response of Strawberry saplings Cv. Albion to spraying with Seaweed Extract, Arginine and Brassinolide. IOP Conference Series: Earth and Environmental Science., 1262(4), 042065.

- [23] Al-Jaf, IHM, Mubarak, AK, Al-Dulaimy, AFZ and ALTaey, DKA 2024. Variance estimation and genotypic, phenotypic correlation and broad heritability percentage in cucumber (*Cucumis sativus* L.). IOP Conf. Series: Earth and Environmental Science., 1371(042020), 1-8.
- [24] Hamza OM and AL-Taey DKA.2020. A study on the effect of glutamic acid and benzyl adenine application up on growth and yield parameters and active components of two Broccoli hybrids. Int. J. Agric. Stat. Sci., 16, Supplement 1: 1163-1167. DocID: <https://connectjournals.com/03899.2020.16.1163>
- [25] Taha, ST and Abdul Kareem, AJMS 2023. Effect of foliar spraying with putrescine and indole acetic acid on growth and flowering of two strains of Snapdragon plant cv. 'Opus III/IV'. IOP Conf. Series: Earth and Environmental Science. 1158 (042054).
- [26] Al-Dulaimy, AFZ and Jubair, N A 2023. Improving chemical content of date palm cv. Khastawi under different levels of spraying with micro-elements, gibberellic acid and salicylic acid. Anbar Journal of Agricultural Sciences., 22(1), 147-159.
- [27] Davies, PJ 2004. Plant Hormones: Their nature, occurrence and function. In Plant hormones biosynthesis, signal transduction, action!, Kluwer Academic Publishers, Dordrecht, The Netherlands; Norwell, MA, USA.
- [28] Alani, OMT and Al-Dulaimy, AFZ 2024. Effect of packaging type and calcium spraying on some chemical traits of seedless pomegranate variety Rawa. IOP Conf. Series: Earth and Environmental Science., 1371(042027), 1-10.
- [29] Kempinski, S and Leyser, O 2005. The Arabidopsis F-box protein TIR1 is an auxin receptor. Nature. 435, 446-451.
- [30] Hardan, ME and Al-Dulaimy, AF 2022. Effect of humic acid addition and spraying with ginger rhizome extract on the growth and some chemical contents of apricot seedlings *Prunus armeniaca* L. cv. Bionatura. 7(4), 1-6.
- [31] Liang, GH and Skinner, DZ 2003. eds. Genetically modified crops, their development, uses, and risks. New York: Haworth Press.
- [32] Alani, MHI, Al-Dulaimy, AFZ, Sekhi, YS, Almehemdi, AF 2021. Chemical analysis of Fig transplants cv Wazeri under with cutting type and humic acid. IOP Conference Series: Earth and Environmental Science. 761(1), 012032.
- [33] Jowkar, M and Salehi, H 2005. Effects of different preservative solutions on the vase life of cut tuberose flowers at usual home conditions. Acta Hort., 411-416.
- [34] Al-Mehmedi, S, and Al-Mehmedi, MFM 2012, Statistics and Experimental Design. Dar Usama for publishing and distributing. Amman- Jordan, 376 pp.
- [35] Goodwin, TW 1976. Chemistry and biochemistry of plant pigment 2nd ed Academic Press London. New York. San Francisco, pp.373.
- [36] Herbert, D, Philips, PJ and Andstang, RE 1971. Methods in Microbiology. Acad press, London.
- [37] Van Dijk, D and Houba, VJG 2000. Homogeneity and stability of materials distributed within the Wageningen evaluating programmes for analytical laboratories. Communications in soil science and plant analysis, 31,(11-14).
- [38] Chapman, HD and Pratt, PF 1961. Methods of analysis for soils. Plants and waters. University of California, Los Angeles, 60, 150-179.
- [39] Tandon, LS 1995. Methods of analysis of soils, plants, waters and fertilizers. India. NewDelhi.
- [40] Rida, D 2019. Effect of spraying with amino acids on the productivity of Strawberry plant. Plant Biology and Pathology. 5(5), 1-8.
- [41] Al-Dulaimy, AFZ, Alani, MHI, Awad, IM, Khudair, RIA, Salim, HA, and Jasem, SM, 2021. Response of Gazania plants (*Gazania Splendens* L.) to seaweed extract (Tecamin Algae) and nano stimulator (Proteck CalBor). IOP Conference Series: Earth and Environmental Science., 910(1), 012052.
- [42] Hassan, HAS, Sarrwy, SMA and Mostafa, EAM 2010. Effect of foliar spraying with liquid arginine fertilizer, some micronutrients and gibberellins on leaf mineral content, fruit set, yield and fruit quality of Hollywood Plum trees. Agriculture and Biology Journal of North America, 1(4),638-643.

- [43] Murad, HJ and Al-Dulaimy, AF 2021. Effects of spraying with urea and seaweed extract (Tecamin Algae) on growth and chemical content of date palm tree cv. Zahdi. IOP Conference Series: Earth and Environmental Science. 904(1), 012065.
- [44] Baqir, HA, Zeboon, NH and Al-behadili, AAJ 2019. The role and importance of amino acids within plants : A review. Plant Archives, 19(2), 1402-1410.
- [45] Mahmood, MM and Al-Dulaimy, AF 2021. Response of Strawberry cv. Festival to culture media and foliar application of nano and normal micronutrients. IOP Conference Series: Earth and Environmental Science. 904(1), 012067.
- [46] Rai, VK 2012. Role of amino acids in plant responses to stresses. Biologia plantarum, 45(4), 481-487.
- [47] Jubair, NA and Al-Dulaimy, AFZ 2023. Response of date palm cv. Khastawi to foliar spray with micro-elements, gibberellic acid and salicylic acid. IOP Conference Series: Earth and Environmental Science., 1158(4), 042063.
- [48] Wiedenhoeft, AC 2006. Plant Nutrition. Chelsea House An imprint of Infobase Publishing, USA. Pp. 153.
- [49] Al-Dulaimy, AFZ, Alalaf, AH, Al-Hayali, REY and ALTaey, DKA 2023. Flowers and fruits abortion in fruit trees ... Causes and solutions: A review. IOP Conference Series: Earth and Environmental Science. 1158(4), 042010.
- [50] Mengel, K and Kirkby, A 2001. Principles Plant Nutrition. Kluwer Academic Publishers.
- [51] Al-Dulaimy, AFZ, Al-Janabi, AMI, Abd, NT and ALTaey, DKA 2024. Fruit pome cracking, causes and solutions: A review. IOP Conf. Series: Earth and Environmental Science., 1371(042025), 1-11.
- [52] Mohammed, AAK 1985. Plant Physiology. Part one, two and three. Ministry of Higher Education and Scientific Research. Mosul University. (1522) p.
- [53] Jubair, NA and Al-Dulaimy, AFZ 2023. Effect of spraying with micro-elements, gibberellic acid and salicylic acid on some yield traits of date palm cv. Khastawi. Bionatura. 8(1), 1-14.
- [54] Al-Khafaji, MA 2014. Plant growth regulators and their horticultural applications. University House for Printing, Publishing and Translation, College of Agriculture, University of Baghdad. The Republic of Iraq.
- [55] Mahmood, MM and Al-Dulaimy, AF 2022. Effect of media culture, nano and normal micronutrients on some flowers and yield traits of Strawberry. AIP Conference Proceedings., 2394(1), 1-13.
- [56] Taiz, L and Zeiger, D 2010. Plant Physiology. Fourth Edition. Sinauer Associates Inc., Publishers Sunderland, MA., USA. pp: 782.
- [57] Murad, HJ and Al-Dulaimy, AFZ 2021. Response of date palms cv. Zahdi to foliar spray with urea and seaweed extract. IOP Conference Series: Earth and Environmental Science. 761(1), 012052.
- [58] Jeffcoat, B 1977. Influence of the cytokinin (6-benzylamino-9- tetrahydropyran-2-yl)-9-11-purine, on the growth and development of some ornamental crops. J. Hort. Sci., 52(1), 143-153.
- [59] Badawi, TM and Hamdi, RF 2020. Effect of seed activation with ascorbic acid and boron foliar fertilization on some morphological characteristics, chlorophyll content and productivity of wheat plant, cultivar (Tamoz 2). Biochemical & Cellular Archives, 20(2), 5015-5020.
- [60] Ziydan, B, Alrawi, M, Almehehdi, AF, and Sekhi, Y 2024. A Growth and seed yield of seven fennel cultivars with two types of ploughing systems. Plant Science Today. 11(2), 300-306.
- [61] Alalwani, AK, AL-Jaf, IH, Abed, BM, Latef, SA and Ahmed, MM 2024. Biosafety and environmental risks of genetically modified plants: A review. Journal of University of Anbar for Pure Science, 18(1), 66-75.
- [62] Ozaga, TA and Reinecke, DM 2003. Hormonal interactions in fruit development. Journal Plant Growth Regulator. 31, 1-15.
- [63] Al-Dulaimy, AFZ, Al-Issawi, MHE, Alani, MHI, and Jubair, NA 2020. Effect of pulp extract and peels powder of Banana in growth and yield of Cucumber cv. Rola. Biochem. Cell. Arch., 20(1), 2221-2227.



AL-KUNOOZE SCIENTIFIC JOURNAL

ISSN 2706-6231 (ONLINE)

ISSN 2706-6223 (PRINT)

6th International Conference of Modern Technologies in Agricultural Sciences,
18-19 December 2024, Kufa, Najaf, Iraq



- [64] Fageria, NK, Barbosa Filho, MP, Moreira, A and Guimaraes, CM 2009. Foliar fertilization of crop plants. Journal of Plant Nutrition, 32, 1044–1064.

Effect of Cultivation Methods, Adding Organic Matter and Spraying Nano-Fertilizers on Some Vegetative Growth Traits of Broad Bean Plant

Ali Karim Hussein¹, Hamid Kadhim Abdel Amir² and Ahmed Hassan Fadel³

Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Babylon, Iraq.

¹E-mail: ali.kareem.tcm@atu.edu.iq

³E-mail: com.ahmd44@atu.edu.iq

Abstract. The cultivation method (Furrow method) excelled in all vegetative growth indicators by recording the highest averages for the studied traits, where the percentage of chlorophyll in leaves (spad) reached (50.15), plant height ((133.12 cm), leaves number (189.18), leaf.plant⁻¹, branches number (16.61). branch Plant⁻¹, the fresh weight of the plant is (1281.95) g and the dry weight of the plant is (384.38) g, respectively. The treatment of adding organic fertilizer to the experiment (40 tons ha⁻¹) excelled in all vegetative growth indicators by recording the highest averages for the studied traits, where the percentage of chlorophyll in the leaves reached (51.44) spad, plant height (134.38) cm, leaves number (195.63) leaf.plant⁻¹, The branches number is (17.38) branches.plant⁻¹, the fresh weight of plant is (1352.02) g and the dry weight of the plant is (410.82) g, respectively. The results of spraying nano-fertilizer showed that nano-iron was significantly excelled in all vegetative growth indicators by recording the highest averages for the studied traits, as the percentage of chlorophyll in the leaves reached (49.62) spad, plant height (131.22 cm), leaves number (187.64) leaf⁻¹. The branches number (15.38) plant branch, the fresh weight of the plant (1279.06) g and the dry weight of the plant (379.52) g, respectively. The bi-interaction treatment between the cultivation methods and the addition of organic fertilizer (the mulch method and the addition of organic fertilizer at a level of 40 tons ha⁻¹) was significantly excelled in all vegetative growth indicators to the rest of the bi-interactions by recording the highest averages for the traits studied above. The results of the bi-interaction between the cultivation method and nanofertilizers showed significantly excelled in all vegetative growth indicators over the rest of the bi-interactions, as (the cultivation method with nano-iron) gave the highest averages for the traits studied above. The results of the bi-interaction between adding organic fertilizers and spraying nano-fertilizers indicate a significantly excelled in all vegetative growth indicators, as the combination between (nano-iron and the level of 40 tons ha⁻¹) gave the highest averages for the traits studied above. The triple combination between the cultivation method, adding organic fertilizers, and spraying nano-fertilizers significantly excelled in all vegetative growth indicators, where the combination between (the mulch method, the level of 40 tons.ha⁻¹, and nano-iron) gave the highest averages for the traits studied above.

Keywords. Cultivation methods, Nano-Fertilizers, Spray.

1. Introduction

Cultivation methods are important for preparing the appropriate cradle for the plant to grow and thus give a good yield. Cultivation methods for crops are known as the processes by which seeds or seeds are placed in such a way that the most appropriate conditions are created for them to germinate and for the seedlings to continue to grow well [1]. There are many methods of manual or mechanical cultivation, depending on the type of crop, the consistency and salinity of the soil, the date of cultivation, the ease of conducting agricultural service operations, the irrigation system, and the level of the land. The method of cultivation, whether (plot or furrow), is also one of the main important factors that may affect the productivity of this crop, which may vary from one region to another and according to the desire of cultivation, as each method has advantages and disadvantages. Organic fertilizers, although their nutrient content is low and their release of nutrients is slow due to the slow conversion to the mineral form, are of great benefit in addition to their ability to create a good nutritional balance between the NPK elements and their ability to retain water and improve the soil's porosity, permeability and biological activity. They also play a role in Soil fertility [2,3]. By using nanofertilizers, plants may be fertilized with less traditional mineral fertilizers, reducing soil contamination from fertilizer and pesticide residues, and protecting the environment. A class of fertilizers known as nanofertilizers is composed of both organic and mineral ingredients that are safe for the environment and plants. They help reduce soil toxicity, increase nutrient efficiency, and decrease the careless use of mineral fertilizers. [4-6]. Recently, there has been a trend to adopt micronutrient fertilizers manufactured using nanotechnology, which is hoped to solve part of the problem, but the issue is still in its infancy and requires further study. Broad bean one of important and used crops of the Fabaceae in many countries of the world, especially the countries of the Near East and North Africa, which ranks second in terms of its economic importance after Poaceae family. Broad bean is one of the oldest and most famous. Cultivated plants have productivity values comparable to all leguminous crops [7,8]. The study aimed to know the effect of cultivation style, addition of organic matter, and spraying of nano-fertilizers, and their interaction on the readiness and absorption of some nutrients in some vegetative growth traits of broad bean plants.

2. Materials and Methods

The experiment was conducted during the winter season 2023-2024 in Al-Saddah district, Al-Mahnawiyah region (21 km) north of Babylon province. The first experiment is located within a circle of latitude 32° 39' 12" north and longitude 44° 16' 40" east. Random samples were taken from different locations of the field soil for both locations. At a depth of (0-30 cm) it was mixed to form a composite sample representing the field soil for each experiment for the purpose of conducting some physical and chemical analyzes on them. Table (1) shows the physical and chemical traits of the field soil for the experiment. The experiment included three factors: the first was the type of cultivation in two methods (plots and furrow), the second was three levels of organic matter (cow manure), namely (0, 20, 40 tons ha⁻¹), and Table (2) shows their traits, and the third was spraying three types of nano-fertilizers (without spraying). Nano-cobalt spraying and nano-iron spraying) at concentrations (0, 4 mg L⁻¹, 40 mg L⁻¹) sequentially. For both experiments, the soil was plowed with two perpendicular plows using a rotary plow, and smoothed with disc harrows. After that, the process of leveling and amending was conducted on it. The land of each experiment was divided into three replicates each replicate containing 18 experimental units, so the number of units for each experiment became (54) experimental units, and it was an area The experimental unit is 12 m² (4 m × 3 m). The experimental units for the cultivation methods were divided into (plots and rows). The distance between one row and another is 0.75 m and between one plant and another is 0.25 m. A distance of two meters was left

as a separation limit between the experimental units and a distance of two meters between one replicate and another in order to Crop service. Broad bean seeds were planted on 10/16/2023. Two seeds were placed in each hole after germination. The hole was reduced to one plant after the plant reached a height of (10-15) cm (Al-Qatrani, 2016). Nitrogen fertilization was carried out in an amount of 80 kg N1 - in the form of 46% urea fertilizer, the first 30 days after cultivation and the second 30 days after cultivation. As for phosphate fertilizer, an amount of 80 kg N1 was added - in the form of triple superphosphate (P2O5). 21%) in one batch before cultivation. As for potassium, it was added at a rate of 120 kg ha⁻¹ in the form of potassium sulfate fertilizer 41.5% K₂O[9] .

Table 1. Shows Some chemical and physical properties for the experiment.

Traits	Units	Values
Soil pH		7.6
Electrical conductivity ECe	DS.m ⁻¹	
Cation exchange capacity (CEC).	centimole.charge.kg ⁻¹	21.4
Soil content of carbonate minerals	%	19.7
Calcium sulfate CASO4.2H2O	%	1.4
Organic matter OM	g.kg ⁻¹	8.3
cobalt available	Mg kg ⁻¹ soil	1.79
iron available	mgkg ⁻¹	3.76
nitrogen available	mgkg ⁻¹	34.2
phosphorus available	mgkg ⁻¹	8.5
potassium available	mgkg ⁻¹	142.6
zinc available	mgkg ⁻¹	0.86
sand	g kg ⁻¹	416
silt	g kg ⁻¹	365
Clay	gkg ⁻¹	219
texture		Clay loam

2.1. Studied Traits

- Leaves' chlorophyll content (SPAD): The chlorophyll content was measured when the plant reached the 100% full flowering stage and as an average of five leaves per plant and five plants from the middle lines using the SPAD device.
- Plant height. cm Ten plants were chosen at random from the center lines of each experimental unit in order to calculate this characteristic. When the plant reached maturity, the height of the plant was measured with a tape measure from the ground to the topmost leaf, and the average was computed [10].
- Leaves number leaf.Plant⁻¹: It was calculated by randomly selecting ten leaves from each plant of the experimental unit and extracting the average.
- Branches number: According to branches number of the five plants whose height was measured from each experimental unit.
- fresh weight Plant (g): Once the plants had reached full maturity, ten were randomly selected from the midlines of each experimental unit, and their weight was recorded.
- Dry weight of Plant (g): Randomly selected from the center lines of each experimental unit, ten plants were cut at the point where the stem contacts the earth, allowed to air dry, and then baked at 65°C until the weight stabilized [11].

3. Results

3.1. The Percentage of Chlorophyll in Leaves (SPAD)

Table (2) The cultivation methods excelled in furrow and gave the highest overall average of 50.15 spad, compared to the cultivation methods in plots, and gave lowest average of 41.72 spad. As for the organic fertilizer factor, the (40) ton-ha⁻¹ treatment significantly excelled by giving the highest average for this trait, amounting to 51.44 spad. While control treatment gave the lowest average of 41.57 spad. As for nano-fertilizers, nano-iron excelled by giving the highest average for the trait, amounting to 49.62 spad, while control treatment gave the lowest average, amounting to 40.32 spad. It is clear from the results of the same table that there is a significant effect of the bi-interaction between cultivation methods and organic fertilizers. (Cultivation with 40 tons ha⁻¹) excelled on the rest of the combinations and gave the highest average of 55.12 spad, while the combination (plots with control treatment) gave the lowest average of 37.83 spad. Significance also appears between cultivation methods and nanofertilizers, as the combination (plots with nano-iron) excelled and gave the highest average of 54.36 spad, while bi-interaction (plots with control treatment) gave the lowest average of 36.92 spad. The bi-interaction between organic and nano-fertilizers gave a significant effect on the trait, where the level (40 tons ha⁻¹ with nano-iron) gave the highest average, amounting to 58.53 spad, while the bi-interaction (between the two control treatments) gave the lowest average, amounting to 39.32 spad. The results of the same table showed a significant effect of the triple interaction, where the combination (cultivation with furrow, organic fertilizer 40 tons ha⁻¹, and spraying with nano-iron) gave the highest average, amounting to 63.32 spad, while the combination (cultivation with plots and control treatments of organic and nano-fertilizer) gave the lowest average, amounting to 36.78 spad.

Table 2. Effect of cultivation methods, addition of organic fertilizers, and spraying of nano-fertilizers on trait of chlorophyll in leaves (SPAD), the first experiment.

Cultivation	Organic fertilizers (tons.h ⁻¹)	Nano fertilizers (mg)			Average
		Without spraying	Cobalt	Nano iron	
furrow	Without adding	41.86	48.92	45.16	45.31
	20	43.59	51.89	54.59	50.02
	40	45.69	56.34	63.32	55.12
plots	Without adding	36.78	37.52	39.19	37.83
	20	37.19	39.82	41.72	39.58
	40	36.79	52.75	53.73	47.76
LSD			1.915		1.106
Nanofertilizers					
/		Without spraying	Cobalt	Nano iron	average
cultivation					
furrow		43.71	52.38	54.36	50.15
plots		36.92	43.36	44.88	41.72
LSD			1.106		0.638
Nanofertilizers					
/		Without spraying	Cobalt	Nano iron	average
organic fertilizers					
Without adding		39.32	43.22	42.18	41.57
20		40.39	45.86	48.15	44.80
40		41.24	54.55	58.53	51.44
LSD			1.354		0.782
average		40.32	47.87	49.62	
LSD			0.782		

3.2. Plant Height(cm)

Table (3) The cultivation method furrow excelled and gave the highest average of 133.12 cm, while the cultivation method in plot had the lowest average of 109.14 cm. Organic fertilizer (40 tons ha⁻¹) also outperformed and recorded the highest average for this trait of 134.38 cm, while the control treatment recorded the lowest average of 109.46 cm. Nano iron also excelled by giving the highest average for the trait of 131.22 cm, while the control treatment recorded the lowest average of 108.06 cm. As for bi- interactions, the combination (furrow method and 40 tons ha⁻¹) excelled and gave the highest average of 149.85 cm, while the plant height was reduced in the treatment (plot method and control treatment) and recorded 99.81 cm. The results showed that the combination (furrow with nano iron) was superior and gave the highest average of 144.87 cm, while (plot method and control treatment) recorded the lowest average of 99.12 cm. treatment (40 tons ha⁻¹ with nano iron) also recorded the highest average of 150.48 cm, while the binary interaction between the two control treatments gave the lowest average of 102.04 cm. While the triple interaction (furrow and organic fertilizer 40 tons ha⁻¹ and nano iron) recorded the highest average of 165.44 cm, while the combination (plot cultivation and the two control treatments of organic and nano fertilizer) gave the lowest average of 93.34 cm.

Table 3. Effect of cultivation methods, addition of organic fertilizers, and spraying of nanofertilizers on the first trait of plant height (cm).

Cultivation	Organic fertilizers (tons.h ⁻¹)	Nanofertilizers (mg)			Average
		Without spraying	Cobalt	Nano iron	
furrow	Without adding	110.74	120.29	126.33	119.12
	20	117.73	130.60	142.85	130.39
	40	122.55	161.55	165.44	149.85
plots	Without adding	93.34	103.37	102.71	99.81
	20	104.00	107.65	114.45	108.70
	40	100.01	121.19	135.52	118.91
LSD		4.068			2.349
Nanofertilizers					
/		Without spraying	Cobalt	Nano iron	average
cultivation					
	furrow	117.00	137.48	144.87	133.12
	plots	99.12	110.73	117.56	109.14
LSD		2.349			1.356
Nanofertilizers					
/		Without spraying	Cobalt	Nano iron	average
organic fertilizers					
	Without adding	102.04	111.83	114.52	109.46
	20	110.87	119.12	128.65	119.55
	40	111.28	141.37	150.48	134.38
LSD		2.876			1.661
	average	108.06	124.11	131.22	
LSD		1.661			

3.3. Leaves Number (leafplant⁻¹)

Table (4) indicate that there are significant differences in the leaves number of the broad bean plant, where the furrow cultivation methods outperformed the highest average for the trait, reaching 189.18 leaves per plant⁻¹, compared to the plot cultivation methods, which gave the lowest average, reaching 157.28 leaves per plant⁻¹. Organic fertilizer (40 tons ha⁻¹) was significantly superior by giving the highest average of 195.63 plant leaves⁻¹, while control

treatment recorded the lowest average of 144.13 plant leaves⁻¹. Nano iron also gave the highest average of 187.64 plant leaves⁻¹, while the comparison treatment had the lowest average of 156.37 plant leaves⁻¹. The treatment (farrow cultivation and 40 tons ha⁻¹ recorded the highest average of 212.03 plant leaves⁻¹, while the combination (plot cultivation and comparison treatment) gave the lowest average of 125.86 plant leaves⁻¹. The treatment (furrow cultivation and nano iron) gave the highest average of 201.91 plant leaves⁻¹, while the binary combination (plot cultivation and comparison treatment) gave the lowest average of 136.89 plant leaves⁻¹. The binary interaction between organic fertilizers gave Nano had a significant effect on the trait, as the level (40 tons ha⁻¹ with nano iron) gave the highest average of 210.68 plant leaves⁻¹, while the triple interaction treatment (furrow cultivation methods, organic fertilizer 40 tons ha⁻¹, and nano iron) recorded the highest average of 224.40 plant leaves⁻¹, while the combination (planting in panels and control treatment of organic and nano fertilizer) gave the lowest average of 115.32 plant leaves⁻¹.

Table 4. Effect of cultivation methods, addition of organic fertilizers, and spraying of nanofertilizers on the leaves number (leaf.plant⁻¹).

Cultivation	Organic fertilizers (tons.h ⁻¹)	Nanofertilizers (mg)			Average
		Without spraying	Cobalt	Nano iron	
furrow	Without adding	147.59	164.80	174.80	162.40
	20	180.64	192.18	206.52	193.11
	40	199.30	212.40	224.40	212.03
plots	Without adding	115.32	125.95	136.31	125.86
	20	134.08	179.33	186.85	166.75
	40	161.28	179.43	196.95	179.22
LSD			3.238		1.869
Nanofertilizers					
/					
cultivation		Without spraying	Cobalt	Nano iron	average
furrow		175.84	189.80	201.91	189.18
plots		136.89	161.57	173.37	157.28
LSD			1.869		1.079
Nanofertilizers					
		Without spraying	Cobalt	Nano iron	average
organic fertilizers					
Without adding		131.45	145.38	155.56	144.13
20		157.36	185.75	196.68	179.93
40		180.29	195.92	210.68	195.63
LSD			2.289		1.322
average		156.37	175.68	187.64	
LSD			1.322		

3.4. Branches Number (branch plant⁻¹)

Table (5). The furrow cultivation methods excelled and gave the highest overall average of 16.61 branch plant⁻¹ compared to the cultivation methods in plot and gave the lowest average of 11.66 branch plant⁻¹. The treatment (40 tons ha⁻¹) was significantly excelled by giving the highest average for this trait amounted to 17.38 branch plant⁻¹, while control treatment gave average amounted to 9.87 branch plant⁻¹. nano-iron excelled by giving the highest average amounted to 15.38 branch plant⁻¹, while control treatment gave the lowest average 12.81 branch plant⁻¹. cultivation in Furrow with 40 tons ha⁻¹) treatment excelled gave the highest average of 19.70

branch plant⁻¹, while the (plot with control treatment) gave the lowest average of 7.43 plant branch⁻¹. the bi-interaction between (the cultivation style on a bed with nano-iron) was excelled and gave the highest average of 17.89 branch plant⁻¹ while bi-interaction between (the cultivation methods in plot with its control treatment) The lowest average was 10.09 branch plant⁻¹. the level of 40 tons ha⁻¹ (with nano-iron) by giving it the highest average amounting to 18.61 branch plant⁻¹, while the bi-interaction (between the two control treatments) gave the lowest average amounting to 9.28 branch plant⁻¹. the combination (cultivation on furrow, organic fertilizer 40 tons ha⁻¹, and nano-iron) gave the highest average, amounting to 21.09 plant branches, while the combination (cultivation in plot and control treatments of organic and nano-fertilizer) gave the lowest average, amounting to 6.40. branch plant⁻¹.

Table 5. Effect of cultivation methods, addition of organic fertilizers, and spraying of nano-fertilizers on the branches number (branch plant⁻¹)

Cultivation	Organic fertilizers (tons.h ⁻¹)	Nanofertilizers (mg)			Average
		Without spraying	Cobalt	Nano iron	
furrow	Without adding	12.15	12.05	12.71	12.31
	20	16.07	17.55	19.88	17.83
	40	18.39	19.62	21.09	19.70
plots	Without adding	6.40	6.99	8.88	7.43
	20	10.41	13.47	13.59	12.49
	40	13.47	15.57	16.14	15.06
LSD		0.544			0.314
Nanofertilizers / cultivation		Without spraying	Cobalt	Nano iron	average
furrow		15.54	16.41	17.89	16.61
plots		10.09	12.01	12.87	11.66
LSD		0.314			0.181
Nanofertilizers / organic fertilizers		Without spraying	Cobalt	Nano iron	average
Without adding		9.28	9.52	10.80	9.87
20		13.24	15.51	16.73	15.16
40		15.93	17.59	18.61	17.38
LSD		0.385			0.222
average		12.81	14.21	15.38	
LSD		0.222			

3.5. The Fresh Weight Plant (g)

Table (6) The cultivation methods excelled on furrow by giving it the highest average capacity of 1281.95 g, while the cultivation methods in plot gave the lowest average 1056.87 g. organic fertilizer (40 tons ha⁻¹) was significantly excelled, giving the highest average amounting to 1352.02 g, the control treatment gave the lowest average amounting to 917.58 g. The nano-iron excelled by giving the highest average 1279.06 g, while control treatment gave the lowest average 1058.98 g. the methods of cultivation (on furrow and 40 tons ha⁻¹) excelled on and gave the highest average amounting to 1467.80 g, while cultivation in plot and control treatment gave the lowest average 869.10 g. the bi-interaction (cropping on mulch with nano-iron) gave the highest average, 1342.92 g, while bi-interaction between the cultivation methods (in plot and the control treatment) gave 928.67 g. The level (40 tons-ha⁻¹ with nano-iron) produced the greatest average amounting to 1588.86 g, while the bi-interaction between (the two control treatments) gave the

lowest average amounting to 876.99 g. These results indicate that the bi-interaction between organic and nano-fertilizers had a substantial impact on the trait. The combination of cultivation methods (on furrow, 40 tons ha⁻¹ of organic fertilizer, and nano-iron) produced the highest average, amounting to 1640.47 g, while the combination (cultivation in plot and control treatments of organic and nano-fertilizer) produced the lowest average, 800.29 g. These results from the same table demonstrate a significant impact of the triple interaction.

Table 6. Effect of cultivation methods, addition of organic fertilizers, and spraying of nano-fertilizers on the fresh weight of plant (g).

Cultivation	Organic fertilizers (tons.h ⁻¹)	Nanofertilizers (mg)			Average
		Without spraying	Cobalt	Nano iron	
furrow	Without adding	953.69	966.64	977.89	966.07
	20	1335.98	1489.56	1410.40	1411.98
	40	1278.21	1484.73	1640.47	1467.80
plots	Without adding	800.29	902.86	904.13	869.10
	20	984.92	1006.73	1204.22	1065.29
	40	1000.79	1170.65	1537.25	1236.23
	LSD		118.752		68.561
	Nanofertilizers / cultivation	Without spraying	Cobalt	Nano iron	average
	furrow	1189.29	1313.64	1342.92	1281.95
	plots	928.67	1026.75	1215.20	1056.87
	LSD		68.561		39.584
	Nanofertilizers / organic fertilizers	Without spraying	Cobalt	Nano iron	average
	Without adding	876.99	934.75	941.01	917.58
	20	1160.45	1248.15	1307.31	1238.64
	40	1139.50	1327.69	1588.86	1352.02
	LSD		83.970		48.480
	average	1058.98	1170.20	1279.06	
	LSD		48.480		

3.6. Dry Weight of Plant (g)

Table (7) shows that the cultivation techniques have a noteworthy impact on this characteristic. The maximum average capacity of 384.38 g was achieved by the cultivation techniques on furrow, while the lowest average of 318.70 g was obtained by the cultivation methods in plot. The same table's data show that organic fertilizer (40 tons ha⁻¹) greatly outperformed other fertilizers, resulting in the highest average for this attribute at 410.82 g. while the (control treatment) gave the lowest average, amounting to 275.59 g. The findings of the nano-fertilizers show a considerable improvement in this feature, with nano-iron showing the greatest improvement with an average of 379.52 g, while the control treatment showed the lowest average of 316.10 g. The bi-interaction of the organic fertilizers and growth techniques produced considerably better outcomes. With an average of 435.10 g, the combination of the cultivation methods (on cultivation and 40 tons ha⁻¹) outperformed the other combinations. while bi-interaction between (cultivation in plot and control treatment) gave the lowest average. It reached 262.48 g. Bi-interaction (cultivation on mulch with nano-iron) produced the highest average, amounting to 403.31 g, while bi-interaction between the cultivation methods (in plot and control treatment) produced the lowest average, amounting to 275.90 g. The results indicated a significant effect

between the cultivation methods and nanofertilizers. The level of 40 tons.ha⁻¹ with nano-iron produced the greatest average, totaling to 465.52 g, while the bi-interaction between (the two control treatments) gave the lowest average, amounting to 257.48 g. These results indicate that the bi-interaction between organic and nano-fertilizers had a substantial impact on the trait. The results of the same table indicate a significant effect of the triple interaction, as the combination of cultivation methods (on furrow, organic fertilizer 40 tons ha⁻¹, and nano-iron) gave the highest average amounting to 471.67 g, while the combination (cultivation in plot and control treatments of organic and nano-fertilizer) gave the lowest average amounting to 231.55 g.

Table 7. Effect of cultivation methods, addition of organic fertilizers, and spraying of nano-fertilizers on the dry weight of plant (g).

Cultivation	Organic fertilizers (tons.h ⁻¹)	Nanofertilizers (mg)			Average
		Without spraying	Cobalt	Nano iron	
furrow	Without adding	283.41	289.98	292.71	288.70
	20	392.58	449.85	445.56	429.33
	40	392.91	440.72	471.67	435.10
plots	Without adding	231.55	277.00	278.88	262.48
	20	281.06	311.25	328.96	307.09
	40	315.08	385.16	459.36	386.54
LSD		13.084			7.554
Nanofertilizers / cultivation		Without spraying	Cobalt	Nano iron	average
furrow		356.30	393.52	403.31	384.38
plots		275.90	324.47	355.73	318.70
LSD		7.554			4.361
Nanofertilizers / organic fertilizers		Without spraying	Cobalt	Nano iron	average
Without adding		257.48	283.49	285.80	275.59
20		336.82	380.55	387.26	368.21
40		354.00	412.94	465.52	410.82
LSD		9.252			5.342
average		316.10	359.00	379.52	
LSD		5.342			

4. Discussion

The results of vegetative growth in Tables (3, 4, 5, 6, 7) demonstrate that the seed cultivation method has a significant impact because it promotes root growth and increases the surface area of the absorption area, which in turn increases the rate of water and nutrient absorption. Additionally, the soil's disintegration plays a role in this process. While cultivation in a plot decreases the amount of air required for root respiration, penetration, and plant development, it also promotes soil cohesion, which allows plants to form strong root growth and facilitates the provision of superior nutritional space. [12]. Due to the activation of several enzymes, including those that produce chlorophyll, the addition of organic fertilizers resulted in a notable improve. The addition of organic fertilizers activated several enzymes, including those that produce chlorophyll, which resulted in a notable improvement in vegetative growth features. Furthermore, by breaking down organic materials into elements that plants can use, tiny creatures like bacteria that live in the midst of the soil carry out vital functions. In addition to facilitating the movement, storage, and release of numerous other mineral elements, carrying water, fixing nitrogen, and

liberating phosphorus from complex compounds into a form that plants can absorb, organic fertilizers also play a significant role in lowering the degree of soil interaction. [13,14]. Due to the activation of several enzymes, including those that produce chlorophyll, the addition of organic fertilizers resulted in a notable improve.

The addition of organic fertilizers activated several enzymes, including those that produce chlorophyll, which resulted in a notable improvement in vegetative growth features. Furthermore, by breaking down organic materials into elements that plants can use, tiny creatures like bacteria that live in the midst of the soil carry out vital functions. In addition to facilitating the movement, storage, and release of numerous other mineral elements, carrying water, fixing nitrogen, and liberating phosphorus from complex compounds into a form that plants can absorb, organic fertilizers also play a significant role in lowering the degree of soil interaction.

Apart from its active role in the formation of many important compounds like cytochromes and ferredoxins in the process of photosynthesis, iron also plays a significant role in the formation of chlorophyll and in stimulating important enzymes in the process of photosynthesis and other vital processes taking place in the plant. All of these processes reflect an increase in cell division and elongation and consequently in plant height. which favorably affects the general development of the plant [15,16].

Conclusions

Spraying with nano-iron fertilizer achieved a significant increase in vegetative growth and yield indicators and had a significant effect on the leaves' content of nutritional elements (Fe, K, P, N, Zn). Adding cow manure as a feed led to a significant increase in all the studied traits. The bi-interaction between the cultivation type using the nano-iron fermentation method at a concentration of (40) mg L⁻¹ achieved a significant increase in the indicators of vegetative growth and yield. The possibility of using organic fertilizers due to their positive effect on the chemical and physical properties of the soil.

References

- [1] Abdel-Gawad, Abdel-Azim Ahmed, Nemat Abdel-Aziz Nour El-Din, and Taher Bahjat Fayed. 1989. Introduction to crop science (basics of production). First edition. Arab Publishing and Distribution House, Cairo. Arab Republic of Egypt.
- [2] Al-Zubaidi, Karim Maayan Rabie (2007). The effect of adding organic and chemical fertilizer on the morphological and physiological traits and the total, seed and oil yield of squash plants. Doctoral thesis. College of Agriculture, University of Baghdad, Iraq.
- [3] AL-Taey, D. K. A., S. S. M. AL-Azawi, M. J. H. AL-Shareefi, and A. R. AL-Tawaha. (2018). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (*Lactuca sativa* var. Parris Island) Research on Crops.19 (3): 441-449. DOI: 10.31830/2348-7542.2018.0001.14
- [4] Naderi, M.R. and Abedi A. 2012. Application of nanotechnology in agriculture and refinement of environmental pollutants. J Nanotechnol. 11(1), 18–26. nano Fe-chelate, Fe-EDDHA and FeSO₄ on vegetative growth, Nutrition.,15(2):333-352.
- [5] Hasan A.A., Aubied I.A. and D.K. A. Al-Taey. (2023). The Effect of Organic Amendments and Minerals on Increasing Soil Fertility (A review). IOP Conf. Series: Earth and Environmental Science. 1262 (2023) 042012. doi:10.1088/1755-1315/1262/4/042012
- [6] Al-Taey, D. K. A., Al-Shareefi, M. J. H., Mijwel, A. K., Al-Tawaha, A. R., & Al-Tawaha, A. R. (2019). The beneficial effects of bio-fertilizers combinations and humic acid on growth, yield parameters and nitrogen content of broccoli grown under drip irrigation system. Bulgarian Journal of Agricultural Science, 25(5), 959–966. <https://agrojournal.org/25/05-16.pdf>
- [7] Mekky R.H., Thabet M.M., Rodríguez-Pérez C., Elnaggar D.M.Y., Mahrous Mengel, K., and E.A. Kirkby. 2001. Principles Of Plant nutrition Dordrecht: Kluwer.



- [8] Al-Taey, D.K.A., Z.N. A. Kamaluddin and H. H. S. Al-Kazemy.(2022). Impact of Zytonic-M and organic fertilizer on growth and yield of hybrid tomato Awahr (F1) under saline conditions, International Journal of Vegetable Science, 28(4) :1-6 DOI: 10.1080/19315260.2022.2070569
- [9] Ali Nour al-Din Shawqi 2012. Fertilizer technologies and uses, College of Agriculture, University of Baghdad. Ministry of Higher Education and Scientific Research. A. p.120.
- [10] Al-Hassani, Ali Rahim Karim. 2018. The effect of feeding with proline and a mixture of nutrients on the growth and yield of broad bean varieties *Vicia faba* L. Doctoral thesis. Field Crops Department. College of Agriculture, Al-Muthanna University.
- [11] Abu Dahi.1989. Practical plant nutrition. Higher Education Press in Mosul. University of Baghdad. Ministry of Higher Education and Scientific Research. Republic of Iraq.
- [12] Ramdhan, A. F., B. H. Abdulallah and F. H. Mekhle (2012). Response of growth and yield soybean plants and its quality to some Agric. practices. Anbar J. Agric. Sci. ,10 (1) : 257 – 272.
- [13] Tilak KVBR, Rauganayaki N, Manoharachari C (2006) Synergistic effects of plant-growth promoting rhizobacteria and Rhizobium on nodulation and nitrogen fixation by pigeonpea *Europ J Soil Sci* 57:67–71.
- [14] Al-Dulaimy, A.F.Z., B. M. Abed, G.I. Abd and D. K. A. ALTaey.2024. Response of *Gazania* (*Gazania splendens* L.) Plants to Foliar Application with Bio Health and Salicylic Acid on some Growth and Flowering Traits. IOP Conf. Series: Earth and Environmental Science 1371 (2024) 042007. doi:10.1088/1755-1315/1371/4/042007
- [15] Abdulgafur , A.H. and J.M . Abas. 2016. Effect of potassium fertilization and foliar nutrition with iron and zinc in qualities of vegetable growth of two genetic structures from *vigna radita* L. Sci. J. Iraqi. Agric. (2) 47: 396 – 411.

Effect of Seed Stimulation by Alpha-Amylase Enzyme, Stimulus Temperature Degree and Sowing Dates on Viability and Vigour of Sorghum Seeds

Jyan Zuhair Abbas¹ and A. H. Saudi²

^{1,2}Field Crops Sciences Department, College of Agriculture Engineering Sciences, University of Baghdad, Baghdad, Iraq.

¹E-mail: jean.abbas2206m@coagri.uobaghdad.edu.iq

²E-mail: ahmed.h@coagri.uobaghdad.edu.iq

Abstract. A laboratory experiment was conducted in the Laboratory Department of the Seed Inspection and Certification Department with the aim of evaluating the strength and vitality of the seeds produced under the influence of the two factors of the field experiment (seed stimulation and planting dates). This experiment was carried out according to factorial experiments with a completely randomized design (CRD) with three replications and two factors (planting dates and stimulation treatments). The main factor included three planting dates (June 25, July 15, and August 5), while the secondary factor included four concentrations of the alpha-amylase enzyme (0.5, 1, 1.5 mg L⁻¹), in addition to the comparison treatment (distilled water). The results showed that the date of 15 June was superior by giving it the highest germination rate in the first count, amounting to 86.00%, the highest average radicle length of 4.18 cm, and the highest average seedling vigor index (158.9), while the date of 5 August gave the highest rate of plumule length of 3.38 cm, while the germination rate was not affected in The final count is significantly different depending on the planting dates. The studied traits were significantly affected by different concentrations of the alpha-amylase enzyme. The concentration of 1 mg L⁻¹ gave the highest germination rate in the final count (90.0%), the highest radicle length reached 4.56 cm, and the highest rate of seedling vigor index reached 170.4, while the concentration recorded 0.5 mg L⁻¹. The highest germination rate in the first count was 85.22%, while the distilled water treatment achieved the highest rate of plumule length of 3.38 cm. The results showed a significant interaction of the study factors in the aforementioned traits, with the exception of the traits of radicle and plumule length.

Keywords. Alpha-amylase enzyme, Food security of sorghum, Seeds germination energy, Wide environmental range.

1. Introduction

Seed strength and vitality are an important indicator of seed quality and their subsequent impact on yield and field establishment. By examining the strength and vitality of the seed, it is possible to predict the quantity of seeds that will be used in agriculture to avoid poor field establishment when the field emergence rate decreases [1], Seed viability is also one of the main tests for seed technology. Seed viability is defined as the degree to which seeds become biologically active and have the ability to germinate and give natural seedlings. Therefore, the term seed viability is used

as a synonym for the ability to germinate [2]. What makes a seed strong is its germination potential relative to the maximum germination strength that a given plant species has achieved in a controlled environment [3]. The capacity of seeds to germinate and develop robust plants in various environmental situations is essential for generating substantial grain yields, which is why they are regarded as the most significant material for crop production [4]. Temperature is one of the important environmental factors, as it regulates the germination process and controls the growth of seedlings to a high degree. Germination temperature also varies with different types of seeds, as temperature affects the percentage and rate of seed germination. When the temperature is higher than the optimum, it affects the germination process. When Lower temperatures reduce the germination rate [5]. [6] mentioned that heat causes low germination rate and poor seedling vigor, or may cause abnormal seedling formation or reduced radicle and plumule growth. [7] explained that the percentage of germination, radicle length, plumule length, and dry weight of the seedling increased with raising the temperature from 15 °C to 25 °C, and decreased at 35 °C. This explains that the most suitable temperature for sorghum germination is 25 °C. Sorghum [*Sorghum bicolor* L. (moench)] is an important fodder and grain crop, and as a result of its large biomass, some of its varieties are used in the production of biofuels [8], It is also a raw material in the extraction of starch, cellulose, and alcohol, and has many other uses [9; 10] Sorghum has been called one of the most important crops because it possesses characteristics that enable it to withstand drought, such as the efficiency of the root system, the large number of fibrous roots, and its leaves with a corky skin that helps reduce the rate of evaporation and transpiration, high rates of carbon assimilation into respiration, and the ability of the leaves to curl [11], Despite its ability to grow quickly after mowing and its abundant grain production, it still suffers from the problem of weak field emergence and thus a decrease in yield. Therefore, it has become necessary to search for technologies and other means to improve the germination or emergence rate [12] Among them is seed stimulation technology, which is a simple technology that has proven its effectiveness in laboratories and experimental fields. It is low-cost and is used in a wide range of crops and under a wide range of environmental conditions [13]. The purpose of stimulation is to partially hydrate the seed to the point where metabolic processes begin to become active before germination [14]. The germination process relies on an efficient enzymatic system, and this has piqued the interest of researchers who are looking for effective enzymes to speed up the process, improve field emergence, and mitigate the harmful effects of high temperatures. Enzymes also simplify complex food materials, which is essential for embryonic development and germination. Soil stimulation is crucial for plant development [15]. Alpha-amylase is an enzyme that aids in embryonic development and germination and boosts product quality due to its significance in the production of carbohydrates. The initial count and final count germination percentages were the greatest for seedlings stimulated by hydrogen peroxide, reaching 94.67% and 95.33%, respectively, according to [16]. The results of [17] indicated differences in the concentrations of seaweed extract among them in the percentage of germination in the first and final count, radicle length, and plumule length, as the concentration exceeded 4 gm, with the highest average for the traits reaching 66.19%, 97.25%, 5.33 cm, and 9.19 cm, respectively. The appropriate sowing date is related to the nature of the climatic conditions accompanying the growth of the crop from planting to harvest. Also, delays in planting dates lead to an acceleration of flowering, and this does not prevent the occurrence of two processes. Pollination and fertilization at high temperatures, as the seed filling rate decreases and the yield decreases [18]. This study aims to evaluate the strength and vitality of the seeds produced under the influence of the two factors of the field experiment (seed stimulation and planting dates).

2. Materials and Methods

A laboratory experiment was carried out in the Laboratory Department of the Seed Inspection and Certification Department with the aim of evaluating the strength and vitality of the seeds produced under the influence of the two factors of the field experiment (sowing dates and seed stimulation). This experiment was designed according to factorial experiments using a (CRD) design with three replications and two factors, the main factor includes three sowing dates (June 25, July 15, and August 5), which are designated as D1, D2, and D3, respectively. The secondary factor includes alpha-amylase stimulation treatments (0.5, 1, and 1.5 mg L⁻¹), which are designated as T1, T2 and T3 sequentially, alongside the distilled water T0 control therapy. Before being rinsed with distilled water to remove chlorine, the seeds were sterilised by soaking them in water mixed with 5% sodium chloride for three minutes. Wearing a mask and paws helped preserve the seeds, and sterilizing the plant and all instruments with alcohol and salt chloride prevented infections. Each copy used the rolling method to plant fifty seeds, which were then placed in a germinator set at 25°C for 10 days.

2.1. Studied Traits

- Standard laboratory germination percentage in the first count (%): After four days in the germinator, we counted the number of natural seedlings and converted the percentage using the calculation in [19].

Germination percentage in the first count = (Number of natural seedlings after 4 days / Total number of seeds) × 100

- Standard laboratory germination percentage in the final count (%): The number of natural seedlings was calculated ten days after placing the seeds in the germinator [19] and the data was converted to a percentage according to the following equation:

Standard laboratory germination rate in the final count % = (Number of natural seedlings after 10 days / Total number of seeds) × 100

- Radicle and Plumule length (cm): Following the conclusion of the examination period (ten days), this attribute was measured. The petiole was severed from its attachment to the middle embryonic peduncle, and ten shoots were harvested from the plant. By removing the root from its seed-contact location, we were able to measure its length and the petiole's length independently with a ruler, from which we calculated their averages [20].
- Initiative strength index: Calculated using the equation mentioned by [20].

Seedling vigor index = Percentage of germination × (Radicle length + Plumule length)

3. Results and Discussion

3.1. Standard Laboratory Germination Percentage in the First Count (%)

The first count is expressed by the percentage of natural seedlings after a limited period less than the period at which the final count takes place [21].

It was clear from looking at Table (1) that the planting dates varied significantly. The laboratory germination rate for the seeds planted on the second date, 15 July, was 86.00%—a figure that differed significantly from the other planting dates—and the lowest percentage, 80.75%, was for the seeds put on the first day, 25 June. According to the same table, there was a notable difference in the germination rates of the different stimulation treatments. For example, the seeds that were

stimulated with 0.5 mg L⁻¹ of alpha-amylase enzyme had the highest germination rate (85.22%), which was not significantly different from the 1 mg L⁻¹ stimulation treatment. In comparison, the seeds that were stimulated with distilled water had the lowest germination rate (80.22%). The interaction between the two study factors was found to be significantly different in the seeds stimulated with the alpha-amylase enzyme at a concentration of 1.5 mg L⁻¹ on the second date, 15 July, compared to the other concentrations on the same date, significantly different from the concentration given at the first appointment, 25 June, with a germination rate of 95.33% (Table 1). There was a 64.00% germination rate at the lowest level in the experiment.

Table 1. The effect of sowing dates and stimulation treatments on the standard laboratory germination rate in first count (%).

Sowing dates (D)	Stimulation treatments (S) (mg L ⁻¹)				Mean (D)
	0 (Control)	0.5	1	1.5	
25 June	77.00	89.00	93.00	64.00	80.75
15 July	82.67	78.67	87.33	95.33	86.00
5 August	81.00	88.00	72.00	83.33	81.08
L.S.D (0.05)		6.61			
Means (S)	80.22	85.22	84.11	80.89	3.30
L.S.D (0.05)			3.81		

3.2. Standard Laboratory Germination Percentage in the Final Count (%)

According to Table 2, the sowing dates do not differ much. Table 2 demonstrated that the stimulation treatments were significantly different from one another. In the second count, the standard laboratory germination rate was 90.0% for seeds stimulated with 1 mg L⁻¹ of alpha-amylase. This concentration was not significantly different from the 0.5 and 1.5 mg L⁻¹ stimulation treatments, and the lowest laboratory germination rate was 79.6% for seeds treated with distilled water. This could be because different concentrations of alpha-amylase soften the seed coat and increase imbibition, which in turn stimulates the cellular metabolic reactions responsible for germination. This improves the seed's performance and increases its ability to germinate. On the second sowing date of 15 July, a concentration of 1.5 mg L⁻¹ of the enzyme alpha-amylase produced the highest germination rate of 95.3%, which was not significantly different from the other concentrations at the same sowing date; on the first sowing date of 25 June, a concentration of 1.5 mg L⁻¹ produced the lowest germination rate of 72.0% (Table 2). The interaction between sowing dates and stimulation treatments was significantly different.

Table 2. The effect of sowing dates and stimulation treatments on the standard laboratory germination rate in final count (%).

Sowing dates (D)	Stimulation treatments (S) (mg L ⁻¹)				Mean (D)
	0 (Control)	0.5	1	1.5	
25 June	80.0	92.0	95.0	72.0	84.8
15 July	84.0	85.3	94.0	95.3	89.7
5 August	74.7	90.0	81.0	85.3	82.8
L.S.D (0.05)		12.0			
Means (S)	79.6	89.1	90.0	84.2	n.s
L.S.D (0.05)			6.9		

3.3. Radicle Length (cm)

This characteristic is an indicator for measuring the strength of the seed, as it is closely linked to field emergence and the seedling's resistance to bad field conditions, any damage to the seedling reflects negatively on field emergence.

The second planting date, 15 July, had the best average radicle length at 4.18 cm (which was not substantially different from the third sowing date), according to Table 3. In contrast, the first sowing date had the lowest average at 3.62 cm. From Table 3, we can see that there was a significant difference in radicle length between the stimulation treatments. At 1 mg L⁻¹, the highest average was 4.56 cm, which was not significantly different from the concentration of 1.5 mg L⁻¹, which reached 4.09 cm. On the other hand, at 0.5 mg L⁻¹, the lowest average for the trait was 3.34 cm, which was stimulated with the alpha-amylase enzyme. For radicle length, there was no statistically significant interaction between the two research variables (Table 3).

Table 3. Effect of sowing dates and stimulation treatments on radicle length (cm).

Sowing dates (D)	Stimulation treatments (S) (mg L ⁻¹)				Mean (D)
	0 (Control)	0.5	1	1.5	
25 June	3.50	3.41	4.27	3.72	3.63
15 July	4.25	3.50	4.31	4.65	4.18
5 August	3.85	3.11	5.09	3.90	3.99
L.S.D (0.05)		n.s			0.49
Means (S)	3.75	3.34	4.56	4.09	
L.S.D (0.05)			0.57		

3.4. plumule Length (cm)

The seeds planted on August 5th had the longest plumules, measuring 3.38 cm; this was not significantly different from the seeds planted on July 15th, which averaged 3.06 cm; and the first sowing date, June 25th, yielded a rate that was significantly lower than the others, according to Table 4. The plumule length rate dropped to 2.75 cm at its lowest point. When it came to plumule length, the distilled water stimulation treatment was head and shoulders above the others; it produced an average of 3.38 cm, which was comparable to the 0.5 and 1.5 mg L⁻¹ treatments, but the lowest average of 2.42 cm was achieved by the 1 mg L⁻¹ treatment (Table 4) The findings were consistent with those of [22]. Table 4 shows that there were no significant variations in plumule length when looking at the interaction between stimulation treatments and sowing dates.

Table 4. Effect of sowing dates and stimulation treatments on plumule length (cm).

Sowing dates (D)	Stimulation treatments (S) (mg L ⁻¹)				Mean (D)
	0 (Control)	0.5	1	1.5	
25 June	2.20	3.00	2.78	3.03	2.75
15 July	2.69	3.21	3.50	2.85	3.06
5 August	2.38	3.86	3.88	3.39	3.38
L.S.D (0.05)		n.s			0.49
Means (S)	3.38	3.35	2.42	3.09	
L.S.D (0.05)			0.57		

3.5. Initiative Strength Index

From Table 5, it was evident that the sowing dates varied significantly. The second sowing date, 15 July, yielded the highest seedling strength index (158.9), which was not significantly different from the third sowing date, 5 August, while the first date recorded the lowest percentage (138.2) in the seedling strength index. According to the same table, the index of the strength of the reaction was significantly affected by the stimulation treatments. The highest percentage in the index of the strength of the reaction was 170.4, which was significantly different from the other treatments, and the lowest percentage for the trait was 130.0, which was achieved with the stimulation treatment with distilled water. [23] also found similar results. On the third date,

August 5, the results demonstrated that the strength index characteristic was best treated with a 1 mg L⁻¹ stimulant, in contrast to the other concentrations tested at the three sowing dates; on the other hand, the trait yielded the lowest percentage (117.1%) when treated with distilled water (Table 5).

Table 5. The effect of sowing dates and stimulation parameters on the seedling strength index.

Sowing dates (D)	Stimulation treatments (S) (mg L ⁻¹)				Mean (D)
	0 (Control)	0.5	1	1.5	
25 June	117.2	146.7	162.9	125.9	138.2
15 July	155.8	145.1	165.0	169.5	158.9
5 August	117.1	160.1	183.4	158.7	154.8
L.S.D (0.05)		10.9			
Means (S)	130.0	150.7	170.4	151.4	4.5
L.S.D (0.05)			6.3		

Conclusion

We conclude from the research that sorghum planted on the date of 15 July was better compared to the dates of 25 June and 5 August. It was found that stimulating seeds with the enzyme alpha-amylase improves seed performance, especially at concentrations of 0.5 and 1 mg L⁻¹.

References

- [1] Hamza, J. H. (2006). Effect Of Seed Size Resulting From Sowing Dates On Seed Vigor And Grain Yield Of Sorghum. [Sorghum bicolor (L) Moench]. D.Ph. thesis, College of Agriculture, University of Baghdad. Iraq.
- [2] Saudi, A. H. (2008). Effect Of Packing Methods And Storage Duration On The Viability And Strength Of Seeds Of Oryza Sativa L. Rice Varieties. D.Ph. thesis, College of Agriculture, University of Baghdad. Iraq.
- [3] Elsahooki, M. & Cheyed, S. H. (2023). Seed Growth Relationships. University of Baghdad. Ministry of Higher Education and Research. Iraq.
- [4] Abbas, J. Z. & Saudi, A. H. (2024). Effect of seed stimulation by alpha-amylase enzyme, stimulus temperature degree and sowing dates on some growth characteristics of sorghum. IOP Conf. Series: Earth and Environmental Science 1371(052074): 1-7.
- [5] Bushama, S. (2014). The Effect Of Salt Stress On Varieties Of The Leguminous Family And The Leguminous Family Treated With Kinetin During The Germination Stage. University of Constantine.
- [6] Kumar, B., Verma S. K. & Singh H. P. (2011). Effect of temperature on seed germination parameters in Kalmegh (Andrographis paniculata Wall. ex Nees.). Industrial Crops and Products, 34(1): 1241–1244.
- [7] Cheyad, S. H., Dawood, S. S., Jasim, R. M. & Hamdon, M. O. (2014). Effect of some plant growth regulators and temperature on seed germination of sorghum. Journal of Wassit for Science and Medicine, 7(3): 74-81.
- [8] Renewable Fuels Association. (2010). The Industry-Statistics, 28: 3-11.
- [9] Himani, B.S. & Tokas, J. (2019). Variation in structural carbohydrates of forage sorghum [Sorghum bicolor (L.) Moench] under saline conditions. Forage Research, 45: 123–127.
- [10] Shi, Y., Wang, X., Wang, J., Niu, J., Du, R. Ji, G. et al. (2022). Systematical characterization of GRF gene family in sorghum, and their potential functions in aphidresistance. Gene 836, 146669.
- [11] Ahmed, S. A. (2007). Response Of Two Varieties Of Sorghum [Sorghum bicolor L. (moench)] To Environmental Stress Under Field Conditions. D.Ph. thesis, College of Agriculture, University of Baghdad. Iraq.
- [12] AL Hade, M. Q. S. (2019). Effect Of Stored Priming With Different Storage Periods On Seed Vigour, Growth, And Yield Of Sorghum bicolor L. (moench). M.Sc. thesis, College of Agriculture, University of Karbala. Iraq.

- [13] Ashraf, M. & Foolad, M. R. (2005). Pre-sowing seed treatment A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Advances in agronomy*, 88: 223-271.
- [14] Al-Jubouri, A. A. A. S. (2023). Effect of Seed Priming on Germination Vigour, Growth and Grain Yield of Maize Genotypes. M. Sc. thesis. College of Agricultural Engineering Science, University of Baghdad, Iraq.
- [15] Damalas, C. A., Koutroubas, S. D. & Fotiadis, S. (2019). Hydro-priming effects on seed germination and field performance of faba bean in spring sowing. *Agriculture*, 9(9): p 201.
- [16] Salman, A. A. D. (2023). Effect Of Seed Priming On Germination Vigour, Growth and Grian Yeld Of Maize Genotypes. Ph. D. Thesis, Coll. Of Agriculture Engineering science, university of Baghdad. Iraq.
- [17] Saudi, A. H. & Abbas, H. A. (2024) Seed quality of two sunflower cultivar by seed priming effect with seaweed extract. *IOP Conf. Series: Earth and Environmental Science* 1371(052058): 1-8.
- [18] Al-Hassani, S. H. J. (2001). Effect Of Planting Dates On The Growth And Yield Of Two Cultivars Of Sorghum, *Sorghum bicolor* L. Moench. M. Sc. Thesis. College of Agriculture, University of Baghdad. Iraq.
- [19] ISTA. (2021). International Rules For Seed Testing. ISTA s contribution to phytosanitary seed testing.
- [20] Murti, G. S. R., Sirohi, G. S. & Upreti, K. K. (2004). Glossary of plant physiology. Daya Publishing house. Delhi. pp: 207
- [21] International Seed Testing Association (ISTA). (2003). International Rules for Seed Testing. Adopted at the Extraordinary Meeting. (2002), Santa Cruz, Bolivia. In The International Seed Testing Association. (ISTA).
- [22] Saudi, A. H., Al-Hilfy, I. H. H., Al-Rawi, A. S. M., Al-Abodi, H. M. K., Mahdi, A. M. & Salman, Kh. A. (2024). Effect of seed stimulation treatments with Hydrogen peroxide and sowing dates on growth traits of sorghum cultivars. *IOP Conf. Series: Earth and Environmental Science* 1371(052021): 1-9.
- [23] Saudi, A. H., & Al-Rawi, A. S. M. (2023). Effect of Seed Priming Duration with Bio-Stimulator (Appetizer) on Germination Characteristics and Seedling Emergence of Sorghum. *IOP Conference Series: Earth and Environmental Science*. (Vol. 1262, No. 5, p. 052034).

Study of Variation in Characteristics of Clay Minerals in Soils North and Southe Al-Handia Dam - Middle Euphrates / Iraq

I. H. Al-Mamoori¹ and S. K. Essa²

^{1,2}College of Agriculture Engineering Sciences, University of Baghdad, Baghdad,
Iraq.

¹E- mail: istabraq.halal1107a@coagri.uobaghdad.edu.iq

²E- mail: salman.essa.52@gmail.com

Abstract. This study was conducted to determine the variation in the properties of clay minerals in the soils of four sites north and south of Al-Hindiyah Dam. The study area was divided into two sections. The first section is located north of Al-Hindiyah Dam and includes two sites for the study soil pedons, which are (Jurf Al-Nasr, Umm Arouk), while the second section is located south of Al-Hindiyah Dam and includes three sites for the study soil pedons, which are (Al-Tabar Village, Al-Mashkhab). After collecting the samples, they were prepared for examination and some physical and chemical analyses were conducted on them, in addition to preparing the samples for mineral examinations. After separating the clay particles from the sand and silt, the clay samples were examined using XRD to determine the type of clay minerals prevalent in the study soil samples. The examination results showed the dominance of chlorite, kaolinite and mica minerals in the soils of the first and second sections north and south of Al-Hindiyah Dam and at both surface and subsurface depths.

Keywords. Clay minerals, XRD, Sediments, Mineralogy.

1. Introduction

River sediments generally consist of solid particles of sand, silt, clay minerals and other organic matter. In river systems, the amount of sediment transported is controlled by both flow transport capacity and sediment supply according to water velocity [1]. Sediments are characterized by a continuous change in their properties as a result of being immersed in water for long periods, and river sediments, especially at dam waters, are subjected to many transformations in their components, which leads to new qualities and products that have a great relationship with the use of land for agricultural purposes [2]. Mineralogical studies of river sediments play an important and fundamental role in evaluating the effect of weathering phenomena on the soil physical and chemical properties and sediments alike. By rivers, one of the geological materials most affected by physical factors, whether physical by rolling, creeping and rubbing as a result of being carried in the course of the river, or chemical as a result of immersing it in water, which is the agent most reactive with these sediments through dissolution, hydration, hydrolysis and oxidation. Due to the different sizes of particles of these sediments, they vary in the extent to which they are affected with weathering, but in general, the intensity of weathering of these sediments increases with the

increase in the finesses of the particles, where they are the finer which, it represents the clay are the most affected and changed by chemical weathering [3]. On the other hand, the clay fraction is the most effective part and most of the physical and chemical properties are attributed to of soil and sediment. Clay particles move in a suspension way from the upper parts to the lower parts of soils. Clay particles move as a suspension from the surface horizons to the lower horizons for the soil profiles, when water is adsorbed on dry lattice units or interposes in the soil, therefore, these units act as a filter to trap the clay particles and accumulate in the subsurface horizons [4].

Dams construction may cause far-reaching influence on hydrological regime and the river ecosystem [5,6]. The alteration of the natural flow regime has led to changes to hydrological, geomorphological and ecological conditions in both upstream and downstream [5,6,7,8]. The loss of natural flow in dammed rivers interferes with the degree and frequency of natural disturbance, and limits sediment sources create new habitats for plant colonization and establishment downstream [9]. The current study aims to know the variation in mineralogical properties of clay particles in the sediments of the Euphrates river in soils north and south of Al-Handia Dam, and to find the effect of NBdam on the process of deposition and distribution and content of clay minerals in these soils.

2. Materials And Methods

Four soil pedons along the Euphrates river were chosen, two of them were north of the Dam (Jurf AL-Nasir and Um-Aruk), when the other two pedons (AL-Tubar village and Almishkhab) were chosen south of Dam, in order to studying the impact of the location of Al-Handia Dam on the distribution and properties of clay minerals in Euphrates river sediments. A collected soil samples were air dried, crashed, and passed through the sieve of 2 mm openings, then some chemical and physical analyzes were done on it (Tables 1 and 2). The particle size distribution was determined according to international pipette method as described in [10]. The Ec and pH of soil were measured using method of [11]. Total CaCO_3 equivalent was determined by method of [12]. While the cation exchange capacity (CEC) was determined according to the method described in [11]. Total organic matter (OM) was determined by using Walkey and Black method as described in [13]. A bulk sample of the soil clay ($< 2 \mu\text{m}$ particle size fractions) was separated by sedimentation and used for mineralogical studies. The mineralogical composition of the clay-sized fraction was determined by X-ray on parallel oriented clay of the Mg^{2+} and K^+ saturated clay samples. The Mg^{2+} saturated clay was treated with the X-ray in the air dry and ethylene glycol salvation. While the K^+ saturated clay was heated to 350°C and 550°C . X-ray analyses were made with a Philips – P.W 1840 diffractometer equipped with a graphite diffracted beam monochromatot using $\text{Cu K } \alpha$ radiation.

3. Results and Discussions

Tables 1 and 2 show some chemical and physical properties of studied soils. Results indicated that the range of pH values was between (7.40 – 8.10), reflecting that the reaction of all pedons was around moderate alkali to neutral, due to effect of calcareous parent material of these soils. Also, results showed there was no specific pattern for pH values with depth in all studied pedons. Results of Electrical conductivity (EC) indicate that all studied soils were non-moderately saline, reflected by their values of EC ($2.19 - 5.21 \text{ dS.m}^{-1}$). Results in Table 1 show that the amount of total CaCO_3 were ranged between ($195.89 - 288.32 \text{ g.kg}^{-1}$). The high content of CaCO_3 reflected a calcareous nature of studied soils. Results also, show that the amounts of total CaCO_3 were increased in soil pedons, north of the Al-Handia Dam reflects the effect of the dam location on sorting and deposition of coarse particles of sediment transported by river. The values of cation exchange capacity (CEC) were ranged between ($7.21 - 27.91 \text{ Cmol.Kg}^{-1}$). In general, the results

showed that the soils located in the north dam have a high values of CEC. These results suggest the effect of organic matter and clay content which both of them were deposited north of the dam were contributed to increases the CEC values of these soils. These results agree with [14], who studied the spatial variation of content and distribution of sediments minerals of Al- Gharraf river and found that both clay and organic matter were deposited in front of the dam, and a high contribution of clay and organic matter in CEC of soil.

Table 1. Some chemical properties of studied soils

Location	Horizon Depth - cm	pH	EC ds . m ⁻¹	CEC Cmol C . kg ⁻¹	T. CaCO ₃ g . kg ⁻¹
Jurf AL-Nasir	0-30	7.90	3.74	27.91	288.32
	31-69	7.80	3.04	16.23	274.70
	70 +	7.70	3.27	18.05	272.09
Um-Aruk	0-48	8.10	5.21	15.28	258.22
	49-80	7.60	4.07	16.39	266.12
	81+	7.70	4.24	16.01	201.18
AL-Tubar village	0-30	7.40	3.31	20.52	230.15
	31-58	7.90	3.57	21.16	198.77
	59+	7.60	5.20	19.76	212.69
Almishkhab	0-33	7.70	2.53	9.89	274.18
	34-61	7.70	2.87	7.21	223.39
	62-90	7.50	2.19	10.02	195.89
	91+	7.60	3.07	9.88	217.33

In general, the values of CEC were increased in soils north of the Dam due to increasing the clay and organic matter. These results suggest the effect of organic matter and clay content and both of them were contributed to increases the CEC values of these soils [15,16,17,18]. Results of particle size distribution in Table 2 show there is a clear difference in the distribution of soil fractions , which caused by the difference in the content of those fractions in the depositional cycles, and the operations of sorting and sedimentation. In general, the results showed that the values of both clay and silt fractions were increased in soils north of Dam, while the values of sand fraction were high in soils located south of Dam. From the results of particle size distribution it is obvious that the texture of studied soils was ranged from silty clay to clay loam.

Table 2. Particle size distribution of soil particles in studied pedons.

Location	Horizon Depth - cm	Sand g . Kg ⁻¹	Silt g . Kg ⁻¹	Clay g . Kg ⁻¹	Texture
Jurf AL-Nasir	0-30	185	379	436	Sic
	31-69	314	448	238	L
	70 +	153	560	287	SiL
Um-Aruk	0-48	163	486	351	SiL
	49-80	113	547	340	SiL
	81+	98	422	480	Sic
AL-Tubar village	0-30	270	601	129	SiL
	31-58	203	578	219	SiL
	59+	328	591	81	SiL
Almishkhab	0-33	680	150	170	SL
	34-61	753	172	75	SL
	62-90	705	208	87	SL
	91+	756	163	81	SL

3.1. Mineralogical Properties of Studied Soils

3.1.1. X – Ray Diffraction

The mineralogical composition of <2 μ fractions for two depths (0-30 and 31-60 cm) in all studied soils (Figs. 1-8) were examined by X-ray diffraction. In Jurf AL-Nasir soil at depth of 0 - 30 cm, results in Fig (1A) show the presence of 14.93 A⁰ reflection, with his third diffraction at d-spacing of 4.62 A⁰ in air-dried, Mg-saturated treatment, and continued presence without changed in all treatments, reflecting the presence of real chlorite in this soil. The presence of 10.40 A⁰ reflection in air-dried, Mg-saturated treatment and remaining without change in all treatments reflecting the presence of mica minerals in this soil, also the appearance of second order of mica with a low intensity at d-spacing of 4.98 A⁰ confirmed the presence of Biotite in this soil. This agrees with results obtained by many studies have been done on Iraqi soils [19] [20], who have shown that the distinguish between two types of mica minerals (Biotite and Muscovite) depending on the second order of mica at 5 A⁰, in Muscovite the peak of second order is always strong and high, while it is disappear or very weak in Biotite. Also, the six order of mica was used for distinguish between mica minerals, so the six order of Muscovite appear at 1.5 A⁰, while it is appear at 1.54 A⁰ in case of Biotite. The increasing of Biotite has a weaker resistance to weathering processes, compared to Muscovite, which has a higher resistance to weathering in clay fraction of surface horizon in Jurf AL-Nasir soil is attributed to dominancy of trioctahedral mica (Biotite) in the parent material of sources of mountains at south of Anatolia in Turkey [21]. Fig (1A) show the presence of 24.54 A⁰ reflection in air-dried, Mg-saturated treatment, and shifts to an 27.61 A⁰ reflection following ethylene-glycol salvation, and then shifts to an 10.40 A⁰ reflection in K-saturated and heating to 350 and 550⁰C, reflecting the presence of regular interstratified (M-S) mineral, This agrees with results obtained by many studies have been done on Iraqi soils [20] [21] [22] [23]. Also, Kaolinite was diagnosed through presence of 7.10 A⁰ reflection in treatments of air-dried, Mg-saturated, ethylene glycol salvation, and K-saturated with heat treatment to 350 ⁰C, and disappeared in K-saturated with heat treatment to 550 ⁰C.

Results in Fig (1B) representing the clay sample of subsurface horizon in Jurf AL-Nasir soil, and show the presence of 14.25 A⁰ reflection, with his third diffraction at d-spacing of 4.77 A⁰ in air-dried, Mg-saturated treatment, and continued presence without changed in all treatments, reflecting the presence of real chlorite in this soil. The presence of 10.16 A⁰ reflection in air-dried, Mg-saturated treatment and remaining without change in all treatments reflecting the presence of mica minerals in this soil, also the appearance of second order of mica with a low intensity at d-spacing of 4.98 A⁰ confirmed the presence of Biotite in this soil. while the appearance of 10.52 A⁰ reflection in treatments of air-dried, Mg-saturated, ethylene glycol salvation, and K-saturated with heat treatment to 350 ⁰C, and disappeared in K-saturated with heat treatment to 550 ⁰C, confirmed the presence of pedogenic palegoreskite, which is characterized by weakly crystalline and not resistant to heat [24].

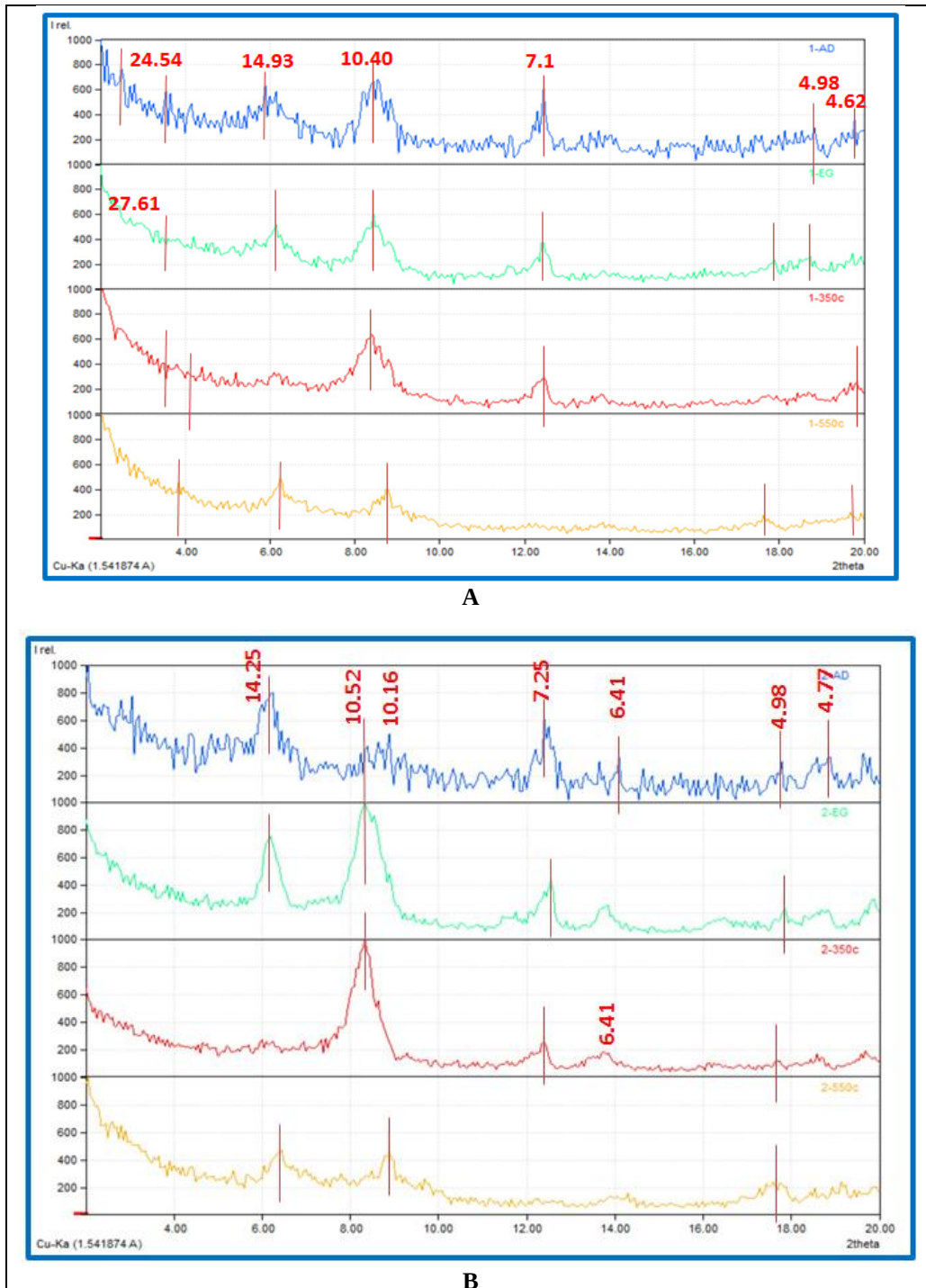
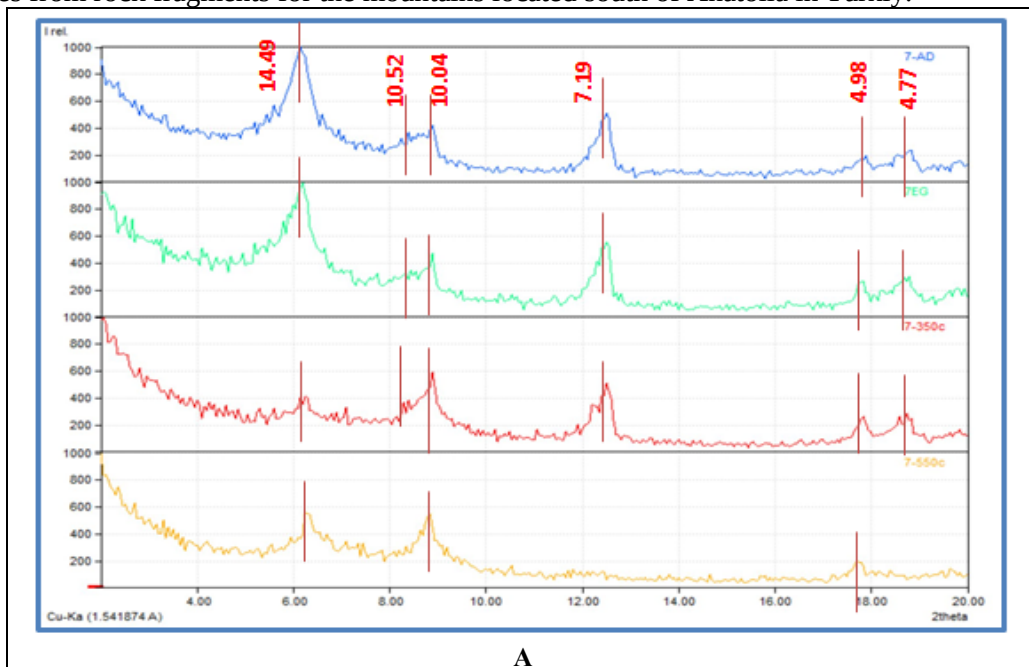


Figure 1. X-ray diffractions of Jurf AL-Nasir soil A. surface horizon , and B. subsurface horizon.

Results in Fig (2A) representing the clay sample of Ap horizon in Um-Aruk soil north of Al-Handia Dam, and show that the presence of 14.49 Å⁰ reflection in air-dried, Mg-saturated treatment, and continued presence without changed in all treatments, reflecting the presence of

real chlorite in this horizon, confirming to that is the appearance of his third diffraction at d-spacing of $4.77 \text{ \AA}$. while the appearance of $10.52 \text{ \AA}$ reflection in treatments of air-dried, Mg-saturated, ethylene glycol salvation, and K-saturated with heat treatment to 350°C , and disappeared in K-saturated with heat treatment to 550°C , confirmed the presence of pedogenic palegoreskite, which is characterized by weakly crystalline and not resistant to heat [24]. The presence of $10.04 \text{ \AA}$ reflection in air-dried, Mg-saturated treatment, and remaining without change in all treatments, reflecting the presence of Mica minerals in the clay fraction of this soil, while the appearance of second order of Mica with a low intensity at d-spacing of $4.98 \text{ \AA}$ confirmed the presence of Biotite in this soil. Also, Kaolinite was diagnosed through presence of $7.19 \text{ \AA}$ reflection in treatments of air-dried, Mg-saturated, ethylene glycol salvation, and K-saturated with heat treatment to 350°C , and disappeared in K-saturated with heat treatment to 550°C .

Results in Fig (2B) representing the clay sample of subsurface horizon in Um-Aruk soil, and show the presence of $14.25 \text{ \AA}$ reflection, with his third diffraction at d-spacing of $4.77 \text{ \AA}$ in air-dried, Mg-saturated treatment, and continued presence without changed in all treatments, reflecting the presence of real chlorite in this soil. The presence of $10.04 \text{ \AA}$ reflection in air-dried, Mg-saturated treatment and remaining without change in all treatments reflecting the presence of mica minerals in this soil, also the appearance of second order of mica with a low intensity at d-spacing of $4.98 \text{ \AA}$ confirmed the presence of Biotite in this soil. while the appearance of $10.52 \text{ \AA}$ reflection in treatments of air-dried, Mg-saturated, ethylene glycol salvation, and K-saturated with heat treatment to 350°C , and disappeared in K-saturated with heat treatment to 550°C , confirmed the presence of pedogenic palegoreskite, which is characterized by weakly crystalline and not resistant to heat [24]. In general, the similarity in mineral types of clays in soils located north of the Dam, is attributed to the fact that the parent material for these soils is same and comes from rock fragments for the mountains located south of Anatolia in Turkiy.



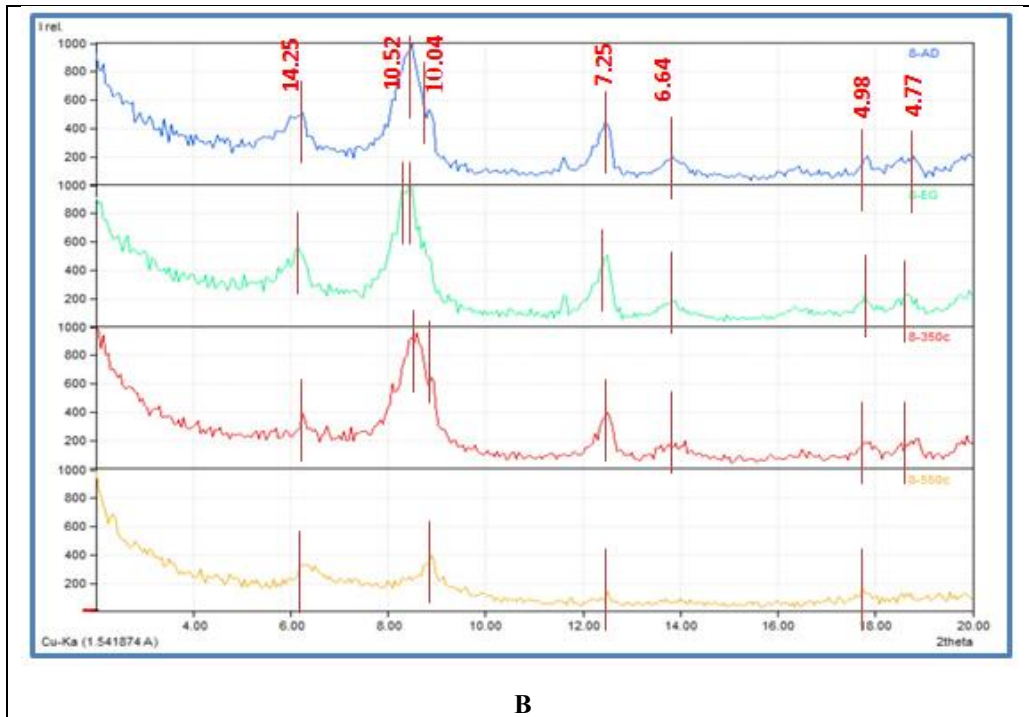


Figure 2. X-ray diffractions of Um-Aruk soil A. surface horizon , and B. subsurface horizon.

