



THE COMBINED EFFECT OF BIOSTIMULANTS AND ANTIOXIDANTS ON THE VEGETATIVE, YIELD CHARACTERISTICS AND ITS COMPONENTS OF SOYBEAN

Sura A. Al -Fahdawi¹ *, Sala B. Ismael Mustafa²

¹Department of Field Crops, College of Agricultural Engineering Sciences, University of Baghdad, Iraq. sura.abd2106m@coagri.uobaghdad.edu.iq

²Assistant Professor PhD., Department of Field Crops, College of Agricultural Engineering Sciences, University of Baghdad, Iraq. sala.b@coagri.uobaghdad.edu.iq

Received 26/ 4/ 2023, Accepted 22/ 6/ 2023, Published 30/ 6/ 2024

This work is licensed under a CCBY 4.0 <https://creativecommons.org/licenses/by/4.0>



ABSTRACT

A field experiment was carried out for the year 2022 at Experimental Station A of the College of Agricultural Engineering Sciences/ University of Baghdad/ Al-Jadriyah, located at latitude 33°N and longitude 44°E. The aim of the experiment was to determine the appropriate amount of bio-stimulants and antioxidants to produce the best chemical content of oil with a good protein ratio compared to seed and oil productivity, antioxidant compounds, and oxidative efficiency of soybean (*Glycine max* L.) seeds. The experiment was conducted according in a factorial arrangement the randomized complete block design (RCBD) for two factors and their interactions with three replication. The first factor included three levels of biostimulants: no spray, humic acid, and folic acid at a concentration of 2 g L⁻¹ for each. The second factor included three levels of antioxidants: no spray, ascorbic acid, and glutathione acid at a concentration of 100 mg L⁻¹ for each. Spraying was applied on vegetative system, first was one month after planting, the second after one month of the first spraying at the formation of branches stage, and the third at the 50% flowering stage. The results showed that bio stimulants had a significant effect on most growth traits, with the treatment of 2 g L⁻¹ humic acid achieving the best results in growth traits, height plant (180.47cm), leaf area (104.15 dm⁻¹), number of branches(12.44 branch plant⁻¹), fresh weight (450.16 g), dry weight (308.03 g). while the treatment of 2 g L⁻¹ folic acid achieved the best results in seed yield (289.10)g and total yield(3.08) g . Antioxidants also had an effect on most growth and yield traits, with the spray of 100 mg L⁻¹ ascorbic acid achieving a significant increase in leaf area(98.01 dm²), while the treatment of 100 mg L⁻¹ glutathione acid outperformed significantly in fresh weight (514.46 g) , dry weight (284.56 g), plant seed yield(307.50 g plan⁻¹), 100 seed weight(19.47 g) , total yield(3.28 t ha⁻¹), and oil yield (0.68 t ha⁻¹). The combined effect of the study factors was significant in most growth and yield traits, but the treatment of 2 g L⁻¹humic acid and 100 mg L⁻¹ascorbic acid achieved a significant increase in plant height (148.53 cm), leaf area (110.95 dm⁻¹). While the treatment of 2 g L⁻¹ humic acid and 100 mg L⁻¹ Glutathione acid outperformed significantly in the number of branches (13.00 branch plant⁻¹), seed yield in the plant (337.80 g), total yield (3.60 g) , and oil yield (0.99 t h⁻¹). The combined effect of the treatment of 2 g L⁻¹ folic acid and 100 mg L⁻¹ Glutathione acid produced a significant increase in fresh weight (562.10 g) and 100 seed weight (22.00 g).

Keywords: Bio-Stimulants, antioxidant yield, yield components, soybean. Top of Form

*Research extracted from a master's thesis by the first researcher.

التأثير المشترك للمحفزات الحيوية ومضادات الاكسدة في الصفات الخضرية والحاصل ومكوناته لفول الصويا

سرى عبد الغفور الفهداوي¹، سلا باسم اسماعيل مصطفى²قسم المحاصيل الحقلية، كلية علوم الهندسة الزراعية، جامعة بغداد، العراق، sura.abd2106m@coagri.uobaghdad.edu.iq
الاستاذ المساعد الدكتور، قسم المحاصيل الحقلية، كلية علوم الهندسة الزراعية، جامعة بغداد، العراق. sala.b@coagri.uobaghdad.edu.iq

