Enhancing the Active Compounds and Antioxidant Content of *Eruca vesicaria* L. Using Biofertilizers and Sprouted Barley Grain Extract

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Abstract

Arugula *Eruca vesicaria* L. contains medically active compounds, and its seeds produce an oil with beneficial effects on overall health and disease prevention. To enhance the quantity and quality of the oil and the bioactive compounds in the leaves, a field experiment was conducted over two seasons: the autumn of 2023–2024 and the spring of 2024.) using a Randomized Complete Block Design (RCBD) with a split-plot system in three replicates. Biofertilization treatments were assigned to the main plots (A) included: $\mathbf{A_1}$ (control, no application), $\mathbf{A_2}$ (*Trichoderma harzianum* at a concentration of 2×10^9 CFU mL^{-1}), $\mathbf{A_3}$ (*Bacillus subtilis* at a concentration of 2.25×10^9 CFU mL^{-1}). Amino acid treatments were assigned to the sub-plots (B) included: $\mathbf{B_1}$ (control, water spray only), $\mathbf{B_2}$ (Arginine 150 mg L^{-1}), $\mathbf{B_3}$ (Tryptophan 150 mg L^{-1}), and $\mathbf{B_4}$ (Phenylalanine 150 mg L^{-1}). The sub-sub plots (C) were allocated to treatments with sprouted barley grain extract included: $\mathbf{C_1}$ (control, water spray only) and $\mathbf{C_2}$ (sprouted barley seed extract at a concentration of 100 g L^{-1})..The application of ***Trichoderma harzianum, Bacillus subtilis,*** amino acids (**Arginine, Tryptophan, Phenylalanine),** and **sprouted barley grain extract,** as well as their interactions, had a significant effect impact on increasing the concentration of bioactive compounds in the leaves, the fatty acids **erucic and linoleic**, and the oil percentage in arugula seeds. This promotes a sustainable agricultural approach that enhances the health benefits of the oil while contributing to environmental conservation, which is of paramount importance.

Keywords: Arugula, Bioactive compounds, Biostimulants**,** fatty acids **,Seed oil.**

Introduction

Arugula (*Eruca vesicaria* L.) Thell. cav. Subsp. *Sativa* (Mill.) is a leafy vegetable crop renowned for its medicinal properties and belongs to the *Brassicaceae* family. It is commonly consumed fresh in salads, while its leaves and flowers are also utilized in Mediterranean regions [30]. In some countries, this plant is cultivated for its oil, which possesses extensive therapeutic properties [40]. The seeds and leaves of arugula exhibit various medicinal attributes, including diuretic, laxative, and stimulant [4]. Additionally, its ethanolic extract demonstrates pharmacological activities against multiple diseases, particularly pancreatic cancer cells [32] as well as anti-inflammatory and neuroprotective properties [20], antibacterial [16] antidiabetic properties [38], and antioxidant activity [31]. Excessive use of chemical fertilizers has significant negative effects on soil and water, necessitating a shift toward sustainable agricultural practices that minimize environmental harm. *Trichoderma* fungi naturally exist in soil as part of the rhizosphere microbiome, where they compete with other microorganisms for resources. *Trichoderma harzianum* is a filamentous fungus widely used as a biological control agent against agricultural pests [50]. Research on its