Results in Fig (3A) representing the clay sample of Ap horizon in AL-Tubar village soil south of Al-Handia Dam, and show that the presence of 14.25 Å⁰ reflection in air-dried, Mg-saturated treatment, and continued presence without changed in all treatments, reflecting the presence of real chlorite in this horizon, confirming to that is the appearance of his third diffraction at d-spacing of 4.77 Å⁰ . while the appearance of 10.52 Å⁰ reflection in treatments of air-dried, Mg-saturated , ethylene glycol salvation , and K-saturated with heat treatment to 350 °C , and disappeared in K-saturated with heat treatment to 550 °C, confirmed the presence of pedogenic palegoreskite, which is characterized by weakly crystalline and not resistant to heat [24]. The presence of 10.40 Å⁰ reflection in air-dried, Mg-saturated treatment, and remaining without change in all treatments, reflecting the presence of Mica minerals in the clay fraction of this soil, while the appearance of second order of Mica with a low intensity at d-spacing of 5.01 Å⁰ confirmed the presence of Biotite in this soil. Also, Kaolinite was diagnosed through presence of 7.50 Å⁰ reflection in treatments of air-dried, Mg-saturated , ethylene glycol salvation , and K-saturated with heat treatment to 350 °C , and disappeared in K-saturated with heat treatment to 550 °C.

Results in Fig (3B) representing the clay sample of subsurface horizon in AL-Tubar village soil, and show the presence of 14.25 Å⁰ reflection, with his third diffraction at d-spacing of 4.72 Å⁰ in air-dried, Mg-saturated treatment, and continued presence without changed in all treatments, reflecting the presence of real chlorite in this soil. The presence of 10.40 Å⁰ reflection in air-dried, Mg-saturated treatment and remaining without change in all treatments reflecting the presence of mica minerals in this soil, also the appearance of second order of mica with a low intensity at d-spacing of 4.98 Å⁰ confirmed the presence of Biotite in this soil. Also, Kaolinite was diagnosed through presence of 7.25 Å⁰ reflection in treatments of air-dried, Mg-saturated , ethylene glycol salvation , and K-saturated with heat treatment to 350 °C , and disappeared in K-saturated with heat treatment to 550 °C.

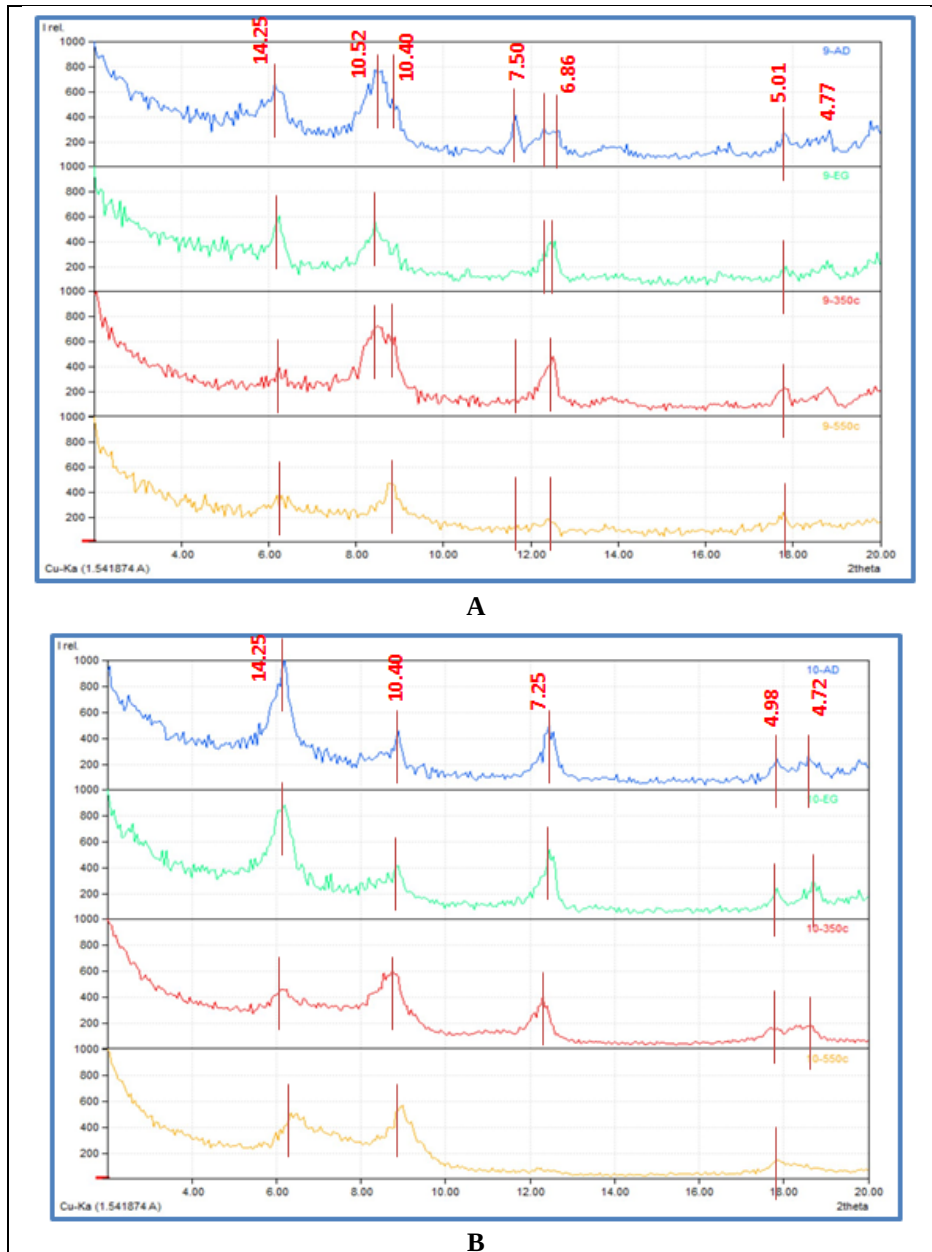
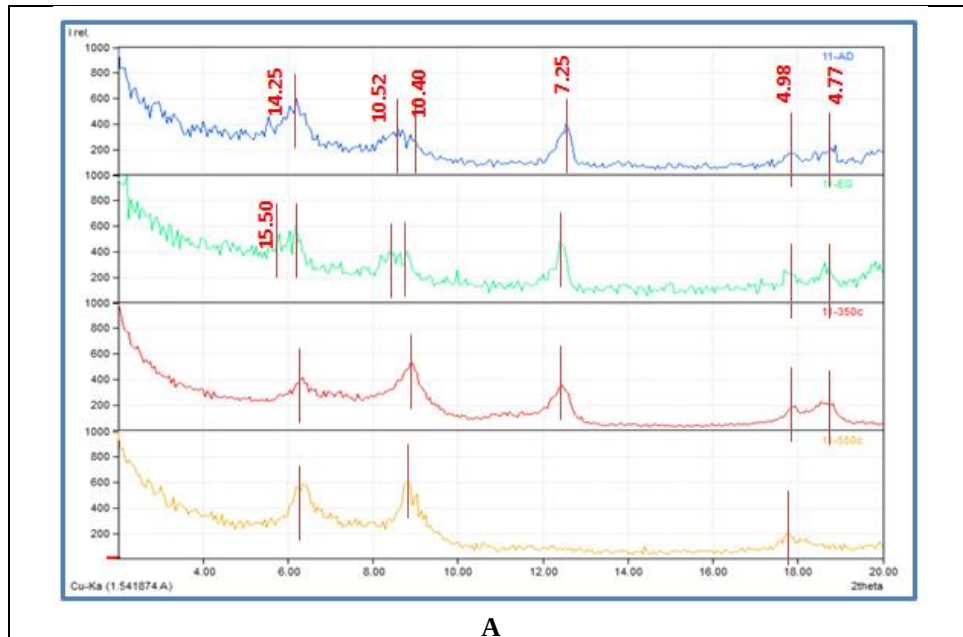


Figure 3. X-ray diffractions of AL-Tubar village soil A. surface horizon , and B. subsurface horizon.

Results in Fig (4A) representing the clay sample of surface horizon in Almishkhab soil south of Al-Handia Dam show the presence of smectite minerals in the clay fraction, which indicated by 14.25 Å⁰ reflection in air-dried, Mg-saturated treatment and shifts to an 15.50 Å⁰ reflection following ethylene-glycol salivation, and then shifts to an 10.40 Å⁰ reflection in K-saturated and heating to 350 and 550°C, and show that the continued presence of 14.25 Å⁰ reflection in all treatments, reflecting the presence of real chlorite in this horizon, confirming to that is the appearance of his third diffraction at d-spacing of 4.77 Å⁰ . while the appearance of 10.52 Å⁰ reflection in treatments of air-dried, Mg-saturated, ethylene glycol salivation, and K-saturated with

heat treatment to 350 °C , and disappeared in K-saturated with heat treatment to 550 °C, confirmed the presence of pedogenic palegoreskite, which is characterized by weakly crystalline and not resistant to heat [24]. The presence of 10.40 Å⁰ reflection in air-dried, Mg-saturated treatment, and remaining without change in all treatments, reflecting the presence of Mica minerals in the clay fraction of this soil, while the appearance of second order of Mica with a low intensity at d-spacing of 4.98 Å⁰ confirmed the presence of Biotite in this soil. Also, Kaolinite was diagnosed through presence of 7.25 Å⁰ reflection in treatments of air-dried, Mg-saturated , ethylene glycol salvation , and K-saturated with heat treatment to 350 °C, and disappeared in K-saturated with heat treatment to 550 °C.

Results in Fig (4B) representing the clay sample of subsurface horizon in Almishkhab soil, and show the presence show the presence of smectite minerals in the clay fraction, which indicated by 14.03 Å⁰ reflection in air-dried, Mg-saturated treatment and shifts to an 15.50 Å⁰ reflection following ethylene-glycol salvation, and then shifts to an 10.40 Å⁰ reflection in K-saturated and heating to 350 and 550°C, and show that the continued presence of 14.03 Å⁰ reflection in all treatments, reflecting the presence of real chlorite in this horizon, confirming to that is the appearance of his third diffraction at d-spacing of 4.82 Å⁰ . while the appearance of 10.50 Å⁰ reflection in treatments of air-dried, Mg-saturated, ethylene glycol salvation, and K-saturated with heat treatment to 350 °C , and disappeared in K-saturated with heat treatment to 550 °C, confirmed the presence of pedogenic palegoreskite, which is characterized by weakly crystalline and not resistant to heat [24]. The presence of 10.40 Å⁰ reflection in air-dried, Mg-saturated treatment, and remaining without change in all treatments, reflecting the presence of Mica minerals in the clay fraction of this soil, while the appearance of second order of Mica with a low intensity at d-spacing of 5.24 Å⁰ confirmed the presence of Biotite in this soil. Also, Kaolinite was diagnosed through presence of 7.25 Å⁰ reflection in treatments of air-dried, Mg-saturated , ethylene glycol salvation , and K-saturated with heat treatment to 350 °C, and disappeared in K-saturated with heat treatment to 550 °C.



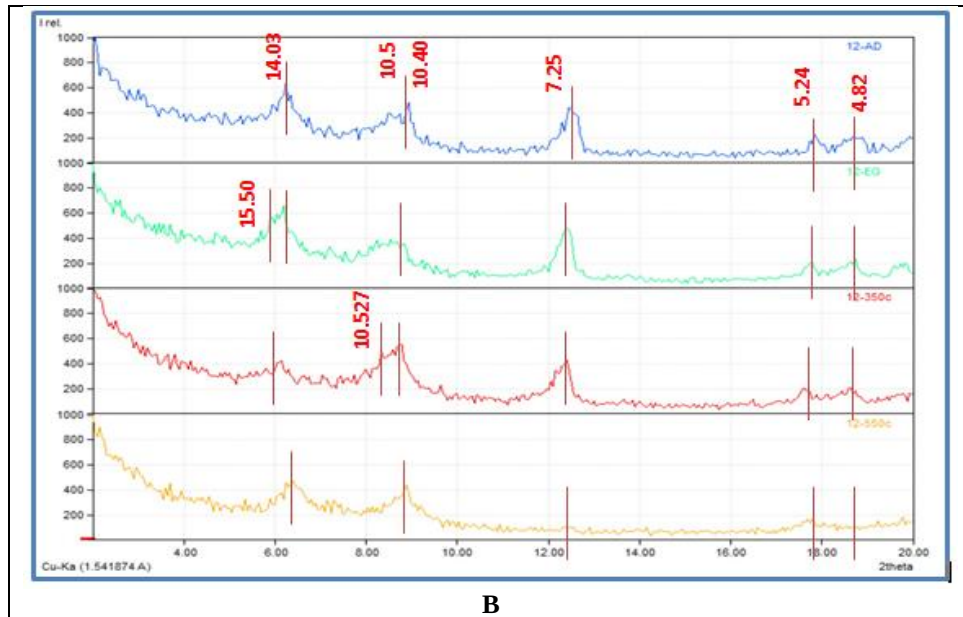


Figure 4. X-ray diffractions of Almishkhab soil A. surface horizon, and B. subsurface horizon.

Conclusions

- We conclude from the results of the x-ray diffraction examinations of the study soils that there is a convergence in the mineral content for clays of these soils, and this is attributed to the fact that the sources of formation of these soils are the same, and that they come from the rock fragments of the mountain ranges in southern Anatolia in Turkey, or what is added to the river load during the river's progress towards Iraqi lands.
- As for the impact of both the location of the Hindiya Dam and the regulators built on the side water branches on the general course of the Euphrates River, these engineering structures were influential in the sorting and sedimentation process of these minerals depending on their sizes and specific weights, as it was noted that the distribution of clay minerals was based on their total, which is a reflection of the severity of their exposure to weathering processes, whether at their sources or during transport and sedimentation processes.
- The sorting and distribution processes of these minerals were also affected by both the speed of water current and the capacity of the river load. It was noted that the content of both montmorillonite and layered minerals (smectite-mica) increased in the river sediments within the soil of the Mishkhab site due to the effect of the decrease in the speed of the water current and the capacity of the river load as the river course moved southward.

References

- [1] Mohammed, A.B., AlTae, M.M.S., F.M. Hassan, F.M. 2009. The Study of some PAHs Compounds in Euphrates River Sediment from Al-Hindiya Barrage to Al-Kifil city, Iraq. 4th Scientific Conference, College of Science, Babylon University. CSASC English Ver. 4(2009): 216–230.
- [2] Vital, H., & Statteger, K. 2000. Major and trace elements of stream sediments from the lowermost Amazon River. Chemical Geology, 168(1-2), 151-168.

- [3] Borger, H., McFarlane, M. J., & Ringrose, S. 2004. Processes of silicate karstification associated with pan formation in the Darwin–Koolpinya area of northern Australia. *Earth Surface Processes and Landforms: The Journal of the British Geomorphological Research Group*, 29(3), 359-371. <https://doi.org/10.1002/esp.1041>.
- [4] Soil Survey Staff .2006. Soil survey manual .USDA. Handbook No.18.US Government Printing Office. Washington , DC.20402.
- [5] Zarfl, C.; Lumsdon, A.E.; Berlekamp, J.; Tydecks, L.; Tockner, K. A.2015. Global boom in hydropower dam construction. *Aquat. Sci.* 2015. 77, 161-170. [Google Scholar] [Cross Ref]
- [6] Van Cappellen, P.; Maavara, T.,2016. Rivers in the Anthropocene: Global scale modifications of riverine nutrient fluxes by damming. *Ecohydrol. Hydrobiol.* 16, 106-111. [Google Scholar] [Cross Ref] [Green Version].
- [7] ICOLD.2021. (International Commission on Large Dams). World Register of Dams.. Available online: <http://www.icold-cigb.org> (accessed on 26 March 2022).
- [8] Zhao, B.; Yao, P.; Li, D.; Yu, Z.2021. Effects of river damming and delta erosion on organic carbon burial in the Changjiang Estuary and adjacent East China Sea inner shelf. *Sci. Total Environ.* 793, 148610.
- [9] Zhang, Y., C. Fang.,Y. Wang., X. Lou., Y. Su., and D. Huang. 2022. Review of Effects of Dam Construction on the Ecosystems of River Estuary and Nearby Marine Areas. *Sustainability*, 14(10), 5974; <https://doi.org/10.3390/su14105974>.
- [10] Day, P.R. 1965. Particle Fractionation and Particle Size Analysis, Ch. 43 in, Black, C.A. (editor), *Method of Soil Analysis*. American Society of Agronomy No. 9.
- [11] Page, A.L., R.H. Miller, and D.R. Kenney .1982. *Methods of Soil Analysis Part (2).Chemical and Microbiological properties* .2nd ed. Agronomy 9 Am. Soc. Agron. Madison, Wisconsin . Physical properties of a group of natural soils of Israel .*Geoderma* 3:185-198.
- [12] Hesse , P.R. 1971. A text book of soil chemical analysis. John murray. LTD. London, british. [https:// twitter.com/Agata](https://twitter.com/Agata) .In calcareous ,Gypsiferous,Saline Soils and Sediments of Bakhtegan lake Bank ,Southern Iran .*Iran Agri.*25(2) and 26(1-2):71-86.
- [13] Blak, G.R. 1965. Chapter 30, Bulk Density. In C.A., Black editor, 1965. *Methods of soil analysis*. Part 1, American Society of Agronomy. No. 9.
- [14] Al-Shahmani, Laith Salman Dawood. 2020. Variation in the distribution of minerals in the Gharraf River sediments in Waist and Dhi Qar governorates. Doctoral thesis. College of Agriculture - University of Baghdad.
- [15] Awad, Kazem Mashhut .1986. *Principles of Soil Chemistry*, Ministry of Higher Education and Scientific Research, University of Basra.
- [16] Asadu, C. L. A., J. Diels. and B. Vanlauwe .1997. a Comparison of the Contributions of Clay, Silt, and Organic Matter to the Effective CEC of Soils of Subsaharan Africa. *Soil Science*, 162 (11), 785-794.
- [17] Zaid, M. S., A. H. Rizk., A. M. A. Mashhour., and MA, W .2017. Interrelationships between some soil quality parameters and wheat productivity for some soils of Monufya Governorate. *Al-Azhar. J. Agric. Res.*, Vol.26, pp441-451.
- [18] Al-Khalil, Shireen Moudhfar Ali. 2020. The effect of precipitation sources on the variation in the characteristics and distribution of feldspar minerals in some soils of Wasit and Maysan governorates. PhD thesis. College of Agricultural Engineering Sciences. Baghdad University.
- [19] Al-jaff .B.O and S.K.Essa.2020. Effect of plant coverage on mineralogical changes of mica in Rhizosphere .*Iraqi journal of Agricultural Sciences* 51(1):493-499.
- [20] AL-Shamari, A.H.D and S.K.Essa.2020. The Effect of sedimentation sources the exchange properties of the clay particles of some soils in waist Maysan governorates. *Plant Archives* Vol.20, Supplement 2, pp.566-573.
- [21] Al-Wotaify, Abbas Sabir. 2012. The effect of montmorillonite to chlorite transformations on the physicochemical properties of some Iraqi soils. PhD thesis. Baghdad University. faculty of Agriculture.



- [22] Al-Khalil, Shireen Moudhfar Ali. 2020. The effect of precipitation sources on the variation in the characteristics and distribution of feldspar minerals in some soils of Wasit and Maysan governorates. PhD thesis. College of Agricultural Engineering Sciences. Baghdad University.
- [23] Shahad. R.F. 2021. Using the center of gravity equation and mineral properties to assess the state of potassium in some soils of the Middle Euphrates. Ph.D. Thesis. College of Agriculture . University of Muthanna.
- [24] Hojati, S., Khademi, H., & Cano, A. F. 2010. Palygorskite formation under the influence of saline and alkaline groundwater in central Iranian soils. Soil science, 175(6), 303-312.

Effect of Planting Date on Vegetative Growth Traits, Yield and its Components of Sweet Corn Hybrids

Firas Ahmed Mohammed¹ and Ammar Hashim Saeed²

^{1,2}Department of Horticulture and Landscape Engineering, College of Agriculture, Tikrit University, Tikrit, Iraq.

¹E-mail: firas.a.m030@st.tu.edu.iq

²E-mail: Dr.ammarhashim@tu.edu.iq

Abstract. This experiment was conducted at the Research Station of the Department of Horticulture and Landscape Engineering/Open Field, College of Agriculture, Tikrit University, in the spring agricultural season of 2024. To study the effect of spraying planting date on the vegetative growth traits and yield of sweet corn hybrids with the aim of determining the best planting date and the best hybrid. It included two factors: The first factor: two planting dates on March 15, April 1 and the second factor hybrid: Sweet Spirit, 007R, SS3006 and 6800R. The experiment was implemented according to the split plot system using randomized complete block design (RCBD) and with three replicates. The planting date was placed on the main plots while the hybrid took sub plots as the most important. The results of the experiment were analyzed using the (SAS) program and the averages were compared statistically according to Duncan's multiple-nomial test at a probability level of 0.05. The results showed that the planting date on April 1 was superior in most of the studied growth and yield traits (field emergence speed, plant height, leaf area, chlorophyll concentration in leaves, total grain yield, percentage of protein in grains, percentage of total sugars in grains and percentage of total soluble solids in grains T.S.S) amounted to 83.61%, 123.1 cm plant⁻¹, 3283.9 cm² plant⁻¹, 0.9155 mg g⁻¹ fresh weight, 4121.0 kg ha⁻¹, 3.574%, 7.752% and 17.72% respectively. As for the effect of hybrid treatments, the results of the study showed that the hybrid SS3006 was superior to the rest of the hybrids for most growth and yield traits, as the field emergence speed reached 88.89%, the leaf area reached 3378.9 cm² plant⁻¹, the chlorophyll concentration in the leaves was 1.136 mg g⁻¹ fresh weight, the total grain yield was 5184.0 kg ha⁻¹, the percentage of protein in the grain was 3.587%, the percentage of total sugars in the grain was 8.226%, while the hybrid 6800R was superior in the plant height trait and recorded 128.2 cm plant⁻¹, and with regard to the trait of the percentage of total soluble solids in the grain, the results indicated the superiority of the hybrid Sweet Spirit and gave 18.48%. As for the bilateral interactions between planting date and hybrid, there were significant differences as a result of these interactions, as the interaction treatment at planting date on April 1 and hybrid SS3006 outperformed in most vegetative growth and yield traits.

Keywords. Planting date, Sweet corn, Hybrid.

1. Introduction

Corn is one of the important crops that ranks first in the world after wheat and rice in terms of cultivated area and grain productivity, due to its nutritional and industrial importance in addition to its medical and feed importance[1], so it is called the miracle crop [2] and at the level of Iraq, the cultivated area amounted to about 360 thousand dunums, and the productivity amounted to 538 thousand tons [3] Sweet corn (*Zea mays* L. var *saccharata*) is one of the groups of field corn and belongs to Poaceae family. It is a monocotyledonous plant, monoecious, and has a chromosome number of $20 = 2n$. The original home of sweet corn is Central America and Mexico. Its plants contain a recessive pair of genes, SU (sugar-specific genes), which prevents the conversion of sugar into starch [4]. Sweet corn is believed to have originated as a mutation of maize at the sugar locus on chromosome 4, both of which belong to the plant species *Zea mays*. Sweet corn differs from field maize in that its grains contain a high percentage of sugar in both the milk stage and the early dough stage, and its dry grains are wrinkled and translucent, and vary based on genetic makeup [5]. The sweet taste of sweet corn grain distinguishes them from the rest of the yellow corn groups, so they are used fresh and often eaten uncooked in salads and are also used in other food fields. Sweet corn kernels contain 70% water, 5-6% sugars, 10-11% starch, 3% soluble sugars, in addition to moderate levels of proteins, vitamins and potassium[6]. Sweet corn plays an important role in the healthy human diet because it contains minerals, vitamins, antioxidants and carotenoids, and it also contains a low percentage of cholesterol [7]. Sweet corn is one of the most popular garden vegetables in Europe and developed countries in the world [8]. The lack of information and scientific research on the cultivation of sweet corn in Iraq prompts us to study many factors that affect the increase in its production, in addition to the fact that this crop is not of interest to producers and researchers in Iraq and has not been introduced as a food crop to contribute to the provision of food. Despite this, the climatic conditions are favorable for the cultivation and production of this crop, as its environmental requirements in terms of temperature and the length of the light period are completely similar to the conditions for growing yellow field corn, in addition to being an economically profitable crop for the farmer due to its short planting season, especially for the early maturing genetic compositions that allow the cultivation of two crops in the same field, in addition to the low production costs compared to other vegetable crops and its effect on reducing weeds in the field when planted in succession with wheat or barley [9]. Therefore, it is possible to successfully cultivate and produce sweet corn in Iraq, which makes the possibility of spreading the cultivation of this crop possible through many factors, including choosing the best and most suitable planting date for the growth of this The crop, which gains special importance in crop planning and management because it affects various growth characteristics and stages, and it is possible to increase the efficiency of using environmental factors that affect the final yield [10]. The planting date can play a major role in determining the plant yield, seed quality and germination and helps in understanding the physiological stages of the plant [11]. Determining the planting date is an important factor in determining the germination date, growth rate, and plant development, and thus increasing the level of vegetative growth, yield and production, which is reflected in increasing the size of the grains in quantity and quality [12,13]. Early planting of plants exposes them to problems of low temperatures, and delaying the planting date exposes plants in the flowering stage to problems of high temperatures, which leads to weak pollination and fertilization [14]. Therefore, when studying the planting date, it is necessary to take into account the environmental conditions of the agricultural area [15].

The results of many researchers indicated the effect of planting dates on the growth and yield of sweet corn plants, as the date of April 25 - May 5 was significantly superior in most of the studied traits, and gave the highest average in the trait of the number of grains in the ear compared to the

rest of the other dates [16] and the planting date on March 21 was superior by recording the highest plant height, while the date of August 22 was excelled in the traits of ear length and ear diameter by recording the highest value for the two traits [17] and the date of July 2 was excelled by giving the highest length and diameter of the ear of sweet corn compared to the rest of the dates [18] and the planting date in mid-June gave the highest plant height and the highest dry weight of the vegetative group, while the date of mid-July recorded the highest production of sweet corn ears compared to the rest of the other dates [19].

Hybrids are one of the most important factors that determine productivity, as genetic factors, regional conditions, and the interaction between them play a major role in determining the degree of growth and development of the organism. Therefore, choosing the hybrid appropriate for the region is one of the basic steps to ensure good growth, which is positively reflected in production. Genetic compositions differ among themselves in terms of growth and yield traits[20]. Choosing the performance of these hybrids is considered essential, as it is based on their distinction in a number of traits that enable them to achieve a high and desirable yield for the consumer, in addition to their tolerance to different environmental conditions and their resistance to different types of pests [21]. One of the main important factors that contribute to increasing production is cultivation hybrids with high productivity and desirable specifications that are suitable for the environmental conditions of a particular region[22]. The variation of genetic compositions in growth and yield traits, such as plant height, cob length and diameter, and the number of vegetative and fruit branches, represents one of the main factors that determine the increase in productivity and quality of the crop. This variation leads to the emergence of clear changes in growth and production traits and reflects the effect of the interaction between genetic factors and environmental conditions in the experimental area, which clearly shows its effect in the phenotypic and production traits [23]. Several studies have indicated the existence of variation in the genetic compositions of sweet corn plants. [24] concluded in a study he conducted on sweet corn plants that there were significant differences between three hybrids of sweet corn Roi Soleal, Seker Misir and Succar, as the hybrid Seker Misir was superior in the trait of plant height, leaf chlorophyll content and plant grain yield compared to the rest of the hybrids. [25] indicated through his study conducted on five genetic compositions of sweet corn plants Hazar, Roi Soleill, Talar, Succir and Zuccherion that the genetic composition Talar was excelled in most of the studied traits, as it gave the highest average in the traits of leaf area, plant height, cob diameter, ear weight, 300-grain weight, and total grain yield compared to other hybrids. [26] showed through their experiment on three genetic compositions of sweet corn Sugar 75, FSCH 75 and CMVL SC genetic composition excelled Sugar 75 significantly by giving the highest rate in the number of cobs, number of grains per cob, cob weight with sheaths, cob length and fresh ear yield. This study aimed to determine the best hybrid of sweet corn plants newly introduced to the country for cultivation in Salah Al-Din province, as well as to determine the appropriate concentration of spraying with gibberellin acid, and the effect of the interaction between the study factors in giving the best traits for vegetative growth, yield and its components.

2. Materials and Methods

The experiment was conducted during the spring season of 2024 at the Horticultural Research Station affiliated to the Department of Horticulture and Landscape Engineering - College of Agriculture - Tikrit University - Open field, and the crop was irrigated in this experiment using the drip irrigation method, and random samples were taken from different places in the experimental field before cultivation at a depth of (0 - 30) cm. Table 1 shows the physical and chemical properties of the soil.

Table 1. Physical and chemical properties of the field soil before cultivation.

Physical and chemical properties of soil	Units	Values	Physical and chemical properties of soil	Units	Values
Phosphorus	mg L ⁻¹	36.2	Ph	-	7.35
Potassium	mg L ⁻¹	96.2	OM	%	0.51
Nitrogen	mg L ⁻¹	325.3	sand	%	38
Gypsum	mg kg ⁻¹	165.2	silt	%	32
Lime	%	16.60	clay	%	30
EC	Ds.m ⁻¹	0.9	soil texture		Sand clay loam

Table 2. Monthly averages of minimum and maximum temperatures and rainfall in Tikrit city during the experimental period for the 2024 season.

Months	Minimum temperature (°C)	Maximum temperature (°C)	Average temperature (°C)	Rainfall rate (mm)
March	9.57	23.07	16.15	26.1
April	17.36	33.29	25.48	14.6
May	20.21	35.16	27.77	12.4
June	24.61	46.86	34.00	0.3

*Iraqi Agricultural Meteorology Network - Tikrit – 2024.

After selecting the area allocated for cultivation, the field land of the horticultural station was plowed with Mold-board plows, then smoothed and leveled. The land was divided into plot (experimental units) with dimensions of 1.25 m x 2.8 m, with an area of 3.5 m², with 36 experimental units distributed over three replicates, where each replicate included 12 experimental units. The distance between each line was 70 cm, and the distance between plants on the same line was 25 cm. The experimental unit included four lines, and in each line five plants, which means that each experimental unit had 20 plants. Spaces were left between the plot at an average of 1 m, as well as a distance of 1 m between the replicates. After that, the ground fertilizer was added according to what was mentioned by [27] nitrogen fertilizer at an average of 320 kg ha⁻¹, triple superphosphate fertilizer at an average of 200 kg ha⁻¹, and potassium sulfate fertilizer at an average of 80 kg ha⁻¹. Mixing the added fertilizers with the soil, followed by installing the drip irrigation system at an average of four pipes inside the experimental unit, then watering the soil before cultivation, then cultivation the sweet corn seeds in the experimental land directly in the early morning by placing three seeds in each hole on the sides of the lines inside the panels (experimental units) according to the scheduled cultivation dates, the first date on 3/15/2024 and the second date on 4/1/2024, followed by the irrigation process directly, the germination and emergence process was monitored, and the necessary agricultural operations were conducted for all plants in a similar manner, the experimental land was netted to avoid bird damage to the planted seeds and the net was lifted after 15 days of germination, the plants were thinned to one plant in each pit, and at the same time the patching process was conducted for the plants in the failed holes after the emergence of 75% of the plants, and all agricultural operations were conducted from removing the bushes, weeding and manual weeding, as well as preventive operations against disease, fungal and insect infections when they appear using the appropriate methods for each case in At that time, the corn stalk borer (*Sesamia cretica*) was controlled locally by feeding the growing tip of the stalk with diazinon (10% active ingredient) at an average of 6 kg/ha⁻¹ and placing it in two batches, the first after 20-25 days from cultivation and the second after 15 days from the first batch [28]

Study factors:

- First factor: Planting date: The first date was on March 15, the second date was on April 1.
- Second factor: Sweet corn hybrids: Four sweet corn hybrids (recently introduced to the country) were used: Sweet Spirit, 007R, SS3006 and 6800R, and the producing company is Syngenta / Switzerland

2.1. Experimental Design

The experiment was carried out according to the Randomized Complete Block Design (RCBD) with a split plot design and three replicates, where the planting date factor took the main plot and the sweet corn hybrids took the sub plot as the most important. The experiment included 8 treatments resulting from the interaction of the study factors (2×4), and at the end of the experiment, the averages of traits were recorded and the results were statistically analyzed using a computer according to the SAS program [29] and the averages were compared according to Duncan's multiple range test at a probability level of 0.05 [30].

2.2. The Studied Traits

Field emergence speed %, plant height cm plant⁻¹, leaf area cm² plant⁻¹, chlorophyll concentration in leaves mg g⁻¹, fresh weight, total grain yield kg ha⁻¹, percentage of protein in grains %, percentage of total sugars in grains % and percentage of total soluble solids in grains %.

3. Results and Discussion

3.1. Effect of Planting Dates on Vegetative Growth Traits, Yield and its Components of Sweet corn Plants

Table 3 shows the superiority of planting date on April 1 for the traits of field emergence speed, plant height, leaf area, chlorophyll concentration in leaves, total grain yield, percentage of protein in grains, percentage of total sugars in grains and percentage of total soluble solids in grains, and recorded the highest values of 83.61%, 123.1 cm plant⁻¹, 3283.9 cm² plant⁻¹, 0.9155 mg g⁻¹ fresh weight, 4121.0 kg ha⁻¹, 3.574%, 7.752% and 17.72% respectively.

Table 3. Effect of planting dates on vegetative growth traits, yield and its components of sweet corn plants.

Planting dates	Traits							
	Field emergence rate %	Plant height cm plant ⁻¹	Leaf area cm ² plant ⁻¹	Chlorophyll concentration in leaves mg gm ⁻¹	Total grain yield kg ha ⁻¹	Percentage of protein in grain %	Percentage of total sugars in grain %	Percentage of total soluble solids in grain %
March 15	38.61 b	121.7 b	3216.3 b	0.8455 b	4080.4 b	2.943 b	7.390 b	17.18 b
April 1	83.61 a	123.1 a	3283.9 a	0.9155 a	4121.0 a	3.574 a	7.752 a	17.72 a

The averages that carry the same alphabetical letters for the single factors and their interactions do not differ significantly from each other according to Duncan's multiple range test at the probability level of 0.05.

3.2. The Effect of Hybrids on Traits of Vegetative Growth, Yield and its Components of Sweet corn Plants

Table 4 shows the occurrence of significant differences between sweet corn hybrids, as the hybrid SS306 significantly outperformed the rest of the other hybrids in traits of field emergence speed, leaf area, chlorophyll concentration in leaves, total grain yield, percentage of protein in grains, percentage of total sugars in grains, and gave the highest values, reaching 88.89%, 3378.9 cm² plant⁻¹, 1.136 mg g⁻¹ fresh weight, 5184.0 kg ha⁻¹, 3.587%, 8.226% respectively, while the hybrid 6800R significantly outperformed the rest of the other hybrids in The plant height trait gave the highest value of 128.2 cm.plant⁻¹, and with regard to the trait of the percentage of total soluble solids in grains, the same table indicated that the hybrid Sweet Spirit was significantly traits to all other hybrids by giving the highest value for the trait of 18.48%.

Table 4. Effect of hybrids on the traits of vegetative growth, yield and its components for sweet corn plants.

Sweet corn hybrid s	Traits							
	Field emergenc e rate%	Plant heigh t cm plant ⁻¹	Leaf area cm ² plant ⁻¹	Chlorophyll concentratio n in leaves mg gm ⁻¹	Total grain yield kg ha ⁻¹	Percentag e of protein in grain%	Percentag e of total sugars in grain%	Percentag e of total soluble solids in grain%
Sweet Spirit	49.17 d	122.0 b	3206. 4 c	07923 c	3416. 2 c	3.154 c	7.286 c	18.48 a
007R	36.94 c	119.9 c	3120. 1 d	0.7292 d	2924. 6 d	2.944 d	7.051 d	17.32 b
SS3006	88.89 a	119.5 c	3378. 9 a	1.136 a	5184. 0 a	3.587 a	8.226 a	17.09 c
6800R	69.45 b	128.2 a	3295. 2 b	0.8650 b	4877. 8 b	3.349 b	7.721 b	16.93 c

The averages with the same alphabetical letters for the single factors and their interactions are not significantly different from each other according to Duncan's multiple range test at the probability level of 0.05.

3.3. Effect of the Interaction Between Planting Dates and Hybrids on the Vegetative Growth Traits , Yield and its Components of Sweet corn Plants

The results of Table 5 indicate that the interaction between the planting date on April 1 and the hybrid SS3006 is superior to most of the interaction traits in traits of field emergence speed, leaf area, chlorophyll concentration in leaves, total grain yield, percentage of protein in grains and percentage of total sugars in grains amounted to 93.89%, 3395.3 cm² plant⁻¹, 1.180 mg g⁻¹ fresh weight, 5208.9 kg ha⁻¹, 3.868% and 8.400% respectively, and the binary interaction between the planting date on April 1 and the hybrid 6800R is significantly traits to most of the interaction traits The interaction in the plant height trait gave the highest value of 128.6 cm plant⁻¹, and the same table showed a significantly excelled for bi-interaction between the planting date on April 1 and the hybrid Sweet Spirit in the trait of the percentage of total soluble solids in grains and gave the highest value of 19.41%.

Table 5. Effect of interaction between planting dates and hybrids on the traits of vegetative growth, yield and its components for sweet corn plants.

Hybrid sweet corn	Planting dates	Traits							
		Field emergence rate%	Plant height cm plant ⁻¹	Leaf area cm ² plant ⁻¹	Chlorophyll concentration in leaves mg gm ⁻¹	Total grain yield kg ha ⁻¹	Percentage of protein in grain%	Percentage of total sugars in grain%	Percentage of total soluble solids in grain%
Sweet Spirit	March 15	12.22 f	121.1 c	3176.3 cd	0.7684 e	3404.5 c	2.808 e	7.126 e	17.54 b
	April 1	86.11 bc	123.1 b	3236.4 c	0.8162 d	3427.9 c	3.500 b	7.446 cd	19.41 a
007R	March 15	11.11 f	119.5 de	3079.3 d	0.7091 f	2889.2 d	2.577 f	6.830 f	17.22 cd
	April 1	62.78 d	120.4 cd	3160.8 cd	0.7493 e	2960.0 d	3.310 c	7.271 de	17.41 bc
SS3006	March 15	83.89 c	118.7 e	3362.5 a	1.091 c	5159.1 a	3.306 c	8.052 b	17.05 d
	April 1	93.89 a	120.3 cd	3395.3 a	1.180 a	5208.9 a	3.868 a	8.400 a	17.13 cd
6800R	March 15	47.22 e	127.8 a	3247.3 bc	0.8135 d	4868.6 b	3.082 d	7.553 c	16.92 d
	April 1	91.67 ab	128.6 a	3343.1 ab	0.9165 c	4887.0 b	3.616 b	7.889 b	16.93 d

The averages with the same alphabetical letters for the individual factors and their interactions do not differ significantly from each other according to Duncan's multiple range test at the probability level of 0.05.

The results of the statistical analysis in each of the field emergence speed, plant height, leaf area of the plant, chlorophyll concentration in the leaves, total grain yield, percentage of protein, percentage of total sugars in the grain and percentage of total soluble solids in the grain in Tables 3, 4, 5 indicated a significantly excelled at the planting date on April 1. This superiority may be attributed to the positive and good compatibility between the ideal environmental factors such as suitable temperatures, sufficient photoperiod and availability of nutrients during the different growth stages, which had a positive effect on increasing the speed of vital processes within plant tissues, which in turn led to improving the physiological performance of the plant, which was positively reflected in flowering and increasing total productivity [31]. Temperature is one of the important factors that affect the vital processes inside the plant. For the sweet corn plant, the ideal temperature for its growth ranges between 21-27 degrees Celsius. Therefore, an increase or decrease in temperature from this ideal range will directly affect the growth of this plant [32]. The results of the study agree with what was reached by [33,34,35] in that the planting dates in the spring season had a significant effect on most growth and yield traits. In addition, the results of the study were consistent with what was found by [36, 37,38,39]. The reason for not excelled the first planting date on March 15 may be due to the fact that the sweet corn plant was exposed to problems of low temperatures during the early growth stages of the plant, as shown in Table 2, which caused a slowdown in the photosynthesis processes and a decrease in the plant growth rate [14]. This is consistent with [35,40] who indicated that early planting in the spring tends to lead to a slowdown in the speed of plant growth and development. The results of Tables 3, 4, and 5 show that there were significant differences in the hybrid factor for growth traits and yield of

plant height, leaf area, chlorophyll concentration in leaves, total grain yield, percentage of protein in grains, percentage of total sugars in grains and percentage of total soluble solids in grains. The reason may be due to the presence of genetic differences between the hybrids under study and that these genetic differences between the hybrids result in a final difference in the traits of vegetative growth and yield, or the reason may be differences in the genetic structure between the hybrids of sweet corn, which differed significantly among themselves[41]. On the other hand, this difference may be related to the size of the root system and its branching between soil particles and the rate of photosynthesis in the area unit of the leaves and the extent of the availability of enzymes necessary for this process in addition to the rate of transfer of manufactured food materials, which depends on the thickness of the leaves and the size of the stomata in them[42]. The results of the study were consistent with the experiences of many researchers through their studies for the purpose of evaluating sweet corn hybrids. The results they obtained indicated that the genetic compositions of sweet corn hybrids differed significantly in the studied vegetative growth and yield traits, including [43,44,45,46]

Recommendations

The results of this study showed that the best results achieved for most of the vegetative growth indicators, yield traits and components were when planting on April 1 and the hybrid SS3006, so it is recommended to conduct more studies in the future and use different planting dates and use other hybrids of sweet corn plants.

References

- [1] FAO. (2019). FAO Statistical Databases. Food and Agriculture Organization of the United Nations, Rome, Italy. available online at: <http://faostat.fao.org/default.aspx>.
- [2] Al-Maliki S., A.Al-Amery., M. Sallal, A. Radhi and D. K.A. Al-Taey.(2021). Effects of Arbuscular Mycorrhiza and Organic Wastes on Soil Carbon Mineralisation, Actinomycete and Nutrient Content in Maize Plants (*Zea Mays L.*). Malaysian Journal of Soil Science Vol. 25 : 107-124.
- [3] Ministry of Planning, Central Statistical Organization (2023). Statistical Abstract issued by the Central Statistical Organization, Statistics and Geographic Information Systems Authority, Ministry of Planning, Iraq.
- [4] Tracy WF, Shuler SL, Dodson-Swenson H. (2020). Sweet Corn. In: Wein HC, Stützel H. The Physiology of Vegetable Crops (eds.). CAB International, Boston.
- [5] Patel R, Memon J, Patel DA, Patil K, Borkhatariya T. (2023). Assessment of genetic diversity of sweet corn (*Zea mays* convar. *saccharata* var. *rugosa*) genotypes using D2 statistics. The Pharma Innovation Journal,12(3):2642-2645.
- [6] Oktem, A. and Oktem, A. (2005). Effect of nitrogen and intra spaces on sweet corn (*Zea mays* *Sachharata* Sturt) ear traits. *Indian Journal of Plant Sciences*, 4(4): 361-363.
- [7] Swapna, G., Jadesha, G., Mahadevu, P. (2020). Sweet Corn - A Future Healthy Human Nutrition Food. *International Journal of Current Microbiology and Applied Sciences*. 9(7): 3859-3865.
- [8] Becerra-Sanchez. F, Taylor G .(2021). Reducing post-harvest losses and improving quality in sweet corn (*Zea mays L.*): challenges and solutions for less food waste and improved food security. *Food Energy Secur* 10:e277. <https://doi.org/10.1002/fes3.277>.
- [9] Sidakova. M. S, E. N. Didanova, A. Y. Kishev, R. Z. Abdulkhalikov. (2020). «Development of the Agro-Industrial Complex in the Context of Robotization and Digitalization of Production in Russia and Abroad», International Scientific and Practical Conference.
- [10] Azadbakht. A, G. Azadbakht. H. Nasrollahi and Z. Bitarafan. (2012). Evaluation of Different Planting Dates Effect on Three Maize Hybrids in Koohdasht Region of Iran. 2 (3).
- [11] Koca,Y.O., and Oner Canavar. (2014). The effect of sowing date on yield and yield components and seed quality of corn (*Zea mays L.*). Scientific Papers. Series A. Agronomy, Vol. LVII.

- [12] Khan, Z. H, S.K. Khalil, Ali, M. Ikramullahc, A. Iqbal, B. Islam, K. Ali, W. Ali, M. Ahmad, S.G. Afridi, F. Shah. (2019). Fresenius Environmental Bulletin, 6568.
- [13] Nazranov, K, E. Didanova, Z.-G. Shibzukhov, M. Orzalieva, B. Nazranov. (2020). «Development of the Agro-Industrial Complex in the Context of Robotization and Digitalization of Production in Russia and Abroad», International Scientific and Practical Conference.
- [14] Garcia, A.G.Y.; L.C. Guerra; G Hoogenboom. (2014). Impact of planting date and hybrid on early growth of sweet corn. Agron. J., 101(1): 193 – 200.
- [15] Holt, J.S. (1995). Plant responses to light: a potential tool for weed management. Weed Sci., 43.474-482.
- [16] Shibzukhov, Zalim-Geri, Kisev, Alim, Shibzukhova, Zalina, Eziev, Murat and Akbar, Ilahun. (2021). Sowing time influence on the sweet corn productivity in the mountainous ecologically clean zone of Kabardino-Balkaria, E3S Web of Conferences 262, 03024, <https://doi.org/10.1051/e3sconf/202126203024>.
- [17] Ibrahim, A. I. A. and Ghada, A. Alfauomy. (2019). “Evaluation of some Sweet Corn Hybrids for Agronomic Traits and Technological Parameters under different Planting Dates,” Suez Canal University Journal of Food Sciences, vol. 6, no. 1, pp. 49–63, doi: 10.21608/scuj.2019.67838.
- [18] Priyatam, Sidhartya and Rani, Sushree. Partikshya. (2021). Effect of sowing dates and variety on dry yield, yield attributes and post-harvest soil fertility in mid hill of Meghalaya under organic condition, The Pharma Innovation Journal 2021; SP-10(12): 1500-1502, doi: <https://dx.doi.org/10.22271/tpi>.
- [19] Momin, Tonya & Ray, Lala I.P. & Singh, Arvind & Swami, Sanjay & Singh, N.J. & Kurkalang, Saphina & Thokchom, Dorendchand. (2024). Agro-economic evaluation of sweet corn under varied dates of sowing and integrated nutrient regimes in mid altitude of Meghalaya. 55. 16969-16983.
- [20] Wahib, Karima Mohammed (2001). Evaluation of the response of some yellow corn genetic compositions to different levels of nitrogen fertilizer and plant densities and estimation of the path coefficient. PhD thesis. College of Agriculture. University of Baghdad. Iraq.
- [21] Al-Shammari, Aziz Mahdi Abdul; Daa Abdul Muhammad Al-Tamimi, Saba Subhi Khamis Junaid (2016). Effect of organic and chemical fertilization on vegetative growth traits and yield in cauliflower. Diyala Journal of Agricultural Sciences 8(2): 214-229.
- [22] Shafiq . Y; A . M . Abou Dahab and J . A. A . Ashoo . (2002) . Effects of different transcultivation Media on growth of crops . mesopotamia of Journal of Agriculture. 13(2) : 167-178.
- [23] Al-Rifai, Abdul Karim Hussein (2016). Response of wheat varieties ((Triticum aestivum)) to foliar feeding with iron and manganese. PhD thesis. College of Agriculture. University of Basra.
- [24] Al-Jubouri, Suleiman Dawood Najm (2021) The effect of cultivation method and foliar fertilization with boron on the growth and yield of sweet corn hybrids in the spring season, Master's thesis. Department of Horticulture and Landscape Engineering - College of Agriculture. University of Diyala. Iraq.
- [25] Al-Qaisi, Mohammed Mahrouz Abdullah (2022). Performance and stability of genetic compositions of sweet corn under different spraying levels of potassium and distance between plants, Master's thesis. Department of Field Crops - College of Agriculture. Tikrit University. Iraq.
- [26] Lone. A. H, T. A. Sheikh, B. A. Lone, T. A. Ahngar, Z. A. Bashir, M. A. Sheikh, M. S. Mir, Z. Rashid, S. Majid, F. Ur-Rasool and Z. A. Dar.(2022). “Performance of Sweet Corn Hybrids (Zea mays saccharata) as Influenced by Soil and Foliar Application of Zinc,” Journal of Experimental Agriculture International, vol. 44, no. 12, pp. 167–174, doi: 10.9734/jeai/2022/v44i122090.
- [27] Al-Abadi, Jalil Esbahi (2011). Guide to the uses of chemical and organic fertilizers in Iraq. Ministry of Agriculture / General Authority for Agricultural Guidance and Cooperation
- [28] Al-Barzanji, Zakaria Mahmoud Muhammad. (2006). The critical period in controlling weeds in yellow corn (Zea mays L.) Master's thesis, College of Agriculture / University of Baghdad.
- [29] SAS (2001) . SAS/STAT User's Guide for personal computers , SAS, Institute Inc, Cary , N.C. USA.

- [30] Al-Rawi, Khashea Mahmoud and Abdul Aziz Mohammed Khalaf Allah (2000). Design and analysis of agricultural experiments, Ministry of Higher Education and Scientific Research, Dar Al-Kutub for Printing and Publishing, Mosul, Iraq.
- [31] Kucharik C. J. (2008). Contribution of Planting Date Trends to Increased Maize Yields in the Central United States. *Agron. J.*, 100(2): 328 - 336.
- [32] Matloub, Adnan Nasser and Ezz El-Din Sultan Mohammed and Karim Saleh Abdul (1989). Vegetable production. Part Two - Revised Edition - Higher Education Press in Mosul / Iraq.
- [33] Gharibo, Gharibo Ahmed and Abdul Mohsen Sayed Omar (2010). Evaluation of the productivity of three yellow corn genotypes under the effect of different planting dates, *Arab Journal of Arid Environments*, 3(1): 4-11.
- [34] Sheikh Qoroosh, Mohammed (2013). Effect of anti-transpiration on physiological and productive indicators of two yellow corn (*Zea mays* L.) cultivars under water stress conditions. PhD thesis, University of Aleppo, Faculty of Agriculture.
- [35] Al-Hassani, Mohammed Hadi Obaid (2016). Response of sweet corn (*Zea mays* L. var *saccharata*) to planting dates and spraying with biostimulants, PhD thesis, College of Agriculture, University of Baghdad.
- [36] Amjadian, M., Farshadfar, M., and M. Gholipoor, (2013). The effects of planting dates on the yield and yield components of corn (*Zea mays* L.) cultivar single cross 704 in Gorgan region. *Annals of Biological Research*, 4(4): 38-41.
- [37] Jasemi, M., Darabi, F., Naseri, R., Naserirad, H., and S. Bazdar, (2013). Effect of Planting Date and Nitrogen Fertilizer Application on Grain Yield and Yield Components in Maize (SC 704). *American-Eurasian J. Agric. & Environ. Sci.*, 13 (7): 914-919.
- [38] Shrestha U, Amgain L P, Karki TB, Dahal K R and Shrestha J. (2016) Effect of Sowing Dates and Maize Cultivars in Growth and Yield of Maize along with their Agro-Climatic Indices in Nawalparasi, Nepal. *Journal of AgriSearch* 3 (1):57-62.
- [39] Haider, Lubna Nasr (2019). Effect of planting date on morphological traits of some sweet corn genotypes, *Syrian Journal of Agricultural Research*, Volume (6), Issue (2): 298-307.
- [40] Sangoi, L., Gracietti, M.A., Rampazzo, C. and P.Biachetti, (2002). Response of Brazilian maize hybrids from different eras to changes in plant population. *Field Crops Res.* 79: 39-51.
- [41] Al-Rifai, Abdul Karim Hussein (2016). Response of wheat varieties ((*Triticum aestivum*)) to foliar feeding with iron and manganese. PhD thesis. College of Agriculture. University of Basra.
- [42] Hassan, Ahmed Abdel Moneim (2017). Principles of vegetable crop breeding. First edition, Faculty of Agriculture, Cairo University, Egypt.
- [43] Subaedah, T. S., Edy, E., Mariana, K. (2021). Growth, Yield, and Sugar Content of Different Varieties Of Sweet Corn And Harvest Time. *International Journal Of Agronomy*. (1):1-7.
- [44] Mustafa. N. R, G. Bin Saleh, and P. Kashiani. (2021). "Genetic potential of tropical sweet corn hybrids and combining ability among parental inbred lines," *Aust J Crop Sci*, vol. 15, no. 10, pp. 1279–1288, doi: 10.21475/ajcs.21.15.10.p3189.
- [45] Ghara, Maya and Mohammed Nael Khattab (2024). Genetic divergence and establishment of selection evidence for sweet corn genotypes, *Syrian Journal of Agricultural Research*, 11(3): 253-264, June, 2024.
- [46] Wahyurini. E, B. Supriyanta and A. Suprihanti. (2023). "Growth and Yield Performance of Genotype Sweet Corn (*Zea mays* L) with Dosage of *Trichoderma* sp", *Earth and Environmental Science*, 1-6, doi:10.1088/1755-1315/1242/1/012001.

Dosing with an Aqueous Extract of Watercress Seeds for Iraqi Lambs and Its Effect on Reproductive and Antioxidants Parameters

Ahmed J. Al-Yasery¹, Hayder Abdullah Talib² and Jassim Kassim Al-Gharawi³

¹⁻³Animal Production Department, Agriculture College, Al-Muthanna University, Al-Muthanna, Iraq.

¹E-mail: ahmeadagr@mu.edu.iq

²E-mail: Agr-grad.haider.abd@mu.edu.iq

³E-mail: jasimirage@mu.edu.iq

Abstract. This study was conducted at Khairat Al-Ittihad Sheep and Cow Breeding Station, at Al-Shomali District, Babylon Governorate, during three months period from 15\12\2023 to 15\3\2024. The aim of conducting the study is to identify the effect of dosing the aqueous extract of watercress seeds *eruca sativa* to Iraqi lambs on reproductive and antioxidants parameters. 40 lambs aged (six months) were used in the experiment. The lambs of the four treatments were dosed with this extract in an amount of 0, 5, 10, 15 ml, for treatments T1, T2, T3 and T4, respectively. The results of the study showed that the aqueous extract of watercress seeds had a positive effect on the reproductive parameters in lambs. A significant increase in T3 at a significance level ($P \leq 0.05$) compared to the control treatment in the volume of semen and sperm concentration. A significant increase in the individual and collective movement of sperm and the percentage of live sperm. A significant decrease in the treatments of the aqueous extract of watercress seeds in deformed sperm and dead sperm. As for the color of semen and its acidity (pH), there were no significant differences among the treatments. As for the effect of the aqueous extract of watercress seeds on antioxidants, T3 was significantly superior compared to the control treatment at a significance level ($P \leq 0.05$) in superoxide dismutase (SOD), while T2 was superior compared to the control treatment in glutathione (GSH), T4 was superior compared to the control treatment in glutathione peroxidase (GPX), as for catalase (CAT) and glutathione transferase (GST), there were no significant differences among the treatments.

Keywords. Watercress seeds, Antioxidants, Dosage, Aqueous extract, Reproductive parameters.

1. Introduction

Watercress is a member of the cruciferous family, scientifically called Cruciferae, its common scientific name in scientific research is *Eruca Sativa*, it is known locally in Iraq as watercress [1]. It has been used in scientific experiments as leaves, seeds, extracts, or powdered seeds or leaves, in a way that ensures that the plant reaches the animal to study its effectiveness [2,3].

Watercress seeds contain a percentage of important active compounds from both the therapeutic and nutritional aspects, which include glycosides, tannins, flavonoids, alkaloids and saponins, in addition to containing proteins, carbohydrates, oils and vitamins, the most important of which are B group vitamins, such as B1, B2 and B12, it also contains minerals, especially sodium (Na), calcium (Ca) and potassium (K) [4,5].

Watercress seeds contain antioxidants that help prevent damage to body cells, repair damage caused by free radicals and the resulting oxidation. One of its most important activities inside the body is to interrupt the chain of reactions of free radicals and their metabolism to prevent or reduce their negative effects. One of the most important and active antioxidants that form a mutual defense system inside the body is superoxide dismutase (SOD) and glutathione peroxidase (GPX). Antioxidants have been divided into two types in terms of their composition: enzymatic and non-enzymatic antioxidants [6,7].

It is known that nutrition plays an effective and important role in terms of reproduction and its development, since the process of sperm production, the growth and development of the reproductive organs, and reaching puberty and sexual maturity, all of these require certain basic nutrients that animals obtain from the feed consumed [8,9], where watercress seeds contribute to reaching sexual maturity early and increasing the size and weight of the testicles better in lambs. They also contribute to giving the first ejaculation early due to their content of carotenoids, vitamin C, and omega-3 fatty acids [10,11].

This study aims to know the effect of administering aqueous extract of watercress seeds to Iraqi lambs on reproductive parameters and antioxidants.

2. Materials and methods:

2.1. Study Treatments

This study was conducted at Khairat Al-Ittihad Station for raising sheep and cows in Al-Shomali District in Babil Governorate, Operation with laboratories in University of Kufa, Ethical approval No. 27086 in 7-12-2022. for a period of three months, from 12/15/2023 to 3/15/2024. The aim of the study is to identify the effect of the aqueous extract of watercress seeds on lambs in their reproductive and antioxidants parameters. 40 lambs aged (six months) were used in the experiment and placed in the place designated for the experiment inside the station. The treatments were divided as follows:

T1: Control group.

T2: They dosed 5 ml of the aqueous extract of watercress seeds.

T3: They dosed 10 ml of the aqueous extract of watercress seeds.

T4: They dosed 15 ml of the aqueous extract of watercress seeds.

2.2. Preparation of Aqueous Extract of Watercress Seeds

Watercress seeds were obtained from local markets, grinded with a home electric grinder to turn them into powder, distributed in plastic bags to protect them from damage and moisture until use, as for the aqueous extract, it was prepared using the method of Hernandez *et al.* [12], at the laboratory of Khairat Al-Ittihad Station by placing 1 kg of powder of watercress seeds in 10 liters of distilled water, then the mixture was placed in a water bath at a temperature of 60 C for an hour, then the mixture was left at room temperature for 24 hours, then filtered with a soft cloth and stored in the designated boxes for use in the experiment.

2.3. Measuring Reproductive Characteristics

2.3.1. Measuring the Physical Characteristics of Semen

2.3.1.1. Volume of Semen Ejaculate

The volume of semen ejaculate of lambs was measured in the station laboratory using a graduated tube from 0.1-5 ml directly after collecting the semen.

2.3.1.2. Mass Activity of Sperm

The mass activity of sperm was calculated according to the Walton [13], by placing one drop of semen after collection on a glass slide, at room temperature 37°C, then it was examined under a microscope with a magnification of 100X, the mass movement was estimated based on the speed and density of the moving waves as a percentage.

2.3.1.3. Individual Motility Estimation

Individual motility measurement of sperm was performed according to the Walton [13], by placing a drop of sodium citrate solution with a concentration of 2.9% on the surface of a glass slide at room temperature (37°C), then a drop of diluted semen is placed on top of this solution and both are covered with a slide cover, then they are examined under a microscope with a magnification of 40x. The individual motility was calculated from 0-100 according to the percentage of sperm moving forward and the speed of their movement.

2.3.1.4. Percentage of Live and Dead Sperm

The percentage of dead and live sperm was calculated based on the method of Swanson [14], a drop of fresh semen was mixed, with the dye (eosin-necrosin) in a ratio of 1:2 on the surface of a glass slide at room temperature (37°C), then this mixture was drawn onto the surface of another glass slide in the form of a smear and examined under a microscope at a magnification of 40X. Live sperm are identified by their lack of a specific color, while dead sperm appear pink.

The percentage of dead sperm was calculated according to Hancock [15] by isolating 200 sperm in different microscopic tubes, placing them on the slide, examining them under a microscope at a magnification of 100X, and then entering them into the equation below:

$$\text{Percentage of dead sperm} = (\text{Number of dead sperm} / \text{Total sperm count (200)}) \times 100$$

2.3.1.5. Abnormal Sperm Percentage

The percentage of abnormal sperm was calculated according to the Hancock [16], from the same slide that estimated the percentage of dead sperm. The abnormalities are divided into abnormalities specific to the sperm head, including Giant head, Small head, Twin, Pyriform head, Detached head and Tapering head. Abnormalities specific to the middle piece of the sperm, which may be lateral or enlarged, abnormalities specific to the tail include Twin tail, Bent tail and Coiled tail. The samples were examined under a microscope at a magnification of 100X. The abnormal sperm were calculated after identifying 200 sperm in different laboratory tubes, entered into the following equation:

$$\text{Percentage of abnormal sperm} = (\text{Number of abnormal sperm} / \text{Total sperm count (200)}) \times 100$$

2.3.1.6. Sperm Concentration Measurement

Using the method of Salisbury *et al.* [17], the sperm concentration was estimated, using a blood cell counter (Neucover Hemocytometer Chamber), where 0.05 ml of semen was placed in 19.05

ml of dilution solution, which contains 0.9% sodium chloride+ 0.1% mercury chloride+ 0.2% eosin dye, as the dye is for coloring sperm, the sperm concentration was calculated in one milliliter using the following equation:

$$C = N / 80 \times 4000 \times 400$$

Where:

C: Number of sperm per milliliter.

N: Number of sperm in the five squares in the device used for calculation.

2.3.2. Measuring the Chemical Properties of Semen

This includes estimating the pH of semen, using acidity testing paper called Litmus paper, where one drop of semen is placed on the Litmus paper and observed, after a short period we notice the color of the Litmus paper changing to another color, it is matched with the specified gradations for each degree and then the readings are recorded.

2.3.2.1. Antioxidant Measurement

2.3.2.1.1. Estimating the Concentration of Glutathione (GSH) in the Blood Serum

The concentration of glutathione in the blood serum was estimated according to the modified method of Al-Zamely *et al.* [18], which depends on the use of a reagent called Ellman's reagent, which contains 5,5 Dithio bis -2-nitrobenzoic acid (DTNB), this reagent reacts quickly with glutathione, it is reduced by the sulfahydral glutathiol group to form a yellow compound, its absorbance is read at a wavelength of (412 Nanometer).

2.3.2.1.2. Estimation of Superoxide Dismutase (SOD) Concentration in Blood Serum

The concentration of SOD was estimated using the modified method of Al-Zamely *et al.* [18], in which its concentration was estimated indirectly, by the change in the optical density of formazine, formed by the reduction of O₂⁻ of the blue chemical dye (NBT), where formazine is generated by irradiation of blood serum samples, and the absorption intensity was measured at a wavelength of (560 Nanometer).

2.3.2.1.3. Estimation of Glutathione Transferase (GST) Concentration in Blood Serum

The estimation of (GST) was done according to the method of Francoise *et al.* [19], where blood serum was initially prepared and the reagents specific to the method were used, then it was placed in a spectrophotometer for the purpose of reading the absorbance and knowing its concentration, note that the absorbance was read according to the wavelength (412 Nanometer).

2.3.2.1.4. Estimation of Catalase Concentration in Blood Serum

The concentration of catalase (CAT) was estimated using the method of Hadwan and Abed [20], by preparing the enzymes in 1 ml of the reactant, which includes 65 mmol of hydrogen peroxide, in 60 mmol of sodium potassium phosphate, at a temperature of 37 °C, and pH = 7.4 for three minutes, after that, the action of ammonium molybdate was stopped, then the absorbance in the yellow compound present in molybdate and hydrogen peroxide was measured at a wavelength estimated at 374 nm.

2.4. Statistical Data Analysis

The statistical analysis was conducted in the statistical program [21], the averages were compared using the Duncan test [22] at a significance level of 0.05, to determine the significance of the

difference between the averages in the study samples, as for the type of design used in the experiment. The complete randomized design (CRD) was used.

3. Results and Discussion

3.1. Reproductive Traits

3.1.1. Some Reproductive Trait of Lambs were Measured to Determine the Effect of Dose of Aqueous Extract of Watercress Seeds

Table (1) shows that there are no significant differences between treatments in the semen color standard (CO) and semen acidity (pH), while there is a significant superiority for the third treatment at a significance level ($P \leq 0.05$) in semen volume (Size), compared to the control group, there were no significant differences between T2 and T3.

The third treatment was significantly superior compared to the control group at a significance level ($P \leq 0.05$) in sperm concentration (CC), the percentage of collective motility (CM%), while the control group was significantly superior to the rest of the treatments in the percentage of dead sperm (DS%) and the percentage of deformed sperm (MS%).

As for the percentage of individual movement (IM%) and the percentage of live sperm (LS%), there was a significant superiority for T3 at a significance level ($P \leq 0.05$) compared to the control group, while there were no significant differences between T2, T4 and T3 because the averages are mathematically close.

The treatments in which the aqueous extract of watercress seeds was used, especially T3 (10 ml of aqueous extract of watercress seeds), improved the reproductive characteristics of semen and spermatozoa, which were represented by the volume of semen, sperm concentration, percentage of individual and group motility, and percentage of live sperm, which were significantly lower in terms of percentage of dead and deformed spermatozoa.

The reason for the improvement in semen quality in the treatment of watercress seeds has been attributed to the seeds containing vitamin E, which increases the secretion of testosterone, which leads to enhancing the secretions of the secondary sex glands, thus preserving sperm and increasing sexual activity, fertility and the number of live sperm [23].

Improving the semen quality may be due to the presence of some nutritional compounds in watercress seeds, such as fatty acids, especially arachidonic and linoleic acids, which have a positive effect on the activity of the testosterone hormone. Fatty acids stimulate the hypothalamus to secrete GnRH and energy, which is the source of watercress seeds, thus improving the quality of semen and increasing sexual desire in lambs [10]. Increasing the percentage of live sperm and reducing the percentage of dead and deformed sperm, because the alcoholic extract of watercress seeds works to preserve the sperm membrane and protect it from harmful effects, due to the presence of antioxidants such as saponins, flavones and glycosides, which work to combat free radicals and harmful fats, in addition to stimulating watercress seeds to the hormones FSH, LH, which stimulate the formation of sperm, increase their concentration and improve their qualities [24].

The reason for semen improving characteristics in watercress seed treatments is the high nutritional value in watercress seeds of mineral elements, such as iron, selenium, zinc, copper, proteins, sugars and vitamins, the most important of which are vitamins C and A, which are characterized by their important role in enhancing the reproductive characteristics of semen [25]. Selenium increases the level of testosterone in the blood serum, by increasing its secretion from the testicle, then testosterone affects the increased effectiveness of the Cooper's glands and the prostate gland [26]. Vitamin C works to protect the testicular tissue from free radical damage,

works to cohesion of the testicular tissue and preserve the DNA of sperm, and increase sperm motility and thus improve sperm quality [27, 28].

Table 1. Effect of dose of aqueous extract of watercress seeds on reproductive parameters (mean $\pm$ standard error).

Treatments	CO	Size (ml)	PH	CC (ml $\times 10^3$)	IM %	CM %	LS %	DS %	MS %
T1	1.40 $\pm$ 0.24	1.22 $\pm$ 0.06 B	6.60 $\pm$ 0.24	2.19 $\pm$ 0.02 C	56.00 $\pm$ 2.92 B	61.00 $\pm$ 2.45 B	57.00 $\pm$ 3.00 B	43.00 $\pm$ 3.00 A	29.00 $\pm$ 2.45 A
T2	1.40 $\pm$ 0.24	1.36 $\pm$ 0.07 AB	6.60 $\pm$ 0.24	2.29 $\pm$ 0.07 C	62.00 $\pm$ 2.55 AB	64.00 $\pm$ 1.87 B	64.00 $\pm$ 4.00 AB	36.00 $\pm$ 4.00 AB	25.00 $\pm$ 2.74 AB
T3	1.20 $\pm$ 0.20	1.52 $\pm$ 0.04 A	6.60 $\pm$ 0.24	3.26 $\pm$ 0.14 A	71.00 $\pm$ 3.32 A	74.00 $\pm$ 1.87 A	74.00 $\pm$ 2.92 A	26.00 $\pm$ 2.92 B	16.00 $\pm$ 1.87 C
T4	1.40 $\pm$ 0.24	1.32 $\pm$ 0.06 B	6.40 $\pm$ 0.24	2.76 $\pm$ 0.13 B	65.00 $\pm$ 3.54 AB	65.00 $\pm$ 3.16 B	66.00 $\pm$ 4.00 AB	30.00 $\pm$ 4.47 B	21.00 $\pm$ 1.87 BC
Sig.	N.S	*	N.S	*	*	*	*	*	*

3.2. Antioxidants

Table (2) shows the extent of the effect of the dose of aqueous extract of watercress seeds on antioxidants, some antioxidants were affected by it and there were significant differences between the treatments, while some other antioxidants were not affected by the aqueous extract. T3 had a significant superiority in superoxide dismutase (SOD) ($P \leq 0.05$) compared to the control group, while the significant superiority in glutathione peroxidase (GPX) was for T4 compared to the control group, while in glutathione (GSH) the significant superiority was for T2 compared to the control group. As for catalase (CAT) and glutathione transferase (GST), there were no significant differences between the treatments despite T4 being mathematically superior to the rest of the treatments.

Table 2. Effect of dose of aqueous extract of watercress seeds on antioxidants (mean $\pm$ standard error).

Treatments	SOD (U/L)	CAT (katal/L)	Gpx (U/L)	GST (U/L)	GSH (μ mole/L)
T1	315 $\pm$ 29.65 C	0.08 $\pm$ 0.002	344.36 $\pm$ 19.67 B	551.67 $\pm$ 17.18	20.90 $\pm$ 0.99 B
T2	513.10 $\pm$ 44.09 B	0.09 $\pm$ 0.01	342.77 $\pm$ 33.60 B	559.28 $\pm$ 17.77	26.37 $\pm$ 1.19 A
T3	909.43 $\pm$ 57.78 A	0.11 $\pm$ 0.01	359.19 $\pm$ 17.24 B	549.46 $\pm$ 67.08	17.68 $\pm$ 2.14 B
T4	617.06 $\pm$ 38.46 B	0.15 $\pm$ 0.06	625.27 $\pm$ 54.78 A	565.95 $\pm$ 13.08	18.03 $\pm$ 0.80 B
Sig.	*	N.S	*	N.S	*

Most antioxidants were significantly superior in the treatment of watercress seeds over the control treatment, which indicates that watercress seeds contribute to raising the concentrations of antioxidants in the blood serum. The reason for this increase in the concentrations of antioxidants is attributed to the seeds' content of antioxidants and active substances [4].

It is worth noting that studies on the effect of the aqueous extract of watercress seeds on antioxidants in blood serum are few. Therefore, it was chosen in our study because of the health and production importance of this topic for lambs. As the results of our study agreed with Al-Rawi [29] in the significant superiority of antioxidants (GPX, GSH) in the treatment of the aqueous extract of watercress seeds compared to the control treatment. The reason for this

significant superiority in Gpx, GSH was attributed to the content of watercress seeds of antioxidants that contribute to raising their concentration in blood serum.

It is worth noting that the results of our study agreed with Alam *et al.* [30] in the increase in the concentration of antioxidants (GPX, SOD) and differed from the results of our study in the significant decrease in the antioxidant catalase CAT, as the reason for this significant increase was attributed to the fact that watercress seeds have a strong protective role in combating oxidative stress as a result of their strong content of antioxidants.

The results of our study differed with El-Sadek *et al.* [31] in the significant decrease in the treatment of watercress seeds compared to the control treatment in antioxidants (Gpx, SOD, CAT). The reason for this significant decrease was attributed to weak liver activity and the presence of a mitochondrial defect.

Conclusion

We conclude from the results of the study that the aqueous extract of watercress seeds contributed positively to improving reproductive characteristics, as the third treatment was mostly superior compared to the control treatment in semen volume, sperm concentration, percentage of live sperm, individual and group motility, while there was a significant decrease in the percentage of dead and deformed sperm. As for antioxidants (SOD, GSH, GpX), the significant increase and superiority were for the treatments in which the lambs were dosed with the aqueous extract of watercress seeds, while there were no significant differences between the treatments in antioxidants (CAT, GST).

Acknowledgments

I would like to extend my thanks and gratitude to everyone who contributed to facilitating the completion of this study and providing me with the necessary assistance, especially the workers at Khairat Al-Ittihad Sheep and Cow Breeding Station, in addition to Dr. Ahmed Jawad Abdul-Ali, my supervisor in the doctoral study, the Deanship of the College of Agriculture, and all the professors and workers at the college for their cooperation. I would also like to extend my thanks and respect to Dr. Muslim Omran, the veterinarian, for helping me provide some of the necessary equipment to draw blood samples and deliver them to the laboratory.

References

- [1] Ibrhim, I.E., M.A. Al-Shuwaili, B.M. AL-Obaidi and H.R. Abed, (2021). Effect of *Eruca Sativa* in Broiler Diet on Productivity. *Medico-legal Update*, 21(2), 383.
- [2] Mossa, J.S., M.A. Al-Yahya, and I.A. Al-Meshal (1987). *Medicinal Plants of Saudi Arabia*. Vol. 1. King Saud Univ. Libraries Publications. Riyadh, KSA.
- [3] Abdul-Jalil, T.Z. (2016). Phytochemicals Screening by GC/MS and Determination of Some Flavonol in Cultivated Iraqi *Eruca sativa* Dried Leaves Extract and its Biological Activity as Antioxidant. *International Journal of Pharmacognosy and Phytochemical Research*, 8(10): 1722-1730.
- [4] Mohammed, W.Y. (2013). Extraction and separation of some active compounds in watercress plant and study of their biological activity. *Anbar University Journal of Pure Sciences*. 7 (3): 3-4.
- [5] Sadiq, A. ; M. Q. Hayat and Mall, S. M.)2014(. Qualitative and quantitative determination of secondary metabolites and antioxidant potential of *Eruca sativa*. *Nat prod Chem Res*, 2(4), p1-7.
- [6] Briggs, O.N., H. Brown, K. Elechi-amadi, F. Ezeiruaku and N. Nduka (2016). Superoxide dismutase and glutathione peroxidase levels in patients with long standing type 2 diabetes in Port Harcourt, Rivers State, Nigeria. *International Journal of Science and Research*, 5(3): 1282-1288.

- [7] Hu, A., Chen, X., Luo, S., Zou, Q., Xie, J., He, D., ...& Cheng, G. (2021). Rhizobium leguminosarum glutathione peroxidase is essential for oxidative stress resistance and efficient nodulation. *Frontiers in microbiology*, P12.
- [8] Al-Gharawi, J.K. and J.A. Ebade (2020). Effect of different levels of cardamom oil in diet on some productive traits of layer hens. *Plant Archives*, 20 (2): 4085-4088.
- [9] Al Salman, N.T.Sh.; and Al-Gharawi. J.K.M. (2019). Effect of Eucalyptus leaves water extract on some productive traits of broilers. *Plant Archives Vol. 19, Supplement 1*, pp. 920-923.
- [10] El-Badawy, M., M. Hafsa, F.H. Youssef, Y.H. Hafez (2018). Effect of Rocket Oil Addition on Productive and Reproductive Performance of Growing Ram Lambs Under Hot Climate Condition. *J. Animal and Poultry Prod., Mansoura Univ., Vol.9 (1): 9-15*.
- [11] El-Fadaly, H.A.A., S.M. El-Kadi, M.M. El-Moghazy, A.A. Soliman and M.S.M. El-Haysha (2017). Glucosinolates Detoxification by Solid State Fermentation as Fungi" Biological" and CoPPer Sulfate Solution" Chemical" Methods. *International Journal of Bioengineering and Biotechnology*, 2(6): 41-53.
- [12] Hernandez, M., R. Lopez, R.M. Abanas, V. Paris and A. Arias (1994). Antimicrobial activity of Visnea mocanera Leaf extracts. *J. Ethnopharmacology* , 41: 115-119.
- [13] Walton, A. (1933). The Technique of Artificial Insemination. Imperial Bureau of Animal Genetics. Edinburgh.
- [14] Swanson, E. W., & Bearden, H. J. (1951). An eosin-nigrosin stain for differentiating live and dead bovine spermatozoa. *Journal of Animal Science*, 10(4), 981-987.
- [15] Hancock, J.L. (1951). A staining technique for the study of temperature-shock in semen. *Nature*, 167(4243), 323-324.
- [16] Hancock, J.L. (1956). The morphology of boar spermatozoa. *Journal of the Royal Microscopical Society*, 76(3), 84-97.
- [17] Salisbury, G.W, G.H Beek, I. Elliot and E.L. Willett (1943). Rapid method of estimation the number of spermatozoa in bull semen. *J. Dairy Sci.*,26: 483-487.
- [18] Al-Zamely, O.Y., M.S. Al-Nimer and R.K. Al-Muslih (2001). Detection the level of peroxynitrite and related antioxidant status in the serum of patients with acute myocardial infraction. *Nation. J. Chem*, 4, 625-637.
- [19] Francoise, C., M. Pierre, R. Jacqueline and J. Henri (2014). plant extracts to accelerate healing and reduce inflammation cosmetics and toiletries. *chemical clinical, Acta* , 209,217.
- [20] Hadwan, M.H. and H.N. Abed (2016). Data supporting the spectrophotometric method for the estimation of catalase activity. *Data in Brief*, 6: 194-199.
- [21] SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
- [22] Duncan, D.B. (1955). Multiple ranges test and Multiple F – test. *Biometrics*, 11: 1-42.
- [23] Al-Moussawi, J.E.Q. (2009). The effect of using ginger (Zingibar Officinale) and mature rocket seeds (Eruca Sativa mill) on some productive, physiological and reproductive traits in Awassi male lambs. Master's thesis - College of Veterinary Medicine - University of Baghdad - Republic of Iraq.
- [24] Hassan, A.F. (2014). Effect of ethyl acetate extract of Eruca Sativa seeds on the reproductive system of male white mice. *Journal of University of Kufa for Biological Sciences*. 6 (3).
- [25] Al-Hasani, A.M.A. (2020). The effect of adding rocket seed powder (Eruca sativa) to the feed on some physiological characteristics of Awassi lambs. Master's thesis - College of Agriculture - University of Kufa - Republic of Iraq.
- [26] Muhand, M.M. and A.D.M. Al-Khashab. (2019). Study of the effect of vitamin E and selenium injections on semen quality and some blood characteristics of Kurdish rams, *Iraqi journal of veterinary sciences*, 33 (1).
- [27] Rizk, N.I., M.S. Rizk, A.S. Mohamed and Y.M. Naguib (2020). Attenuation of sleep deprivation dependent deterioration in male fertility parameters by vitamin C. *Reproductive Biology and Endocrinology*, 18(1), 1-13.



- [28] Cabrita, E., S. Ma, P. Diogo, S. Martínez-Páramo, C. Sarasquete and M.T. Dinis (2011). The influence of certain aminoacids and vitamins on Post-thaw fish sPerm motility, viability and DNA fragmentation. *Animal reProduction science*, 125(1-4), 189-195.
- [29] Al-Rawi, A.A.K. (2021). The preventive and therapeutic role of the aqueous extract of celery and watercress on induced anemia in white rats. A physiological and biochemical study. Master's thesis, Department of Life Sciences, College of Education, University of Iraq.
- [30] Alam, M.S.,G. Kaur, Z. Jabbar, K. Javed and M. Athar (2007). *Eruca sativa* seeds Possess antioxidant activity and exert a Protective effect on mercuric chloride induced renal toxicity. *Food and chemical toxicology*, 45(6), 910-920.
- [31] Elsadek, M.F., M.M.E. El-Din and B.M. Ahmed (2021). Evaluation of anticarcinogenic and antioxidant properties of *Eruca sativa* extracts versus Ehrlich ascites carcinoma in mice. *Journal of King Saud University-Science*, 33(4), 101435.

Impact of Watercress Seed Aqueous Extract Dosing on Productive Traits and Testicle Dimensions of Iraqi Lambs

Ahmed J. Al-Yasery¹, Hayder Abdullah Talib² and Jassim Kassim Al-Gharawi³

¹⁻³Animal Production Department, Agriculture College, Al-Muthanna University, Al-Muthanna, Iraq.

¹E-mail: ahmeadagr@mu.edu.iq

²E-mail: Agr-grad.haider.abd@mu.edu.iq

³E-mail: jasimiraqe@mu.edu.iq

Abstract. This study was conducted at Khairat Al-Ittihad Sheep and Cow Breeding Station, at Al-Shomali District, Babylon Governorate, during three months period from 15\12\2023 to 15\3\2024. The aim of conducting the study is to identify the effect of administering aqueous extract of watercress seeds to lambs on productive traits and testicle dimensions. 40 lambs aged (six months) were used in the experiment. The lambs of the four treatments were dosed with this extract in an amount of 0, 5, 10, 15 ml, for treatments T1, T2, T3 and T4, respectively. The results of the study indicated that dosing lambs with aqueous extract of watercress seeds, led to a significant superiority in weight gain for T3 compared to the control treatment in most weeks of the study, while the body dimensions, which include breast circumference and abdominal circumference, did not have significant differences among the treatments, unlike the dimensions, which include forehead height, hindquarter height and body length, T3 was significantly superior compared to the control treatment in the last weeks of the study. The dimensions of the testicles, which were the length and width of the testicle, in addition to the circumference of the testicle, were significantly superior in T4 compared to the control treatment, while T3 and T4 were superior in some weeks of the study regarding the dimensions of the testicles.

Keywords. Watercress seeds, Aqueous extract, Iraqi lambs, Testicle dimensions.

1. Introduction

As a result of the increase in the world population, researchers must find solutions that contribute to increasing animal production with the aim of achieving local and global food security. Sheep are among the animals that are widely used in the nutritional aspect to meet the need for animal protein from their meat and milk [1,2].

Watercress seeds are one of the solutions that researchers are trying to know its productive role for lambs, the seeds contain different active compounds, as is the case with most parts of medicinal plants, they have a positive physiological and therapeutic effect in addition to the preventive effect against various diseases, that affect the animal's body organs, therefore, they enhance the animal's health and maintain its production [3,4].

The content of *Eruca Sativa* seeds of crude protein (27.4-40.89%), crude fat (19.81-35%), crude fiber (2.6-12%) and the ash (6-6.62%). This variation in the proportions is due to the nature of the soil in which the plant grows and environmental factors [5-7].

Watercress seeds are important from the production side because they contain important nutrients, most notably minerals such as sodium, potassium and amino acids, such as linoleic and linolenic and various types of fats and various types of vitamins, so they should be included in animal feed [8]. Watercress seeds improve growth in animals and increase body weight, by stimulating the secretion of digestive enzymes and increasing the animal's appetite, in addition to increasing intestinal movement and raising the efficiency of food conversion [9,10].

Content of watercress seeds of amino acids, active groups, vitamins and minerals, it is certain that this stimulates the growth and maturation of the testicles and the differentiation of sperm, maintaining the testicular tissue and preserving it and protecting it from damage [11,12]. It has also become clear to some researchers that watercress contributes to significant differences in the size and weight of the testicle and the increase in the circumference of the scrotum, due to the fact that watercress seeds contain fatty acids, carotenoids and vitamin C [13-15].

The purpose of this study is to know the effect of administering aqueous extract of watercress seeds to Iraqi lambs on productive traits and testicle dimensions.

2. Materials and Methods

2.1. Study Treatments

This study was conducted at Khairat Al-Ittihad Station for raising sheep and cows in Al-Shomali District in Babil Governorate, Operation with laboratories in University of Kufa, Ethical approval No. 27086 in 7-12-2022. for a period of three months, from 12/15/2023 to 3/15/2024. The aim of the study is to identify the effect of the aqueous extract of watercress seeds on lambs in their reproductive and antioxidants parameters. 40 lambs aged (six months) were used in the experiment and placed in the place designated for the experiment inside the station. The treatments were divided as follows:

T1: Control group.

T2: They dosed 5 ml of the aqueous extract of watercress seeds.

T3: They dosed 10 ml of the aqueous extract of watercress seeds.

T4: They dosed 15 ml of the aqueous extract of watercress seeds.

2.2. Preparation of Aqueous Extract of Watercress Seeds

Watercress seeds were obtained from local markets, grinded with a home electric grinder to turn them into powder, distributed in plastic bags to protect them from damage and moisture until use, as for the aqueous extract, it was prepared using the method of Hernandez *et al.* [16], at the laboratory of Khairat Al-Ittihad Station by placing 1 kg of powder of watercress seeds in 10 liters of distilled water, then the mixture was placed in a water bath at a temperature of 60 C for an hour, then the mixture was left at room temperature for 24 hours, then filtered with a soft cloth and stored in the designated boxes for use in the experiment.