الخلاصة

نفذت تجربة حقلية لعام ٢٠٢٢ في محطة التجارب A التابعة لكلية علوم الهندسة الزراعية/ جامعة بغداد/ الجادرية الواقعة عند خط عرض ٣٣° شمالاً وخط طول ٤٤° شرقاً بهدف تحديد كمية المحفزات الحيوية ومضادات الاكسدة المناسبة لإنتاج أفضل محتوى كيميائي من الزيت مع نسبة جيدة من البروتين بالمقارنة مع إنتاجية البذور والزيت والمركبات المضادة للاكسدة والفعالية التأكسدية لبذور فول الصويا *Glycine max L.* طبقت التجربة وفق ترتيب التجارب العاملية ويتصميم القطاعات العشوائية الكاملة RCBD لعاملين وتداخلاتها وبثلاث مكررات ، شمل العامل الأول ثلاث مستويات من المحفزات الحيوية هي بدون رش و Humic acid و Folic acid بتركيز ٢ غم لتر⁻¹ لكل منهما، والعامل الثاني ثلاث مستويات من مضادات الاكسدة هي بدون رش و Ascorbic acid و Glutathion acid بتركيز ١٠٠ ملغم لتر⁻¹ لكل منهما استخدمت رشاً على المجموع الخضري الأولي بعد شهر من الزراعة والثانية بعد شهر من الرشة الأولى مرحلة نشوء وتكون الافرع والثالثة مرحلة ٥٠ % تزهير. أظهرت النتائج ان المحفزات الحيوية اثرت معنوياً في اغلب صفات النمو وقد حققت المعاملة ٢ غم لتر⁻¹ Humic acid افضل النتائج في مؤشرات النمو، ارتفاع النبات (١٨٠,٤٧ سم) المساحة الورقية (١٠٤,١٥ دسم^٢)، عدد الافرع (١٢,٤٤ فرع نبات⁻¹)، الوزن الرطب (٤٥٠,١٦ غم)، والوزن الجاف (٢٠٨,٠٣ غم) الا ان ٢ غم لتر⁻¹ Folic acid سجل افضل النتائج في حاصل البذور في النبات (٢٨٩,١٠ غم) والحاصل الكلي (٣,٠٨ طن هـ⁻¹)، كما اثرت مضادات الاكسدة في معظم صفات النمو والحاصل فقد حقق الرش ١٠٠ ملغم لتر⁻¹ Ascorbic acid زيادة معنوية في صفة المساحة الورقية (٩٨,٠١ دسم^٢) بينما تفوقت المعاملة ١٠٠ ملغم لتر⁻¹ Glutathion acid معنوياً في صفة الوزن الرطب (٥١٤,٤٦ غم)، الوزن الجاف (٢٨٤,٥٦ غم) ، حاصل البذور في النبات (٣٠٧,٥٠ غم، وزن ١٠٠ بذرة (١٩,٤٧ غم) ،الحاصل الكلي (٣,٢٨ طن هـ⁻¹) وحاصل الزيت (٠,٨٦ طن هـ⁻¹). كان التأثير المشترك لعوامل الدراسة معنوياً في اغلب صفات النمو والحاصل، إلا ان المعاملة ٢ غم لتر⁻¹ Humic acid و ١٠٠ ملغم لتر⁻¹ Ascorbic acid زيادة معنوية في صفة ارتفاع النبات (١٨٤,٥٣ سم) والمساحة الورقية (١١٠,٩٥ دسم^٢). بينما تفوقت المعاملة ٢ غم لتر⁻¹ Humic acid و ١٠٠ ملغم لتر⁻¹ Glutathion acid وسجلت اعلى النتائج في عدد الافرع (13.00 فرع نبات⁻¹)، حاصل البذور في النبات (337.80 غم)، الحاصل الكلي (٣,٢٨ طن هـ⁻¹) وحاصل الزيت (٠,٩٩ طن هـ⁻¹). وأنتجت معاملة التأثير المشترك ٢ غم لتر⁻¹ Folic acid و ١٠٠ ملغم لتر⁻¹ Glutathion acid زيادة معنوية في صفة الوزن الرطب (٥٦٢,١٠ غم) ووزن ١٠٠ بذرة (٢٢,٠٠ غم).

الكلمات المفتاحية: المحفزات الحيوية، مضادات الاكسدة، الحاصل، مكونات الحاصل، فول الصويا.

INTRODUCTION

Soybean (*Glycine max L.*) is considered one of the most important oil crops used in the food and pharmaceutical industries worldwide, as well as in the dye industry (Al-Karawi, 2022). It belongs to the fabaceae family and is distinguished from other legume species by containing all eight essential amino acids necessary for the human body to produce protein and oil. This makes it an excellent source of complete plant protein, with a content of no less than 37%. Soybean seeds also contain oil with a value that exceeds 27%, as well as sugars, saponins, and sterols. The fatty acids are the active essence of the plant, as crude soybean oil contains oleic acid, linoleic acid, and lenolenic acid, which give soybean oil greater stability, making it an antioxidant. It also contains palmitic acid, stearic acid, myristic acid, arachidonic acid, tocopherol, delta-tocopherol, and alpha-tocopherol, which work alone or together to reduce triglyceride levels in the body and lower blood sugar levels. It is considered a primary building block for muscles, bones, and nerves, and is a powerful stimulant and restorative for



the body, achieving balance between cells (**Arab Organization for Agricultural Development, 2014**).