genetic traits has led to patented applications in biopesticides and plant breeding strategies due to its strong ability to combat plant pathogens and stimulate plant growth [42]. The effectiveness of *T. harzianum* in promoting plant growth has been demonstrated in various vegetable crops by reducing disease incidence through root colonization and competition with harmful pathogens [33]. A study on the impact of beneficial fungi and bacteria on plant growth and nitrate content in hydroponically grown arugula revealed that inoculation with *Azospirillum brasilense* and *Trichoderma harzianum* at different electrical conductivity levels significantly increased root biomass and improved crop yield and nutrition while reducing nitrate levels in the leaves. Furthermore, *T. harzianum* inoculation enhanced phosphorus content in leaves, reaching 10.8 g kg^{-1} [36]. This highlights its positive role in supporting plant nutrition, increasing germination rates, and boosting production. Plant growth-promoting rhizobacteria (PGPR) are considered pioneers of the next-generation green revolution, offering an eco-friendly alternative to chemical fertilizers. *Bacillus subtilis* is one of the most widely used PGPR species, effectively enhancing plant growth and yield [29,45]. This bacterium colonizes the rhizosphere and stimulates plant growth by producing phytohormones such as auxins [12]. In an arugula study, foliar application of *B. subtilis* at a concentration of 50 mL L^{-1} significantly increased the active compound content in leaves, with phenolic compounds reaching 1.083 mg g^{-1} dry weight, flavonoids at 35.98 mg g^{-1} dry weight, and tannins at 1.086 mg g^{-1} dry weight [8]. **Arginine** serves as a precursor for polyamine and nitric oxide synthesis, both of which regulate key physiological and biochemical processes, helping plants adapt to stress conditions [52]. It is one of the most abundant amino acids in plant proteins and acts as a primary nitrogen source and transporter, constituting about 50% of total nitrogen in seeds and, in some cases, over 90% of free nitrogen in vegetative tissues [5]. **Tryptophan** accelerates plant growth by promoting tissue formation and facilitates the breakdown of dead cells into proteins, thereby stimulating development and enhancing flowering [3,26,11]. **Phenylalanine** is a precursor to *phenylpropanoids*, a diverse group of secondary metabolites in dicotyledonous plants, including flavonoids and phenolic acids, which play significant roles in plant defense and adaptation [15]. A promising alternative to chemical fertilizers is the use of plant extracts, particularly those derived from newly sprouted seeds. Recently, the application of sprouted seed extracts has gained attention due to their richness in bioactive compounds that are absent in mature plants. These extracts are easily absorbed, contain high levels of gibberellins, and have lower abscisic acid content [48]. The stored nutrients in seeds, including starch and proteins, play a fundamental role in supporting embryo growth. These reserves, found in the endosperm or cotyledons, undergo enzymatic hydrolysis into soluble units for easy uptake by seedlings. The aleurone layer secretes enzymes that break down starch and proteins into sugars and amino acids, which are then transported to the embryo. Gibberellins, secreted by the embryo, regulate the production of these hydrolytic enzymes. Proteolytic enzymes are crucial in degrading stored proteins and are categorized into four types based on their activity sites. *Thioredoxin* proteins also regulate the release of these stored [10]. In recent years, research has increasingly focused on environmentally friendly biostimulation techniques to enhance crop performance within sustainable agricultural systems. The integration of advanced agricultural technologies based on scientific principles offers an effective strategy for improving arugula productivity, enhancing oil quality, increasing bioactive compound

concentrations, and ultimately boosting its nutritional and medicinal value.

Materials and Methods

The experiment was conducted in the fields of the Department of Horticulture and Landscape Engineering, College of Agriculture, Al-Qasim Green University, to enhance the content of arugula (*Eruca vesicari*) in oil, bioactive compounds, and antioxidants using biofertilizers and sprouted barley seed extract.

Experimental Design

The experiment investigated three factors: Biofertilizer (A): A_1: Control (without application), A_2: *Trichoderma harzianum* at a concentration of $2*10^9$ CFU mL^{-1} (applied at 5 g per planting hole), A_3: *Bacillus subtilis* at a concentration of $2.25*10^9$ CFU mL^{-1} (applied at 5 g per planting hole). The biofertilizers were provided by the Agricultural Research Directorate / Biotechnology Center / Ministry of Science and Technology. Amino Acid Foliar Sprays (B): B_1: Control (sprayed with water only), B_2: Arginine at 150 mg L^{-1}, B_3: Tryptophan at 150 mg L^{-1}, B_4: Phenylalanine at 150 mg L^{-1}. Plants were sprayed twice: the first spray was applied at the 4-6 true leaf stage, and the second spray was applied 14 days later. Spraying was performed in the morning until full wetting, using a spreading agent to reduce water surface tension. Sprouted Barley Seed Extract (C): C_1: Control (sprayed with water only), C_2: Sprouted barley seed extract at 100 g L^{-1} prepared according to [6]. The extract was applied twice, the day after the amino acid foliar sprays.