2.3. Analysis of the Ratios of Active Compounds of the Aqueous Extract of Watercress Seeds Before and After Extraction

The watercress seeds were analyzed before and after the aqueous extraction process in the laboratories of the Department of Environment, Water and Renewable Energy of the Ministry of Science and Technology in Baghdad, to identify the extent of the effect of the extraction process on the ratios of active compounds and their concentration in watercress seeds, it was found that

the extraction process has a slight effect on the ratios of active compounds in watercress seeds (Table 1).

Table 1. The proportions and concentrations of some active compounds in watercress seeds before and after extraction.

Items	Seeds	Aqueous extract
Phenols (mg/gm)	290	220.5
Flavonoids (mg/gm)	201.9	165.9
Alkaloids (%)	9.5	5.9
Glycosides (%)	4.6	3.6
Tanins (%)	5.7	4.8
Saponins (%)	3.5	1.3

2.4. Measurement of Body Dimensions, Weight and Testicle Dimensions

Body dimensions including front height, rear height, chest circumference, abdominal circumference, body length were measured, using a measuring tape in centimeters, these measurements were taken with high accuracy on a weekly basis throughout the experiment, they were recorded in the data table for statistical analysis, while testicle dimensions including (testicle length, testicle width, testicle circumference) were taken using an Electronic Calper vernia device, with one measurement every two weeks to find out the difference between the treatments. As for body weights and weight gain, the weights of the lambs were recorded using an electronic scale measuring up to 100 kg, made in China, on a weekly basis, with the initial weight measured before starting the experiment. The weight gain was extracted from the difference between the following and previous two weeks.

2.5. Statistical Data Analysis

The statistical analysis was conducted in the statistical program [17], the averages were compared using the Duncan test [18] at a significance level of 0.05, to determine the significance of the difference between the averages in the study samples, as for the type of design used in the experiment. The complete randomized design (CRD) was used.

3. Result and Discussion

3.1. Body Weight and Weight Gain

3.1.1. Body Weight

Table (2) shows us that there are no significant differences among the treatments in body weight during the study weeks throughout the experiment period, despite T3 being mathematically superior to the rest of the treatments, it indicates that the administration of the aqueous extract of watercress seeds, improved the body weight of the lambs mathematically compared to the control treatment. The best dose given to the lambs is 10 ml.

Table 2. Effect of dose of aqueous extract of watercress seeds on body weight (mean± standard error).

Treatments	Age (week)						
	1	2	4	6	8	10	12
T1	22.30±1.27	23.00±1.30	24.76±1.43	26.79±1.70	27.53±1.73	27.97±1.81	29.06±1.83
T2	21.50±0.96	22.42±0.96	24.64±1.24	26.78±1.41	27.38±1.44	28.12±1.51	29.27±1.53
T3	22.67±0.91	23.44±0.94	26.08±1.14	27.21±1.10	28.99±1.16	30.62±1.15	32.32±1.09
T4	22.62±0.54	23.41±0.56	24.46±0.66	26.50±0.96	26.40±0.95	26.87±1.05	28.01±1.07

Sig.	N.S	N.S	N.S	N.S	N.S	N.S	N.S

The results of Table (2) explain that there were no significant differences among the treatments throughout the study weeks, all lambs went through two periods, the first was the end of the growth phase and the second is the beginning of sexual maturity. Both periods are considered to be within the growth periods, as the study lambs are six months old. Al-Wakadh [19] indicated that sexual maturity was not distinct in lambs as in females, however, the activity of male hormones and sperm production begins at the age of 4-6 months, these results agreed with Al-Sayegh and Al-Qass [20], that lambs continue to grow until they reach puberty and then sexual maturity. As for after sexual maturity, the results agreed with Al-Hasani [21] in that watercress seeds did not contribute to significant differences between treatments in the first weeks of sexual maturity. The results also differed from what was reached by Shams El-Din *et al.* [10] and Al-Hasnawi, [22] as there was a significant superiority in their study for the treatment in which watercress seeds were used compared to the control group.

3.1.2. Weight Gain

Table (3) shows that there were significant differences in the weight gain of lambs, that were given the aqueous extract of watercress seeds in most of the study weeks, T2 and T3 were significantly superior at a significance level ($P \leq 0.05$) compared to the control treatment in the fourth week, while the significant superiority was T2 in the sixth week of the study, there were no significant differences between T2 and T3 for the same week.

There was a significant increase at a significance level ($P \leq 0.05$) for T3 in 10, 12 and 13 weeks respectively compared to the control group, while we did not notice any significant differences among the four treatments in the second and eighth weeks.

Table 3. The effect of dose of aqueous extract of watercress seeds on body weight gain (mean $\pm$ standard error).

Treatments	Age (week)						
	2	4	6	8	10	12	13
T1	0.40 $\pm$ 0.03	0.81 $\pm$ 0.10 B	1.47 $\pm$ 0.16 B	0.94 $\pm$ 0.26	0.56 $\pm$ 0.10 BC	0.78 $\pm$ 0.10 B	0.58 $\pm$ 0.05 BC
T2	0.51 $\pm$ 0.04	1.49 $\pm$ 0.21 A	2.90 $\pm$ 0.29 A	0.59 $\pm$ 0.13	0.88 $\pm$ 0.16 B	0.89 $\pm$ 0.11 B	0.80 $\pm$ 0.08 B
T3	0.47 $\pm$ 0.05	1.75 $\pm$ 0.22 A	2.18 $\pm$ 0.37 AB	1.13 $\pm$ 0.21	1.52 $\pm$ 0.08 A	1.57 $\pm$ 0.13 A	1.13 $\pm$ 0.14 A
T4	0.47 $\pm$ 0.05	1.30 $\pm$ 0.24 AB	1.43 $\pm$ 0.21 B	1.16 $\pm$ 0.27	0.40 $\pm$ 0.07 C	0.98 $\pm$ 0.10 B	0.52 $\pm$ 0.05 C
Sig.	N.S	*	*	N.S	*	*	*

The second week of the experiment did not have significant differences among the treatments in weight gain, because the lambs in all treatments were in the growth stage and their weight gain was equal. As for the rest of the weeks, significant differences for the treatments in which rocket seeds were used, because rocket seeds improve growth by stimulating bile secretions and saliva secretion, these secretions work to break down large molecules into smaller molecules, then they are absorbed and metabolized into muscle tissue, in addition to the high nutritional value of these seeds [12, 23], these results are consistent with Al-Hasani [21] and Shams Al-Din *et al.* [10]. It is worth noting that rocket seeds increase appetite due to their nutritional components, they also contain flavonoids and glucosinolates that enhance the health of lambs, it increases the weights of lambs and causes significant differences in the treatments in which rocket seeds are used, in

addition to the fact that they work to increase the efficiency of food conversion, this study agreed with Al-Hasnawi [22]; Al-Moussawi [24]; Al-Jubouri [25].

3.2. Body Dimensions

3.2.1. Breast Circumference

Table (4) shows that there were no significant differences at a significance level ($P \leq 0.05$) between the four treatments in all weeks of the study except for the first week, T3 and T4 outperformed the rest of the treatments.

Table 4. Effect of dose of aqueous extract of rocket seeds on chest circumference (mean $\pm$ standard error).

Treatments	Age (week)						
	1	3	5	7	9	11	13
T1	± 2.57 71.98 AB	73.56 $\pm$ 2.56	79.11 $\pm$ 2.04	77.34 $\pm$ 2.33	76.71 $\pm$ 2.35	77.28 $\pm$ 2.31	78.22 $\pm$ 2.33
T2	67.24 $\pm$ 1.20 B	70.43 $\pm$ 1.13	80.65 $\pm$ 1.15	81.94 $\pm$ 1.27	79.68 $\pm$ 0.74	80.64 $\pm$ 0.81	81.88 $\pm$ 0.89
T3	72.99 $\pm$ 1.70 A	73.58 $\pm$ 1.51	79.22 $\pm$ 1.94	81.41 $\pm$ 1.96	78.24 $\pm$ 1.54	79.79 $\pm$ 1.53	81.49 $\pm$ 1.47
T4	72.85 $\pm$ 1.66 A	73.80 $\pm$ 2.06	79.40 $\pm$ 1.92	81.15 $\pm$ 2.12	79.39 $\pm$ 1.94	80.03 $\pm$ 1.96	80.91 $\pm$ 1.96
Sig.	*	N.S	N.S	N.S	N.S	N.S	N.S

3.2.2. Abdominal Circumference

Table (5) show that there were no significant differences between the treatments at a significance level ($P \leq 0.05$) compared to the control treatment, as growth was at the same level in all treatments and for all study weeks.

Table 5. Effect of dosing the aqueous extract of rocket seeds on abdominal circumference (mean $\pm$ standard error).

Treatments	Age (week)						
	1	3	5	7	9	11	13
T1	82.28 $\pm$ 2.47	85.38 $\pm$ 2.43	94.33 $\pm$ 2.47	93.12 $\pm$ 2.61	92.64 $\pm$ 2.42	93.38 $\pm$ 2.43	94.24 $\pm$ 2.42
T2	78.91 $\pm$ 1.27	81.84 $\pm$ 1.06	91.20 $\pm$ 1.81	90.41 $\pm$ 1.90	92.53 $\pm$ 1.70	93.64 $\pm$ 1.70	94.72 $\pm$ 1.69
T3	82.38 $\pm$ 1.93	84.10 $\pm$ 1.96	90.67 $\pm$ 2.40	94.30 $\pm$ 2.14	92.08 $\pm$ 1.84	93.71 $\pm$ 1.82	95.56 $\pm$ 1.74
T4	80.60 $\pm$ 1.40	83.24 $\pm$ 1.22	91.05 $\pm$ 1.94	85.78 $\pm$ 8.81	92.81 $\pm$ 1.92	93.57 $\pm$ 1.94	94.37 $\pm$ 1.95
Sig.	N.S	N.S	N.S	N.S	N.S	N.S	N.S

3.2.3. Forefoot Height

Table (6) shows that the dose of aqueous extract of rocket seeds had a positive effect in the last weeks of the experiment on the forefoot height of the study lambs. At the ninth week of the study that T3 had a significant superiority at a significance level ($P \leq 0.05$) compared to the control group, while there were no significant differences among T2, T3 and T4 treatment. There was a significant increase in the third treatment in the eleventh and thirteenth weeks compared to the control group, while there were no significant differences in the other weeks.

Table 6. The effect of dose of aqueous extract of rocket seeds on the height of the forelimb (mean $\pm$ standard error).

Treatments	Age (week)						
	1	3	5	7	9	11	13
T1	52.67 $\pm$ 1.76	53.92 $\pm$ 1.24	63.43 $\pm$ 1.30	65.29 $\pm$ 0.97	65.03 $\pm$ 1.02	65.54 $\pm$ 1.02	66.51 $\pm$ 1.05

					B	B	B
T2	51.72±1.12	53.95±1.23	64.30±1.19	66.69±1.13	65.61±1.23 AB	66.31±1.21 B	67.50±1.22 B
T3	52.44±0.26	53.74±0.45	64.44±0.94	67.56±0.62	68.78±0.59 A	70.53±0.57 A	72.07±0.59 A
T4	52.21±1.16	54.57±1.05	64.40±1.20	60.65±6.15	65.72±1.16 AB	66.32±1.15 B	67.80±1.37 B
Sig.	N.S	N.S	N.S	N.S	*	*	*

3.2.4. Height of the Hindquarters

Table (7) shows us that there were significant differences at the significance level ($P \leq 0.05$) among the treatments in the first week, where T4 was significantly superior compared to the control group in this week, while T3 was significantly superior compared to the control group in the thirteenth week, but there were no significant differences among T2, T3 and T4 treatments. There were no significant differences among the treatments in the remaining weeks.

Table 7. Effect of dosing the aqueous extract of rocket seeds on the height of the hindquarters (mean $\pm$ standard error).

Treatments	Age (week)						
	1	3	5	7	9	11	13
T1	52.36±0.86 B	56.46±2.27	67.22±1.91	68.36±2.19	66.77±1.99	67.28±2.09	68.31±2.05 B
T2	52.36±0.68 B	54.49±0.79	67.05±1.45	68.90±1.42	66.78±1.34	67.60±1.26	68.78±1.22 AB
T3	53.64±0.70 AB	55.47±0.73	66.67±1.25	68.71±1.21	70.17±1.06	71.73±1.06	73.12±1.11 A
T4	56.56±1.01 A	58.68±1.54	66.41±1.34	69.45±1.39	67.79±1.32	68.35±1.29	69.36±1.30 AB
Sig.	*	N.S	N.S	N.S	N.S	N.S	*

3.3. Body Length

Table (8) show that there were significant differences at the significance level ($P \leq 0.05$) in T3 in 9, 11 and 13 weeks, as it was significantly superior compared to the control group, but for the weeks from the first week to the seventh week, there were no significant differences among the treatments.

Table 8. Effect of dosing the aqueous extract of rocket seeds on body length (mean $\pm$ standard error).

Treatments	Age (week)						
	1	3	5	7	9	11	13
T1	58.51±1.47	60.80±1.76	74.33±1.24	73.94±1.08	75.53±1.13 B	76.31±1.23 B	77.41±1.22 B
T2	57.79±1.70	59.52±1.64	74.75±1.31	76.90±1.77	78.41±1.82 AB	79.87±1.83 AB	81.18±1.82 AB
T3	60.26±1.42	61.28±1.28	75.11±1.73	78.00±1.13	81.17±1.01 A	83.29±0.92 A	85.01±0.85 A
T4	56.14±0.91	59.43±0.75	72.40±1.72	76.40±1.77	77.52±1.80 AB	78.39±1.82 B	79.54±1.85 B
Sig.	N.S	N.S	N.S	N.S	*	*	*

The results of the tables for body dimensions that most of the body dimensions, showed significant differences in the last weeks of the experiment, while in the first weeks they were free

of significant differences and were mostly equal in growth in all treatments except for the breast circumference, which showed significant differences in the first week only in T3 and T4 treatments were significantly superior. The height of the buttocks in T4 was significantly superior compared to the control and the abdominal circumference, there were no significant differences among treatments throughout the weeks of the study.

These results agreed with Al-Moussawi [24] who explained that the reason for this is that the growth of lambs is in all directions of the body (body dimensions) equally, which positively affects the increase in body weight and that the relationship between body dimensions and body weight is a direct relationship, therefore, there were no significant differences between the treatments, it also agreed with Al-Fityan [26] who used mature rocket seeds on Awassi lambs, showed significant differences in the last weeks of the study, especially in the height of the foreleg, while did not show any significant differences at the beginning of the study because the growth was equal between the treatments in body dimensions.

These results differed from the study of Shams El-Din *et al.* [10] who used rocket seed powder in the diets of Awassi lambs and found that the treatments in which rocket seeds were used were significantly superior compared to the control group in body dimensions. This was attributed to the presence of a strong and positive correlation between the live body weight of lambs and the measurements of body dimensions.

From the above results, we find that the dose of the aqueous extract of rocket seeds improves the productive characteristics of body dimensions, morally or mathematically, when compared to the control group.

3.4. Testis Dimensions

3.4.1. Testis Length

Table (9) shows that there were significant differences in testis length between the four treatments ($P \leq 0.05$) in all study weeks, except for the first week in which there were no significant differences among treatments. At the weeks third-ninth, T4 outperformed the rest of the treatments in testis length, while in the eleventh week T3 and T4 treatments outperformed the rest of the treatments with averages 10.11 and 10.14, respectively, while in the thirteenth week, T3 outperformed the rest of the treatments significantly, there were no significant differences between T3 and T4 treatments.

Table 9. Effect of dose of aqueous extract of rocket seeds on testis length (mean $\pm$ standard error).

Treatments	Age (week)						
	1	3	5	7	9	11	13
T1	3.67 $\pm$ 0.29	4.10 $\pm$ 0.30 AB	4.96 $\pm$ 0.28 B	5.89 $\pm$ 0.28 C	9.53 $\pm$ 0.14 AB	9.87 $\pm$ 0.11 AB	10.22 $\pm$ 0.08 BC
T2	3.19 $\pm$ 0.28	3.84 $\pm$ 0.30 B	5.06 $\pm$ 0.29 B	6.00 $\pm$ 0.28 C	9.28 $\pm$ 0.16 B	9.70 $\pm$ 0.15 B	10.06 $\pm$ 0.13 C
T3	2.92 $\pm$ 0.27	3.77 $\pm$ 0.27 B	5.36 $\pm$ 0.25 B	6.99 $\pm$ 0.24 B	9.54 $\pm$ 0.15 AB	10.11 $\pm$ 0.14 A	10.58 $\pm$ 0.09 A
T4	3.61 $\pm$ 0.18	4.66 $\pm$ 0.17 A	6.62 $\pm$ 0.15 A	7.78 $\pm$ 0.13 A	9.77 $\pm$ 0.09 A	10.14 $\pm$ 0.07 A	10.54 $\pm$ 0.05 AB
Sig.	N.S	*	*	*	*	*	*

3.4.2. Testicular Display

Table (10) show that there were significant differences ($P \leq 0.05$) in all treatments and for all weeks of the study, whereas the fourth treatment, in which 15 ml of the aqueous extract of rocket seeds was used as a dose for the lambs, was significantly superior compared to the control treatment to the rest of the treatments in all weeks from the first week to the last week.

Table 10. The effect of dosing the aqueous extract of rocket seeds on testicular display (mean $\pm$ standard error).

Treatments	Age (week)						
	1	3	5	7	9	11	13
T1	2.76 $\pm$ 0.20 AB	2.99 $\pm$ 0.21 B	3.52 $\pm$ 0.17 C	4.10 $\pm$ 0.15 C	6.79 $\pm$ 0.09 C	7.20 $\pm$ 0.07 C	7.51 $\pm$ 0.04 C
T2	2.48 $\pm$ 0.19 B	2.91 $\pm$ 0.19 B	3.77 $\pm$ 0.18 BC	4.32 $\pm$ 0.17 C	7.13 $\pm$ 0.09 B	7.52 $\pm$ 0.09 B	7.74 $\pm$ 0.06 B
T3	2.27 $\pm$ 0.16 B	2.91 $\pm$ 0.17 B	4.17 $\pm$ 0.14 B	5.57 $\pm$ 0.14 B	6.92 $\pm$ 0.14 BC	7.48 $\pm$ 0.10 B	7.72 $\pm$ 0.07 B
T4	3.08 $\pm$ 0.19 A	4.02 $\pm$ 0.19 A	5.82 $\pm$ 0.17 A	6.87 $\pm$ 0.16 A	7.53 $\pm$ 0.10 A	7.79 $\pm$ 0.07 A	8.04 $\pm$ 0.06 A
Sig.	*	*	*	*	*	*	*

3.4.3. Testicular Circumference

Table (11) show that there were significant differences between the treatments in all weeks of the study, where we notice a significant increase ($P \leq 0.05$) in T4 compared to the rest of the treatments from the first week to the ninth week, while T3 and T4 outperformed the rest of the treatments significantly in the eleventh week with averages of 13.51, 14.10 for the third and fourth treatments respectively, and in the thirteenth week also with averages of 14.33 and 14.58 for T3 and T4 treatments respectively.

Table 11. The effect of dosing the aqueous extract of rocket seeds on the circumference of the testicle (mean $\pm$ standard error).

Treatments	Age (week)						
	1	3	5	7	9	11	13
T1	5.30 $\pm$ 0.29 AB	5.88 $\pm$ 0.30 B	6.92 $\pm$ 0.29 B	8.02 $\pm$ 0.29 C	11.04 $\pm$ 0.26 C	12.02 $\pm$ 0.26 B	13.14 $\pm$ 0.28 B
T2	4.90 $\pm$ 0.34 B	5.72 $\pm$ 0.34 B	7.32 $\pm$ 0.34 B	8.43 $\pm$ 0.33 C	11.28 $\pm$ 0.28 BC	12.40 $\pm$ 0.29 B	13.35 $\pm$ 0.23 B
T3	4.44 $\pm$ 0.38 B	5.50 $\pm$ 0.36 B	7.38 $\pm$ 0.36 B	9.49 $\pm$ 0.35 B	11.99 $\pm$ 0.32 B	13.51 $\pm$ 0.23 A	14.33 $\pm$ 0.11 A
T4	5.88 $\pm$ 0.20 A	7.23 $\pm$ 0.20 A	9.35 $\pm$ 0.17 A	10.84 $\pm$ 0.17 A	13.23 $\pm$ 0.12 A	14.10 $\pm$ 0.07 A	14.58 $\pm$ 0.05 A
Sig.	*	*	*	*	*	*	*

Through the tables that show us the effect of dosing the aqueous extract of rocket seeds on the dimensions of the testicle, which is represented by (testicle length, testicle width, testicle circumference), it became clear to us that the moral superiority was for T3 and T4, the aqueous extract improved the dimensions of the testicle, as this leads to the lambs reaching sexual maturity early, where Al-Wakadh [19] indicated that the length of the testicles in lambs at sexual maturity reaches 10 cm, the width of the testicles reaches 7.5 cm, and the circumference of the testicles reaches 14 cm. The reason for improving the dimensions of the testicles is due to the

content of rocket seeds of minerals, vitamins, proteins and antioxidants that contribute to the growth of the testicles in a healthy and sound manner [3,12,27].

These results agreed with Al-Moussawi [24], who used rocket seeds in his study and found that they improved the dimensions of the testicles, especially in the last period of his study. They also improved the dimensions of the testicles mathematically at the beginning of the experiment and showed that the increase in the circumference of the testicles and the dimensions of the body is an indicator of improving reproductive efficiency in lambs, attributed the reason to the components of rocket seeds with high nutritional and health value. They also agreed with Al-Fityan [26], who studied the effect of mature rocket seeds on Awassi lambs and showed a significant superiority in the dimensions of the testicles in the treatment in which rocket seeds were used compared to the control treatment.

Conclusion

We conclude from the results of the study that feeding the lambs with the aqueous extract of rocket seeds contributed to the superiority of T3 over the rest of the treatments in most of the study weeks in weight gain, while the superiority in body weight was arithmetic superiority. As for body dimensions, we find that the breast and abdominal circumferences did not have significant differences between the treatments, while we find that the aqueous extract leads to the superiority of T3 compared to the control treatment in the last weeks of the study in the height of the forefoot, height of the hindquarters and body length. As for the dimensions of the testicles (testicle length, testicle width, testicle circumference), we find that the aqueous extract of rocket seeds contributed to the moral superiority of T4 compared to the control treatment in most of the study weeks, while we find that the superiority was for the third and fourth treatments together in other weeks.

Acknowledgments

I am pleased to thank those who provided me with the facilities to complete this study and take the necessary measurements and analyses to extract the results, especially the staff of Khairat Al-Itihad Station in Babylon Governorate, the administrative assistant Ahmed Hans, and Ms. Zahraa Jalil, the laboratory manager at the station. I also extend my thanks to the supervisor of the doctoral experiment, Professor Dr. Ahmed Al-Yasiri, the Deanship of the College of Agriculture-Al-Muthanna University, and all the staff working there from teaching and administrative staff.

References

- [1] Awda, H.K. (2010). Economics of sheep farming, a field study in Babil Governorate. *Al-Furat Journal of Agricultural Sciences*, 2 (2), 120-127.
- [2] Taha, N.D., N.N. Ahmed and S.F. Al-Dabbagh. 2011. Estimation of phenotypic correlation and recurrence coefficient for some economic traits in Awassi and Hamdaniya ewes. *Al-Rafidain Journal of Agriculture*, 39 (4), 139-145.
- [3] Mohammed, W.Y. (2013). Extraction and separation of some active compounds in watercress plant and study of their biological activity. *Anbar University Journal of Pure Sciences*. 7 (3): 3-4.
- [4] Khoobchandani, M., B.K. Ojeswi, N. Ganesh, M.M. Srivastava, S. Gabbanini, R. Matera and L. Valgimigli (2010). Antimicrobial Properties and analytical profile of traditional *Eruca sativa* seed oil: Comparison with various aerial and root plant extracts. *Food Chemistry*, 120(1): 217-224.
- [5] Al-Ali, J.T.A., Al-Jobory, N.M.A. and A.M. Al-Khazaei (2015). Effect of Adding Different Levels of Powder of Rocket Seeds (*Eruca sativa*) to the Diets on Some Productive and Reproductive Traits of Japanese Quail. *Iraqi J. Poultry Sci.* 9 (2): 86-103.

- [6] El-Gengaihi, S.E., A. Salem, S.A. Bashandi, N.A. Ibrahim and S.A. ElHamid (2004). Hypolipidemic effect of some vegetable oils in rats. *Journal of food Agriculture and Environment*, 2, 88-93.
- [7] Flanders, A. and S.M. Abdulkarim (1985). The composition of seed and seed oils of Taramira (*Eruca sativa*). *Journal of the American Oil Chemists' Society*, 62(7): 1134-1135.
- [8] Jaafar, N.S. and I.S. Jaafar (2019). *Eruca sativa* Linn. Pharmacognostical And Pharmacological Properties And Pharmaceutical Preparations. *Asian J Pharm Clin Res*, 12(3), 39-45.
- [9] Giannenas, I., E. Bonos, E. Christaki and P. Florou-paneri (2018). *Oregano: A Feed Additive with Functional Properties*. In *Therapeutic Foods* (PP. 179-208). Academic Press.
- [10] Shams Al-Din, Q.Z., E.A. Gerges, H.A. Suleiman, M.H. Ali and Y.I. Hamad. (2016). Study of the effect of using rocket seed powder in the diets of male Awassi lambs on some growth and blood traits. *Journal of Kirkuk University for Agricultural Sciences*. 7 (4): 94-104.
- [11] Raouf, C.G. and M.O. Ahmad, (2019). Suspected Protective Effect of *Eruca Sativa* Leaves Extract on Sperm Abnormalities in Male Albino Mice Treated with *Hypericum triquetrifolium* aqueous crude extract. *UHD Journal of Science and Technology*, 3(2), 87-92.
- [12] Gulfraz, M., A. Sadiq, H. Tariq, M. Imran, R. Qureshi and A. Zeenat (2011). Phytochemical analysis and antibacterial activity of *Eruca sativa* seed. *Pak. J. Bot*, 43(2), 1351-1359.
- [13] El-Badawy, M.M., F.H. Hafsa, J. Youssef and Y.H. Hafez (2018). Effect of Rocket Oil Addition on Productive and Reproductive Performance of Growing Ram Lambs Under Hot Climate Condition. *J. Animal and Poultry Prod., Mansoura Univ.*, Vol.9 (1): 9-15.
- [14] Al-Gharawi, J.K. and J.A. Ebade (2020). Effect of different levels of cardamom oil in diet on some productive traits of layer hens. *Plant Archives*, 20 (2): 4085-4088.
- [15] Al Salman, N.T.Sh.; and Al-Gharawi. J.K.M. (2019). Effect of Eucalyptus leaves water extract on some productive traits of broilers. *Plant Archives* Vol. 19, Supplement 1, pp. 920-923.
- [16] Hernandez, M., R. Lopez, R.M. Abanas, V. Paris and A. Arias (1994). Antimicrobial activity of *Visnea mocanera* Leaf extracts. *J. Ethnopharmacology*, 41 ; 115-119.
- [17] SAS (2012). *Statistical Analysis System, User's Guide*. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
- [18] Duncan, D.B. (1955). Multiple ranges test and Multiple F – test. *Biometrics*, 11: 1-42.
- [19] Al-Wakadh, I.Y.H. (2018). Morphological, histological and hormonal study of the testicles and epididymis of Awassi and Arabi sheep at different stages of sexual maturity. Master's thesis-Al-Muthanna University-College of Agriculture-Republic of Iraq.
- [20] Al-Sayegh, M.N.R. and J.E. Al-Qass. (1992). *Sheep and Goat Production*. Dar Al-Hikma Press, University of Basra, Iraq.
- [21] Al-Hasani, A.M.A. (2020). The effect of adding rocket seed powder (*Eruca sativa*) to the feed on some physiological characteristics of Awassi lambs. Master's thesis - College of Agriculture - University of Kufa - Republic of Iraq.
- [22] Al-Hasnawi, M..Z. (2014). The effect of substituting *Eruca Sativa* seed and/or flaxseed in the feed on some productive, biochemical and slaughter characteristics of crossbred calves (Friesian × Southern). Master's thesis – College of Agriculture – University of Basra – Republic of Iraq.
- [23] Fratianni, F., S. Pepe, F. Cardinale, T. Granese, A. Cozzolino, R. Coppola and F. Nazzaro (2014). *Eruca sativa* might influence the growth, survival under simulated gastrointestinal conditions and some biological features of *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* strains. *International journal of molecular sciences*, 15(10), P.17790-17805.
- [24] Al-Moussawi, J.E.Q. (2009). The effect of using ginger (*Zingibar Officinale*) and mature rocket seeds (*Eruca Sativa* mill) on some productive, physiological and reproductive traits in Awassi male lambs. Master's thesis - College of Veterinary Medicine - University of Baghdad - Republic of Iraq.
- [25] Al-Jubouri, A.S.K. (2013). The effect of adding rocket seeds and/or ground ginger roots to the feed of Awassi ewes during the lactation period on some productive and physiological traits. Master's thesis, College of Veterinary Medicine, University of Baghdad.



- [26] Al-Fityan, M.H.S. (2008). The use of mature rocket seeds (*Eruca sativa*) and vitamin E in feeding male Awassi lambs and its effect on some productive traits. Master's thesis, College of Veterinary Medicine, University of Baghdad, Republic of Iraq.
- [27] Marwat, S.K., F.U. Rehman and A.A. Khan (2016). Phytochemistry and Pharmacological Values of Rocket (*Eruca sativa* Miller)--A Review. *International Journal of Horticulture*, 6.

Study the Effect of Adding (*Punica granatum*) Extract to the Tris Extender on the Characteristics of Goats Sperm Epididymal Under Cooling Conditions

Mohammed Hatem Abd¹, Nawal Juma Shansul², Eqbal Awadh Gatea³, Ali Abdullah Zairi⁴ and Hadi Awad Hassooni⁵

¹⁻⁵Department of Animal Production, Al-Muthanna University, Samawah, Iraq.

¹E-mail: Mohammed.hatem@mu.edu.iq

²E-mail: nawal.jumaah@mu.edu.iq

³E-mail: eq_bio2013@mu.edu.iq

⁴E-mail: Ali.abdz@mu.edu.iq

⁵E-mail: Hadi_habeb2000@mu.edu.iq

Abstract. This experiment was conducted to identify the effectiveness of *Punica granatum* seed extract (PJ) as a natural antioxidant in protecting goat epididymal sperm from the side effects of cooling preservation. Nine samples (testicles) of adult male goats was collected from the slaughterhouse, and transferred to the laboratory at a low temperature using a thermos bottle containing ice. Then, the tail of the epididymis was washed and cut into small pieces and the previously prepared diluent (TCYF) was added at a dilution ratio of (10:1 ml). The epididymal sperm were evaluated before cooling to ensure their activity. Then, the samples were divided into five treatments (1 ml for each treatment), and each treatment contained a different concentration of PJ, as follows: positive control treatment T1 (after cooling) 0% PJ, second treatment T2 5% PJ, third treatment T3 10% PJ, fourth treatment T4 15% PJ and fifth treatment T5 20% PJ. Then, the samples were placed in the refrigerator at a temperature of 5°C and then evaluated after 24 hours (1day), 48 hours (2day), 72 hours(3day), 96 hours(4day) and 120 hours(5day). The results of the experiment showed a significant superiority ($P<0.05$) for T3. On all treatments during T1, T2, T4, and T5 in the progressive motility type A, which is considered one of the most important sperm movements that help the sperm reach the fertilization site. T3 also significantly outperformed the rest of the treatments in non-progressive motility type D and C. additionally, the research results demonstrated a notable superiority ($P>0.05$) of T2 over T1, T4 and T5 in non-progressive motility type C. It was also noted that T3 was significantly superior in protecting sperm from deformities.

Keywords. *Punica granatum* seed extract (PJ), Cooling preservation, Epididymal sperm.

1. Introduction

Cooling preservation of sperm is the simplest method that can successfully reduce the rate of sperm metabolism, and thus prolong the survival of sperm [1]. Oxidative damage occurs naturally to sperm during the period of cooling preservation or freezing, which is a major cause of the production of fatty acid peroxides.

These peroxides are a major factor in the decrease in sperm motility and the loss of their ability to fertilize, especially sperm of mammals[2] because sperm of mammals are very sensitive to low temperatures, especially the plasma membrane part of the sperm, as freezing leads to changes in the membrane, including a change in the effectiveness of proteins, and then the permeability of water and dissolved materials changes, and this will lead to a loss in the vitality of sperm [3]. To avoid these problems, substances are added to semen diluents that protect the sperm and increase their storage period, and thus have a major role in increasing fertility [4]. Modern sources have proven that the most important main causes of semen deterioration after cooling preservation or freezing are peroxidation (formation of toxic substances including hydrogen peroxide) and the production of reactive oxygen species (ROS). One of the best ways to prevent this is to use antioxidants such as PJ. Pomegranate fruit contains polyphenols, which are considered one of the most important antioxidants and represent half of the antioxidant capacity of fruits to combat ROS [5]. In addition, several experimental studies have described that pomegranate seed extract improves semen quality [6], [7] and erectile dysfunction in male patients [8]. (El-Ghazzawy et al). reported that pomegranate seed extract has the ability to combat structural and physiological changes in the epididymis of rats caused by the plasticizer Bisphenol A, which interferes with its function and contributes to infertility, by increasing the number of sperm in the tail of the epididymis, reducing sperm abnormalities and improving male fertility [9]. To avoid many problems of cooling preservation of sperm, this study came to achieve the following objectives:

- Identify the role of PJ as an antioxidant when added to TCYF during the process of cooling preservation of epididymal sperm at a temperature of 5 degrees Celsius.
- Know the best concentration of PJ in the process of improving the epididymal sperm characteristics studied in this experiment (progressive motility of type A, progressive motility of type B, non-progressive motility of type C, non-progressive motility of type D, and the integrity of epididymal sperm from deformities) when cryopreserved for five consecutive days.

2. Material and Methods

This study was conducted at the postgraduate laboratory of the Animal Production Department, College of Agriculture, Al-Muthanna University operation with laboratories in The Ministry of Science and Technology Ethical approval No. ISO 17025, with the aim of determining if the properties of goat epididymal sperm could be improved by adding different amounts of PJ.

2.1. Semen Collection and Preparation

Nine adult male goat testicles were used to create the samples (testicles), which were then brought to the lab at a low temperature in a thermos container. The epididymal sperm tail was then washed and cut into small pieces, and a 10:1 ml dilution ratio of the previously prepared diluent (TCYF) was created using the Moce et al. [10] procedure. Lastly, the samples were split up into five treatments, each with a single milliliter and a different PJ concentration as follows: T1 (after cooling) 0% PJ T2 5%, T3 10% PJ, T4 15% PJ and T5 20% of PJ, then the samples were placed in the refrigerator at a temperature of 5°C and then evaluated after (1day), (2day), (3day), (4day) and (5day).

2.2. Pomegranate Seed Extract Preparation

Pomegranates were purchased from an Iraqi market (Yemeni type) and the outer and inner peels were removed to obtain only the seeds. Then the seeds were washed with distilled water and mixed using a blender to obtain pomegranate juice (PJ). After that, the extract was filtered using a clean medical gauze and placed in a centrifuge at a rotation rate of 5000/rpm to get rid of the seed

residues and obtain an extract free of impurities. It was stored at a temperature of -18°C until use [11].

2.3. Studied Characteristics

A few characteristics of the semen were evaluated, the most important as follows:

2.3.1. Sperm Motility

According to WHO [12] Sperm motility can be classified into four categories: tail or axial circular motility (shown by C), circular or irregular motility (represented by B), high progressive motility (represented by A), and inactive or non-moving (represented by D)., the method of measuring motility involved putting a droplet of semen on a glass slide and covering it with the slide cover. After one minute at 30–35°C, live sperm were routinely counted for each microscopic field under a light microscope.

2.3.2. Sperm Abnormalities

Total sperm abnormalities were measured using eosin-necrosin stain (5%, 10%) such as head abnormalities, detached head, abnormal midpiece and head defects. Total normal sperm and total abnormal sperm were also measured, to extract the percentage of each based on Sidhu [13].

2.4. Statistical Analysis

Using the ready-made statistical software SPSS, 2008 [14], the data were statistically examined to see how the concentrations of pj and Storage period. The Duncan (1955) multinomial test was used to analyze the significant differences between the averages, and the completely randomized design (CRD) was employed to examine the interaction between time and concentration at a probability threshold of 0.05.

3. Results

3.1. Effect of Adding Different Concentrations of PJ on Epididymal Sperm Motility of Goats (%)

The results of the experiment shown in Table (1) showed a significant effect ($P \leq 0.05$) as a result of adding different concentrations of PJ on four types of goat epididymal sperm motility (A high progressive motility, B slow progressive motility and circular motility only, C tail motility only and D inactive sperm). The results of the experiment showed that the progressive motility of type A was significantly superior ($P \leq 0.05$) when adding 10% (T3) of PJ during the first, second, third, fourth and fifth days when stored in the refrigerator compared to the T1, T2, T4, and T5 respectively. The results of T1, T2, T3, T4 and T5 were 28.68, 26.94, 24.22, 21.42, 19.38 during the first, second, third, fourth and fifth days, respectively.

Table 1. Effect of adding different concentrations of PJ on the percentage of progressive motility (A) of goat epididymal sperm after cooling preservation (mean $\pm$ standard error).

PJ (%)	Periods Storage (day)				
	1	2	3	4	5
0	1.17 $\pm$ 19.72 ^{fed}	1.08 $\pm$ 16.99 ^{feh}	1.19 $\pm$ 14.42 ^{ih}	1.18 $\pm$ 12.37 ^{fhg}	0.91 $\pm$ 10.00 ^j
5	1.03 $\pm$ 21.47 ^{cd}	0.81 $\pm$ 20.22 ^{ed}	0.73 $\pm$ 18.34 ^{fed}	0.65 $\pm$ 16.37 ^{feh}	0.63 $\pm$ 14.85 ^{ihg}
10	1.71 $\pm$ 28.68 ^a	1.56 $\pm$ 26.94 ^{ab}	1.72 $\pm$ 24.22 ^{cb}	0.85 $\pm$ 21.42 ^{cd}	0.98 $\pm$ 19.38 ^{fed}
15	1.16 $\pm$ 18.04 ^{feh}	0.82 $\pm$ 14.41 ^{ih}	0.96 $\pm$ 17.09 ^{feh}	0.96 $\pm$ 14.65 ^{ih}	1.02 $\pm$ 12.89 ^{ij}
20	0.69 $\pm$ 16.38 ^{fhg}	0.69 $\pm$ 16.38 ^{fhg}	0.74 $\pm$ 14.33 ^{ih}	0.67 $\pm$ 11.64 ^{ij}	0.72 $\pm$ 10.18 ^j

The presence of significant differences ($P \leq 0.05$) between the treatments is shown by averages with different letters within a single column.

As for the progressive movement of type B, the results in Table (2) showed a significant superiority ($P \leq 0.05$) for the T3 during the fifth day of cold storage compared to T1, T2, T4 and T5, while the T4 was significantly superior ($P \leq 0.05$) during the first, third and fourth days. A significant superiority was also observed for T1 and T2 during the second day of cold storage, as the results of the T1, T2, T3, T4 and T5 were 28.39, 26.29, 27.21, 24.14, 21.83, respectively, throughout the cold storage period.

Table 2. Effect of adding different concentrations of PJ on the percentage of progressive motility (B) of goat epididymal sperm after cooling preservation (mean $\pm$ standard error).

PJ (%)	Periods Storage (day)				
	1	2	3	4	5
0	1.54 $\pm$ 27.59 ^{ab}	1.02 $\pm$ 26.29 ^{cadb}	1.06 $\pm$ 23.11 ^{fcgdb}	0.96 $\pm$ 21.32 ^{fgde}	0.75 $\pm$ 19.61 ^g
5	1.38 $\pm$ 27.34 ^{ab}	1.33 $\pm$ 26.52 ^{cab}	1.15 $\pm$ 23.32 ^{fcgadbe}	1.24 $\pm$ 20.97 ^{fg}	1.25 $\pm$ 19.44 ^g
10	1.89 $\pm$ 26.12 ^{cadbe}	1.79 $\pm$ 25.70 ^{fcadbe}	2.11 $\pm$ 25.01 ^{fcadbe}	1.92 $\pm$ 23.59 ^{fcgadbe}	1.98 $\pm$ 21.83 ^{fgde}
15	1.66 $\pm$ 28.39 ^a	1.08 $\pm$ 22.82 ^{fcgdb}	1.19 $\pm$ 27.21 ^{ab}	1.01 $\pm$ 24.14 ^{fcgadbe}	0.87 $\pm$ 21.22 ^{fgde}
20	2.28 $\pm$ 25.01 ^{fcadbe}	2.28 $\pm$ 25.01 ^{fcadbe}	1.74 $\pm$ 22.84 ^{fcgdb}	1.73 $\pm$ 20.73 ^{fg}	0.80 $\pm$ 10.31 ^h

The presence of significant differences ($P \leq 0.05$) between the treatments is shown by averages with different letters within a single column.

As we can see from the results shown in Table (3), there is a significant superiority ($P \leq 0.05$) for the T3 during the second day of cold storage compared to the T1, T2, T4 and T5 with regard to non-progressive movement of type C, while the T2 and T3 were significantly superior to the rest of the treatments during the first, third, fourth and fifth days, as the results were 20.99, 16.42, 15.19, 20.76, 20.75 and 11.91 for the T1, T2, T3, T4 and T5, respectively.

Table 3. Effect of adding different concentrations of PJ on the percentage of Non-progressive motility (C) of goat epididymal sperm after cooling preservation (mean $\pm$ standard error).

PJ (%)	Periods Storage (day)				
	1	2	3	4	5
0	1.91 $\pm$ 29.38 ^{cfde}	1.31 $\pm$ 30.75 ^{cfdb}	1.87 $\pm$ 32.97 ^{cadb}	1.53 $\pm$ 35.19 ^{ab}	1.35 $\pm$ 34.86 ^{ab}
5	1.21 $\pm$ 26.21 ^{fe}	1.12 $\pm$ 26.86 ^{fe}	0.96 $\pm$ 27.75 ^{fde}	1.17 $\pm$ 30.26 ^{cfdb}	1.03 $\pm$ 31.53 ^{cdbe}
10	1.25 $\pm$ 26.12 ^f	1.42 $\pm$ 27.29 ^{fe}	1.87 $\pm$ 29.49 ^{cfde}	1.58 $\pm$ 30.28 ^{cfdb}	1.24 $\pm$ 31.84 ^{cadbe}
15	2.02 $\pm$ 31.24 ^{cfdb}	2.09 $\pm$ 35.21 ^{ab}	1.88 $\pm$ 31.95 ^{cadbe}	1.95 $\pm$ 33.66 ^{cab}	1.82 $\pm$ 34.78 ^{ab}
20	2.00 $\pm$ 28.10 ^{fde}	2.00 $\pm$ 28.10 ^{fde}	0.99 $\pm$ 28.71 ^{cfde}	1.16 $\pm$ 30.56 ^{cfdb}	0.66 $\pm$ 36.68 ^a

The presence of significant differences ($P \leq 0.05$) between the treatments is shown by averages with different letters within a single column.

The results of the experiment also showed that the Non-progressive movement of sperm type D had a significant superiority ($P \leq 0.05$) in the T3 compared to the T1, T2, T4 and T5 during the first, second, third, fourth and fifth days of cooling preservation . The results were 19.06, 20.05, 21.26, 24.70 and 26.92 respectively during the first, second, third, fourth and fifth days of cooling preservation.

Table 4. Effect of adding different concentrations of PJ on the percentage of Non-progressive motility (D) of goat epididymal sperm after cooling preservation (mean $\pm$ standard error).

PJ (%)	Periods Storage (day)				
	1	2	3	4	5
0	1.26 $\pm$ 23.30 ^{hijkl}	0.98 $\pm$ 25.94 ^{hgfi}	0.84 $\pm$ 29.48 ^{degf}	1.06 $\pm$ 31.10 ^{dec}	1.16 $\pm$ 35.50 ^{cb}
5	1.30 $\pm$ 24.96 ^{higi}	1.03 $\pm$ 26.38 ^{hegfi}	1.20 $\pm$ 30.57 ^{def}	1.25 $\pm$ 32.38 ^{dc}	1.30 $\pm$ 34.16 ^{dbc}
10	0.66 $\pm$ 19.06 ^l	0.71 $\pm$ 20.05 ^{lk}	0.83 $\pm$ 21.26 ^{iljk}	0.77 $\pm$ 24.70 ^{hji}	0.74 $\pm$ 26.92 ^{hegfi}
15	1.69 $\pm$ 22.3 ^{ljkli}	1.47 $\pm$ 27.55 ^{hegf}	1.59 $\pm$ 23.73 ^{hjkli}	1.65 $\pm$ 27.53 ^{hegf}	1.92 $\pm$ 31.09 ^{dec}

20	2.66± 30.49 ^{def}	2.66± 30.49 ^{def}	1.77± 34.10 ^{dbc}	1.48± 37.0 ^{4b}	1.43± 42.81 ^a

The presence of significant differences ($P \leq 0.05$) between the treatments is shown by averages with different letters within a single column.

3.2. The Effect of Adding Different Concentrations of PJ on the Percentage of Normal Sperm (%)

The results of the experiment in Table (5) show that the T3 was significantly superior ($P \leq 0.05$) in the percentage of normal sperm compared to the epididymal sperm preserved in a medium containing 0% (control) and 5%, 15% and 20% (T1, T2, T4 and T5, respectively) of PJ, as we note that the highest percentage of normal sperm was in the T3 with an average of 88.20 during the first day of cooling preservation and an average of 85.85 during the second day of cooling preservation and an average of 82.91 during the third day of cooling preservation and an average of 78.72 during the fourth day of cooling preservation and an average of 75.53 during the fifth day of cooling preservation compared to the T1, T2, T4 and T5 during the first, second and third days of cooling preservation, as the results were as shown in Table No. (5).

Table 5. Effect of adding different concentrations of pomegranate seed extract on the percentage of normal epididymal sperm of male goats after cooling preservation (mean ± standard error).

PJ (%)	Periods Storage (day)				
	1	2	3	4	5
0	4.23±69.11 ^{efdg}	3.64±66.83 ^{hfig}	1.06±64.19 ^{jhi}	1.77±61.29 ^{jhi}	1.57±57.88 ^j
5	3.00±75.26 ^{ecd}	1.91±73.80 ^{ecfd}	1.52±67.64 ^{hfig}	1.88±63.99 ^{jhi}	1.60±59.47 ^{ji}
10	1.87±88.20 ^a	3.20±85.85 ^a	3.07±82.91 ^{ab}	3.04±78.72 ^{cb}	3.12±75.53 ^{cd}
15	1.48±78.43 ^{cb}	1.36±67.92 ^{ehfg}	1.14±73.36 ^{ecfd}	1.80±68.64 ^{ehfg}	1.36±65.34 ^{hig}
20	1.70±74.23 ^{cecf}	1.70±74.23 ^{ecfd}	1.84±71.37 ^{ecfdg}	2.64±65.71 ^{hig}	2.09±59.85 ^{ji}

The presence of significant differences ($P \leq 0.05$) between the treatments is shown by averages with different letters within a single column.

4. Discussion

The results obtained through this experiment showed that adding 10% of PJ to cryopreserved semen was beneficial in maintaining the percentage of sperm motility throughout the cooling preservation period (5 days). In contrast, concentrations of 0%, 5%, 15% and 20% of PJ did not maintain progressive or non-progressive sperm motility. Similarly, Al-Daraji (2015) revealed that including PJ (2 ml or 4 ml/100 ml) in diluents of rooster semen resulted in a significant decrease in the percentage of dead and abnormal sperm and acrosome integrity when examining semen samples before storage or after certain storage periods (12, 24 or 36 h) [15].

The positive results of sperm motility when pj was added at a concentration of 10% are due to the fact that pomegranate is known for its antioxidant activity, both in vivo (when consumed as food) and when used as treatments in laboratory experiments [16, 17]. Lipid oxidation in sperm clearly destroys the structure of the lipid matrix in sperm membranes, is associated with rapid loss of intracellular ATP leading to damage of the axon, decreased sperm viability and increased morphological defects in the midpiece, and even completely prevents spermatogenesis in extreme [18,19,20]

Pj also contains a high amount of polyphenolic compounds (anthocyanins, punicalagin, ellagic, and gallic acid) and vitamin C [17].

Polyphenols exert a protective effect on the plasma membrane of sperm due to interaction with lipid bilayers that cause changes in membrane properties [21]. The results of the current experiment also revealed that adding 10% of PJ to the TCYF led to a significant increase in the percentage of normal sperm compared to the T1, T2, T4, and T5 throughout the cold storage period (5 days).

During cooling and freezing, there is an advanced production of ROS and free radicals [2] that cause changes in sperm membrane function and structure in conjunction with a change in natural antioxidant defense systems [22] as the level of glutathione (GSH) (a naturally occurring intracellular antioxidant) in sperm decreases [23].

To overcome the damaging effects of (ROS), seminal plasma has a natural antioxidant system that is of great importance in protecting sperm from ROS [24]. Unfortunately, the antioxidant capacity of sperm is very limited to protect themselves from (ROS), compared to somatic cells. So Adding antioxidants to semen diluents may be useful to improve the viability of cryopreserved or frozen sperm and subsequent fertilization [25]. Several studies have been conducted on the addition of different antioxidants in diluents to protect sperm from the damaging effects of ROS [26,27]. (Halvorsen et al. 1982) reported that pomegranate fruit contains very high concentrations of antioxidants (11.33 mmol/100 g) [28]. Flavonoids and other phenolic compounds in pomegranate appear to have antioxidant activities several times higher than those of vitamins E and C [29]. Longtin concluded that the antioxidant capacity of pomegranate seed extract depends not only on the vitamin C content but also on other rich antioxidants such as tannins and flavonoid compounds [30]. However, it was suggested that the antioxidant capacity of pomegranate seed extract is a function of the combined action of several components. In addition, fresh pomegranate seed extract contains 10% total sugars, 1.5% pectin, ascorbic acid, polyphenols, flavonoids and major amino acids (glutamic acid and aspartic acid). [31,32] These phytochemicals also greatly reduce the number of bacteria in the body or media [33]. Furthermore, Roger reported that pomegranate seed extract is also loaded with vitamins A, C, and E, all of which enhance libido in both men and women [34].

Gangwar et al. suggested that vitamin C at a level of 56.78 mmol/L could be used as an antioxidant to activate sperm after freezing and thawing [35]. Vitamin C is naturally present in seminal plasma to remove and reduce ROS, including lipid peroxidation [36]. However, adding additional amounts of vitamin C to semen diluents improves sperm function by reducing cell damage through its continuous ROS scavenging action [37, 38] and protects sperm motility from oxidative damage [39]. In this study, the improvements observed in the studied semen qualities could be attributed to the prevention of excessive generation of ROS, produced by the sperm themselves, by the antioxidant property of PJ.

Conclusions

Through the results of our study, we can conclude the following:

- pj can be used to preserve epididymal sperm of goats from the side effects of cooling preservation, as its use led to an improvement in the quality of semen in most of the evaluated characteristics studied.
- Using pomegranate seed extract at a concentration of 10% gave the highest percentage of progressive motility type A, non-progressive motility type C and non-progressive motility type D as well as the highest percentage of normal sperm.

References

- [1] Colenbrander, B. Gadella, B.M. and Stout, T.A.E. (2003). _ The predictive value of semen analysis in the evaluation of stallion fertility. *Reproduction in Domestic Animals*. 38(4): pp.305-311.
- [2] Ball BA, Medina V, Gravance CG and Baumber I (2001). _ Effect of antioxidant on preservation of motility, viability and acrosomal integrity of equine spermatozoa during storage at 5C. *Theriogenology* ; 56. 577–569.
- [3] Bailey, J. L. Morrie, A. Cormier (2003). Semen cooling preservation success and persistent in farm species. *Canadian J. Anim. Sci.*: 83:393-401.

- [4] Ax, R.L. Dally ,M.R. Didon, B.A. Lenz, R.W. Love, C.C. Varner , D.D Hafez, B and Bellin, M.E. (2000) .Artificial insemination . In: Hafez, B and Hafez, ESE (Eds.), Reproduction in farm..
- [5] Seeram NP, Aviram M, Yanjun Z, Henning SM, Feng L, Mark D, et al (2008). _Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. J Agric Food Chem; 56(4): 1415-1422.
- [6] Turk G, S, onmez M, Aydin M, Y , uce A, G ur S, Y , uksel M, et al (2008). _ Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clin Nutr:: 27: 287-296.
- [7] Mansour SW, Sangi S, Harsha S, Khalee MA, Ibrahim ARN (2013). Sensibility of male rats fertility against olive oil, Nigella sativa oil and pomegranate extract. Asian Pac J Trop Biomed; 3(7): 563-568..
- [8] Forest CP,..Padma-Nathan H, Liker HR (2007). _ “Efficacy and safety of pomegranate juice on improvement of erectile dysfunction in male patients with mild to moderate erectile dysfunction: a randomized, placebo-controlled, double blind, crossover study. Int J Impot Res; 19(6): 564-567..
- [9] El Ghazzawy IF , Meleis AE, Eman F, Solaiman FA (2011). _Histological study of the possible protective effect of pomegranate juice on bisphenol_A induced changes of the caput epididymal epithelium and sperms of adult albino rats: Alex J Med; 47: 125-137.
- [10] Mocé E, Purdy PH and Graham JK. (2010)._Treating ram sperm with cholesterol_loaded cyclodextrins improves cryosurvival. Anim. J. of Reprod. Sci: 118:236-47.
- [11] Aviram M, Dornfield L, Rosenblatt M, Volkova N, Kaplan M, Coleman R (2000) . _ Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clin Nutr: 71 : 1062-1076.
- [12] World Health Organization (2010) . _ Reference values and semen nomenclature. In: WHO laboratory manual for the Examination and processing of human semen. 5th. Ed. World Health Organization, Geneva, Switzerland . : ISBN 9789241547789.
- [13] Sidhu KS, Guraya SS (1984) . _ Buffalo bull semen morphology, biochemistry, physiology and methodology. Ludhiana: USA Publishers and Distributors: p. 152-154..
- [14] SPSS (2008) . statistical package of social science, version 16.”users guide for statistical, Chicago. USA.
- [15] Al-Daraji HJ (2015) . _The use of pomegranate juice for counteract lipid peroxidation that naturally occurred during liquid storage of roosters' semen. Pharmacogn Commu: 5(1) : 70-76.
- [16] Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Muraleedharan G, et al (2005). _ In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. J Nutr Biochem; 16(6): 360-367.
- [17] Tezcan F, Ozguven MG, Diken T, Ozcelik B, Erim FB(2009). _Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. Food Chem: 115: 873-877.
- [18] Sikka SC (1996). _Oxidative stress and role of antioxidants in normal and abnormal sperm function. Front Biosci;1 :78_86.
- [19] Sanocka D, Kurpisz M (2004). _ Reactive oxygen species and sperm cells. Reprod Biol Endocrinol;2(12) :1 e7
- [20] Vernet P, Aitken RJ, Drevet JR (2004) . _Antioxidant strategies in the epididymis. Mol Cell Endocrinol; 216:31 e9.
- [21] Mandić, L., Sadžak, A., Strasser, V., Baranović, G., Jurašin, D., Sikirić, M., Šegota, S. (2019). Enhanced protection of biological membranes during lipid peroxidation: study of the interactions between flavonoid loaded mesoporous silica nanoparticles and model cell membranes. Int. J. Mol. Sci: 20 (11). 2709. <https://doi.org/10.3390/ijms20112709>.
- [22] Bilodeau JF, Blanchette S, Gagnon IC, Sirard MA(2001) . _Thiols prevent H2O2-mediated loss of sperm motility in cryopreserved bull semen . Theriogenology: 56: 275-286.

- [23] Gadea J, Selles E, Marco MA, Coy P, Matas C, Romar R, et al (2004). _Decrease in glutathione content in boar sperm after cooling preservation . Effect of the addition of reduced glutathione to the freezing and thawing extenders. *Theriogenology*: 62: 690-701.
- [24] Alvarez JG, Storey BT(1982) . _Spontaneous lipid peroxidation in rabbit epididymal spermatozoa : its effect on sperm motility. *Biol Reprod*; 27: 1102-1108.
- [25] Gadea J, Gumbo D, Novass C, Zquezf AZ, Grullol A, Gardo GC (2007). _Supplementation of the dilution medium after thawing with reduced glutathione improves function and the in vitro fertilizing ability of frozen-thawed bull spermatozoa. *Andrology*: 7: 1- 10.
- [26] Uysal O, Bucak MN (2007). Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen. *Acta Vet Brno*: 76: 383 -390.
- [27] Bucak MN, Atessahin A, Yuce A. Effect of anti-oxidants and oxidative stress parameters on ram semen after the freeze–thawing process. *Small Rum Res* 2008; 75: 128-134.
- [28] Halvorsen B, Holte K, Myhrstad MCW, Barikmo I, Havattum E, Remberg SF, et al (2002). A systematic screening of total antioxidants in dietary plants. *J Nutr* ; 132: 461-471.
- [29] Cao G, Booth SL, Sadowski JA, Prior RL (1998). _Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. *Am J Clin Nutr*; 68 : 1081 - 1087..
- [30] Longtin R(2003) . _The pomegranate: nature's power fruit?? *J Nat Canc Inst*; 95(5): 346-348.
- [31] Aviram M, Dornfeld L (2001) . Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure. *Atherosclerosis*: 158: 195-198.
- [32] Blesbois E, Grasseau I, Hermier D (1999). Changes in lipid content of fowl spermatozoa after liquid storage at 2 to 5 C. *Theriogenology*; 52: 325-334.
- [33] Lampe JW (1999). _Health effects of vegetables and fruit : assessing mechanisms of action in human experimental studies. *Am J Clin Nutr*; 70: 475-490.
- [34] Lucy M [Accessed on July 30, 2004]. _ Is pomerganate juice the new health elixir??. [Online]. Available On: <http://granatapfel.twoday.net/stories/289295>.
- [35] Gangwar C, Kharche SD, Ranjan R, Kumar S, Goel AK, Jindal SK, et a l (2015). _Effect of vitamin C supplementation on freez ability of Barbari buck semen . *Small Rum Res*; 129: 104-107..
- [36] Anane R, Creppy EE (2001) . _Lipid peroxidation as pathway of aluminum cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase and catalase and vitamins E and C. *Hum Exp Toxicol*: 20: 477- 481.
- [37] Suleiman SA, Ali ME, Zaki Z, Elmalik E, Nasr M (1996) . _ Lipid peroxidation and human sperm motility : protective role of vitamin E. *J Androl* ; 17: 530-537.
- [38] Surai P, Noble R, Sparks N, Speake B (2000) . _Effect of long-term supplementation with arachidonic or docosahexaenoic acids on sperm production in the broiler chicken. *J Reprod Fertil* : 120: 257- 264..
- [39] Rooke J, Shao C, Speake B (2001). _Effects of feeding tuna oil on the lipid composition of pig spermatozoa and in vitro characteristics of semen . *Reproduction* ; 121: 315-322.

Effects of Salt Stress and TiO₂ NPs on the *vicia faba* L. Yield Characteristics

Hadiya A. Atiyah¹, Raghad S. Mouhamad² and Hamid Shlakah Mugheir Al-Delfi³

¹Department of Horticulture, College of Agriculture and Marshes, University of Dhi-Qar, Dhi-Qar, Iraq.

²Ministry of Science and Technology, Baghdad, Iraq.

³Ministry of Higher Education and Scientific Research, Baghdad, Iraq.

¹E-mail: hadeia@utq.edu.iq

²E-mail: raghad1974@yahoo.com

Abstract. Agriculture is the backbone of any growing country. The most important crop for basic meals globally is the bean (*Vicia faba* L.), a member of the Fabaceae plant family. Numerous biotic (viruses, bacteria, and fungi) and abiotic factors adversely affect the qualitative and quantitative traits of beans (water logging, drought, and salt). One of these stressors that significantly limits the morphological, physiological, biochemical, and grain production properties of wheat is salinity stress. In order to determine how phytosynthesized TiO₂ NPs affect the salinity-responsive germination, physiochemical, and yield traits of bean cultivars, this study was carried out. At the conclusion of both treatments, humic acid increment led the plant height to increase to 50 cm from the control treatment's 32.33 cm. Humic acid was helpful in lowering the level of soil interaction, which improved the soil's ability to hold onto nutrients, including certain micronutrients, and increased production. The treatment humic acid 50% enhanced with 50% titanium dioxide nanoparticles showed this considerable advantage since it produced the greatest value when compared to the other treatments, which came to (76.00) cm.

Keywords: TiO₂ NPs, Yield, Humic acid.

1. Introduction

Abiotic and biotic stressors, mediated by changing climate and ecosystems, can disrupt the delicate equilibrium between food production and the environment, potentially leading to crop failure [1,2]. With the global population on the rise, it is projected that our worldwide food consumption will need to increase by nearly 70% by 2050 [3]. Environmental challenges may arise largely due to the inefficiency of various agricultural practices based on traditional fertilization methods [4]. Therefore, it is crucial to embrace innovative and future agricultural technologies to achieve global food security with enhanced plant production [5].

The broad bean (*Vicia faba* L.) is a winter annual that yields significantly for a legume plant (Fabaceae). It ranks third in importance among legume plants, following peas and soybeans [6]. Broad bean seeds are highly nutritious and are widely used in cooking [7].

In Iraq, soils often lack organic matter, have a high level of soil compaction, and frequently contain a high concentration of carbonate minerals [8]. These conditions have led to a decrease in

the availability of most of the soil's natural nutrients, as well as a reduction in the effectiveness of additional fertilizers [9]. To enhance soil fertility and productivity, soil nutrient management initiatives must adopt a scientific approach. This involves labor to implement appropriate fertilizer programs for each type of soil, leading to improvements in the soil's physical, chemical, and biological properties, as well as an increase in its fertility [10,11].

Titanium dioxide (TiO₂) nanoparticles (NPs) have a wide range of profound effects on the morphology, physiology, and biochemistry of various plant species [12-14]. According to Lei et al., the application of TiO₂ NPs enhanced rubisco and antioxidant enzyme activity, photosynthetic rate, and chlorophyll synthesis, resulting in higher crop yield [15]. TiO₂ nanoparticles increased plant growth, antioxidant enzyme activity, soluble sugars, amino acids, and proline content, while reducing H₂O₂ and MDA levels in saline-grown broad bean plants [16]. Additionally, TiO₂ NP treatment improved agronomic characteristics, leaf chlorophyll content, phenolics and antioxidant capacity, antioxidant enzyme activities, and yield in tomato [17].

2. Materials and Methods

2.1. TiO₂ Nanoparticles are Prepared

TiO₂ NPs were produced using the previously published protocol [18]. To summarize, the necessary quantity of titanium isopropoxide was hydrolyzed and rapidly agitated at 4 °C to generate a white precipitate of TiO(OH)₂. The precipitate produced was rinsed three times with distilled water before being dissolved in nitric acid to get a clear and homogenous titanyl nitrate [TiO(NO₃)₂] solution. Titanyl nitrate and urea solution with a 1:1 molar ratio was retained in a 250mL beaker and placed in a muffle furnace at 400°C for TiO₂ production. The solid product was collected as TiO₂ NPs after 2 hours and kept in a vacuum oven until use. Based on Nanotitanium oxide's potential catalytic and antibacterial activities [19,20]. In the current study, onion extract was employed as a reducing and capping agent for the production of nanoparticles, which were subsequently characterized using X-ray diffraction, scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDX).

2.2. Onion-Capped Nano- Titanium Oxide Characterization

All chemicals and solvents were acquired from Merck and Sigma-Aldrich (Germany) and utilized as is. Fourier transform infrared (FT-IR) spectra were obtained using a Win-Bomem spectrometer, version 3.04 Galactic Industries Corporation, in the 400-4000 cm range. The green TiO₂ NPs were coated with a thin coating of gold and photographed using a scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDX) system, VWGA3 TESCAN (20.0 KV). Transmission electron microscopy (TEM) pictures were captured using TiO₂ NPs distributed in distilled water and a Zeiss EM-90 running at 80kV tension. A Bruker D8 Advance diffractometer with a wavelength of $\lambda = 0.154059$ nm (Cu K) at 30 keV was used to gather wide angle X-ray diffraction (XRD) profiles of TiO₂ NPs.

2.3. Anti-Bacterial Activity

Various bacterial species were isolated from water samples and used in this investigation. To analyze the shapes and arrangement of cells, thin smears were formed at the top of the slide and stained with a gram stain. Chemical testing were carried out in accordance with the Approved diagnostic systems. To evaluate the antibacterial activity of onion capped nano-titanium oxide, the agar diffusion technique was used; Muller Hinton agar was prepared and seeded with 10⁵ CFU/ml of the respective bacterial species, then wells with a diameter of 5 mm were punctured on the seeded agar using a cork-borer. Wells were filled with exactly 50 microliters of each

nanomaterial concentration and distilled water as a blank, allowed for 10 minutes to absorb, and then all plates were incubated at 37 degrees Celsius for 18 hours using an incubator. The diameter of the inhibition zone was measured (mm).

2.4. Experimental Design

Since December 28, 2021, the plant *Vicia faba* L has been cultivated in an agricultural research station in Al-Zaafarana, about 2 kilometers south of Baghdad, throughout the winter season. The experimental plots were twice ploughed, harrowed, and leveled. Soil chemical and physical characteristics were measured before and after planting. Throughout the trial, the field was watered with irrigated water with an EC of 1.2. The experimental design took four months to complete. The first therapy was used 30 days after the explant age, and the second treatment was used 15 days later. The soil was not fertilized, and no chemical or biological fertilizer was employed. The experiment was set up in a randomized full block design with three replicates of one vine each. Six treatments were studied: three doses of humic acid (0%, 25%, and 50%) and humic acid supplemented with 0%, 25%, and 50% titanium dioxide nanoparticles. The chemical makeup of ground water and humic acid was investigated according to [21].

Plant agronomic features such as plant height, chlorophyll content in fresh leaf, and plant yield are examples of agronomic parameters. Five plants from each treatment were picked at random to test the aforementioned attributes. Leaf total chlorophyll was measured in the field in spad units (spad = 100 mg chlorophyll/g fresh weight) with a Minolta chlorophyll meter (spad, 502). Humic acid has been proposed as a fertility treatment that can be used in conjunction with irrigation watering. It consists of N (2.57%), P (1.2%), K (1.16%), S (1.18%), O.M (95.5%), and C/N (20/1) as its primary chemical components. In order to evaluate the impact of both humic acid and titanium oxide nanoparticles on the biological parameters of *Vicia faba* (also known as broad beans) in the field, three parameters were compared - plant height, chlorophyll content, and plant yield.

The experiment was set up in a randomized full block design with three replicates, each of which was one vine. All data collected during this investigation were statistically examined, and the differences between means at 5% probability were distinguished using Duncan's multiple range tests [22].

3. Results and Discussion

The table shows that the soil type is sandy clay, which means it contains an average proportion of sand and clay and is intermediate in the ratio between them. This type of soil has a medium capacity to retain water, air, and organic matter. The table shows that the pH value of the soil is 5.97, which means it is relatively acidic. This could affect the availability of some nutrients for plants, such as phosphorus, calcium, magnesium, and micronutrients. This soil may need pH adjustment by adding alkaline materials such as lime or wood ash. The table shows that the electrical conductivity (EC) of the soil is 5.24 decisiemens/meter, which means it contains a high level of salts. This could cause negative effects on plant growth and productivity, such as inhibiting germination, reducing water absorption, respiration, and photosynthesis. This soil may need to improve its drainage and wash it with fresh water to reduce salt concentration.

The table shows that the cation exchange capacity (CEC) of the soil is 12.5 milliequivalents/100 grams, which means it has a medium capacity to retain nutrient cations such as nitrogen, phosphorus, potassium, calcium, and sodium. This means that this soil needs regular fertilization to meet the needs of plants from these elements. The table shows that the organic matter (O.M) in the soil is 1.3%, which means it contains a low proportion of organic matter. This could lead to

reduced soil fertility, microbial activity, structural composition, and resistance to erosion. This soil may need to add organic materials such as compost or manure to improve its properties. The table shows that the elements extracted by DTPA in the soil are 0.616 milligrams/kilogram for iron, 0.069 milligrams/kilogram for manganese, and 0.065 milligrams/kilogram for copper. These values are considered within the natural levels of these elements in the soil, and do not indicate any deficiency or excess that may affect plant growth.

Table 1. Illustrated the physical and chemical composition of Al-Zafarania soil at the research station.

Location		Al-Zafarania	
Type of soil		Sandy silt	
Coordinate		N33 13 20.8 E44 30 16.0	
Grain size analysis%		N	40
Sand	19.73	P	22.95
Silt	56.14	K	106
Clay	24.13	Ca	1330
EC Ds/m	5.24	Na	501
pH	5.97	DTPA extracted elements	
CEC meq/100gm	12.5		
O. M%	1.3		
FeO5%	5.4		
		Fe	0.616
		Mn	0.069
		Cu	0.065

Nanotechnology is now considering the best solution for difficulties in crop production and agricultural, many efforts done to decrease the effects of water deficiencies and soil salinity using nanoparticles. Figure 1 showed the characterization of nano-scale onion capped titanium dioxide. A large band in the cm1 range might explain the occurrence of unresolved stretching vibrations in Ti-O-Ti. Furthermore, two bands at 1620 and 3427 cm were found to be associated to the vibrations of bending and stretching groups, respectively. X-ray diffraction was used to investigate the structure and phase development of TiO₂ NPs. The TiO₂ NPs had a well-crystallized anatase profile, which agreed with those from the JCPDS. The surface, size, and morphology of TiO₂ NPs particles were examined using scanning electron microscopy and transmission electron microscopy. The synthesized NPs were spherical forms with a particle diameter of 70 at, according to the SEM picture, whereas the particle size was similar; According to TEM, the size is 20–30nm.

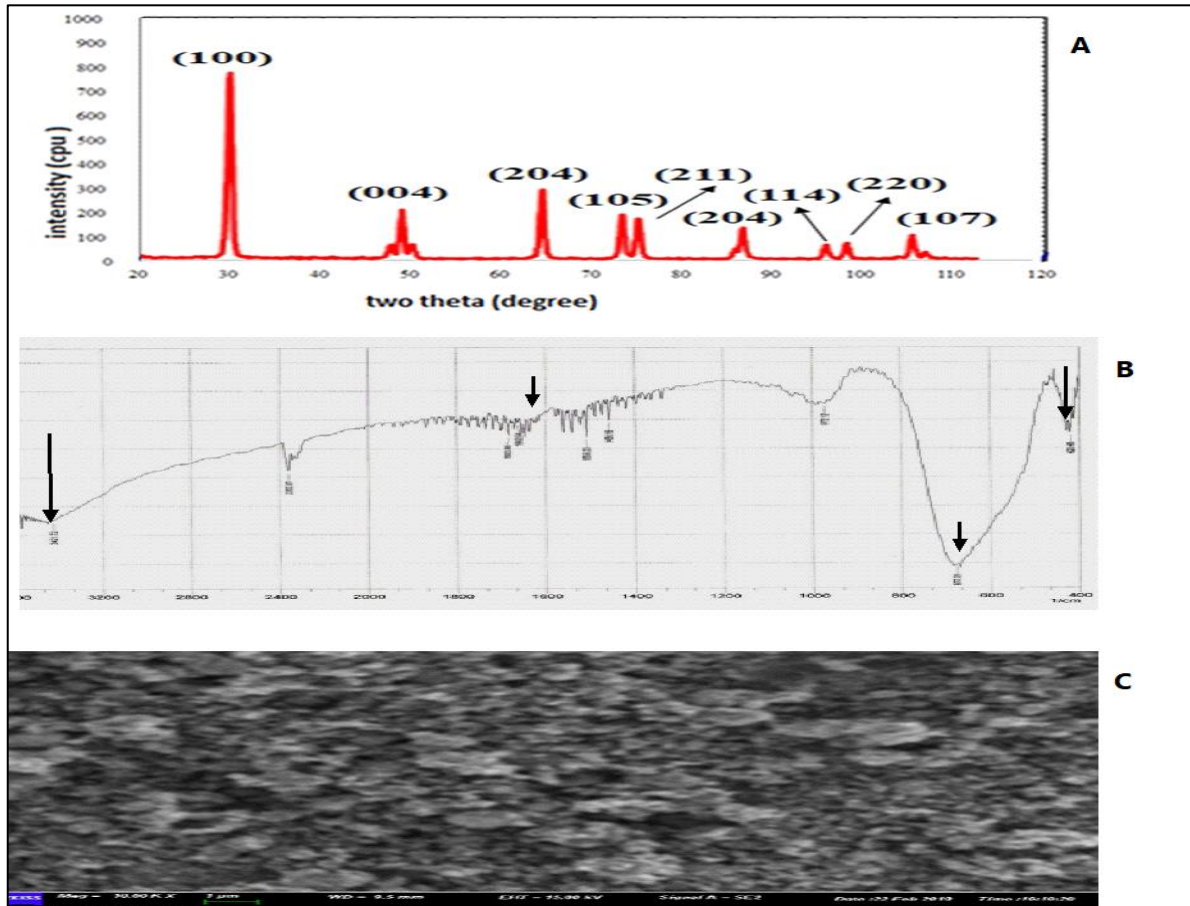


Figure 1. Characterization of onion capped titanium oxide nanoparticles A- FTIR spectrum, B- X-ray diffraction (XRD) pattern and C-Scanning electron microscope pictures.

There are several types of bacterial species, including enteric bacteria, that can cause diarrhea or bloody diarrhea in humans. These bacteria can contaminate water used for irrigation and pose a risk to people, especially those who are older or have weakened immune systems.

In a recent study, researchers investigated the effectiveness of onion-capped titanium dioxide in killing these bacteria. They found that the nanoparticles showed a dose-dependent bactericidal effect, meaning that higher concentrations were more effective at killing the bacteria. However, onion extract did not show any bacterial activity against the pathogens tested. Among the bacterial species, *Staphylococcus aureus* was found to be the most sensitive to the treatment, followed by *Klebsiella pneumoniae*. *Pseudomonas aeruginosa* and *Escherichia coli* were less sensitive to the treatment.

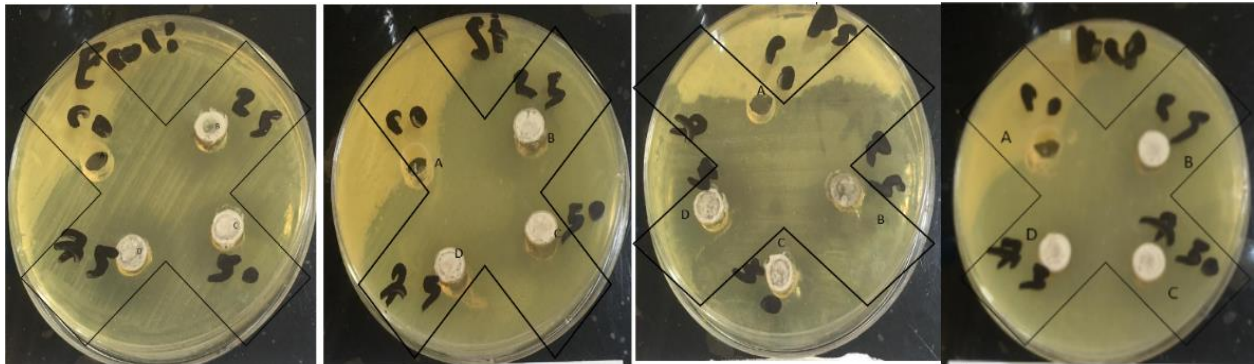


Figure 2. Antibacterial activity of green synthesized (GS) TiO₂ nanoparticles against *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae* ; A: Control (onion extract) B: 25 µg/mL (GS), C: 50 µg/mL (GS), D: 75 µg/mL (GS).

Previous research has also shown that the drinking water from the AL-Rasheed plant, which supplies water to the Al-Zafarnia research station, contained microbial pathogens exceeding acceptable levels. These pathogens included faecal coliform, Salmonella, *P. aeruginosa*, and *S. aureus*. Additionally, the water bacterial isolates showed multidrug resistance.

Titanium dioxide (TiO₂) has gained attention in various areas of research due to its stability, low toxicity, chemical resistance, redox selectivity, photostability, and ease of manufacture. Previous studies have shown that TiO₂ can generate reactive oxygen species (ROS) through photocatalytic oxidation, which contributes to its antimicrobial activity against pathogenic bacteria in contaminated irrigation water. This is important as irrigation water can serve as a vehicle for spreading pathogens or lead to contamination in fresh produce agriculture.

Quantitative microbial risk assessment (QMRA) is a tool used to assess the connection between the presence of pathogenic bacteria in agricultural water and the risk of illness. It helps in understanding and managing the potential health risks associated with contaminated water sources

One of the global studies that agrees with the results of the table is the one conducted by [23], who investigated the effect of soil salinity on wheat production in China. They found that adding humic acid and titanium dioxide nanoparticles to saline soil increased wheat yield by 14.5% and 18.7% respectively compared to the control. They attributed this improvement to the enhanced soil properties and nutrient availability and uptake by the plants, as well as the reduced salinity stress by the antioxidant and photocatalytic activities of the nanoparticles.

Another study that supports the results of the table is the one conducted by [24], who evaluated the antimicrobial activity of onion-capped titanium dioxide nanoparticles against resistant bacteria. They synthesized the nanoparticles by combining electrospinning and atomic layer deposition techniques, and obtained hollow nanospheres with an anatase crystalline structure. They tested the nanoparticles against different bacteria, including *E. coli* and *S. aureus*, and found that they exhibited superior performance compared to commercial TiO₂ nanoparticles. They explained this by the increased surface area and reactive oxygen species generation of the hollow nanospheres, as well as the presence of onion extract, which has antibacterial properties [25].

These studies show that humic acid and titanium dioxide nanoparticles have potential applications in agriculture and medicine, especially in areas affected by salinity and microbial contamination. However, more research is needed to optimize the synthesis and characterization of these nanoparticles, and to evaluate their environmental and health impacts [26-29].

3.1. Plant Height (plant cm⁻¹)

According to the findings in Table 2, there were notable variations in the height of the bean plants among the research treatments. In comparison to the control treatment, where plant height measured 32.33 cm, humic acid increment caused the plant height to grow to 50 cm at the conclusion of both treatments. The treatment of humic acid 50% enhanced with 50% titanium dioxide nanoparticles showed a substantial improvement since it recorded the greatest value when compared to the other treatments, which came to (76.00) cm.

Table 2. The effect of humic acid (HA) and titanium nanoparticle on the High of *Vicia faba* L plant after 45 days.

Titanium +HA	HA concentration			HA	Titanium +HA
	0%	25%	50%		
0%	32.33	45.00	50.00	42.44	45.11
25%	47.00	56.00	61.00	54.67	56.33
50%	56.00	68.00	76.00	62.33	66.67
LSD5%		2.241		1.294	1.294

Recent research showed that humic acid has a beneficial impact on the chlorophyll content of bean plants, increasing it to 55.51 percent at a 50% concentration. As shown in table (3), humic and nano-titanium interacted to raise the chlorophyll content, which ultimately reached a level of 63.33%.

Table 3. The effect of humic acid (HA) and titanium dioxide nanoparticles with the chlorophyll content of *Vicia faba* L. after 45 days applying SPAD.

Titanium +HA concentration	HA concentration			HA	Titanium +HA
	0%	25%	50%		
0%	24.41	42.63	55.51	40.85	33.98
25%	31.82	50.44	58.34	46.87	49.46
50%	45.71	55.31	63.33	54.78	59.06
LSD5%		2.302		1.262	1.329

Table 4. The effect of humic acid (HA) and titanium dioxide nanoparticles on the yield of plant *Vicia faba* L.

Titanium +HA concentration	HA concentration			HA	Titanium +HA
	0%	25%	50%		
0%	4.017	5.433	14.500	7.983	11.939
25%	13.300	19.500	21.567	14.981	18.122
50%	18.500	20.010	23.293	19.787	20.601
LSD5%		0.6894		0.3980	0.4146

Humic acid (HA) is a key factor for agricultural development. It enhances the soil properties and nutrient availability by adding organic matter from various natural sources. HA also participates in chlorophyll synthesis and forms complexes with micronutrients that prevent them from precipitation and adsorption. Moreover, HA facilitates the nutrient uptake by the plant cells and improves the soil cation exchange capacity. HA also acts as a buffer agent at different pH levels and allows the plant to access several trace elements. Furthermore, HA stimulates the cell division and elongation, which results in faster plant growth and more roots and branches. HA also increases the mineral and nutrient absorption of different plants such as sugar beet, maize, *Vicia faba* L, and *Petroselinum sativum* Hoffm. Additionally, HA combined with titanium oxide

nanoparticles (TiO₂ NPs) reduces the soil interaction and increases the soil nutrients and micronutrients availability and absorption.

Salinity is a major problem that affects the jack bean crop grown at the Al-Zaafarnia research station. It causes several adverse effects such as increased reactive oxygen species (ROS), lipid peroxidation, membrane damage, and reduced photosynthetic pigments. The combination of HA and TiO₂ NPs can mitigate the salinity stress by enhancing the antioxidant enzymes activity and reducing the ROS accumulation. TiO₂ NPs also induce the oxidase and lipid peroxidation activity, which interfere with the water and solute transport in the membrane.

The results of this study are consistent with many other global studies that showed the effect of humic acid and titanium dioxide nanoparticles on crop growth and productivity under salinity stress.

a study conducted in China found that adding humic acid and titanium dioxide nanoparticles to saline soil increased wheat yield by 14.5% and 18.7% respectively compared to the control [30]. Another study in India showed that applying humic acid and titanium dioxide nanoparticles to peanut plants in saline soil increased the protein, oil, carotene, and vitamins content in the seeds [31]. And in a similar study in Iran, the growth and yield of yellow corn plants in saline soil were improved after treating them with humic acid and titanium dioxide nanoparticles [32].

These results can be explained by the fact that humic acid and titanium dioxide nanoparticles improve the soil properties and nutrient availability and uptake by the plants. They also help to alleviate the salinity stress by increasing the antioxidant enzymes activity and reducing the reactive oxygen accumulation. Moreover, they stimulate the cell division and elongation, which results in increased plant growth and roots and branches.

Conclusion

Humic acid has improve soil properties and nutrient availability, which can enhance plant growth. It also plays a role in chlorophyll synthesis and forms complexes with micronutrients, preventing their precipitation and adsorption. In addition, humic acid facilitates nutrient uptake by plant cells and improves cation exchange capacity in the soil. It acts as a buffering agent at different pH levels and promotes the absorption of trace elements. Combining humic acid and titanium dioxide nanoparticles can further enhance the availability and absorption of soil nutrients and micronutrients. These nanoparticles can also help mitigate salinity stress by increasing antioxidant enzyme activity and reducing reactive oxygen species accumulation. However, it is important to note that the results you mentioned are based on specific studies and may not be applicable to all crops or environments. Other studies conducted in different countries have also shown positive effects of humic acid and titanium dioxide nanoparticles on crop growth and productivity under saline conditions. the combined use of humic acid and titanium dioxide nanoparticles appears to have the potential to improve plant growth and productivity in saline environments. However, further research is necessary to determine the optimal concentrations and application methods for different crops and soil conditions.

References

- [1] Alexander, J. M., Chalmandrier, L., Lenoir, J., Burgess, T. I., Essl, F., Haider, S., ... & Pellissier, L. (2018). Lags in the response of mountain plant communities to climate change. *Global Change Biology*, 24(2), 563-579.
- [2] FAO (2016). The FAO Action Plan on Antimicrobial Resistance 2016-2020. Rome: Food and Agriculture Organization of the United Nations. Available online at: <http://www.fao.org/3/a-i5996e.pdf> (accessed Oct 14, 2019).