All of these vital compounds for plants and humans can be produced by this medicinal oil plant in economical quantities. However, their concentrations in the soybean crop can be affected by the levels of organic acids and their different types, in addition to vitamins, which represent the basic structures in the secondary plant metabolism, energy compounds, enzymes and their accompaniments, and their companions. Therefore, it is a major and determinant factor in the growth and development of the crop, its transition from one stage to another until completing its life cycle and producing the seed yield. It is important to determine the appropriate amount of bio-stimulants and antioxidants for optimal production. For example, bio-stimulants are encouraged in plant growth because they are carbon-based substances or compounds that build plant tissues, and they work within mechanisms that reducing the harmful biotic and abiotic stress to which the plant is exposed to during its growth period (**Saheed & Darwesh, 2021 & Mustafa, 2022**). Spraying antioxidants on plants plays a major role in stimulating physiological and vital processes, producing oil and proteins, and manufacturing carbohydrates by building chlorophyll and stimulating the process of photosynthetic and amino acid metabolism, which contributes to the formation of proteins and other components, such as the aforementioned compounds that this crop is famous for. In combination with bio-stimulants, the production of antioxidant compounds is encouraged, including glutathione (**Conklin & Barth, 2004**). Ascorbic acid is known for its ability to revive its production, and a group of vitamins including thiamine (vit. B1), biotin (vit. H), lipoic acid, and the enzymatic co-factors coenzyme A (**Barth et al., 2006**), as well as compounds like thioredoxins and sulfolipids that play an important role in the plant's resistance to pests and diseases (**Suleiman, 2017**), These factors, with their individual or interacting effects, reflect on the effectiveness of the plant's biological defense system (as they work within antioxidant mechanisms), which depends entirely on the metabolism of active compounds and their production levels. At the same time, the level of antioxidant effectiveness is determined to resist biotic stress (pest infestation) and abiotic stress (unfavorable environmental conditions) that can cause damage or disturbances in the plant cell, such as damage to DNA and RNA nucleic acids or ribosomes, resulting in the production of ineffective proteins or enzymes or damage to the cell membrane, which loses its selectivity and eventually dies.

MATERIALS AND METHODS

The field experiment was conducted at the research station A of the College of Agricultural Engineering Sciences / University of Baghdad in a sandy soil with the aim of (knowing the joint effect of bio stimulants and antioxidants on the vegetative traits, yield and its components of soybean plant) Shimaa cultivar. The first factor included biostimulants: no spray, humic acid, and folic acid at a concentration of 2 g L⁻¹ for each. The second factor included three levels of antioxidants without spraying, Ascorbic acid and Glutathion acid at a concentration of 100 mg L⁻¹ each. Field experiment was conducted at Research Station A, of the College of Agricultural Engineering Sciences / University of Baghdad, in sandy loam soil with the aim of determining the combined effect of bio-stimulants and antioxidants on the vegetative traits, yield, and yield components of Shimaa soybean variety.

The experimental field plowed twice perpendicular to each other and divided it into 27 experimental units, each consisting of 4 rows with a spacing of 0.75 cm between rows and 0.25

cm between plants, resulting in a plant density of 53,333 plants ha⁻¹. Superphosphate fertilizer was added at a rate of 80 kg ha⁻¹ of triple superphosphate P₂O₅ in one application before planting, while urea fertilizer at a rate of 160 kg ha⁻¹ of 46% N was added in two applications, the first at the vegetative stage and the second at the beginning of the flowering stage (Ali, 2012). Crop management practices including irrigation and weeding were carried out as needed, and the crop was harvested at maturity. Statistical analysis was performed using the Genstat software, and the least significant difference (LSD) test was used to compare means at a probability level of 0.05 Steel & Torri (1980)

The studied traits are:

- 1- Plant height (cm): The average of five plants was calculated using the metric tape from the soil surface to the top of the plant
- 2- Number of branches per plant.: According to the average number of branches on the main stem and the bearer of the pods
- 3- Leaf area (dm² plant⁻¹): LA = 0.624 + (0.723) (L .W) (Wiersma & Bailey, 1975)
- 4- Fresh weight of the plant (g) :Using the sensitive scale for five plants and calculating their average
- 5- Dry weight of the plant (g) .:Using the sensitive scale of five dried plants and calculating their average
- 6- Seed yield per plant (g):. The seeds of five plants were weighed and averaged
- 7- 100-seed weight (g):. After mixing the seeds, I weighed and weighed 100 seeds using the sensitive scale and calculated their average
- 8- Total seed yield (t ha⁻¹):. Five plants were randomly harvested from the two central markers of the experimental unit, weighed, their average extracted, and converted from plant⁻¹ gm to ton hectare by multiplying them by the plant density and dividing the result by 10⁶.
- 9- Plant oil yield (t ha⁻¹): According to the oil yield × total seed yield