Preparation of Sprouted Barley Seed Extract

One kilogram of barley seeds (Ibaa 265 variety) was obtained from the Agricultural Research Directorate / Suwaira Research Station. The seeds were washed and soaked in water for 24 h, then drained and spread in a single layer on cheesecloth in trays, kept in the dark, and misted with water as needed to maintain moisture. After 72 h, when the radicle emerged, the germinated seeds were blended with water until fully homogenized, then filtered and diluted to a final volume of 10 L. The extract was sprayed on the plants in the early morning. A separate extract from dormant barley seeds was prepared for comparison, following the same procedure but using dry seeds instead of germinated ones. Table 1 presents the chemical and physical properties and nutrient availability of both extracts [6].

Experimental Layout

The factorial experiment (3×4×2) was arranged in a split-split plot design using a Randomized Complete Block Design (RCBD) with three replications. The main plots were assigned to biofertilizer treatments. The sub-plots were assigned to amino acid foliar sprays. The sub-sub plots were assigned to sprouted barley seed extract treatments. Data analysis was conducted using GenStat V.12, and treatment means were compared using the Least Significant Difference (LSD) test at a 0.05 probability level. The field was divided into three blocks, with a 50 cm space between blocks. Each block contained 24 experimental units, with a 50 cm space between units. Each experimental unit consisted of two rows (20 cm apart); one row was used for vegetative measurements, and the other was reserved for seed and oil analysis. Ten plants per row were planted, with 20 cm spacing between plants, resulting in 20 plants per experimental unit.

Planting and Agronomic Practices

Egyptian arugula seeds were obtained from a certified agricultural supplier. A germination test was performed by

randomly selecting 100 seeds, placing them on filter paper moistened with water, and incubating them at room temperature for 48 hours. The germination rate was 98%.

The recommended N-P-K fertilizer was applied at 100-100-10 kg ha^{-1}, following the recommendations of [46]. Soil samples were collected from three locations at a 30 cm depth before planting, mixed thoroughly, and analyzed for physical and chemical properties at the Soil and Water Laboratory, Directorate of Agriculture, Al-Qadisiyah. Table 2 presents the soil analysis results.

The field was sown on October 1, 2023 (first season) and January 15, 2024 (second season). Three seeds were placed in each planting hole and covered with vermiculite. Drip irrigation was applied immediately after sowing. Thinning was performed after the emergence of true leaves, leaving one plant per hole. Weeds were manually removed throughout the growing season.

Table 1. Chemical and Physical Properties and Nutritional Conversion Factor of the Water Extract of Sprouted Barley Grains.

Trait	Unit of Measurement	Water Extract of Dormant Barley Grains	Water Extract of Sprouted Barley Grains	*Nutritional Availability Ratio (Nutrient Conversion)
pH	———	7.00	6.90	———
EC1:1	dc.cm	1.70	1.80	———
N	g L^{-1}	6.18	8.07	1.30
P	mg L^{-1}	219	222	1.01
K	mg L^{-1}	278	266	0.95
Ca	mg L^{-1}	29.5	34.1	1.15
Mg	mg L^{-1}	76.3	80.7	1.05
Fe	mg L^{-1}	2.50	6.00	2.40
Zn	mg L^{-1}	2.00	4.01	2.00
Gibberellin	µg L^{-1}	2	304	152

***The conversion ratio was calculated by dividing the values of the elements in the extract from sprouted seeds by the values of the elements in the extract from dormant seeds.**

Table 2. Physical and Chemical Properties of the Field Soil.

Soil Texture			N%	P%	K ppm	Organic Matter	EC dc.cm	pH
Silt	Clay	Sand						
50	12.5	37.5	0.021	0.351	13.1	0.42	2.43	**7.2**

Experimental Readings and Measurements

Leaf Content of Selected Bioactive Compounds

Leaf samples were collected from the first harvest to determine the bioactive compound content.