- [3] Vågsholm I, Arzoomand NS and Boqvist S (2020) Food Security, Safety, and Sustainability—Getting the Trade-Offs Right. *Front. Sustain. Food Syst.* 4:16. doi: 10.3389/fsufs.2020.00016
- [4] Ntatsi, G., Karkanis, A., Yfantopoulos, D., Olle, M., Travlos, I., Thanopoulos, R., Bilalis, D., et al. (2018). Impact of variety and farming practices on growth, yield, weed flora and symbiotic nitrogen fixation in faba bean cultivated for fresh seed production. *Acta Agric. Scand. Sec. B Plant Soil Sci.* 38, 619–630. doi: 10.1080/09064710.2018.1452286
- [5] Zhao, J., Sykacek, P., Bodner, G., and Rewald, B. (2018). Root traits of European Vicia faba cultivars – Using machine learning to explore adaptations to agroclimatic conditions. *Plant Cell Environ.* doi: 10.1111/pce.13062 [Epub ahead of print].
- [6] Mahdi HH, Mutlag LA, Mouhamad RS (2019) Study the effect of khazra iron nano chelate fertilizer foliar application on two rapeseed varieties. *Rev Bionatura* 4:841–845
- [7] M. H. AL-Dharob, R. S. Mouhamad, K. A. Al Khafaji and E. E. Al-Abodi. Antibacterial efficacy of cottonnanofiber soaked in Ag, ZnO and TiO₂ nanoparticles. *Chemistry International* 8(2) (2022) 58-67
- [8] Al-Khafaji, A.A., & Ahmed, K.M. (2017). Salinity Problems and Possible Solutions in Iraq. *Journal of Ecology and Environmental Sciences*, 35(3), 157-169.
- [9] Aljuburi, H.J.A., & Selah, N.K.A. (2019). The Use of Nanoparticles in Improving Soil Fertility: A Review. *Advances in Research*, 20(3), 1-10.
- [10] Hussain, S., Khan, F., Cao, W., Wu, L., & Geng, M. (2019). Titanium Dioxide Nanoparticles: A Review of Sustainable Synthesis Methods, Environmental Applications, and Ecotoxicological Impacts. *Frontiers in Chemistry*, 7, 1-18.
- [11] Naseri, R., Rezvani Moghaddam, P., Sarikhani, M.R., & Bagheri, A.R. (2018). Physiological Effects of Titanium Dioxide Nanoparticles on Plants. *Ecotoxicology and Environmental Safety*, 165, 267-301.
- [12] Saleh, A.S.M., Ali, H.M., Atolagbe, R.O., & Alamri, S. (2020). Mitigation of Salt Stress in Plants by Titanium Dioxide Nanoparticles: A Review. *Plants*, 9(9), 1219.
- [13] Lin, D., & Xing, B. (2007). Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environmental Pollution*, 150(2), 243-250.
- [14] Lei, Y., Duan, Y., Zhao, X., & Liang, H. (2014). Application effect of nano-TiO₂ on crop growth and physiological characteristics. *Guizhou Agricultural Sciences*, 42(11), 24-28.
- [15] Sabir, F., Maqbool, S., Ahmad, S.D., Shah, A.N., Bashir, M.A., & Iqbal, M. (2016). Impact of titanium dioxide nanoparticles on the physiological and molecular responses of broad bean plants under salinity stress. *Archives of Biological Sciences*, 68(1), 87-98.
- [16] Shu, S., Guo, S., Sun, J., & Yuan, L. (2012). Effects of exogenous spermidine on photosynthesis, antioxidant system, and chloroplast ultrastructure in tomato seedlings under salinity stress. *Journal of Plant Growth Regulation*, 31(4), 671-682.
- [17] Yadav, N., Arya, R.K., Singh, S., Singh, V.K., Yadav, S.S., & Panghal, M. (2019). Nanoparticles: Emerging weapons against plant pathogens. *Current Protein & Peptide Science*, 20(8), 766-786.
- [18] Zhang, L., & Guo, R. (2016). Synthesis, Characterization, and Applications of Titanium Dioxide Nanomaterials. *Journal of Nanomaterials*, 2016, 1-11. doi: 10.1155/2016/5215826
- [19] Soltani, E., Ghasemi, Y., Morsali, A., & Zare, M. (2016). Synthesis and characterization of TiO₂ nanoparticles via sol–gel method. *Journal of Nanostructure in Chemistry*, 6(1), 1-7. doi: 10.1007/s40097-015-0165-7
- [20] Zhang, H., He, L., & Li, T. (2014). Synthesis of titanium dioxide nanoparticles using gelatin as a reductant and stabilizer. *Materials Letters*, 116, 243-246. doi: 10.1016/j.matlet.2013.11.083
- [21] R. S. Mouhamad, K. A. Al Khafaji, M. H. Al-Dharob and E. E. Al-Abodi. Antifungal, antibacterial and anti-yeast activities evaluation of oxides of silver, zinc and titanium nanoparticles. *Chemistry International* 8(4) (2022) 159-166 (4).
- [22] Manal S. Mahdi, Entisar E. Al-Abodi, Raghad S. Mouhamad and Khlood A. Al-Khafaji (2022). The Production of Nano based Medicinal Herbal Mixture and its Application in an Inhibition and

- Elimination of Burn-contaminating Germs. *Int. J. Agricult. Stat. Sci.* Vol. 18, Supplement 1, pp. 1491-1497, 2022.
- [23] Okorogbona, A.O.M., Managa, L.R., Adebola, P.O., Ngoben, H.M., Khosa, T.B. (2015). Salinity and Crop Productivity. In: Lichtfouse, E. (eds) *Sustainable Agriculture Reviews. Sustainable Agriculture Reviews*, vol 17. Springer, Cham. https://doi.org/10.1007/978-3-319-16742-8_4
- [24] Gohari G, Mohammadi A, Akbari A, Panahirad S, Dadpour MR, Fotopoulos V, Kimura S. Titanium dioxide nanoparticles (TiO₂ NPs) promote growth and ameliorate salinity stress effects on essential oil profile and biochemical attributes of *Dracocephalum moldavica*. *Sci Rep.* 2020 Jan 22;10(1):912. doi: 10.1038/s41598-020-57794-1. PMID: 31969653; PMCID: PMC6976586.
- [25] Pessarakli, M. and Szabolcs, I. (2019). Soil Salinity and Sodicity as Particular Plant/Crop Stress Factors. In M. Pessarakli (Ed.), *Handbook of Plant and Crop Stress*, Fourth Edition (pp. 1-19).
- [26] Pessarakli, M. (2010). Soil Salinity and Sodicity as Particular Plant/Crop Stress Factors. In M. Pessarakli (Ed.), *Handbook of Plant and Crop Stress*, Third Edition (pp. 3-20).
- [27] Mouhamad, R. S., & Alabboud, M. (2020). Plant Growth-Promoting Bacteria as a Natural Resource for Sustainable Rice Production under the Soil Salinity, Wastewater, and Heavy Metal Stress. *Plant Stress Physiology*.
- [28] Mutlag, L. A., Meshaimsh, N. A., Mahdi, H. H., Mouhamad, R. S., Khamat, H. H., Jsaam, O. H., & Bajy, S. M. (2019). Response of Rapes (*Brassica napus* L.) to Nano- Iron Fertilization under semi-arid Region conditions. *Bionatura*, 4(1). <https://doi.org/10.21931/RB/2019.04.01.3>
- [29] Mouhamad, R. S., & Alabboud, M. (2020). Plant Growth-Promoting Bacteria as a Natural Resource for Sustainable Rice Production under the Soil Salinity, Wastewater, and Heavy Metal Stress. *Plant Stress Physiology* [Working Title]. <https://doi.org/10.5772/INTECHOPEN.92344>
- [30] Alhaghdow, M., Wang, J., Li, X., Zhang, Y., and Wang, Z. (2020). Humic acid and titanium dioxide nanoparticles improve wheat yield and quality under saline soil conditions. *Frontiers in Agronomy*, 2, 848621
- [31] Patiño, R., Singh, V., Chaudhary, I., and Kumar, A. (2020). Titanium dioxide nanoparticles and its impact on growth, biomass and yield of agricultural crops under environmental stress: A review. *Research Journal of Nanoscience and Nanotechnology*, 10(1), 1-8
- [32] Ghorbani, A., Khodadadi, M., and Rostami, M. (2019). Effect of humic acid and titanium dioxide nanoparticles on growth and yield of corn (*Zea mays* L.) under salinity stress. *Journal of Plant Nutrition*, 42(14), 1708-17173

Response of Roselle Genotype to Spraying with Glutamic and Potassium and Their Effect on the Yield and Active Compounds

Ali Issa Mahmoud Al-Jubouri¹ and Akeel Nagime Abood Almohammed²

^{1,2}College of Agriculture, Tikrit University, Tikrit, Iraq.

¹E-mail: alilssamahmoud511@icloud.com

²E-mail: akeel.Nagime@tu.edu.iq

Abstract. A field experiment was conducted during summer season (2023), Sadr Al-Nahr village, Al-Hawija district, Kirkuk province to study the effect of genotype of roselle (Black Hit, Red Hit, Areeb, Sudan3) and spraying with different concentrations of acid (Sifro Glutamic Acid 250 mg.L⁻¹ and potassium element 600 mg.L⁻¹ and Glutamic 250 mg.L⁻¹ + potassium 600 mg.L⁻¹) on the yield and active compounds of roselle genotype. This study used a randomized complete block design (RCBD) within the factorial experiment system with three replicates, each replicate containing 16 experimental units, each experimental unit area 2*2 m, for a total of 48 experimental units. The data were analyzed to determine the effect of genotype, concentration of glutamic acid, potassium and the interaction between them, with the exception of the active compounds, which were analyzed in duplicate. The results of the statistical analysis showed significant differences in all the studied traits: The genotype Black Hit was excelled through the statistical analysis of most of the results of the studied traits, including the traits of the yield and its components, including dry weight per plant (482.15) g plant⁻¹, number of nuts.plant⁻¹ (148.16) nuts .plant⁻¹, the number of seeds per nut (25.54) seeds . nut⁻¹, the weight of 1000 seeds (41.08) g seeds, trait seed yield (899.89) kg ha⁻¹, and trait sepals leaf yield (720.51) kg ha⁻¹. As for the content of sepals leaves and active compounds, the genotype Black Hit was excelled and recorded the highest value for the active substances Quercetin (19.79) ppm and Ferulic acid (13.74) ppm and Chlorogenic acid (15.64) ppm and Caffeic acid (14.35) ppm and Rutin (26.79) ppm and Gallic acid (36.16) ppm, the combination of Glutamic acid 250 mm.L⁻¹ + Potassium element 600 mm.L⁻¹ was significantly excelled in all traits in the number of nuts.plant⁻¹ (149.58) nuts.plant⁻¹ and the fresh weight of one nut (11.37) g nut⁻¹ and the number of seeds per nut (26.92) nut seeds and the dry weight of one nut (5.54) g nut⁻¹ and the weight of 1000 seeds (45.43) g and the seed yield trait (1003.21) kg ha⁻¹ and the sepals leaf yield trait (800.37) kg ha⁻¹ Quercetin and Ferulic acid and Chlorogenic acid Caffeic acid, Rutin and Gallic acid (21.61, 15.37, 17.21, 16.88, 28.71, 37.79), as for the interaction between the combination of amino acid and potassium element (250+600 mg.L⁻¹) with the genetic structure (Black Hit) recorded the highest values in most of the studied traits in the yield and the content of the sepals leaves of active compounds, while the genetic structure (Areeb with the combination of glutamic 250 mg.L⁻¹ + potassium element 600 mg.L⁻¹) was excelled in the trait of weight of 1000 grains.

Keywords. Roselle plant, Genotype, Glutamic, Potassium.

1. Introduction

The roselle plant *Hibiscus sabdariffa* L. is one of the important plants of the Malvaceae family (Malvaceae) medically and economically, as its cultivation is widely spread in tropical and subtropical regions, including India, Australia, Indonesia and Africa [1]. The global roselle cultivation area reached 1.8 million hectares in 2022, an increase of 2.5% on 2021. China is one of the largest roselle producing countries in the world, with a cultivated area of about 1.1 million hectares, as global roselle production reached about 1.2 million tons in 2022, an increase of 3% over 2021. China is also one of the largest roselle producing countries in the world, as China's roselle production reached about 700 thousand tons, and India comes in second place globally with a production of about 300 thousand tons, according to statistics from [2]. As for the Arab world, the area of roselle cultivation is about 2 million acres, equivalent to 8,000 square kilometers, and Egypt and the Gulf countries are at the forefront of the Arab countries. The Arab world's production of roselle is about 1 million tons annually [2]. The area planted with roselle in Iraq is about 400 thousand acres, equivalent to 1,600 square kilometers. Iraq's production rate of roselle is about 200 thousand tons annually [3]. Its medical importance is completed by the fact that its cup leaves contain high levels of Hibiscin glycoside. Roselle also contains (Hibsin glycoside), which plays a major role in reducing blood viscosity, strengthening the heartbeat, and calming the nerves [4]. In addition to citric acid, malic acid and protocatechuic acid, which is an antioxidant for its effective role in treating many cancerous diseases [5]. The roselle plant has several names in the Arab countries. In Iraq, it is called roselle, after the Roselle region known for its production in India. The use of old and traditional methods in agriculture, production and extraction of active compounds when cultivating this plant has led to a deterioration in production per unit area. Therefore, modern methods must be used in agriculture, including spraying the plant with nutrients and introducing modern varieties to obtain the highest production. Spraying potassium in the form of potassium sulphate on roselle plants led to an increase in the productivity index, and potassium has a significant effect on the number of fruits [6]. Glutamic acid is one of the amino acids that contribute to building proteins in the plant. When glutamic is sprayed on the plant, it leads to a decrease in the content of proline and antioxidant enzymes, thus increasing the plant's ability to absorb and balance essential elements. It has an important role in transporting nitrogen between the roots, leaves, fruits and other parts of the plant. Glutamic acid is converted into alpha-ketoglutaric acid, which enters the Krebs cycle and contributes to energy production and the production of intermediate compounds for various biosynthetic processes [7]. Glutamic is also considered a buffer solution for the cytoplasm of cells, which improves the process of opening and closing the stomata and a source of carbon and affects the construction and formation of the chlorophyll molecule, which affects the increase in the rate of carbon assimilation of the plant and thus increases the construction of carbohydrates, in addition to helping to maintain the required osmotic pressure in the cells and protect the plant in the event of an increase in salt concentration and reduce plant stresses [8]. Genetic structures are one of the important factors that have a direct impact on productivity and quality traits, and the reason for this is their ability to adapt to various surrounding environmental conditions. Many recent scientific studies and research have proven that the interaction between environmental conditions, genotype, and balanced nutrient management has a significant impact on improving production in terms of quantity and quality [9].

2. Materials and Methods

The experiment was conducted during the summer agricultural season of 2023 in the village of Sadr al-Nahr , Al-Hawija District , Kirkuk province, which is located at point G P S (L E 84038 19647), in a soil with a silty clay texture, with the aim of studying the effect of spraying with

different concentrations of potassium and glutamic acid on the growth, yield, and active compounds of genotype of roselle. A sample was taken randomly from the surface soil 0-30 cm before planting and air-dried, after which the chemical and physical properties were estimated in the laboratory of the Department of Soil and Water Resources - College of Agriculture - Tikrit University, the factors studied: The first factor - four genotype of the roselle plant, which are (black hit, red hit, Areeb, Sudan 3) which were obtained from the Department of Field Crops in the College of Agriculture, Tikrit University, the second factor - spraying with potassium and glutamic as follows: The first treatment (control) distilled water and the third treatment (spraying with potassium 600 mg/L) and the third treatment (spraying with glutamic 250 mg/L) and the fourth treatment (spraying with potassium 600 mg/L + spraying with glutamic 250 mg/L) The spraying process was carried out when the plant reached the age of four leaves or a height of 10 cm and in the early morning.

The following characteristics were studied:

- Number of nuts.plant⁻¹ (nut.plant⁻¹) and the number of nuts set in six plants from the experimental unit for each replicate was calculated, then the average number of nuts.plant⁻¹ was taken.
- Weight of fresh nuts (g.plant⁻¹): The weight of five nuts was calculated (to increase the accuracy of the weight) taken randomly from each of the marked plants for each experimental unit and then the average was calculated.
- Number of seeds in one nut (nut seed⁻¹): The number of seeds contained in each nut was calculated, five nuts were taken from the specified plants in each experimental unit and then the average number of seeds per nut was taken.
- Weight of 1000 seeds (g.seed⁻¹): A thousand seeds were counted from the marked plants in the experimental unit and then weighed using a sensitive balance.
- Seed yield (kg ha⁻¹): The weight of the seed yield for one experimental unit was calculated using a sensitive balance and the following equation was applied to extract the seed yield per hectare.

$$(\text{Seed yield of experimental unit}) / (\text{Seed yield (ha)}) = (\text{Experimental unit area (4m)}) / (\text{Area per hectare (10000 m)})$$

Content of the sepals leaves of some active compounds: Some active compounds were extracted from the dry sepals leaves of the roselle plant and these compounds are.

(Ferulic acid, Quercetin, Gallic acid, Rutin, Caffeic, and Chlorogenic) were the active chemicals extracted from the sepal leaves of the roselle plant using an aqueous extraction technique. This procedure was executed using the approach of [10], by weighing one gram of dried sepal leaves from each experiment treatment and soaking it for three hours in fifty milliliters of boiling water (100 °C). Filter sheets No. 1 were then used to filter the extract once it had cooled (1). In order to perform measurements on the filtrate using the HPLC apparatus, it was gathered and put in firmly sealed glass tubes containing the extract of the active ingredient. The White Fields Company performed measurements on the active chemicals. Using a fully randomized block design, the experiment was examined as a factorial (D.B.C.R). Duncan's multiple range test was used to compare the averages at a probability threshold of 5% [11].

3. Results and Discussion

3.1. Number of nuts.plant⁻¹ (nut plant⁻¹)

The results of Table (1) showed significant differences between the genotype, as the plants of the genotype Black Hit excelled, as they recorded the highest average for the trait (148.16) nuts plant⁻¹ compared to the plants of the genotype Sudan3, which gave the lowest average of (131.18) nuts plant⁻¹. The results of Table (1) showed a significant effect of spraying the plants with different concentrations of the amino acid glutamic acid and the element potassium on the trait. Spraying the plants with the amino acid glutamic acid and the element potassium led to significant differences in achieving the highest average for the trait of (149.58) nuts plant⁻¹ compared to the lowest average for the trait when treated with the control sprayed with water only, which amounted to (123.36) nuts plant⁻¹. It is noted in the same table that the plants of the genotype Black Hit with the fourth treatment, glutamic and potassium, gave the highest average for the trait of (161.80) nut plant⁻¹, as the plants of the genotype Sudan 3 sprayed with water recorded the lowest average for the trait (115.23) nut plant⁻¹.

Table 1. Effect of genotype and spraying with different concentrations of glutamic acid and potassium on the number of nuts.plant⁻¹ trait.

Genotype / Concentrations	Black Hit	Sudan 3	Red Hit	Areeb	Average
control	131.70i	115.23k	124.30j	122.23j	123.36d
Potassium 600 mg.L ⁻¹	151.03b	135.10gh	143.63de	138.26f	142.36b
Glutamic 250 mg.L ⁻¹	148.13c	132.23hi	139.90f	134.16gh	138.60c
Potassium 600 mg.L ⁻¹ + Glutamic 250 mg.L ⁻¹	161.80a	142.16e	149.30bc	145.06d	149.58a
Average	148.16a	131.18d	139.28b	134.93c	

3.2. Fresh Nut Weight (g.plant⁻¹)

The results of Table (2) showed significant differences between the plants of the genotype, as the plants of the genotype Red Hit excelled and recorded the highest average for the trait (10.73) g.plant⁻¹, while the plants of the genotype Hit Black gave the lowest average for the trait, reaching (9.80) nuts.plant⁻¹. The reason may be due to the variation in genotype and the ability to respond Torres-Moran. The results of Table (2) also indicate the presence of a significant effect when spraying roselle with concentrations of amino acid and potassium. Spraying the treatment with amino acid and potassium showed significant differences in the average of the trait, reaching (11.37) nuts.plant⁻¹, compared to the lowest average in the control treatment sprayed with water, which reached (8.57) nuts.plant⁻¹. The excelled may be due to the role of the combination of amino acid and potassium, which works to activate the plants by increasing the level of [12]. Table (2) shows that the plants of the red Hit genotype with the fourth treatment of glutamic and potassium gave the highest average for the trait, which was (12.30) nuts/plant⁻¹, while the plants of the black Hit genotype sprayed with water recorded the lowest average for the trait, which was (8.40) nuts.plant⁻¹.

Table 2. Effect of genotypes and spraying with different concentrations of potassium and glutamic on the trait of fresh weight of one nut (g. plant⁻¹).

Genotype / Concentrations	Black Hit	Sudan 3	Red Hit	Areeb	Average
control	8.40i	8.40i	8.60hi	8.90h	8.57d
Potassium 600 mg.L ⁻¹	10.30d-f	10.10fg	11.60b	10.90c	10.72b
Glutamic 250 mg.L ⁻¹	9.90g	9.95g	10.45de	10.20e-g	10.12c
Potassium 600 mg.L ⁻¹ + Glutamic 250 mg.L ⁻¹	10.60cd	10.90c	12.30a	11.70b	11.37a
Average	9.80c	9.83c	10.73a	10.42B	

3.3. Number of Seeds in One Nut (nut seed⁻¹)

The results of Table (3) indicate the presence of significant differences between the genotype, as the plants of the genotype Black Hit outperformed with the highest average for the trait, which amounted to (54.25) nut seeds⁻¹, while the plants of the genotype Sudan 3 gave the lowest average for the trait, which amounted to (22.50) nut seeds⁻¹. The reason for the increase in the number of seeds in one nut may be due to the genetic nature of the variety and in response to nutrition and environmental conditions specific to growth, Atta et al. (3201). The results showed significant differences when spraying plants with concentrations of glutamic acid and potassium in the trait. It was found that spraying plants with glutamic acid and potassium resulted in significant differences in the averages of this trait, which amounted to (26.92) nut seeds⁻¹ compared to the control treatment that was sprayed with water only, which gave the lowest average of (20.08) nut seeds⁻¹. This may be attributed to the role of glutamic acid and potassium in building chloroplasts, which have an important role in increasing the process of carbon metabolism in the leaves, which increases their products of carbohydrates, proteins and nutrients, then transferring these products, elements and dry materials from the leaves to their storage place in the reproductive parts represented by the nut and the formed seeds. The glutamic acid and potassium also have an important role in stimulating physiological processes in the flowering stages, which increases the amount of pollen grains and reduces growth inhibitors affecting the growth and division of cells in the plant, which increases From the amounts of pollen grains formed in the nut. The same table also showed that the plants of the genotype Black Hit with the fourth treatment spraying with the amino acid glutamic acid and the element potassium gave the highest average for the trait, reaching (29.98) seeds nut⁻¹ compared to the plants of the genotype Sudan3 sprayed with water only, which had the lowest average for the trait, reaching (18.83) seeds nut⁻¹.

Table 3. Effect of genotype and spraying with different concentrations of potassium and glutamic acid on the trait of the number of seeds in one nut with a nut seed⁻¹.

Genotype / Concentrations	Black Hit	Sudan 3	Red Hit	Areeb	Average
control	21.40f-g	18.83h	20.50gh	19.60h	20.08d
Potassium 600 mg.L ⁻¹	23.50d-f	23.80d-f	22.60e-g	24.70c-e	23.65c
Glutamic 250 mg.L ⁻¹	27.30a-d	23.30d-g	23.43d-f	26.26b-d	25.07b
Potassium 600 mg.L ⁻¹ + Glutamic 250 mg.L ⁻¹	29.98a	24.10d-f	28.20ab	25.40b-e	26.92a
Average	25.54a	22.50c	23.68bc	23.99b	

3.4. Weight of 1000 Seeds (g seed^{-1})

The results of Table (4) indicate that there are significant differences between the compositions, as the genotype Areeb was excelled and recorded the highest average for the trait, reaching $(44.04) \text{ g seed}^{-1}$, while the genotype Black Hit gave the lowest average for the trait, reaching $(41.08) \text{ g seed}^{-1}$. The variation of the varieties in the trait of weight of 1000 seeds indicates differences in the amount of dry matter accumulated in different parts of the plant between the different compositions. This variation may be due to genetic factors between the genotype or the adaptation of one of the genotype to environmental conditions that affect plant growth [12]. The treatment sprayed with amino acid and potassium led to a significant increase in the trait, recording the highest rate for the trait, reaching $(45.43) \text{ g seed}^{-1}$, compared to the lowest averages in the control treatment sprayed with water, reaching (38.89) . 1 gm seed, and this may be attributed to an increase in net photosynthesis rates, or the variation in the response of genotype to the effect of amino acid and potassium in the stage that led to an increase in the vegetative group is due to the net photosynthesis rates that increase the weight of 1000 seeds [13]. As for the bi-interaction between the genotype, amino acid and potassium, the plants of the Areeb genotype excelled and recorded the highest rate at the fourth addition, reaching $(48.13) \text{ gm seed}^{-1}$, as the control treatment with the plants of the Black Heat genotype gave the lowest average for the trait $(37.37) \text{ gm seed}^{-1}$.

Table 4. The effect of genotype and spraying with different concentrations of potassium and glutamic acid on the trait of the weight of 1000 seeds (gm seed^{-1}).

Genotype / Concentrations	Black Hit	Sudan 3	Red Hit	Areeb	Average
Control	37.37i	38.70hi	39.12hi	40.40gh	38.89c
Potassium 600 mg.L^{-1}	41.90e-g	43.1c-f	45.25bc	42.56d-g	43.20b
Glutamic 250 mg.L^{-1}	41.15f-h	43.00c-f	45.00b-d	45.10b-d	43.56b
Potassium 600 mg.L^{-1} + Glutamic 250 mg.L^{-1}	43.90b-e	45.80b	43.89b-e	48.13a	45.43a
Average	41.08c	42.65b	43.31ab	44.04a	

3.5. The Effect of Study Factors on the Active Compounds of Roselle Plant

3.5.1. Quercetin (ppm)

The results of Table (5) In addition, there were notable genotype differences: plants with the genotype Black Hit performed better than those with the genotype Areeb, recording the highest trait average of 19.79 ppm, while plants with the genotype Black Hit recorded the lowest trait average of 19.23 ppm. These results are presented in Table 5, which demonstrates the significant effects of study factors and bilateral interaction in increasing the quercetin trait. The trait reached $(21.61) \text{ ppm}$ when the spraying treatment including potassium and amino acid combined to give superiority. The phenotypic average for the addition-free treatment was the lowest, coming in at 16.57 ppm. The plants of the genotype Black Hit with glutamic and potassium element were superior in the bilateral interaction between the genotype and the amino acid and potassium element, as evidenced by the highest trait value of 21.95 ppm that they recorded, whereas the plants of the genotype Areeb with control treatment (without addition) had the lowest trait value of 16.42 ppm.

Table 5. Effect of genotype and spraying with different concentrations of potassium and glutamic acid on the trait of quercefin in the sepals leaves.

Genotype / Concentrations	Black Hit	Sudan 3	Red Hit	Areeb	Average
Control	16.70m	16.54q	16.62n	16.42p	16.57d
Potassium 600 mg.L ⁻¹	19.68i	19.25k	19.46j	19.10l	19.37c
Glutamic 250 mg.L ⁻¹	20.82e	20.32g	20.67f	20.14h	20.49b
Potassium 600 mg.L ⁻¹ + Glutamic 250 mg.L ⁻¹	21.95a	21.53c	21.74b	21.25d	21.61a
average	19.79a	19.41c	19.62b	19.23d	

3.5.2. Ferulicacid (ppm)

The genotype Black Hit plants recorded a significant superiority with the highest average of the trait, which amounted to 13.74 ppm, compared to the genotype Areeb plants, which gave the lowest average of the trait, which amounted to 13.32 ppm. The results of Table (6) demonstrated the significant effects of the study factors and the binary combination in increasing the rates of the trait. The application of potassium and the amino acid glutamic by spraying resulted in a higher rate of the trait, with the addition of glutamic and potassium recording the highest rate of the trait at (15.37) ppm. and there was a notable difference in the control treatment, which was 10.68 ppm. Regarding the bi-interaction of genotype, amino acid, and potassium, the Black Hit genotype plants outperformed the fourth treatment, exhibiting the highest trait rate of 15.68 ppm, while the Areeb genotype plants in the control treatment displayed the lowest trait average of 10.55 ppm.

Table 6. Effect of genotype and spraying with different concentrations of potassium and glutamic acid on the trait (Ferulicacid) ferulic in the sepals leaves. ppm.

Genotype / Concentrations	Black Hit	Sudan 3	Red Hit	Areeb	Average
Control	10.82j	10.64jk	10.73jk	10.55k	10.68d
Potassium 600 mg.L ⁻¹	13.56h	13.26i	13.44hi	13.30i	13.39c
Glutamic 250 mg.L ⁻¹	14.89e	14.57f	14.74e	14.38g	14.64b
Potassium 600 mg.L ⁻¹ + Glutamic 250 mg.L ⁻¹	15.68a	15.27c	15.47b	15.07d	15.37a
Average	13.74a	13.43c	13.43b	13.32d	

3.5.3. Chlorogenic Acid (ppm)

Table 7's results demonstrated the significant effects of the binary combination and the study factors on raising the rates of chlorogenic acid. The plants exhibiting the genetic combination Black Hit and Red Hit were significantly better than the plants exhibiting the genetic combination Areeb, which exhibited the lowest average of the trait at (14.63, 15.47) ppm. The plants exhibiting the genetic combination Black Hit and Red Hit gave the highest rate of the trait, reaching (15.64) ppm. The rate of chlorogenic acid increased in the spraying treatment with glutamic acid and potassium as well. The maximum rate was recorded when adding (glutamic 250 mg.L⁻¹ + potassium 600 mg.L⁻¹), reaching 17.21 ppm. The control treatment yielded the

lowest value, 13.23 ppm, indicating a substantial difference as well. The plants of the genotype Black Hit exhibited the highest rate of bi-interaction between the genetic combinations and the amino acid potassium (18.15 ppm) when glutamic 250 mg.L⁻¹ + potassium 600 mg.L⁻¹ was added, whereas the plants in the genotype control treatment yielded the lowest average for the trait (13.09 ppm).

Table 7. Effect of genotype and spraying with different concentrations of potassium and glutamic on the trait (Chlorogehi acid) in the sepals leaves.

Genotype / Concentrations	Black Hit	Sudan 3	Red Hit	Areeb	Average
Control	13.19h	13.50h	13.16h	13.09h	13.23d
Potassium 600 mg.L ⁻¹	14.93ef	14.55g	14.85ef	14.15g	14.62c
Glutamic 250 mg.L ⁻¹	16.32d	15.83d	16.25d	15.23e	15.91b
Potassium 600 mg.L ⁻¹ + Glutamic 250 mg.L ⁻¹	18.15a	17.03c	17.64b	16.04d	17.21a
Average	15.64a	15.22b	15.47a	14.63c	

3.5.4. Caffeic Acid (ppm)

According to Table (8), the study factors and the binary combination had a significant impact on the rate of caffeic acid production. The genotype Black Hit plants outperformed the genotype Areeb plants, whose trait average was the lowest at 13.35 ppm, while the genotype Black Hit plants excelled with the highest trait average of 14.35 ppm. The spraying treatment that included potassium and amino acid increased the rate of caffeic acid, resulting in the greatest rate of the trait (16.88) ppm), whereas the treatment that did not include any additions produced the lowest average of the trait (10.69) ppm). The plants of the genotype Black Hit performed very well in the bi-interaction between the genotype, amino acid, and potassium (glutamic 250 mg.L⁻¹ + potassium 600 mg.L⁻¹) with the highest average of the trait, which amounted to (17.56) ppm. In contrast to the plants with the Areeb genotype and the control treatment, which produced the trait's lowest average of 10.56 ppm.

Table 8. Effect of genotypes and spraying with different concentrations of potassium and glutamic acid on the trait (Caffeic acid) in the sepals leaves.ppm

Genotype / Concentrations	Black Hit	Sudan 3	Red Hit	Areeb	Average
control	10.86k	10.63k	10.71k	10.56k	10.69d
Potassium 600 mg.L ⁻¹	13.63h	12.95i	13.23i	12.09j	12.97c
Glutamic 250 mg.L ⁻¹	15.33e	14.83Fg	15.08ef	14.57g	14.95b
Potassium 600 mg.L ⁻¹ + Glutamic 250 mg.L ⁻¹	17.56a	16.72c	17.03b	16.19d	16.88a
Average	14.35a	13.78c	14.015b	13.35d	

3.6. Rutin (ppm)

The plants with the genotype Black Hit recorded the greatest average for the characteristic, reaching 26.69 ppm, while the plants with the genotype Areeb produced the lowest average, reaching 25.82 ppm. These results demonstrated the substantial impacts of the research parameters. Regarding the application of amino acid spraying and potassium squeezing, the fourth treatment (glutamic 250 mg.L⁻¹ + Potassium 600 mg.L⁻¹) shown exceptional performance, yielding the greatest trait average of (28.71) ppm, whereas the treatment without addition produced the lowest trait average of (23.11) ppm. The use of potassium and amino acid spray therapy resulted in the greatest mean trait value when glutamic 250 mg was added, hence

increasing the trait rate. The plants in the control treatment, which had plants with the Areeb genetic structure, produced the lowest mean for the class, reaching 22.87 ppm. L⁻¹ + potassium 600 mg.L⁻¹, achieving (29.15) ppm.

Table 9. Effect of genetic structures and spraying with different concentrations of potassium and glutamic on the trait rutin in the sepals leaves. ppm.

Genotype / Concentrations	Black Hit	Sudan 3	Red Hit	Areeb	Average
Control	23.42m	23.02o	23.13n	22.87p	23.11d
Potassium 600 mg.L ⁻¹	26.35i	25.84k	26.04j	25.18l	25.85c
Glutamic 250 mg.L ⁻¹	27.83e	27.33e	27.63f	27.03h	27.45b
Potassium 600 mg.L ⁻¹ + Glutamic 250 mg.L ⁻¹	29.15a	28.63c	28.85b	28.21d	28.71a
Average	26.69a	26.20c	26.41b	25.82d	

3.6.1. Gallic Acid (ppm)

According to Duncan's multiple range test, Table (10) shows that the effect of genotype was significant. The plants with the genotype Black Hit demonstrated a significant superiority for the trait, reaching an average of 36.13 ppm, while the plants with the genotype Areeb demonstrated the lowest average, reaching 35.49 ppm. Regarding the spraying of potassium and amino acids, the addition of glutamic acid (250 mg.L⁻¹ + potassium 600 mg.L⁻¹) was important since it produced the greatest trait average (37.79 ppm) as opposed to the treatment in which no addition was applied, which produced the lowest trait average (33.19 ppm). The bi-interaction between genotype, amino acid and potassium, the plants of the genotype Black Hit excelled and recorded the highest rate when adding (Glutamic 250 mg.L⁻¹ + Potassium 600 mg.L⁻¹) reaching (38.23) ppm, while the control treatment with the plants of the genotype Areeb gave the lowest average for the trait reaching (33.04) ppm.

Table 10. Effect of genotype and spraying with different concentrations of potassium and glutamic on the trait gallic in the sepals leaves. ppm.

Genotype / Concentrations	Black Hit	Sudan 3	Red Hit	Areeb	Average
Control	33.34m	33.13o	33.23n	33.04p	33.19d
Potassium 600 mg.L ⁻¹	35.93i	35.46k	35.62j	35.23l	35.56c
Glutamic 250 mg.L ⁻¹	37.03e	36.53g	36.78f	36.26h	36.65b
Potassium 600 mg.L ⁻¹ + Glutamic 250 mg.L ⁻¹	38.23a	37.60c	37.93b	37.42d	37.79a
Average	36.13a	35.68c	35.89b	35.49d	

The results of Tables (5, 6, 7, 8, 9, 10) show that there are significant differences between the genotype of roselle plants, glutamic acid, potassium and the interaction between them. There are significant differences in the highest traits of the active compounds (Quercetin, Ferulic acid, Chlorogenic acid, Caffeic acid, Rutin and Gallic acid). The genotype Black Hit plants recorded the highest averages in all active compounds, and the genotype Areeb plants recorded the lowest averages for the active compounds, which are (19.79- 19.23) (13.74- 13.32) (15.64- 14.63) (14.35- 13.35) (26.69- 25.82) (36.13- 35.49). As for the concentration of glutamic acid and potassium, the sample with the concentration (glutamic 250 mg L⁻¹ + potassium 600 mg L⁻¹) recorded an average in all active compounds, and the control treatment recorded the lowest average (21.61-16.57) (15.37-10.68) (17.21-13.23) (16.88-10.69) (28.71-23.11) (37.72-33.19). As for the interaction between the genotype and concentrations, the plants of the genotype Black Hit

recorded with the concentration treatment (glutamic 250 mg L⁻¹ + potassium 600 mg L⁻¹) an average in all active compounds, while the control treatment with the plants of the genotype Areeb recorded the lowest average for the active compounds (21.95-16.42) (15.68- 10.55) (18.15-13.09) (17.56-10.56) (29.69-22.87) (37.03-33.04).

Conclusions

We can conclude from the results: The genotype Black Hit was excelled through the statistical analysis of most of the results of the studied traits, genotype Black Hit was excelled and recorded the highest value for the active substances Quercetin and Ferulic acid and Chlorogenic acid and Caffeic acid and Rutin and Gallic acid, the combination of Glutamic acid 250 mm.L⁻¹ + Potassium element 600 mm.L⁻¹

References

- [1] Rabo, S.A.; B.S. Mustapha, A.B. Simon and I.L. Hama. 2015. Influence of NP fertilizer on the performance of Roselle (*Hibiscus Sabdariffa* L.) insamaru Zaria. Niger. J. Agric. Food Environ. 11(3): 61-64.
- [2] Food and agriculture organization of the United Nations—FAO(2022). In The Europa directory of international organizations 2021 (pp. 297-305). Routledge.
- [3] Iraqi Ministry of Agriculture, 2023. Statistics of the Ministry of Agriculture, Iraqi Ministry of Agriculture.
- [4] Saad, Shukry Ibrahim. 1988. Medicinal, aromatic and poisonous plants in the Areeb world. Khartoum, Dar Misr for Printing.
- [5] Kilic, C. S.; Aslan, S.; Kartal, M. and Coskun, M. 2011. Fatty acid composition of *Hibiscus trionum* L. (Malvaceae). Rec. Nat. Prod. 5(1): 65-69.
- [6] Al-Hilfy, I. H., Nasseralla, A. Y., & Al-Abodi, H. M. K. (2017). Effect of nitrogenous and phosphorus fertilizers on growth and yield of roselle *Hibiscus sabdariffa* L. Anbar J. Agric Sci, 15, 199-207.
- [7] El-Desouki, Heshmat Soliman. 2008. Fundamentals of Plant Physiology. Mansoura University. Egypt
- [8] Haruna, I. M, H. Y, Ibrahim and S. A. Rahman, (2010) The yield and profitability of roselle (*Hibiscus sabdariffa* L.) at varying poultry manure and nitrogen fertilizer rates in the southern guinea savanna of Nigeria. J. Agric. Tech. 7(3): 605 – 60
- [9] Maes, H., and Dudareva, N. (2016). The shikimate pathway and aromatic amino acids biosynthesis in plants. Annu. Rev. Plant Biol. 63, 73- 105. doi: 10.1146/annurev-arplant-042811-105439.
- [10] Ranganna, S. 1977. Manual Analysis of Fruit And Vegetable Products . Tata mcgraw – Hill publishing Company Limited, New Delhi.
- [11] Al-Rawi, Khashe' Mahmoud and Khalaf Allah Abdul Aziz Mohammed 1980. Design and Analysis of Agricultural Experiments Dar Al-Kutub Printing and Publishing Establishment. College of Agriculture and Forestry, University of Mosul. Mosul Education Press. Republic of Iraq.
- [12] Kandil, A. A., A.E.M.Sharief., S.E.Seadh and D.S.K.Altai.2016. Role of humic acid and amino acids in limiting loss of nitrogen fertilizer and increasing productivity of some wheat cultivars grown under newly reclaimed sandy soil. Int. J. Adv. Res.Biol. Sci. 3(4): 123- 136.
- [13] Elsayed .A.G.(2018).Improving Nutritional Quality of hot pepper (*capsum annua* L.) plant via Foliar Application With Arginine or Tryptophan or Octoben Journal.Applied Sciences .2(4) 1390-1403.

Role of Biofertilization on the Growth and Yield of Strawberry Plants (*Fragaria x ananassa* Duch.) cv.Camarosa

Amina D. Z. Aldabagh¹ and Jassim M. A. Al-A'reji²

^{1,2}Department of Horticulture and Landscape Design, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq.

¹E-mail: amina.ag24@student.uomosul.edu.iq

²E-mail: jassim@uomosul.edu.iq

Abstract. The study was conducted from 1/10/2023 to 1/5/2024 on strawberry seedlings of the Camarosa variety, which were planted in one of the plastic houses belonging to the Department of Horticulture and Garden Engineering / College of Agriculture and Forestry / University of Mosul, with the aim of determining the effect of adding Bio fertilizers, Azotobacter, which were added at three levels (0, 2, 4 g plant⁻¹), and Azospirillum at three levels as well (0, 2, 4 g plant⁻¹), and two levels of mycorrhizal fungi (0, 5 g plant⁻¹) In the concentration of some nutrients in the leaves and some vegetative growth characteristics, including the chlorophyll content in the leaves and the leaf area of Camarosa strawberry. All fertilizers from bacteria and fungi were added at once and distributed randomly. The study was executed according to the design of the Randomized Complete Block Design (RCBD) with three replications and 8 plants per experimental unit. The results were statistically analyzed according to the design used, and the means were compared using the Duncan's multiple range test at a significance level of 0.05. The results showed that the addition of the fertilizer Azotobacter bacteria, Azospirillum bacteria, and mycorrhizal fungi each individually, as well as all the interactions among them, especially the treatment level of 4 g plant⁻¹ of Azotobacter with the treatment of 4 g plant⁻¹ of Azospirillum + 5 g plant⁻¹ of Mycorrhizae fungi, led to a significant increase in the concentrations of nitrogen, phosphorus, and potassium elements, as well as an increase in the chlorophyll content of the leaves and an increase in the leaf area of the plant compared to the control treatment.

Keywords. Strawberry, Mycorrhiza, Azotobacter, Azospirillum.

1. Introduction

Strawberry (*Fragaria x ananassa* Duch) is one of the economically important perennial herbaceous fruits, widely distributed around the world, belonging to the order Rosales, subfamily Rosaideae, and family Rosaceae [1] [2] It is believed that the strawberry's native habitat is North America, but it is also cultivated in Europe, Asia, Africa, and the Americas [3,4]. Some strawberry varieties have the ability to grow in subtropical regions and even up to 70° north of the equator, but they are predominantly found between the latitudes of 15-55°N [5] Globally, strawberries rank fourth in consumption and preference among consumers, following apples,

oranges, and bananas [1] [6] The area cultivated with strawberries worldwide is estimated to be approximately 389,665 hectares, with a production of 9,175,384 tons. China is the largest country in terms of cultivated area and production of strawberries, with a production of about 3,380,478 tons and a cultivated area of 111,132 hectares. The United States follows, with a production of approximately 1,211,090 tons and a cultivated area of 19,919 hectares [7]. As for Iraq, there are no statistics available regarding the cultivated areas and production. In recent years, the use of bio fertilizers containing microorganisms (beneficial bacteria and fungi) has increased. Among the most important bio fertilizers are those containing *Azotobacter* bacteria, which are among the most effective free-living nitrogen fixers. This genus includes several species, the most important and widespread of which is *Azotobacter Chroococcum*. This bacterium promotes plant growth and production by converting nitrogen from its elemental form (N_2) to ammonium (NH_4^+) with the help of the enzyme Nitrogenase [8]. It also decomposes organic matter, produces chelating compounds, and reduces ethylene, and is used in biological control. This bacterium contributes in the root zone by providing protection to the plant from various pathogenic agents present in the soil through its direct and indirect effects by competing with pathogenic organisms for space and nutrients and preventing the pathogen from reaching the infection sites. Additionally, *A. Chroococcum* induces significant changes in the root system, including promoting the formation of lateral roots and increasing the root surface area, which is mainly attributed to its secretion of the auxin IAA. These changes are related to improving water and nutrient absorption by the inoculated plant [9] [10].

The bacteria *Azospirillum* are also used in bio-fertilization, which are considered microorganisms that can live in a symbiotic manner with plant roots [11]. They can be used as a bacterial inoculant that fixes atmospheric nitrogen for plants and secretes certain plant hormones that enhance the growth and development of the root system, thereby increasing the plants' capacity to absorb nutrients such as phosphorus and potassium from the soil [12]. Additionally, mycorrhizal fungi live in a mutualistic relationship with the roots of many plants and play an important role in dissolving insoluble phosphates present in the soil, transferring them through the fungal hyphae to the plant. They also have the capability to increase the absorption of several other nutrients besides phosphorus, along with improving soil structure and increasing plants' resilience to drought, soil salinity, and diseases. Fungi taken from soils infected with these fungi are directly utilized in the inoculation process [13,14].

This study aims to improve the vegetative and fruiting growth of Camarosa strawberry plants, and to determine the suitable concentrations of *Azotobacter* bacteria, *Azospirillum* bacteria, and mycorrhizal fungi that should be added to the soil of these plants to achieve this, due to the lack of similar studies on this variety in the city of Mosul.

2. Materials and Methods

This study was conducted on Camarosa strawberry plants grown in one of the unheated greenhouses belonging to the Department of Horticulture and Landscape Engineering / College of Agriculture and Forestry / University of Mosul during the agricultural season 2023 – 2024, where some physical and chemical properties of the soil were estimated before planting, as shown in Table (1).

Table 1. Some chemical and physical properties of plastic house soil.

Parameter	Unit	Value
Electrical	(dsm. m ⁻¹)	2.71
pH		7.44
Organic	gm kg ⁻¹	6.31

sand	gm kg ⁻¹	617.9
clay	gm kg ⁻¹	143.1
silt	gm kg ⁻¹	240.8
soil		Mixity
Available nitrogen	mg kg ⁻¹	42
Available phosphorus	mg kg ⁻¹	25
Available potassium	mg kg ⁻¹	124

*The analysis was conducted in the central laboratory of the College of Agriculture and Forestry / University of Mosul.

The Camarosa strawberry seedlings were chosen from the nurseries at the College of Agriculture and Forestry at the University of Mosul, ensuring they were nearly uniform in growth. The seedlings were carefully uprooted from the soil, with damaged roots and large leaves removed, leaving just two new leaves intact. Before planting, a compound NPK fertilizer was applied according to recommended guidelines. The fertilizer was spread across the top of the planting beds and thoroughly mixed with the soil, followed by immediate irrigation. Each bed was then covered with black polyethylene, with openings created at the top spaced 25 cm apart for planting. This planting occurred on October 1, 2023. The seedlings were arranged in three rows, with 25 cm between each row and 25 cm between each plant. Fertilization treatments included *Azotobacter* bacteria at three different levels (0, 2, and 4 grams per plant) and *Azospirillum* bacteria at the same three levels, along with mycorrhizal fungi at two levels (0 and 5 grams per plant). These were all added to the soil on September 30, 2023. The study utilized a completely randomized block design (RCBD) with three factors, three replications, and eight plants per experimental unit, leading to a total of 432 plants included in the research. The following characteristics were measured:

- Nitrogen concentration in leaves (%): This was measured using a Micro-Kjeldahl device, following the method described by [15].
- Phosphorus concentration in leaves (%): This was determined using a colorimetric method, with light absorption measured at a wavelength of 882 nanometers using a Spectrophotometer model 303 – PD – APPL, as outlined by [16].
- Potassium concentration in leaves (%): This was assessed using a Flame-photometer, according to the method detailed by Horneck and [17].
- Total chlorophyll content in leaves (mg g⁻¹ fresh weight): The chlorophyll content was estimated using the methods of [18,19].
- Leaf area (cm² per plant): The number of leaves per experimental unit, consisting of 8 plants, was counted, and the total was divided by 8 to find the average number of leaves per plant. The area of a single leaf was calculated using a method of ratios and proportions. Six leaves from each experimental unit were randomly selected, and then twenty leaf discs were taken using a cork borer with a known area of 1 cm². These discs and leaves were dried in an electric oven at 70[°] ± 1 until they reached a stable weight. The dry weight of the discs and leaves, along with the area of the discs, were used to determine the area of a single leaf through the ratio and proportion method. The total leaf area for each plant was then calculated as follows:

$$\text{Total leaf area for the plant} = \text{Area of a single leaf} \times \text{Number of leaves per plant}$$

3. Results and Discussion

3.1. Impact of Azotobacter

The findings presented in Tables (2 – 6) reveal that applying Azotobacter bacteria, especially at a rate of 4 grams per plant, significantly enhanced several key characteristics. This treatment resulted in the highest averages for nitrogen content in the leaves at 2.135%, phosphorus at 0.3504%, potassium at 1.841%, total chlorophyll content at 14.79 mg per gram of fresh weight, and leaf area at 3045.2 cm² per plant. In contrast, the control group exhibited the lowest values for these traits, measuring 1.978%, 0.3109%, 1.682%, 14.03 mg per gram of fresh weight, and 2723.0 cm² per plant, respectively.

Table 2. Effect of Biofertilization with Azotobacter and Azospirillum bacteria and mycorrhizal fungi and their interactions on nitrogen concentration in the leaves (%) of Camarosa strawberry plants*.

Mycorrhiza Fertilizer means	Interaction between Azotobacter and Mycorrhiza	Azospirillum Fertilizer Levels (gm plant ⁻¹)			Mycorrhiza fertilizer levels (gm plant ⁻¹)	Azotobacter Fertilizer Levels (gm plant ⁻¹)	
		4	2	0			
1.7670 b	1.651 e	1.990 hi	1.727 k	1.237 n	0	0	
	1.771 d	2.083 gh	1.860 j	1.370 m		2	
	1.879 c	2.083 gh	1.960 ij	1.593 l		4	
	2.306 b	2.417 bc	2.317 c-e	2.183 fg		0	
2.3452 a	2.339 ab	2.450 ab	2.350 b-d	2.217 ef	5	2	
	2.391 a	2.540 a	2.383 bc	2.250 d-f		4	
	Azotobacter Fertilizer means	2.052 d	1.849 e	1.400 f		0	Interaction between Azospirillum and Mycorrhiza
		2.469 a	2.350 b	2.217 c		5	
1.978 c		2.203 bc	2.022 e	1.710 h	0	Interaction between Azotobacter and Azospirillum	
2.055 b	2.267 b	2.105 d	1.793 g	2			
2.135 a	2.312 a	2.172 cd	1.922 f	4			
		2.261 a	2.099 b	1.808 c	Azospirillum Fertilizer means		

* The averages that share a common letter of the alphabet for each factor and each interaction do not show significant differences when analyzed using the Duncan multiple range test at a 0.05 significance level.

Table 3. Effect of bio-fertilization with Azotobacter and Azospirillum bacteria and mycorrhizal fungi and their interactions on the phosphorus concentration in the leaves (%) of Camarosa strawberry plants *.

Mycorrhiza Fertilizer means	Interaction between Azotobacter and Mycorrhiza	Azospirillum Fertilizer Levels (gm plant ⁻¹)			Mycorrhiza fertilizer levels (gm plant ⁻¹)	Azotobacter Fertilizer Levels (gm plant ⁻¹)
		4	2	0		
0.2709 b	0.2472 d	0.2963 f-i	0.2623 hi	0.1830 j	0	0
	0.2790 c	0.3057 e-h	0.2820 hi	0.2493 i		2
	0.2864 c	0.3213 e-g	0.2860 f-i	0.2520 i		4
	0.3746 b	0.4117 b	0.3753 b-d	0.3367 d-f		0
0.3925 a	0.3887 ab	0.4200 b	0.3920 bc	0.3540 c-e	5	2
	0.4143 a	0.4867 a	0.4017 bc	0.3547 c-e		4
		0.3078 d	0.2768 e	0.2281 f		
Azotobacter Fertilizer means		0.4394 a	0.3897 b	0.3484 c	0	Interaction between Azospirillum and Mycorrhiza
0.3109 b		0.3540 bc	0.3188 cd	0.2598 e	0	Interaction between Azotobacter and Azospirillum
0.3338 a		0.3628 b	0.3370 b-d	0.3017 d	2	
0.3504 a		0.4040 a	0.3438 bc	0.3033 d	4	
		0.3736 a	0.3332 b	0.2883 c	Azospirillum Fertilizer means	

* The averages that share a common letter of the alphabet for each factor and each interaction do not show significant differences when analyzed using the Duncan multiple range test at a 0.05 significance level.

Table 4. Effect of bio-fertilization with Azotobacter and Azospirillum bacteria and mycorrhizal fungi and their interactions on the potassium concentration in the leaves (%) of Camarosa strawberry plants*.

Mycorrhiza Fertilizer means	Interaction between Azotobacter and Mycorrhiza	Azospirillum Fertilizer Levels (gm plant ⁻¹)			Mycorrhiza fertilizer levels (gm plant ⁻¹)	Azotobacter Fertilizer Levels (gm plant ⁻¹)
		4	2	0		
1.467 b	1.421 c	1.590 h-j	1.443 i-l	1.230 l	0	0
	1.461 c	1.617 g-i	1.457 i-l	1.310 kl		2
	1.519 c	1.670 g-i	1.537 i-k	1.350 j-l		4
2.055	1.942	2.197	1.943	1.687	5	0

a	b	bc	d-f	g-i		
	2.060	2.313	2.063	1.803		2
	ab	ab	c-e	f-h		
	2.162	2.477	2.150	1.860		4
	a	a	b-d	e-g		
		1.626	1.479	1.297	0	Interaction between
		d	e	f		Azospirillum and
Azotobacter Fertilizer means		2.329	2.052	1.783	5	Mycorrhiza
		a	b	c		
1.682		1.893	1.693	1.458	0	Interaction between
b		bc	d-f	g		Azotobacter
1.761		1.965	1.760	1.557	2	and
ab		ab	c-e	fg		Azospirillum
1.841		2.073	1.843	1.605	4	
a		a	b-d	e-g		
		1.977	1.766	1.540		
		a	b	c		Azospirillum Fertilizer means

* The averages that share a common letter of the alphabet for each factor and each interaction do not show significant differences when analyzed using the Duncan multiple range test at a 0.05 significance level.

Table 5. Effect of biofertilization with Azotobacter and Azospirillum bacteria and mycorrhizal fungi and their interactions on the total chlorophyll content in the leaves (mg g⁻¹ fresh weight) of the Camarosa strawberry plant *.

Mycorrhiza Fertilizer means	Interaction between Azotobacter and Mycorrhiza	Azospirillum Fertilizer Levels (gm plant ⁻¹)			Mycorrhiza fertilizer levels (gm plant ⁻¹)	Azotobacter Fertilizer Levels (gm plant ⁻¹)
		4	2	0		
	12.92	14.18	12.98	11.60		0
	d	d-f	f-h	i		
13.263	13.22	14.32	13.33	12.02	0	2
b	cd	d-f	e-g	hi		
	13.65	14.44	13.87	12.63		4
	c	de	d-g	g-i		
	15.14	16.02	14.88	14.53		0
	b	bc	cd	de		
15.542	15.55	17.09	15.02	14.55	5	2
a	ab	ab	cd	de		
	15.93	17.87	15.15	14.76		4
	a	a	cd	cd		
		14.31	13.39	12.09	0	Interaction between
		b	c	d		Azospirillum and
Azotobacter Fertilizer means		16.99	15.02	14.61	5	Mycorrhiza
		a	b	b		
14.03		15.10	13.93	13.06	0	Interaction between
b		bc	d-f	f		Azotobacter
14.39		15.70	14.17	13.28	2	and
ab		ab	de	ef		Azospirillum
14.79		16.15	14.51	13.70	4	
a		a	cd	d-f		
		15.65	14.21	13.35		Azospirillum Fertilizer means

Mycorrhiza Fertilizer means	Interaction between Azotobacter and Mycorrhiza	Azospirillum Fertilizer Levels (gm plant ⁻¹)			Mycorrhiza fertilizer levels (gm plant ⁻¹)	Azotobacter Fertilizer Levels (gm plant ⁻¹)
		4	2	0		
		a	b	c		

* The averages that share a common letter of the alphabet for each factor and each interaction do not show significant differences when analyzed using the Duncan multiple range test at a 0.05 significance level.

Table 6. Effect of biofertilization with Azotobacter and Azospirillum bacteria and mycorrhizal fungi and their interactions on leaf area (cm² plant⁻¹) of Camarosa strawberry plants*.

Mycorrhiza Fertilizer means	Interaction between Azotobacter and Mycorrhiza	Azospirillum Fertilizer Levels (gm plant ⁻¹)			Mycorrhiza fertilizer levels (gm plant ⁻¹)	Azotobacter Fertilizer Levels (gm plant ⁻¹)
		4	2	0		
	2329.4	2727.6	2574.8	1685.8		0
	c	e-g	fg	h		
2559.6	2642.8	2862.3	2613.6	2452.7	0	2
b	b	d-g	e-g	g		
	2706.8	2915.6	2675.2	2529.6		4
	b	c-g	e-g	g		
	3116.7	3421.8	2849.8	3078.6		0
	a	a-c	d-g	b-f		
3216.8	3150.2	3460.8	2869.9	3120.1	5	2
a	a	ab	d-g	a-f		
	3383.6	3616.0	3379.0	3155.8		4
	a	a	a-d	a-e		
		2835.1	2621.2	2222.7	0	Interaction between
		bc	c	d		Azospirillum and
Azotobacter Fertilizer means		3499.5	3032.9	3118.1	5	Mycorrhiza
	a	a	b	b		
	2723.0	3074.7	2712.3	2382.2	0	Interaction between
b	a-c	cd	d			Azotobacter
2896.5	ab	ab	c	c	2	and
3045.2	a	a	a-c	bc	4	Azospirillum
		3167.3	2827.0	2670.4		
		a	b	b		Azospirillum Fertilizer means

* The averages that share a common letter of the alphabet for each factor and each interaction do not show significant differences when analyzed using the Duncan multiple range test at a 0.05 significance level.

3.2. Effect of Azospirillum Bacteria

The results mentioned in Tables (2-6) showed that fertilization with Azospirillum bacteria, especially at the rate of 4 g plant⁻¹, led to a significant increase in the studied traits, which yielded the highest averages for nitrogen content in the leaves at (2.261%), phosphorus in the leaves (0.2883%), potassium in the leaves (1.977%), total chlorophyll content in the leaves (15.65 mg g⁻¹ fresh weight), and leaf area per plant (3167.3 cm² plant⁻¹) compared to the control treatment,

which provided the lowest values for these traits, measuring 1.808%, 0.2883%, 1.540%, 13.35 mg g⁻¹ fresh weight, and 2670.4 cm² plant⁻¹, respectively.

3.3. The Effect of mycorrhizal fungi

It is observed from the results shown in tables (2-6) that fertilization with mycorrhizal fungi at a rate of 5 g per plant led to a significant increase in the studied traits, which provided the highest averages for nitrogen in the leaves, reaching (2.3452%), phosphorus in the leaves (0.3925%), potassium in the leaves (2.055%), total chlorophyll content in the leaves (15.542 mg per g fresh weight), and leaf area per plant (3216.8 cm² per plant) compared to the control treatment, which gave the lowest values for these traits, measuring 1.7670%, 0.2709%, 1.467%, 13.263 mg per g fresh weight, and 2559.6 cm² per plant, respectively.

3.4. The Effect of Interactions Among the Studied Factors

The results (Tables 2-6) indicate that all the binary interactions and the ternary interaction among Azotobacter, Azospirillum, and mycorrhizal fungi significantly affected all the studied traits. The highest levels of these interactions (4 g plant⁻¹ and 4 g plant⁻¹ and 5 g plant⁻¹) yielded the highest values for the studied traits, especially the ternary interaction, which resulted in nitrogen values in the leaves (2.540%), phosphorus in the leaves (0.4867%), potassium in the leaves (2.477%), total chlorophyll in the leaves (17.87 mg g⁻¹ fresh weight), and leaf area per plant (3616.0 cm plant⁻¹), compared to the control treatment, which gave the lowest values for these traits, recorded at 1.237%, 0.1830%, 1.230%, 11.60 mg g⁻¹ fresh weight, and 1685.8 cm plant⁻¹. This is attributed to the synergistic effect of both Azotobacter and Azospirillum and mycorrhizal fungi in enhancing vegetative and root growth traits.

3.5. The Effect of Bio-Fertilization on the Leaf Content of Nutrients, Total Chlorophyll, and Leaf Area

The results mentioned in tables (2-6) indicate that bio-fertilization with both types of bacteria and mycorrhizal fungi led to a significant increase in the concentration of nitrogen, phosphorus, and potassium elements in the leaves. This aligns with the findings of [20-23]. This may be attributed to the increased availability of these elements in the soil, as Azotobacter and Azospirillum bacteria play an effective role in accumulating nutrients and converting them into readily available forms, facilitating their absorption by plants through several biological processes [24]. This may also affect the development of a robust root system that enhances the efficiency of nutrient absorption, including nitrogen, phosphorus, and potassium. Furthermore, inoculation with these bacteria increases nitrogenous compounds and the activity of the enzyme Nitrogenase. [14,25] also mentioned that these two types of bacteria have the ability to produce hormones such as auxins, gibberellins, and cytokinin's, which may enhance root growth in plants, thereby increasing nutrient absorption from the soil.

The fertilization with Mycorrhizae fungi may lead to an increase in the root surface area, which has a significant impact on enhancing the absorption of mineral elements. The roots serve as the pathway through which these elements travel to the rest of the plant. The fungal hyphae have the ability to penetrate between soil particles and reach areas that root hairs cannot access, thus absorbing a larger quantity of nutrients [26]. The increase in the total chlorophyll content and leaf area can be attributed to the increased concentration of certain nutrients in the leaves, including nitrogen, phosphorus, and potassium. Nitrogen contributes to the formation of this pigment as it is involved in the structure of Porphyrins, which are part of this pigment's composition. Additionally, phosphorus helps provide the energy necessary for building this pigment, as it is involved in the formation of energy-carrying compounds such as ATP and ADP.

Furthermore, potassium activates a number of enzymes that may play a role in the synthesis of chlorophyll [27]. This may lead to an increase in the manufactured materials in the leaves, which are carbohydrates, and a portion of these may be used in the process of photosynthesis, thereby enhancing the vegetative growth of the plant.

Conclusions

The study shows that adding *Azotobacter* bacteria, *Azospirillum* bacteria, and mycorrhizal fungi to the Camarosa strawberry plant, especially at high levels (4 g plant⁻¹ *Azotobacter*, 4 g plant⁻¹ *Azospirillum*, and 5 g plant⁻¹ mycorrhiza), especially when added together, has greatly improved the plants' root and leaf growth, which may have a positive effect on their yield.

References

- [1] Al-Saidi, Ibrahim Hassan Muhammad (2015). Production of Strawberry (Strawberry - Friz) Dijla Library for Printing, Publishing, and Distribution, Mosul, Iraq.
- [2] Lateef, M.A. A.M. Noori, Y. M Saleh, D.K.A. Al-Taey.2021. The effect of foliar spraying with salicylic acid and calcium chloride on the growth, yield, and storage traits of two Strawberry cultivars, *Fragaria* × *ananassa* Duch. Int. J. Agricult. Stat. Sci.,17(2):611-615. DocID: <https://connectjournals.com/03899.2021.17.611>
- [3] Jamal Uddin, A. F. M., Hossan, M. J., Islam, M. S., Ahsan, M. K., & Mehraj, H. (2012). Strawberry growth and yield responses to gibberellic acid concentrations. *Journal of Experimental Biosciences*, 3(2), 51-56.
- [4] Habib, Hussein Mahdi, and Ali Abdul Hadi Al-Faraon (2013). Strawberry, Ministry of Agriculture, Horticulture Department, Al-Jazeera Press, page38, Iraq.
- [5] Ceausescu, E.; R. Vieru and A. Teodorescu (1982). Culture și valorificarea capsunului. Ed. Ceres București:p.195.
- [6] Sonam and S. K. Singh (2018). A review on impact of GA3 application on strawberry cultivation. *Intern. J.Chem. Studies*, 6(3):1382-1387.
- [7] FAO(2023).The Worlds production and Harvested Areas of Strawberries From 1980 to 2021.Food and Agriculture Organization of the United Nations,2023.
- [8] AL-Taey, D. K. A., S. S. M. AL-Azawi, M. J. H. AL-Shareefi, and A. R. AL-Tawaha. (2018). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (*Lactuca sativa* var. Parris Island) *Research on Crops*.19 (3) : 441-449. DOI : 10.31830/2348-7542.2018.0001.14
- [9] Gomare, S. ; M.Mese and Y. Shetkar (2013). Isolation of *Azotobacter* and cost effective production of biofertilizer . *India J. Appli. Res.*, 3(5) : 54 – 56 .
- [10] Kaur , I. (2014). Effect of nitrogen fixing bacteria *Azotobacter* and *Azospirillum* on the growth of *rosa polyantha*. *Inter. J. Emerg. Trend Sci. & Tech.*, 1(7): 1073 – 1080 .
- [11] Okon, Y. ; E. Falik ; S. Sarig ; E. Yahelom and S. Tal (1988). Plant growth promoting effect of *Azospirillum* In : Both, H. De Bagen , F.F. and Newton W. E.(eds). *Nitrogen Fixation , Hundred Year after*. pp: 741-746 .
- [12] Romos , H. J. ; L. D. Rancoto- Maccari ; E. M. Souza ; J. R. Soares – Romos ; M. Hungria and F.O. Pedrosa (2002). Monitoring *Azospirillum* wheat interaction using the *gfp* and *gus* genes constitutively expressed from a new broad – hostrange vector.*J.Biotech.*, 97 : 243 – 252 .
- [13] Alwan, Jasem Mohammed and Raida Ismail Abdullah Al-Hamdani (2012). *Organic Agriculture and the Environment*. Dar Al-Kutub for Printing and Publishing. University of Mosul. Iraq.
- [14] AL-Taey , D.K.A. , Alftlawi, S. S. and Sahib, M. R.(2022). EFFECT OF ZYTONIC-M, PALM WASTES COMPOST AND NPK ON THEGROWTH AND YIELD OF TOMATO UNDER SALT STRESSCONDITIONS. *Int. J. Agricult. Stat. Sci.*, 18(2): 829-836. DocID: <https://connectjournals.com/03899.2022.18.829>
- [15] Black, C.A. (1965). *Methods of Soil Analysis*. Part 2. Chemical and Microbiological Properties. Amer. Soc. Agron., Inc. Madison, Wisconsin,USA.

- [16] Matt. J. (1970). Colorimetric determination of phosphorus in soil and plant material with ascorbic acid. *Soil Sci.*, 109 : 214 – 220.
- [17] Horneck, D. A., and D. Hanson(1998). Determination of Potassium and Sodium by Flame Emission Spectrophotometry. pp. 153-155. In: Kalra, Y. P. (ed.). *Handbook of Reference Methods for Plant Analysis*. Soil and Plant Analysis Council, Inc. CRC Press. FL. USA. pp. 287.
- [18] Mackinney, G. (1941). Absorption of light by chlorophyll solutions. *Journal of biological chemistry*, 140(2), 315-322
- [19] Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant physiology*, 24(1), 1.
- [20] Tomic, Jelena M., Jasminka M. Milivojevic, and Marijana I. Pesakovic. "The response to bacterial inoculation is cultivar-related in strawberries." *Turkish Journal of Agriculture and Forestry* 39.2 (2015): 332-341.
- [21] Apu, S. C., Biswas, M. S., Bhuiyan, M. A. B., Gomasta, J., Easmin, S., & Kayesh, E. (2022). Effect of organic amendments and arbuscular mycorrhizal fungi on plant growth, yield and quality of strawberry. *Ann. Bangladesh Agric*, 26(2), 71-82.
- [22] Lachinani, A., Tabatabaei, S. J., Bostani, A., Abdossi, V., & Rezaee, S(2023). Effects of Mycorrhizal (*Rhizophagus irregularis*) and *Trichoderma Harzianum* Fungus on Strawberry cv. Camarosa Quality under Different Selenium Levels.
- [23] Demir, S., Durak, E. D., Güneş, H., Boyno, G., Mulet, J. M., Rezaee Danesh, Y., & Porcel, R. (2023). Biological control of three fungal diseases in strawberry (*Fragaria× ananassa*) with arbuscular mycorrhizal fungi. *Agronomy*, 13(9), 2439.
- [24] Singh, S. R., Zargar, M. Y., Singh, U., & Ishaq, M. (2010). Influence of bio-inoculants and inorganic fertilizers on yield, nutrient balance, microbial dynamics and quality of strawberry (*Fragaria× ananassa*) under rainfed conditions of Kashmir valley. *Indian Journal of Agricultural Sciences*, 80(4), 275.
- [25] Coronado, M. H.; R. L. Carrillo Romo; M. S. Cerecedo, Melgoza Castillo, A.; M. H. Royo Márquez,,and Jiménez Castro, J. 2002. Emergence and survival of grasses inoculated with biofertilizers in a greenhouse study. *Téc. Pec. Méx.*, 42: 459-475.
- [26] AL-Taey D.K.A. , K.M.N.AL-Dirbil and S.S.M.AL-Azawi.(2021). Study The Effect of Water Quality, Bio Booster (Zytonic F) and Nano NPK fertilizer on Tomato Growth and Yield. *IOP Conf. Series: Earth and Environmental Science* 910 (2021) 012063. doi:10.1088/1755-1315/910/1/012063
- [27] Havlin , J.L. ; J. D. Beaton ; S. L. Tisdale and W. L. Nelson (2005). *Soil Fertility and Fertilizers* .7th ed.Upper Saddle River , New Jersey 07458.

Effect of Vermicompost Fertilizer and Spraying with Salicylic Acid and Gibberellin on *camellia japonica* Plant

Hawraa K. A.¹ and Ban M. A. Abood²

^{1,2}Department of Horticulture and Landscape, College of Agriculture, University of Baghdad, Baghdad, Iraq.

¹E-mail: hawraa.enaaid2015@coagri.uobaghdad.edu.iq

²E-mail: dr.banmohamad@gmail.com

Abstract. The experiment over two consecutive seasons (2022-2023) aimed to improve the growth and flowering of *Camellia japonica* by applying vermicompost fertilizer (0%, 7.5%, 15%) and its symbols V_0, V_1, V_2 sequentially, and foliar application plant growth regulators (salicylic acid in concentration 0,100,200 mg L⁻¹ and its symbols S_0, S_1, S_2 and gibberellic acid in concentration 0,150,300 mg L⁻¹ and its symbols G_0, G_1, G_2 resleeetively). The plants were cultivated under a shade net structure of the Department of Horticulture and Garden Engineering - College of Agricultural Engineering Sciences - University of Baghdad .Using randomized Complete Block Design(RCBD). The $V_2S_2G_2$ treatment showed significant improvements in vegetative and flowering growth indicators. leaves number were 95.5 and 145.5 leaves plant⁻¹, and the percentage of dry in the leaves was 17.90% and 18.81% , respectively, in the two seasons. Flowering indicators included a reduction in the days to flowering (252.9 and 239.6 days), an extended flowering duration of 87.3 and 89.6 days,.The percentage of each of(Nitrogen was 4.03% and 3.36%, Phosphorus 0.46% 0.48%, potassium 3.21% and 3.23% in both seasons.

Keywords. Growth regulator, Ornamental plants, *Camellia japonica*.

1. Introduction

Camellia japonica L. It is one of the flowering ornamental shrubs belonging to the tea family Theaceae and includes about 250 species naturally spread from East and South Asia from the Himalayas in the east to Japan, Indonesia and the coasts of Korea [1,2]. Japanese camellia is characterized by its large, variety of colors flowers, including red, white and pink. *Camellia japonica* is used for ornamental purposes, where it is grown as flowering potted plants and in outdoor gardens due to its distinctive and attractive shape and as picking flowers and its leaves are used in the manufacture of green tea [3,4].

Agriculture is moving at the present time to the use of organic fertilizers because they provide the soil with nutrients and improve the properties of the soil and from these fertilizers is Vermicompost fertilizer one of the organic fertilizers that have received the attention of researchers in recent years because of its excellent role in increasing plant productivity by improving soil fertility and tissue and its content of nutrients and the activity of bacteria, enzymes and microorganisms [5-7].

Plant growth regulators are chemical compounds added at low doses that cause a change in plant growth and development, and these compounds are known to control many physiological and

biochemical processes through primary and secondary metabolic processes. Salicylic acid SA is an internal growth regulator, a phenolic compound that regulates plant growth and increases its tolerance to stress by regulating ion absorption, hormonal balance, increased photosynthesis and enzyme activity, and has a role in inhibiting ethylene synthesis [8-10].

Gibberelins are one of the plant hormones that are manufactured and produced within various plant tissues, including (growing tops, advanced embryos, seeds, young leaves) and plays an important role in activating and encouraging the process of elongation of cells and increases the size of cells and stimulate the formation and development of flowers and the number of leaves [11,12].

Given the limited studies on this plant in Iraq, the study aimed to improve the growth and flowering of *Camellia japonica* by applying different amount of vermicompost and concentrations of gibberellic acid, and salicylic acid.

2. Materials and Methods

This study was conducted at the Agricultural Research Station (B) of the College of Agricultural Engineering Sciences – University of Baghdad, Jadriyah campus, in a shade net structure (covered with saran cloth) on *Camellia japonica* saplings over two consecutive seasons, 2022/2023. The *Camellia japonica* saplings, obtained from a nursery in Baghdad, were transplanted into 12 kg plastic pots on 1/6/2022. The pots contained a garden sand substrate mixed with three levels of vermicompost, the first factor: 0%, 7.5%, and 15%, labeled as V₀, V₁, and V₂, respectively. One month after planting, the second factor, salicylic acid, was applied at concentrations 0, 100, and 200 mg L⁻¹, labeled as S₀, S₁, and S₂. Four days later, the third factor, gibberellic acid, was foliar application concentrations 0, 150, and 300 mg L⁻¹, labeled as G₀, G₁, and G₂. A total of three foliar application were applied at 21-day intervals. Control plants were foliar application with distilled water only. Half of the recommended NPK fertilizer (20-20-20) was applied to the camellia saplings every 30 days throughout the research period. The factorial experiment (3*3*3) Randomized Complete Block Design (RCBD) with three replicates, each experimental unit pot containing 3 plants. The total number of saplings used for the two seasons (2022-2023) was 243.

Table 1. Some chemical and physical properties of the planting soil and vermicompost fertilizer.

Properties	Soil Soil0.80	Vermicompost V2	Unit
EC 1:1	0.80		ds m ⁻¹
ph	7.2	7.5	-----
Available Nitrogen (N)	25.01	2.33	mg kg ⁻¹ soil
Available Phosphorus (P)	8.25	0.48	
Available Potassium (K)	78.50	1.65	
Organic Matter (O M)	14.3	-----	g kg ⁻¹
	347.5		
Soluble Calcium (Ca ⁺²)	-----	0.99	%
	4.03	0.45	
Magnesium (Mg ⁺²)	2.52		meq L ⁻¹
	1.25		
	1.53	0.25	
	4.82		
	0.54		
Soil Separates	Sand	952	-----
	Silt	20	-----
	Clay	28	-----
			g kg ⁻¹ soil

Soil Texture

Sand

The soil and Fertilizer analyzed in the laboratories of the Soil Department at the College of Agriculture – University of Baghdad.

2.1. Study Indicator

- Main stem diameter (mm): The diameter of the main stem of each plant of the experimental unit was measured 3 cm from the pot soil surface by Vernia and recorded its rate
- Percentage of dry matter in leaves (%) [13].
- Number of Days to Flowering (days): The number of days was calculated from the date of recycling plants 1/6/2022 until the first flower opens in each plant of the experimental unit and recorded its rate
- Flowering Duration (days) :The number of days was calculated from the beginning of the opening of the first flower until the loss of the last flower to its coordination value for all plants of the experimental unit, then the average was recorded.
- Percentage of nitrogen in leaves (%):Using a Microkjeldahl device by distillation [14].
- Percentage of phosphorus leaves (%): Using a spectrophotometer [15].
- Percentage of potassium leaves (%):Using a flamephotometer [13].

3. Results and Discussion

3.1. Number of Leaves (leaves plant⁻¹)

Table (2) showed that the V_2 treatment significantly increased the number of leaves, with an average 88.2 and 137.8 leaves plant⁻¹, compared to the control plants, which had 83.4 and 132.4 leaves plant⁻¹. The salicylic acid spray treatment S_2 also significantly increased the number of leaves, 89.3 and 139.5 leaves plant⁻¹, compared to the control, which gave 81.6 and 130.1 leaves plant⁻¹ for both seasons. Similarly, gibberellic acid treatment G_2 resulted a significant increase in the number of leaves for two consecutive seasons, 90.3 and 140.7 leaves plant⁻¹. Regarding the two-way interactions, the V_2S_2 treatment had a significant effect in both seasons, with 91.5 and 141.5 leaves plant⁻¹, compared to the control V_0S_0 , which gave 78.7 and 125.4 leaves plant⁻¹. The interaction between vermicompost and gibberellic acid also had a significant effect in both seasons, with the number of leaves ranging between the highest value of 92.8 and 143.2 leaves plant⁻¹, and the lowest value 78.0 and 125.3 leaves plant⁻¹. Additionally, the interaction between salicylic acid and gibberellic acid had a significant impact, with the S_2G_2 treatment giving 93.4 and 143.5 leaves plant⁻¹ for both seasons.

The three-way interaction between the study factors was significant for this trait, with the V₂S₂G₂ treatment showing the highest number of leaves, reaching 95.5 and 145.5 leaves plant⁻¹ for both seasons.

Table 2. Effect of vermicompost fertilizer, salicylic acid, gibberellic acid sprays on the number of leaves (leaves plant⁻¹) of *Camellia japonica* for both seasons 2022-2023.

V	S	2022			VxS	2023			VxS
		G				G			
		G ₀	G ₁	G ₂		G ₀	G ₁	G ₂	
V ₀	S ₀	75.1	80.4	80.5	78.7	120.3	126.5	129.4	125.4
	S ₁	79.4	87.5	89.2	85.4	126.4	139.4	140.4	135.4
	S ₂	79.5	89.2	89.5	86.1	129.2	139.5	140.5	136.4

V	S	2022				VxS	2023			VxS
		G			G					
		G ₀	G ₁	G ₂	G ₀		G ₁	G ₂		
V ₁	S ₀	75.4	86.4	87.2	83.0	122.4	136.2	138.4	132.3	
	S ₁	83.4	90.4	93.2	89.0	131.2	141.4	143.4	138.7	
	S ₂	85.4	90.2	95.1	90.2	134.4	143.2	144.5	140.7	
V ₂	S ₀	75.5	86.5	87.4	83.2	122.4	136.4	138.5	132.5	
	S ₁	83.5	90.5	95.4	89.8	131.4	141.5	145.4	139.5	
	S ₂	85.6	93.5	95.5	91.5	134.5	144.4	145.5	141.5	
L.S.D	0.05	0.288			0.166	0.277			0.160	
	V	V × G			V	V × G			V	
	V ₀	78.0	85.7	86.4	83.4	125.3	135.2	136.8	132.4	
	V ₁	81.4	89.0	91.8	87.4	129.3	140.3	142.1	137.2	
	V ₂	81.5	90.2	92.8	88.2	129.5	140.8	143.2	137.8	
L.S.D	0.05	0.166			0.096	0.160			0.092	
	S	S × G			S	S × G			S	
	S ₀	75.3	84.4	85.0	81.6	121.7	133.1	135.4	130.1	
	S ₁	82.1	89.5	92.6	88.1	129.7	140.8	143.1	137.9	
	S ₂	83.5	91.0	93.4	89.3	132.7	142.4	143.5	139.5	
L.S.D	0.05	0.166			0.096	0.160			0.092	
	G	80.3	88.3	90.3		128.0	138.7	140.7		
L.S.D	0.05	0.096				0.092				

3.2. Percentage of Dry Matter in Leaves (%)

The results of Table (3) showed that the addition of vermpost fertilizer led to the superiority of the V2 treatment in giving the highest percentage of dry weight in the leaves amounted to (16.43 and 16.67) % respectively and for both seasons. As for salicylic acid spraying, the treatment at S2 concentration significantly exceeded the percentage of dry weight in the leaves amounted to (16.29 and 17.06) % compared to the comparison plants, as it reached (14.35 and 14.76) % respectively and for both seasons. The effect of spraying with gibberellin acid was significant, as the G2 treatment plants excelled in the percentage of dry weight in the leaves and a significant difference for the two consecutive seasons amounted to (16.43 and 17.26) .

The two-way interaction between manure and salicylic acid had a significant impact in both seasons, with V2S2 treatment resulting in (16.94 and 17.65)%. As for the interference between vermicpost and gibberellin acid, significant differences were shown for both seasons in the percentage of dry weight in the leaves at the treatment V2G2 amounted to (17.09 and 17.97)%, while the interaction between salicylic acid and gibberellin acid gave a significant effect at the treatment S2G2 amounted to (17.27 and 18.20)%.

The three-way interaction V2S2G2 resulted in the percentage of dry matter in the leaves reaching (17.90 and 18.81)%.

Table 4. Effect of vermicompost fertilizer, salicylic acid, gibberellic acid sprays, on Percentage of dry matter in leaves (%) of *Camellia japonica* for both seasons 2022-2023.

V	S	2022				2023			
		G			VxS	G			VxS
		G ₀	G ₁	G ₂		G ₀	G ₁	G ₂	
V ₀	S ₀	13.30	13.87	14.33	13.83	13.37	14.46	14.76	14.20
	S ₁	13.78	15.66	15.95	15.13	14.21	16.53	16.83	15.86
	S ₂	14.16	15.77	16.12	15.35	14.54	16.72	17.26	16.17
V ₁	S ₀	13.68	14.61	15.28	14.52	13.42	15.33	16.12	14.96
	S ₁	14.46	16.53	17.15	16.05	15.06	17.45	17.92	16.81
	S ₂	15.07	16.87	17.78	16.57	15.76	17.74	18.54	17.35
V ₂	S ₀	13.73	14.83	15.53	14.70	13.56	15.54	16.33	15.14
	S ₁	14.74	16.70	17.83	16.42	15.15	17.75	18.76	17.22
	S ₂	15.49	17.43	17.90	16.94	15.87	18.28	18.81	17.65
L.S.D 0.05		0.023			0.013	0.019			0.011
V		V × G			V	V × G			V
V ₀		13.75	15.10	15.47	14.77	14.040	15.902	16.282	15.41
V ₁		14.40	16.01	16.74	15.87	17.527	16.839	17.527	16.37
V ₂		14.65	16.32	17.09	16.43	14.860	17.189	17.967	16.67
L.S.D 0.05		0.013			1.081	0.011			0.006
S		S × G			S	S × G			S
S ₀		13.57	14.44	15.05	14.35	13.45	15.11	15.74	14.76
S ₁		14.33	16.30	16.98	15.87	14.81	17.24	17.84	16.63
S ₂		14.91	16.69	17.27	16.29	15.39	17.58	18.20	17.06
L.S.D 0.05		0.013			0.008	0.011			0.006
G		14.27	15.81	16.43		14.55	16.64	17.26	
L.S.D 0.05		0.008				0.006			

3.3. Number of Days to Flowering (days)

The results showed a significant effect of vermicompost on the number of days to flowering Table (4). The V₂ treatment resulted the highest number of days to flowering, 295.8 and 274.0 days, respectively compared to the control 319.0 days and 297.1 days. Salicylic acid spraying also had a significant effect, the S₂ treatment reducing the number of days to flowering to 288.6 and 268.2 days for both seasons compared to the control 333.8 days and 305.8 days. Gibberellic acid spraying played a crucial role in further reducing the number of days to flowering, the G₂ treatment giving 282.3 and 264.9 days for both seasons compared to the control 3389 days and 309.5 days.