RESULTS AND DISCUSSION

1. Plant height

The results indicate that plants treated with biological stimulants significantly outperformed untreated plants in terms of plant height, specifically with 2 g L⁻¹ Humic acid treatment mean at 180.47 cm compared to untreated plants averaging of 164.46 cm, while 2 g L⁻¹ of Folic acid treatment mean at 167.78 cm (Table 1). The results showed that the antioxidant concentrations of 100 mg L⁻¹ Glutathione acid and 100 mg L⁻¹ Ascorbic acid did not differ significantly from untreated plants, as the latter achieved the highest mean plant height at 173.91 cm. Regarding the combined effect of the study factors, the results revealed that plant height was significantly affected by the 2 g L⁻¹ Humic acid treatment with both untreated and treated plants, as well as by 100 mg L⁻¹ Ascorbic acid with an increase of 11.47% and 10.27%, respectively, compared to untreated plants. The latter also achieved an increase of 7.60% with 100 mg L⁻¹ Ascorbic acid. This is attributed to the effective role of Humic acid in increasing membrane permeability and nutrient transfer, which helps activate the serine with indole ring to form tryptophan, which is the source of the hormone auxin (IAA) that leads to cell division and elongation, thus increasing plant height (Abid Al-Ameen , 2010). This is consistent with the findings of Abdul Aziz *et al.*, (2018) & (Mahmood and Zeboon(2019),

&Abdul Qadir *et al.*, (2022) on the role of Ascorbic acid in activating photosynthesis and increasing cell division and expansion, as concluded by Al-Aboudi *et al.*, (2016).

Table (1): The combined effect of biostimulants and antioxidants and the interaction between them on plant height (cm) of Soybean (2022)

Bio stimulants g L ⁻¹	Antioxidants mg L ⁻¹			Mean Bio stimulants
	Control	Ascorbic acid 100 mg	Glutathion acid 100 mg	
Con.	167.33	155.50	171.11	164.64
Humic acid 2gm	186.53	184.53	170.37	180.47
Folic acid 2gm	167.87	168.40	167.00	167.78
LSD _{0.05}	8.11			4.68
Mean antioxidants	173.91	169.5	169.49	
LSD _{0.05}	N.S			

2. Leaf area (dm²)

The results indicate that plants treated with 2 g L⁻¹ Humic acid achieved the highest mean leaf area of 104.15dm² (Table 2), while 2 g L⁻¹ of Folic acid treatment mean at 88.44 dm², compared to untreated plants which had the lowest mean of 86.97 dm². The same table shows that plants treated with 100 mg L⁻¹Ascorbic acid had a significant difference in mean leaf area of 98.01 dm², followed by plants treated with 100 mg L⁻¹Glutathione acid with mean of 92.24 dm², compared to untreated plants with mean of 89.31 dm². Regarding the combined effect of the study factors, Table 2 revealed that plants sprayed with 2 g L⁻¹ Humic acid and 100 mg L⁻¹ Ascorbic acid achieved a significant increase of 30.18% in leaf area compared to untreated plants. Plants treated with 2 g L⁻¹Humic acid with distilled water also achieved a significant increase of 27.16% compared to untreated plants. This is attributed to the effective role of Humic acid in the biological processes involved in photosynthesis, respiration, and the plant's ability to utilize solar energy, which positively influenced the increase in green biomass, including leaf area Danta (2007), consistent with the findings of Abdul Qader *et al.* (2022). Ascorbic acid also works to stimulate cell division and expansion, as well as protect chloroplasts from oxidation Al-Alaf (2017). This is consistent with what was found by Alak and Al-Sabagh, (2020) .

Table (2): The combined effect of bio stimulants and antioxidants and the interaction between them on leaf area (dm²) of Soybean (2022)

Bio stimulants g L ⁻¹	Antioxidants mg L ⁻¹			Mean Bio stimulants
	Control	Ascorbic acid 100 mg	Glutathion acid 100 mg	
Con.	85.23	85.49	90.21	86.97
Humic acid 2gm	108.38	110.95	93.13	104.15
Folic acid 2gm	74.34	97.59	93.39	88.44
LSD _{0.05}	5.94			3.43
Mean antioxidants	89.31	98.01	92.24	
LSD _{0.05}	3.433			

3. Number of branches (branch plant⁻¹)

Results show that plants treated with 2 g L⁻¹ Humic acid achieved the highest mean number of branches with 12.44 branch plant⁻¹ (Table 3), while the 2 g L⁻¹ of Folic acid treatment mean at 10.67 branch/plant⁻¹, compared to untreated plants which had the lowest mean of 9.78 branch plant⁻¹. The results indicates that the general behavior of antioxidant-treated plants differed significantly. Plants treated with 100 mg L⁻¹ Glutathione acid had the highest mean of 11.22 branch plant⁻¹ compared to untreated plants with an mean of 11.34 branch plant⁻¹, while plants treated with 100 mg L⁻¹ Ascorbic acid had the lowest mean of 10.33 branch plant⁻¹. Regarding the combined effect of the study factors, the same table revealed that treatment with 2 g L⁻¹ Humic acid with distilled water and 2 g L⁻¹ Humic acid with 100 mg L⁻¹ Glutathione acid with distilled water achieved an increase of 62.36% and 59.90%, respectively, compared to untreated plants. This is attributed to the efficient distribution of products of photosynthesis between different plant parts, which plays a major role in increasing cytokinin's that counteract auxins, leading to the differentiation of the vascular connection area between lateral buds and stem and the growth of more vegetative branches. Additionally, Glutathione acid plays a role in the process of cell division and differentiation in flowers **Noctor et al., (2011)** This is consistent with what was found by **Al-Hasani (2018)**.