Preparation of Leaf Extract

The extract was prepared by weighing 2 g of dried and ground leaf powder following the method described by [19]. A total of 40 mL of 80% ethanol (C_2 H_6 O) was added to a 100 mL glass flask, which was then placed in a water bath at 32°C for 72 hours. The total phenolic content (mg kg^{-1} dry weight) in the leaves was determined according to the method outlined by [28]. Similarly, the total flavonoid content (mg g^{-1} dry weight) was estimated following [28]. while the total tannin content (mg kg^{-1} dry weight) was measured using the method adopted by [35].

Oil Extraction

The seeds were extracted from the siliques, cleaned of impurities, and ground into a fine powder. A 10 g sample of the powder was wrapped in filter paper and placed in a Soxhlet apparatus, where 250 mL of hexane was added to a round-bottom flask. The temperature was set to 200°F, and after running the apparatus, the yellow color observed in the round-bottom flask indicated the presence of extracted oil mixed with the solvent. Upon completion of the extraction, the oil-solvent mixture was removed, and the solvent was separated. The extracted oil was stored in a flask at room temperature to evaporate the residual solvent before weighing using a precision balance. The oil samples were then refrigerated until further analysis [22]. The percentage of oil in the dried ground leaf samples was determined following the method described by [21]. using the following equation:

Oil Percentage=(Weight of Extracted Oil (g) /Sample Weight (g))×100

Determination of Active Compounds in the Oil

Esterification of Fats

The sample preparation followed the standard method outlined [1]. which involved esterifying the fats by reacting them with methanolic potassium hydroxide. A methanolic potassium hydroxide solution was prepared by dissolving 11.2 g of potassium hydroxide in 100 mL of methanol. A 1 g fat sample was mixed with 8 mL of methanolic potassium hydroxide and 5 mL of hexane, then shaken vigorously for 30 seconds and left to separate into two layers. The upper hexane layer, containing the esterified fats, was collected for further analysis.

Gas Chromatographic Analysis

The analysis was conducted at the laboratories of the Ministry of Science and Technology / Environment and Water Directorate. Fatty acid compounds were analyzed using a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector (FID) and a capillary column (SE-30) with dimensions (30 m × 0.25 mm) following the conditions outlined by [55].

According to the following conditions:

Table 3. Conditions for Analyzing Fatty Acid Compounds Using Gas Chromatography (GC – 2010)

Paragraph Title	Temperature
Injection Area Temperature	**280 C**
Detector Temperature	**310 C**
Column Temperature	**)10 C^0/MIN (290 –120**
Gas Flow Rate	**100 Kpa**