The two-way interaction between vermicompost and salicylic acid showed a significant effect, the V₂S₂ treatment outperforming the control V₀S₀, which took 278.9 and 257.3 days to flower. The interaction between vermicompost and gibberellic acid V₂G₂ led to a significant reduction in the number of days to flowering, reaching 268.2 and 253.7 days for both seasons while the lowest values 348.7 days and 322.0 days The interaction between salicylic acid and gibberellic acid S₂G₂ also showed a significant effect, reducing the number of days to flowering to 264.1 and 251.0 days for both seasons. The three-way interaction V₂S₂G₂ recorded the lowest number of days to

flowering, 252.9 and 239.6 days for two consecutive seasons while the lowest values 358.9 days and 330.9 days.

Table 4. Effect of vermicompost fertilizer , salicylic acid, gibberellic acid sprays on number of days to flowering (days) of *Camellia japonica* for both seasons 2022-2023.

V	S	2022				2023			
		G			VxS	G			VxS
		G ₀	G ₁	G ₂		G ₀	G ₁	G ₂	
V ₀	S ₀	358.9	343.8	335.3	346.0	330.9	317.7	308.7	319.1
	S ₁	347.3	292.7	284.3	308.1	321.4	274.4	268.7	288.2
	S ₂	339.8	288.9	279.8	302.8	313.8	272.4	265.9	284.0
V ₁	S ₀	355.4	326.7	313.3	331.8	329.2	292.7	279.0	300.3
	S ₁	331.7	276.7	263.7	290.7	298.4	262.8	252.8	271.3
	S ₂	319.6	272.9	259.7	284.0	285.9	256.4	247.6	263.3
V ₂	S ₀	351.7	322.9	296.4	323.7	327.0	289.4	277.9	298.1
	S ₁	329.8	269.9	255.2	285.0	295.7	259.9	243.8	266.4
	S ₂	315.9	267.8	252.9	278.9	282.7	249.8	239.6	257.3
L.S.D 0.05		1.61			0.93	1.18			0.68
V		V × G			V	V × G			V
V ₀		348.7	308.4	299.8	319.0	322.0	288.2	281.1	297.1
V ₁		335.6	292.1	278.9	302.2	304.5	270.6	259.8	278.3
V ₂		332.4	286.9	268.2	295.8	301.8	266.4	253.7	274.0
L.S.D 0.05		0.932			0.538	0.681			0.392
S		S × G			S	S × G			S
S ₀		355.3	331.1	315.0	333.8	329.0	299.9	288.5	305.8
S ₁		336.3	279.7	267.7	294.6	305.2	265.7	255.1	275.3
S ₂		325.1	276.5	264.1	288.6	294.1	259.6	251.0	268.2
L.S.D 0.05		0.932			0.538	0.681			0.392
G		338.9	295.8	282.3		309.5	275.1	264.9	
L.S.D 0.05		0.538				0.392			

3.4. Flowering Duration (days)

Table (5) showed a significant effect of vermicompost on flowering duration. The V₂ treatment resulted the longest flowering duration, 76.4 and 78.6 days, respectively compared to the control 68.3 days and 71.4 days. Salicylic acid spraying also had a clear and significant effect, the S₂ treatment giving 78.3 and 80.0 days for both seasons. Gibberellic acid spraying further extended the flowering duration, the G₂ treatment resulting 79.0 and 81.2 days for both seasons compared to the control 63.7 days and 67.2 days.

The two-way interaction between vermicompost and salicylic acid had a significant effect, the V₂S₂ treatment outperforming the control V₀S₀, reaching 82.4 and 83.9 days. The interaction between vermicompost and gibberellic acid V₂G₂ also had a significant effect, the flowering duration reaching the highest values 82.9 and 84.9 days, while the lowest values were observed in the control (59.4 and 64.0 days) for both seasons.

The interaction between salicylic acid and gibberellic acid S₂G₂ showed a significant effect, resulting 83.7 and 85.8 days for both seasons while the lowest values 57.4 days and 62.4 days. The three-way interaction V₂S₂G₂ recorded the longest flowering duration, reaching 87.3 and 89.6 days for both seasons while the lowest values 56.3 days and 61.3 days .

Table 5. Effect of vermicompost fertilizer, salicylic acid, gibberellic acid sprays, on flowering duration (days) of *Camellia japonica* for both seasons 2022-2023.

V	S	2022				2023			
		G			VxS	G			VxS
		G ₀	G ₁	G ₂		G ₀	G ₁	G ₂	
V ₀	S ₀	13.30	13.87	14.33	13.83	13.37	14.46	14.76	14.20
	S ₁	13.78	15.66	15.95	15.13	14.21	16.53	16.83	15.86
	S ₂	14.16	15.77	16.12	15.35	14.54	16.72	17.26	16.17
V ₁	S ₀	13.68	14.61	15.28	14.52	13.42	15.33	16.12	14.96
	S ₁	14.46	16.53	17.15	16.05	15.06	17.45	17.92	16.81
	S ₂	15.07	16.87	17.78	16.57	15.76	17.74	18.54	17.35
V ₂	S ₀	13.73	14.83	15.53	14.70	13.56	15.54	16.33	15.14
	S ₁	14.74	16.70	17.83	16.42	15.15	17.75	18.76	17.22
	S ₂	15.49	17.43	17.90	16.94	15.87	18.28	18.81	17.65
L.S.D 0.05		0.023			0.013	0.019			0.011
V		V × G			V	V × G			V
V ₀		13.75	15.10	15.47	14.77	14.04	15.90	16.28	15.41
V ₁		14.40	16.01	16.74	15.87	17.53	16.84	17.53	16.37
V ₂		14.65	16.32	17.09	16.43	14.86	17.19	17.97	16.67
L.S.D 0.05		0.013			0.008	0.011			0.006
S		S × G			S	S × G			S
S ₀		13.57	14.44	15.05	14.35	13.45	15.11	15.74	14.76
S ₁		14.33	16.30	16.98	15.87	14.81	17.24	17.84	16.63
S ₂		14.91	16.69	17.27	16.29	15.39	17.58	18.20	17.26
L.S.D 0.05		0.013			0.008	0.011			0.006
G		14.27	15.81	16.43		17.26	16.64	14.55	
L.S.D 0.05		0.008				0.006			

3.5. Percentage of Nitrogen in Leaves (%)

Vermicompost application significantly increased leaf nitrogen concentration for two seasons Table (6). The V₂ treatment produced the highest nitrogen percentages, reaching 2.91% and 2.85%. Foliar application with salicylic acid led to a considerable rise in nitrogen concentration, the S₂ treatment reaching 3.00% and 2.94% in both seasons compared to the control 2.12% and 2.42%. Gibberellic acid spraying further enhanced the nitrogen percentage, the G₂ treatment gave 3.12% and 2.98% for both seasons compared to the control 2.03% and 2.38%.

The two-way interaction between vermicompost and salicylic acid V₂S₂ resulted the highest nitrogen percentage, reaching 3.34% and 3.09% for both seasons. Similarly, the interaction between vermicompost and gibberellic acid V₂G₂ had a significant effect, on nitrogen concentration reaching 3.50% and 3.13% for two consecutive seasons while the lowest values 1.86% and 2.23% .

The interaction between salicylic acid and gibberellic acid S₂G₂ significantly increased nitrogen content in the leaves, reaching 3.55% and 3.15%.

The three-way interaction V₂S₂G₂ resulted the highest nitrogen percentage, reaching 4.03% and 3.36% for two consecutive seasons while the lowest values 1.72% and 1.95%.

Table 6. Effect of vermicompost fertilizer, salicylic acid, gibberellic acid sprays on percentage of nitrogen in leaves (%) of *Camellia japonica* for both seasons 2022-2023.

V	S	2022				2023			
		G			VxS	G			VxS
		G ₀	G ₁	G ₂		G ₀	G ₁	G ₂	
V ₀	S ₀	1.72	1.91	2.11	1.91	1.95	2.29	2.46	2.23
	S ₁	1.88	2.76	2.86	2.50	2.28	2.89	2.93	2.70
	S ₂	1.97	2.83	2.97	2.60	2.45	2.91	2.94	2.77
V ₁	S ₀	1.78	2.21	2.57	2.19	1.97	2.65	2.85	2.49
	S ₁	2.14	3.20	3.46	2.93	2.47	2.96	3.13	2.85
	S ₂	2.28	3.28	3.65	3.07	2.80	2.98	3.14	2.97
V ₂	S ₀	1.83	2.37	2.63	2.27	2.03	2.66	2.88	2.52
	S ₁	2.18	3.34	3.83	3.12	2.63	3.03	3.15	2.94
	S ₂	2.48	3.51	4.03	3.34	2.83	3.09	3.36	3.09
L.S.D 0.05		0.025			0.014	0.019			0.011
V		V × G			V	V × G			V
V ₀		1.86	2.50	2.65	2.33	2.23	2.70	2.78	2.57
V ₁		2.06	2.90	3.22	2.73	2.41	2.86	3.04	2.77
V ₂		2.16	3.07	3.50	2.91	2.49	2.93	3.13	2.85
L.S.D 0.05		0.014			0.008	0.011			0.006
S		S × G			S	S × G			S
S ₀		1.77	2.16	2.44	2.12	1.98	2.53	2.73	2.42
S ₁		2.06	3.10	3.38	2.85	2.46	2.96	2.07	2.83
S ₂		2.24	3.21	3.55	3.00	2.69	2.99	3.15	2.94
L.S.D 0.05		0.014			0.008	0.011			0.006
G		2.03	2.82	3.12		2.38	2.83	2.98	
L.S.D 0.05		0.008				0.006			

3.6. Percentage of Phosphorus in Leaves(%)

The results in Table (7) showed that the addition of worm fertilizer significantly affected the percentage of phosphorus in the leaves of Japanese camellia. V2 treatment resulted in the highest percentage of phosphorus, reaching 0.32% and 0.34% for two consecutive seasons, compared to the V0 treatment, which had the lowest phosphorus content of 0.23% and 0.25%.

The foliar application of salicylic acid also showed a significant effect, with the S2 treatment giving the highest phosphorus content of 0.34% and 0.36% for both seasons. Similarly, the foliar application of gibberellic acid led to a significant increase in phosphorus, with G2 treatment reaching 0.36% and 0.37% for both seasons compared to control 0.18% and 0.19%.

The two-way interaction between manure and salicylic acid (V2S2) significantly increased the phosphorus content, reaching 0.38% and 0.40% for both seasons. In addition, the reaction between vermicompost and gibberellic acid (V2G2) resulted in the highest phosphorus content, reaching 0.32% and 0.42% for two consecutive seasons while the lowest values were 0.15% and 0.16%.

The interaction between salicylic acid and gibberellic acid (S2G2) also showed a significant effect, with phosphorus reaching 0.42% and 0.43% for both seasons while the lower values were 0.12% and 0.13%.

The three-way interaction (V2S2G2) recorded the highest phosphorus, reaching 0.46% and 0.48% for two consecutive seasons while the lowest values were recorded 0.11% and 0.12%.

Table 7. Effect of vermicompost fertilizer, salicylic acid, gibberellic acid sprays on percentage of phosphorus in leaves (%) of *Camellia japonica* for both seasons 2022-2023.

V	S	2022				2023			
		G			VxS	G			VxS
		G ₀	G ₁	G ₂		G ₀	G ₁	G ₂	
V ₀	S ₀	0.11	0.16	0.19	0.15	0.12	0.18	0.21	0.17
	S ₁	0.15	0.31	0.33	0.26	0.16	0.33	0.35	0.28
	S ₂	0.18	0.32	0.35	0.28	0.19	0.34	0.36	0.30
V ₁	S ₀	0.12	0.23	0.28	0.21	0.13	0.24	0.26	0.21
	S ₁	0.21	0.36	0.43	0.33	0.22	0.38	0.45	0.35
	S ₂	0.25	0.38	0.44	0.36	0.27	0.42	0.46	0.38
V ₂	S ₀	0.13	0.24	0.28	0.22	0.14	0.25	0.31	0.23
	S ₁	0.22	0.41	0.45	0.36	0.23	0.43	0.47	0.37
	S ₂	0.26	0.42	0.46	0.38	0.28	0.44	0.48	0.40
L.S.D 0.05		0.021			0.012	0.026			0.015
V		V × G			V	V × G			V
V ₀		0.15	0.21	0.23	0.23	0.16	0.28	0.31	0.25
V ₁		0.19	0.36	0.30	0.30	0.21	0.35	0.39	0.31
V ₂		0.23	0.37	0.32	0.32	0.22	0.37	0.42	0.34
L.S.D 0.05		0.012			0.007	0.015			0.009
S		S × G			S	S × G			S
S ₀		0.12	0.21	0.25	0.19	0.13	0.22	0.26	0.20
S ₁		0.19	0.36	0.40	0.32	0.20	0.38	0.42	0.34
S ₂		0.23	0.31	0.42	0.34	0.25	0.40	0.43	0.36
L.S.D 0.05		0.012			0.007	0.026			0.009
G		0.18	0.31	0.36		0.19	0.33	0.37	
L.S.D 0.05		0.007				0.015			

3.7. Percentage of Potassium in Leaves(%)

The use of vermicompost significantly increased the potassium content in the leaves for two seasons Table (8). V2 processing produced the highest potassium ratios, reaching 2.69% and 2.75%. Foliar application with salicylic acid led to a significant rise in potassium, with S2 treatment reaching 2.76% and 2.82% in both seasons compared to control 2.27% and 2.36%. with G2 treatment reaching 2.82% and 2.89% for both seasons compared to control 2.22% and 2.30%. The two-way reaction between manure and salicylic acid V2S2 resulted in the highest potassium content, reaching 2.93% and 2.98% for both seasons. Similarly, the interaction between manure and gibberellic acid V2G2 had a significant effect, with phosphorus reaching 2.99% and 3.03% for two consecutive seasons while the minimum values were 2.12% and 2.19%. The interaction between salicylic acid and gibberellic acid S2G2 significantly increased the percentage of potassium in the leaves, reaching 3.04% and 3.08%.

The V2S2G2 triple reaction resulted in the highest potassium content, reaching 3.21% and 3.23% for two consecutive seasons while the lowest values were 2.07% and 2.11%.

Table 8. Effect of vermicompost fertilizer, salicylic acid, gibberellic acid sprays on percentage of potassium in leaves (%) of *Camellia japonica* for both seasons 2022-2023.

V	S	2022				2023			
		G			VxS	G			VxS
		G ₀	G ₁	G ₂		G ₀	G ₁	G ₂	
V ₀	S ₀	2.07	2.15	2.18	2.13	2.11	2.24	2.31	2.22
	S ₁	2.13	2.64	2.71	2.49	2.19	2.71	2.79	2.56

V	S	2022			VxS	2023			VxS
		G				G			
		G ₀	G ₁	G ₂		G ₀	G ₁	G ₂	
V ₁	S ₂	2.17	2.67	2.76	2.53	2.28	2.75	2.83	2.62
	S ₀	2.08	2.33	2.53	2.31	2.13	2.46	2.63	2.41
	S ₁	2.25	2.81	3.13	2.73	2.35	2.87	3.15	2.79
	S ₂	2.42	2.83	3.15	2.80	2.52	2.89	3.17	2.86
V ₂	S ₀	2.11	2.38	2.59	2.36	2.17	2.49	2.67	2.44
	S ₁	2.28	2.89	3.16	2.78	2.38	2.93	3.19	2.83
	S ₂	2.47	3.12	3.21	2.93	2.57	3.13	3.23	2.98
L.S.D	0.05		0.017		0.010		0.018		0.010
	V		V × G		V		V × G		V
	V ₀	2.12	2.49	2.55	2.39	2.19	2.56	2.62	2.47
	V ₁	2.25	2.66	2.94	2.61	2.33	2.79	2.98	2.69
	V ₂	2.29	2.80	2.99	2.69	2.37	2.83	3.03	2.75
L.S.D	0.05		0.010		0.005		0.010		0.006
	S		S × G		S		S × G		S
	S ₀	2.09	2.29	2.43	2.27	2.14	2.40	2.54	2.36
	S ₁	2.22	2.78	3.00	2.67	2.31	2.84	3.04	2.73
	S ₂	2.35	2.87	3.04	2.76	2.46	2.92	3.08	2.82
L.S.D	0.05		0.010		0.005		0.010		0.006
	G	2.22	2.65	2.82		2.30	2.72	2.89	
L.S.D	0.05		0.005				0.006		

The results showed that the addition of vermicompost fertilizer to the soil caused a significant increase in the content of the leaves of mineral elements, which can be attributed to its high solubility in water, which makes the growth medium of a plant suitable for absorbing essential nutrients such as N, P, K and Ca [16,17], in addition to its role in improving root growth through its positive impact on the physical, chemical and biological properties of the soil and works to provide moisture and sufficient mineral elements around Root system improving vegetative and flowering growth characteristics [18-20]. This is consistent with the findings of Khatun *et al.*, 2018 about the response of the white pea plant *Basella alba* and West Alhassan *Ipomea* to vermicompost fertilizer, which led to a significant increase in the plant's content of potassium, phosphorus and sulfur for two plants [21], as found by Dhakar *et al.*, 2019 When adding vermicompost fertilizer to the cladiolis plant, it increased the percentage of mineral elements N and P K [22].

As for spraying with salicylic acid, it is the cause of an increase in the content of the leaves of nutrients N, K, Mg and Fe, and this may be due to its role in the formation of chlorophyll and carotene pigments and accelerate the process of photosynthesis and activates the work of some important enzymes in physiological processes within the plant [23] and the increase in carbohydrates leads to an increase in the leaf area, which leads to an increase in the number, size and diameter of the roots and thus increase the absorption of nutrients [24]. This is consistent with the findings of Abdolmaleki *et al.*, 2015 that spraying *Rosa hybida* with salicylic acid increased the percentage of nitrogen in the leaves [25], and Jaddo and Rabee, 2016 indicated that the effect of salicylic acid increased the content of the leaves of mineral elements (potassium, phosphorus and nitrogen) [26].

While the physiological effect of gibberellins in the content of the basic mineral elements in the leaves is attributed to its role in activating the construction of RNA and protein and the construction of special enzymes as well as its role in encouraging vegetative and root growth and its branches, which is reflected in its ability to absorb nitrogen, phosphorus and potassium from

the soil and increase its concentration in the plant and its transition to the leaves [27], and as a result of increasing the process of transpiration and absorption and the transfer of mineral ions from the soil solution to the plant [28] thus increasing the absorption rate of iron and magnesium [29,30]. Dhanasekaran, 2018 found that gibberellin acid had a significant effect on plant height, leaf count, and increased number of flowers of jasmine, *Jasminum sambac* [31].

Conclusion

The current study demonstrated that the application of vermicompost, salicylic acid, and gibberellic acid significantly enhanced the growth, flowering, and physiological traits of *Camellia japonica*. Vermicompost, due to its high humus content and beneficial nutrients, improved soil structure, water retention, and nutrient availability, resulting in increased plant height, leaves number, chlorophyll concentration, and overall biomass. The addition of salicylic acid promoted vegetative growth, chlorophyll production, and nutrient uptake by activating growth regulators like auxins and cytokinins. Gibberellic acid contributed to cell elongation, biomass accumulation, and increased photosynthetic activity, further improving the flowering traits and nutrient absorption, particularly for iron and magnesium. Overall, the combined treatments of vermicompost, salicylic acid, and gibberellic acid significantly optimized plant growth, flowering duration, and nutrient content, suggesting their potential as effective tools for enhancing ornamental plant productivity in sustainable horticultural practices.

References

- [1] Park, H. 2016. Plant Growth Promoting Rhizobacteria Affect Soil Fertility and Growth Environment of *Camellia japonica* Seedlings in Saemangeum Coastal Reclaimed Land of Korea." Master's thesis, Chonnam National University, 1-27.
- [2] Wang, J -H, Cai, Y-F. , Li, S-F. and Zhang, S-B. 2020. Differences in leaf physiological and morphological traits between *Camellia japonica* and *Camellia reticulata*. *Plant Diversity*, 42(3):181-188.
- [3] Dole, J. M., and H. F. Wilkins. 1999. *Floriculture Principles and Species*. New Jersey 07458.
- [4] Guo, N., T. Tong, N. Ren, Y. Tu, and B. Li. 2018. Saponins from Seeds of Genus *Camellia*: Phytochemistry and Bioactivity. *Phytochemistry* 149(): 42–55.
- [5] Joshi, R., and A.P. Vig. 2010. Effect of vermicompost on growth, yield, and quality of tomato (*Lycopersicon esculentum* L). *African Journal of Basic & Applied Sciences* 2, (3-4): 117-123.
- [6] Aziz, M. N., and B. M. Alwan 2022. Effect of ammonium sulfate and vermicompost on nitrogen and phosphorus availability and the zucchini squash yield. *Iraqi Journal of Soil Sciences* 22(2): 132-141.
- [7] Al-Hlfie, R. G. and W. A. Hussein. 2024. effect of organic fertilizers and nutrients on anatomical traits of red beetroots. *Iraqi Journal Of Agricultural Sciences*, 55(Special), 151-161.
- [8] Hoopkins, W. G. and N. P. Muner. 2008. *Introduction to Plant Physiology*. 4th ed. Wiley and Sons. USA
- [9] Pacheco, A.C., da Silva Cabral, C., da Silva Fermino, E.S. and Aleman, C.C. 2013. Salicylic acid-induced changes to growth , flowering and flavonoids production in marigold plants. *J. Med. Plants Res.*, 7: 3158-3163
- [10] Aljassani, I. F., Alqaisi, M. R. M., & Al-Ahbab, A. J. (2022). effect of the hydrogen peroxide and salicylic acid on induction the sod gene expression of date palm (*Phoenix dactylifera* L.) as defense factor against salinity stress. *Iraqi Journal Of Agricultural Sciences*, 53(5), 1099-1106.
- [11] Alkhafaji, M.A. 2014. *Plant Growth Regulators: Horticultural Uses and Applications*. College of Agriculture, University of Baghdad: Al-Mosul University Press.
- [12] Al-Hasnawi, H.A., J.K. Hussein and T.H. Khaleel. 2019. effect of growth regulators and preservative solution on vase life and water relation of *Gladiolus hybrida* L. after cut flowers. *Iraqi Journal Of Agricultural Sciences*, 50(Special), 182-191.

- [13] Jackson, M.L. 1958. Soil Chemical analysis. Prentice Hall, Inc. Englewood Cliff, N.J. USA.225-276.
- [14] Olsen, S.R. and L.E. Sommers. 1982. Phosphorus in A.L Page, (Ed). Methods of soil analysis. Part2. Chemical and Microbiological properties 2nd edition, Amer. Soc. of Agron. Inc. Soil Sci. Soc. Amer. Inc. Madision . Wis. U.S.A.
- [15] Al-Sahhaf, F. H.1989. Applied plant nutrition. Baghdad University . Ministry of Higher Education and Scientific Research House of Wisdom for publication, translation and distribution. Higher Education Press in Mosul. Iraq,
- [16] Smith, S. E and D. J. Read. 2008 . Mycorrhizal symbiosis , 3rd ed ; Academic Press. London 42 (4) : 203
- [17] Alkobaisy,J.S. and Mutlag, N.A. 2021. Effect Of The Use Of Vermicompost And Rhizobial Inoculation On Some Soil Characteristics, Growth And Yield Of Mung Bean Vigni Radiate L. Iraqi Journal Of Agricultural Sciences :52(1):163-169
- [18] ALmamori, H. A and H A. AbdulRatha.2020. Effect Of Addition Of Vermicompost, Bio And Mineral Fertilizer On The Availability Of Some Nutrients In Soil And Potato Yield . Iraqi Journal of Agricultural Sciences.pp: 644-656.
- [19] Al-Obaidi, S. M. J and H. A. AbdulRatha.2021. Evaluation Of The Combination Of Bacterial Biofertilizer And Vermicompost In The Availability Of N, P, K And Some Of Plant Parameters Of Beans (PHASEOLUS VULGARIS L.). Iraqi Journal of Agricultural Sciences.pp:960-970.
- [20] Al-Silmawy, N A. J.K. and H. A. Abdul- Ratha. 2023. Effect of biofertilizer,vermicompost and phosphate fertilizer on growth and yield of cauliflower(Brassica oleraceae Var.botrytis). Iraqi Journal of Agricultural Sciences :54(2):505- 515.
- [21] Khatun, R.; M. D. Zulfikar khan; S. Dey and S. Motasim Billah (2018). Effect of vermicompost and chemical fertilizer on the growth of yield and nutrient content of Ipomoea aquatica and Basella alba. Journal of Agriculture and Ecology Research International, 16 (2): 1-9.
- [22] Dhakar, S. ; K.Swaroop ; K.P.Singh ; V.K.Sharma ; N.Singh and S.Singh .2019. Effect of organic and inorganic sources of fertilizer on corm yield and nutrient uptake of Gladiolus. Int.J.Curr. Microbiol . App .Sci . 8(2): 2628-2633.
- [23] Khattab, E. A.; E. A. El-Housini, and H. H. Khedr .2019. EFFECT OF SOME ANTIOXIDATS (ASCORBIC ACID, PROLINE, AND SALIC ACID) ON JOJOBA PLANTS UNDER CIRCUMSTANCE OF SINAI. Iraqi Journal of Agricultural Sciences :50(4):1086- 1093
- [24] Ahmed, T. S. and A. Y. Salih. 2023. Effect of foliar application of salicylic acid, magnesium, and iron on the seedlings of Citrus medica L., 54(2):388-398.
- [25] Abdolmaleki, M. ; M . Khosh-Khui ; S. Eshghi. And A. Ramezani . 2015. Improvement in vase life of cut rose cv. "Dolce Vita" by preharvest foliar application of calcium chloride and salicylic acid. Int. J. Hort. Sci. Technol. 2(1): 55-66
- [26] Jaddo, Z. I. and K. M. Rabee. 2016. Effect Of Salicylic And Humic Acid On Vincristine And N, P, K Content In Madagascar Periwinkle Leaves. The Iraqi Journal of Agricultural Sciences – 47(2): 543-551.
- [27] Taiz, L. and Zeiger ,E. 2010.Plant Physiology. 4th edition. Annals of Botany Company. Publisher: Sinauer Associates. Tissue & Org. Cult., 53:79 - 84.
- [28] Haroun, S. A.; H. S. Aldesouquy; Abo-Hamed and El-Said, A.A. 2003. Kinetin induced modification in growth criteria, ion contents and water relations of sorghum plants treated with cadmium chloride. Acta Botan. Hunga, 45, 113 - 126.
- [29] Zainaldeen, M. A. and I. J. Abdul Rasool.2018. EFFECT OF FOLAIR APPLICATION OF GIBBERELLIN AND NUTRIENTS ON GROWTH AND YIELD OF POTATO VAR. "BURREN". Iraqi Journal of Agricultural Sciences:49(2):168-176
- [30] Salih, A.Y.; Ibraheem, H.K. Effect Of Gibbrillen Spraying And Soil Fertilizing With Humic Acid At Vegetative And Root System Growth At Mandarin Saplings Citrus reticula L. Revis Bionatura 2023;8 (2) 68.
- [31] Dhanasekaran, D. 2018 . Influence of growth regulating chemicals on growth and flowering in Jasmine (Jasminum sambac.Ait.). J. Hortl. Sci. Vol. 13(2) : 221-226.

Knowledge Guidance Needs of Rural Women in the Field of Raising Domestic Chickens in Al-Elm District / Salah Al-Din Governorate

Walaa Hussein Muhaimed Aljubouri¹ and Sahab Ayed Al-Ajili²

^{1,2}Department of Economics and Agricultural Extension, Faculty of Agriculture, Tikrit University, Tikrit, Iraq.

¹E-mail: walaa.h.m0370@tu.edu.iq

²E-mail: Sahabalyousif@tu.edu.iq

Abstract. The study aimed to identify the level of cognitive guidance needs of rural women in the field of raising domestic chickens in the district of science / Salah al-Din Governorate, as well as to identify the cognitive guidance needs of rural women in each of the following areas (housing construction, hatching, nutrition, health care, productivity of domestic chickens), as well as finding the descending order of the fields according to the percentage weight of the cognitive extension needs, and identifying the correlation between training needs and a number of independent variables related to the respondents. Included Research Community All rural women in the district of science numbered (1100) women, a simple random sample of them was elected by 10% with a size of 110 respondents, a questionnaire consisting of two parts was prepared, the first part included questions Related to the independent variables related to the respondents The second part is such as the dependent factor, which is a measure of the cognitive guidance needs of rural women in the field of domestic chicken breeding, which consisted of five areas measured by 47 test items distributed on those areas were measured honesty in both types of virtual and sincerity of the content by presenting the questionnaire to a group of experts and specialists As for stability, the Alpha Cronbach method was used to extract it Where the value of the stability coefficient was (0.86). And the validity coefficient is 0.927, and thus the data collection tool was characterized by high stability and validity, and many statistical means were used to analyze the study data. The study concluded that there is a great need for rural women to educational and extension courses in order to raise the level of their knowledge in the field of raising domestic chickens in the study area. The study recommended the need for the Agricultural Extension Authority, in cooperation with the Agricultural Division and the veterinary departments in the district of science, to organize activities and extension and training programs that focus on increasing the knowledge and skills of rural women in the field of home breeding for chickens.

Keywords. Agricultural extension, Rural women, Chicken.

1. Introduction

Agricultural extension is considered an essential pillar of agricultural development and an effective tool. It is the main channel for the transfer of agricultural research results and modern

technical methods into application and implementation in farmers' fields in order to improve and increase agricultural productivity [1]. The development of the agricultural sector plays a vital role in ensuring food security, achieving economic diversification, alleviating poverty at the global level, as well as achieving balance between food resources and population growth, and the provision of inputs from the industrial, productive and other service sectors [2]. In addition, it is the most important sector in the economies of most developing countries [3]. The human element is the main goal of agricultural extension through its efforts for the purpose of making a directional change, knowledge, and skills among rural people, and agricultural extension directs its activities and programs to all members of the rural community and does not exclude a category of women, men, children and the elderly [4]. It is taken for granted in many countries of the third world that rural women constitute half of the rural society, if not more than that, and this shows the importance of rural women as one of the groups that must be developed in order to advance the rural community, so it pays special attention to providing extension services to them according to their tasks in the fields of home and farm economics [5]. Women have a major role in improving the standard of life within their families and in the society in which they live if they have the appropriate capabilities, where rural women represent a high percentage of the total population, and supervise an important part of the elements of life in the countryside, so they are an important human wealth [6]. During many productive projects such as food processing and domestic chicken farming, they often replace men when they are outside the home [7].

The field of animal production is one of the basic components and main pillars in the agricultural sector, where livestock is one of the most important areas of animal production, but the first pillar of it, and poultry production (poultry breeding) represents the second source of animal production, as it contributes to many food industries and provides agricultural land with organic fertilizers, which is an important element to achieve food security, especially from animal protein, as it provides job opportunities for many citizens. It contributes to increasing their incomes [8]. Poultry (Poultry)) One of the main sources of animal protein, it provides the consumer with eggs (eggs) and meat (meat), in addition to that, its meat has a high percentage of protein compared to the meat of other animals. [9] The domestic chicken has a high nutritional conversion efficiency compared to the rest of the animals, where it reaches (1:2), meaning that the bird consumes (2 kg) feed to produce (1 kg) meat, while the efficiency of food conversion in sheep is (1:5.5), meaning that the animal consumes (5.5 kg) feed to produce (1 kg) meat. Rural women are mainly responsible for the domestic breeding of domestic chickens and the associated production of meat and eggs, and this is one of their most important roles in agricultural production [10]. Rural women work in agriculture in all its branches, but their work in the field of plant production and animal husbandry far exceeds their work in any other field, and despite the importance of the role of rural women in raising domestic birds, the vast majority of them still use primitive methods in raising poultry and domestic birds [11]. Which leads to many problems, including causing the spread of diseases, which confirms the need for these rural women to use safe means to raise chickens in a way that ensures commitment to health aspects during the education process and this cannot be achieved without involving them in training programs based on the proper identification of their training needs and training is one of the areas of development of the general individual, through which the individual is activated mentally and skillfully and made in a state of readiness to face working conditions to reach the goals that the institution seeks to reach, [12]. Studies [13], [14] and [15] have agreed that the rural women who were all included in their studies that they need information and guidance on raising domestic chickens to improve their productive reality and make them able to adapt to the developments and changes that occur in raising domestic chickens.

In the province of Salah al-Din, the judiciary of science is one of the distinct districts in agricultural production, both plant and animal, where rural women, who represent half of the community in the male judiciary, participate in the majority of agricultural activities and events [16]. Therefore, it was necessary to conduct the current study to identify the cognitive guidance needs of rural women in the judiciary of science in the field of raising domestic chickens and the research problem can be highlighted through the following research questions:

- What are the knowledge guidance needs of rural women in the field of raising domestic chickens in the district of science in general / Salah al-Din Governorate?
- What are the knowledge guidance needs in each field of domestic chicken breeding?
- What is the correlation between the cognitive guidance needs of rural women in the field of domestic chicken breeding and a number of personal variables?

1.1. Research Objectives

- Identify the knowledge guidance needs of rural women in the field of raising domestic chickens in Al-Alam District / Salah Al-Din Governorate in general.
- Identify the knowledge guidance needs of rural women in each of the following areas (housing construction, hatching, nutrition, health care, domestic chicken productivity).
- Descending order of research fields according to the percentage weight of cognitive guidance needs.
- Identify the correlation between the cognitive guidance needs of rural women in the field of raising domestic chickens and each of the personal variables such as (age, educational level, marital status, family type, number of family members, type of family work studied, purpose of breeding, number of years of breeding, sources of information on raising domestic chickens, trend towards raising domestic chickens).

1.2. Statistical Hypotheses

- There is no significant correlation between the cognitive guidance needs of rural women in the field of raising chickens and the age.
- There is no significant correlation between the cognitive guidance needs of rural women in the field of raising chickens and the educational level.
- There is no significant correlation between the cognitive guidance needs of rural women in the field of chicken breeding and social status.

1.3. Procedural Definitions

- Cognitive extension needs: It means in this research the extent of rural women's knowledge in the implementation of technical guidance practices in the field of raising domestic chickens.
- Rural women: It is the woman who lives in rural areas in Al-Alam District / Salah Al-Din Governorate and raises domestic chickens.
- Field of breeding: It is the processes practiced by the respondent in the process of raising domestic chickens.

2. Materials and Methods

2.1. Research Methodology

The descriptive approach was used in the current research to achieve the objectives of the study, as this approach is based on describing the phenomenon in a quantitative or qualitative way in a certain period of time in order to identify the phenomenon in terms of content and content and reach results and generalizations that help in understanding and developing reality [17].

2.2. Search Area

The district of science in Salah Al-Din province was chosen as a region not due to research operation with laboratories in The Ministry of Science and Technology Ethical approval No. ISO 17025, as it includes a large number of rural women who perform various agricultural tasks in the agricultural and animal field, especially raising domestic chickens, as it is one of the sources of income for many rural families in the district, in addition to benefiting from meat and eggs as foods preferred by many people in the region.

2.3. Research Community and Sample

The research community included all rural women in the district of science, numbering (1100) women, a simple random sample of them was elected by 10%, the size of which reached 110 respondents.

2.4. Preparation of the Data Collection Tool

A questionnaire consisting of two parts was prepared as follows:

- Includes questions related to the independent variables of the respondents, namely (age, educational level, marital status,).
- Represents the dependent factor, which is a measure of the cognitive guidance needs of rural women in the field of raising domestic chickens, which consisted of five areas measured by 47 test items distributed on those areas.

2.5. Measuring the Variables of the Study

The variables of the study were measured as follows: Personal variables, which included: Age: It was measured by the number of years of age of the respondent at the time of data collection. Educational level: The educational level of the respondents was measured according to the following levels (illiterate, primary, intermediate, preparatory, bachelor's, with a higher certificate), and the following symbols were given to her (1, 2, 3, 4, 5, 6) respectively and finally marital status: The social status was measured according to the following levels (married, widowed, divorced) and was given the following symbols (3, 2, 1) and respectively .as for the dependent factor, the cognitive guidance needs of rural women were measured through (47) standard test items distributed over five areas and the degree (100) prepared the standard degree on which the degrees of research fields were distributed according to the relative importance of the fields, where the theoretical range of the field of housing construction (0-10) was while the theoretical range of the field of hatching (0-14) The theoretical range of the field of nutrition (0-28) while the theoretical range of the field of health care (0-22) and the theoretical range of the field of productivity of domestic chickens (0-26).

3. Results and Discussion

3.1. The First Objective: The Level of Knowledge Guidance Needs of Rural Women in the Field of Raising Domestic Chickens in the District of Science / Salah Al-Din Governorate

The results showed that the lowest degree expressing the cognitive guidance needs of rural women in the field of raising domestic chickens in Al-Alam District / Salah Al-Din Governorate is 30.40 degrees and the highest degree is 68.20 degrees with an average of 53.42 degrees, and a standard deviation of 7.00, the respondents were distributed into three categories using the range law and it appeared that the highest percentage within the medium need category, as shown in Table (1).

Table 1. Distribution of respondents according to categories of cognitive guidance needs in general.

Categories of need	Number	%	Arithmetic mean of need
Low (30.40-42.9)	12	10.9	39.22
Medium (43-55.59)	59	53.6	50.07
High (55.60-68.20)	39	35.5	59.43
Total	110	100%	SD=7.00

It is clear from Table (1) that the highest percentage of 53.6% of the respondents came within the medium need category with an average knowledge need of 50.07 degrees, followed by the high need category by 35.6% and an average knowledge need of 59.43 degrees, so the level of need of the respondents is described as medium tends to rise, and this means that the largest percentage of respondents lack the necessary experience and knowledge in raising domestic chickens, and the reason may be due to the fact that the respondents follow traditional inherited experiences in raising chickens, so they need Knowledge extension activities to provide them with experiences and information to improve the level of their knowledge towards raising chickens and follow the guidance recommendations related to raising chickens.

3.2. The Second Objective: Identify the Level of Knowledge Guidance Needs in Raising Domestic Chickens in the District of Science / Salah Al-Din Governorate in Each of the Following Research Areas : (Construction of Chicken Houses, the Field of Hatching, the Field of Nutrition, the Field of Health Care, the Field of Productivity of Domestic Chickens)

3.2.1. The First Field: The Field of Housing Construction

The results showed the lowest degree expressing the cognitive guidance needs of rural women in the field of housing construction (2) degree and the highest degree 8 degree with an arithmetic average of 3.63 degrees and a standard deviation of 1.82, the respondents were distributed into three categories using the law of range and the highest percentage of respondents appeared within the low category of need, as shown in Table (2).

Table 2. Distribution of respondents according to categories of knowledge extension needs in the field of chicken housing construction.

Categories	Number	%	Arithmetic mean of need
Low (2-3)	53	48.2	2.00
Medium (4-5)	36	32.7	4.00
High (6- and more)	21	19.1	6.57
Total	110	100%	SD=1.82

It is clear from Table (2) that the highest percentage of 48.2% of the respondents came within the low category with an average need of 2.00 degrees, followed by the intermediate category by

32.7% with an average need of 4.00 degrees and indicates that the respondents are those with low cognitive guidance needs tend to average, and the reason for this may be due to the fact that the process of building domestic chicken houses is one of the inherited processes that do not need experience and knowledge in its construction and that the respondents have good information and experience. On the field of building chicken houses, and this reflects positively on their lack of need for cognitive extension activities, and that there are only 19% of the respondents who need experiences and guidance information in the field of raising domestic chickens in the field of housing construction.

3.2.2. Second Area: Hatching

The results showed that the lowest degree expressing the level of needs in the field of hatching 4 degrees and the highest degree 10 with an arithmetic average of 6.07 degrees, and a standard deviation of 1.68, the surveys were distributed into three categories using the law of range and it appeared that the highest percentage of respondents within the medium category, as shown in Table (3).

Table 3. Distribution of respondents according to categories of knowledge guidance needs in the field of hatching.

Categories	Number	%	Arithmetic mean of need
Low (4-5)	33	30.0	4.00
Medium (6-7)	41	37.3	6.00
High (8- and above)	36	32.7	8.17
Total	110	100%	SD=1.68

It is clear from Table (3) that the highest percentage of 37.3% of the respondents fall within the medium need category with an average need of 6.00 degrees, followed by the high need category by 36% and an average need of 8.17 degrees, and the result indicates that 70% of the respondents are with medium and high cognitive guidance needs, which is a large percentage, and the reason for this may be due to the weak information of rural women in the field of hatching, which is an indicator of the rural woman's need for cognitive extension experiences in the hatching process.

3.3. The Third Objective: The Descending Order of the Research Fields According to the Percentage Weight of the Cognitive Guidance Needs

The results of the descending order of the research fields came with the highest percentage weight of need 26.02 and the lowest percentage weight of 13.03, as shown in Table (4).

Table 4. Descending order of indicative needs areas according to the percentage weight of need.

The soil	Celsius weight	Maximum degree	Rate of need	Domains	t
1	61.07	28	17.10	Nutrition	1
2	53.38	26	13.88	Productivity of domestic chickens	2
3	53.27	22	11.72	Healthcare	3
4	43.35	14	6.07	Breed	4
5	36.3	10	3.63	Construction of chicken housing	5

It is clear from Table (4) that the field of nutrition came in first place when arranging the fields according to the percentage weight of the fields and the reason for this may be the weakness of the respondents' knowledge of feeding domestic chickens in terms of quantities and timings of providing food, as well as the difference in quantities and quality for young chicks and what are

the necessary additions to the diet as well as factors affecting the quantity of the diet, and the respondents may not know these quantities and their difference between adult and middle-aged chickens, as well as laying hens and what must be provided from the diet of chicks Neologians, which is an indicator of their need for theoretical and applied guidance information, and in second place came the field of domestic chicken productivity, lack of knowledge of laying hens varieties or lack of knowledge of the importance of chicken exposure to sunlight, as well as neglect of some factors that negatively affect the productivity of domestic chickens, while the field of health care came in third place, and the reason for this may be that health care is one of the curative and preventive measures To protect chickens from rampant diseases and ways to apply these procedures, which may be the reason for the weakness of the information and expertise of the respondents in the field of health care, and in fourth place came the field of hatching and the reason may be the weakness of the information and experiences of the respondents in the quality of eggs prepared for hatching and their size and what are the factors affecting the process of hatching eggs, while the field of building chicken housing ranked fifth and may be the reason for this that the respondents have experiences on how to build chicken housing and materials used in construction and cleaning housing.

3.4. Fourth Objective: Identify the Correlation Between Training Needs and the Independent Variables Studied

3.4.1. Age

The results of the research showed that the lowest years of age of the respondents 21 years and the oldest years of age 64 years, with an arithmetic mean of 38.74 and a standard deviation of 10.71, classified the surveys into three categories using the law of range, and it appeared that the highest percentage of respondents within the category of young years of age, as shown in Table (5).

Table 5. Distribution of Respondents by Age Variable Categories.

T	Categories	Number	%	Average	r-Value	t-Value	Moral
1	Kids (21-34) years	46	41.8	54.09	0.10-	1.049-	N.S
2	Averages (35-48) years	33	30.0	54.14			
3	Adults (49- and above) years	31	28.2	50.97			
	Total	110	100%	N.S = Insignificant			

To find between the cognitive guidance needs and the variable of the years of life of the respondents, Pearson correlation coefficient was used, which amounted to -0.10 and indicates a negative relationship between the two variables, and to calculate the significance of the relationship, the t-test was used, which amounted to 1.049 - and when compared with the tabular value of t-1.658, it was found to be insignificant, so the imposition of nothingness is accepted, and the reason for this may be that the respondents of all age groups do not differ in terms of their cognitive guidance need, Therefore, they have knowledge guidance needs in the field of raising domestic chickens. This finding is consistent with what I found [18] and [19].

3.4.2. Educational Level

The educational level variable was classified into six categories, and the highest percentage was within the category of reading and writing, and the highest percentage appeared within the category of reading and writing, as shown in Table (6).

Table 6. Distribution of Respondents by Educational Level Variable Categories.

T	Categories	Number	%	Average	r-Value	t-Value	Moral
1	Mom	11	10.0	55.40	0.32**-	3.52-	0.01
2	He reads and writes	36	32.7	54.96			
3	Primary	23	20.9	53.68			
4	Medium	18	16.4	53.10			
5	Bachelor	18	16.4	49.19			
6	Higher degree	4	3.6	45.05			
	Total	110	100%	**Significant at probability level 0.01			

To find between the correlation between cognitive guidance needs and the educational level variable, use the Spearman's rank correlation coefficient, which amounted to -0.32, which indicates a negative relationship between the two variables, and to ensure the significance of the relationship, use the t-test, which amounted to -3.52, and when compared with the value of t. The tabular of 2.358 was found to be significant for the probability level of 0.01, so it rejects the imposition of nothingness and accepts the alternative hypothesis, which states (there is a significant relationship between the two variables), and the reason for this may be that the respondents domestic chicken breeders with a higher educational level are more experienced and informed in raising domestic chickens and thus they are less in need of experience and information than their peers with a low educational level. This result is consistent with what I found [18] and [19].

3.4.3. Marital Status

The marital status variable was classified into four categories and the highest percentage was among married women, as shown in Table (7).

Table 7. Distribution of Respondents by Categories of Marital Status Variable.

t	Categories	reiteration	%	Average	-Value r	Moral
1	I am single	38		47.94	0.05.-	N.S
2	Married	54		48.96		
3	Widow	10		47.82		
4	Divorced	8		46.78		
	Total	100	100%	N.S= Insignificant		

To find the correlation between the cognitive guidance needs and the social status variable, use the Spearman correlation coefficient ordinal, which amounted to 0.05 - and indicates a negative apparent relationship between the two variables, and the reason for this may be that the respondents from all categories of social status do not differ from each other in the extent of their need for information and experiences guidance in the field of raising domestic chickens, and thus may all be similar in the extent of their need for information and experiences guidance.

Conclusions

- The level of cognitive guidance needs is average and tends to be high overall. It follows from this that there is a great need for educational and guidance courses in order to raise the level of knowledge of rural women in the study area in the field of raising domestic chickens.
- The results of the order of the levels of cognitive guidance need in descending order "according to" the areas of research showed that the respondents have a cognitive weakness in raising domestic chickens, where the results came as follows: nutrition in the first place, productivity in second place and health care in third place, and therefore it can be concluded that there is a great need for extension and veterinary training activities to

- provide them with information, knowledge and experiences that help satisfy their training needs in the mentioned areas.
- The high level of extension needs of rural women in the aspect of raising domestic chickens indicates a weakness or deficiency in the extension and veterinary services provided to nannies in the district of science.

References

- [1] Khairy, Mohamed, Essam Sayed Shaheen and Sherif Abdel Hadi Mohamed Hassan, (2015), Efficiency and Effectiveness of the Agricultural Extension Authority in Menoufia Governorate (Case Study), Journal of Economics and Social Sciences, Mansoura University, Volume VI (3).
- [2] Abdel Halim, Mohamed Fathi Ismail, (2022), Rural Women's Participation in Some Agricultural Production Activities in Assiut Governorate, Journal of the Agricultural Extension Association, Volume (26), Issue (4).
- [3] Al-Zoubi, Saja Taha, Abdul Ghani Abdul Latif and Ahmed Hassan Jadoua, (2013), Determining the Impact of Economic and Social Variables on the Level of Rural Women's Contribution to Agricultural Work, Jordanian Journal of Agricultural Sciences, Issue (2), Volume (9).
- [4] Muharram, Ibrahim, Ibrahim Rihan, Magdy Yahya and Abdel Aziz Shafiq (2018). Fundamentals of Rural Development, Ain Shams University, Faculty of Agriculture, Department of Rural Community and Agricultural Extension.
- [5] Al-Khodarji, Manal Mohamed Ali, (2019), The Role of Agricultural Extension in Achieving Family Food Security in New Lands from the Point of View of Researchers at the Agricultural Extension Department, Economic and Social Studies Division, Desert Research Center, Journal of Agricultural Sciences and Environment, Damanhour University, Issue (1), Volume (18).
- [6] Al-Khouli, Amani Saeed Abdel Hamid and Iman Mustafa Ibrahim, (2022), Rural Development Efforts in Improving the Quality of Life of Rural Women Breadwinners in the Villages of Ashmoun Center in Menoufia Governorate, Arabic Journal of Science and Research Publishing - Journal of Humanities and Social Sciences, Issue Twelve, Volume VI.
- [7] Moawad, Mahmoud Mustafa, Samia Abdel Azim Mahrous and Salah Mohamed Amer (2019). Agricultural Extension, Ain Shams University, Faculty of Agriculture, Department of Rural Community and Agricultural Extension.
- [8] Salem, Abdel Hamid Salem, Mervat Shehata and Shaima Abdel Majeed El-Khouly, (2023), The Role of Rural Women in the Development of Animal and Poultry Production in Some Villages of Menoufia Governorate, Journal of Agricultural, Environmental and Veterinary Sciences, Issue (2), Volume (7).
- [9] Ministry of Planning - Agricultural Department (1996), The reality of the fields and production of broilers in the Iraq and the role of the state in their development,
- [10] Ramadan, Mahdi Ahmed and Ebtisam Bassiouni Radi El-Meligy, (2015), The behavior of rural women in the field of safe breeding of domestic birds in Kafr El-Sheikh Governorate, Egyptian Journal of Agricultural Research, Issue (93), Volume (3).
- [11] Al-Shal , Ahmed Mohamed Ibrahim Ahmed, (2018), An analytical study of a conflict over the actual roles of rural women in one of the villages of Dakahlia Governorate, J. Agrik Magazine, Economics and Social Sciences, Issue (3), Volume (9).
- [12] Al-Zaidi, Abdullah Awad, Muhammad Suleiman Sakran, Hajj Ahmed and Abdul Aziz Darwish (2001), trainees' attitudes towards extension training courses implemented by the Ministry of Agriculture and Water in the Kingdom of Saudi Arabic Arabia, Research Center of the College of Agriculture, King Saud University.
- [13] Salim, Najm al-Din and Roaa Mohamed Hamed (2011), The contribution of rural women in agricultural work and their relationship to some variables in some villages in Nineveh Governorate, Researches of the Fifth Scientific Conference of the Faculty of Agriculture (26-27) April, Tikrit University



- [14] El-Deeb Sherine Mahmoud Hamdi (2004), The Cognitive Extension Needs of Rural Women in the Field of Poultry Production in Kafr El-Sheikh Governorate, Master's Thesis, Faculty of Kafr El-Sheikh, Tanta University.
- [15] Al-Hafez, Asma Zuhair Younis (2005), the level of knowledge of rural women in some agricultural fields and family care and its relationship to some variables, a field study in the villages of Al-Kibbeh and Al-Shuraykhan, Nineveh Governorate, Master's thesis, College of Agriculture and Forestry, University of Mosul.
- [16] Al-Jubouri, Khalaf Jassim Saleh (2020), Participation of rural women in local community activities in Al-Alam District - Salah Al-Din Governorate
- [17] Mahmoud, Heba Mohsen, (2021), Descriptive Approach, Postgraduate Lectures, College of Education for Girls, University of Mosul.
- [18] Al-Janabi, Nour Mohamed Abdullah, (2010), The level of knowledge of rural women in the field of raising domestic chickens in Baiji District - Salah Al-Din Governorate and their relationship to some factors, Faculty of Agriculture, University of Tikrit.
- [19] Kazim, Ahlam Talib, (2013), Cognitive needs of rural women in raising laying hens in cages in the central region of Iraq, Department of Extension and Transfer of Agricultural Techniques, Faculty of Agriculture, University of Tikrit.

Isolation and Identification of *Bifidobacterium* Sp. Bacteria in the Stomach of Honey Bees

Mohammed Shakir Manjy¹ and Kamila Ward Shaher²

^{1,2} Plant Protection Department ,College of Agricultural Engineering Sciences,
University of Baghdad, Baghdad, Iraq.

¹E-mail: mohammed.shakir@coagri.uobaghdad.edu.iq

²E-mail: kamila.shaher@coagri.uobaghdad.edu.iq

Abstract. This study was conducted in the apiaries of the College of Agricultural Engineering Sciences / University of Baghdad, and 15 packages of *Apis mellifera* honey bees were selected. As *Bifidobacterium* Sp. bacteria were isolated from the stomach of honey bees, where samples were taken from the bees at the rate of (50) bees for each treatment , the stomach of the bees was withdrawn, Then, it was mashed and inoculated on MRS agar solid culture medium in plates, and the plates were incubated in the incubator at 37°C for 48 hours. The results of the molecular diagnosis by PCR technique showed that the proportions of matching the nucleotide sequence of a part of the 16S ribosomal RNA gene of the bacteria *Bifidobacterium* Sp. isolated from honey bees in Baghdad province, which were multiplied using the primer kit (16 SrRNA) and its equivalent sequences retrieved from the gene bank. The phylogenetic tree, which was drawn by the (Tamura) program of the type of macrophages, shows the genetic relationship that was built from the nucleotide sequences of part of the genetic region 16 SrRNA ribosomal gene belonging to the bacteria *Bifidobacterium* Sp. isolated from honey bees in Baghdad Governorate. They were duplicated using primers set (16 srRNA) and their equivalent sequences retrieved from the GenBank.

Keywords. *Apis mellifera*, Beneficial microbes, *Bifidobacterium*, Honeybees.

1. Introduction

The western honey bee *Apis mellifera* L. is a social insect that lives in groups and works as a single unit to preserve its species or contribute to improving the ecosystem in the environment in which it lives. The importance of all types of honey bees lies in their ability to pollinate many crops and vegetables, as well as bee products such as Honey, wax, propolis and royal jelly are important in human nutrition and treatment [1-3]. Studies have shown that honeybees are the most economically valuable pollinator of crops in the world and that the pollination of some types of fruits, nuts and seeds decreases by more than 90% in the absence of honeybees [4,5]. It was also found that the abundance and diversity of wild plant species Insect pollinators decrease in areas with low honey bee hives [6-8].

There is a close connection between honey bees and the microscopic organisms that live inside their intestines, as these organisms, including lactic acid bacteria, digest food as well as detoxify harmful or toxic molecules, and also provide essential nutrients to the bees [9-12] , Bees' gut microbial populations play a crucial role in their well-being because they protect the bees from

parasites and diseases, supply growth factors, and boost immunity [13-16]. There are various bacteria found in honey bees' digestive tracts, such as the species *Bifidobacterium* Sp., and their presence is influenced by their preferences and how they interact. Which make up around 15% of the gut bacteria [17-19], settle in the bees' stomachs, and are transported by pollen and flower nectar. *Bifidobacterium* bacteria are members of the Actinobacteria class; they are Gram-positive, anaerobic, non-spore-forming, lactate-and acetate-producing bacteria. They are an essential part of the honey bee's gastrointestinal community, right alongside lactobacilli [20,21]. These bacteria have an important role in multiple fields related to technology and food vitality, as well as nutrition, health, safety, and protection of honey bees from pathogens [22,23]. These bacteria are of great importance to honey bees through their intrinsic symbiotic importance because they help in Digesting food also protects bees from pathogens, increases their immunity, and removes toxins from the body. It is transmitted between members of the colony, from adult bees to newly emerging bees, via Trophallosis [24-27]. Two species of *Bifidobacterium* bacteria—*Bifidobacterium* asteroides and *Bifidobacterium* coryneforme—have been identified from bee guts, and both play a crucial role in bee health and disease prevention [28-31].

Nine bifidobacterial species have been identified so far from various Apidae bee species' digestive tracts; the four most recent additions to this list are *Bifidobacterium* mizhiense, *Bifidobacterium* choladohabitans, and *Bifidobacterium* polysaccharoliticum, all of which are honey bee species. [32-34]. There were many types of *Bifidobacterium* bacteria, where 108 species of bacteria were isolated and identified from bees [35-37]. The study aimed to diagnose the genus of beneficial bacteria *Bifidobacterium* Sp. Found in the digestive system of honey bee workers using PCR technology.

2. Materials and Methods

2.1. Partial Diagnosis by PCR Technique (Sample Preparation)

For each treatment, (20) bees collected samples from the bees. The bee stomach was removed, crushed, and inoculated in plates on MRS agar. 48 hours were spent incubating the plates in the incubator at 37 °C.

2.2. DNA Extraction

1. Colonies growing on MRS agar were washed by adding distilled water 100 µl for the purpose of obtaining bacteria. In tubes with covers, the germs were scraped off and put. The tubes underwent a 5-minute centrifugal treatment in a centrifuge. A precipitate was ignored while a filter was developed. The filtrate was added to the precipitate 750 µl of DNA EiuTion Buffer and mixed well.
2. Add 50-100 mg (wet weight) of the bacteria that had previously been suspended in 200 microliters of water or isotonic solution (PBS). or approximately 200 mg of tissue into a ZR Bashing Baed Tm lysis tube (0.1 mm and 0.5 mm) and 750 µL of lysis solution was added to tube 2.
3. The tube was installed in a granules beater attached to a 2 ml tube holder, then the beater was operated at maximum speed for 5 minutes.
4. The centrifugation process was conducted on the tube containing the decomposition medium ZR Bashing Baed Tm in the centrifuge at a speed of 1000 cycles for one minute.
5. Place 400 µL of the supernatant into the collecting tube's Zymo Spin TM IV spin filter Corang ToP and centrifuge at 7,000 rpm for one minute.
6. 1200 microliters of bacterial DNA binding buffer solution was added to a filter in the collection tube, step (5).

7. 800 microliters of the mixture in step (6) was transferred to an IIC column3 Zymo Spin
8. TM in the collection tube, and the centrifugation process was carried out at 10,000 cycles for one minute.
9. J- Discard the supernatant from the collection tube and repeat step (7)
10. Add 200 microliters of DNA wash Buffer solution to the IIC Zymo Spin TM column. In the new collection tube, it was centrifuged at 10,000 revolutions for one minute.
11. The IIC Zymo Spin TM column was transferred to a clean centrifuge tube with a capacity of (1.5) ml, and 100 microliters of DNA buffer solution was added directly to the column (Column matrix) and a centrifugation process was performed at a speed of 10,000 cycles for 30 minutes to extract DNA.

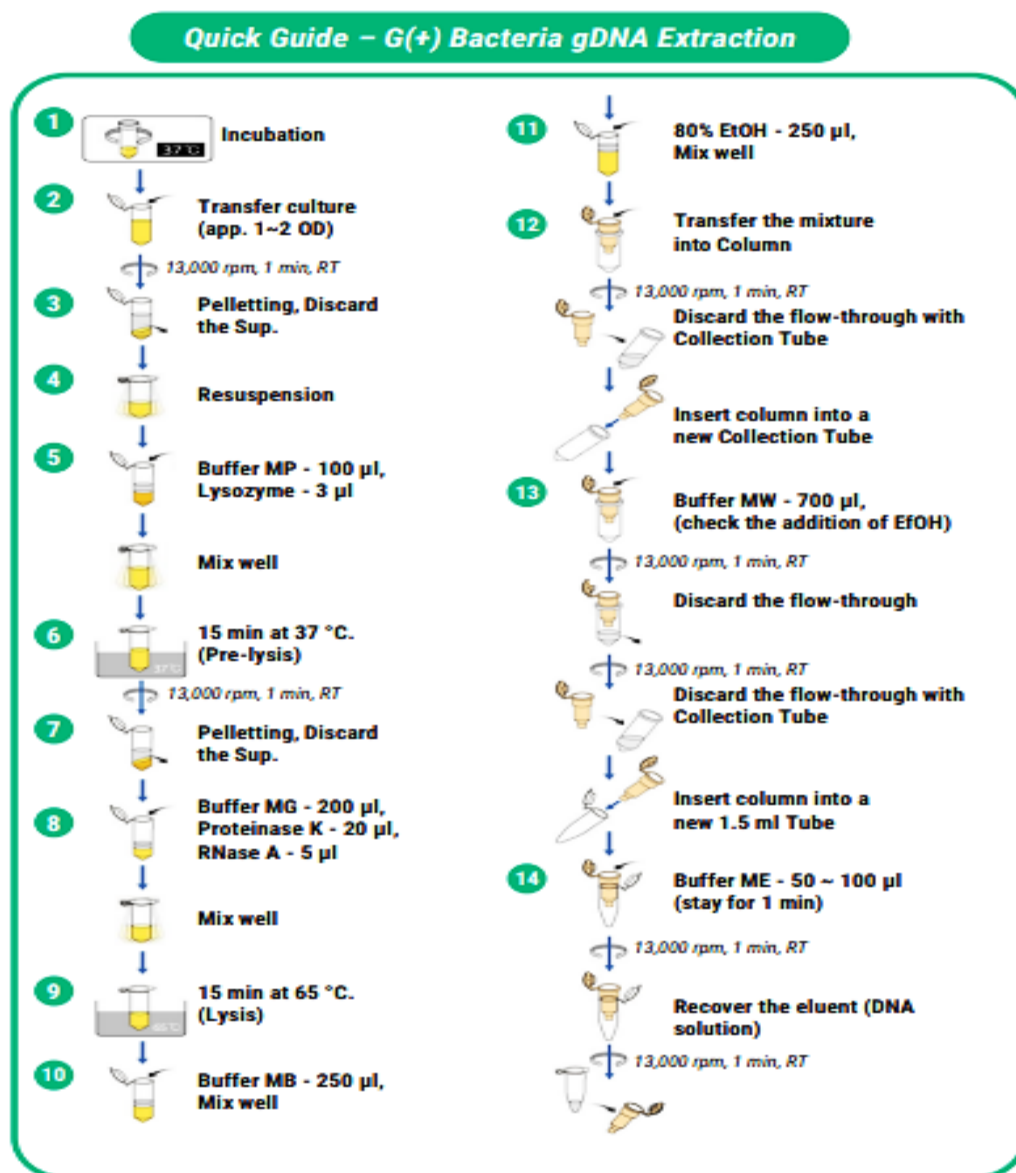


Figure1. DNA extraction of bacteria.

2.3. The Primers Used in the Interaction

(Alph DNA / USA/Canada) conducted the investigation by lyophilising the primers, dissolving them in free ddH₂O to achieve a final concentration of 100 pmol/μl as a stock solution, and then keeping a stock at -20 to prepare a 10 pmol/μl concentration as a work primer suspended. The final volume of the mixture was 100 μl.

Table 1. Specific primers pbi targeting the specific sequence of the 16srRNA gene.

Primer	Sequence	T _m (°C)	GC (%)	Product size
Forward	5'-CCGGAATAGCTCC - 3'	46.05	50	914
Reverse	5'-GACCATGCACCACCTGTGAA - 3'	56.83	50	base pair

2.4. Maxime PCR PreMix kit (i-Taq) 20μlrxn (Cat. No. 25025)

The PCR mixture was made with a 2X Master mix solution and several different types of PreMix Kits from the Maxime PCR PreMix Kit, which are designed for different types of experiences. One tube contains all the ingredients for one round of PCR in the Maxime PCR Pre Mix Kit (i-Taq), including i-Taq DNA Polymerase, dNTP mixture, reaction buffer, and more. You won't find a more convenient system or better product than this one. For starters, it comes with everything you need to run a polymerase chain reaction (PCR); all you need is a template DNA, primer set, and D.W. to finish the process. The second justification is that it comes with electrophoresis buffer specifically for gel loading, allowing us to perform gel loading procedures untreated. Its quick and easy-to-use procedure makes it suited for a wide range of sample experiences.

Table 3. Components of the Maxime PCR PreMix kit (i-Taq).

Components	Concentration
Taq PCR PreMix	5μl
Forward primer	10 picomols/μl (1 μl)
Reverse primer	10 picomols/μl (1 μl)
DNA	1.5μl
Distill water	16.5 μl
Final volume	25μl

Table 4. The Components of the Monoplex PCR master mix.

Material	Volume
i-Taq DNA Polymerase	5U/μl
DNTPs	2.5mM
Reaction buffer (10X)	1X
Gel loading buffer	1X

2.5. Agarose Gel Electrophoresis of DNA

Using electrophoresis, DNA fragments can be identified following extraction or the interaction between PCR and standard DNA can be detected, allowing for the differentiation of bundle size on an Agarose gel.

2.6. Prepare of the Agarose Gel

Sambrook et al. [38] states that 1.5 g of agarose melted in 100 ml of TBE buffer that has been previously produced yields a 1.5% condensation agarose gel. It has been allowed to cool down from 45 to 50°C after the agarose has been heated to boiling. After preparing the agarose support

plate by fastening the comb to produce holes that would hold the samples, the gel has been poured into the pour plate. After carefully pouring the gel so as not to create air bubbles, it was allowed to cool for half an hour. Gentle removal of the comb from the solid agarose has been accomplished. The electrophoresis tank, which stands for the horizontal unit, has the plate securely fastened to its platform. The surface of the gel has been covered with TBE buffer, which has been added to the tank [39-48].

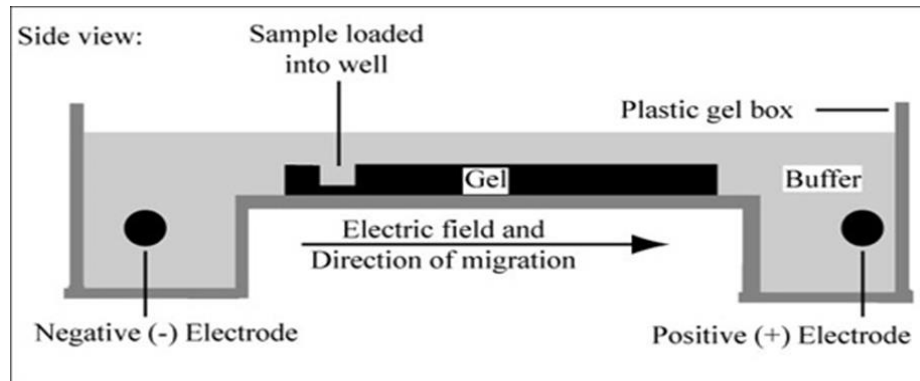


Figure 2. The electrophoresis system.

3. Results and Discussion

3.1. Gel Electrophoresis

The Korean corporation used the (BLAST) tool from the National Centre for Biotechnology (NCBI) to analyse the SKONSC data, and they also used the program BioID to incorporate them [49].

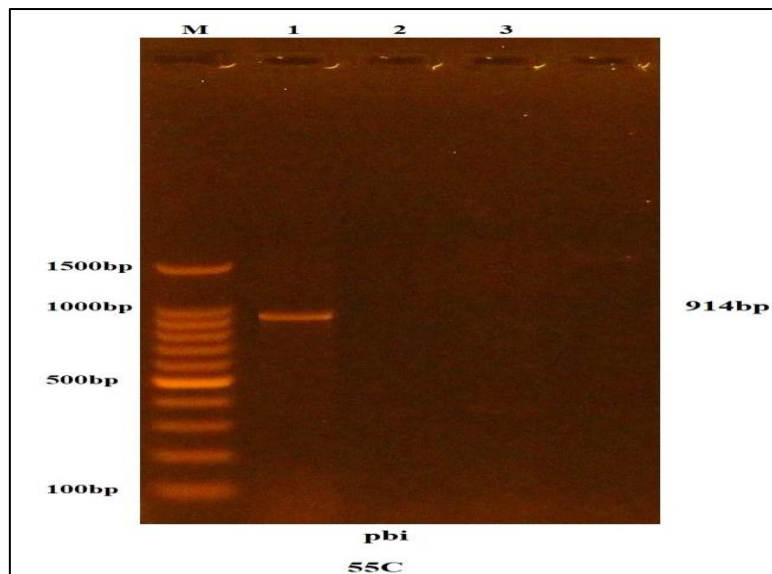


Figure 3. The DNA duplicate product using crystal chain reaction (PCR) isolated from the stomach of the honey bee *Apis mellifera*.

3.2. Trees of *Bifidobacterium* Sp. and Relationship with Other Isolates Recorder in NCBI

The phylogenetic tree of the type connective tissue adjacent to it shows the genetic relationship. It was built from the nucleotide sequences of a part of the 16S ribosomal RNA gene region belonging to the bacterium *Bifidobacterium* Sp. isolated from honey bees in Baghdad province which were replicated using the primer set (16 SrRNA) and its counterparts, this tree was drawn using the MEGA11 program[49], of its equivalent sequences retrieved from the Gen Bank. The nucleotide sequence of the 16S ribosomal RNA gene of *Bifidobacterium* Sp. was used as an out group comparison [50-53].

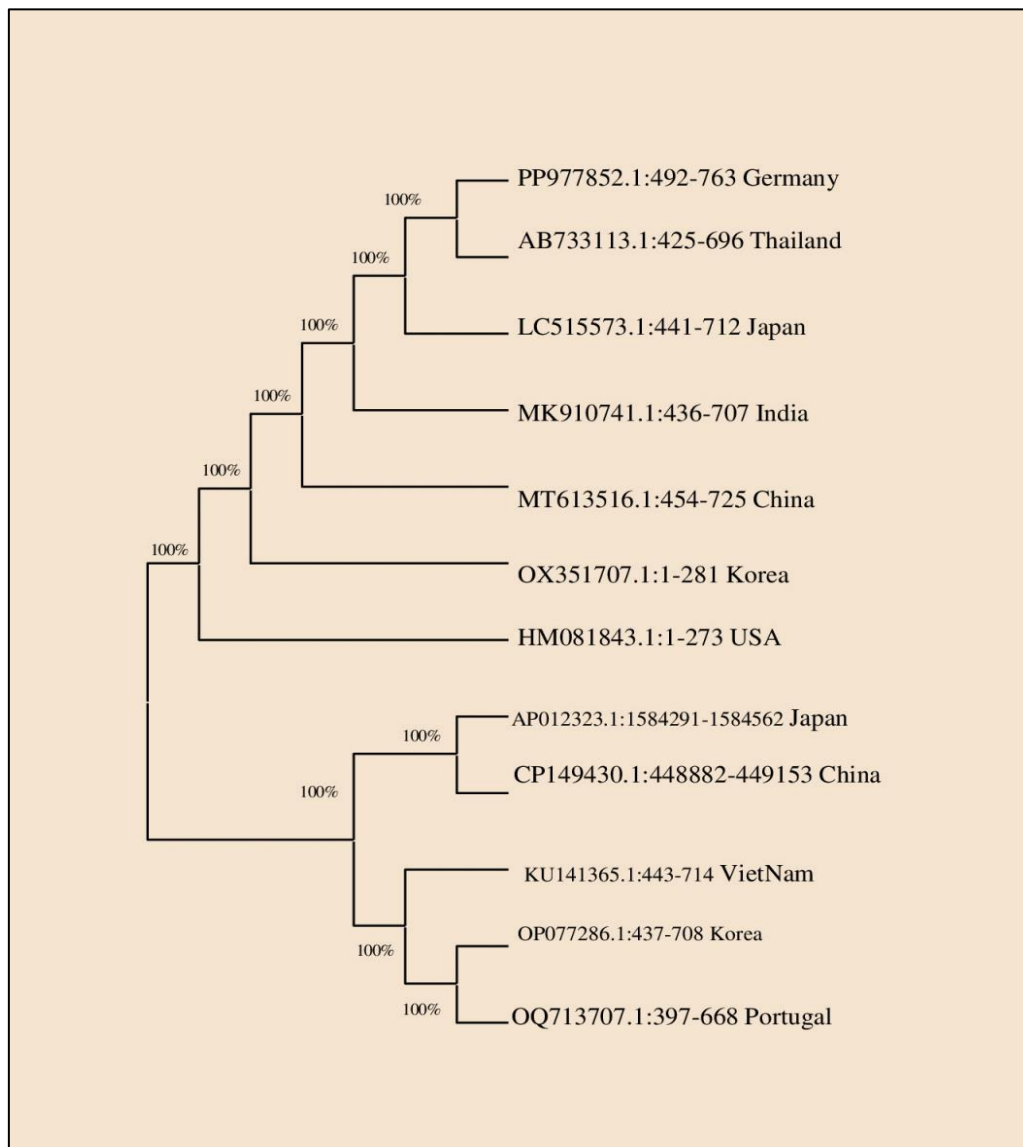


Figure 4. Trees of *Bifidobacterium* Sp. and relationship with other isolates recorder in NCBI.


An official website of the United States government
[Here's how you know](#)

Nucleotide

GenBank

Bifidobacterium sp. strain MS1 16S ribosomal RNA gene, partial sequence

GenBank: PP916620.1

[FASTA](#) [Graphics](#)

[Go to:](#)

LOCUS	PP916620	281 bp	DNA	linear	BCT 20-JUN-2024
DEFINITION	Bifidobacterium sp. strain MS1 16S ribosomal RNA gene, partial sequence.				
ACCESSION	PP916620				
VERSION	PP916620.1				
KEYWORDS	.				
SOURCE	Bifidobacterium sp.				
ORGANISM	Bifidobacterium sp. Bacteria; Actinomycetota; Actinomycetes; Bifidobacteriales; Bifidobacteriaceae; Bifidobacterium.				
REFERENCE	1 (bases 1 to 281)				
AUTHORS	Manjy,M.S., Shaher,K.W. and Al- Sa'ady,A.J.				
TITLE	Molecular identification of bacteria isolated from the digestive tract of Honey bees in Iraq				
JOURNAL	Unpublished				
REFERENCE	2 (bases 1 to 281)				
AUTHORS	Manjy,M.S. and Shaher,K.W.				
TITLE	Direct Submission				
JOURNAL	Submitted (15-JUN-2024) plant production, college of Agricultural Engineering Sciences, Baghdad university, Al-Jadriya, Al-Jadriya, Baghdad 10071, Iraq				
COMMENT	##Assembly-Data-START## Sequencing Technology :: Sanger dideoxy sequencing ##Assembly-Data-END##				
FEATURES	Location/Qualifiers				
source	1..281 /organism="Bifidobacterium sp." /mol_type="genomic DNA" /strain="MS1" /db_xref="taxon:41200" /geo_loc_name="Iraq" /collection_date="01-Jan-2023"				
rRNA	<1..>281 /product="16S ribosomal RNA"				
ORIGIN	1 tatggtaatg tgcccgccgc cgcggtaata ctagggcgc aagcgttatc cggatttatt 61 gggcgtaaaag ggctcgtagg cggctcgtcg cgtccggtgt gaaagtcctat cgcttaacgg 121 tggatctgcg ccgggtacgg gcgggctgga gtgcggtagg ggagactgga attcccggtg 181 taacggtgga atgtgtagat atcggaaga acaccgatgg cgaaggcagg tctctgggccc 241 gtactgacg ctgaggagcg aaagcgtggg gagcgaacag g //				

Figure 5. Bifid bacterium sp. strain MS1 16S ribosomal RNA gene, partial sequence. Gen Bank: PP916620.1.

References

- [1] Al-Hujaimi, K.W., & Al-Taie, H.K. (2008). Training needs of beekeepers and their problems in Iraq. *Iraqi Journal of Agricultural Sciences*, 13(2): 173-187.
- [2] Zheng, H., Powell, J.E., Steele, M.I., Dietrich, C., & Moran, N. A. (2017). Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. *Proceedings of the National Academy of Sciences*, 114(18): 4775-4780.
- [3] Al-Badri, B.H. (2017). Economies of beekeeping in Iraq. *Iraq Journal of Agricultural Sciences*, 48(Special Issue): 126-137.
- [4] Al-Hujaimi, K.W. (2000). Environmental study of American foul brood disease on honey bee and its control by plant extracts and artificial swarm method. M.Sc., Thesis, College of Agriculture, University of Baghdad. pp. 74.
- [5] Hamed, A.H., Dhahir, S.A., & Abid, F.M. (2016). Determination of water soluble vitamins in Iraqi honey bee and compare with others types by high performance liquid chromatography. *Baghdad Science Journal*, 13(25): 447-457.
- [6] Al-Jourani, R.S., Shaher, K.W., & Abbas, A.S. (2004). Evaluating the efficiency of some means for controlling American Foul Brood disease (AFB) on honeybees. *Iraqi Journal of Agricultural Sciences*, 35(4): 95-100.
- [7] Villalba, A., Maggi, M., Ondarza, P.M., Szawarski, N., & Miglioranza, K.S.B. (2020). Influence of land use on chlorpyrifos and persistent organic pollutant levels in honey bees, bee bread and honey: Beehive exposure assessment. *Science of the Total Environment*, 713: 136554.
- [8] Abdul – Kareem, A.A. (2021). Isolation and identification of a pathogen *Nosema spp.* in honey bee workers *Apis mellifera* and efficacy evaluation of some plant extract in its control. M.Sc. Thesis, College of the Agricultural Engineering Sciences, University of Baghdad. pp. 81.
- [9] Al-Kinani, M.A. (2000). Study of European foulbrood disease on honey bees and controlling it using plant extracts. Ph.D. dissertation. College of Agricultural Engineering Sciences, University of Baghdad. pp. 89.
- [10] Bourtzis, K., & Miller, T.A. (2010). *Crepess*, Bocaraton. Insect Symbiosis. CRC Press, 1st Edition. London, 12: 301.
- [11] Al-Hujaimi, K.W., & Taher, I.J. (2012). Contamination of honey of Bee Honey With Cd and Pb. *Iraqi Journal of Agricultural Sciences*, 43(5): 63-68.
- [12] Okaily, R.A., Mouhamed, A.J., Alqeragouly, A.A., Hamad, B.S., Kasem, E.M., Al-Taweel, A.A.E.A., & Mahmood, E.A. (2015). Comparative between Populations of Honey Bee Worker *Apis mellifera* L. for Baghdad and Babylon Province by Using Outline – Based Geometric Morphometric Technique. *Iraqi Journal of Biotechnology*, 14(2): 108-118.
- [13] Engel, P., & Moran, N.A. (2013). The gut microbiota of insects — diversity in structure and function. *FEMS Microbiol. Rev.*, 37: 699–735.
- [14] Kwong, W.K., Engel, P., Koch, H., & Moran, N.A. (2014). Genomics and host specialization of honey bee and bumble bee gut symbionts. *Proc. Nat. Acad. Sci. USA.*: 111-115.
- [15] Nowak, A., Szczuka, D., Gorczynska, A., Motyl, I., & Kregiel, D. (2021). Characterization of *Apis mellifera* Gastrointestinal Microbiota and Lactic Acid Bacteria for Honeybee Protection—A Review. *Cells*, 10(701): 1-29.
- [16] Al-Baghdadi, S.A., & Sadiq, F.H. (2023). New records of Halictid bees (Hymenoptera: Halictinae) from Iraq. *Bulletin Iraq National History Museum*, 17(4): 679-697.
- [17] Flint, H.J., Scott, K.P., Louis, P., & Duncan, S.H. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews. Gastroenterology and Hepatology*, 9(16): 577-589.
- [18] Wang, H., Lei, L., Chen, W., Chi, X., Han, K., Wang, Y., Ma, L., Liu, Z., & Xu, B. (2022). The Comparison of Antioxidant Performance, Immune Performance, IIS Activity and Gut Microbiota Composition between Queen and Worker Bees Revealed the Mechanism of Different Lifespan of Female Casts in the Honeybee. *Insects*, 13: 1-15.
- [19] Awwad, W.H., & Shaher, K.W. (2023). Study on the Effect of Bee Strain and Pollination Method on the Hygienic Behavior of Honey Bee Colonies Tested by Liquid Nitrogen Freezing Method. *Earth and Environmental Science*, 1262(3): 032028.

- [20] Martinson, V.G., Danforth, B.N., Minckley, R.L., Rueppell, O., & Tingek, S.A. (2011). simple and distinctive microbiota associated with honey bees and bumble bees. *Mol. Ecol.*, 20:619–628.
- [21] Al-Obaydi, W.H., S & haheer, K.W. (2023). Study on the effect of bee strain and insemination method on the hygienic behavior of bee colonies tested using the pricking method. *Earth and Environmental Science*, 1252:1-7.
- [22] Widyastuti, Y., Rohmatussolihat, R., & Andi, F. (2014). The role of lactic acid bacteria in milk fermentation. *Food and Nutrition Sciences*, 5(4):435-442.
- [23] Hadi, H.A. (2023). Evaluation of some plant extracts and probiotic *Lactobacillus* spp. to control on the fungus *Nosema ceranae* in honey bee workers, *Apis mellifera* L. M.Sc. Thesis, College of the Agricultural Engineering Sciences. University of Baghdad, pp.145.
- [24] Kwong, W.K., & Moran, N.A. (2016). Gut microbial communities of social bees. *Nat. Rev. Microbiol.*, 14:374-384.
- [25] Khan, K.A., Ansari, M.J., Al-Ghamdi, A., & Nnuru, A. (2017). Investigation of gut microbial communities associated with indigenous honey bee *Apis mellifera jemenitica* from two different eco-regions of Saudi Arabia. *Saudi Journal of Biological Sciences*, 24(5):1061-1068.
- [26] Kim, M.J., Ku, S., Kim, S.Y., Lee, H.H., Jin, H., Kang, S., Li, R., Johnston, T.V., Park, M.S., & Ji, G.E. (2018). Safety Evaluations of *Bifidobacterium bifidum* BGN4 and *Bifidobacterium longum* BORI. *International Journal of Molecular Sciences* 2018 Dec;19:1-22.
- [27] Najm, A.D., & Al-Jassany, R.F. (2023). A checklist of Megachilid bees (Hymenoptera: Megachilidae), with a new record genus from Iraq. *Bulletin Iraq National History Museum* 17(4):571-588.
- [28] Alberoni, D. (2018). Beneficial Microorganisms for Honey Bees Health. Thesis, M.Sc. Alma Mater Studiorum – Università di Bologna, pp.161.
- [29] Pan, M., Nethery, M.A., Hidalgo-Cantabrana, C., & Barrangou R. (2020). Comprehensive Mining and Characterization of CRISPR-Cas Systems in *Bifidobacterium*. *Microorganisms*, 8:720.
- [30] Pino, A., Benkaddour, B., Inturri, R., Amico, P., Vaccaro, S.C., Russo, N., Vaccalluzzo, A., Agolino, G., Caggia, C., & Miloud, H. (2022). Characterization of *Bifidobacterium asteroides* Isolates. *Microorganisms*, 10:1-17.
- [31] Al-Hasnawi, N.K. (2023). Identification of viruses infesting honey bee *Apis mellifera* L. in some Iraqi Provinces and their vectors. Ph.D. dissertation. College of Agricultural Engineering Sciences. University of Baghdad, pp.152.
- [32] Saleh, G.M. (2020). Isolation and characterization of unique fructophilic lactic acid bacteria from different flower sources. *Iraq Journal of Agricultural Sciences*, 51:508-518.
- [33] Chen, J., Wang, J., & Zheng, H. (2021). Characterization of *Bifidobacterium apousia* sp. nov., *Bifidobacterium choladohabitans* sp. nov., and *Bifidobacterium polysaccharolyticum* sp. nov., three novel species of the genus *Bifidobacterium* from honey bee gut. *Syst Appl Microbiol.*, 44:126247.
- [34] Awwad, I.H. (2023). The efficiency of some plant extracts in controlling the Varroa mite *Varroa destructor* and their effects on the activity of honey bee *Apis mellifera* colonies. M.Sc. Thesis, College of Agriculture. University of Baghdad, pp.98.
- [35] Logue, J.B. C. A., Stedmon, A.M., Kellerman, N. J., Nielsen, A.F., Andersson, H., Laudon, E.S., Lindstro, M., & Kritzberg, E.S. (2016). Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *The ISME. J.*, 10: 533-545.
- [36] Olofsson, T.C., Modesto, M., Pascarelli, S., Scarafile, D., Mattarelli, P., & Vasquez, A. (2024). *Bifidobacterium mellis* sp. nov., isolated from the honey stomach of the honey bee *Apis mellifera*. *International Journal Systematic Evolutionary Microbiology*, 73:1-8.
- [37] Luo, S., Xue, Z., & Zhou, X. (2024). Temporospatial dynamics and host specificity of honeybee gut bacteria: Review. *Cell Reports*, 43:1-14.
- [38] Sambrook, J., Fritsch, E.R., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual* (2nd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, p.p.1659-1989.