Table (3): The combined effect of bio stimulants and antioxidants and the interaction between them on Number of branches (branch plant⁻¹) of Soybean(2022)

Bio stimulants g L ⁻¹	Antioxidants mg L ⁻¹			Mean Bio stimulants
	Control	Ascorbic acid 100 mg	Glutathion acid 100 mg	
Con.	8.13	10.11	11.10	9.78
Humic acid 2gm	13.20	11.13	13.00	12.44
Folic acid 2gm	12.33	9.76	9.93	10.67
LSD _{0.05}	1.361			0.78
Mean antioxidants	11.22	10.33	11.34	
LSD _{0.05}	0.78			

4. Plant fresh weight (g)

The results indicate that the bio-stimulants achieved significant differences in the fresh weight of the plant (Table 4). The treatment with 2 g L⁻¹ Humic acid and the treatment with 2 g L⁻¹ Folic acid recorded the highest mean for the trait, reaching 450.16 g and 446.50 g, respectively, while the untreated plants recorded the lowest mean of 366.93 g. As for the effect of antioxidants, Table 4 shows that the treatment with 100 mg L⁻¹ Glutathione acid achieved the highest mean of 514.46 g, followed by the treatment with 100 mg L⁻¹ Ascorbic acid with an mean of 417.43 g, compared to the untreated plants which recorded the lowest mean of 331.70 g. The combined treatment showed significant differences, especially the treatment with 2 g L⁻¹ Folic acid with 100 mg L⁻¹ Glutathione acid, and the treatment with 2 g L⁻¹ Humic acid with 100 mg L⁻¹ Glutathione acid, with an increase of 81.91% and 80.58%, respectively compared to the untreated plants. The reason for the increase is attributed to the role of Humic acid as a biological enhancer, which increased the plant's ability to efficiently carry out carbon metabolism and absorb water and nutrients, which reflected an increase in plant size and thus an increase in fresh weight (**Ferrara & Brunetli, 2010**). The positive role of Glutathione acid

and its cycle in overall biological processes within the plant, including increasing the rate of cell division, also contributed to the increase in fresh weight **Noctor, 2011**).

Table (4): The combined effect of bio stimulants and antioxidants and the interaction between them on plant fresh weight (g) of Soybean(2022)

Bio stimulants g L ⁻¹	Antioxidants mg L ⁻¹			Mean Bio stimulants
	Control	Ascorbic acid 100 mg	Glutathion acid 100 mg	
Con.	309.00	368.60	423.20	366.93
Humic acid 2gm	385.80	406.60	558.10	450.16
Folic acid 2gm	300.30	477.10	562.10	446.50
LSD _{0.05}	30.86			0.78
Mean antioxidants	331.70	417.43	514.46	
LSD _{0.05}	17.82			

5- Dry weight (g)

The results shows that the biostimulants have significantly increased the dry weight of the plants (Table, 5), particularly the treatment with 2 g L⁻¹ Humic acid which recorded the highest mean of 308.03 g compared to the untreated plants which had the lowest mean of 234.63 g. The plants treated with 2 g L⁻¹ Folic acid also recorded a significantly higher mean of 249.30g compared to the untreated plants. The results also indicate that the antioxidants have significantly increased the dry weight, with the treatment of 100 mg L⁻¹ Glutathione acid recording the highest mean of 284.56 g, followed by the treatment of 100 mg L⁻¹ Ascorbic acid with an mean of 262.36 g, while the untreated plants recorded the lowest mean of 245.03 g.

Regarding the combined effect of the study factors, it was observed from the same table that the plants treated with 2 g L⁻¹ Humic acid with distilled water and the plants treated with 2 g L⁻¹ Humic acid with 100 mg L⁻¹ Ascorbic acid achieved an increase percentage of 61.26% and 59.95%, respectively, compared to the untreated plants. The increase in dry weight can be attributed to the positive effect of the biostimulants on the growth indicators, including plant height, leaf area, and number of branches as shown in Tables (1), (2), and (3), which positively affected the dry weight of the plants. This is consistent with the findings of **Baqer & Zboun, (2019)** in their study on the response of wheat to foliar spraying with humic acid.