Results

The results presented in Table (4) indicate that the application of biofertilizers, sprouted barley seed extract, and their combined treatments had a significant effect on the content of bioactive compounds in leaves and seeds, as well as the oil percentage across both growing seasons. The treatment with *Bacillus subtilis* resulted in the highest mean values for phenolics and tannins, recording 0.945, 0.890, and 1.208, 1.233 mg kg^{-1} DW for both seasons, respectively, compared to the control treatment, which exhibited the lowest values. The highest flavonoid content (34.742 mg kg^{-1} DW) was observed in the second season under this treatment. Plants treated with the amino acid Phenylalanine exhibited the highest values for phenolics, flavonoids, and tannins across both seasons, recording 0.987, 0.913, 35.705, 35.006, and 1.281, 1.334 mg kg^{-1} DW, respectively. Additionally, plants treated with sprouted barley seed extract showed significantly higher mean values of phenolics, flavonoids, and tannins, reaching 0.952, 0.890, 34.845, 35.655, and 1.134, 1.167 mg kg^{-1} DW, respectively. The same table illustrates the effect of treatments on the oil percentage. The highest oil content was observed in plants treated with *Trichoderma harzianum* in the first season (30.77%) and with *Bacillus subtilis* in the second season (24.94%). Regarding the effect of amino acids on oil percentage, plants treated with Arginine exhibited the highest values in both seasons, reaching 31.28% and 26.73%, respectively. Furthermore, *Trichoderma harzianum* significantly enhanced the fatty acid composition in arugula seeds, with Linoleic acid (19.94%) and Erucic acid (37.52%) in the first season, and 15.47% and 31.18% in the second season, respectively. Similarly, sprouted barley seed extract application led to an increase in Linoleic acid (19.99%) and Erucic acid (37.56%) in the first season and 15.48% and 31.19% in the second season, outperforming the control treatment. The results presented in Table (5), which detail the interaction effects among treatments, indicate that plants treated with *Bacillus subtilis* + Phenylalanine + Sprouted barley seed extract exhibited the highest mean values of phenolics, flavonoids, and tannins, reaching 1.033, 36.539, and 1.480 mg kg^{-1} DW in the first season, and 0.943, 35.205, and 1.543 mg kg^{-1} DW in the second season, compared to the control treatment, which recorded lower values (0.853, 0.676, 34.976 mg kg^{-1} DW in the first season and 0.821, 34.310, 0.613 mg kg^{-1} DW in the second season). Regarding oil-related parameters, the highest oil percentage was recorded in plants treated with *Trichoderma harzianum* + Arginine + Sprouted barley seed extract in the first season (33.90%) and Bacillus subtilis + Arginine + Sprouted barley seed extract in the second season (30.93%). The treatment combination of *Trichoderma harzianum* + Tryptophan + Sprouted barley seed extract exhibited a significant improvement in the fatty acid composition of arugula seeds, outperforming all other treatment interactions.

Table 4. Effects of the treatments on the content of some active compounds in arugula and the oil percentage in its seeds.

Treatment	Phenols (mg kg^{-1}DW)		Flavonoids (mg kg^{-1}DW)		Tannins (mg kg^{-1}DW)		Oil%		Lenolic%		Erucic%	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
A1	0.889	0.873	35.272	34.695	0.797	0.822	26.27	20.33	18.32	14.05	36.06	29.83
A2	0.941	0.876	35.608	34.733	1.161	1.194	30.77	24.11	19.94	15.47	37.52	31.18
A3	0.945	0.890	35.565	34.742	1.208	1.233	29.02	24.94	19.33	15.13	36.95	30.82
L.S.D (A)	0.002	N.S	0.038	N.S	0.027	0.048	0.142	0.229	0.013	0.007	0.016	0.027
B1	0.900	0.863	35.331	34.483	0.816	0.820	26.15	18.21	18.43	14.23	36.10	29.95
B2	0.901	0.867	35.438	34.569	1.055	1.070	31.28	26.73	19.61	15.17	37.23	30.88
B3	0.913	0.875	35.452	34.836	1.068	1.109	28.90	22.63	19.25	14.76	36.90	30.52
B4	0.987	0.913	35.705	35.006	1.281	1.334	28.43	24.93	19.51	15.37	37.15	31.09
L.S.D (B)	0.003	0.004	0.034	0.022	0.017	0.024	0.130	0.203	0.018	0.012	0.017	0.011
C1	0.898	0.870	35.308	34.602	0.976	0.999	27.44	22.35	18.41	14.29	36.13	30.03
C2	0.952	0.890	35.655	34.845	1.134	1.167	29.94	23.91	19.99	15.48	37.56	31.19
L.S.D (C)	0.001	0.003	0.021	0.018	0.013	0.020	0.113	0.100	0.012	0.006	0.012	0.006
A1B1	0.877	0.852	35.210	34.386	0.712	0.700	23.76	15.90	17.77	13.70	35.51	29.49
A1B2	0.907	0.849	35.197	34.566	0.770	0.768	28.55	24.16	18.21	14.00	35.93	29.73
A1B3	0.854	0.886	35.304	34.803	0.781	0.811	25.60	18.38	18.35	13.82	36.08	29.67
A1B4	0.918	0.904	35.378	35.025	0.923	1.009	27.18	22.90	18.96	14.69	36.72	30.43
A2B1	0.879	0.883	35.315	34.497	0.924	0.906	27.13	19.81	18.94	14.63	36.55	30.33
A2B2	0.912	0.846	35.840	34.598	1.152	1.223	33.11	26.45	20.48	15.92	38.08	31.62
A2B3	0.958	0.874	35.684	34.938	1.069	1.141	29.70	25.10	20.13	15.61	37.70	31.33
A2B4	1.016	0.904	35.593	34.898	1.497	1.506	33.16	25.08	20.23	15.72	37.78	31.45
A3B1	0.944	0.855	35.468	34.565	0.812	0.853	27.55	18.93	18.58	14.36	36.26	30.04
A3B2	0.88	0.90	35.27	34.54	1.243	1.21	32.1	29.58	20.14	15.60	37.6	31.2