- [39] Hickson, T., & Polson, A.(1968).Some physical characteristics of the agarose molecule. *Biochimica Et Biophysica Acta (BBA) - General Subjects*,165, (1):43-58.
- [40] Lee, P.Y., Costumbrado, J., Hsu, C., & Kim,Y. H.(2012).Agarose Gel Electrophoresis for the Separation of DNA Fragments. *Journal of Visualized Experiments*, 20(62):1-5.
- [41] Smith,S.,Aldridge,P., & Callis,J.(1989).Observation of individual DNA molecules undergoing gel electrophoresis. *Science*,243(Issue 4888):203-206.
- [42] Stellwagen,N.C.(2009).Electrophoresis of DNA in agarose gels, polyacrylamide gels and in free solution. *Electrophoresis*, (Suppl 1): 188-95.
- [43] Ellegaard, K.M.,Tamarit, D.,Javelind, E.,Olofsson,T.C., Andersson,S.G., & Vasquez, A. (2015).Extensive intra-phylo-type diversity in *Lactobacilli* and *Bifidobacteria* from honey bee gut.*BMC.Genomics* ,16(1):1-22.
- [44] AL- Kafage, N.JM., & AL – Bedre, A.A.N.(2016).Extension agricultural innovation for keepers to develop and increase productivity in the central region of Iraq.*Iraqi Journal of Agricultural Sciences*, 63(5):1202-1210.
- [45] Modesto,M.,Michelini,S.,Oki,K.,Biavati,B.,Watanabe,K., & Mattarelli, P.(2018). *Bifidobacterium catulorum* sp. nov ., a novel taxon from the faeces of the baby common marmoset (*Callithrix jacchus*). *International Journal Systematic Evolutionary Microbiology*,68:575–581.
- [46] Bleau, N., Bouslama, S., Giovenazzo, P., & Derome, N.(2020).Dynamics of the honeybee (*Apis mellifera*) gut microbiota throughout the overwintering period in Canada. *Microorganisms* 2020 Oct; 29: 1146.
- [47] Yang, J.,Zhong,Y., Xu, L., Zeng, B., Lai, K.,Yang, M., Li, D., Zhao, Y., Zhang, M., & Li, D.(2021). The dominating role of genetic background in shaping gut microbiota of honeybee queen over environmental factors. *Front. Microbiol.*,12:722901.
- [48] Lugli, G.A.,Fontana, F.,Tarracchini, C.,Mancabelli, L.,Milani, C.,Turroni, C., & Ventura, M.(2022).Exploring the biodiversity of *Bifidobacterium asteroides* among honey bee microbiomes. *Environmental Microbiology*,24:5666–5679.
- [49] Tamura,K.,Stecher,G., & Kumar,S.(2021).MEGA11:Molecular Evolutionary Genetics Analysis Version11.*Molecular Biology and Evolution*,38(Issue 7): 3022–3027.
- [50] Li,M.,Jin,Y.,Wang,Y.,Meng,L.,Zhang,N.,Sun,Y.,Hao,J.,Fu,Q., & Sun, Q.(2019).Preparation of *Bifidobacterium breve* encapsulated in low methoxyl pectin beads and its effects on yogurt quality. *Journal of Dairy Science*,102 (6):4832-4843.
- [51] Kusharyati, D.F., Hendrati, P.M., Ryandini, D., Manshur, T.A.M., Dewi, A., Khatimah,K., & Rovik, A.(2020).Isolation of *Bifidobacterium* from Infant’s Feces and Its Antimicrobial Activity. *10th Asian Conference of Lactic Acid Bacteria*;1-7.
- [52] Motta, E.V.S., Powell, J.E., Leonard, S.L., & Moran, N.A.(2021).Prospects for probiotics in social bees. *Philosophical Transactions*,:1-10.
- [53] Ewert, A.M., Finstrom, M. S. F. Read, Q., Husseneder, C., Ricigliano, V.(2023). Effects of ingested essential oils and propolis extracts on honey bee (*Hymenoptera: Apidae*) health and gut microbiota. *Journal of Insect Science*,23(6):1–13.

A micro Morphological Study of Pollen Grains and Male Reproductive Organs of Two Species of the Genus *Senna* in Iraq

Shaymaa Mahde Khadhum¹ and Khansaa Abdul Alee Shaheed², Neepal Imtair Algaraawi³

^{1,2, 3} Department of Biology, College of Science, University of Karbala, Karbala, Iraq.

¹E-mail: shaymaa.m@s.uokerbala.edu.iq

²E-mail: khansaah@uokerbala.edu.iq

³ E-mail : neepal.i@uokerbala.edu.iq

Abstract. The genus *Senna* belongs to the family Leguminosae and includes species with monosymmetric flowers and species with various asymmetric flowers. We explored the morphology of the male reproductive organs in two species of the genus *Senna* and the symmetric type and studied the dimensions of the reproductive organs represented by the androecium of the species *Senna alata* and *Senna didymobotrya*. The morphological difference of Androecium of the two species was examined using LM and SEM respectively, exine ornamentation and morphological study of pollen grains represented by the length of the equatorial and polar axis, size, type and shape of grains, in addition to the precise morphological study of the exine ornamentation of pollen grains and the method of secretion of pollen grains from two apical openings in the anther. We identified patterns of floral asymmetry, in which the male reproductive organs participate and which result from different combinations of elements, and patterns of reticulated and striped exine ornamentation of the reproductive organs and pollen grains for the first time in Iraq for the species *Senna alata* and *Senna didymobotrya*, which were considered important characteristics for species identification.

Keywords. Genus *senna*, Pollen grains, LM and SEM.

1. Introduction

The Fabaceae, also known as the legume family, is a large and economically important family of flowering plants. It includes trees, shrubs and herbaceous perennials or annuals, which are easily recognized by their fruits (legumes) and compound, pointed leaves. The group is widespread and is the third largest plant family in the world in terms of number of species, after the Asteraceae and Orchidaceae, with 19,500 species and 770 genera [1]. Currently distributed into six subfamilies: Cercidoideae, Detarioideae, Duparquetioideae, Dialioideae, and Papilionoideae [2,3]. Based on their differences in some floral characters related to the male organ and the corolla, they were divided into three subfamilies: Caesalpinioideae, Mimosoideae, and Papilionoideae. A number of researchers agreed on this division, including [4,7], and the scientist [8].

The subfamily Caesalpinioideae is among the recognized families within the legume family and includes approximately 2000-3000 species of flowering plants [9]. The subfamily includes 180 genera and 3000 species of tropical and subtropical trees and shrubs. The largest genus is Cassia with about 600 species, mainly located in tropical regions, followed by the genus Mimosa with 500 species, the genus Senegalia with 220 species, the genus Chamaecrista with 300 species, and the genus Vachellia with 160 species [10].

In terms of its importance to humans, this family is a source of a wide range of products, such as (foods and medicines, foods, oils, fibers, fuel, wood), and although their number is less than 50 types of legumes, the species provide 90% of the world's current needs [11].

The genus Senna includes about 250 species of flowering plants in the family Caesalpinioideae in tropical regions [12]. Miller (1754) is considered the first to classify the genus. The origin of the word cassia is derived from senna, meaning fragrant herbs [13] where it is considered the second genera of the family [14]. Cassia was previously isolated from the genus Cassia by [15]. Linnaeus (1753) was the first to name the genus Cassia.

Two species of plants in the genus Senna were studied, namely Senna alata, which is locally known as Candle brush or winged Senna in Asian and African countries [16] and Senna didymobotrya, a shrub with golden-yellow flowers with a distinctive peanut butter or popcorn scent that blooms from brown buds [17,18] presented the pollen morphology of 353 species from Karachi, belonging to 67 angiosperm families, distributed among 58 dicotyledons and 9 monocotyledons, as well as 15 species of the genus Lycopodium examined [19]. It was shown that the pollen grains were hexagonal, medium to small in size, and their shape was flattened to swollen [20]. Undertook the task of preparing a taxonomic key based on the morphological characteristics of most families. The most recent studies are those in which he referred to the phenotypic differences in pollen grains of forty-nine species belonging to twenty-five families, where the results showed significant differences and great diversity in shape, size, decoration and openings in pollen grains for most families and genera, which have their own distinctive pollen types. Pantoporate pollen grains appeared in several families such as the Chenopodiaceae family, the Cockscomb family and Amaranthaceae, and the number of grooves varied. Most species contain three colpi, as in Bacopa monniera, while Myriophyllum spicatum contains [4][5] grooves [21].

Differences in the size and length of reproductive organs may lead to different cell numbers. The structure of reproductive organs in different embryonic types of Phaseolus vulgaris of the legume family was compared [22].

When determining the type of symmetry of the flowers of the genus Senna, the asymmetric type of flowers was identified, as the male reproductive organs participate in the asymmetry of the flowers of this genus, from a study conducted on the species S. corymbosa [23].

2. Materials and Methods

Pollen grains were studied based on fresh samples collected during field trips in different areas of Iraq, which were preserved in 70% ethyl alcohol, in addition to herbarium samples. Light microscope preparations.

2.1. Direct Method

Mature anthers were taken from the flowers of each of the studied plant species and placed in a watch glass containing 96% ethyl alcohol. After that, the anthers were opened with two dissection sheets under the dissection microscope of the type WildMB3. Then, the pollen grains were transferred by means of a dissection needle to a clean glass slide containing a drop of safranin-glycerin stain. The slide was covered with a cover slip and examined with a Novex light

microscope at magnification (40X). The measurements were made using a graduated Ocular lens coupled with the microscope [24].

2.2. Dyeing Method using Fusion Basic Dye

The mature anthers were taken from the flowers of each type of the studied plant species, and were placed in a watch glass and the anther was opened using T-needles for dissection under a dissecting microscope, and the pollen grains were extracted from them and the impurities represented by the anther sheaths were removed, and then drops of ethyl alcohol at a concentration of 96% were added to them for the purpose of washing them (in case they evaporate quickly, other drops can be added). It was observed that the fats spread quickly to the edge of the watch glass and were removed using blotting paper, then drops of dye were added and left for a period of 15 minutes, after which a clean glass slide was taken and a drop of glycerin and a drop of dye containing pollen grains were placed on it, and then the slide was covered with a slide cover, and examined with a light microscope at a power of 40X [25].

2.3. Electron Microscope Preparations

The microscopic study of pollen grains was carried out by scanning electron microscope if the samples were prepared by direct method and dry samples were used which were placed on a metal holder known as Stub and the samples were coated with a thin layer of gold Using a coating machine of the type Coating machine specifications and photographed by scanning electron microscope SEM (scanning electron microscope) at Amir Kabir University in the Islamic Republic of Iran Many shots were taken at different magnifications for each sample so that the dimensions and shapes of pollen grains, the number of germination holes, the type of pollen grains, and the shape of the polar and equatorial view of each type of the species under study were investigated As for the terms specific to pollen grains mentioned in the current study, they are according to [26-28].

2.4. Microscopic Study of Reproductive Organs

Samples were taken from two species of flowering plants with heterogeneous stamens of the genus *Senna* L. belonging to the legume family from three governorates in Iraq then the precise morphology of the male reproductive organs Androecial Organs represented by the stamen that includes the anther and the filament was studied.

2.5. Measurement of the Floral Parts

The length and width of the anthers were measured by a digital lens attached to a Novex light microscope. Ten flowers of five individuals of each species were filled when fully opened. The flowers were collected and numbered. One stamen of each species was randomly selected for measurement. The anther sac was measured from the tip of the anther to the apex of the anther. The width of the anther was measured in the middle of the antheridia. The length and width of the anther stalk were measured in the same way for all stamen species. The anther and stalk were then placed in special tubes.

2.6. Electron Microscope Preparations

The microscopic study of the male reproductive organs was carried out by scanning electron microscope. The male reproductive organs of the two plants were prepared and previously prepared, which were placed on a metal stand known as Stub. The samples were coated with a thin layer of gold and photographed by scanning electron microscope (SEM) at Amir Kabir University in the Islamic Republic of Iran. Several shots were taken at different magnifications

for each sample, so that the type of covering of the organs and the surface decorations of each part of the organs were investigated.

3. Results and Discussion

3.1. Microscopic Morphological Characteristics of Male Reproductive Organs

A typical flower is divided into four rings: the calyx, the corolla, the androsium, and the gynoecium. The androsium is the third flower and arises from the inner side of the corolla. It is the male reproductive part of the flower and consists of stamens. They are the male reproductive organs in flowering plants with the primary function of producing pollen grains [29,30] they consist of an anther and a filament, which varies in length between plant species. The anthers are usually double-lobed.

3.2. Morphological Characteristics and Differentiation of the Male Reproductive Organ's Androecium

The extent and patterns of differentiation in androecium varied among the species examined, even in the same genus Irwin & Barneby in the genus *Senna* Table (1): Figure (1) *S.alata* (A) and *S.didymobotrya* (B) androecium have four types of stamens with marked morphological differentiation. The three axial stamens are sterile and flattened. No pollen grains are produced (Figure1: A4, B4,) with an anther whose maximum length was (250) micrometers between 300-155 and whose maximum width was (145) micrometers between 155-128 for *S.didymobotrya*, while in *S.alata* the anther length was between 200-140 at a rate of (174.2) micrometers and its width was an average of (75) micrometers between 85-55, while the *S.alata* filament recorded the highest average length of 280 micrometers between 300-210 and its width was an average of 23.8 micrometers between 30-20 for *S.didymobotrya*, whose average length was (247) micrometers between 255-245 and its maximum width ranged between 30-20 at a rate of 25.1 micrometers.

In the center of the flower there are four short stamens with erect filaments and an erect anther only at the apex slightly curved which is usually suggested to be the larval feeding stamens with the function of providing pollen Fig1:A3, B3. The highest average length 440 μm of the anther in *S.alata* was between 410-340 and the lowest average width was 132.4 μm ranging between 140-100 compared to the average length of the anther in *S.didymobotrya* which was (408) μm between 442-300 and the highest average width was 142.8 μm between 190-105 while the filament recorded the highest average length in *S.alata* which was (328) μm ranging between 390-270 and the lowest average width was (21.4) μm between 25-20 compared to the filament of the plant *S.didymobotrya* which had the lowest average length of 223.5 μm between 250-190 and the highest display rate width was 29.2 micrometers between 40-30.

The three axial stamens (pollinated stamens) often form two groups, the axial lateral stamen belongs to the inner flower while the semi-axial stamen belongs to the outer flower Fig1:A1, B1, A2, and B2. The lateral stamens in *S.alata* contain strong filaments with a maximum length of 542 between 400-600 and a maximum width of (61) micrometers between 32-60, while the length of the filaments in *S.didymobotrya* recorded the lowest average length of 365.7 micrometers between 300-400 and a minimum average width of (27.2) micrometers between 30-35 and it contains large and slanted anthers (one another to the right and the other to the left). The maximum average length in *S.didymobotrya* reached (1105) micrometers between 925-1000 and a maximum width of (199.5) micrometers ranging between 205-190 while the length of the *S.alata* recorded the lowest average length of 892 μm between 705-1000 and the lowest average width of 198.5 μm between 230-160.

While the third central stamen has a much smaller anther but slightly longer filaments, the highest average anther length in *S. didymobotrya* was (544.5) μm between 570-512 and the lowest average width was 94.5 μm between 105-90, while the lowest average anther length in *S. alata* was 454.4 μm between 450-400 and the highest average width was 153.5 μm between 140-125, while the filaments *S. alata* recorded the highest average length and width of 605.7 μm between 600-550 and the width was (45.7 μm between 70-40, , while the *S.alata* filaments the highest length and width rate of 605.7 micrometers between 600-550 and width rate of (45.7) micrometers between 70-40, while the *S.didymobotrya* filaments the lowest length and width rate of (440) micrometers between 500-400 and width rate of (29.2) micrometers between 38-25.

All anthers and filaments appeared light yellow in *S.alata* while in *S.didymobotrya* the anthers and filaments tended to be yellowish brown. No study on the dimensions of anthers and filaments of the studied species was available except [23]. They explained the importance of measuring the dimensions of the male reproductive organs of one of the species of the genus *Senna* and the results of the study agreed with [31] when measuring the dimensions of anthers of one of the studied species, *S.alata*.

And about 20,000 species of flowering plants offer only pollen as a reward to pollinators [32]. The widespread characteristic of these pollen-offering flowers is stamen dimorphism, involving two sets of stamens that differ in position, size, and color [33]. Darwin was the first to postulate the "division of labor" among the stamens, that some stamens provide pollen as food for the larvae while others provide pollen for fertilization [32]. Some studies have shown that one set of stamens always acts as the "feeder" stamen, while the other stamens act as the "fertilizing" stamens in the genus *Senna* [31]. According to [34] *Senna* species are classified into three types: (1) monosymmetric flower (2) asymmetric flower in which only the female reproductive organs participate in the asymmetry of the flowers (3) asymmetric flower in which the petals and stamens also participate in the asymmetry of the flowers. Thus, *S.alata* and *S.didymobotrya* were classified into asymmetric flower containing four sets of stamens that differ in color, size, and position. In the present study, the anther color variation between the two species is under consideration. The study, however, generally showed brown color on *S. didymobotrya*, and yellow on *S.alata*.

Stamens containing yellow anthers, whether large (AL), medium (AM) or small (MI), act as "feeder" stamens and the brownish yellow is supposed to be an effective colour attractant for pollinators. Thus, pollen is the main reward for insects and the colouration of the corolla and anthers is the primary attractant [23].

The results of the current study agree with [31] when studying the four stamen groups of one of the species, *S. alata*, while no study was available on the species *S.didymobotrya*, but explained in his study the importance of studying the dimensions of the reproductive organs of species of the genus *Senna*(35).

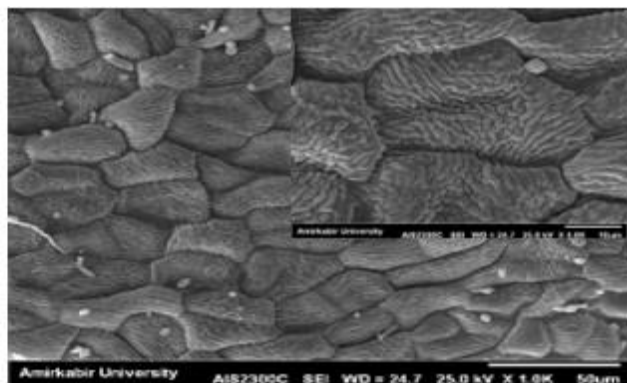
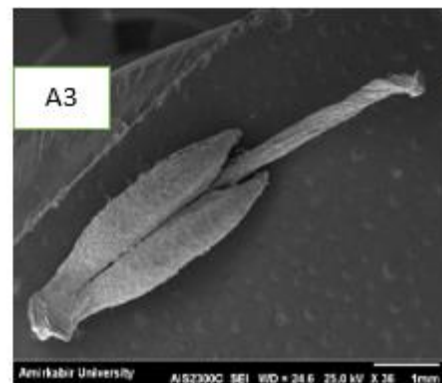
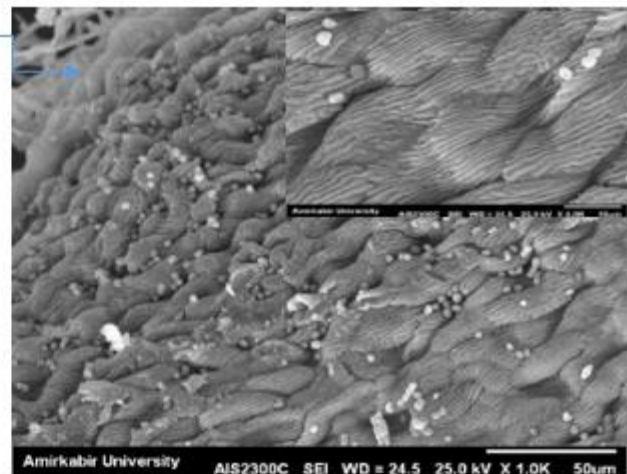
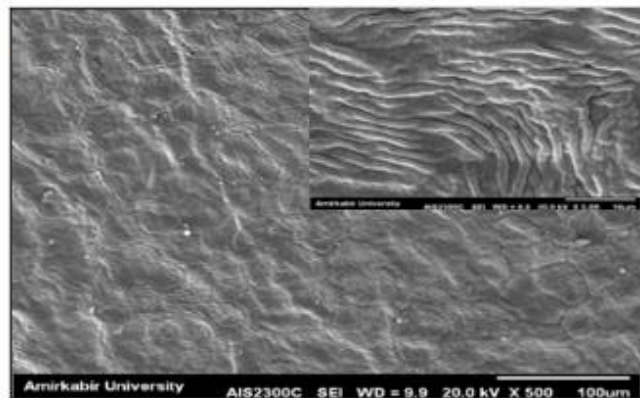
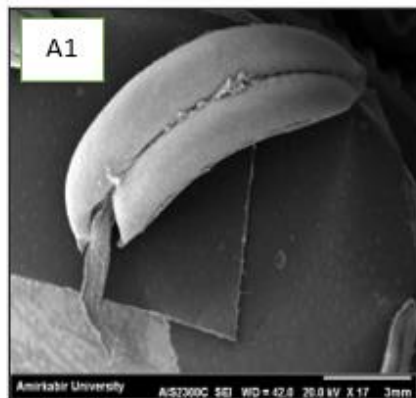
Pollen is also released through two openings located at the tip of the curved anther of both species (Fig. 1). The research agreed with [31]. When studying the way pollen is released from the tips of the anthers using a Sem for the plant species *S. alata*.

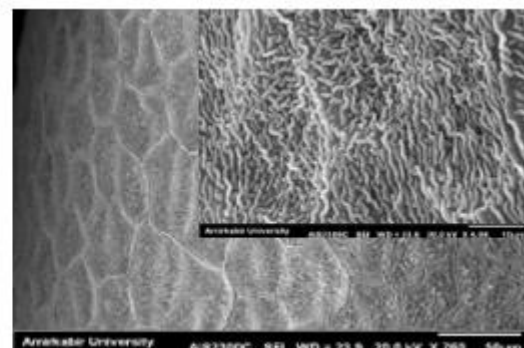
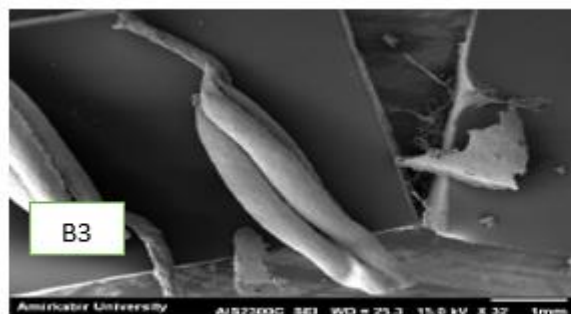
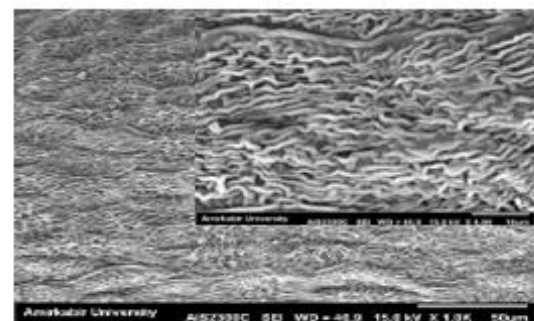
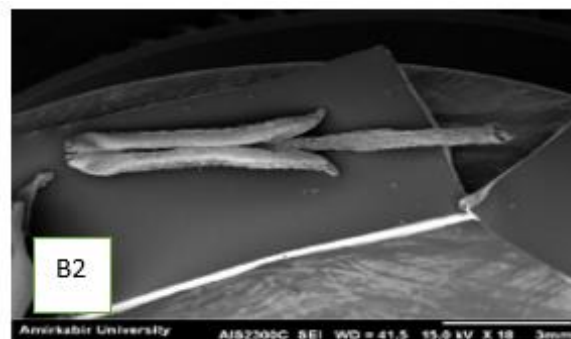
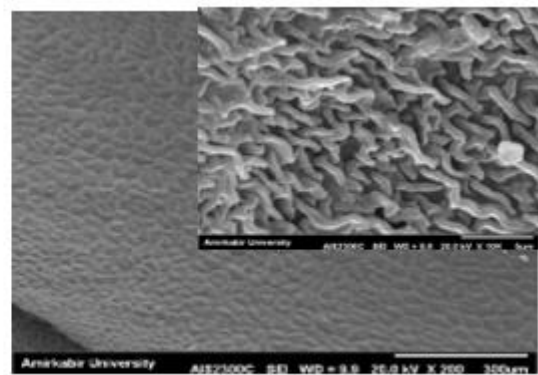
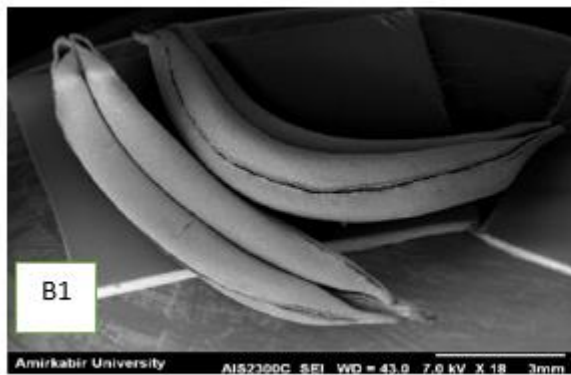
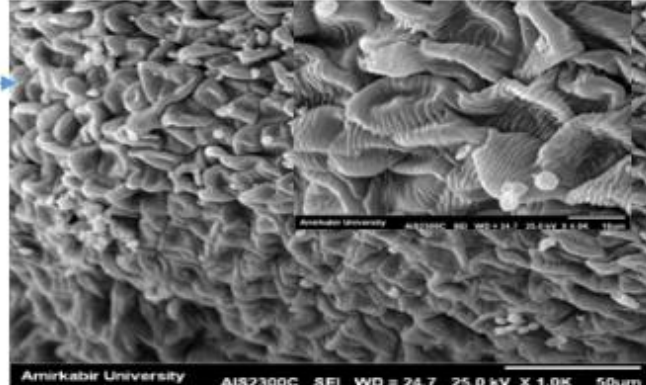
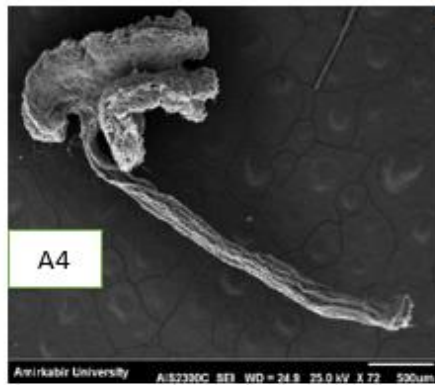


Figure 1. Androecium of two species of plants (A) *Senna alata* and B) *Senna didymobotrya* - consists of 7 stamens and 3 stamens of unequal size (A4, B4; very small, no pollen). The fertile stamens are arranged in 3 levels two of which A1, B1 are long, one is medium-sized (A2, B2) and the remaining four are small (A3, B3). The anthers of the longest stamens are curved while those of the others, consisting of five stamens, are straight. The anther is fixed, biconcave. The apical pores detach the anther.

The exine ornamentation of the four groups of stamens was also studied using the scanning electron microscope (SEM), where the exine ornamentation of the large anther in the yellow candlestick *S. alata* appeared striped with sunken walls, while the exine ornamentation in the medium anther of the same species was striped with prominent and convex cell walls. As for the small anthers, the exine ornamentation was striped and cellular with sunken walls, while it appeared in a papillary or semi-papillary shape with a striped decoration in the group of the smaller anther. While the exine ornamentation in the brown candlestick *S. didymobotrya* took on shapes different from the first type, where the exine ornamentation of the large anther of the brown candlestick appeared cellular reticulate with prominent cell walls, while the walls of the medium anther took on a sunken and cellular shape and the exine ornamentation pattern was reticulate. As for the small anthers with four groups, their walls appeared prominent and regular polygonal and regular cellular and reticulate decoration, while the smaller anther groups took on a striped and cellular decoration with sunken walls.

Thus, it was possible to isolate the four groups of stamens of each of the two species under study. (Figure 2) table. 2 There was no study of the exine ornamentation of the species under study,





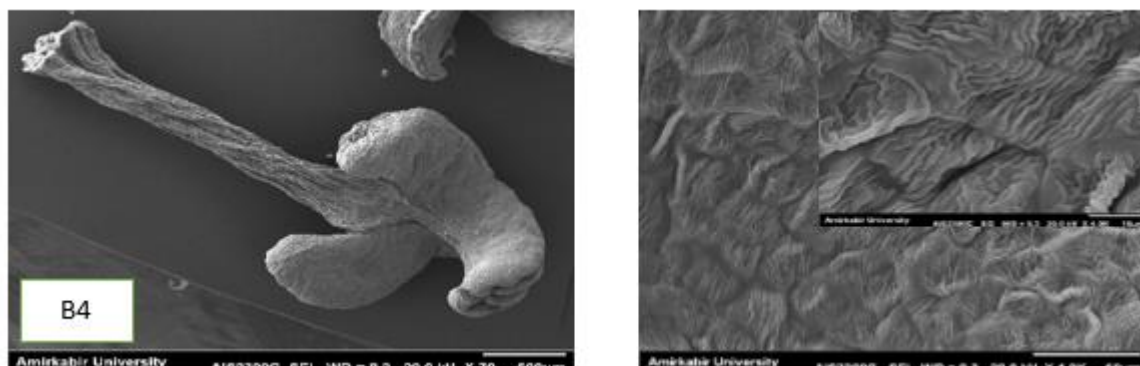


Figure 2. Variations in the exine ornamentation of the four stamen groups of the yellow candlestick *Senna alata*(A1) Large anther with striated decoration and sunken cell walls(A2) Medium anther with raised convex walls and striated The pattern of the striated squamous (A3) small cellular squamous with recessed walls and reticulated exine ornamentation (A4) smaller squamous papillary or semi-papillary with reticulated exine ornamentation and brown candlestick *Senna didymobotrya*(B1) large cellular squamous with raised walls and reticulated exine ornamentation (B2) medium cellular squamous with reticulated walls and reticulated exine ornamentation (B3) small cellular squamous with regular and raised polygonal walls and reticulated exine ornamentation (B4) smaller cellular squamous with reticulated walls and reticulated exine ornamentation From the legumenacea family.

Table 1. Quantitative and qualitative characters of the male reproductive organs androecium of the yellow candlestick *Senna alata* and the brown candlestick *Senna didymobotrya* of the legume family Legumenacea under 40x magnification.

Types	Large stamen(AL,BL)				Medium stamen(AM,BM)				small stamen(MIA,MIB)				Smallest stamen(ST)			
	Anther		Filament		Anther		Filament		Anther		Filament		Anther		Filament	
	length	Width	length	Width	length	Width	length	Width	length	Width	length	Width	length	Width	length	Width
<i>Senna alata</i>	100	230	600	60	450	140	600	70	41	140	390	25	200	85	30	30
	0	(19	(542	(61	(	(	(	70	(	(	(	(	(	(75	(	(
	(89	8)	)	)	454.	153.	605.	45.	44	132.	(328	21.	174.	)	28	23.
	2)	160	400	32	4)	5)	7	40	0)	4)	270	4)	2)	55	0)	8)
	705				400	125	550		34	100	20	140		21	20	
<i>Senna didymobotrya</i>	100	205	400	35	570	105	500	38	44	190	250	40	300	15	25	30
	0	(199.	(	(	(	(	(	(	2	(	(	(	(	5	5	(
	(11	5)	365.	27.	544.	94.5	440	29.	40	142.	223.	29.	(250	(	24	25.
	05)	190	7)	2)	5)	)	)	2)	8)	8)	5)	2)	)	5)	7)	1)
	925		300	30	512	90	400	25	30	105	190	30	155	12	24	20
									0					8	5	

The values inside the parentheses represent the average, the number above the average represents the upper limit, and the number below the average represents the lower limit.

Table 2. Variations in the sculptural decorations of the male reproductive organs androecium of the yellow candlestick *Senna alata* and the brown candlestick *Senna didymobotrya* of the legume family Legumenacea using scanning electron microscopy (SEM)

Cells	Smaller-mat decoration	Cells	Small mat decoration	Cells	Medium sized ornament	Cells	Big mat decoration	Types
papillar or semi-papillary	Planned	Barren walls and empty	Planned	protruding and convex walls	planned	sunken walls	Planned	<i>Senna alata</i>
Cellular with sunken walls	Planned	Regular polygonal and regular cell walls	Reticulated	Barren walls and empty	Reticulated	Cellular with prominent walls	Reticulated	<i>Senna didymobotrya</i>

3.3. Microscopic Study of Pollen Grains

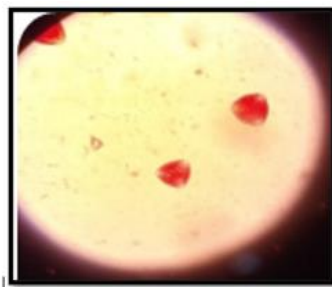
Each pollen type of two plant species belonging to the genus *Senna*, family Fabaceae, subfamily Gaesalpinioideae, namely *S. alata* and *S. didymobotrya*, were studied and analyzed. The morphological characters investigated include, for example, aperture type, morph type, morph type thickness, morph type, polarity, symmetry, shape, and pollen size. An average of ten (10) pollen grains were used to obtain the dimensions and other morphological characteristics of the pollen grains, which helped in the ease of describing and identifying the pollen grains that were examined. The results of the study showed that all pollen grains had a fixed tricolporate opening type, which is a distinctive feature of the species belonging to the legume family, and were generally radially symmetrical. The shape of the pollen grains of the two species belonging to the genus *Senna* under study appeared circular or swollen spherical and triangular in the polar view, while in the equatorial view it appeared widely elliptical. The diameters of the equatorial and polar axes differed across the species that were studied. It was noted that the longest equatorial axis of the pollen grains was in the species *S. didymobotrya*, reaching an average of (43) micrometers, ranging between (37.5 - 50) micrometers, and the shortest equatorial axis of the pollen grains was in *S. alata*, reaching (39) micrometers, ranging between (37.5 - 42.5). Micrometer indicating the presence of differences.

In addition to the variation in the lengths of the polar axis between species, where the longest polar axis of pollen grains in the species *S. didymobotrya* reached an average of (37.25) micrometers, ranging between (25.5-47.5) micrometers, and the shortest polar axis of pollen grains was in the species *S. alata*, reaching an average of (32.75) micrometers, ranging between (22.5-37.5), as the variation in the dimensions of the axes became clear and according to the criteria mentioned by [26] and [36] shown in the table below to determine the size of the grain, the pollen grains of the two species recorded sizes that reached an average of 127.7 micrometers for the species *S. didymobotrya*, and the size of the grain reached an average of 160 micrometers, which is within the limits of the sizes of very large pollen grains. As for the sculptural decorations of the surfaces of the pollen grains in both species, the slit-like reticulate and the projections in it are elongated rods appeared. In general, comparing the pollen morphology of the two studied species of the Fabaceae family creates differences between species. The type of aperture is usually constant within the family. The Fabaceae family under the class Caesalpinioidea is mostly tricolporate in the aperture, is polar, and radially symmetrical [37]. The genus *Cassia* is considered a heterogeneous taxon [38]. Pollen characteristics can be useful in determining affinity and classifying taxa. This research is expected to be crucial for identifying these plants and enhancing their economic conservation status.

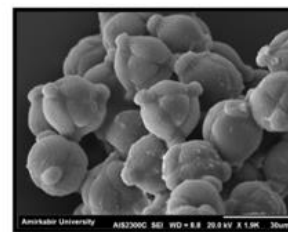
The study agreed with the study of [37] for one of the species of the genus *Senna*, *S. alata*, in that the type of opening is constant, but the study differed in the length of the equatorial axis and the type of exine ornamentation of the grain surface, which is granular, which appeared in the current study as a pitted reticulate and the projections in it are rod-shaped, which confirmed the reticulate decoration by both [31]. Our study added the shape of the projections extending from the walls in which an elongated rod appeared.

Table 3. Morphological characteristics of pollen grains of the yellow candlestick *Senna alata* and the brown candlestick *Senna didymobotrya* of the legume family under 40x magnification.

Ornamen t type	Equatori al shape	Size P*E*10 0	Polar Shape	Equatorial axis			Polar axis				Types
				Ma x	Mea n	Mi n	Ma x	Mea n	Mi n		
Reticulate d Pincus with rod- like projection s	Elliptical	127.7 Very large pollen	triangula r circle	42.5	39	37. 5	37.5	32.75	22. 5	length	<i>Senna alata</i>
				30	23.5	20	37.5	32.5	22. 5	width	
Reticulate d Pincus with rod- like projection s	Elliptical	160 Very larg pollen	triangula r circle	50	43	37. 5	47.5	37.25	25	length	<i>Senna didymabatrya</i>

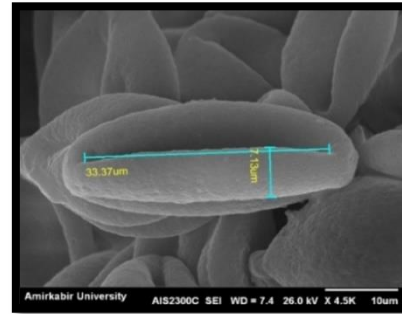
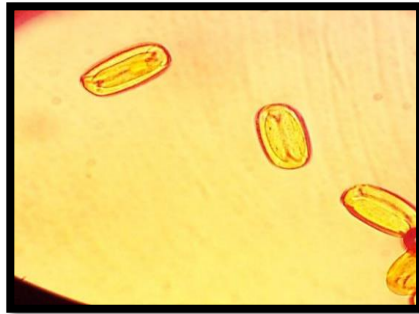


S. alata

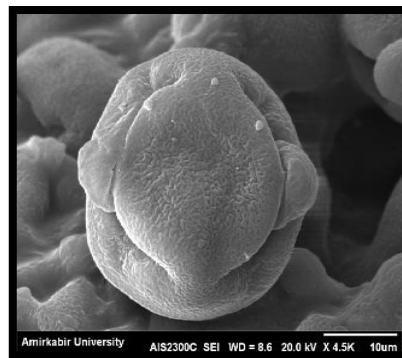
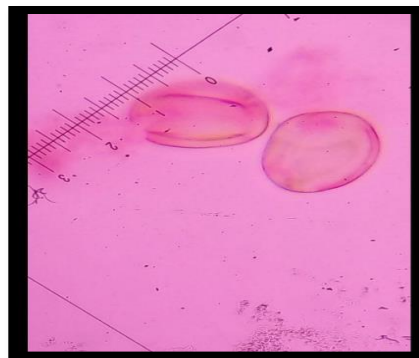


S. didymobotrya

Figure 3. Polar view of pollen grains of some species of the legume family. The scale of the two images using an electron microscope is 10 μ m.

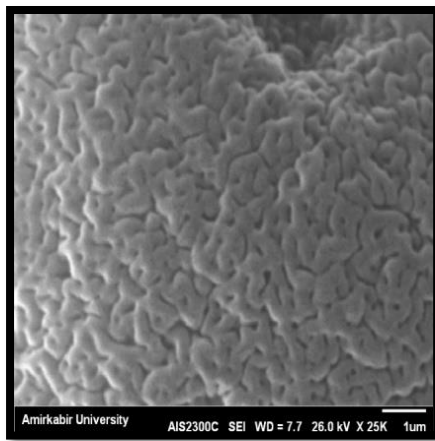


S. alata

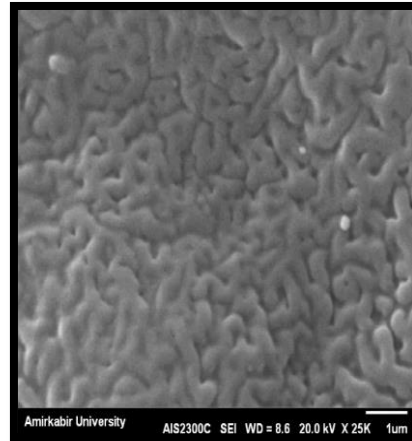


S. didymobotrya

Figure 4. Equatorial view of pollen grains of some species of the legume family. Scale of the two electron microscope images: 10 μ m.



S. alata



S. didymobotrya

Figure 5. Variation in the exine ornamentation of the pollen wall of the yellow candlestick *Senna alata* and the brown candlestick *Senna didymobotrya* of some species of the Leguminaceae family using scanning electron microscopy (SEM).

Conclusions

The present study showed that the morphological characters have a taxonomic importance in diagnosing and distinguishing between the two species under study, in addition to the quantitative and qualitative variations in the dimensions of the polar and equatorial axes of the pollen grains,

as well as the dimensions of the male reproductive organs. The study using the scanning electron microscope also proved the existence of important variations in the fine morphological characters of the surfaces of the reproductive organs and the surfaces of the pollen grains, which are useful in isolating and diagnosing the two species.

References

- [1] Judd, W.S., Campbell, C.S., Kellog, E.A., Stevens, P.F., Donoghue, M.J. Plant Systematics: a phylogenetic approach Sinauer Axxoc, 2002; 287-292, ISBN 0-87893-403-0.
- [2] Banks H, Lewis G. Phylogenetically informative pollen structures of 'caesalpinoid' pollen (Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae and Duparquetioideae: Fabaceae). Botanical Journal of the Linnean Society. 2018 Apr 23;187(1):59-86.
- [3] Hughes, C. E., & Legume Phylogeny Working Group. (2017). A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. Taxon, 66(1), 44-77.
- [4] Hughes CE, Legume Phylogeny Working Group. A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. Taxon. 2017;66(1):44-77.
- [5] Lawrence GH. An introduction to plant taxonomy. Central Book Depot; 1955.
- [6] Sinjushin AA. Evolutionary history of the leguminous flower. Biology Bulletin Reviews. 2021 Jul;11(4):400-13.
- [7] Thorne RF. The classification and geography of the flowering plants: Dicotyledons of the class Angiospermae: Subclasses Magnoliidae, Ranunculidae, Caryophyllidae, Dilleniidae, Rosidae, Asteridae, and Lamiidae. The Botanical Review. 2000 Oct; 66:441-647.
- [8] Martin AC. The comparative internal morphology of seeds. The American Midland Naturalist. 1946 Nov 1;36(3):513-660.
- [9] Estrada-Castillón E, Villarreal-Quintanilla JÁ, Cuéllar-Rodríguez G, Encina-Domínguez JA, Martínez-Ávalos JG, Mora-Olivo A, Sánchez-Salas J. The Fabaceae in Northeastern Mexico (Subfamily Caesalpinioideae, Mimosoideae Clade, Tribes Mimoseae, Acacieae, and Ingeae). Plants. 2024 Jan 30;13(3):403.
- [10] Bruneau A, Forest F, Herendeen PS, Klitgaard BB, Lewis GP. Phylogenetic relationships in the Caesalpinioideae (Leguminosae) as inferred from chloroplast trnL intron sequences. Systematic Botany. 2001 Jul;26(3):487-514.
- [11] Semba RD, Ramsing R, Rahman N, Kraemer K, Bloem MW. Legumes as a sustainable source of protein in human diets. Global Food Security. 2021 Mar 1; 28:100520.
- [12] Oladeji OS, Adelowo FE, Oluyori AP. The genus Senna (Fabaceae): A review on its traditional uses, botany, phytochemistry, pharmacology and toxicology. South African Journal of Botany. 2021 May 1; 138:1-32.
- [13] Parsons WT, Cuthbertson EG. Noxious weeds of Australia. CSIRO publishing; 2001.
- [14] Lock M. Legumes of the World. Lewis GP, Schrire B, Mackinder B, editors. Kew: Royal Botanic Gardens; 2005.
- [15] Irwin HS, Barneby RC. The American Cassiinae: a synoptical revision of Leguminosae tribe Cassieae subtribe Cassiinae in the new world.
- [16] Asmah N, Amna U. Analisa kandungan senyawa metabolit sekunder ekstrak daun ketepeng cina (Cassia alata L.) dari Bireum Bayeun, Aceh Timur. QUIMICA: Jurnal Kimia Sains dan Terapan. 2020 Oct 6;2(2):7-10.
- [17] Nodza GI, Tochukwu E, Igbari AD, Onuminya TO, Ogundipe OT. Application of IUCN Red List Criteria for Regional Assessment of Some Northern Savanna Trees of Nigeria, West Africa.
- [18] PERVEEN A. Pollen Characters and Their Evolutionary Significance with Special Reference to the Flora of Karachi. Turkish Journal of Biology. 2000;24(2):365-78.
- [19] Moon HK, Hong SP. Pollen morphology of the genus Lycopodium (Lamiaceae). In Annales Botanici Fennici 2003 Jan 1 (pp. 191-198). Finnish Zoological and Botanical Publishing Board.
- [20] Faegri K, Iversen J. Textbook of pollen analysis.
- [21] Al-Saadi SA, Al-Mayah AR. Pollen morphological study of the dicot's wetland plants of southern marshes of Iraq. Marsh Bulletin. 2012;7(2):169-88.

- [22] Tekdal D, Can SS, Küçükrecep A. Comparison of reproductive organs structure in various genotypes of *Phaseolus vulgaris* grown under the same conditions in Mersin, Turkey. *Plant introduction*. 2021 Aug 25(91-92):3-9.
- [23] Lin CM, Li YY. Floral syndrome and breeding system of *Senna (Cassia) corymbosa*. *African Journal of Biotechnology*. 2011;10(25):4988-95.
- [24] Sass JE. *BOTANICAL MICROTECNIAQUE*.
- [25] Smoljaninova LA, Gollubkova VF. LAK Metodike issledovani pilci. *Doklady Akademii Nauk SSSR T*. 1953;88(1):125-6.
- [26] Erdtman G. *Pollen morphology and plant taxonomy: Angiosperms (an introduction to palynology)*. Brill; 2023 Nov 27.
- [27] Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A. Glossary of pollen and spore terminology. Review of palaeobotany and palynology. 2007 Jan 1;143(1-2):1-81.
- [28] Moore PD, Webb JA. *An illustrated guide to pollen analysis*.
- [29] Walker-Larsen J, Harder LD. The evolution of stamens in angiosperms: patterns of stamen reduction, loss, and functional re-invention. *American Journal of Botany*. 2000 Oct;87(10):1367-84.
- [30] Scott RJ, Spielman M, Dickinson H. Stamen structure and function. *The plant cell*. 2004 Jun 1;16(suppl_1): S46-60.
- [31] LUO ZL, Gu L, ZHANG DX. Intrafloral differentiation of stamens in heterantherous flowers. *Journal of Systematics and Evolution*. 2009 Jan;47(1):43-56.
- [32] Luo Z, Zhang D, Renner SS. Why two kinds of stamens in buzz-pollinated flowers? Experimental support for Darwin's division-of-labour hypothesis. *Functional Ecology*. 2008 Oct;22(5):794-800.
- [33] Buchmann SL. Buzz pollination in angiosperms.
- [34] Marazzi B, Endress PK, De Queiroz LP, Conti E. Phylogenetic relationships within *Senna* (Leguminosae, Caesalpinieae) based on three chloroplast DNA regions: patterns in the evolution of floral symmetry and extrafloral nectaries. *American journal of botany*. 2006 Feb;93(2):288-303.
- [35] Torres MW, Aximoff IA, Ferreira MH, Guerra TJ. ASPECTS OF FLORAL BIOLOGY OF BUZZ POLLINATED *SENNA CANA* (LEGUMINOSAE: CAESALPINIOIDEAE) IN A RUPESTRAN FIELD IN MUCUGÊ, BA.
- [36] Kremp GO. *Encyclopaedia of pollen morphology*.
- [37] Doty MK, Rashid P, Shethi KJ. Study of petiole anatomy and pollen morphology of five species of *Senna* Mill. from Bangladesh. *Dhaka University Journal of Biological Sciences*. 2020 Aug 26;29(2):245-52.
- [38] Lock JM. *Cassia sens. lat. (Leguminosae: Caesalpinioideae) in Africa*. *Kew Bulletin*. 1988 Jan 1:333-42.

Evaluating the Impact of Farm Ownership on Agricultural Production by Estimating the Superior Efficiency of Baghdad Governorate Farms

Z. A. Al-Haboobi¹ and E. H. Ali²

^{1,2}Department of Agriculture Economics, College of Agriculture Engineering Science, University of Baghdad, Baghdad, Iraq..

¹E-mail: zahraa.ali@coagri.uobaghdad.edu.iq

²E-mail: eskander.hussain@coagri.uobaghdad.edu.iq

Abstract. The research aimed to identify the impact and relationship of holdings on agricultural production by estimating super-efficiency. The study requirements were met from the cross-sectional data in light of a questionnaire form distributed randomly and through personal interviews with (134) farmers in the agricultural divisions of Baghdad Governorate on both sides of Karkh and Rusafa for the production year (2021-2022), as it included detailed information about holdings, their nature, and all cultivated crops with their details in terms of areas, revenues, and costs, as four inputs and one output were entered, namely (manual work hours, automated work hours, the amount of chemical fertilizer, and the cultivated area), while the output was the total revenue. The data were analyzed using the (EMS) program, and the analysis results showed that there were (15) farms out of a total of (134) farms that achieved full efficiency starting from (100%) and above, and that the average efficiency at the sample level reached (66.24%), which means that it is far from full efficiency by (33.76%) due to the lack of optimal use of resources. We conclude from this that approximately 90% of the farms in the sample are inefficient farms, whether in terms of not optimally using resources or not allocating resources correctly between projects, or the inappropriate mix of projects and outputs. The research recommends the necessity of using modern technologies in agriculture and emphasizing the importance of following appropriate agricultural cycles, as well as monitoring the progress of agricultural operations that contribute to improving land yields such as regular irrigation, integrated pest control, and regular fertilization

Keywords. Data Envelope Analysis, Decision-Making Unit, Sensitivity Analysis.

1. Introduction

The concept of efficiency occupies some thinking and management practices, and one of the most important concerns of management is achieving efficiency measured by scientific methods, which is one of the most important determinants of food production in the agricultural sector, as efficiency greatly helps in diagnosing agricultural production problems, in addition to providing appropriate solutions according to experimental, theoretical and economic practices [1,2]. It can also be a driving force for investment that is witnessing a reluctance in the agricultural sector, especially with the widening food gap, and it can absorb labor and a resource for technology and

thus contribute to the development of the agricultural sector [3]. Achieving economic efficiency is done by mixing the elements of production and obtaining the largest amount of output [4], and it is one of the important indicators by which the extent of management's efficiency in directing various economic resources can be known [5]. Data envelopment analysis was initially developed to measure the relative efficiency of decision-making units for a number of inputs and outputs in 1978 by the researcher Charnes et al. [6]. The CCR model (Charnes, Cooper and Rhodes) was the first DEA model. The goal of DEA models is to evaluate the overall efficiencies of decision-making units (DMUs) responsible for transforming a set of inputs into a set of outputs. Efficient DMUs are not comparable to each other in the CCR model and other DEA models. Efficient DMUs are assigned a score of 1.0 and inefficient DMUs are assigned a score of less than 1.0. The DMU score is measured by combining a set of efficient DMUs from DEA that are part of the segments on the efficiency frontier. Banker and Chang [7], have shown that using the super-efficiency model to rank efficient DMUs is inappropriate. However, the concept of super-efficiency has been used in other situations such as sensitivity analysis, numerical data analysis, etc. [8-11]. In the last decade, some DEA researchers have started a new field called super-efficiency to classify effective DMUs in DEA management and have developed different models. Although the models developed are interesting and useful, they are generally not without drawbacks, as they lack stability or feasibility [12].

2. Theoretical Framework

2.1. Super-Efficiency: LJK–CCR Model

The super-efficiency model is called LJK (Li, Jahanshahloo, and Khodabakhshi). Although the model aims to rank the most efficient DMUs in terms of DEA obtained through the CCR model, it can be used to evaluate efficient units directly. That is, without solving the CCR model, one can rank efficient DMUs by solving the super-efficiency model alone. Highly efficient DMUs have a super-efficiency score greater than or equal to 1, while inefficient DMUs have a super-efficiency score less than 1 [13]. Lovell and Rowse (LR) proposed a modification of the data envelopment analysis (DEA) model that overcomes the inefficiency problem, often encountered in super-efficiency in the LR procedure, where the producer expands the observed input vector (minimizes the output vector) of the super-efficient facility, thus resulting in an inefficient replacement. Teschen proposed a different procedure that replaces the input-output bundles that were found to be ineffective in farm management through their effective expectations in an alternative procedure proposed that uses the directional distance function and the resulting (Nerlove Luenberger) measure of super-efficiency. The directional distance function by definition combines the features of both the input-oriented model and the output-oriented model that leads to a complete ranking of observations and can be easily interpreted [14].

The tenure is linked to efficiency by estimating the super-efficiency for each size, and here the use of super-efficiency must be justified, since efficiency is not only sought but also performance, meaning if we assume that we have 50 economic units and 15 of them achieved 100% complete efficiency, the decision maker prefers any of these units with complete efficiency, hence the need to know the performance of these units by estimating super-efficiency using the data envelopment analysis (DEA) method, and ranking them according to their potential capacity in the event of increasing inputs and decreasing outputs based on SLACK resources, which is similar to sensitivity analysis or post-optimization analysis. According to the following model [15,16]:

$$\begin{aligned}
 \text{[SBM]} \quad \min \quad & \rho = \frac{1 - \frac{1}{m} \sum_{i=1}^m s_i^- / x_{io}}{1 + \frac{1}{s} \sum_{i=1}^s s_i^+ / y_{io}} \\
 \text{subject to} \quad & x_o = X\lambda + s^- \\
 & y_o = Y\lambda - s^+ \\
 & \lambda \geq 0, s^- \geq 0, s^+ \geq 0.
 \end{aligned}$$

Tone in his study came up with a proposal for a high-efficiency scale to measure super-efficiency using data envelopment analysis (DEA), and the study distinguished between two types of efficiency calculation methods:

- The (SDM) approach that depends on stagnation in inputs/outputs.
- The radial measurement method.

Super-efficiency presents the potential ability of efficient farms to expand their inputs or reduce their outputs without becoming inefficient [17], and Banker and Chang [18] exploited the super-efficiency model to detect and remove outliers (Banker and Chang, 2006, 1320), and Chen proposed using the integrated super-efficiency obtained from VRS super-efficiency models directed towards inputs and outputs, and super-efficiency has been repeatedly applied to evaluate farm efficiency, and efficiency has been widely measured using super-efficiency in many studies [17].

2.2. Super-Efficiency Models

- The radial super-efficiency model presented by Andersen and Petersen in 1993 [19]: The super-efficiency model was used to determine the efficient values, which indicates the cancellation of units that provide efficiency scores equal to one after each exclusion of the unit. The $n-1$ units were evaluated and the number of efficiency scores that changed was calculated and the efficient unit measure equal to one (the average change in the measured efficiency) resulting from the elimination of the efficient unit was calculated until it became super-efficient.
- The super-efficiency model for a stagnation-based measure SBM presented by Tone in 2002 [20]: Tone proposed the super-efficiency model as a measure of efficiency based on stagnation, also called the mathematical complement or unutilized production requirements, as it measures the efficiency of the units under evaluation using only the stagnation variables S^+ and the super-efficient SBM model removes the evaluated unit for inefficient decision-making units from the set of completely efficient units (100%) only if both the inputs and outputs are equal to one That is, each slowdown $S^+ \geq 0$.
- The efficiency model of the directional distance function proposed by Ray in 2008: Ray developed the directional distance function model for super-efficiency that works to minimize inputs at the same rate while increasing outputs to reach the limits of data envelope analysis.

3. Materials and Methods

The primary data were obtained by creating a questionnaire designed for this purpose, and it was distributed randomly to farmers, as information was collected from (134) farms that included all crops grown in them and belonging to the agricultural people of the Karkh and Rusafa sides of Baghdad Governorate for the year (2021 - 2022), which is located in the central part of Iraq and is bordered by Diyala Governorates to the east, Wasit and Babylon to the south, Anbar to the west, and Salah al-Din Governorates to the north. The area of Baghdad Governorate is (5169) square

kilometers, and includes (32) administrative units (districts) that form (10) districts. It is one of the most densely populated governorates, as it represents the capital of Iraq and its economic, administrative and cultural center. The total population is about (8,126,755) people for the year 2017, with a population growth rate of (2.1%), as the percentage of urban population in the governorate constituted (87%), while their percentage in the countryside amounted to (13%). As for economic activity, the rate for the same year reached (43.9%), while the unemployment rate reached (9.3%). The EMS program was used to analyze data and measure super-efficiency by determining the optimal combination of a group of inputs and one output.

4. Results and Discussion

In this research, the super-efficiency model was used to classify highly efficient DMU farms in DEA, as this efficiency measures the potential ability of efficient farms to expand their inputs or reduce their outputs without becoming inefficient. In order to know which farms achieved super efficiency among the efficient sample farms, the data (collected from the questionnaire forms after tabulation) was analyzed using the (EMS) program, where four inputs and one output were entered, which are:

X1: Number of manual work hours (hour).

X2: Number of automated work hours (hour).

X3: Quantity of chemical fertilizer (kg).

X4: Cultivated area (dunum).

And one output, which is: -

TR: Total revenue (thousand dinars).

The results of the analysis showed that there were (15) farms out of a total of (134) farms that achieved a complete efficiency of 11% starting from (100%) and above, and they were indicated by shading, and this can be observed through Table 1. It was shown that the highest percentage of super-efficiency was for farm No. (66), which achieved (117%). This means that the producer who owns the farm can increase the use of resource X2, which represents the number of hours of automated work by (0.25) hours, and increase the use of resource X3, which represents the amount of chemical fertilizer by (0.75) kg at each fertilization process, in addition to reducing the output TR, which represents the total revenue by (1.17) thousand dinars, and yet this farm remains efficient. The lowest super-efficiency rate was for farms (13, 78, 103, 115) which achieved a rate of (100%), which means that the owners of farms (13, 78, 103) can increase the number of manual labor hours by (0.88) working hours, increase the amount of chemical fertilizer by (0.11) kg, and increase the cultivated area by (0.01) dunums for each production process, in addition to reducing the total revenue by (1) thousand dinars, and these farms remain efficient. As for farm number (115), which means that when the number of manual labor hours increases by (0.32) working hours, the amount of chemical fertilizer increases by (0.09) kg, and the cultivated area increases by (0.59) dunums, and the total revenue decreases by (1) thousand dinars, this farm remains efficient. It is also clear from the table that the average efficiency at the sample level reached (66.24%), which means that it is far from full efficiency by (33.76%) due to the lack of optimal use of resources.

Table 1. Efficiency ratios achieved by the study sample farms for the year (2021-2022).

DMU	Score	X1	X2	X3	X4	TR	DMU	Score	X1	X2	X3	X4	TR
f1	82.02%	0.58	0	0	0.42	0.82	f68	45.31%	0.82	0.16	0	0.01	0.45
f2	52.48%	0.67	0.25	0	0.08	0.52	f69	87.15%	0.81	0	0	0.19	0.87
f3	87.15%	0.81	0	0	0.19	0.87	f70	57.77%	0.84	0	0	0.16	0.58
f4	57.77%	0.84	0	0	0.16	0.58	f71	32.64%	0.42	0.41	0.16	0	0.33
f5	34.49%	0.42	0.41	0.17	0	0.34	f72	51.60%	0.35	0.39	0.07	0.19	0.52

DMU	Score	X1	X2	X3	X4	TR	DMU	Score	X1	X2	X3	X4	TR
f6	53.60%	0.35	0.39	0.07	0.19	0.54	f73	43.10%	0.42	0.27	0.32	0	0.43
f7	47.02%	0.42	0.27	0.32	0	0.47	f74	99.13%	0.43	0	0.02	0.54	0.99
f8	100.88%	0.44	0	0.02	0.54	1.01	f75	85.97%	0.57	0	0	0.43	0.86
f9	85.97%	0.57	0	0	0.43	0.86	f76	92.83%	0.72	0.27	0	0.01	0.93
f10	90.64%	0.83	0.15	0	0.01	0.91	f77	63.15%	0.42	0.55	0.03	0	0.63
f11	92.83%	0.81	0	0	0.19	0.93	f78	100.00%	0.88	0	0.11	0.01	1
f12	63.55%	0.42	0.55	0.03	0	0.64	f79	67.30%	0.4	0.6	0	0	0.67
f13	100.00%	0.88	0	0.11	0.01	1	f80	72.51%	0.83	0.16	0	0.01	0.73
f14	59.58%	0.41	0.59	0	0	0.6	f81	63.57%	0.74	0.26	0	0	0.64
f15	58.96%	0.82	0.16	0	0.01	0.59	f82	51.00%	0.77	0	0.13	0.1	0.51
f16	55.66%	0.74	0.26	0	0	0.56	f83	71.71%	0.83	0.16	0	0.01	0.72
f17	57.57%	0.77	0	0.13	0.1	0.58	f84	55.25%	0.54	0	0	0.46	0.55
f18	71.71%	0.83	0.16	0	0.01	0.72	f85	47.15%	0.46	0.26	0.16	0.12	0.47
f19	56.21%	0.37	0	0.25	0.38	0.56	f86	66.12%	0.47	0.33	0.07	0.13	0.66
f20	67.82%	0.39	0	0.26	0.36	0.68	f87	68.51%	0.48	0.36	0.16	0	0.69
f21	49.95%	0.09	0	0	0.91	0.5	f88	60.28%	0.46	0.25	0.16	0.14	0.6
f22	40.50%	0	0.5	0.5	0	0.41	f89	86.54%	0.47	0.35	0.18	0	0.87
f23	37.91%	0.49	0	0.22	0.29	0.38	f90	43.24%	0.47	0.26	0.14	0.12	0.43
f24	60.78%	0.3	0.36	0.35	0	0.61	f91	42.46%	0.43	0	0.28	0.29	0.42
f25	48.07%	0.84	0.06	0	0.09	0.48	f92	46.51%	0.43	0	0.28	0.29	0.47
f26	48.44%	0.31	0.37	0.32	0	0.48	f93	41.50%	0.45	0.28	0.14	0.13	0.42
f27	98.08%	0	0.06	0.58	0.35	0.98	f94	39.19%	0.38	0.32	0.15	0.15	0.39
f28	100.25%	0.2	0	0.45	0.34	1	f95	43.53%	0.56	0.26	0.08	0.1	0.44
f29	100.30%	0	0	0.62	0.38	1	f96	54.54%	0	0.48	0.52	0	0.55
f30	88.65%	0.82	0	0	0.18	0.89	f97	105.00%	0	0	0	1	1.05
f31	51.56%	0.35	0.07	0.24	0.34	0.52	f98	87.15%	0.79	0	0	0.21	0.87
f32	44.88%	0.37	0.39	0.06	0.18	0.45	f99	31.91%	0.45	0.29	0.12	0.14	0.32
f33	61.09%	0.57	0.26	0.07	0.1	0.61	f100	43.10%	0.42	0.27	0.32	0	0.43
f34	52.77%	0.37	0.39	0.06	0.18	0.53	f101	85.97%	0.57	0	0	0.43	0.86
f35	112.50%	0	1	0	0	1.12	f102	92.83%	0.81	0	0	0.19	0.93
f36	92.19%	0.72	0	0.23	0.05	0.92	f103	100.00%	0.88	0	0.11	0.01	1
f37	69.88%	0.8	0	0	0.2	0.7	f104	58.96%	0.82	0.16	0	0.01	0.59
f38	66.77%	0.3	0.36	0.34	0	0.67	f105	51.00%	0.77	0	0.13	0.1	0.51
f39	55.96%	0.53	0	0	0.47	0.56	f106	53.65%	0.54	0	0	0.46	0.54
f40	87.15%	0.79	0	0	0.21	0.87	f107	47.67%	0.09	0	0	0.91	0.48
f41	86.13%	0.44	0	0.25	0.31	0.86	f108	36.66%	0.49	0	0.22	0.29	0.37
f42	43.41%	0	0	0.47	0.53	0.43	f109	47.28%	0.84	0.06	0	0.09	0.47
f43	60.94%	0.26	0.31	0.43	0	0.61	f110	89.27%	0	0.05	0.61	0.34	0.89
f44	52.49%	0.45	0.36	0.07	0.13	0.52	f111	90.06%	0	0.07	0.58	0.35	0.9
f45	58.17%	0.81	0	0	0.19	0.58	f112	84.82%	0.22	0	0.46	0.32	0.85
f46	45.33%	0.49	0.24	0.15	0.13	0.45	f113	50.79%	0.35	0.07	0.24	0.34	0.51
f47	52.57%	0.4	0.06	0.28	0.26	0.53	f114	62.00%	0.57	0.26	0.07	0.1	0.62
f48	92.44%	0.32	0.31	0.37	0	0.92	f115	100.00%	0.32	0	0.09	0.59	1
f49	62.16%	0.27	0.32	0.41	0	0.62	f116	69.88%	0.79	0	0	0.21	0.7
f50	35.26%	0.41	0.06	0.23	0.3	0.35	f117	55.53%	0.53	0	0	0.47	0.56
f51	38.13%	0.45	0.06	0.21	0.28	0.38	f118	86.16%	0.45	0	0.24	0.31	0.86
f52	86.50%	0.3	0.36	0.35	0	0.87	f119	44.65%	0.63	0.35	0.02	0	0.45
f53	71.85%	0.26	0.31	0.43	0	0.72	f120	53.57%	0.41	0.3	0.14	0.16	0.54
f54	111.70%	0	0.52	0.48	0	1.12	f121	44.53%	0.68	0.32	0	0	0.45
f55	50.60%	0.82	0.16	0	0.01	0.51	f122	50.13%	0.57	0.41	0.02	0	0.5
f56	45.97%	0.58	0	0	0.42	0.46	f123	39.24%	0.45	0.06	0.22	0.27	0.39
f57	66.98%	0.34	0.06	0.3	0.31	0.67	f124	71.85%	0.26	0.31	0.43	0	0.72
f58	63.84%	0	0.05	0.62	0.33	0.64	f125	50.60%	0.82	0.16	0	0.01	0.51
f59	33.51%	0.51	0	0.22	0.26	0.34	f126	67.15%	0.34	0.06	0.3	0.31	0.67
f60	113.53%	0.37	0.47	0.16	0	1.14	f127	33.29%	0.52	0	0.22	0.26	0.33
f61	107.85%	0.34	0	0.66	0	1.08	f128	101.69%	0.29	0.36	0.36	0	1.02
f62	49.48%	0.05	0	0	0.95	0.49	f129	60.25%	0.4	0	0.24	0.36	0.6

DMU	Score	X1	X2	X3	X4	TR	DMU	Score	X1	X2	X3	X4	TR
f63	68.02%	0.4	0	0.24	0.36	0.68	f130	87.15%	0.81	0	0	0.19	0.87
f64	87.76%	0.42	0	0.25	0.34	0.88	f131	32.76%	0.42	0.41	0.16	0	0.33
f65	86.45%	0.03	0	0.88	0.09	0.86	f132	42.87%	0.47	0	0.2	0.33	0.43
f66	117.26%	0	0.25	0.75	0	1.17	f133	85.97%	0.57	0	0	0.43	0.86
f67	104.52%	0.47	0.38	0	0.15	1.05	f134	64.82%	0.41	0.56	0.03	0	0.65
						Average							66.24%

*Source: Researcher's work using EMS program.

4.1. Efficiency Levels of the Study Sample Farms

In order to identify the efficiency levels achieved by the studied farms, the efficiency levels were divided into three categories as in Table 2. The first category included farms whose efficiency rates ranged between (1-49) %, which are inefficient farms, amounting to (38) farms, representing an importance rate of (27%) of the total sample farms. The second category included (83) farms whose efficiency rates ranged between (50-99) %, and the highest importance rate exceeded half, which is (62%) of the total sample farms. The farms within these two categories are inefficient farms, while the third and final category occupied the last place in terms of the number of farms, which reached (15) farms, representing (11%) of the total sample farms. The farms of this category are considered efficient farms that achieved complete or superior efficiency, as their efficiency rate reached 100% or more.

Table 2. Number of farms according to efficiency levels for the study sample farms for the year (2021-2022).

Proficiency Level Categories %	Number of farms	Relative importance %
(49-1)	36	27
(99-50)	83	62
(or more 100)	15	11
the total	134	%100

*Source: Researcher's work based on the questionnaire and EMS program results.

4.2. The Relationship Between Efficiency and Holding Size for the Study Sample Farms

Table 3 shows the holding size of the sample farmers and the average level of efficiency. It was also divided into three categories. The first category includes small cultivated areas ranging from (1-40) dunums, which achieved an average efficiency of (64.56%), which is the lowest percentage among the three categories. It is followed by the second category with medium areas (41-80) dunums, which achieved an efficiency rate of (71.09%), which is the highest category among the three categories. As for the third category with large areas (81 or more), it achieved an average efficiency rate of (69%). It is noted that medium-sized farms are the ones that achieved the best levels of efficiency and were able to exploit their resources with better efficiency.

Table 3. Average efficiency for the holding size categories for the study sample farms for the year (2021-2022).

Holding Size Categories	Average proficiency level
Small (1-40)	% 64.56
Medium (41-80)	% 71.09
Large (81+)	% 69.00

*Source: Researcher's work based on the questionnaire and EMS program results.

4.3. Inputs and Revenues Aspects According to Efficiency

4.3.1. Inputs

In order to know the reasons that led some farms to achieve high efficiency, and others less than that, the inputs, which include (manual labor cost, mechanical labor cost, chemical fertilizers, pesticides) were divided into two sections: inputs related to efficient farms, and inputs related to inefficient farms, as shown in Table 46. The average inputs of efficient farms, which numbered (15) farms that achieved an efficiency rate of (100%) or more, and inefficient farms, which numbered (119) farms that achieved an efficiency rate of less than (100%) out of a total of (134) farms, as we note that the averages of all inputs except the cost of mechanical labor for efficient farms were higher than their counterparts for inefficient farms.

Table 4. Average inputs by efficiency.

Inputs	Average inputs of efficient farms (thousand dinars /dunum)	Average inputs of inefficient farms (thousand dinars/dunum)
Manual labor cost	22.68	20.09
Automated labor cost	22.39	25.99
Chemical fertilizers	123.85	123.53
Pesticides	21.14	14.45

*Source: Researcher's work based on the questionnaire and EMS program results.

4.3.2. Revenues

Table 5 includes the average revenue per dunum for efficient and inefficient farms. The table shows the large difference between them, as efficient farms achieved an average revenue per dunum of (1488.96) thousand dinars/dunum, while inefficient farms achieved an average revenue of (974.38) thousand dinars per dunum.

Table 5. Average revenue by efficiency.

Farm Efficiency	Average farm income (thousand dinars/dunum)
Efficient Farms	1488.96
Inefficient Farms	974.38

*Source: Researcher's work based on the questionnaire and EMS program results.

From Tables 4 and 5, the reason for their efficiency can be attributed to the fact that they use modern technologies in agriculture or to the element of efficient management and organization or both, in addition to other factors such as regular irrigation and the quality of seeds used in agriculture and others.

Conclusions and Recommendations

The study showed that 11% of the sample farms achieved super efficiency, so we conclude from this that approximately 90% of the sample farms studied are inefficient farms, whether in terms of the lack of optimal use of resources or the failure to allocate resources correctly between projects, or the inappropriate mix of projects and outputs. Also, medium-sized holdings are the most stable or more tolerant of change, whether in terms of inputs or outputs. The research recommends the necessity of using modern technologies in agriculture and emphasizing the importance of following appropriate agricultural cycles, as well as monitoring the progress of agricultural operations that contribute to improving land yields such as regular irrigation, integrated pest control and regular fertilization, training and qualifying workers in the agricultural sector at

various levels, supporting agricultural research, encouraging cooperation and coordination in agricultural work, and activating the information systems structure.

References

- [1] Ali, E. H., & Hamza, M. A. (2018). An economic analysis for the factors effecting on net farm income using panel data. *The Iraqi journal of agricultural science*, 49(3), 410-417.
- [2] Ali, E. H., & Lafta, A. H. (2020). Measuring the economic efficiency and total productivity of resource and the technical change of agricultural companies in Iraq using SFA and DEA for the period 2005-2017. *Iraqi Journal of Agricultural Sciences*, 51(4).
- [3] Al-Jubory, Z. A. G., & Ali, E. H. (2019). Estimating constant elasticity of substitution production function for agricultural companies in Iraq. *Iraqi Journal of Agricultural Sciences*, 50.
- [4] Ali, E. H. (2016). Measuring cow farms efficiency by using the quality response. *Iraqi Journal of Agricultural Sciences*, 47(2).
- [5] Ali, E. H., & El-Eezi, J. M. H. (2015). Estimating farm technical efficiency by using stochastic frontier approach. *Iraqi Journal of Agricultural Sciences*, 46(2).
- [6] Charnes, A., Cooper, W. W., & Rhodes, E. (1978). Measuring the efficiency of decision-making units. *European journal of operational research*, 2(6), 429-444.
- [7] Banker, R. D., & Chang, H. (2000). Evaluating the super efficiency procedure in data envelopment analysis for outlier identification and for ranking efficient units. *School of Management, The University of Texas at Dallas, Richardson, TX*, 75083-70688.
- [8] Charnes, A., Haag, S., Jaska, P., & Semple, J. (1992). Sensitivity of efficiency classifications in the additive model of data envelopment analysis. *International Journal of Systems Science*, 23(5), 789-798.
- [9] Zhu, J. (1996). Data envelopment analysis with preference structure. *Journal of the Operational Research Society*, 47(1), 136-150.
- [10] Zhu, J. (2001). Super-efficiency and DEA sensitivity analysis. *European Journal of operational research*, 129(2), 443-455.
- [11] Seiford, L. M., & Zhu, J. (2005). A response to comments on modeling undesirable factors in efficiency evaluation. *European Journal of Operational Research*, 161(2), 579-581.
- [12] Shanling Li, Jahanshahloo, G. R., & Khodabakhshi, M. (2007). A super-efficiency model for ranking efficient units in data envelopment analysis. *Applied mathematics and computation*, 184(2), 638-648.
- [13] Li, S., Jahanshahloo, G. R., & Khodabakhshi, M. (2007). A super-efficiency model for ranking efficient units in data envelopment analysis. *Applied mathematics and computation*, 184(2), 638-648.
- [14] Ray, S. C. (2008). The directional distance function and measurement of super-efficiency: an application to airlines data. *Journal of the Operational Research Society*, 59(6), 788-797.
- [15] Tone, K. (2001). A slacks-based measure of efficiency in data envelopment analysis. *European journal of operational research*, 130(3), 498-509.
- [16] Tone, K., & Sahoo, B. K. (2003). Scale, indivisibilities and production function in data envelopment analysis. *International Journal of Production Economics*, 84(2), 165-192.
- [17] Chen, Y. (2005). Measuring super-efficiency in DEA in the presence of infeasibility. *European journal of operational research*, 161(2), 545-551.
- [18] Banker, R. D., & Chang, H. (2006). The super-efficiency procedure for outlier identification, not for ranking efficient units. *European journal of operational research*, 175(2), 1311-1320.
- [19] Andersen, P., & Petersen, N. C. (1993). A procedure for ranking efficient units in data envelopment analysis. *Management science*, 39(10), 1261-1264.
- [20] Tone, K. (2002). A strange case of the cost and allocative efficiencies in DEA. *Journal of the Operational Research Society*, 53(11), 1225-1231.

An analytical Economic Study of the Relationship Between Individual Food Security and Possession Security in Baghdad Governorate

Z. A. Al-Haboobi¹ and E. H. Ali²

^{1,2}Department of Agriculture Economics, College of Agriculture Engineering Science, University of Baghdad, Baghdad, Iraq.

¹E-mail: zahraa.ali@coagri.uobaghdad.edu.iq

²E-mail: eskander.hussain@coagri.uobaghdad.edu.iq

Abstract. The aim of the research was to evaluate the effects and relationship of tenure security on individual food security using the ordinary least squares OLS method. Data were collected through a questionnaire form distributed randomly to (134) farms affiliated with the agricultural divisions on both sides of Karkh and Rusafa in Baghdad Governorate for the year (2021-2022). It included detailed information about the holdings, their nature, and the crops grown in detail in terms of areas, revenues, and costs, as well as the composition, household spending, food information, livestock, assets, and others. The results of the standard analysis using the EViews program showed that the model was free of standard problems through a set of tests used to detect the presence or absence of second-degree problems. As for the percentage of self-sufficiency of plant food from the farm, we note that the highest percentage was in small farms, reaching (35.6%). As for animal production, the average self-sufficiency percentage of the agricultural family is completely complete in the third category (41 or more) heads. It also showed that with the increase in the number of members of the agricultural family, individual food security increases to (20) individuals, then begins to decrease. It was shown that the value of farm assets has a positive impact on individual food security to a certain extent, then begins to decrease. The research concluded that the size of the holding has a significant impact on the food security of the farmer, and that large farms rely on the cultivation of cash crops to achieve their profit goals, while small farms cultivate various crops to achieve self-sufficiency in food. The size of the family is also linked to the size of the project. By increasing the size of the family with the increase in the size of the project, individual food security is achieved, but to some extent, and that investment in the value of assets, especially medium-term ones, is positively reflected in the food security of the holding. Accordingly, the research recommends determining the specifications and characteristics of agricultural holdings, activating the legislation and laws related to the uses of agricultural lands and following up on their implementation in order to stop encroachments on fertile agricultural lands and limit their erosion and conversion into residential lands, in addition to stopping the fragmentation of agricultural holdings, which represents an obstacle to the adoption of modern technologies, which leads to a negative impact on agricultural production and thus on individual food security.

Keywords. Body mass, Self-sufficiency, Modern technologies.

1. Introduction

The vast diversity and complexity of land tenure practices, which has led to the emergence of many areas of specialization for land tenure researchers, has led to similarities between trends in tenure security and trends in individual food security, despite them being separate areas of scientific research. The term food security has received great attention from international organizations, experts and researchers [1]. Food security can be defined as “secure access at all times to sufficient food for a healthy life.” The concepts of vulnerability and poverty are linked to food insecurity. Vulnerability can be defined as the inability to protect oneself from shocks [2,3]. It remains a major humanitarian concern in many contexts [4]. The complexity of defining food insecurity makes it difficult to measure it empirically in a complete and objective manner [5]. Therefore, through this research, we try to reach and know the extent of the impact of tenure security on individual food security and the extent to which it is affected by it by evaluating the dynamic effects of enhancing tenure security on the availability of calories, as they are considered basic pillars for ensuring the livelihood of rural families, especially the poor ones, and what happened to the agrarian reform law, which targeted the social status of the Iraqi farmer without his economic status, as we notice that rural families have become producers and consumers at the same time.

The lack of tenure security, separately or in combination with other obstacles, restricts agricultural performance and makes tenure a complex and multidimensional relationship [6], as is the case in some countries that have undergone radical land reforms in response to the problems of tenure insecurity due to repeated land redistribution and weak property rights that undermined incentives for investment [7-10]. Reducing the property rights of owners and enhancing the rights of tenants, which are often called "land for the farmer" reforms or rental reforms, were important policy interventions in many Asian countries [11]. Landowners may prefer to rent to less efficient tenants or not to rent at all [12]. Land registration can create uncertainty and conflicts on land rights rather than reduce them [13-17]. Due to the limited cultivated areas, scarce water resources, low crop productivity and low livestock productivity [18], and high food prices, new threats to land tenure security are introduced, especially in areas abundant in land and exposed to new high demands for land [19]. Land tenure and food security have been two separate areas of research, each with a vast and complex area, especially tenure due to the great heterogeneity, complexity and legislation in land tenure systems [20]. There are several dependent variables that represent indicators of individual food security, which represent the nutritional needs of individuals and households and the availability of food, including adult calories as a variable at the household level, and body mass index for household members. There are indicators developed by FAO to calculate both variables. The explanatory variables are (holding size, years of land exploitation, gender of the household head (qualitative variable), size of the exploited farm, farm characteristics, age, education... [21], and there are other indicators such as the availability of safety nets, the ability to access credit and insurance markets such as informal insurance, the number of meals and food consumed at specific times of the year (for example, before harvest), the nutritional status of children, food stocks in households, and indicators of asset poverty [22]. Climate risks and change also have serious impacts on family vulnerability and food security [23]. Accelerating the adoption of technologies is a major requirement to increase agricultural productivity, enhance food security, and reduce poverty, especially in economically poor communities [24]. Materials and methods of work: To meet the requirements of the research and achieve its objectives, data were collected from their primary sources through a questionnaire designed for this purpose and distributed randomly to farmers. Information was collected from (134) farms affiliated with the agricultural divisions on both sides of Karkh and Rusafa in Baghdad Governorate for the year (2021-2022), and it included detailed information about The

tenure, its nature, and the crops grown in detail in terms of areas, revenues, and costs, as well as the composition and household expenditures, food information, livestock, assets, and other assets. To know the impact of social and economic factors affecting individual food security, by using the regular least squares method using the EViews program.

2. Results and discussion

2.1. First: Description of the Economic and Mathematical Model

The description and formulation of the economic mathematical model is based on the data of the economic theory and any other information related to the phenomenon under study, as the description stage is considered one of the most important and most difficult stages of standard research and is considered the weak point in standard applications and research if the model is described incorrectly, and most standard estimation methods are sensitive to description errors.

Based on this, we can clarify the relationships between possession, production, and household or individual food security and how to link them by estimating the following model:

$$Y=b_0+b_1 X_1+b_2 X_2+b_3 X_3+b_4 X_4+b_5 X_5+b_6 X_6+b_7 X_7+b_8 X_8+u_i$$

Dependent variable (Y): It is one of the indicators expressing food security for individuals, which represents an indicator of body mass for family members. It was obtained from dividing the weight in kilograms by the square of the height in meters for the farmer. This indicator may not be fully expressive because the farmer's food did not depend entirely on his farm, which is what was relied upon from foreign research and previous sources.

B: Independent variables: The independent variables included the following:

X1: A quantitative variable representing the size of the farmer's possession (dunum).

X2: A quantitative variable representing the number of years the farmer has exploited the land (year).

X3: A qualitative or dummy variable (the gender of the household head), where it is (1) for males and (0) for females.

X4: A variable representing the number of years of land exploitation * the gender of the household head.

X5: A quantitative variable representing the size of the exploited farm / the size of the farmer's holding (dunums).

X6: A quantitative variable representing the years of experience in agriculture for the farmer (years).

X7: A quantitative variable representing farmer age (years).

X8: A quantitative variable representing the distance between the farm and the market (km).

The function was estimated to represent the relationship between body mass as a dependent variable and the independent variables affecting body mass. After several attempts to formulate the appropriate mathematical model, including the linear function, the double logarithmic function, the inverse logarithmic function, and the semi-logarithmic function, in order to obtain the model that is adopted in studying the body mass index using the ordinary least squares (OLS) method through the standard analysis program ((EViews), and the results of the study analysis showed that the double logarithmic function is one of the best functions in terms of evaluating the estimated coefficients and representing the relationship according to the logic of economic theory, as shown in fig. 1. In addition to passing the statistical and standard tests.

After estimating the function, we know the effect of the studied variables on body mass through:

2.1.1. Holding Size

It is an independent quantitative variable that represents the total holding of the farmer, as its parameter reached (0.135), and came with a positive sign and consistent with the logic of economic theory. This confirms the direct relationship between holding and body mass, as when increasing the holding size by (1%), it will lead to an increase in body mass by (0.135%) when the other variables are constant at the average. As for the statistical aspect and through the table above and based on the t-test, we note that it is significant at the level of (5%), which means that it is representative of the sample community, i.e. when increasing the holding size, the cultivated areas will increase, which leads to an increase in the amount of production, which in turn increases the amount of food present in the crop.

2.1.2. Years of Land Exploitation

It is a quantitative variable that represents the number of years the farmer has exploited the agricultural land. Its parameter reached (0.637), and it came with a positive sign, meaning the relationship is direct and consistent with economic logic, where it indicates that when the number of years of land exploitation increases by (1%), it will lead to an increase in body mass by (0.637%) when the other variables are constant at the average. Based on the statistical test t, we note that it is significant at a significance level of (5%), and the relationship between them can be interpreted as the more years of land exploitation by the farm owner, this leads to an increase in the farmer's experience and thus an increase in his stability on the land and an increase in investment in assets, which leads to an increase in the amount of production and then to an increase in the amount of food on the farm, which leads to an increase in body mass.