Table (5): The combined effect of bio stimulants and antioxidants and the interaction between them on plant dry weight (g) of Soybean(2022)

Bio stimulants g L ⁻¹	Antioxidants mg L ⁻¹			Mean Bio stimulants
	Control	Ascorbic acid 100 mg	Glutathion acid 100 mg	
Con.	199.00	234.00	270.90	234.63
Humic acid 2gm	320.90	318.30	284.90	308.03
Folic acid 2gm	215.20	234.80	297.90	249.30
LSD _{0.05}	49.96			28.84
Mean antioxidants	245.03	262.36	284.56	
LSD _{0.05}	28.84			

6- Seed yield per plant (g).

The results indicate a significant effect of the biological stimulants on the seed yield of the plants (Table 6). Treatment with 2 g L⁻¹ Folic acid produced the highest mean seed yield of 289.10 g plant⁻¹, while treatment with 2 g L⁻¹ Humic acid resulted in an mean of 272.03 g plant⁻¹. The control treatment had the lowest mean seed yield of 196.33 g plant⁻¹. The same table also shows that treatment with antioxidants, especially 100 mg L⁻¹ Glutathion acid, significantly outperformed the control group with an mean seed yield of 307.50 g plant⁻¹. Treatment with 100 mg L⁻¹ 1 Ascorbic acid resulted in an average seed yield of 257.90 g plant⁻¹, which was also significantly higher than the control group with an mean seed yield of 192.06 g plant⁻¹.

Regarding the combined effect of the factors, treatment with 2 g L⁻¹ Humic acid and 100 mg L⁻¹ Glutathion acid, as well as treatment with 2 g L⁻¹ Folic acid and 100 mg L⁻¹ Glutathion acid, both showed a significant increase in seed yield compared to the control treatment, with percentage increases of 150.22% and 145.48%, respectively. The reason for the increase can be explained by the interaction of factors with each other, which led to an increase in plant yield.

Table (6): The combined effect of bio stimulants and antioxidants and the interaction between them on plant seed yield (g plant⁻¹) of Soybean(2022)

Bio stimulants g L ⁻¹	Antioxidants mg L ⁻¹			Mean Bio stimulants
	Control	Ascorbic acid 100 mg	Glutathion acid 100 mg	
Con.	135.00	200.70	253.30	196.33
Humic acid 2gm	189.50	288.80	337.80	272.03
Folic acid 2gm	251.70	284.20	331.40	289.10
LSD _{0.05}	30.53			17.62
Mean antioxidants	192.06	257.90	307.50	
LSD _{0.05}	17.62			

7- The weight of 100 seeds (g)

The results indicate non-significance effect between treatments of biostimulants. The same table shows a significant effect when plants are treated with antioxidants, as the treatment of 100 mg L⁻¹ Glutathion acid achieved the highest mean of 19.47 g compared to the treatment of 100 mg L⁻¹ Ascorbic acid, which recorded an mean of 15.63 g, while non-treated plants recorded the lowest mean at 13.23 g. The combined effect of the study factors was significant, and the plants treated with 2 g L⁻¹ Folic acid and 100 mg L⁻¹ Glutathion acid exceeded non-treated plants by a percentage increase of 69.62%, followed by plants treated with 100 mg L⁻¹ Glutathion acid with distilled water, which recorded a significant increase over non-treated plants of 43.95%. This trait is related to the efficiency of the photosynthetic process and the interrelated relationship between the source and the sink and the activation of physiological activities within the plant, as the weight of the seeds is determined according to the activity of the plant and the quantity and quality of the primary and secondary metabolic substances formed for it. This is consistent with what was reached by **Mahmoud (2019)** on the maize plant.

Table (7): The combined effect of bio stimulants and antioxidants and the interaction between them on weight of 100 seeds (g) of Soybean(2022)

Bio stimulants g L ⁻¹	Antioxidants mg L ⁻¹			Mean Bio stimulants
	Control	Ascorbic acid 100 mg	Glutathion acid 100 mg	
Con.	12.97	16.57	18.67	16.07
Humic acid 2gm	13.33	16.67	17.75	15.91
Folic acid 2gm	13.40	13.66	22.00	16.35
LSD _{0.05}	1.24			N.S
Mean antioxidants	13.23	15.63	19.47	
LSD _{0.05}	0.71			

8- Total grain yield (t ha⁻¹)

The results show a significant effect of biostimulants (Table 8). Plants treated with 2 g L⁻¹ Folic acid achieved mean of 3.08 t ha⁻¹, while plants treated with 2 g L⁻¹ Humic acid recorded mean of 2.90 t ha⁻¹ compared to non-treated plants that gave the lowest mean at 2.09 t ha⁻¹. The results also showed that plants treated with 100 mg L⁻¹ Glutathione acid achieved mean of 3.28 t ha⁻¹ compared to plants treated with 100 mg L⁻¹ Ascorbic acid, which recorded mean of 2.75 t ha⁻¹, while non-treated plants recorded the lowest mean at 2.04 t ha⁻¹. The combined effect of the study factors was significant, as plants treated with 2 g L⁻¹ Humic acid and 100 mg L⁻¹ Glutathione acid and plants treated with 2 g L⁻¹ Folic acid and 100 mg L⁻¹ Glutathione acid showed a significant increase of 151.75% and 146.85%, respectively, compared to non-treated plants for the two treatments respectively. Total yield is the final result of the biological activities carried out by the plant, and the effective role of biostimulants and Glutathione acid in improving physiological traits has contributed to increasing plant efficiency in carrying out the photosynthetic process and increasing its metabolic products, which effectively contributed to increasing the total yield. This is consistent with what was reached by **Al-Saeedi (2018)** when spraying Humic acid on fenugreek plants and spraying Folic acid on maize plants (**Yassin, 2020**).