	3	7	9	2		9	8				8	9
A3B3	0.927	0.866	35.367	34.768	1.352	1.375	31.41	24.43	19.27	14.86	36.93	30.57
A3B4	1.027	0.932	36.146	35.095	1.424	1.488	24.95	26.83	19.33	15.71	36.94	31.37
L.S.D (A.B)	0.004	0.008	0.058	0.037	0.033	0.053	0.221	0.347	0.028	0.019	0.028	0.028
B1C1	0.856	0.842	35.143	34.379	0.732	0.715	25.64	17.55	17.79	13.70	35.54	29.44
B1C2	0.944	0.884	35.518	34.586	0.900	0.924	26.65	18.87	19.07	14.76	36.67	30.47
B2C1	0.864	0.859	35.294	34.401	0.988	0.972	29.62	25.48	19.00	14.68	36.70	30.41
B2C2	0.938	0.876	35.582	34.736	1.122	1.169	32.94	27.97	20.22	15.65	37.76	31.35
B3C1	0.884	0.873	35.304	34.723	0.968	1.016	27.40	21.86	18.25	14.08	35.97	29.83
B3C2	0.942	0.877	35.599	34.949	1.167	1.202	30.41	23.41	20.26	15.45	37.83	31.22
B4C1	0.988	0.905	35.492	34.903	1.215	1.295	27.10	24.48	18.61	14.69	36.31	30.46
B4C2	0.986	0.921	35.919	35.109	1.347	1.374	29.76	25.38	20.41	16.06	37.98	31.71
L.S.D (B.C)	0.003	0.006	0.044	0.033	0.025	0.036	0.200	0.241	0.024	0.015	0.023	0.013
A1C1	0.874	0.863	35.104	34.535	0.732	0.718	23.80	19.48	17.47	13.47	35.33	29.30
A1C2	0.903	0.883	35.440	34.855	0.890	0.926	28.74	21.19	19.17	14.63	36.79	30.35
A2C1	0.895	0.861	35.450	34.633	1.072	1.100	30.10	23.16	19.16	14.85	36.82	30.55
A2C2	0.987	0.891	35.765	34.833	1.249	1.288	31.45	25.05	20.73	16.09	38.23	31.81
A3C1	0.924	0.885	35.371	34.637	1.153	1.179	28.40	24.40	18.60	14.54	36.25	30.24
A3C2	0.967	0.895	35.759	34.848	1.253	1.287	29.64	25.49	20.07	15.72	37.66	31.39
L.S.D (A.C)	0.002	0.006	N.S	0.029	0.028	0.048	0.176	0.231	0.018	0.009	0.019	0.027

A2**:** *Trichoderma harzianum* $2*10^9$ CFU mL^{-1}**,**A3**:** *bacillus subtilis* $2.25*10^9$ CFU mL^{-1}**,**B2**:** Arginine **150mgl^{-1}** B3**:** Tryptophan **150mgl^{-1}** ,B4: Phenylalanine **150mgl^{-1}** C2: Sprouted barley grain extract at a concentration of 100 gl^{-1},A1,B1,C1 Without addition, **N.S**: Non-significant, S1: first season , S2: second season.

Table 5. Effects of the triple interactions of the treatments on the content of some active compounds in arugula and the oil percentage in its seeds.