Dependent Variable: LY				
Method: Least Squares				
Date: 07/11/24 Time: 22:04				
Sample: 1 134				
Included observations: 134				
Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	3.693687	0.243181	15.18905	0.0000
LX1	0.135358	0.066906	2.023108	0.0282
LX2	0.637082	0.253552	2.512630	0.0329
LX3	0.302241	0.111704	2.705720	0.0482
LX4	0.304263	0.081629	3.727383	0.0084
LX5	0.212168	0.100180	2.117867	0.0362
LX6	0.112167	0.069783	1.607376	0.1105
LX7	-0.068568	0.110907	-0.618247	0.5375
DUMMY	0.033050	0.012737	2.594795	0.0231
R-squared	0.769012	Mean dependent var	3.353834	
Adjusted R-squared	0.751655	S.D. dependent var	0.130380	
S.E. of regression	0.128300	Akaike info criterion	0.204091	
Sum squared resid	2.057606	Schwarz criterion	1.009460	
Log likelihood	89.67412	Hannan-Quinn criter.	0.125000	
F-statistic	20.54347	Durbin-Watson stat	1.906110	
Prob(F-statistic)	0.000000			

Figure 1. Analysis of explanatory variables affecting body mass. Source: Researcher's work using EViews 11.

2.1.3. Gender of the Head of the Household

It is a qualitative variable that indicates the gender of the head of the household or the owner of the farm. It is a binary variable, as it takes the value (1) for the male and (0) for the female. Its parameter reached (0.033), and it came with a positive and significant sign at the level of (5%). This means that the shift to the male gender or when the male gender will increase the amount of production and then the amount of food and thus increase the dependent factor.

2.1.4. Years of Land Exploitation × Gender of the Head of the Household

It is a quantitative variable that represents the number of years the farmer has exploited the land, whether male or female, and its parameter reached (0.302) and came with a positive sign, i.e. the relationship is directly proportional to the dependent variable, as it indicates that when the number of years of land exploitation increases by (1%), it will lead to an increase in body mass by (0.302%) when the other variables are constant at the average, and based on the statistical t test, we note that it is significant at a significance level of (5%).

2.1.5. Size of the Exploited Farm / Size of the Holding

It is a quantitative variable that indicates the cultivated volume from the cultivated area to its total volume, and its parameter reached (0.304), and came with a positive sign, which means that if it increases by (1%), this will lead to a corresponding change in the same direction in body mass by (0.304%) when the other variables are constant at the average, and through the statistical t test, we find that it is significant at a level of (1%). The higher this percentage, meaning the greater the cultivated area of land is compared to the total area, will certainly lead to an increase in the quantities of crops and vegetables produced and thus to an increase in the quantity of food on the farm.

2.1.6. Years of Experience

It is a quantitative variable that indicates the number of years of experience that the farmer has gained as a result of cultivating his land. Its parameter reached (0.212) and came with a positive and significant sign at the level of (5%). This means that when the number of years of experience of the farmer increases by (1%), this will lead to an increase in body mass by (0.212%) when the other variables are constant at the average. The more experienced the farmer is in agricultural matters, the more this will lead to an improvement in his production in terms of quantity and quality, and this will contribute to an increase in the amount of food on the farm and thus an increase in body mass.

2.1.7. Farmer Age

It is a quantitative variable that represents farmer age in years. Its parameter reached (0.112) and came with a positive sign. This means that when farmer age increases by (1%), this will lead to an increase in body mass by (0.112%) when the other variables are constant at the average. Through the statistical t-test, we find that it is not significant at the level of (5%).

2.1.8. The Distance Between the Farm and the Market

It is a quantitative variable that indicates the distance that the farmer travels from his farm to the market or (the upper floor), and it came with a negative sign to indicate the inverse relationship between distance and mass, as in the case of a change (increase or decrease) by (1%), this leads to a corresponding change and opposite direction in body mass by (0.068%) when the other

variables are constant at the average, and through the statistical t test, we find that it is not significant at a significance level of (5%).

As for the value of the coefficient of determination R^2 , which is (0.769), it indicates that about (77%) of the fluctuations in body mass were caused by the independent variables included in the model, while (23%) of those fluctuations are due to other variables that were not included in the model and their effect was absorbed by the random variable U_i .

The same is the case when testing the function as a whole based on the (F) test, which amounted to (20.543), as it was significant and at a very high level, which indicates the importance of the independent variables included in the model and the quality of the fit of the regression line.

The research showed that there were no standard problems (second-degree problems).

2.2. Second: Food Sufficiency from the Farm

2.2.1. Plant Production

In order to identify the percentage of farmers' sufficiency in food produced by their farms, the cultivated areas were divided into five holding categories, in addition to collecting the answers of a sample of farmers through the questionnaire form about the extent of agricultural families' sufficiency in food, as shown in Table 1. It is noted that the first holding category (1-20) dunums obtained the highest percentage of food sufficiency, which amounted to (35.6%) with a number of farms amounting to (55) farms, on the contrary, in the fifth category (81 or more) dunums, where the lowest sufficiency rate amounted to (10.9%) with a number of farms amounting to (11) farms. This is because the larger the farm holdings, the more the farm specializes in growing one or two main crops to facilitate farm administrative operations. There are also other reasons, as the farmer may grow the most profitable crop and not the most needed, so he relies on the farm to meet his profit goals and on the market to meet his food needs. We note that the farm is not relied upon to meet food needs completely, and as we note in the table below, the highest self-sufficiency rate reached (35%) in small farms because these farms are diverse and considered family farms.

Table 1. Categories of average food self-sufficiency rate from plant products on the farm for the study sample for the year (2021-2022).

Number of farms	Average farm food self-sufficiency ratio %	Categories (dunams)
55	35.6	20-1
41	22.805	40-21
15	16.067	60-41
12	22.917	80-61
11	10.909	81 or more
134		total

Source: Researcher's work based on the questionnaire form.

2.2.2. Animal Production

In order to identify the extent of farmers' self-sufficiency in animal products (meat and milk), livestock of all types (sheep, cows, goats, buffaloes) were divided into four categories based on the number of heads. It is noted from Table 2 that the average self-sufficiency ratio of the agricultural family is completely complete in the third category (41 or more) heads and the number of farms reached (103) farms that own livestock.

Table 2. Categories of average food self-sufficiency ratio of animal products on the farm for the study sample for the year (2021-2022).

Number of farms	Average food sufficiency ratio of animal products %	Livestock Categories (Head)
61	54.59	20-1
21	91.19	40-21
21	100	41 or more
103		total

Source: Researcher's work based on the questionnaire form.

2.2.3. Animal Income

It is the sum of the animal incomes of each farm, whether (sheep, cows, goats, buffaloes, poultry, beehives), as shown in Table 3. Animal income categories are divided into five categories according to the total area, as the total animal income for the study sample reached (618306) thousand dinars, as the first category was the first, which amounted to (240275) thousand dinars, with a relative importance of (38.86%) of the total animal income, while the lowest category was for the fourth category (61-81) dunums, as it reached (10000) thousand dinars, which constituted a percentage of (1.62%) of the total animal income. The reason for this is that the owners of small areas depend heavily and clearly on animal production.

Table 3. Animal income categories for the study sample for the year (2021-2022).

Relative importance %	Animal income (thousand dinars)	Categories (dunams)
38.86	240275	20-1
38.51	238125	40-21
7.08	43781	60-41
1.62	10000	80-61
13.93	86125	81 or more
%100	618306	total

Source: Researcher's work based on the questionnaire.

2.3. Third: Economic and Social Characteristics and the Effects on Individual Food Security for the Study Sample

2.3.1. The Size of the Agricultural Family and its Impact on Individual Food Security

There are some economic and social factors and indicators surrounding the farm that have a direct and indirect impact on the food security of the agricultural family, including the size of the agricultural family. Through conducting the questionnaire and extracting the body mass index for farmers, and after dividing the number of members of the agricultural family into five categories, it was found that the fourth category, which includes agricultural families consisting of (16-20) individuals with a number of farms amounting to (5) farms out of the total sample farms, had the highest average farmer mass of (30.16) kg/m², while the lowest average farmer mass of (27.81) kg/m² was for the fifth category (21 or more) individuals with a number of (10) farmers out of the total study sample farms. This means that with the increase in the number of members of the agricultural family, individual food security increases until the number reaches (20) individuals, after which it begins to decrease. This can be seen from Table 4.

Table 4. Categories of agricultural household size and average farm mass for the study sample for the year (2021-2022).

Number of farms	Average farmer's mass (kg/m ²)	Household size categories (individual)
28	28.55	5-1
72	28.77	10-6
19	29.89	15-11
5	30.16	20-16
10	27.81	21 or more
134		total

Source: Researcher's work based on the questionnaire form.

2.3.2. Educational Level and its Impact on Individual Food Security

Among other factors affecting household food security is the educational level or academic achievement of the farmer, as shown in Table 5. We note the categories of educational achievement (illiterate, primary, intermediate, preparatory, bachelor's) divided into five categories, as it was found that the lowest average mass was (27.4) kg/m² in the fifth category (bachelor's) with a number of (16) farms, while the second category (primary) was the highest category for the average mass of farmers, reaching (29.3) kg/m² with a number of (40) farmers. This may be attributed to the fact that those who have a higher educational qualification do not depend on the farm to provide food.

Table 5. Educational level categories and average farm mass for the study sample for the year (2021-2022).

Number of farms	Average farm mass (kg/m ²)	Academic achievement categories
6	28.53	I can't read or write
40	29.30	Elementary
41	29.15	Middle
31	28.73	Preparatory
16	27.40	Bachelor's
134		total

Source: Researcher's work based on the questionnaire form.

2.3.3. The Value of Farm Assets and Their Impact on Individual Food Security

Farm assets have a clear and explicit impact on the farming family and thus on their food security, as we note from Table 6. There are also five categories for the values of farm assets and their relationship with the mass of farmers, as the highest average mass was in the third category (41-60) million with a number of farms of (19) farms, while the lowest average was in the fourth category (61-80) million with a number of (4) farms out of the total sample farms.

Table 6. Categories of farm asset values and average farm mass for the study sample for the year (2021-2022).

Number of farms	Average Farm Mass (kg/m ²)	Asset Value Categories (Million)
43	28.26	20-1
61	28.91	40-21
19	30.03	60-41
4	28.19	80-61
7	29.37	81 or more
134		total

Source: Researcher's work based on the questionnaire form.

2.3.4. Type of Farm Holding and its Impact on the Level of Farm Income

Table 7 shows the types of farm holdings in three categories (rent, contract, ownership), and the average income level per dunum, as it is noted that the highest average income level is (1283.51) thousand dinars per dunum for the rental category with a number of farms reaching (21) farms, while the category of owned holdings occupied the lowest level of average income, reaching (1167.33) thousand dinars per dunum with a number of farms reaching (37) farms.

Table 7. Categories of farm holding type and farm income level for the study sample for the year (2021-2022).

Number of farms	Average income level (thousand dinars/dunum)	Tenure Type Categories
21	1283.51	Rent
76	1225.30	Contract
37	1167.33	Own
134		total

Source: Researcher's work based on the questionnaire.

2.3.5. Size of Farm Holdings and Educational Level of Farmers

In order to identify the relationship between the educational level of farmers and the size of their agricultural land holdings, the size of the holdings was divided into five categories, each category divided into the educational levels of farmers, as shown in Table 8. We note that the first educational level (cannot read or write) included the smallest number of the total sample farmers, amounting to (6) farmers, while the second educational level (elementary) included (40) farmers from the total sample farmers, reaching a maximum of (17) farmers in the second holding category (21-40) dunums, and the third educational level (intermediate) included a total of (41) farmers from the total sample farmers, which is the largest level in terms of the total number of farmers, reaching a maximum in the first category (1-20) dunums, while the fourth educational level (preparatory) included (31) farmers, reaching a maximum in the first category (1-20) dunums, and finally the fifth level included (16) Farmers out of the total sample farmers were mostly in the first category as well (1-20) dunums.

Table 12. Categories of farm holding size and educational level of the study sample for the year (2021-2022).

Farmer numbers	Postgraduate studies	Preparatory	Middle School	Primary school	He can't read or write.	Holding size categories (dunums)
51	8	10	19	12	2	20-1
39	2	9	11	17	0	40-21
17	2	3	7	5	0	60-41
6	1	2	1	2	0	80-61
21	3	7	3	4	4	81 فأكثر
134	16	31	41	40	6	total

Source: Researcher's work based on the questionnaire.

Conclusions and Recommendations

The research concluded with a set of conclusions, including: The size of the holding has a significant impact on the food security of the farmer, as it came with a positive sign and is consistent with economic logic, as increasing the size of the holding leads to an increase in the cultivated areas, which leads to an increase in the amount of production and thus an increase in the amount of food available on the farm, and the percentage of farmers' self-sufficiency in food produced by their farms was higher in small holding categories and lower in large holding

categories, and we conclude from this that large farms rely on the cultivation of cash crops to achieve their profit goals, while small farms cultivate various crops to achieve self-sufficiency in food, and the research concluded that the size of the family is linked to the size of the project, as increasing the size of the family with the increase in the size of the project achieves individual food security but to some extent, as after increasing this number the relationship is inverse, and the results of the questionnaire showed that families with low education (primary) are the most food secure, unlike their peers who hold a bachelor's degree, and this may be attributed to the fact that farmers who have an academic qualification do not rely on the farm primarily to provide food, and investment in The value of assets, especially medium-term ones, is positively reflected on the food security of the holding, as the increase in investments increases the net farm income. Accordingly, the study recommends determining the specifications and characteristics of agricultural holdings, activating the legislation and laws related to the uses of agricultural lands and following up on their implementation in order to stop encroachments on fertile agricultural lands and limit their erosion and conversion into residential lands, in addition to stopping the fragmentation of agricultural holdings, which represents an obstacle to the adoption of modern technologies, which leads to a negative impact on agricultural production and thus on individual food security, and emphasizing the importance of following appropriate agricultural cycles, as well as monitoring the progress of agricultural operations that contribute to improving land yields, such as regular irrigation, integrated pest control, and maintaining the sustainability of the productive properties of agricultural soils by relying on organic fertilization as a basis for preserving them, stopping their deterioration, and their future exit from agricultural investment.

References

- [1] Jbara, O. K. (2024). An Economic Study Of The Factors Affecting The Food Security Coefficient Of The Rice Crop In Iraq For The Period (2003-2020). *Iraqi Journal Of Agricultural Sciences*, 55(3), 1209-1220.
- [2] Maxwell, S., & Frankenberger, T. R. (1992). Household Food Security: Concepts, Indicators, Measurements: A Technical Review. Pt. 1. Household Food Security, A Conceptual Review/Simon Maxwell, Marisol Smith--Pt. 2. Indicators And Data Collection Methods For Assessing Household Food Security/Timothy R. Frankenberger--Pt. 3. Household Food Security, Concepts And Definitions--An Annotated Bibliography/Marisol Smith, Judy Pointing, Simon Maxwell--Pt. 4. A Selected Annotated Bibliography On Indicators With Application To Household Food Security/Barbara
- [3] Maxwell, D., & Wiebe, K. D. (1998). Land Tenure And Food Security: A Review Of Concepts, Evidence, And Methods. Land Tenure Center, University Of Wisconsin-Madison.
- [4] Elbushra, A. A., & Ahmed, A. E. (2020). Food Security In Sudan: A Historical Analysis Of Food Availability. *Iraqi Journal Of Agricultural Sciences*, 51(1).
- [5] Maxwell, D. G. (1996). Measuring Food Insecurity: The Frequency And Severity Of "Coping Strategies". *Food Policy*, 21(3), 291-303.
- [6] Roth, M., & Haase, D. (1998). Land Tenure Security And Agricultural Performance In Southern Africa. Boarding Access And Shirghening Input Market Systems:1-18.
- [7] Holden, S. T., Deininger, K., & Ghebru, H. (2009). Impacts Of Low-Cost Land Certification On Investment And Productivity. *American Journal Of Agricultural Economics*, 91(2), 359-373.
- [8] Holden, S. T., & Ghebru, H. (2011). Household Welfare Effects Of Low-Cost Land Certification In Ethiopia (No. 03/11). Centre For Land Tenure Studies Working Paper.
- [9] Deininger, K., Ali, D. A., & Alemu, T. (2011). Impacts Of Land Certification On Tenure Security, Investment, And Land Market Participation: Evidence From Ethiopia. *Land Economics*, 87(2), 312-334.
- [10] Khai, L. D., T. Markussen, S. McCoy, And F. Tarp. 2012. "Impacts Of Land Rights Reforms On Land Transactions In Vietnam." Draft Paper.



- [11] Otsuka, K. (2007). Efficiency And Equity Effects Of Land Markets. *Handbook Of Agricultural Economics*, 3, 2671-2703.
- [12] Aryal, J. P., & Holden, S. T. (2011). Caste Discrimination, Land Reforms And Land Market Performance In Nepal (No. 01/11). Centre For Land Tenure Studies Working Paper.
- [13] Atwood, D. A. (1990). Land Registration In Africa: The Impact On Agricultural Production. *World Development*, 18(5), 659-671.
- [14] Benjaminsen, T. A., Holden, S., Lund, C., & Sjaastad, E. (2009). Formalisation Of Land Rights: Some Empirical Evidence From Mali, Niger And South Africa. *Land Use Policy*, 26(1), 28-35.
- [15] Green, J. K. (1987). Evaluating The Impact Of Consolidation Of Holdings, Individualization Of Tenure, And Registration Of Title: Lessons From Kenya.
- [16] Bruce, J. W. (1986). Land Tenure Issues In Project Design And Strategies For Agricultural Development In Sub-Saharan Africa.
- [17] Mackenzie, F. (1993). 'A Piece Of Land Never Shrinks': Reconceptualizing Land Tenure In A Smallholding District, Kenya (Pp. 194-221).
- [18] Gasim, O. A., & Al-Hani, A. S. (2019). An Economical Study Of Some Indicators Of Food Security In Iraq During The Period (1990-2015)/Wheat As Case Study. *The Iraqi Journal Of Agricultural Science*, 50(1), 456-464.
- [19] Deininger, K., & Byerlee, D. (2012). The Rise Of Large Farms In Land Abundant Countries: Do They Have A Future?. *World Development*, 40(4), 701-714.
- [20] Holden, S., Otsuka, K., & Deininger, K. (Eds.). (2013). *Land Tenure Reform In Asia And Africa: Assessing Impacts On Poverty And Natural Resource Management*. Springer.
- [21] Ghebru, H., & Holden, S. (2013). Links Between Tenure Security And Food Security: Evidence From Ethiopia (No. 02/13). Centre For Land Tenure Studies Working Paper.
- [22] Dercon, S. (2002). Income Risk, Coping Strategies, And Safety Nets. *The World Bank Research Observer*, 17(2), 141-166.
- [23] Holden, S. T. (2020). Policies For Improved Food Security: The Roles Of Land Tenure Policies And Land Markets. *The Role Of Smallholder Farms In Food And Nutrition Security*, 153-169.
- [24] Ali, E. H., Duaila, D. A., & Mohammed, M. K. (2023). A Study Of Economic, Social, And Institutional Factors Affecting The Adoption Of The High-Rank Wheat Seeds. *Iraqi Journal Of Agricultural Sciences*, 54(1), 161-175.

Effect of Planting Distances Between Pits and Lines on the Growth and Yield of Broad Bean (*vicia faba* L.)

Heba Star Naeem Easal

Al-Musaib Technical College, Al-Furat Al-Awsat Technical University, Babylon, Iraq.

E-mail: hbhstar276@gmail.com

Abstract. A field experiment was conducted on broad bean (*Vicia faba* L.) crop for the winter season 2023-2024 in Babylon province/Al-Mashrou' District to know: The effect of planting distances between pits and lines on the growth and yield of broad bean *vicia faba* L.). According to the design of randomized complete blocks (RCBD) and with three replicates. The experiment included studying the effect of two factors: the distance between lines: three distances were used for planting between lines (30, 40 and 50) cm and the distance between pits: three distances were used (15, 20 and 25) cm. The results showed a significant effect of planting distances between pits. The distance of 25 cm was excelled in giving the highest average in the trait of number of branches, number of pods and number of seeds, which reached (5.36 branches/plant⁻¹, 13.61 pods/plant⁻¹ and 4.75 seeds/pod⁻¹), while the distance of 15 cm gave the highest average in the trait (plant height and weight of 100 seeds reached (108.28 cm and 120.00 g). The planting distances between lines showed a significant effect. The distance of 50 cm gave the highest average in the trait of number of branches, The number of pods and the number of seeds reached (5.64 branches/plant⁻¹, 16.05 pods/plant⁻¹ and 5.36 seeds/pod⁻¹), while the distance of 30 cm gave the highest average in the trait of plant height and weight of 100 seeds reached (123.82 cm and 140.82 g). The results of bi-interaction showed a significant effect, as the combination (50 and 25) excelled in the trait of number of branches, number of pods and number of seeds, as it gave the highest average of (6.10 branches/plant⁻¹, 17.80 pods/plant⁻¹ and 6.15 seeds/pod⁻¹), while the combination (30 and 15) excelled in the trait of plant height and weight of 100 seeds, giving the highest average of (133.46 cm and 156.35 g).

Keywords. Broad bean, Growth and yield, Agricultural operations.

1. Introduction

Broad bean production is affected by many environmental and field factors, including plant density or changing the number of plants and the way they are distributed in a unit area, as they have a direct impact on the strength of plant growth and increase or decrease crop production[1]. Solar radiation is the factor that controls the final yield of any plant community, especially when temperature, water and nutrients are available in sufficient quantities. Here, light becomes one of the environmental factors that determine the growth and production of crops. Increasing plant density or changing the distribution of plants to the optimal level is an important way to improve light interception and use energy efficiently to obtain the best production[2]. Plant density plays an important role in determining the shape of the plant because of its relationship to branching and leaf area. Low plant density leads to a decrease in stem elongation, thus preventing lodging, while close distance is accompanied by a reduction in the light penetrating to the base of the plant, causing the lower internodes to elongate. When plant density increases, the height of the

plant increases, while the seed yield increases linearly with an increase in plant density to some extent. Broad bean yield is affected by many genetic and environmental factors, including plant distribution systems in the field that give different plant densities in the optimal distribution method. Due to the lack of studies on the optimal distribution of plants in order to give the highest yield and the best quality. Broad bean is one of the main food crops in Iraq. It is grown in large areas to produce green pods, fresh and dry grains, in addition to green fodder used in concentrated animal feed, in addition to its entry as a main crop in the agricultural cycle, as it works to improve the fertility properties of the soil through the process of fixing atmospheric nitrogen. Broad bean is one of the oldest and most famous cultivated plants and has productivity values compared to all leguminous crops [3]. The study aims to know the best planting distance between lines and the best distance between pits, as well as the best combination of plant distribution among line and pit distances that achieve the best vegetative growth yield per unit area.

2. Materials and Methods

2.1. Experiment Location

A field experiment was conducted for the broad bean (*Vicia faba* L.) crop for the winter season 2023-2024 in Babylon province/Al-Mashrou' District to know: The effect of the distances between pits and lines on the growth and yield of the broad bean *Vicia faba* L. plant.

2.2. Experiment Parameters

The experiment included studying the effect of two factors:

- First: The distance between lines:- Three distances were used between lines (30, 40 and 50) cm.
- Second: The distance between pits:- Three distances were used between pits (15, 20 and 25) cm.

2.3. Agricultural Operations

The land was plowed using a rotary plough, then smoothing, leveling and division operations were carried out into three replicates, each replicate containing 12 panels, and the area of the panel was (6=3x2 m²), then the panels were divided into several lines according to the mentioned distances Above. Leaving a distance of 50 cm between each panel, broad bean seeds (Luz de Otono) were planted on 10/20/2023 at a rate of 3 seeds per hole, which was thinned to one plant two weeks after emergence. Nitrogen fertilizer (urea 46% N) was added at a rate of 50 kg N/ha in two batches, the first at planting and the second 45 days after planting (beginning of flower bud formation). Phosphate fertilizer in the form of (superphosphate P₂O₅) at a rate of 200 kg P/ha in one batch before planting[4] . patching operations were carried out two weeks after planting. The plants of the middle lines of each experimental unit were harvested on 4/15/2024.

2.4. Experimental Design

A factorial experiment (4×3) m was implemented using the randomized complete block design (R.C.B.D) with three replicates. Each block included (9) factorial treatments resulting from the use of three distances between lines with three distances between pits, so the experiment included 36 experimental units. The experimental unit designated for the distance of 30 cm included six lines, the experimental unit designated for the distance of 40 cm included five lines, and the experimental unit designated for the distance of 50 cm included four lines. Statistical analysis After collecting the data related to the above-studied traits, they were statistically analyzed. The

data were analyzed and the averages were compared using the least significant difference at a probability level of 0.05 [5]. Using the Genstat program.

Table 1. Some chemical and physical characteristics of the soil of the experimental field before planting.

Traits	Values	Units
PH	7.54	
EC electrical conductivity	3.1	DS.m ⁻¹
Ca ⁺⁺	17.8	Meq/L
K ⁺	2.3	Meq/L
dissolved positive ions	Mg ⁺⁺	15.5 Meq/L
	NH ₄ ⁺	34.7 ppm
	Na ⁺	39.1 Meq/L
	HCO ₃ ⁻	4.6 Meq/L
	CL ⁻	54.7 Meq/L
dissolved negative ions	SO ₄ ⁼	14.7 Meq/L
	NO ₃ ⁻	40.1 ppm
	CO ₃ ⁼	0.00 Meq/L
available phosphorus	P	6.3 ppm
Soil Separators	sand	86
(silty clay loam)	silt	532 g/kg
	clay	382

2.5. Studied Traits

Ten random plants were selected from the midlines to calculate the following characteristics:

2.5.1. Field Emergence Percentage %

The emergence percentage was calculated according to the following equation:

$$\text{Field emergence percentage} = [\text{Number of sprouted pits} / \text{Total number of pit}] \times 100$$

2.5.2. Plant Height (cm)

It was calculated from the base of the plant to the end of the main branch when the plants reached the stage of maturity [6].

2.5.3. Number of Branches in the Plant

This traits was measured from the same plants for which the height was measured.

The yield and its components

2.5.4. Number of Pods in the Plant

According to the number of pods for the ten plants that were harvested, the average number of pods in the plant was extracted.

2.5.5. Number of Seeds in the Pod

The number of seeds for the ten plants was calculated, and the average number of seeds in the pod was calculated according to the following equation:

$$\text{Average number of seeds in the pod} = \text{Number of seeds in the plant} / \text{Number of pods in the plant}$$

2.5.6. Weight of 100 Seeds

100 seeds were counted, air-dried and weighed on a sensitive balance until the weight was stable, then the weight was adjusted to 15% humidity.

3. Results and Discussion

3.1. Field Emergence Rate (%)

The results of the statistical analysis in Table (2) showed that there was no significant effect on the field emergence rate of the planting distances between the lines and also the distances between the pits, as well as the interaction between them.

We note from the results shown in Table (2) that the emergence rate was high in all parts of the experiment and no significant differences were recorded. This is consistent with what [7] reached, as they indicated that there were no significant differences in the field emergence rate according to plant densities. The reason for this is that the plants are still young, meaning that there is no competition between them for water, light and nutrients.

Table 2. Effect of distances between lines and pits and their interaction on the percentage of field emergence (%).

Distance between lines(cm)	Distance between pits(cm)			Average distance between lines
	15	20	25	
30	98.16	98.08	98.15	98.13
40	98.4	97.7	97.32	97.87
50	96.91	94.99	98.57	96.47
Average distance between pits	97.82	96.92	98.01	
L. S. D 0.05		T=N.S	V=N.S	V*T=N.S

3.2. Plant Height (cm)

The results shown in Table (3) indicate that there are significant differences in plant height depending on the distances used between lines. Reducing the distance between lines led to a significant increase in plant height, as the distance of 30 cm between lines was significantly excelled in this trait, as it recorded the highest rate of plant height, reaching 123.82 cm, compared to the distance of 50 cm between lines, which gave the lowest rate of plant height, reaching 71.76 cm. This may be due to the increased competition between plants to obtain sufficient light to manufacture their food, which leads to an increase in plant height as a result of the elongation of the internodes. These results are consistent with what [8] reached, as they noted that there is a direct relationship between plant height and increased plant density. The results also showed that changing the distances between pits had a significant effect on plant height. The distance of 15 cm gave the highest average plant height of 108.28 cm compared to the distance of 25 cm, which recorded the lowest average plant height of 91.93 cm. This is a natural result due to competition for growth factors, the most important of which is light, as plant height increases at high plant densities. These results are consistent with what [9] reached. While bi- interaction between the planting distances between the lines and the planting distances between the pits, Table (3) indicates a significant effect, as the combination (30 and 15) recorded the highest rate of plant height, reaching 133.46 cm, while the combination (50 and 25) gave the lowest rate of plant height, reaching 61.12 cm. The increase in plant height at high densities may be due to the increase in the concentration of auxins at high densities due to the lack of photooxidation, which leads, in cooperation with gibberellins, to increased cell division and elongation, and then increased elongation of the internodes, and thus increased plant height.

Table 3. Effect of the distances between the lines and pits and the interaction between them on plant height (cm).

Distance between lines (cm)	Distance between pits (cm)			Average distance between lines
	15	20	25	
30	133.46	126.49	111.51	123.82
40	109.43	108.22	103.17	106.93
50	81.94	72.23	61.12	71.76
Average distance between pits	108.28	102.31	91.93	
L. S. D 0.05		T=0.69	V=0.69	V*T=1.38

3.3. Number of Branches (plant branch⁻¹)

From the results shown in Table (4), it is clear that the number of branches differs according to the distances used between the lines, as increasing the distance between the lines led to a significant increase in the number of branches, as the distance of 50 cm between the lines was significantly excelled in achieving the highest rate of the number of branches, recording 5.64 branches/plant⁻¹ compared to the 30 cm treatment between the lines, which recorded the lowest rate for this trait, reaching 4.34 branches/plant⁻¹. This may be attributed to the availability of appropriate environmental factors at wide distances and the lack of competition between plants for these factors due to their small number, which makes them increase the number of their branches and have the largest possible leaf area. These results are consistent with what [10] reached, as they indicated that increasing the distances between the lines led to an increase in the number of branches for the plant. As for the effect of planting distances between pits, the results of Table (4) indicate that there is a significant effect of the distances used between pits, as increasing the distance between plants led to a significant increase in the number of branches, as the distance of 25 cm was significantly excelled in achieving the highest rate of the number of branches, as it recorded 5.36 branches per plant⁻¹, while the distance of 15 between pits recorded the lowest rate, reaching 4.40 branches per plant⁻¹. These results are consistent with what [11] reached. The interaction between the planting distances between the lines and the planting distances between the pits showed significant differences in the interaction between them in the number of branches, as the combination (25 and 50) gave the highest average number of branches, reaching 6.10 branches/plant⁻¹, as it significantly excelled on all other combinations, and the combination (30 and 15) gave the lowest average number of branches, reaching 3.72 branches/plant⁻¹. From this, we can conclude that the number of branches/plant-1 increases the greater the distance between the plants and the lines, as this allows the plants to grow well and provides environmental factors such as heat, light and humidity to the plants better than in high densities, and thus competition between plants decreases. These results are consistent with what was found by [12].

Table 4. The effect of the distances between the lines and pits and the interaction between them on the number of branches in the plant.

Distance between lines (cm)	Distance between pits (cm)			Average distance between lines
	15	20	25	
30	3.72	4.34	4.95	4.34
40	4.31	4.42	5.02	4.58
50	5.17	5.65	6.10	5.64
Average distance between pits	4.40	4.80	5.36	
L. S. D 0.05		T=0.17	V=0.17	V*T=0.34

3.4. Number of Pods (pod/plant^{-1})

Table (5) shows a significant effect of the distances used between the lines on the number of pods in a plant, as the distance of 50 cm recorded the highest rate of $16.05 \text{ pods/plant}^{-1}$ compared to the lowest rate of $8.60 \text{ pods/plant}^{-1}$ given by the distance of 30 cm. This may be due to the increase in the number of plant branches at wide distances, or that the high density of plants (resulting from narrowing the planting distances between the lines) leads to an increase in the number of falling flowers and a reduction in the number of flowers within a single node, which results in a decrease in the average number of pods per plant, or perhaps due to shading, which leads to a decrease in light penetration and an imbalance in the vital processes within the plant. These results were similar to what [9] reached, as they indicated that expanding planting distances leads to a significant increase in the number of pods for the broad bean crop. We also note from Table (5) the significant effect of planting distances between pits on the number of pods per plant, as the distance of 25 cm was superior with the highest rate of pods per plant, amounting to $13.61 \text{ pods per plant}^{-1}$, compared to the lowest number of pods per plant, $10.84 \text{ pods per plant}^{-1}$, given by the distance of 15 cm. This means that plants planted at low densities form the largest number of pods per plant. The reason for this is that low densities of plants provide suitable conditions of lighting, ventilation, and suitable temperatures to fertilize the largest number of flowers formed on the plant. These results are consistent with what [8] reached. The effect of bi-interaction between the planting distances between the lines and the distances between the pits had a significant effect on the number of pods per plant, as the combination (25 and 50) was superior and gave $17.80 \text{ pods . plant}^{-1}$, while the combination that gave the least number of pods per plant was (30 and 15) as it gave $7.63 \text{ pods . plant}^{-1}$. The reason for this is that the shading of plants to each other at high density reduces the intensity of light in the lower parts of the plant that flower first, so the effect is indirect to the intensity of light controlling the amount of sugar transferred to the meristematic areas capable of forming flowers slightly, or perhaps due to the increase in the number of branches per plant at low density. The reason may also be that high density leads to increased shading on the flowers and may lead to their falling, which in turn reduces the number of pods, and these results are similar to what was found by [13].

Table 5. The effect of the distances between lines and pits and the interaction between them on the number of pods (pod.plant^{-1}).

distance between lines (cm)	Distance between pits (cm)			Average distance between lines
	15	20	25	
30	7.63	8.50	9.68	8.60
40	10.27	11.43	13.35	11.68
50	14.62	15.74	17.80	16.05
Average distance between pits	10.84	11.89	13.61	
L. S. D 0.05		T=0.05	V=0.05	V*T=0.1

3.5. Number of Seeds (pod seed^{-1})

We note from the results shown in Table (6) that the distance of 50 cm between the lines is superior in the number of seeds in the pod, as it reached 5.36 seeds of pod^{-1} compared to the distance of 30 cm, which gave the lowest rate of the number of seeds in the pod, which reached 3.06 seeds of pod^{-1} . The reason may be that the plant at the greater distance takes its need for water, light and nutrients necessary to form the largest number of seeds, i.e. there is no competition between plants. On the contrary, at high density, competition increases, and this affects the number of seeds in the pod. This is consistent with what [13] reached. From the same table, we find that the trend of the number of seeds in the pod was in favor of the wider distance between pits, as the distance of 25 cm gave the highest rate of 4.75 seeds per pod^{-1} and the lowest

rate at the distance of 15 cm, which amounted to 3.85 seeds per pod⁻¹. The reason for this was mentioned as the plant trying to reduce the effect of competition on nutrients at the beginning of the fruiting growth stage by dropping a number of its flowers or young pods, so its effect on the seed formation stages is small [14]. Bi-interaction between the distances used between the lines and the distances used between the pits showed through Table (6) the significant effect on the number of seeds in the pod, as the combination ((50 and 25)) recorded the highest rate of the number of seeds, which amounted to 6.15 seeds per pod⁻¹, while the combination (30 and 15) recorded the lowest rate of the number of seeds, which amounted to 2.84 seeds per pod⁻¹. Perhaps because the plant gets enough water, light and other nutrients, which leads to an increase in the number of pods as well as the number of seeds in the pod.

Table 6. Effect of the distances between the lines and pits and the interaction between them on the number of pod seeds (pod seed¹).

distance between lines (cm)	Distance between pits (cm)			Average distance between lines
	15	20	25	
30	2.84	3.05	3.30	3.06
40	3.92	4.77	4.80	4.49
50	4.78	5.15	6.15	5.36
Average distance between pits	3.85	4.32	4.75	
L. S. D 0.05		T=0.10	V=0.10	V*T=0.2

3.6. Weight of 100 seeds.g

The results shown in Table (7) showed that this trait took the opposite direction in the weight of 100 seeds from the traits of number of pods in a plant and number of seeds in a pod, as the distance of 30 cm gave the highest average weight of 100 seeds, reaching 140.82 g, compared to the lowest average at a distance of 50 cm, 83.14 g. The reason for this may be that at high plant density, both the number of pods in a plant and the number of seeds in a pod decreased, which led to an increase in the weight of 100 seeds, i.e. a small number of seeds, and the collection of nutrients became more, but their size was large, which led to an increase in the weight of 100 seeds. We also note from the table mentioned below the significant effect of planting distances between pits on the weight of 100 seeds, as the distance of 15 cm was superior in giving the highest average weight of 100 seeds, which reached 120.00 g, compared to the lowest average weight of 100 seeds, which was 99.22 g, given by the distance of 25 cm. This result is consistent with [15]. The interaction between planting distances between lines and pits showed a highly significant effect on the weight of 100 seeds, as the combination (30 and 15) was superior and gave 156.35 g, while the combination that gave the lowest weight of 100 seeds was (50 and 25), which reached 77.93 g. This may be due to the increase in the number of branches and the number of pods, as well as the increase in the number of seeds per pod, which negatively affected the weight of 100 seeds.

Table 7. Effect of distances between lines and pits and interaction between them on the weight of 100 seeds (g).

distance between lines (cm)	Distance between pits (cm)			Average distance between lines
	15	20	25	
30	156.35	144.14	121.98	140.82
40	111.76	102.68	97.73	104.06
50	91.90	79.59	77.93	83.14
Average distance between pits	120.00	108.80	99.22	
L. S. D 0.05		T=0.38	V=0.38	V*T=0.76

Conclusions

We can conclude the distance of 25 cm excelled in giving the highest average in all trait.

References

- [1] Hassan, Zainab Ahmed Abdul Razzaq. (2010). Effect of row spacing and weed control methods on growth and yield of broad bean. *Vicia faba L.* Master's thesis. College of Agriculture. University of Basra. Republic of Iraq 4 pp.
- [2] Issa, Talib Ahmed. 1984. Crop cultivation and growth, first edition. Mosul University Press. Iraq. (Translated) by Roger Magill.
- [3] Mekky R. H., Thabet M. M., Rodríguez-Pérez C., Elnaggar D. M. Y., Mahrous Mengel, K, and E. A. Kirkby . 2020. Principles Of Plant nutrition Dordrecht:Kluwer.Academic publishers.
- [4] Abdul Hamid Ahmed Al-Younes and Wafiqi Shaker Al-Shamaa. (1980). Cereal and legume crops. Department of Field Crops - College of Agriculture. University of Baghdad.
- [5] Al-Sahouki, Medhat Majeed and Karima Waheeb. 1990. Applications in design and analysis of experiments. Ministry of Higher Education and Scientific Research. University of Baghdad. Iraq.
- [6] Al-Rifai. Yahya Ali. 2001. Effect of planting dates and plant density on two lines of local broad bean under the conditions of the central region of the Kingdom of Saudi Arabia. *Journal of the Federation of Arab Universities for Agricultural Studies and Research*. Volume (9): Issue (1). Pp. 93-79.
- [7] Tuarira, M. and M. Moses .(2014). Effect of plant density and planting arrangement in green bean seed production. *J. Glob. Innov. Agric. Soc. Sci.* 2(4):152-157.
- [8] Al-Jubouri, Rashid Khadir and Ayad Hussein Ali. (2012). Study of the effect of seed size and plant density on the yield and components of broad bean *Vicia faba L.*. *Al-Furat Journal of Agricultural Sciences*. 4(1): 86-95.
- [9] Bakry, B.A.;T.A. Elewa; M.F. El karamany; M.S. Zeidan and M.M. Tawfik,(2011).Effect of row spacing on yield and its components of some faba bean varieties under newly reclaimed sandy soil condition, *World Journal of Agricultural Sciences*. 7(1):68-72.
- [10] Kakahy, A. N., Ahmad, D., & Abdullahi, A. S. (2012). The effect of planting distance on yield of beans (*Vicia faba L.*) under drip irrigation system. *African Journal of Agricultural Research*, 7(46), 6110-6114.
- [11] Mekkei, M.E.R.(2014) . Effect of intra-row spacing and seed size on yield and seed quality of faba bean (*Vicia faba L.*).*Intl J Agri Crop Sci.* 7(10):665-670
- [12] Attiya, H. J(1985). The Effects of Plant Population, and Yield of Spring Sown Field Beans(*Vicia faba L.*) Ph. D. Thesis. Univ. of Canterbury, New Zealand(C. F. Abbas, A. G., 1989. Effect of Hole Spacing and Number of Plants Per Hole on the Yield, Yield Components and Protein Percentage of Two Broad Bean Cultivars. M. Sc. Thesis. Collage of Agriculture Univ. of Baghdad) pp.
- [13] Derogar, N. and M. Mojaddam.(2014). Effect of plant density on grain yield and yield components in faba bean. *International Journal of Plant, Animal and Environmental Sciences*.5(1):267-274
- [14] Al-Farih, Lamia Mahmoud and Rawafed Hadi Al-Obaidi. 2003. Effect of pit spacing and number of plants in pits on growth characteristics, yield and its components of broad bean *Vicia faba L.* in Basra region. *Basra Journal of Agricultural Sciences*. Volume (16): Issue (2).
- [15] Sharaan A.N. E. A. Megawer, H.A. Saber and Z.A. Hemida.(2004).Seed yield, yield components and quality characters as affected by cultivars, sowing dates and planting distances in faba bean. *Fayoum*.

Algal Antimicrobial Power: Investigating the Influence of *Spirogyra varians* Extract on *Staphylococcus aureus* Biofilm Gene Expression

Nagham Dawood Salman¹, Ahmed Sahi Dwaish² and Sawsan Mohammed Kareem³

¹⁻³Department of Biology, College of Science, University of Mustansiriyah, Baghdad, Iraq.

¹E-mail: nagham.dawood24@uomustansiriyah.edu.iq

²E-mail: ahmedsahi@uomustansiriyah.edu.iq

³E-mail: stsqa@uomustansiriyah.edu.iq

Abstract. Resistance to methicillin that is capable of producing biofilms is *Staphylococcus aureus* (MRSA). This has significantly impeded patient treatment; between 20 and 30 percent of individuals have these germs but do not exhibit any symptoms. This research aimed to determine MRSA genes expression levels, following treatment with alga (*Spirogyra*) extract in order to develop new techniques for eliminating the impact of these bacteria on healthcare. After the algae (*Spirogyra*) was isolated and identified, it was extracted using soxhlet, a methanol-based extract of the *Spirogyra varians* was prepared, and the *Spirogyra* methanol extract's (MIC) against *S. aureus* was determined. Real-time PCR was performed using the PCR technique to assess the intended genes' expression levels (*icaA, icaD*) at concentrations lower than the minimum inhibitory concentration (MIC). The impact of the algal extract on genes significantly changed the expression of genes involved in the development of MRSA biofilms; its minimum inhibitory concentration (MIC) against *S. aureus* was 200 µg/ml. Following treatment with *Spirogyra* extracts, *icaA* and *icaD* expression levels were 0.480 and 0.688, respectively, with *icaD* expression being similarly high. In summary, our investigation demonstrated that methanolic *Spirogyra* extract inhibits the formation of biofilms by *S. aureus*.

Keywords. *Spirogyra varians*, Biofilm, Gene expressions, *S. aureus*.

1. Introduction

The main cause of many serious issues that cause physical disabilities, health problems, and mortality worldwide on a daily basis is bacterial infection. *Staphylococcus aureus* is a significant human pathogen that is capable of adapting to a wide range of hosts and environmental conditions in order to cause a wide range of illnesses.

It also plays a major role in the spread of illnesses that occur in the community and in hospitals [1]. Osteomyelitis, endocarditis, and central line-associated bloodstream infections (CLABSI) are among the severe, systemic illnesses that can result from this [2]. Other likely results are lower respiratory tract infections, skin and soft tissues, bloodstream, and medical equipment. With a wide range of virulence factors and toxins, *Staphylococcus aureus* can cause staphylococcal toxic

shock syndrome [3] and spiral skin syndrome among other toxin-mediated illnesses. *S. aureus* is mostly problematic since it can grow resistant to most drugs; hence another main obstacle to the application of alternative therapeutic agents is the development of bacterial resistance to antibiotics. Furthermore, the sometimes strong adverse effects of many antibiotics are enough to induce dislike of many synthetic medications [4]. The creation of novel medications utilizing necessary resources like algae is in great demand. Algae are gaining greater attention in response to the increased demand for biodiversity because of their more targeted effects on conventional therapies. Traditional medicine has long benefited from evidence that some algae have bactericidal, bacteriostatic, antiviral, antifungal, and anticancer effects [5]. The filamentous green algae *Spirogyra*, which is a member of the Zygnematales order, possesses novel physiologically active compounds with antiviral, antioxidant, anti-inflammatory, and antibacterial characteristics [6]. Conversely, Gram-positive bacterium *Staphylococcus aureus* and human pathogen can lead to several disorders including bacteremia, endocarditis, and infections of the soft tissues and skin [7]. A resistant bacterium strain called MRSA can develop when antibiotics, such as penicillin, are administered incorrectly or in excess against *Staphylococcus aureus* [8]. Colonies of microbes, called microbial biofilms, can form on inanimate or alive solids. A self-generating network ensnares them. Cells that are part of biofilms can attach themselves permanently to many different surfaces, including living tissues and implanted medical devices (e.g., valves, prostheses, catheters, etc.) [9]. Viewed as a main virulence component causing persistent, recurrent infections, biofilms are remarkably resistant to both human immune systems and drugs [10]. Exopolysaccharides found in biofilms can increase the resistance of bacteria up to 1,000 times against a treatment over free-floating planktonic cells [9]. This has major effects on therapy and makes alternate cure search much more challenging. A number of elements can lead to antibiotics causing biofilm resistance, including type IV secretion systems, evolution of multidrug efflux pumps, low permeability, and strong enzyme activity. The need of new control measures to be developed is highlighted by the growing biofilm resistance to present therapies [11]. Many bacterial and fungal illnesses are thought to be treatable by inhibiting the production of biofilms, and pharmacological research is now being done to create these drugs. Since the mechanism of action of these state-of-the-art antibiofilm drugs is significantly less susceptible to the development of resistance, several non-lethal, environmentally friendly solutions for reducing biofilms have been discovered lately [12].

None of these strategies have been entirely effective, so even while they have promise, they all have some downsides [13]. One strategy is looking for naturally occurring molecules produced by algae that stop biofilm development [14]. Long valued natural products with substantial impact on human health diagnosis, maintenance, and treatment are the physiologically active components of plant extracts [15]. Their allure stems from the fact that they have been used for thousands of years in traditional medicine to cure various ailments and conditions. People think they are safe as well [16]. This study aimed to assess the efficacy of organic extracts from algae against methicillin-resistant *Staphylococcus aureus* and to draw attention to the importance of new materials as potential antibiotics.

2. Materials and Techniques

2.1. Collecting and Preparing Ready for Algal Samples

Many samples were gathered from different parts of Baghdad and the surrounding area. In the summer of 2022, the University of Baghdad-aljadria isolated just one species. Samples of different algae were manually collected from the water. To be stored, the gathered macro algae were transported to the laboratory in polypropylene bags. Voucher specimens of a number of

species were compressed and stored in 5% formalin for identification, per Bellis *et al.* (1986) [17]. The biomass was rinsed with fresh water to remove any extraneous materials like as sand, shells, etc. After being stored in the laboratory and allowed to dry at room temperature, the algae were blended into a powder.

2.2. *Spirogyra varians* Extract

Using a soxhlet system, the active chemicals were extracted in six hours using 20 grams of algal and 500 milliliters of methanol as the extraction solvent [18]. Following the incubator's drying process, the dry extract was weighed, diluted in DMSO, then stored in the refrigerator until needed.

2.3. Culture Conditions and Bacterial Strain

Using a standard strain of MRSA gathered from the bacteriology lab at Mustansiriya University/College of Science, *Spirogyra* extract's antibacterial qualities were assessed on bacterial biofilm. First produced in Muller Hinton Broth (MHB) and Brain Heart Agar (BHA/India), the strain was the isolate was then grown for 24 hours at 37°C before being used for more investigation.

To evaluate the biofilm formation, a modified version of the Micro Titer Plate technique was employed [19]. In listed below:

- 2% sucrose was used to make agar for the infusion of the brain and heart.
- The plastic plate was sterilized using alcohol and UV light. After adding medium to the wells and adding 75 micrometers of bacterial broth, the plate was incubated at 37°C for twenty-four hours.
- To get rid of any excess, the liquid combination was drained after incubation and washed three times with PBS (sterile phosphate buffer).
- The plate was immersed in crystal violet dye and left for a minute before being cleaned with 200 liters of 95% ethanol.
- The plate was read at a wavelength of 620 nanometers. [20–21].

Biofilm production can be non or weak -existent at 0.1 nm, intermediate or good at 0.5 nm, and strong or powerful at more than 0.500 nm.

2.4. The Minimum Inhibitory Concentration (MIC) and Antibigram

The following six antimicrobial agents were tested for antibiotic susceptibility using a disc diffusion method test: ceftazidime (30 µg), levofloxacin (5 µg), tetracycline (10 µg), imipenem (10 µg), erythromycin (15 µg), and gentamicin (10 µg), all in order to comply with CLSI 2022 guidelines.

The antibacterial activity of the *Spirogyra* metabolic extract was determined by micro dilution technique. Bacterial suspensions were prepared by dissolving one colony from an MRSA culture on BHA that had been incubated for 24 hours in MHB. Half of the McFarland standard concentrations (0.5) were supplied, and they were all the same turbidity. The algal extracts were first diluted in a serial fashion, with a starting concentration of 200 mg/L. The first step was to fill up every well on the microtiter plate with 100 µl of algal extract and 100 µl of culture media. The next step was to shift 100 microliters to the second well, 100 microliters out of the second, and so on until the last well of the micro titer plate was covered. With the exception of the last well, which served as a positive control and did not contain any bacterial culture, 50 µl of microbial suspension was subsequently added to every well. The next step was to incubate the wells at 37 degrees for a whole day. Results from the turbidity test revealed the existence of microbes in the

positive control well. The "MIC" is the minimum inhibitory concentration (MIC) at which no growth was observed. For each experiment, three rounds were conducted [22].

2.5. Assessed Gene Expression in Biofilms

The biofilm genes (*icaA* and *icaD*) were evaluated for expression levels using real-time PCR. Specifically, we want to know whether and how MRSA isolates' *icaA* and *icaD* biofilm gene expression changes after extract treatment. The process consisted of multiple steps, including:

2.5.1. Extracting RNA

Two groups (one with algal extract present, the other without) had their total RNA extracted using the TransZol Up Kit (Trans/China) and a centrifuge set to 8000 rpm for two minutes. After discarding the bacterial solution and suspending the pellet and supernatant in a 1000 µl TransZol Up Kit, the eluted RNA was extracted as directed by the manufacturer and stored at -80°C. Nanodrop then measured the concentration and purity of the RNA.

2.5.2. Removing Genomic DNA

The Easy Script® One-Step gDNA Removal and cDNA Synthesis Super Mix (Trans/China) Kit system was used for cDNA synthesis.

2.5.3. Real-Time PCR Conditions and Primer Oligonucleotide Sequences

Primers from the list in Table 1 were used for this process. Ten microliters of Master Mix syper green, three microliters of cDNA, one microliter of each primer, and twenty microliters of nuclease-free water made up the RT-PCR mixture. CFX96 (Bio-Rad/USA) was used to amplify RT-PCR. Denaturation at 94 C° for one minute; followed by forty times repetitions of denaturation at 94 C° for ten seconds, annealing at 58 C° for fifteen seconds, and extension at 72 C° for twenty seconds. While the proportional amount of amplified product appeared on Y-axis, the RT-PCR the image below on X-axis showed fluorescence from the amplification reaction and cycle numbers.

Table 1. Sequence of primer oligonucleotides.

Primer	Sequence (5'→3' direction)
<i>icaA</i>	
Forward	GAGGTAAAGCCAACGCACTC
Reverse	CCTGTAACCGCACCAAGTTT
<i>icaD</i>	
Forward	ACCCAACGCTAAAATCATCG
Reverse	GCGAAAATGCCCATAGTTTC
<i>gyrB</i>	
Forward	GTCGAAGGGGACTCTG
Reverse	GCTCCATCCACATCGG

2.6. Statistical Evaluation

A DMRTs test was used to evaluate gene expression. This test considered the 95% CI and considered a P-value less than 0.01 as a significant outcome.

3. Results

3.1. Structure Morphologically of *Spirogyra* Variants

In shallow waters, unbranched filamentous green algae of the species *Spirogyra varians* form mats. It often grows in canals and ponds, meandering streams, and the shady littoral zones of lakes. Because of the unique form of spirally coiled chloroplasts seen in their cells, they may be clearly distinguished as having a vegetative structure (Figure 1).



Figure 1: *Spirogyra* spp. under microscope.

Because the genus *Spirogyra* is photosynthetic, it contributes to the process of fixing carbon dioxide in their surroundings, increases the amount of oxygen in the air, and is consumed by a wide range of aquatic life [23].

3.2. Algal Crude Extracts' Antibacterial Properties

The antibiogram test findings showed that every isolate had multidrug resistance (Figure 2). The rate of resistance to imipenem (32 mm), levofloxacin (28 mm), and gentamicin (20 mm) was found to be very sensitive; however, resistance to erythromycin (18 mm), ceftazidime (1 mm), and tetracycline (12 mm) was shown to be both sensitive and moderately resistant. Because bacteria are becoming increasingly resistant to antibiotics, alga was used in the hunt for natural antibacterial substitutes.

Algae demonstrated the effectiveness of *Spirogyra* methanol extracts at 250 µg/ml against human pathogenic bacteria, with an inhibitory zone of 28 mm. Figure (3) reports on a number of investigations conducted in the last few decades about the antibacterial activity of algal extract.

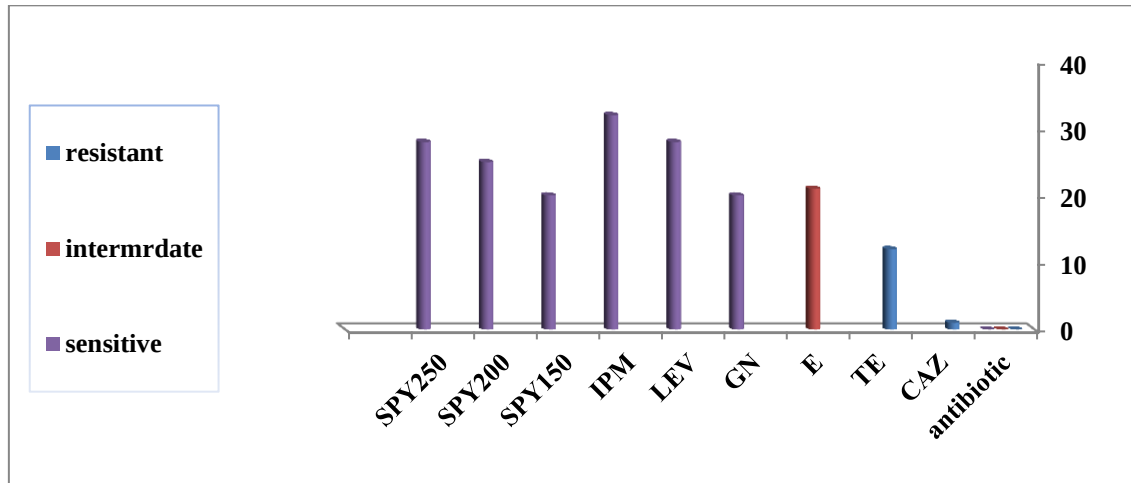


Figure 2. *S. aureus*'s profile of antibiotic resistance is shown in Figure (2). Ceftazidime (CAZ), imipenem (IMP), levofloxacin (LEV), erythromycin (E), tetracycline (TET), gentamicin (GEN), and spirogyra extract (SPI).

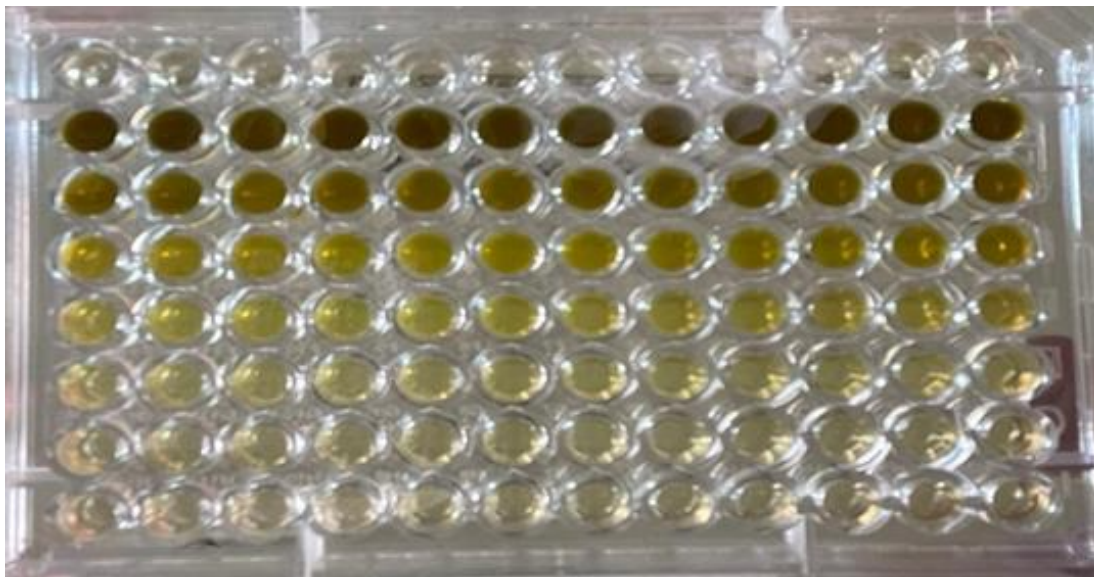


Figure 3: *Spirogyra*'s impact on *S. aureus* biofilm on a microtiter plate MIC determination utilizing algae extracts in opposition to six isolates of *S. aureus*.

3.3. The Results of Gene Expression

The MIC approach was applied to ascertain the sensitivity of MRSA toward the impacts of *Spirogyra* extracts. The lowest inhibitory concentration of the methanol extract of *Spirogyra* turned out to be 200 mg/ml. After 18 hours of treatment with herbal extracts of *Spirogyra*, the expression levels of the biofilm generating genes (*icaA* and *icaD*) showed statistically significant alterations ($p < 0.01$) compared to the housekeeping gene (*gyrB*). Actually, the genes under investigation exhibited a decrease in expression as concentration raised. Tables 2 and 3 show the respective reaction following *Spirogyra* treatment.

The genes were expressed using three copies of the target genes, *icaA*, *icaD*, and *gyrB*—thought to be major resistance genes in *S. aureus*.

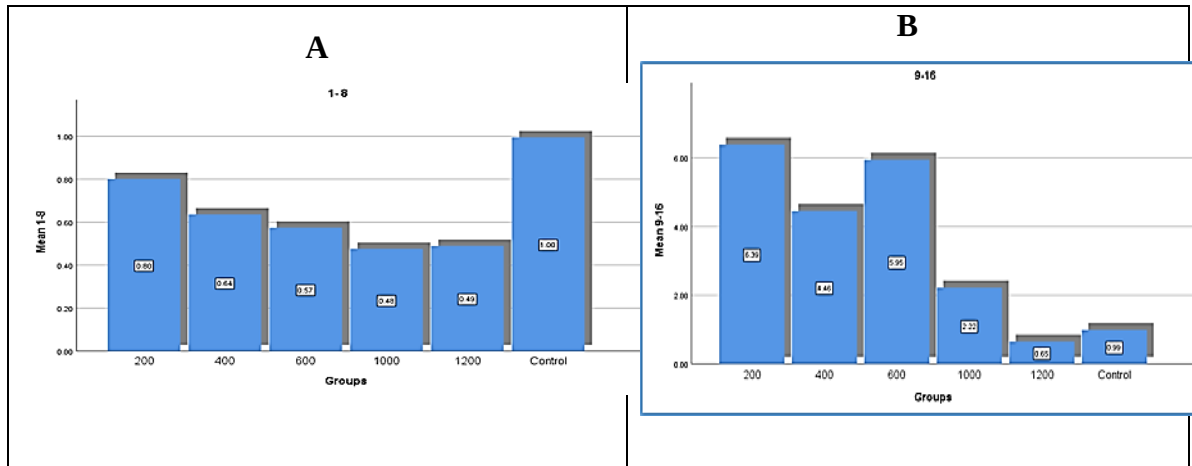


Figure 4. Show the mean of C_t values, A is the mean in a Agenes C_t values while B is the mean in a D genes C_t value.

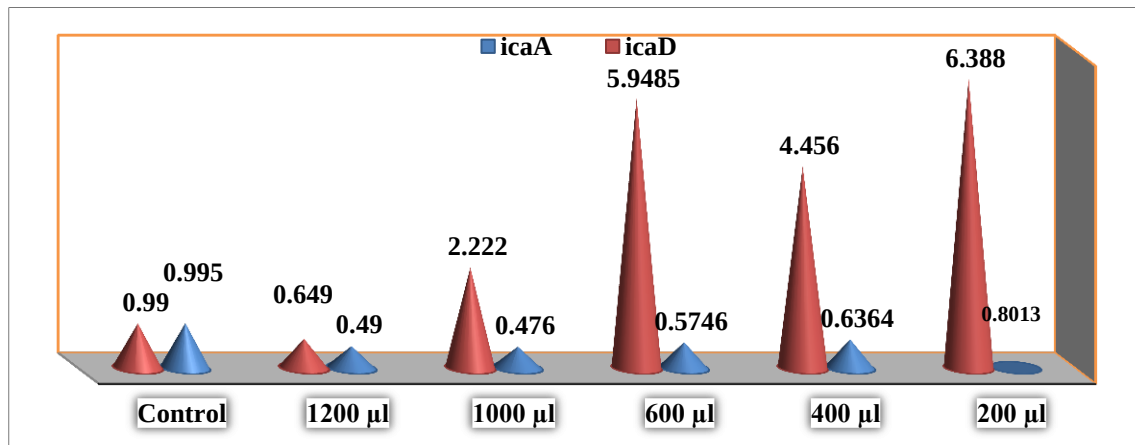


Figure 5. Comparison of the three groups' average declines in gene expression (*icaA*, *icaD*, and control) at different concentration of extracts.

In the past, real-time PCR techniques for RNA expression analysis have used reference or housekeeping genes to account for sample variance [27]. This approach is under research since it is becoming clearer that some housekeeping genes could vary significantly among different biological samples. Using RT-PCR, genotypic expression of the genes *icaA*, *icaB*, and *gyrB* was ascertained from RNA extraction from *S. aureus*. Whereas the mean values for the *icaA* and *icaD* genes vary from 0.8013 to 0.4900 in Figure 4-A and 6.3880 to 0.6490 in Figure 4-B, respectively, the average reduction in gene expression in the two groups, *icaA* and *icaD*, is compared in Figure 5.

Table 2. Fold of *icaA* Gene expression Depending on $2^{-\Delta C_t}$ Method.

Groups	Mean	Std. Deviation	Std. Error of Mean	p-value
200 µl	0.8013 b	0.14142	0.10000	0.01**
400 µl	0.6364 c	0.07071	0.05000	
600 µl	0.5746 c	0.02828	0.02000	
1000 µ	0.4760 c	0.01414	0.01000	
1200 µ	0.4900 c	0.01414	0.01000	
Control	0.9950 a	0.00707	0.00500	

Table 3. Fold of *icaD* Gene expression Depending on $2^{-\Delta Ct}$ Methods.

Groups	Mean	Std. Deviation	Std. Error of Mean	p-value
200 μ l	6.3880 a	0.15839	0.11200	0.01**
400 μ l	4.4560 b	0.48649	0.34400	
600 μ l	5.9485 a	0.07283	0.05150	
1000 μ	2.2220 c	0.11031	0.07800	
1200 μ	0.6490 d	0.05515	0.03900	
Control	0.9900 d	0.01414	0.01000	

4. Discussion

The emergence of multi-drug resistant bacteria has accelerated in recent years [28, 29], leading to a rise in public health issues that impact not only the health sector but also the economy [30]. Using natural materials, such as medicinal herbs, seems to be the most practical approach to treating various illnesses. Until recently, a great deal of study has been conducted on this topic and the effects of medicinal herbs on many bacteria, including *H. pylori*, *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, and many others [31]. This study has shown that therapeutic herbs, such as *Spirogyra*, change the cell wall of bacteria and stop them from growing. Financial loss and resource waste arise from the misuse of antibiotics, which accounts for 20–50% of hospital pharmaceutical expenses. [32]. Recent studies in different industries have focused on the potential of algal chemicals to manage biofilm. This is in response to the increasing interest in these compounds and their usefulness in treating bacterial infections. There have been a plethora of studies published in the last several decades detailing the antibacterial properties of algae extract. It is challenging to compare the results because many factors can influence the antimicrobial activity of an algal extract. These factors include the type of algae used, the extraction method, the testing protocol, the extraction solvent, the season or time of year the samples were collected, and the location of the study. As a result, the results from these studies varied in terms of the tested microorganisms. [24-26]. Applying *Spirogyra* extracts reduces the genes for the methicillin-resistant *S. aureus* bacteria to develop biofilms, according to the results of this study looking at an MRSA strain with the *icaA*, *icaD*, and *gyrB* genes. In 2015 Yang Chio investigated the efficacy of an ethanolic extract from *Artemisia princeps* against MRSA. Reported outcomes of the study included suppression of genes linked to pathogenicity, biofilm growth, and acid generation [11]. Chio investigated the esteemed genes *sea*, *agrA*, and *sarA*—not the same as our target genes—in *sea*. Furthermore, the concentrations we utilized in our study and Choi's vary; Choi used an *Artemisia princeps* extract concentration of 2 mg/mL, but the effective amount we used for our research was 200 mg/mL of *Spirogyra* extract.

Aloe vera leaf aqueous extract showed antibacterial action against six strains of *S. aureus* resistant to methicillin in a 2018 study by Saddiq *et al.* After 24 and/or 48 hours of exposure, the dosages of plant extract 15-20 mg/ml considerably decreased the dry weights of most *S. aureus* strains [32]. Our study's findings showed that because these extracts more strongly suppressed the expression of the target gene, they had a more potent effect on MRSA biofilm development. Overall, the investigation's findings support the claim that the medicinal algal extract from *Spirogyra* contains strong chemicals that inhibit the gene expression required for the formation of MRSA biofilms.

We found that the gene expression of biofilm growth was positively impacted by algal extract, which suggests that this finding merits more research.

Conclusion

Multiple genes are involved in biofilm formation, a major pathogenic strategy for microbes. Conversely, novel factors that could influence the expression of these genes are an exciting prospect. Researchers are keen to find out how different substances, both natural and man-made, influence gene expression in biofilms. We found that algae and respectable algae extracts significantly affect genes linked to the MRSA biofilm-forming strain; this suggests that further research into this topic is warranted.

Recognitions

The biology department of Mustansiriyah University / College of Science (<https://uomustansiriyah.edu.iq>), Baghdad, Iraq, was much appreciated by the authors for helping them finish this study.

Author Contributions

Each author made an equal contribution to this work.

After reading it, each author decided to publish this version of the work.

Funds

The writers did not receive funding for this project.

Consent to Participate (Ethics)

This work was authorized and evaluated by the Mustansiriyah University/College of Science/Biology Department Ethics Committee.

Consent to publish (Ethics)

This work's publication is agreed upon by all writers.

Research with participants who are humans or animals

Not relevant.

In terms of conflicts of interest, none exist.

Information Availability Statement

The manuscript contains no related data.

References

- [1] D. A. Edwar, I. N. Naji, and H. M. Aboul-Ela, "Investigation the Role of Various Antiseptics on the Prevalence of Skin Microbiota and Post Cesarean Surgery Infections", *Al-Mustansiriyah Journal of Science*, vol. 34, no. 3, pp. 1–9, Sep. 2023.
- [2] Kareem SM, Sajid aljubori S, Ali MR, The spa gene diversity and molecular typing of *Staphylococcus aureus* clinical and environmental isolates in Iraq, *New Microbes and New Infections*, <https://doi.org/10.1016/j.nmni.2020.100653>.
- [3] Lakhundi S., Zhang K. Methicillin-resistant *Staphylococcus aureus*: Molecular characterization, evolution, and epidemiology. *Clin. Microbiol. Rev.* 2018;31:e00020-18.
- [4] Thwaites, G. E., Scarborough, M., Szubert, A., Nsutebu, E., Tilley, R., Greig, J., et al. (2018). Adjunctive rifampicin for *Staphylococcus aureus* bacteraemia (ARREST): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet* 391, 668–678. doi: [10.1016/S0140-6736\(17\)32456-X](https://doi.org/10.1016/S0140-6736(17)32456-X).

- [5] Das, B.K., Pradhan, J., Pattnaik, P., Samantaray, B.R. & Samal, S. K. 2005. Production of antibacterials from the freshwater alga *Euglena viridis* (Ehren). *W. J. Microbiol. Biotech.*, 21: 45–50.
- [6] Takano, T., Higuchi, S., Ikegaya, H., Matsuzaki, R., Kawachi, M., Takahashi, F., & Nozaki, H. (2019). Identification of 13 *Spirogyra* species (Zygnemataceae) by traits of sexual reproduction induced under laboratory culture conditions. *Scientific reports*, 9(1), 1-11.
- [7] Shariati, A., Dadashi, M., Chegini, Z., van Belkum, A., Mirzaii, M., Khoramrooz, S. S., et al. (2020). The global prevalence of Daptomycin, Tigecycline, Quinupristin/Dalfopristin, and linezolid-resistant *Staphylococcus aureus* and coagulase-negative staphylococci strains: a systematic review and meta-analysis. *Antimicrob. Resist. Infect. Control* 9:56. doi: [10.1186/s13756-020-00714-9](https://doi.org/10.1186/s13756-020-00714-9)
- [8] Karakostas S, Kalemaki D. Antimicrobial overuse and misuse in the community in Greece and link to antimicrobial resistance using methicillin-resistant *S. aureus* as an example. *J Infect Public Health*. 2019 Jul-Aug;12(4):460-464. doi: 10.1016/j.jiph.2019.03.017. Epub 2019 Apr 10. PMID: 30981652.
- [9] Shang, Y., Guo, J., Zhao, Y., Chen, J., Meng, Q., Qu, D., et al. (2022). Clemastine inhibits the biofilm and hemolytic of *Staphylococcus aureus* through the GdpP protein. *Microbiol. Spectr.* 10:e0054121. doi: [10.1128/spectrum.00541-21](https://doi.org/10.1128/spectrum.00541-21)
- [10] Heim, C. E., Bosch, M. E., Yamada, K. J., Aldrich, A. L., Chaudhari, S. S., Klinkebiel, D., et al. (2020). Lactate production by *Staphylococcus aureus* biofilm inhibits HDAC11 to reprogramme the host immune response during persistent infection. *Nat. Microbiol.* 5, 1271–1284. doi: [10.1038/s41564-020-0756-3](https://doi.org/10.1038/s41564-020-0756-3)
- [11] Choi NY, Kang SY, Kim KJ. *Artemisia princeps* inhibits biofilm formation and virulence-factor expression of antibiotic-resistant bacteria. *Biomed Res Int* 2015; 2015:239519.
- [12] Simões, M., Simões, L.C., & Vieira, M.J. (2010). A review of current and emergent biofilm control strategies. *Lwt - Food Science and Technology*, 43, 573-583.
- [13] F. Sun, F. Qu, Y. Ling et al., “Biofilm-associated infections: antibiotic resistance and novel therapeutic strategies,” *Future Microbiology*, vol.8, no.7, pp.877–886, 2013.
- [14] J.A.Lizana,S.L´opez,A.Marchal,U.Serrano,D.Velasco,and M. Espinosa-Urgel, “Use of plant extracts to block bacterial biofilm formation,” in *High School Students for Agricultural Science Research, Proceedings of the 3rd Congress PIISA*, pp.43– 50, 2013.
- [15] P. Karuppiyah and M. Mustaffa, “Antibacterial and antioxidant activities of *Musa* sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection, ”*Asian Pacific Journal of Tropical Biomedicine*, vol.3, no.9, pp.737–742, 2013.
- [16] T. Rasamiravaka, Q. Labtani, P. Duez, and M. El Jaziri, “The formation of biofilms by *Pseudomonas aeruginosa*: a review of the natural and synthetic compounds interfering with control mechanisms,”*BioMed Research International*, vol.2015, Article ID759348, 17pages, 2015.
- [17] Bellis, V. J., & McLarty, D. A. (1967). ECOLOGY OF CLADOPHORA GLOMERATA (L.) KÜTZ IN SOUTHERN ONTARIO I. *Journal of Phycology*, 3(2), 57-63.
- [18] Wong YC, Shahirah R (2019). Effect of different solvent and ratio towards microalgae oil production by ultrasonic assisted soxhlet extraction techniques. *Orient Journal of Chemistry* 35(4):1377-1383.
- [19] Babapour E, Haddadi A, Mirnejad R, Angaji SA, Amirmozafari N. Biofilm formation in clinical isolates of nosocomial *Acinetobacter baumannii* and its relationship with multidrug resistance. *Asian Pac J Trop Biomed*. 2016;6(6):528–533. doi: 10.1016/j.apjtb.2016.04.006.
- [20] A. Hassan, J. Usman, F. Kaleem, M. Omaid, A. Khalid, and M. Iqbal, "Evaluation of different detection methods of biofilm formation in the clinical isolates," *Brazilian journal of infectious diseases*, vol. 15, pp. 305-311, 2011.
- [21] S. Stepanović et al., "Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci," *Apmis*, vol. 115, no. 8, pp. 891-899, 2007.

- [22] Baghini GS, Sepahi AA, Tabatabaei RR, Tahvildari K. The combined effects of ethanolic extract of *Artemisia aucheri* and *Artemisia oliveriana* on biofilm genes expression of methicillin resistant *Staphylococcus aureus*. Iran J Microbiol. 2018 Dec;10(6):417-423. PMID: 30873270; PMCID: PMC6414741.
- [23] Dwaish, A. S., Yousif, D. Y., & Lefta, S. N. (2016). Use of *Spirogyra* sp. Extract against multi drug resistant bacterial pathogens. International Journal of Advanced Research, 4(7), 575-579.
- [24] Al-Khazraji, R.A.A. (2018). Evaluation the antibacterial activity of *Cladophora glomerata* extracts. M.sc.thesis.Al-Mustansiriyah University. College of Science
- [25] Magnusson, M., Yuen, A. K., Zhang, R., Wright, J. T., Taylor, R. B., Maschmeyer, T., & de Nys, R. (2017). A comparative assessment of microwave assisted (MAE) and conventional solid-liquid (SLE) techniques for the extraction of phloroglucinol from brown seaweed. Algal research, 23, 28-36.
- [26] Salvador, N., Gomez-Garreta, A., Lavelli, L. and Ribera, M.A. (2007). Antimicrobial activity of Iberian macroalgae. Scientia Marina, 71: 101- 114.
- [27] Yourick MR, Sandkam BA, Gammerdinger WJ, Escobar-Camacho D, Nandamuri SP, Clark FE, Joyce B, Conte MA, Kocher TD, Carleton KL. (2019). Diurnal variation in opsin expression and common housekeeping genes necessitates comprehensive normalization methods for quantitative real-time PCR analyses. Molecular Ecology Resources. 19(6):1447-1460.
- [28] Ali MG, Almoneim ZA, Kareem SM. Evaluated gene expressions of Metallo beta lactamase genes GIM and , VIM, SPM in *Pseudomonas aeruginosa* clinical isolates. Mol Biol Rep. 2023 Dec;50(12):10111-10120. doi: 10.1007/s11033-023-08883-7. Epub 2023 Nov 2. PMID: 37917414.
- [29] Hussein NH, Mohammed Kareem S, Hussein AL, Taha BM (2022) The predominance of *Klebsiella pneumoniae* carbapenemase (KPC-type) gene among high-level carbapenem-resistant *Klebsiella pneumoniae* isolates in Baghdad. Iraq Mol Biology Rep 49(6):4653–4658. [https:// doi. org/ 10. 2147/ IDR. S2338 08](https://doi.org/10.2147/IDR.S233808).
- [30] Abid SA, Aziz SN, Saeed NA-H, A A, Mizil SN, Al-Kadmy IM, Hussein NH, Al-Saryi N, Ibrahim SA, Hussein JD (2022) Investigation of virulence factors in microbial organisms that associated with public health risk isolates from different environmental regions. Al-Mustansiriyah J Sci 33(5):1–7. [https://doi. org/ 10. 1016/j. jgar. 2020. 01. 011](https://doi.org/10.1016/j.jgar.2020.01.011)
- [31] Kareem SM, Mahmood SS, Hindi NK (2020) Effects of curcumin and Silymarin on the *Shigella dysenteriae* and *Campylobacter jejuni* in vitro. J Gastrointest Cancer 51:824–828. [https://doi. org/ 10. 1007/ s12029- 019- 00301-1](https://doi.org/10.1007/s12029-019-00301-1)
- [32] Saddiq AA, Al-Ghamdi H. A novel antimicrobial and antibiofilm against methicillin resistant *Staphylococcus aureus* strains. Pak J Pharm Sci 2018; 31(5):2123- 2130.

Effect of Soaking with Glycine Betaine Acid on some Physiological Qualities of Sesame Under the Influence of Salt Stress

Elaf K. Suleiman¹ and Waleed A.T. El-Fahdawi²

^{1,2}Department of Field Crops, College of Agriculture, University of Anbar, Ramadi, Iraq.

¹E-mail: ela21g3009@uoanbar.edu.iq

²E-mail: ag.waleed.abdul@uoanbar.edu.iq

Abstract. A laboratory experiment was carried out in the phycology Laboratory of the field crops Department, Faculty of Agriculture –Anbar University for the year 2023, to find out the effect of soaking with Glycine Betaine acid in some phycological qualities of sesame under the influence of salt stress. It used the fully randomized design C. R.D in the order of the factorial experiment and with three repetitions. The experiment included two factors, the first factor is salt stress and three concentrations of sodium chloride (NaCl) are (1.5, 3 and 4.5) desisimens M-1, as well as a measurement treatment (distilled water) and a code for these coefficients in the codes S1, S2, S3 and S0 respectively. The second factor involved soaking sesame seeds with three concentrations of Glycine Betaine (GB) acid: 50, 100 and 150 mg L-1, coded GB1, GB2, GB3 respectively, as well as two treatments of soaking seeds with distilled water(DW) and seeds without soaking(dry seeds)(DS). The results showed the superiority of salt stress treatment S1 in most physiological traits: feather length, Root Length, gesture strength and gesture dry weight . The results also recorded the superiority of soaking treatment with Gb3 Glycine Betaine acid in most physiological characteristics: feather length, Root Length, Bud strength, dry weight of the bud, chlorophyll content in the leaves and Proline content. The results also recorded significant differences between the interference coefficients, as dry seed plants (DS) grown under the influence of a high level of salt stress (S3) showed significant superiority in most of the studied traits.

Keywords. Sesame harvest, Seed soaking, Salt straining, Glycine Betaine, Physiological qualities.

1. Introduction

Sesame L is prepared. *Sesamum indicum* is an important oil crop widely cultivated in many parts of the world, and the percentage of oil in it ranges between 60-50%, and its oil is one of the finest types of oils . The percentage of protein, carbohydrates and minerals is 20-25%, 15% and 5-7%, respectively [9]. As for Kasbah, it is rich in phosphorus and calcium elements and also contains vitamins such as vitamin B12, and its oil is also used as an alternative in the treatment of superficial burns compared to common medicines[1]. Despite the economic importance of the Sesame crop, its productivity in Iraq is still low.Iraq suffers today, as in most other countries of the world, from the lack of rainfall and the scarcity of its resources, as well as the misuse of these water sources in agriculture by following the random method in the number of irrigations during

the growing season, and this problem will worsen in the coming years, especially if we know that fresh water on the surface of the globe does not exceed 3%, and in front of this great challenge, many efforts around the world have focused on using saltier water sources such as eyes, wells and agricultural wastewater to irrigate crops. Salt stress is one of the important factors affecting the growth and production of crops in the world, and the high level of salinity often leads to a decrease in agricultural production and land development [6]. pointed out that Sesame has varying degrees of tolerance to abiotic stresses by stimulating various response mechanisms, and works to control the transport of ions to the leaves and due to the strong induction of antioxidant enzymes accumulate osmotic regulators, in addition to the regulation of plant hormones and photosynthesis[2]. In general, when plants are exposed to salt stress, changes occur in metabolic processes, and plants and their ability to cope with the harmful effects of salt stress were evaluated through various examinations of both physiological and biochemical properties such as inorganic nutrients and water relationships, in addition to hormonal regulation, solutes and oxidative defense metabolism. Therefore, it is necessary to make some ways to alleviate the effect of salt stress by soaking sesame seeds with amino acids, including glycine Betaine, an amino acid consisting of quaternary ammonium compounds, which is also known as Tri-methyltrimethylglycine and has functions to a large extent in high-end plants, where it works to protect plant cells during stress, in other words, preserving cells osmologically in plants exposed to drought and high salinity [4]. found that salinity has a significant impact on vegetative growth due to its effect on the absorption of water by the plant, so it reduces and facilitates its absorption, or it is due to the toxic effect of some ions such as chlorine and sodium, and this causes slow or stop the meristematic activity and thus leads to reduced plant growth, and the plant remains short below the normal limit[5]. The delay or failure in germination of seeds in saline environments is due to the toxic effect caused by ions that cause salinity such as (sodium), as its increased accumulation inside the seed will lead to an impact on the vital activities of the embryo and seed [3]. As pointed out by [7], the increase in salinity in the growth medium of the plant leads to a decrease in the percentage of germination in addition to this lengthening the time period necessary for the completion of the germination process. stated that claicin-betaine acid, in addition to other amino acids, is an important nitrogenous source involved in the construction of enzymes and proteins, and all this has a positive yield in the vegetative growth of the plant, as well as claicin-betaine acid is easily absorbed by the plant due to the fact that its partial weight is small, in addition to its role in affecting the permeability of cell membranes. Based on the above, this study was conducted to find out the effect of soaking with Glycine Betaine acid in some physiological qualities of sesame under the influence of salt stress[8].

2. Material and Methods

A laboratory experiment was conducted in the phycology Laboratory of the Department of field crops, Faculty of Agriculture-Anbar University for the year 2023, to find out the effect of soaking with Glycine Betaine acid in some phycological qualities of sesame under the influence of salt stress. It used the fully randomized C design.R.D in the order of the factorial experiment and with three repetitions. The experiment included two factors, the first factor is the salt stress with three concentrations of sodium chloride (NaCl) (1.5, 3 and 4.5 decisimens M-1), as well as the measurement treatment (distilled water)and a code for these coefficients in the codes S1, S2, S3 and S0 respectively. The second factor involved soaking sesame seeds with three concentrations of Glycine Betaine (GB) acid: 50, 100 and 150 mg L-1, coded GB1, GB2, GB3 respectively, as well as two treatments of soaking seeds with distilled water(DW) and seeds without soaking(dry seeds)(DS). The seeds were soaked in distilled water and glycine Betaine acid for (8) hours, and

then the seeds were placed in plastic dishes with (60) plates and arranged on filter paper, and then treated with the above-mentioned salt concentrations.

2.1. Studied Qualities

- Standard laboratory germination percentage examination (%): The germinated seedlings were calculated eight days after placing the seeds in plastic dishes and calculated according to the following law:

$$\text{Standard laboratory germination \%} = \left(\frac{\text{number of natural seedlings after eight days}}{\text{total seeds}} \right) \times 100$$

- Root Length (cm): (10) seedlings were selected at random, where the roller was separated from the point of their connection and the root lengths were measured by an inserted ruler.
- The length of the roller (cm): (10) random gestures were selected and the lengths of the roller were measured by an inserted ruler.
- Measuring the power of the gesture: where its value was measured by the following law:

$$\text{Germination percentage\% (Root Length + Blade length)}$$

- Measuring the dry weight of the gesture (g): 5 random gestures were taken for each sample and dried, and the dry weight of each sample was measured with a sensitive device, and the total dry gestures were divided by their number.
- Chlorophyll content in leaves ($\text{mg } 100\text{g}^{-1}$ soft weight): The plants were measured using a spectrophoto meter by taking three readings of the three types of chlorophyll (A, B, T) with a wavelength of 663, 645, 652 sequentially using acetone, where a sample of the plant weighing 2.0 G was taken and crushed with 10 ml of acetone, then left in the dark for 24 hours, then the next day the extract was placed in Special Tubes in a centrifuge(centri Fuge) for 10 minutes at a speed of 3000 rpm, then after the samples were completed from the centrifuge, the extract was placed in the qvit of the spectrophotometer and the readings were taken according to the mentioned wavelengths.
- Proline(mg GM^{-1}): half a gram of the sample was taken and digested with (4)ml of Sulfosalicylic and then placed in the centerfuge for (10) minutes at a degree (rpm1000), after which (1)ml of fragrant was taken and added to (2) ml of Ninhydrin and (2) ml of glacial acetic Auel, and then placed in the strings for an hour at a temperature of 60°/ and then I put it directly in the ice and added (4)ml of toluene to the samples, and then I read on (520).