Table (8): The combined effect of bio stimulants and antioxidants and the interaction between them on total grain yield (g) of Soybean(2022)

Bio stimulants g L ⁻¹	Antioxidants mg L ⁻¹			Mean Bio stimulants
	Control	Ascorbic acid 100 mg	Glutathion acid 100 mg	
Con.	1.43	2.14	2.70	2.09
Humic acid 2gm	2.02	3.08	3.60	2.90
Folic acid 2gm	2.68	3.03	3.53	3.08
LSD _{0.05}	0.32			0.18
Mean antioxidants	2.04	2.75	3.28	
LSD _{0.05}	0.18			

9- oil yield (t ha⁻¹)

The results showed that the biostimulants achieved a significant increase, and the treatment of 2 gm L⁻¹ Humic acid with the highest yield of oil (0.81 ton ha⁻¹), which outperformed the untreated plants with mean of 0.61 ton ha⁻¹. Antioxidants also had a significant effect, as Treatment with 100 milligrams per liter of glutathione acid recorded the highest mean of 0.86 tons ha⁻¹, while untreated plants recorded the lowest mean at 0.57%. There was a significant interaction between the study factors, as Treatment with 2 grams per liter of humic acid and 100 milligrams per liter of glutathione acid achieved the highest value of 0.99 tons ha⁻¹, followed by Treatment with 2 grams per liter of humic acid and 100 milligrams per liter of ascorbic acid with a value of 0.87 tons ha⁻¹, while untreated plants recorded the lowest value for the trait at 0.43 tons ha⁻¹. The oil yield is a final result of the percentage of oil and the total yield of the plant, so the increase is the result of the positive relationship between them.

Table (9): The combined effect of bio stimulants and antioxidants and the interaction between them on oil yield (t ha⁻¹) of Soybean(2022)

Bio stimulants g L ⁻¹	Antioxidants mg L ⁻¹			Mean Bio stimulants
	Control	Ascorbic acid 100 mg	glutathione acid 100 mg	
Con.	0.43	0.63	0.76	0.61
Humic acid 2gm	0.57	0.87	0.99	0.81
Folic acid 2gm	0.71	0.76	0.82	0.76
LSD _{0.05}	0.09			0.18
Mean antioxidants	0.57	0.75	0.86	
LSD _{0.05}	0.01			

CONCLUSIONS

- 1- Treatment with 2 g L⁻¹ of humic acid outperformed in most vegetative growth traits. However, Treatment with 2 g L⁻¹ of folic acid achieved the highest mean for seed yield in the plant and total yield.
- 2- Treatment with 100 mg L⁻¹ of glutathione acid resulted in an increase in most growth traits, including fresh weight, dry weight, seed yield, 100-seed weight, total yield, and oil yield, while Treatment with 100 mg L⁻¹ of ascorbic acid recorded a significant increase only in the leaf area.
- 3- The interaction between the study factors had a significant effect on most study traits in the desired direction. Treatment with 2 g L⁻¹ of humic acid and 100 mg L⁻¹ of glutathione acid and Treatment with 2 g L⁻¹ of folic acid and 100 mg L⁻¹ of glutathione acid resulted in a significant increase in most vegetative growth traits and yield.