Treatment (A.B.C)	Phenols (mg kg^{-1}DW)		Flavonoids (mg kg^{-1}DW)		Tannins (mg kg^{-1}DW)		Oil %		Lenolic%		Erucic%	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
A1B1C1	0.853	0.821	34.976	34.310	0.676	0.613	23.43	15.30	17.19	13.28	35.11	29.18
A1B1C2	0.900	0.882	35.445	34.462	0.748	0.786	24.10	16.50	18.36	14.13	35.92	29.80
A1B2C1	0.868	0.840	34.910	34.322	0.676	0.637	25.50	23.03	17.75	13.66	35.55	29.43
A1B2C2	0.946	0.857	35.484	34.810	0.863	0.899	31.60	25.30	18.67	14.33	36.31	30.03
A1B3C1	0.840	0.885	35.220	34.596	0.632	0.653	22.10	17.20	17.39	13.40	35.24	29.25
A1B3C2	0.867	0.887	35.388	35.011	0.930	0.969	29.10	19.56	19.32	14.23	36.92	30.09
A1B4C1	0.936	0.904	35.309	34.912	0.826	0.969	24.20	22.40	17.56	13.54	35.42	29.36
A1B4C2	0.899	0.904	35.446	35.137	1.019	1.048	30.16	23.40	20.36	15.85	38.03	31.50
A2B1C1	0.842	0.848	35.097	34.417	0.761	0.713	26.26	18.80	18.18	14.02	35.81	29.64
A2B1C2	0.915	0.917	35.532	34.578	1.087	1.099	28.00	20.83	19.69	15.25	37.28	31.02
A2B2C1	0.844	0.838	35.778	34.434	1.099	1.159	32.33	25.20	20.16	15.65	37.87	31.36
A2B2C2	0.980	0.853	35.902	34.762	1.205	1.288	33.90	27.70	20.80	16.18	38.30	31.88
A2B3C1	0.889	0.869	35.514	34.868	0.976	1.046	29.13	24.20	18.82	14.61	36.48	30.33
A2B3C2	1.028	0.878	35.854	35.007	1.162	1.236	30.26	26.00	21.44	16.61	38.91	32.33
A2B4C1	1.006	0.891	35.413	34.811	1.451	1.482	32.70	24.46	19.47	15.13	37.10	30.89
A2B4C2	1.026	0.916	35.773	34.985	1.543	1.530	33.63	25.70	20.99	16.31	38.45	32.01
A3B1C1	0.872	0.856	35.357	34.411	0.760	0.817	27.23	18.56	17.99	13.81	35.70	29.50
A3B1C2	1.016	0.854	35.579	34.719	0.865	0.888	27.86	19.30	19.18	14.91	36.81	30.58
A3B2C1	0.878	0.898	35.196	34.447	1.187	1.119	31.03	28.23	19.08	14.74	36.69	30.44
A3B2C2	0.888	0.917	35.362	34.637	1.299	1.318	33.33	30.93	21.21	16.45	38.67	32.14
A3B3C1	0.924	0.866	35.178	34.706	1.296	1.349	30.96	24.20	18.53	14.22	36.18	29.90
A3B3C2	0.93	0.86	35.55	34.82	1.40	1.400	31.8	24.66	20.02	15.50	37.6	31.2

	1	6	6	9	9		6				8	3
A3B4C1	1.02	0.92	35.75	34.98	1.36	1.432	24.4	26.60	18.79	15.40	36.4	31.1
	2	1	3	5	8		0				2	4
A3B4C2	1.03	0.94	36.53	35.20	1.48	1.543	25.5	27.06	19.87	16.02	37.4	31.6
	3	3	9	5	0		0				7	1
L.S.D	0.00	0.01	0.075	0.075	0.04	0.070	0.34	0.414	0.040	0.024	0.04	0.03
(A.B.C)	6	0			6		5				0	1

A2**:** *Trichoderma harzianum* $2*10^9$ CFU mL^{-1}**,**A3**:** *bacillus subtilis* $2.25*10^9$ CFU mL^{-1}**,**B2**:** Arginine **150mgl^{-1}** B3**:** Tryptophan **150mgl^{-1}** ,B4: Phenylalanine **150mgl^{-1}** C2: Sprouted barley grain extract at a concentration of 100 gl^{-1},A1,B1,C1 Without addition, N.S: Non-significant, S1: first season , S2: second season.