2.2. Material Preparation

- Take (1.25) g of Ninhydrin and dissolve it in (30) ml of iced acetic acid.
- Take (20) ml phosphoric acid with Vortex .(3)g of sulfosalic acid was prepared in (80)ml distilled water and then the volume was completed to (100)ml distilled water.

3. Results and Discussion

3.1. Standard laboratory germination percentage examination

The results in Table 1 showed that there were significant differences between the average concentrations of glycine betaine acid in the standard laboratory germination percentage, as the treatment of soaking seeds with distilled water (DW) gave the highest average of 96.15%, which

did not differ significantly from the DS, GB2 and GB3 transactions, giving averages of 95.46, 93.94 and 94.93% sequentially. While the treatment of seeds with glycine acid gave betaine at the GB1 treatment with the lowest average of 71.93%

Table 1. Effect of soaking seeds with betaine glycine acid in the characteristic of the germination ratio (cm) under the influence of salt stress and their interference.

Salt stress(S)	Acid concentrations Glycine Betaine (GB)					
	DS	DW	GB1	GB2	GB3	Average
S0	97.50	95.67	95.00	95.67	93.67	95.50
S1	96.67	95.50	94.67	93.33	92.33	94.50
S2	93.83	96.83	91.33	95.08	95.33	94.48
S3	93.83	96.58	93.83	94.83	94.42	94.70
Average	95.46	96.15	93.71	94.93	93.94	
L.S.D 0.05	Salt stress(S)		Acid concentrations(GB)		Interference (S×GB)	
	N.S		2.341		N.S	

3.2. Length of the Shuttlecock

The results in Table 2 indicated the presence of significant differences between the averages of salt stress coefficients and the soaking of seeds with glycine betaine acid and the overlap between them for the characteristic of the feather length, where the salt stress treatment S1 excelled in giving the highest average of 2.109 cm, while the treatment S3 recorded the lowest average of this characteristic of 1.794 cm. The results of the same table also indicated that there were significant differences between the averages of maceration coefficients with glycine betaine acid, where the acid concentration exceeded GB3 by giving the highest average of 2.064 cm, while the GB1 concentration gave the lowest average for this trait and amounted to 1.818 cm. The results of the same table showed a significant difference in the mean overlap between the salt stress coefficients and the concentration of glycine betaine acid. the interference treatment between S3 and GB2 recorded the highest average roll length of 2.423 cm, which did not differ significantly with the interference treatment S3 and DW recorded 2.400 cm, while the interference between the salt stress treatment S1 and acid concentration GB1 gave the lowest average roll length of 1.700 cm.

Table 2. Effect of soaking seeds with claicin-betaine acid in the length of the blade (cm) under the influence of salt stress and the interference between them.

Salt stress(S)	Acid concentrations Glycine Betaine (GB)					
	DS	DW	GB1	GB2	GB3	Average
S0	2.233	2.060	1.800	1.823	2.050	1.993
S1	2.073	1.650	1.700	1.723	1.823	2.109
S2	2.100	1.800	1.973	1.873	2.000	1.949
S3	1.850	2.400	1.800	2.423	2.073	1.794
Average	1.987	1.978	1.818	1.961	2.064	
L.S.D 0.05	Salt stress(S)		Acid concentrations(GB)		Interference (S×GB)	
	0.0902		0.1009		0.2017	

3.3. Root Length

The results in Table 3 showed that there are significant differences between the salt stress coefficients and glycine betaine acid and the overlap between them for the Root Length trait, where the salt stress treatment S1 exceeded by giving the highest average for this trait of 1.569 cm, and the treatment S2 recorded the lowest average for the Root Length trait of 1.314 cm. The results of the table also indicated that the concentration of glycine betaine acid exceeded GB3, as

it gave the highest average for this trait of 1.593 CM, while the concentration of GB1 gave the lowest average of 1.387 cm. The reason for the superiority of soaking treatment with GB3 concentration is due to the fact that high concentrations of glycine betaine acid promote cell division and elongation of the root and these results agreed with [10]. The same table also showed the significance of the overlap between the salt stress coefficients and the chelicin-betaine acid soaking coefficients, as the interference treatment S3 and DW exceeded the highest average of 2.300 cm, while the interference treatment S3 and DDS gave the lowest average for this trait of 1.023 cm.

Table 3. Effect of soaking with glycine betaine acid for the characteristic Root Length (cm) under the influence of salt stress and interference between them.

Salt stress(S)	Acid concentrations Glycine Betaine (GB)					
	DS	DW	GB1	GB2	GB3	Average
S0	2.050	1.673	1.450	1.150	1.523	1.519
S1	1.600	1.323	1.150	1.223	1.273	1.569
S2	1.573	1.073	1.773	1.623	1.550	1.314
S3	1.023	2.300	1.173	1.673	1.300	1.494
Average	1.562	1.412	1.387	1.418	1.593	
L.S.D 0.05	Salt stress(S)		Acid concentrations(GB)		Interference (S×GB)	
	0.1681		0.1879		0.3759	

3.4. Power of Gesture

The results of the data confirmed the existence of significant differences between the salt stress coefficients and the claicin-betaine acid coefficients (Table 4), as the salt stress treatment1 was significantly superior by giving the highest average for the strength of the gesture, amounting to 391.8, while the S3 treatment gave the lowest rate of 294.7. The table also pointed to the significant superiority of the concentration of claisen betaine Gb3 acid, as it gave the highest average for the prodromal strength trait of 385.7, while the treatment of dry seeds (DS) had the lowest average of 320.8, which did not differ significantly from the treatment of GB2, which averaged (320.9). The same table also showed the significance of the overlap between the salt stress coefficients and the coefficients of soaking seeds with glycine betaine acid, where the combination between the coefficients S0 and DS gave the highest average, at 557.2, while the overlap between the coefficients S1 and GB1 gave the lowest average, at 267.8.

Table 4. Effect of soaking seeds with glycine betaine acid in the character of prodromal strength under the influence of salt stress and interference between them.

Salt stress(S)	Acid concentrations Glycine Betaine (GB)					
	DS	DW	GB1	GB2	GB3	Average
S0	557.2	365.4	307.2	275.8	353.5	361.5
S1	347.2	276.1	267.8	291.0	291.5	391.8
S2	355.4	303.2	347.4	332.4	325.4	332.8
S3	283.2	444.1	383.5	384.7	312.2	294.7
Average	320.8	347.2	326.5	320.9	385.7	
L.S.D 0.05	Salt stress(S)		Acid concentrations(GB)		Interference (S×GB)	
	52.58		58.79		117.57	

3.5. Dry Weight of the Gesture

The results in Table 5 showed that there were significant differences between the salt stress coefficients and the soaking of seeds with glycine betaine acid, as the salt stress treatment S1 gave the highest average dry weight characteristic of the seed, amounting to 0.0014 g, while the treatment S3 gave the lowest average for this characteristic, amounting to 0.0007 g. The superiority of the salt stress treatment S1 is due to its superiority in the length of the Blade (2), the length of the root(3) and the strength of the gesture (4), which led to an increase in the dry weight of the gesture, the same table also showed a moral superiority of the concentration of glycine betaine acid GB3 with the highest average of this trait, reaching 0.0013 G, while the treatment gave DW the lowest average, reaching 0.0010 g, which did not differ significantly from the concentrations GB1, GB2, reaching their average is 0.0011 g. The superiority of Gb3 acid concentration in these is due to its superiority in the length of the blade (table2), the length of the root (table3) and the strength of the gesture (table4).The same table also showed the significance of the overlap between the coefficients of salt stress and the coefficients of soaking seeds with glycine betaine acid, where the combination between S1 and DDS recorded the highest average dry weight of the gesture, which amounted to 0.0019 g, while the combination between S1 and GB3 recorded the lowest average for this trait, which amounted to 0.0005 g.

Table 5. Effect of soaking seeds with glycine betaine acid for the dry weight of the seedling (g) under the influence of salt stress and interference between them.

Salt stress(S)	Acid concentrations Glycine Betaine (GB)					Average
	DS	DW	GB1	GB2	GB3	
S0	0.0014	0.0008	0.0016	0.0011	0.0009	0.0012
S1	0.0019	0.0016	0.0009	0.0014	0.0005	0.0014
S2	0.0011	0.0007	0.0009	0.0006	0.0006	0.0011
S3	0.0009	0.0012	0.0013	0.0013	0.0027	0.0007
Average	0.0012	0.0010	0.0011	0.0011	0.0013	
L.S.D 0.05	Salt stress(S)		Acid concentrations(GB)		Interference (S×GB)	
	0.0002560		0.0002862		0.0005724	

3.6. Content of Chlorophyll in the Leaves

The results of Table 6 indicate that there are significant differences between the averages of soaking seeds with glycine betaine acid in the chlorophyll content in the leaves, as the treatment of dry seeds (DS) gave the highest average for this trait, amounting to 16.45 mg g⁻¹, which did not differ significantly from the concentrations GB1 and GB3, averaging 15.65 and 15.16 mg g⁻¹ respectively. While the lowest average for this trait was reported for the concentration of glycine betaine GB2 acid and amounted to 14.71 mg g⁻¹, this is not significantly different from the treatment of distilled water (DW), averaging 14.77 mg g⁻¹.

Table 6. Effect of soaking seeds with claicin-betaine acid for the content of chlorophyll in the leaves (mg 100 g⁻¹ soft weight) under the influence of salt stress and interference between them.

Salt stress(S)	Acid concentrations Glycine Betaine (GB)					Average
	DS	DW	GB1	GB2	GB3	
S0	24.63	20.63	21.33	20.23	18.23	15.97
S1	16.63	18.1	16.17	14.07	14.87	21.01
S2	13.93	10.93	13.13	13.53	14.33	13.17
S3	10.06	9.43	11.97	11	13.02	11.24
Average	16.45	14.77	15.65	14.71	15.16	
L.S.D 0.05	Salt stress(S)		Acid concentrations(GB)		Interference (S×GB)	
	N.S		1.308		N.S	

3.7. Proline Content

The results in Table 7 showed that there were significant differences between the concentrations of glycine betaine acid in the ProLine content, with the highest average for this trait at the concentration of Gb3 acid reaching 0.595 mg g^{-1} while the lowest average for this trait at the concentration GB2 reached 0.560 mg g^{-1} this does not differ significantly from the average concentration GB1 (0.570). As for the overlap between the averages of the salt stress coefficients with the averages of the concentrations of glycine betaine acid, the same table indicated the presence of significant differences between these averages, as the highest average of this characteristic was reached when treating the overlap S3 and DS, and it amounted to 0.783 mg g^{-1} , while the lowest average of the overlap between the combination S0 and DS, averaging 0.230 mg g^{-1} .

Table 7. Effect of soaking seeds with claicin-betaine acid under the influence of salt stress and the overlap between them in the characteristic of proline content (mg g^{-1}).

Salt stress(S)	Acid concentrations Glycine Betaine (GB)					
	DS	DW	GB1	GB2	GB3	Average
S0	0.230	0.293	0.350	0.383	0.393	0.330
S1	0.460	0.473	0.530	0.507	0.553	0.743
S2	0.423	0.553	0.640	0.710	0.683	0.602
S3	0.783	0.780	0.760	0.780	0.610	0.505
Average	0.474	0.525	0.570	0.560	0.595	
L.S.D 0.05	Salt stress(S)		Acid concentrations(GB)		Interference (S×GB)	
	N.S		0.715		0.1429	

Conclusion

conclude from this study that soaking sesame seeds with different concentrations of claicin-betaine acid has reduced the effect of salt stress in some physiological traits, especially at low levels of salinity.the study also concluded that the resistance of dry seeds to this effect was evident in most physiological traits at high levels of salt stress.

References

- [1] Hussain, M., Ul-Allah, S. and Farooq, S. (2023). Sesame (*Sesamum indicum* L.). In *Neglected and Underutilized Crops* (pp. 733-755). Academic Press.
- [2] Kadkhodaie, A., Zahedi, M., Razmjoo, J. and Pessarakli, M. (2014). Changes in some anti-oxidative enzymes and physiological indices among sesame genotypes (*Sesamum indicum* L.) in response to soil water deficits under field conditions. *Acta Physiologiae Plantarum*, 36(3), 641-650.
- [3] Kafi.M.. and Goldani.M.. (2001): Effect of water potential and type of osmoticum on seed germination of three crop species of wheat, sugarbeet, and chickpea. *Agric. Sci. and Tech* . 15: 121-33.
- [4] Lachli, A. and Lutge, U. 2004. *Salinity : Environment- Plants Molecules*. Kluwer Academic Publishers, 552p.
- [5] Mohr, H. and Schopfer, P. (2006). *Plant Physiology*. The Biological Institute of the University of Freiburg, Germany.
- [6] Mudgal, V., N. Madaan and A. Mudgal. 2010. Biochemical mechanisms of salt tolerance in plants. A Review. *Int. J. Bot.*, 6: 136-143.
- [7] Othman.Y., Al-Karaki .G., Al- Tawaha. A.R., and Al-Horani.A..(2006): Variation in germination and ion uptake in genotype barley under salinity conditions. *World J. Agric. Sci.* 2: 11-15.
- [8] Rai, V. K. 2002. Role of amino acids in plant responses to stresses. *Biol. Plantarum*, 45: 481-487.
- [9] Sheehy, T., Carey, E., Sharma, S., and Biadgilign, S. 2019. Trends in energy and nutrient supply in Ethiopia: a perspective from FAO food balance sheets. *Nutrition journal*, 18, 1-12.



- [10] Wang, G.P., Zhang, X. Y., Luo, F.L. and Wang, W., 2010. Photosynthetic wheat Agron. J. 70: 563-565.

Effect of Partial Replacement of Dietary Corn by Soaked Barley with Water or Supplemental Enzymes on the Productive Characteristics of Broilers

Alaa Salim Abd¹ and Ali J. Hammod²

^{1,2}Department of Animal Production, Faculty of Agriculture, University of Kufa, Kufa, Iraq.

²E-mail: alij.alhemaidawi@uokufa.edu.iq

Abstract. The current experiment was conducted in poultry farm belongs to Animal production department/ Faculty of Agriculture/ University of Kufa from November /10/2023 until December /15/2023. To study the effect of partial replacement of barley soaked in alone water or without enzymes instead of corn on the productive performance of broiler chickens. Using 300 chicks Ross 308 unsexed, It was purchased. from the Al-Sharq Hatchery in Babylon Governorate. The chicks were distributed randomly for five treatments with three replicates for each treatment where each treatment included 60 chicks with 20 chicks for each replicate through a period of 35 days. Treatments were divided as follows: T1: Control treatment (Basal Ration), T2: Replacement of barley instead of corn by 50%, T3: Replacement of barley soaked in water instead of corn by 50%, T4: Replacement of barley instead of corn by 50% with the addition of enzymes to the feed without soaking, T5: Replacement of barley soaked in water with enzymes instead of corn by 50%. The chicks were fed with starter diet from 1-10 days of age, grower diet from 11-24 days of age, and finisher diet from 25-35 days of age. The data for the studied characteristics were analyzed using a completely randomized design, and the significance differences between treatments were tested using the Duncan test at the probability level of 0.05. Statistical program (SAS) was used in the statistical analysis. The most important results obtained were the following: The final live body weight, cumulative weight gain, total feed intake, final feed conversion ratio, and economic figure of the birds in the fifth treatment (which substituted 50% of the corn with barley soaked in water with enzymes) did not differ significantly from those of the birds in the first treatment (T1). The final live body weight, total weight gain, total feed consumption and economic figure of the second, third and fourth treatments (50% replacement of barley with corn), and fourth treatments (50% replacement of barley with soaked barley with corn with addition of enzymes to the feed without soaking) decreased significantly compared to the first treatment (T1). The results also showed a significant deterioration in the final feed conversion ratio of T2 and T4 compared to T1.

Keywords. Broiler, Barley, Productive Characteristics, Enzymes.

1. Introduction

Because it has minimal levels of saturated fats and a high percentage of vital amino acids, vitamins, and minerals, poultry meat is known for having a high nutritional value [1,2]. Traditional grains and their by-products constitute the largest proportion of feed components, as

their use in poultry feed ranges from 50-80%, and corn is the most widely used feed material, as its percentage in production feed reaches about 75% [3]. Due to the increase in global demand for corn for use in the production of ethanol, or what is known as biofuel [4; 5], this has led to a shortage in its supply and an increase in its global prices [6]. Therefore, researchers turned their attention to finding acceptable alternatives to replace corn in poultry feed. Barley is one of the feed components, Because of its high fiber content (10%), its usage in chicken feed is restricted, low amino acid content, especially lysine and methionine, low energy content (2800 kcal/kg), and high beta-glucan content [3,7]. High levels of beta-glucan lead to increased intestinal viscosity and decelerate the migration and nutrition absorption, as high digestive viscosity has a detrimental effect on starch and protein digestion, which affects the health of birds and increases the cost of production. Therefore, solutions must be found to these problems. Exogenous feed enzyme supplements in poultry used in high-no-starch polysaccharide diets have shown an improvement in nutrient availability and digestibility [8-10]. Many studies have confirmed that external feed enzyme supplements improve growth performance, intestinal health, and increase nutrient digestibility in chickens [11-14]. Therefore, the present study aimed to replace corn instead barley soaked in alone water or without enzymes in the order of broiler diets to improve the nutritional value of barley and reduce the harmful effects of anti-nutrients while increasing the percentage of digestible nutrients, especially fiber.

2. Materials and Methods

2.1. Design of Experiment

The Department of Animal Production, Faculty of Agriculture poultry farm served as the site of the experiment / University of Kufa for the period from November 10/2023 to December 15/2023, Operation with laboratories in University of Kufa, Ethical approval No. 27157 in November 7/2023. For the purpose of knowing the results of replacing barley partially soaked in alone water or without enzymes rather than corn on the broiler productive performance. 300 unsexed Ross 308 hybrid chicks, one day old, with mean initial weight of 44 g, were used. They were prepared from the Al-Sharq Hatchery in Babylon Governorate and were raised in a semi-closed house divided to pens. The chicks were randomly distributed into five treatments with 3 replicates for each treatment, since there were 60 chicks in each treatment. The division of treatments is as follows:

T1: Control treatment (Basal Ration).

T2: Replacement of barley instead of corn by 50%.

T3: Replacement of barley soaked in water instead of corn by 50%.

T4: Replacement of barley instead of corn by 50% with the addition of enzymes to the feed without soaking.

T5: Replacement of barley soaked in water with enzymes instead of corn by 50%.

2.2. Contents of the Enzyme Mixture Used

Xylanase 11000000 IU; Cellulase 7000000 IU; Beta-glucanase 800000 IU; Protease 3500000 IU; Alpha-Amylase 800000 IU; Pectinase 100000 IU; Lipase 12000 IU and Phytase 150000 IU.

2.3. Soaking Method

The barley was finely crushed and the enzyme mixture was dissolved according to the manufacturer's recommended ratios (0.3 g/ kg feed) in the required amount of water (50%) to soak the barley. The water was stirred well to dissolve the enzymes and the crushed barley was

soaked in the resulting solution. It was then packed in nylon bags and left for 24 hours. It was then dried well and mixed with the feed at a 50% replacement rate instead of corn.

2.4. Animals and Experimental Design

The chicks were grown in pens with 5-8 cm of dry sawdust covering the floor. During the first week, one 38-cm-diameter plastic feed dish was used for each pen. As the chicks grew older, they were gradually replaced by 45-cm-diameter hanging circular feeders. They were gradually raised upwards and at the level of the bird's chest to facilitate feeding and ensure reducing feed scattering. The lighting system was adopted (23 hours of light and 1 hour of darkness/ day). Water was freely available in five-liter inverted plastic waterers, one trough for each pen. During the trial phase, the rearing house was heated at night by gas incubators. Four huge mercury thermometers that were positioned throughout the home were used to measure the temperature inside. The chicks were fed three diets: Starter, grower and finisher. The nutritional requirements for each period were calculated as mentioned in the Ross Nutrition Specifications [15]. After that, the components of the feed, and the feed was formulated using a computer after it was provided with detailed information about the feed ingredients, their chemical composition, and the standard nutritional requirements of the birds using a program prepared for formulating the feed, as displayed in Table 1.

Table 1. Feed formulation and chemical composition of diets.

Ingredients	Starter diet%	Grower diet%	Finisher diet%
Corn	50	25	25
Barley	-	25	25
Soybean meal (48% CP)	36	31	24
Wheat	8.0	12	17
Premix ^(*)	2.5	2.5	2.5
Sun flower oil	2	3	5
Dicalcium phosphate	0.1	0.1	0.1
Limestone	1.1	1.1	1.1
Salt	0.3	0.3	0.3
Total	100	100	100
Calculated composition ^(**)			
Crude protein%	22.43	21.29	18.92
Metabolizable energy (Kcal/kg)	2916	2967	3149
Energy: Protein	130.14	139.36	166.44
Calcium %	1.42	1.40	1.39
Avi. Phosphorus%	0.44	0.45	0.52

(*)The following components are included in each kilogram of the Premix Jordanian Origin Type Provimi 3110: 2750 Kcal of metabolizable energy, 10% crude protein.

(**)Using NRC [16] Tables, the nutritional components of each diet are determined.

3. Results and Discussion

3.1. Live Body Weight

Table 2 shows an effect of partial replacement of barley soaked in alone water or water without enzymes instead of corn on mean live body weight. We note from the results of day 11 that there are no significant differences ($P \leq 0.05$) for all experimental treatments compared to T1. We also note from the results of day 24 that there is a significant decrease ($P \leq 0.05$) for T2, T3 and T4

compared to T1, with no significant differences ($P \leq 0.05$) in T5 compared to T1. It is also clear from the results of day 35 that there are no significant differences between T5 and T1 with a significant decrease ($P \leq 0.05$) between T2, T3 and T4 compared to T1.

Table 2. Partial replacement of soaked barley in alone water or without enzymes instead of corn on the mean live body weight (gm/ bird), in broiler. (Mean $\pm$ SEM).

Treatments	Live body weight at 11 days	Live body weight at 24 days	Live body weight at 35 days
T1	173.8 4.17 $\pm$	1059.6a $\pm$ 34.08	2155.0a $\pm$ 31.08
T2	170.7 4.25 $\pm$	977.6b $\pm$ 12.41	1861.0c 6.65 $\pm$
T3	171.5 2.75 $\pm$	978.6b 13.96 $\pm$	1957.0b $\pm$ 10.01
T4	174.8 3.34 $\pm$	978.6b 1.85 $\pm$	1844.6c $\pm$ 7.17
T5	175.6 2.33 $\pm$	1047.6a $\pm$ 19.05	2139.0a $\pm$ 30.80
Significant level	N.S	*	*

NS: No-significant, * Different letters within a column indicate significant differences ($P < 0.05$), T1: Control treatment (basal ration), T2: Replacement of barley instead of corn by 50%, T3: Replacement of barley soaked in water instead of corn by 50%, T4: Replacement of barley instead of corn by 50% with the addition of enzymes to the feed without soaking, T5: Replacement of barley soaked in water with enzymes instead of corn by 50%.

3.2. Weight Gain

Table 3 show the effect of partial replacement for barley soaked in water with or without enzymes instead of corn on weight gain (gm/bird) for the experimental periods. We note from the results of the grower period (11-24) days that there were no significant differences ($P \leq 0.05$) between T2, T3 and T5 compared to T1, with a significantly lower in T4 than to T1. We also note from the results of the finisher period (25-35) days that there was a significant decrease ($P \leq 0.05$) T2, T3 and T4 compared to T1 and T5, with no significant difference for T5 compared to T1. However, the overall period findings (11-35) days demonstrated a significant decrease ($P \leq 0.05$) for T2, T3 and T4 compared to T1, with T5 and T1 not differing significantly from one another.

Table 3. Partial replacement of soaked barley in alone water or without enzymes instead of corn on the mean weight gain (gm/ bird), in broiler. (Mean $\pm$ SEM).

Treatments	Weight gain gm/bird (11-24) days	Weight gain gm/bird (25-35) days	Weight gain gm/bird (11-35) days
T1	885.8a $\pm$ 37.94	1095.3a $\pm$ 30.60	1981.1a $\pm$ 32.10
T2	806.9ab $\pm$ 14.08	883.3c $\pm$ 6.43	1690.2c $\pm$ 9.64
T3	807.1ab $\pm$ 11.87	978.3b $\pm$ 7.35	1785.5b $\pm$ 7.28
T4	803.8b $\pm$ 5.01	866.0c $\pm$ 6.24	1669.83c 8.16 $\pm$

T5	872.0ab ±16.82	1091.3a ±44.34	1963.3a ±32.62
Significant level	*	*	*

* Different letters within a column indicate significant differences ($P < 0.05$), T1: Control treatment (Basal Ration), T2: Replacement of barley instead of corn by 50%, T3: Replacement of barley soaked in water instead of corn by 50%, T4: Replacement of barley instead of corn by 50% with the addition of enzymes to the feed without soaking, T5: Replacement of barley soaked in water with enzymes instead of corn by 50%.

The decrease in live body weight and accusative weight gain in treatments containing barley, soaked barley, or barley with enzymes without soaking by 50% may be due to the presence of the insoluble β -glucan substance in water, which leads to an increase in the viscosity of feed ingredients in the bird's digestive tract [17], and thus reduces the mixing of the contents of the digestive tract and thus reduces their absorption through the intestinal mucosa. As a result, there is an increase in the number of microorganisms that compete with the bird for these feed ingredients and the adhesion of feces to the exit opening. This compound also causes digestive disorders when consumed and reduces the benefit from nutrients, thus weakening the growth of birds [18]. Since the external β -glucanase enzyme attacks the β -glucan present in the cell wall of the barley seed, the reason why the treatment that added barley soaked in enzymes performed better than the other treatments from the start of the experiment to the end of the experiment could be because of the role of enzymes in increasing the benefit that the birds received from the feed that contained barley [18]. Enzymes also play a role in reducing the gelatinous ingredients formed by the effect of β -glucan, as well as reducing the effect of anti-nutritional substances in it. This was confirmed by Alhasan [19] when studying the effect of feeding broilers on a feed to which barley was added at levels of 20, 25 and 30% with the enzyme preparation (Ro Fabio-Advance) for the experimental treatments at level of 100 g/ton feed, which led to an increase in the live body weight and weight gain for the treatment that contained 25% barley with the enzyme. This was attributed to the fact that adding enzymes to barley led to a decrease in viscosity and an improvement in the value of the digestion coefficient of nutrients and the role of enzymes in reducing anti-nutritional in barley and thus improving growth performance and utilization of nutrients. In a similar study on the possibility of using β -glucanase enzyme in poultry feed with the aim of increasing the digestive efficiency of barley, It was utilized as a corn substitute in broiler feed; the results demonstrated that adding the enzyme at doses of 1 and 1.5 kg/ton to barley, which replaced corn 100% of the time, improved weight gain.

3.3. Consumption of Feed

The results of Table 4 show the effect of partial replacement of barley soaked in alone water or without enzymes instead of corn on the mean of feed intake (gm /bird) for the experiment periods. It is clear from the feed intake during the grower period (11-24) days that there were no significant differences for all experimental treatments compared to the T1, with a significant decrease ($P \leq 0.05$) for feed intake during the finisher period (25-35) days for each of T2, T3 and T4 compared to T1 and T5, with no significant variations for the T5 compared to T1. While a significant decrease ($P \leq 0.05$) is clear for the total feed intake for T2, T3 and T4 compared to T1 and T5.

Table 4. Partial replacement of soaked barley in alone water or without enzymes instead of corn on the mean Intake feed (gm/ bird), in broiler. (Mean $\pm$ SEM).

Treatments	Intake feed gm/bird (11-24) days	Intake feed gm/bird (25-35) days	Intake feed gm/bird (11-35 days)
T1	1118.6 ± 35.87	1795.6a ± 48.36	2914.3a ± 43.67
T2	1084.0 ± 10.58	1539.0bc ± 6.42	2623.0b ± 16.77
T3	1078.3 ± 37.95	1567.3b ± 11.86	2645.6b ± 48.92
T4	1098.6 ± 15.43	1452.6c ± 15.32	2551.3b ± 23.48
T5	1111.0 ± 9.53	1806.6a ± 42.98	2917.6a ± 38.16
Significant level	N.S	*	*

NS: No-significant, * Different letters within a column indicate significant differences ($P < 0.05$), T1: Control treatment (basal ration), T2: Replacement of barley instead of corn by 50%, T3: Replacement of barley soaked in water instead of corn by 50%, T4: Replacement of barley instead of corn by 50% with the addition of enzymes to the feed without soaking, T5: Replacement of barley soaked in water with enzymes instead of corn by 50%.

The decrease in feed intake in T2 and T3 compared to T1 may be due to the high percentage of barley used and thus the birds' unpalatability of it with the effect of anti-nutrients, especially β -glucan found in barley in high proportions with the absence of digestive enzymes for these two treatments. As for the fourth treatment, the reason for the decrease in feed intake may be attributed to the lack of sufficient time for the enzymes to work as well as the high percentage of undigested fibers. We note an increase in feed intake for the fifth treatment compared to the second, third and fourth treatments. This may be due to the fact that adding enzymes and soaking led to increased fiber decomposition and improved feed palatability and reduced anti-nutrients, which work to increase viscosity in the intestines, which slows down the passage of the feed mass. In a study conducted by Ibrahim and Younis [18] when replacing untreated local black barley with β -glucanase enzyme instead of corn in the feeding of laying hens, it was noted that there were no significant differences in daily feed intake between the control diet and the diets containing 30 or 60% barley treated with the enzyme. Scout et al. [20] also found that adding β -glucanase enzyme to laying hens' diets containing barley led to improved feed intake compared to diets not to which the enzyme was added. Ahmed [21] also found when conducting an experiment on the possibility of using the enzyme β -glucanase in poultry feed with the aim of increasing the digestive efficiency of barley, which was used as a substitute for corn in broiler feed, a decrease in the consumption of feeds in which barley was replaced by 100% without enzyme or at an enzyme level of 0.5 kg/ton was observed, while it increased when the enzyme was added at a level of 1 and 1.5 kg/ton without significant differences with the birds of the control treatment. In the feeds containing 50% barley, the feed not containing the enzyme was the least consumed compared to the feeds to which the enzyme was added at a level of 0.5, 1 and 1.5 kg/ton.

3.4. Feed Conversion Ratio and Economic Figure

The results of Table 5 show the impacts of partial replacement of barley soaked in alone water or without enzymes instead of corn for the experimental periods. We notice through the feed conversion ratio during the grower period (11-24) days that, when comparing all experimental

treatments to T1, there are no appreciable variations. With a significant deterioration ($P \leq 0.05$) for T2 compared to T1 and the rest of the experimental treatments except for T4, which did not differ significantly during the finisher period (25-35), T3, T4, and T5 did not differ significantly from the control therapy throughout the same time period.. As for the total feed conversion ratio (11-35) days, we notice a significant deterioration ($P \leq 0.05$) for T2 and T4 compared to T1, with no significant difference for T3 and T5 compared to T1. The results of the economic figure show a significant decrease in T2, T3 and T4 compared to T1 and T5, with no significant difference between T5 and T1.

Table 5. Partial replacement of soaked barley in alone water or without enzymes instead of corn on the mean feed conversion ratio and economic figure, in broiler (Mean $\pm$ SEM).

Treatments	Feed conversion ratio (11-24) days	Feed conversion ratio (25-35) days	Feed conversion ratio (11-35) days	Economic figure
T1	1.26 ± 0.036	1.64b ± 0.034	1.47c ± 0.005	416.4a ± 7.24
T2	1.34 ± 0.023	1.74a ± 0.016	1.55a ± 0.013	342.6c ± 3.72
T3	1.33 ± 0.029	1.60b ± 0.020	1.48bc ± 0.020	377.5b ± 4.47
T4	1.36 ± 0.024	1.67ab ± 0.013	1.52ab ± 0.008	344.9c ± 1.31
T5	1.27 ± 0.024	1.65b ± 0.029	1.48bc ± 0.014	411.3a ± 9.21
Significant level	N.S	*	*	*

NS: No-significant, * Different letters within a column indicate significant differences ($P < 0.05$), T1: Control treatment (basal ration), T2: Replacement of barley instead of corn by 50%, T3: Replacement of barley soaked in water instead of corn by 50%, T4: Replacement of barley instead of corn by 50% with the addition of enzymes to the feed without soaking, T5: Replacement of barley soaked in water with enzymes instead of corn by 50%.

The reason for the improvement of T5 over the rest of the experimental treatments may be due to the role the added enzymes in analyzing the components of the fibers that make up the cell walls of the endosperm of the cereals used in the diets. The enzymes may contribute to increasing the surface area of the cellulose and protein contents of the endosperm cells in the feed ingredients [22]. Alternatively, the group of enzymes contained in the mixture Phytase, Amylase, Protase, and Xylanases, may have worked together to execute the joint activity that resulted in the superiority attained upon introducing the enzyme mixture. The Xylanases enzyme works to load the arabinosilane compound, thus reducing the viscosity of the intestine, and the Amylase enzyme analyzes the starch, part of which is linked to phytate after it is released by the phytase enzyme [23]. Also, replacing corn with enzyme-treated barley in the diets of Japanese quails led to an improvement in the feed conversion ratio in the treatments in which barley with added enzymes was used, while the feed conversion ratio declined significantly for the two treatments that contained (40% and 60% barley not treated with enzymes) and to which enzymes were without added. The reason for the deterioration of the feed conversion ratio is due to the high viscosity of the untreated barley and the high percentage of barley in the feed and the presence of β -glucan, which acts as a protective shield that protects the starch granules from decomposition by digestive enzymes [24]. High intestinal viscosity also leads to retention of nutrients and hinders their absorption [25]. Al-Asha,ab et al., [26]stated that enzyme preparations can play a vital role in feed digestion, as they work to increase the nutritional value of the feed ingredients by increasing

the digestibility of the feed by improving digestibility and releasing and liberating beneficial nutrients from feed components in addition to eliminating the harmful nutritional effects of some complex compounds found in grains. While increasing efficiency is due to the soaking process with the addition of feed enzymes, as the soaking process increased the activity and effectiveness of enzymes in releasing nutrients and increasing the birds' benefit from them, thus improved of feed conversion ratio [27].

Conclusions

For the majority of the traits under study, including final live body weight, cumulative weight gain, total feed intake, final feed conversion ratio, and economic figure, substituting 50% of barley soaked in water with enzymes for corn in broiler diets produced the best results. This is because the results did not differ significantly from the control treatment.

References

- [1] Oreamuno, M. A. A. and Siddig, K., 2020. What does the OECD-FAO Agricultural Outlook 2017-2026 imply for income distribution in the Sudan and Ethiopia.. Working Paper 99. HU Berlin.
- [2] Al-Wasity, R. T., Mahmood, Z. H., and Al-Sammarraie, M. H. 2023. Measuring efficiency of broiler breeding projects using stochastic cost limit in Iraq (Baghdad governorate). Iraqi Journal of Agricultural Sciences, 54(2): 535-541.
- [3] Al-Yaseen A A and Abdul-Abass M H 2009. Nutrition of poultry. University of Baghdad. College of Agriculture. Iraq.
- [4] Hameed, K. K., Musa, K. M., and Allawi, S. J. 2015. The Production of Biofuels From Grain Corn. Journal of Babylon University/ Engineering Sciences. 2(23): 329-335.
- [5] Sharma, A., Sharma, S., Verma, S., and Bhargava, R. 2016. Production of biofuel (ethanol) from corn and co product evolution: A review. Int. Res. J. Eng. Technol, 3: 745-749.
- [6] Hamed, A. F.; Soliman, I. Amer, M. G. and Noreldeen, A. M. E., 2009. Impact of production bio-fuel on poultry feed market. Zagazig J. Agric. Res. 36(3): 661-684.
- [7] Al-Kaisey, M. T.; Mohammad, M. A.; Abou-Tubik, S. M.; Al-Fadhli, M. K. M. and Abdul-Abass, M. M., 2007. Effect of germination in improving the feed value of two local barley cultivars in broiler chick diets. Mesopotamia Journal of Agriculture, 35(2): 75-83.
- [8] Zhou, Y., Jiang, Z., Lv, D., Wang, T., 2009. Improved energy-utilizing efficiency by enzyme preparation supplement in broiler diets with different metabolizable energy levels. Poultry Sci. 88 (2): 316-322.
- [9] Saleh, A. A., Ali, H., Abdel-Latif, M. A., Emam, M. A., Ghanem, R., El-Hamid, H. S. A., 2018. Exogenous dietary enzyme formulations improve growth performance of broiler chickens fed a low-energy diet targeting the intestinal nutrient transporter genes. PLoS One 13 (5): e0198085.
- [10] Bedford, M. R., and Apajalahti, J. H. 2022. The role of feed enzymes in maintaining poultry intestinal health. Journal of the Science of Food and Agriculture, 102(5): 1759-1770.
- [11] Li, J.; Bai, G.; Gao, Y.; Gao, Q.; Zhong, R.; Chen, L.; Wang, Y.; Ma, T. and Zhang, H., 2024. Solid-state fermentation pro-enzymes supplementation benefits growth performance, health, and intestinal microbiota of broiler chickens fed wheat-based diet. Animal Research and One Health. DOI: 10.1002/aro2.55
- [12] Ibrahim, D.; El-Sayed, H. I.; Mahmoud, E. R.; El-Rahman, G. I. A.; Bazeed, S. M.; Abdelwarith, A. A.; Elgamal, A.; Khalil, S. S.; Younis, E. M.; Kishawy, A. T. Y.; Davies, S. J. and Metwally, A. E., 2023. Impacts of Solid-State Fermented Barley with Fibrolytic Exogenous Enzymes on Feed Utilization, and Antioxidant Status of Broiler Chickens. Vet Sci. 10(10):594.
- [13] Zubaidah, S., Ariyadi, B., Hanim, C., and Baskara, A. P. 2024, June. Performance of broiler chickens fed palm kernel cake with enzyme supplementation. In IOP Conference Series: Earth and Environmental Science: 1360, 1: 012012.
- [14] Bawish, B. M., Ismael, E., Abdelfatah, S. H., Kamel, S., and Fahmy, K. N. 2024. Effectiveness of phytase and nostarch polysaccharides-degrading enzymes on performance, bone mineralization,

- litter, and gene expression in broiler chickens fed nutritionally reduced diets. *Journal of Advanced Veterinary Research*, 14(6): 1033-1041.
- [15] Ross Nutrition Specification. 2019. Retrieved from https://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerNutritionSpecifications2022-EN.pdf
- [16] NRC, 1994. *Nutrient Requirements of Poultry*. National Research Council. 9. rev. ed. Natl. Acad. Press Washington. DC.
- [17] Al-Gharawi, J. K. M.; Al-Zamili I.F.B. and Al-Janab, H. K. H., 2017. Effect of barley cultivated for different times as supplemented diet in some productive traits of broiler chickens. *The Iraqi Journal of Agricultural Sciences*, 48(1): 360-367.
- [18] Ibrahim, S. I. and Younis, R. Z., 2011. Effect of using local untreated black barley or treated with β -glucanase enzyme or germinated instead of corn in laying hens nutrition. *Mesopotamia Journal of Agriculture*. 39(3): 55-61.
- [19] Alhasan, Y. 2022. Studying the effect of broiler feeding on a diet supplemented with barley and Ro Fabio-Advance compound on some productivity indicators in broilers. Master thesis. College of Agriculture Science. Al Baath University. Syria.
- [20] Scout, T. A., Leslie and A. Karimi 2001. Measurements of enzyme response with hullless barley based diets full- fed to leghorn and broiler chicks or restricted – for broiler chicks *Can. Canadian Journal of Animal Science*. 81(3): 403-410.
- [21] Ahmed, H. I. N. 2008. The possibility of economically benefiting from barley after adding beta-glucanase enzyme to the performance of broiler as an alternative to corn grains. Master thesis. College of Agriculture. Al-Fateh University. Libya.
- [22] Johnston, D. and Freeman, F., 2005. Dietary Preference and Digestive Enzyme Activities as Indicators of Trophic Resource Utilization by Six Species. *Biological Bulletin*., 208(1): 36-46.
- [23] Cowieson, A.J.V. Ravindran, and P. H. Selle, 2008. Influence of dietary phytic acid and source of microbial phytase on ileal endogenous amino acid flows of broiler chickens. *Poultry Sci.* 87(11): 2287-2299.
- [24] Al-Fadley, M. K. ; Salman, A. H. and Al-Ashaab, M. H., 2012. Replacement of Enzyme Treated Barley Instead of Corn in the Diets of *Coturnix coturnix japonica*. *Iraqi Journal of Science and Technology*. 3(2): 91-99.
- [25] Potter, L. M.; Stutz, M. W. and Matersson, L. D., 1965. Metabolizable Energy and Digestibility Coefficient of Barley for Chicks as Influenced by Water Treatment or by Presence of Fungal Enzyme. *Poultry Sci.* 44: 565-573.
- [26] Al-Ashaab, M. H.; Salman, A. A.; Mohammad, S. D.; Mutar, A. J. 2007. Effect of enzymes supplement (Safizyme XP1000) on growth performance and some physiological characteristics for common carp *Cyprinus carpio* L. fingerlings reared in warm temperatures. *Iraqi J. Agri.* 12(2): 82-89.
- [27] Yasar, S. and J. M. Forbes , 2000. Enzyme supplementation of dry and wet wheat-based feeds for broiler chickens: performance and gut responses. *Br. Journal of Nutrition*, 84: 297-307.

The Effect of Spraying Thiamine and Nano-Potassium in the Growth and Flowering of *Catharanthus roseus*

Ameer A. A. Hassan¹, Thaer R. Hmood², Safaa Sh. Naeem³ and Mushtaq T. H. Al-Zurfi⁴

¹Department of Physical Planning, Faculty of Physical Planning, University of Kufa, Najaf, Iraq.

^{2,4}Department of Horticulture and Landscape, Faculty of Agriculture, University of Kufa, Najaf, Iraq.

³Agriculture Services Section, University of Kufa, Najaf, Iraq.

¹E-mail: ameera.alfaham@uokufa.edu.iq

²E-mail: thaer.84rah@gmail.com

³E-mail: safairaq1984@gmail.com

⁴E-mail: mushtaq.alzurfi@uokufa.edu.iq

Abstract. An experiment was conducted under green shade conditions in a commercial nursery during the 2023 growing season to study the effect of spraying thiamine and Nano-potassium on the growth and flowering of the *Catharanthus roseus* plant. The experiment was carried out in a randomized complete block design (R.C.B.D) with three replications with two factors: the first was thiamine at three concentrations (0.20 and 40) mg L⁻¹ and the second factor was Nano-potassium at three concentrations (0.1 and 2 g.L⁻¹). The means were compared according to the least significant difference (L.S.D) test at the 0.05 probability level. The results showed that spraying thiamine at a concentration of 40 mg L⁻¹. with the addition of Nano-potassium at a concentration of 1 g.L⁻¹ significantly increased growth and flowering indicators, as it increased (plant height, number of leaves, leaf content of total chlorophyll, number of flower inflorescences, number of florets, and floret diameter). I reached 27cm, 82.67 leaf. plant⁻¹, 62.10 mg.100 g⁻¹, 12.66 inflorescences plant⁻¹, 44.00 florets inflorescence⁻¹, and 2.46 cm compared to the control treatment, which gave the lowest rate of 35.90 cm and 55.33 leaf. plant⁻¹ and 48.34 mg.100g⁻¹ and 5.66 inflorescences. Plant⁻¹ and 23.67 florets. inflorescence⁻¹ and 1.23 cm, respectively.

Keywords. Cut flowers, Ornamentals, Nano-fertilizers, Vitamins.

1. Introduction

The *Catharanthus roseus* L. plant is one of the perennial plants belonging to the oleander family, Apocynaceae [1], which is important as cut flowers and commonly used worldwide [2]. It is one of the medically important plants, as it contains many alkaloids, the most important of which are the compounds Vinblastine and Vincristine, which are used in the treatment of cancer [3]. Flowering and flowers quality may be affected by extreme cold, so its upper stems dry up, but it grows again in the spring and dies in severe freezing [4,5].

Vitamins participate and work as biological auxiliary factors or enzymatic companions in most vital processes [6]. Thiamine is an enzymatic factor that follows the carboxyl group. It is considered a ketoacid, such as keto-glutamic acid. This acid has an important role in the metabolism of fats and carbohydrates [7]. Thiamine is a crystalline substance with a White color dissolve in water but not in organic solvents such as ether and chloroform [8]. It is essential in the plant to transfer the products of the photosynthesis process from the leaves to the growing tips to other plant parts [9].

Thiamine is commercially produced and widely used to encourage root development, and ensures the success of cultivation and plant rotation. Its unique content of nitrogen and sulfur, has a major role in the formation and manufacture of many important plant hormonal and enzymatic compounds that enhance plant growth and vigor [10,11].

Potassium on the other hand possesses the most important cation in plant physiology due to its physiological and biochemical functions. It represents the only positively charged single ion (K^+) that all plants need, even though it is not included in any organic compound and the absorption of this element is absorption. It is active because it accumulates in plant tissues against the concentration gradient with the external environment [12].

Potassium is one of the important elements in the plant, as it controls stomata movements beside of being necessary for the transport of metabolic products, protein formation, increasing the thickness of cell walls, increasing the stiffness of the stems, and increasing the plant's resistance to low temperatures [13]. It participates in ionic balance, regulation of osmotic potential, an auxiliary factor in the process of opening stomata, and nutritional conversion of carbohydrates or protein [14]. To that end, this research set out to determine how spraying *Catharanthus roseus* plants with thiamine and Nano-potassium affected their development and blossoming.

2. Materials and Methods

A study was carried out in the 2023 growing season at the nursery of the Agricultural Division / University of Kufa to assess the impact of potassium and nano-thiamine spraying on the development and blossoming of the *Catharanthus roseus* plant. The seeds were sown on January 10, 2023, and as soon as the first true leaves emerged, the seedlings were transferred to plastic containers with a diameter of 28 cm and a height of 25 cm. Each container contained 5 kg of soil. Table 1 displays the physical and chemical properties of potting soil.

Table 1. Physical and chemical characteristics of potting soil used in the study.

Characteristics	Units	Value
Acidity of soil Ph		7.21
EC	Ds cm^{-1}	1.72
N	mg L^{-1}	32.4
P	mg L^{-1}	15.6
K	mg L^{-1}	9.3
Mg ⁺⁺	mlMol L^{-1}	1.8
Organic matters	%	0.75
Soil texture	Sandy	
Clay	%	21
Silt	%	18
Sand	%	61

The experiment utilized a factorial design with a completely randomized block layout. There were two factors: the first was the concentration of thiamine, which could be 0, 20, or 40 mg. L^{-1} , and the second was the concentration of Nano-potassium, which could be 0, 1, or 2 g. pot⁻¹. The

first application of the two components occurred after the plant's actual leaves emerged, and the second occurred twenty-one days later.

Every experimental unit had all service operations performed on it as needed, including watering and weeding. Among the metrics used to track vegetative growth were plant height (cm), leaf/plant number, branch/plant number, shoot dry weight (g), and total chlorophyll concentration ($\text{mg}/100 \text{ g}^{-1}$) in the leaves. Indicators of flower growth include the following: elapsed time till first blooming, flower stem length in centimeters, inflorescence to plant ratio, floret to flower ratio, and flower diameter in centimeters. A significance level of 0.05 was used for the comparison of means using the least significant difference (S.D.L) test.

3. Results

It is noted from the results (Table2) that spraying thiamine at 40 mg.L^{-1} with applying of Nano-potassium at a concentration of 1 g.pot^{-1} led to a significant increase in plant height, the number of total leaves, the number of branches, the shoot dry weight, the leaf content of total chlorophyll, recording 56.27 cm , $82.67 \text{ leaf.plant}^{-1}$ and $10.66 \text{ branche.plant}^{-1}$, 8.51 g and $62.10 \text{ mg.100 g}^{-1}$, compared to the control treatment, which gave the lowest rate of 35.90 cm , $55.33 \text{ leaves.plant}^{-1}$, $5.00 \text{ branch.plant}^{-1}$, 4.35 g , and $48.34 \text{ mg.100 g}^{-1}$, respectively.

Table 2. Effect of nano-spraying thiamine and nanopotassium on the vegetative characteristics of the *Catharanthus roseus* L. plant.

Treatments		Plant height (cm)	No. of leaf.plant ⁻¹	No. of branch.plant ⁻¹	Shoot Dry weight (g)	Leaf's total chlorophyll (100 mg. ⁻¹)	
Thiamine mg L ⁻¹	0	37.59	59.11	5.66	4.75	50.02	
	20	45.03	71.78	7.77	6.60	53.83	
	40	54.63	78.89	10.11	8.05	58.97	
	L.S.D. 0.05	0.767	1.058	0.366	0.348	0.688	
Nano-potassium g pot ⁻¹	0	43.24	67.00	7.44	6.06	52.68	
	1	46.27	70.67	8.11	6.54	55.16	
	2	47.74	71.89	8.00	6.78	54.98	
	L.S.D. 0.05	0.767	1.058	0.366	0.348	0.688	
Thiamin mg. L ⁻¹ X Nano-K g. pot ⁻¹	0	35.90	55.33	5.00	4.35	48.34	
		1	37.57	57.67	5.66	4.51	49.79
		2	39.30	64.33	6.33	5.10	51.69
	20	0	42.33	67.00	7.00	5.83	53.08
		1	44.97	71.67	8.00	6.54	53.58
		2	47.80	76.00	8.33	7.43	54.82
	40	0	51.50	78.76	10.33	8.01	56.65
		1	56.27	82.67	10.66	8.51	62.10
		2	54.13	75.33	9.41	7.58	58.14
	L.S.D. 0.05	1.329	1.832	0.634	0.603	1.191	

Table 3 also shows that there was a significant decrease in the number of days needed for flowering as well as an increase in the length of the flower stem, the number of flower inflorescences, the number of florets, and the diameter of the florets (53 days, 25.40 cm, 12.66 inflorescences) when 40 mg.L^{-1} of thiamine was sprayed in combination with Nano-potassium at a concentration of 2 g. pot^{-1} . Compare this to the control treatment, which produced the lowest

rates at 66.33 days, 15.37 cm, and 5.66 inflorescences: plant⁻¹, 44.00 florets.inflorescence⁻¹, and 2.46 cm. For each plant, there are 1.23 cm of inflorescence and 23.67 florets.

Table 3. Effect of spraying with thiamine and Nano-potassium on the flowering characteristics of the *Catharanthus roseus* L. plant.

Treatments		No. of days to 1st flowers	Flower stem length (cm)	No. of inflorescence plant ⁻¹	No. of floret inflorescence ⁻¹	Flower diameter (cm)	
Thiamine Mg L ⁻¹	0	65.00	16.14	6.55	26.78	1.43	
	20	58.67	19.20	8.44	35.56	1.93	
	40	55.22	24.08	11.55	42.48	2.63	
	L.S.D. 0.05	1.058	0.529	0.366	0.917	0.107	
Nano-potassium (g pot ⁻¹)	0	60.78	18.71	8.11	32.69	1.80	
	1	58.33	20.08	9.22	35.87	1.90	
	2	55.22	20.63	9.89	36.78	2.03	
	L.S.D. 0.05	1.058	0.529	0.366	0.917	0.107	
Thiamin mg L ⁻¹ X Nano-potassium g pot ⁻¹	0	0	66.33	15.37	5.66	23.67	1.23
		1	64.33	16.07	6.33	27.00	1.36
		2	64.33	17.00	7.66	29.67	1.70
	20	0	61.33	17.97	7.33	32.00	1.80
		1	57.67	18.77	8.66	35.78	1.86
		2	57.00	20.89	9.33	39.00	2.13
	40	0	54.59	22.08	11.33	42.00	2.36
		1	53.00	25.40	12.66	44.00	2.46
		2	58.00	24.03	10.77	41.67	2.26
	L.S.D. 0.05	1.832	0.916	0.634	1.589	0.187	

4. Discussion

The study's indicators showed a notable improvement after spraying with thiamine, as shown in Tables 2 and 3. The amount of plant hormones, particularly photosynthetic pigments, is directly influenced by thiamine levels in the plant cell, which may explain why thiamine is so crucial to many physiological functions. It regulates biosynthesis and organic transport mechanisms, which in turn boosts the plant's adaptive capacity and health by making photosynthesis and nutrient manufacturing more efficient and by increasing ion absorption from the nutrient medium [15,16]. This, in turn, causes cells to grow larger and divide faster, which promotes greater vegetative growth, which in turn increases the plant's height and leaf count. Additionally, it increases internal growth hormones, specifically cytokinins and gibberellins, which boost the rate of vegetative growth[17,18].

It also has a significant effect on the flowering growth characteristics of the plant. This may be due to the role of thiamine in increasing the efficiency of the photosynthesis process, absorbing nutrients, and increasing cytokinin and gibberellin [19,20]. Beside the enzymatic activity of thiamine in increasing the products of photosynthesis, resulting in an increase of sugars that are used for shoot growth and improving flower characteristics to reduce the number of days required for flowering. The other thing is the availability of appropriate environmental conditions, such as temperature, humidity, and good lighting, and then to early flowering [19,21]. It was also found

that a significant increase in the studied characteristics when potassium was added. It is due to the fact that the element potassium has an important role in influencing the physiological functions within the plant, despite the claim that it is not included in the synthesis of any organic compound or plant cell. It plays the role of opening and closing stomata and is considered an essential activator for the enzymes that accompany the assimilation of carbohydrates and peptide bonds. It also plays the role of a catalyst in many vital processes. Including regulation of solvent movement and absorption of nutrients [11,22].

There was also an increase in the study indicators when applying Nano-potassium. The number of leaves was increased mostly due to the effect of potassium, which contributes to increasing the efficiency and process of photosynthesis, activating enzymes, and accumulating carbohydrates, which affects the increase in the number of leaves, increasing production, and improving its quality [23]. It may also be attributed to the introduction of Nitrogen is an element in the formation of proteins, amides, nucleic acids, and amino acids, and is involved in the synthesis of hormones, especially IAA, which plays an important role in increasing cell division and stimulating growth [10,24]. In addition to the role of potassium in activation of enzymes and the accumulation of carbohydrates as a result of its involvement in the processes of protoplasmic construction, and that a lack of potassium leads to the destruction of chloroplasts [26].

Conclusion

The results of the study generally indicate that spraying plants with thiamine, especially at a concentration of 40 mg L⁻¹, led to a significant improvement in the vegetative and floral indicators of *Catharanthus roseus* flowers. It was also found that treating the potted soil with potassium at a level of 2 g pot⁻¹ achieved a clear increase in all study indicators. In general, findings of the study concluded that it is possible to obtain the best vegetative growth and qualitative floral yield by using foliar spraying with thiamine 40 mg L⁻¹ in combination with treating the potted soil with nano potassium 1 g pot⁻¹.

References

- [1] Welch, C. W. (2002). Breeding New Plants and Flowers. Crowood Press (UK).
- [2] Salhi, H. and A. Karim. (2017). The Effect of brassinolide growth regulator and mineral fertilizer on some characteristics of vegetative growth and active substance of *Catharanthus roseus* plant. Master Thesis, College of Agriculture - University of Tikrit. Republic of Iraq. pp. 6-9.
- [3] Ferreres, F.; Pereira, D. M.; Valentão, P., Andrade, P. B., Seabra, R. M., & Sottomayor, M. (2008). New phenolic compounds and antioxidant potential of *Catharanthus roseus*. Journal of Agricultural and Food Chemistry, 56(21), 9967-9974.
- [4] Khattak, A. M., Ahmad, I., Amin, N. U., Wahid, F., & Rahman, H. U. (2011). Effects of different amended organic media on the growth and development of *Vinca rosea* 'Victory'. Sarhad J Agric, 27, 201-5.
- [5] Al-Janabi NTA, Al-Zurfi MTH (2022). Snapdragon (*Antirrhinum majus* L.) response to foliar application of arginine and nano-iron. SABRAO J. Breed. Genet. 54(4): 927-934. <http://doi.org/10.54910/sabrao2022.54.4.23>.
- [6] Piedrafito, G.; M.A. Keller and Ralser, M. 2015. The impact of non-enzymatic reactions and enzyme promiscuity on cellular metabolism during (oxidative stress conditions. Biomolecules, 5: 2101–2122.
- [7] Oertli, J.J. (1987). Exogenous application of vitamins as regulators for growth and development of plants-a review. Z. Planzer Nahr. Boden, 15: 375 – 391.
- [8] Rindi, G. (1996). Thiamin. In E.E Ziegler and L.J. filer (eds). Present knowledge in nutrition. ILSI. Press, Washington, D.C. Rotation Crop Effects on the Recovery of soil organic matter D.C.PP.160-166.
- [9] Routh, J. I (ed.) (1978). Introduction to Biochemistry. Second Edition. Routh W.B. Saunders Co, Inc. Toronto. USA.

- [10] Tylicki, A.; Z. Lotowski.; M. Siemieniuk and Ratkiewicz, A. (2018). Thiamine and selected thiamine antivitamins—Biological activity and methods of synthesis. *Biosci., Rep.*, 38(1):1-23.
- [11] Al-janabi, A. S.;Alpresem,W.F.and Kadhemi,D.A.(2021). Influence of Foliar Spraying with Potassium, Zinc, and Copper Nano-fertilizers on Some Vegetative Growth Characteristics and Anthocyanin Gene Expression of Pomegranate Transplants. *Annals of the Romanian Society for Cell Biology*, V25,ISSU3 8152–8160. <https://annalsofrscb.ro/index.php/journal/article/view/2355>.
- [12] Morard, P., Lacoste, L., & Silvestre, J. (2004). Effect of oxygen deficiency on mineral nutrition of excised tomato roots. *Journal of plant nutrition*, 27(4), 613-626. <https://doi.org/10.1081/PLN-120030371>
- [13] Taiz L. and E. Zeiger. (2010). *Plant Physiology*.5th Ed. Sinauer Associates. Sunderland. MA. USA.
- [14] Dong, W., Zhang, X., Wang, H., Dai, X., Sun, X., Qiu, W. and Yang, F. (2012). Effect of different fertilizer application on the soil fertility of paddy soils in red soil region of southern China. *PloSOne*, 7(9), 1-9.
- [15] Smirnoff, N. and G.L Wheeler. (2000). Ascorbic Acid in plant: biosynthesis and function. *Biochemistry and Molecular Biology*, 35(4) : 291 – 314.
- [16] Sajjad, Y.; M.J.Jaskani.; M.Qassim.; G. Akhtar and Mehmood, A. (2015). Foliar application of Growth Bioregulators Influences floral traits. correlative traits and chemical constituents in *Gladiolus grandiflorus* L. *Korean.J.Hortic. Sci. Technol.*, 33(6): 12-819.
- [17] Bocobza, S.E.; S Malitsky.; W.L .Araújo.; A.Nunes-Nesi.; S .Meir.; Shapira, M.; A.R .Ferne and Aharoni, A. (2013). Orchestration of thiamin biosynthesis and central metabolism by combined action of the thiamin pyrophosphate riboswitch and the circadian clock in *Arabidopsis*. *Plant Cell*, 25 :288–307.
- [18] Noordally, Z.; C. Trichtinger.; I. Dalvit.; M.Hofmann.; N. Zamboni.; L. Pourcel.; E.Gas.; A.Gisler and Fitzpatrick,T.B. (2020).The coenzyme thiamine diphosphate displays a nuclear rhythm independent of the circadian clock. *Commun. Biol.*, 3: 209.
- [19] Peng, C., S. Uygun.; S.H. Shiu and Last, R.L.(2015). The impact of the branched-chain ketoacid dehydrogenase complex on amino acid homeostasis in *arabidopsis*. *Plant Physiol.*, 169: 1807–1820.
- [20] Mohammed AJ, Al-Janabi ASA (2022). Irrigation water quality and antioxidants effects on the chemical composition of sweet orange. *SABRAO J. Breed. Genet.* 54(5): 1241-1253. <http://doi.org/10.54910/sabrao2022.54.5.25>.
- [21] AL-Zurfi MTH, K M. Bhiah and Jamal A A (2023). The effect of spraying with amino acids and iron on some characteristics of vegetative and flowering growth of *petunia* *Bionatura* <http://dx.doi.org/10.21931/RB/2023.08.04.47>
- [22] Atal, H. L., & Kumar, K. P.(2021). Role of Plant Growth Regulators in Floriculture.
- [23] Mohammed AJ, Al-Janabi ASA (2022). Liquefied water and antioxidants influence on vegetative growth and physiological traits of sweet orange. *SABRAO J. Breed. Genet.* 54(4): 917-926. <http://doi.org/10.54910/sabrao2022.54.4.22>.
- [24] AL-Zurfi MTH, Inas MT and Jamal A A (2022). Effect of Spraying Putrescine and Roselle Extract on the Growth and Flowering of Chinese Carnation (*Dianthus chinensis*). *International Journal of Agricultural and Statistical Sciences*. DocID: <https://connectjournals.com/03899.2022.18.2207>.
- [25] Kareem KhA, Alojany ZOO, Al-Janabi ASA (2022). Marine algae extracts, and nano fertilizer with zinc and copper effects on growth, and macro- and micronutrients composition of apple trees. *SABRAO J. Breed. Genet.* 54(2): 389-396. <http://doi.org/10.54910/sabrao2022.54.2.14>.
- [26] Kareem, M. A., Raheem, A. A., Oriola, K. O., & Abdulwahab, R. (2022). A review on application of oil palm shell as aggregate in concrete-Towards realising a pollution-free environment and sustainable concrete. *Environmental Challenges*, 8, 100531.

Evaluation of the Diffusion Results of the Dairy Industry Techniques in Ninevah Province

Asmaa Zuhair Al-Hafidh

Department of Agricultural Extension, College of Agriculture and Forestry,
University of Mosul, Mosul, Iraq.

E-mail: asmaa_alhafidh@uomosul.edu.iq

Abstract. The current research aims to calculation evaluation the diffusion results of the dairy industry techniques in Ninevah province , as well prearrangement to the fields that concerned with evaluation the diffusion results of dairy industry techniques in Ninevah province are: dairy diffusion range capacity, land possession area , economic arrival , maintainable , types of dairy products, customers satisfaction. assessment the grade of fields questions followed to evaluation of diffusion results of the dairy industry techniques in Ninevah province ,recognize differences between evaluation of diffusion results of the dairy industry techniques in Ninevah province according to the factors : (education grade, agricultural activity importance , type service in the dairy factory , number of working years in dairy industry ,source of milk gains) . To accomplish those aims , prepared area questioner verified (virtual validity & content validity) as well as reading many publishes , books & done interviews with the specialists ,while research community consisting of (100%) which equal (57) of producers in Nineveh province, then most important of results in diffusion in dairy industry techniques to the producers were (medium) tend to (few), also most imperative conclusion were :all of the research factors performs a weak role in assessment Dairy industry techniques diffusion by the Producers except source of milk gains performs a strong role in Governorate of Nineveh

Keywords. Dairy industry, Evaluation, Diffusion, Ninevah province, Techniques.

1. Introduction

Development to any humanity hasn't perfect that because of accidental effort , but through agricultural officers activities to aims & necessity data gains to accomplish these aims vital information requirements , sureness it that to attempt providing it that to different of us by different of communication implements , but this information should strong, common, & help us to transfer and diffusion evaluation The dairy techniques at best receivers [1], techniques diffusion was lucky about a last period years ,but production remained in low level, it didn't any improvement at its efficiency [2] from view of relationship among agricultural development and it's society growth that be levied on us that to be great efficient Agricultural technologies producers , these requisite of full satisfaction [3] there appearing relative failure in increase production of the cultivators because absence area extension work [4] new information be very essential in strengthening scientific research & making problem solutions be built on sufficient knowledge [5] the modern indicators agreement to spread instruction , exercise, growth of professionals rate , also division of knowledge [6] many agricultural dairy techniques were

transfer to the producers basically on feedback technologies & were proved realization, understood provided great output, in general the dairy techniques technologies do not be achievement unless adept in space situations under training unless appropriate to the economic, physical, mental abilities of Agricultural technologies producers and appropriate to diffusion requirements [7] diffusion requirements be crucial, important on level of scientific expected result as controlled of other events [8] some of the assessments been exposed and increasing their chance of production (50 %) since existing manufacture estimate using application satisfactory amount of the agricultural technologies [9] in one of the studies it was fended following there is several essential reasons be an significance to participation of an progress serves, arrangement of sureness & encourage their on physical effort conditions [10] so this means obstacles are n't in agricultural technologies but, in the current obstacles effect in the diffusion process, indicate to the big gap among researchers & productivity rates by producers in area [11] so to assure on the organization situation in agricultural institutions, that is because affected to the increase of motivations of agricultural technologies producers or, stop it [12] we should involving the programs training and qualification effort ethics in the Agricultural technologies producers & follow it that to increase work coefficient in agricultural organization [13] agricultural organization must to be appropriately cultured about how reach to highest level of apply in new view or applies their rural actions that to counter really to the new knowledge [14] therefor work weakness & low level of the motivation will drop of staffs efficient efforts that due to faintness in performance and at end will failed to translate agricultural technologies to producers, when essential transfer methods have been done of dairy techniques agricultural techniques their isn't be success then results will not be satisfied, one of studies refers to that most applied studies careful that led to the necessity & priority the identify with the progress program [15] in another study showed that weakness of the communication among extension & research institution, there was a hole between certified criteria, firm in the study and the extension work & not improvement, all of these reasons led to different opinions [16] technical administrations these which monitored by the ministry of agriculture stalemates to affixed approaches and be dependable on process of research and technology diffusion [17] in addition to study [18], Showed large faintness in the technologies introduction progression, there are many difficulties associated by technologies transfer to cultivators, as well as to study of [19] it cleared a high level of the obstacles size, and most important of the obstacles were be management, also appeared in the study of [20] as an improvements in responsibility of the agricultural extension for maintaining on speed the modern technologies development to deliver beneficiaries to exact logical knowledge & need from them on right trail that to decrease mistakes of the agriculture products about consume great amounts [21] so research indicating to design modern & lengthened political program viewed with agricultural extension and design platform too that for encouraging active discussion [22] we should consideration with the extension managers in addition to our attention with the extension agricultural technologies producers, that is because of the multiple work stresses of them & there required to involve him in training course to reduce work stresses also developing their efficiency [23] that for reaching to real concepts, sustainable development, active strategies to improve the Agricultural technologies producers [24] dairy technique is the method to transfer isn't success, one researches indicates to that some studies be implemented without watchful recognising to necessity & priority that to recognise with progress program [25] so since there is basic required because of decline gap between attendance of the dairy techniques agriculture and it's applied in add to that, it is a essential to transfer majors of Agricultural technologies producers about agricultural dairy techniques to vital requires useful in the preparation planning and application to technique diffusion then we reaching to the highest equal of production and productivity.

1.1. Research Hypotheses

There is not a significant alteration in evaluation of diffusion result to the dairy industry according to (education grade-agricultural activity importance-type service in the dairy factory - number of working years in dairy industry -source of milk gains).

1.2. Research Objective

The essential object from this research: evaluation of the diffusion results of the dairy industry, therefore we should follow these subordinate objectives.

- Calculation evaluation the diffusion results of the dairy industry techniques in Ninevah province.
- Prearrangement to the fields that concerned with evaluation the diffusion results of dairy industry techniques in Ninevah province are: dairy diffusion range capacity, land possession area , economical arrival , maintainable , types of dairy products, customers satisfaction.
- Assessment the grade of fields questions followed to evaluation of diffusion results of the dairy industry techniques in Ninevah province.
- Recognize differences between evaluation of diffusion results of the dairy industry techniques , in Ninevah province according to the factors: (education grade, agricultural activity importance, type service in the dairy factory, number of working years in dairy industry, source of milk gains).

2. Research Methodology

Approach that using in this research is: descriptive approach because of it the best way appropriate to this study , fields correlated with evaluation of the diffusion results of dairy industry techniques had preparation affording to Mean , as well as a questionnaire was prepared ,it had examined an apparent validity as well as content validity, then value of validity 0.93 in the sample of pre_test, was (12) out of final sample , then grade of stability examined of it when using Alpha test was 0.751 this score due to high dependability factor also we can be dependent to this questionnaire fields in final sample . Target community involved of (100 %) final sample full equal (45) dairy industry techniques producers , that is a various statistical means used such as : Z-score ,Kruskal Wallis, percentage , Man-Witney, Mean, Frequency [26]. Prepared research questionnaire to collected required data , it have two parts : The first part personal factors are: education grade was measured by given(1) illiteracy grade (2) Primary grade (3) Secondary grade (4)Institute grade (5) College grade (6)master grade, agricultural activity importance:(1) important activity (2) not important activity , type work in the dairy factory: (1) Individual work (2) Work with family (3)Workers (from out of family) , number years of practicing in the dairy industry : (1) Small number(2) Median number (3) Big number, source of milk gains : Family farm (1), nearby farms (2) nearby villages (3) .While second part limited to evaluation the diffusion results the dairy industry techniques of (34) questions focused on six fields, against each field from these fields triple measure (too much, medium, few) that was at the first form then give the following degree (3,2,1) respectively, but at the final form completed (27) questions, distant because of not appropriate its validity .

3. Results

3.1. Calculation Evaluation the Diffusion Results of the Dairy Industry Techniques in Ninevah Province

And as illustration in chart(1) ratio (73.33%) from dairy industry producers gained a large of evaluation diffusion results to the dairy industry, the ratio (15.55%) in the second & the last ratio (11.11%) from the dairy industry.

Table 1. Distribution of categories according evaluation calculation of diffusion results.

Categories	Z Score	Number	The ratio
Few	Less From - 1	7	15.55%
Medium	(- 1 , + 1)	33	73.33%
High	more Than + 1	5	11.11%

Mean = 59.35 S.D= 3.77 Total number = 45 Max.= 66 Min.= 53.

Then to faraway measurement errors , pure data exchanged to Z Score (Standard Scores) through dependent on standard Score (Z) ,then at the next of it divided in to three categories ,in the beginning (High) increase than (+1), while the second (medium) between (+1,-1), while the third (Low) decrease than (-1) , as it clear in character (1) the category of medium to few score is being a big category , then this consequence agreement by research [27], disagreement by research of [28], agreement by research (29) also . These mean of evaluation the diffusion results the dairy industry techniques was good to progress that means them information and skills and data about agricultural technologies transfer and diffusion to the others.

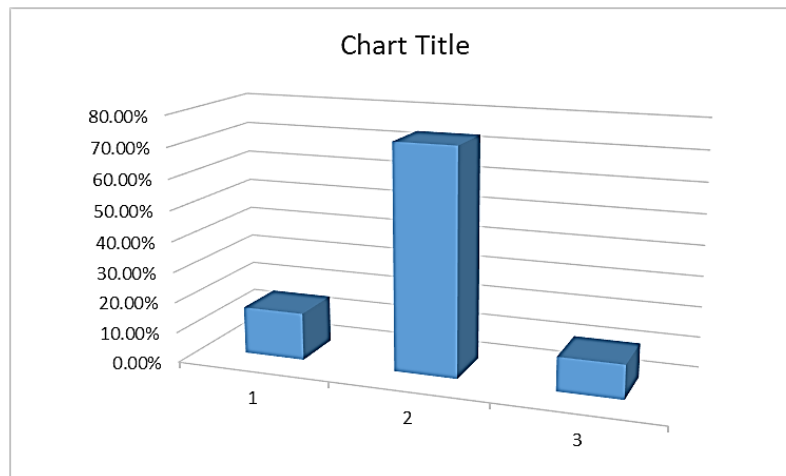


Figure 1. Ratio of the distribution categories according to evaluation diffusion results of dairy industry techniques.

3.2. Prearrangement to the Fields that Concerned with Evaluation the Diffusion Results of Dairy Industry Techniques in Ninevah Province

Dairy diffusion range capacity, land possession area , economical arrival , maintainable , types of dairy products, customers satisfaction:

As illustrated in the chart (2) the higher of evaluation of the diffusion results of dairy industry techniques is, The land of factory (possession) dependent in productivity value of Mean to this domain is (17.06) which get the first order as showed in the following chart:

Table 2. Arrangement of measurement fields according to mean.

Fields	Means	Orders
The land of factory (possession) dependent in productivity	17.06	1
The diffusion range capacity	7.28	6
The economic material return	8.37	4
The producers satisfaction about dairy products	8.02	5
Maintainable	8.82	3
Milk healthy	9.77	2

3.3. Assessment the Grade of Fields Questions Followed to Evaluation of Diffusion Results of the Dairy Industry Techniques in Ninevah Province

Table 3. Arrange the grade of fields questions according to their means.

Fields questions	Means	Orders
The speed of diffusion(period achieved which in it diffusion the dairy industry techniques)	2.62	1.5
Satisfaction producer about the results obtained	2.62	1.5
Satisfaction producer about the extension activity	2.6	3
Satisfaction producer about the dairy industry techniques appropriate	2.42	4
Satisfaction producer about the agricultural equipment's manufacturing related	2.55	5.5
The ratio of mix to the milk with other components	2.53	5.5
The types of mix components produced to the human consumption	2.53	7.5
The extent of nutritional value to the dairy products	2.46	7.5
Mount of product of milk during productivity season	2.46	9
The price of manufactured products sale	2.4	10
The cost of using milk	2.28	11
Different types of the manufactured milk	2.28	12
Extent impossibility of application dairy techniques in different milk types	2.28	13
Extent possibility adding a different materials types to the milk	2.28	14
Extent palatability manufactures products by humans	2.26	15
The possibility of controlling nutritional gap in markets by manufacture these products	2.24	16
The range of efficiency the manufactured products	2.2	17
The range of it's extent the diffusion to the others	2.13	18
The mount of increase of quantity of manufactured production	2.08	19
Maintaining the nutritional value to the milk products	2.08	20
Increasing the material profit of products	1.95	22
Decreasing the ratio of the loss in manufacturing	1.91	22
Solving problems related with productivity	1.91	22
Extent increasing in the area used with techniques	1.53	24
Area of factory used for manufactured	1.51	25
The productivity which achieved from the dairy factory	1.66	26
The extent application of sustainability & health rules of these techniques	1.46	27

3.4. Recognize Differences Between Evaluation of Diffusion Results of the Dairy Industry Techniques in Ninevah Province

According to the factors : (education grade, agricultural activity importance ,type service in the dairy factory , number of working years in dairy industry ,source of milk gains) as presented in chart (4):

Table 4. Differences in evaluation of diffusion results of the dairy industry techniques affording to some factors.

Factors	No.	%	Median	Average rank	Calculated h	Calculated w	p-Value
The education grade							
Illiteracy grade (1)	12	26.66%	58.5	20.6	5.52	-----	0.35
Primary grade (2)	17	37.77%	60.0	26.9			
Secondary grade (3)	5	11.11%	59.0	22.0			
Institute grade (4)	2	4.44%	61.0	33.0			
College grade (5)	5	11.11%	57.0	14.2			
master grade (6)	4	0.19%	58.5	21.0			
Agricultural activity importance							
Important activity (1)	33	73.33%	59.0	59.72	-----	807.0	0.21
Not importance activity (2)	12	26.66%	57.5	58.33			
Type work in the dairy factory							
individually work (1)	5	11.11 %	58	17.2	-----	1.55	0.46
with family work (2)	27	60%	59	24.7			
Workers work (3)	13	28.88%	59	21.8			
Number of practicing years in dairy industry							
Small number (1) year	6	13.33%	58.5	18.3	1.36	-----	0.50
Median number (2) year	12	26.66%	58.0	21.6			
Big number (3) year	27	60%	59.0	24.7			
Source of milk gains							
Family farm (1)	29	64.44%	59	26.1	10.25	-----	**
Nearby farms (2)	10	22.22%	57	11.4			0.006
Nearby villages (3)	6	13.33%	62	27.2			

** significant at 1%.

Conclusions

Majority of the producers in this research have a great level in fields of diffusion of the dairy industry techniques, for this results we can conclude producers of the dairy poor to scientific knowledge about dairy industry techniques diffusion, the scientific knowledge and there is a needing to review of different data in this field, also considerate, adapting with new changes, increasing awareness about this matter.

The dairy producers had been poor considerably to diffusion extensional facts of each of education grade, agricultural activity importance, type service in the dairy factory, number of working years in dairy industry, that is a hole need to required and ask more technologies and info. Source of milk gains was important factor and had been perform strong role to assessment dairy techniques diffusion to Producers in research area.

Recommendations

Watchful with diffusion a dairy industry techniques to producers in Nineveh Governorate, also achieving the sustainability to face ag. products prices great, dealing with an action absence of correlation between scientific research and dairy producers, as well as hole among the focus on the particular training regarding producers and related with dairy industry techniques advisers in addition of hole among the funding of researches , techniques of research ,funding application

methods at field Dairy industry techniques diffusion also presence of a very big of dairy industry techniques according to different courses, achieving mobile transports, online communications by dairy industry techniques officers, rapid him to increase training and exercise courses in Dairy industry techniques diffusion, adding a largest imaginable number of producers according to their variegated courses, transmission new ideas as well as technics to agriculture officers by online that to facilitate their practicing, submitting the dairy industry techniques with reinforced prices that to help and facilitate by producers and facilitate process of diffusion .

References

- [1] Shams AL-Din , Sh. A .(2001).Theorist of information , terms and essential issues , knowledge Magazine , Syria ,No.(450).
- [2] Lafta, A.H. 2009. Constraints to technology transfer as viewed by extension producers . Iraqi Journal of Agricultural Sciences, 40(4), 86-91
- [3] Lafta, A.H., Jasem, S. D., & Hamad, T. N 2007. Job satisfaction of agricultural extension workers in Iraq. Iraqi Journal of Agricultural Sciences, 38(5), 24-33
- [4] Mahmoud, Y. A. Lafta, A.H., & Fayadh, S. U. 2009. Knowledge level of side damages of horticultural growers when using excess fertilizers. Iraqi Journal of Agricultural Science, 40(6), 101-107.
- [5] Qazzanje , Y.f.(2001). The in for motion Zane its role in making the scientific knowledge and it's in fluency to Arabic countries , Iraqi Magazine to information , folder(7),NO.(2).
- [6] Kremmydas ,D.,Athanasiadis ,I.N.Rozakis S.(2018).Agricultural Systems ,Contents lists available at Science Direct, journal homepage : www.elsevier.com/locate/agsy 95-1
- [7] Shideed , K.H. and Mohammad El-Mourid (2006).Adoption and impact assessment of improved technologies crop and livestock production systems in the dry area, ICARDAIFAD .
- [8] Jasem, M. S., Norsida Man, J. M., Lafta, A.H., Saleh, M. H., Salim, H., Nolila, M. N. and Bassim H. K. 2016. A review: Training requirement of agriculture extension officers in Iraq. Asian Journal of Applied Science, 9(2), 34-40.
- [9] Arab organization for agr. development , (2001). National symposium for strengthening the links among users the water and it's organizations and institutions of research's and extension .
- [10] Lafta, A.H. 2011. Factors affecting work motivations and their relationship to performance. Iraqi Journal of Agricultural Sciences, 42(2), 103-110.
- [11] FAO (1997) . Management of agricultural research , attaining manual , module 8 , research – extension linkage , Rome.
- [12] Lafta, A.H., Mazher, S. H., & Khamees, A. A. 2007. The level effect of organizational climate on workers in the general board of agricultural extension and cooperative in Iraq. Iraqi Journal of Agricultural Sciences, 38(5), 15-23
- [13] Khamis, A.I., Hasan, A.M.A., & Lafta, A.H. 2022. WORK ETHICS AND ITS IMPACT ON EFFICIENCY OF WORKERS PERFORMANCE OF AGRICULTURAL EXTENSION ZONE. Annals of Forest Research, 65(1), 4468-4491.
- [14] Challob, M., Lafta, A.H., & Ridha, B. 2020. Study on major constraints and problems in transfer of technology by agricultural extension organization. Indian Journal of Ecology, 47(12), 373-375.
- [15] Arab organization for agriculture development , (1997). National symposium for strengthening the links among users the water and it's organizations and institutions of research's and extension, <http://www.un.org>
- [16] Lafta, A.H. 2007. The level of job performance of agricultural extension workers in the general board of agricultural extension and cooperative. Iraqi Journal of Agricultural Science, 38(4), 10-16
- [17] Al- Shaar , Y.S. (2006). The relationship reality among some of research institutions in Iraq from side the communicational and extension research standards, Doctoral certificate, college, of agr. and forstry , University of Mosul .
- [18] Al- Aboody , A.J. (2002) . The process reality of research and age . technologies transfer which implemented by the general companies which followed to the age. Ministry and its relationship with agricultural extension work , Master Thesis , college of agr . 6 University of Baghdad

- [19] Al- jobbery , A . A . G h . (2011). Produsers views towards agricultural technologies transfer problems to the producers in Salah – Aldine Governorate , Iraqi agricultural sciences Magazine , Agriculture college , University of Tikrit Volume (42) , No.(3).
- [20] Al- Hafidh , A.Z.Y. (2012)Obstacles of agr. research implementation from view of the researchers in Nineveh Governorate , Mesopotamia journal of agriculture , University of Mosul , Volume (24), No (1).
- [21] Adnan, S.A., & Lafta, A.H. (2022). Environmental Obstacles In The Process Of Rationalizing Irrigation Water And Treatment Methods From The Point Of View Of Producers In Baghdad Province. International Journal of Agricultural and Statistical Sciences, 18(1), 2281-2286.
- [22] Adnan, S.A., & Lafta, A.H. (2022). Obstacles to extension service in the process of rationalizing irrigation water and treatment methods from the view point of producers in Baghdad governorate. NeuroQuantology, 20(6), 7324-7334
- [23] Ridha, B. A. J., Challob, M. A., & Lafta, A.H. (2020). Agricultural extension system industrial managers in the light of their training - Necessities to face job stress-A field study. AIP Conference Proceedings, 2292(1), 030010 - 030011.
- [24] Lafta, A.H., & AL Khafaji, N.J.M. (2022). THE WORK ENVIRONMENT OF AGRICULTURAL EXTENSION WORKERS IN THE AGRICULTURAL EXTENSION ORGANIZATION IN IRAQ. International Journal of Agricultural and Statistical Sciences, 18(1), 2435-2438.
- [25] Al-Hafidh ,Asmaa Zuhair Younis & Ahmed Hamdan Lafta (2023). IOP Conf. Ser.: Earth Environ. Sci. 1259 012136 ,Suggestion Patron to Diffusion Evaluation the Fodder Agricultural Technologies in Ninevah Province, IOP Conference Series, doi :10.1088/1755-1315/1259/1/012136
- [26] Al-Abbasi , A.F.(2015). Scientific research approaches and statistical analysis in behavioral sciences , University of Mosul ,First print.
- [27] AI- Bahr , A .H.M.& Kh. AI-Taie (2010). knowledge needs of the officers in the diffusion process management of the agr technologies organization , implementation , preview, and evaluating , Iraqi agr. sciences magazine , 41(1) : 93-97 .
- [28] Al-Hafidh ,Asmaa Zuhair Younis & Ahmed Hamdan Lafta ,(2024) Evaluation of the diffusion results of the fodder Azolla crop in Ninevah – Governorate, <https://www.springer.com/gp/authors-editors/conference-proceedings/conference-proceedings-guidelines>, Sustainability - Proceeding of International Conference on Environment and Sustainability,2023, A Springer book series Springer Proceedings in Earth and Environmental Sciences
- [29] Hassan , W.S.A.(2013).the constraints of transferring the agr. cultural technologies from agricultural extension staffs viewpoint and the instructor of the college of agriculture Tikrit university ,Agricultural economic extension Department , college of agriculture , university of Tikrit .