REFERENCES

1. Abdel Aziz, M., Ashram, M., & Harba, R. (2018). The effect of plant distribution and spraying with humic acid on some productive traits of *Trigonella foenum-graecum* grown in citrus orchards. *Tishreen University Journal for Research and Scientific Studies - Biological Sciences Series*, 40(5), 255-271.
2. Abdel Qader, S. M., Ibrahim, Z. R., & Nabi, H. S. (2022). Growth response of almond seedlings (*Prunus amygdalus*) to spraying amino plasma, humic acid, and boron. *Iraqi Journal of Agricultural Sciences*, 53(2), 415-428.
3. Al-Aboudi, H. M. K., Nasrallah, A. Y., & Al-Hilfi, I. H. (2016). Response of some soybean genotypes to ascorbic acid spraying. *Iraqi Journal of Agricultural Sciences*, 47(5), 1188-1195.
4. Alak, M. K., & Al-Sabagh, T. M. H. B. (2020). Role of soaking seeds with cobalt and ascorbic acid in alleviation of mung bean under water stress effect. *Plant Archives*, 20(1), 253-259.
5. Al-Alaf, I. H. (2017). The use of ascorbic acid in improving the growth of bitter orange seedlings. Retrieved from <http://alfallahalyoum.news/news>
6. Alewi, E. Y. (2020). *Response of some genotypes of sunflower to spraying with folic acid (vitamin B9)*. Master's thesis, Department of Field Crops, College of Agricultural Engineering Sciences, University of Baghdad.
7. Al-Hassany, M. A., & M.Z.F.Al Shammari. (2019). The effect of humic acid and brassinolide and their interaction on the growth, yield and active compounds of *Trigonella foenum-graecum* L. *PLANT. plant Archives* 19(1):18-23.
8. Ali, N. S. (2012). *Fertilizer technologies and their uses*. University House for Printing and Publishing, Ministry of Higher Education and Scientific Research, College of Agriculture, University of Baghdad.
9. Al-Jumaili, J. M. A. (2014). *Growth, yield and quality of soybean cultivars under the effect of cyclosoel and foliar feeding with nitrogen and boron*. PhD thesis, College of Agriculture, University of Baghdad.
10. Al-Karawi, A. W. R., & Al-Jumaily, J. M. M. A. (2022). Study of some growth criteria and yield of soybean crop with the effect of boron and some growth regulators. *Iraqi Journal of Market Research and Consumer Protection*, 14(1), 137-145.
11. Al-Saeedi, H. A. M. (2018). The effect of planting distances and spraying with humic acid on the growth and yield characteristics of fenugreek (*Trigonella foenum-graecum*). *Kirkuk University Journal of Agricultural Sciences*, 9(4), 118-125.
12. Arab Organization for Agricultural Development. (2014). *Development of Oilseed Crops and Oilseeds in The Arab World*.
13. Baqir, H. A. R., & Zeboon, N. H. (2019). Response of some growth characteristics of wheat crop to foliar spraying with humic and glutamic acid. *Iraqi Journal of Agricultural Sciences*, 50(6), 1455-1464.
14. Barth, C., De-Tullio, M., & Conklin, P. L. (2006). The role of ascorbic acid in the control of flowering time and the onset of senescence. *Journal of Experimental Botany*, 57, 1657-1665.



15. Conklin, P. L., & Barth, C. (2004). Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens and the onset of senescence. *Plant and Cell Environment.*, 27, 959–971.
16. Ferrara, G., & Brunetli, G. (2010). Effect of the times of application of a soil humic acid on berry quality of table grape (*Vitis vinifera* L.) cv Italia. *Vitis vinifera Spanish Journal of Agricultural Research*, 8(3), 817-822.
17. Goodwin, T. W. (1976). Chemistry and biochemistry of plant pigment. 2nd Ed. Academic Press, London, N. Y., San Francisco. USA, p. 373.
18. Mahmood, R. S. H., & Zeboon, N. H. (2019). Effect of foliar spraying with gibberellic and humic acid on wheat growth. *Int. J. Agricult. Stat. Sci*, 15(2), 621-625.
19. Mahmoud, R. H. (2019). *Response of growth and yield of maize cultivars to spraying with glutathione*. Master thesis, Department of Field Crops, College of Agricultural Engineering Sciences, University of Baghdad.
20. Mustafa, S. I. Jaafar, M. S., & Alnaimi, S. B. I. M. (2022, July). The Combined Effect of Bio-Fertilizers, Coconut Endosperm Fluid and Amino Acids Tryptophan on the Vegetative Growth Characteristics of Cumin (*Cuminum cyminum* L.). In *IOP Conference Series: Earth and Environmental Science* (Vol. 1060, No. 1, p. 012113). IOP Publishing.
21. Noctor, G., Queval, G., Mhamdi, A., Chaouch, S., & Foyer, C. H. (2011). *Glutathione*. The Arabidopsis Book. *The American Society of Plant Biologists* 9, 1-32.
22. Saheed, S. A., & Darwesh, D. A. (2021). Effect of Humic Acid on Tolerance indexes of Barley plant to Cadmium Toxicity. *Zanco Journal of Pure and Applied Sciences*, 33(3), 12-17.
23. Steel, R. G. D., & Torrie, J. H. (1980). *Principles and procedures of statistics, a biometrical approach* (No. Ed. 2). McGraw-Hill Kogakusha, Ltd.
24. Suleiman, N. S. (2017). Foliar spray of organic humic and amino fertilizers effect on plants growth and yield in greenhouse. *International Journal of Agronomy and Agricultural Research*, 10(3), 16-23.
25. Wiersma, J. V., & Bailey, T. B. (1975). Estimation of leaflet, trifoliolate and total leaf area of soybean. *Agron. J.*, 67, 26-30.
26. Zeboon, N. H. (2018). Response Of Growth And Yield Of Zea Maize For Foliar Spraying With Humic Acid. *Int. J. Agricult. Stat. Sci. Vol*, 14(1), 153-157.