Discussion

The results presented in Table 4 highlight the superiority of the individual and combined treatments in the bioactive compound content of arugula. This superiority can be attributed to the role of biofertilizers, which enhance soil microorganisms' ability to mobilize unavailable nutrients in the soil and promote plant growth, making them essential for sustainable agriculture [24]. The plant growth-promoting rhizobacteria (PGPR) yielded positive results for sustainable agriculture through processes such as plant hormone synthesis, nitrogen fixation, phosphate solubilization, and biological control of plant diseases. These processes likely contributed to the increased production of phenolics, flavonoids, tannins, oil, and bioactive compounds [23,25]. by producing plant hormones like auxins and cytokinins, which contribute to plant growth and development [56,34]. These hormones work synergistically to activate comprehensive plant growth and stimulate the formation of bioactive compounds and oil. The joint stimulation of auxins and cytokinins can improve lateral and adventitious root branching and increase the absorption of nutrients, thus activating metabolic [43]. directly enhancing the quality of arugula seed oil. PGPR bacteria play a vital role in enhancing plant growth through various mechanisms, including the production of siderophores (molecules that help plants absorb iron) [14,18]. These siderophores assist enzymes involved in the conversion of Acetyl-CoA to Malonyl-CoA, a crucial step in the fatty acid biosynthesis pathway, as demonstrated by [53]. Regarding the foliar application of amino acids, their significant effect was evident, with Phenylalanine contributing to an increased content of phenolics, flavonoids, and tannins. This is likely due to its role in enhancing photosynthesis and the production of primary metabolites, which are subsequently converted into secondary metabolites through enzymatic pathways. Phenylalanine's involvement in secondary metabolism, particularly in the production of phenolic compounds and flavonoids via the phenylpropanoid pathway, involves the enzyme phenylalanine ammonia-lyase (PAL). This enzyme deaminates phenylalanine into cinnamic acid, which is then converted into coumaric acid, the precursor for phenolic compounds [51,39,37].The effect of Arginine, a precursor for polyamine synthesis, was also significant. Polyamines are vital for nitrogen-carbon balance within the cell, which is crucial for fatty acid biosynthesis and oil content in seeds, as shown by [41] in sunflower seeds, and [2] in maize, and [54] in canola. Tryptophan's effect was also notable in increasing fatty acid content due to its nitrogen content, which the plant directly absorbs during foliar application. This, in turn, stimulates

root growth and improves nutrient uptake, which enhances carbon assimilation, ultimately increasing fatty acid production. The foliar application of sprouted barley extract contributed positively due to its high gibberellin content, known to promote cell division and elongation, resulting in larger tissues containing oil, such as seeds. This increase in biomass may contribute to higher oil production [13]. Furthermore, the extract also stimulates metabolic processes, including fatty acid biosynthesis, and increases the activity of enzymes responsible for fatty acid production, the primary component of oils [17]. The extract contains soluble nutrients that are easy for the plant to absorb, including nitrogen in the form of amino acids and phosphates, which are readily available to the plant, as shown in Table 1. This synergy of nutrients enhances the plant's biological activities and increases the production of bioactive compounds [6]. The first season's results were better than those of the second season, likely due to more favorable environmental conditions for physiological processes, especially photosynthesis, which led to increased bioactive compounds[49]. found similar variations in fatty acid content due to different growing periods in arugula.

Conclusion:

The fertilization program used in this study showed promising results, encouraging the use of eco-friendly resources for clean agriculture and cost reduction. The treatments applied showed statistically significant differences, particularly in fertilization, which opens up new prospects for sustainable agriculture in Iraq.

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