Available online at http://bjas.bajas.edu.iq
https://doi.org/10.37077/25200860.2024.38.1.14
College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

ISSN 1814 – 5868 Basrah J. Agric. Sci., 38(1), 170-182, 2025 E-ISSN: 2520-0860

The Role of Biofertilizers and Sprouted Barley Grain Extract in Enhancing the Active Compound Content of Arugula *Eruca vesicaria* Mill Seeds Oil to Achieve Agricultural Sustainability

Hawraa H. Al-Jibawi*& Abbas K. Mijwel
Department of Horticulture and Landscape Engineering, College of Agriculture, Al-Qasim Green University, Iraq

***Corresponding author email**: **H.H.M.**: hawaljibawi@gmail.com, **A.K.M.**: mijwelabbas@gmail.com

Received 22nd December 2024; Accepted 13th April 2025; Available online 30th June 2025

Abstract: Arugula contains compounds with therapeutic and preventive roles against many diseases, and it promotes overall health. Additionally, its seeds contain promising medicinal oil that general health and disease prevention. An experiment was conducted over two agricultural seasons (autumn 2023-2024 and spring 2024) using a Randomized Complete Block Design (RCBD) with a split-plot system in three replicates to enhance the quantity and quality of the oil in the seeds. The biostimulant treatments (A) were applied to the main plots, with A_1 as the control (no addition), A_2 treated with *Trichoderma harzianum*, and A_3 treated with *Bacillus subtilis*. Amino acids (B) were organized within the sub-plots, including B_1 as the control (water spray), B_2 with Arginine 150 mg L^{-1}, B_3 with Tryptophan 150 mg L^{-1}, and B_4 with Phenylalanine 150 mg L^{-1}. The treatment with sprouted barley seed extract (C) was applied in the sub-sub-plots, with C_1 as the control, and C_2 involved spraying the sprouted barley seed extract at a concentration of 100 g L^{-1}. The results showed a positive and significant effect of the biostimulant treatments *Trichoderma harzianum*, *Bacillus subtilis*, amino acids Arginine, and Tryptophan, Phenylalanine, and sprouted barley seed extract, and their interactions, in increasing the concentrations of fatty acids in arugula seed oil, including Erucic, Palmitic, Stearic, Oleic, Lenolic, and Linolenic acids. This encourages the trend towards sustainable agriculture to enhance the health value of the oil while preserving the environment and the ecosystem.

Keywords: amino acids, arugula seed oil, biostimulants, sprouted grains.

Introduction

Arugula (*Eruca vesicaria* Thell. Cav. Subsp. Sativa (Mill.) L.) is an annual herbaceous winter plant belonging to the Brassicaceae family. It has gained significant medicinal and nutritional importance. Arugula is edible and widely cultivated in temperate regions throughout the year, except during extreme cold and hot months (Mohammed & Rafiq, 2009). Originating from the Mediterranean region, the plant is rich in vitamin C, known for its diverse health-promoting effects, including improving circulation and anti-inflammatory properties (Fuentes *et al*., 2014). Despite the growing demand for food production today, it is essential to focus on sustainable agricultural practices without harming the environment Plant Growth-Promoting .

Rhizobacteria (PGPR) are considered pioneers of the next-generation green

revolution and serve as excellent alternatives to chemical fertilizers. Among the most common PGPR species is *Bacillus subtilis*, which has been effectively used to enhance plant growth and increase yields (Mahapatra *et al*., 2022; Suhag, 2016; Haran & Thaher, 2019). These bacteria inhabit the rhizosphere and promote growth by producing plant hormones such as auxins (Bhat *et al*., 2019). Since *Trichoderma* naturally occurs in the soil, it is an integral component of the rhizosphere, with the ability to grow, multiply, and compete with other microorganisms.

This gives it a positive role in supporting plant nutrition, enhancing germination rates, and boosting productivity. Foliar feeding has become an essential aspect of modern agricultural practices. Foliar feeding programs are widely adopted for their numerous benefits and effectiveness in meeting plant needs directly, bypassing soil-related challenges such as nutrient fixation, leaching, and slow nutrient mobility. It allows for the efficient and precise application of nutrients tailored to the plant's actual requirements at each growth stage, especially during rapid growth phases, with a particular focus on micronutrients and growth regulators (Taiz *et al*., 2014). The foliar application of amino acids as biofertilizers plays a significant role in altering the osmotic potential within plant tissues. This reduces water potential, enhancing the ability of cells to absorb water and nutrients, thereby promoting vegetative growth and increasing the uptake of key nutrients such as nitrogen, phosphorus, and potassium (Azza & Yousf, 2015). *Arginine* is a precursor to polyamines and nitric oxide, both of which are involved in almost all physiological and biochemical processes, helping plants adapt to stress conditions (Yang & Gao, 2007). It also plays a role in plant flowering, a feature it shares with other amino acids (Mohammed & Khalil, 1992). The significance of the amino acid *Phenylalanine* lies in its role as a precursor to phenylpropanoids, a broad spectrum of important compounds. These include numerous secondary metabolites in most dicot plants, such as flavonoids and phenolic acids (Deng & Shanfa, 2017). On the other hand, one of the most complementary alternatives to chemical fertilizers is the use of plant extracts, particularly from freshly germinated seeds. Recently, these extracts have gained popularity in various fields due to their richness in compounds absent in mature plants, their simple composition, and ease of absorption. They are rich in gibberellins and have a reduced abscisic acid content (Taiz *et al*., 2014). Currently, there is a growing focus on eco-friendly biostimulation to improve crop performance under sustainable agricultural systems. Therefore, this research aims to integrate the use of advanced agricultural technologies, based on sound scientific principles, to provide effective outcomes in enhancing the productivity and quality of arugula plants, improving oil quality, and increasing the concentration of bioactive compounds. This ultimately elevates its nutritional and medicinal value.

Materials & Methods

The experiment was conducted in the fields of the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Al-Qasim Green, to study the role of biofertilizers and sprouted barley grain extract in enhancing the active compound content of arugula (*Eruca vesicaria*) seed oil. The experiment included the study of three factors**:** Factor 1: Biofertilizer (A): A_1: Control treatment (no addition), A_2: Treatment with the fungus

Trichoderma harzianum at a concentration of 10^8, applied at a rate of 5 g per hole during planting and A_3: Treatment with the bacterium *Bacillus subtilis* at a concentration of 10^8, applied at a rate of 5 g per hole during planting. The biofertilizers were prepared by the Agricultural Research, Department at the Biotechnology Center, Ministry of Science and Technology, Factor 2: Spraying with Amino Acids (B): B_1: Control treatment (spraying with water only), B_2: Spraying with Arginine at 150 mg L^{-1}, B_3: Spraying with Tryptophan at 150 mg L^{-1} and B_4: Spraying with *Phenylalanine* at 150 mg L^{-1}. The plants were sprayed twice: the first spray was applied when 4–6 true leaves had emerged, and the second spray was conducted 14 days after the first. Spraying was performed in the morning until complete wetting of the plants, using a surfactant to reduce water surface tension**.** Factor 3: Spraying with Sprouted Barley Grain Extract (C): C_1: Control treatment (spraying with water only)**.** C_2: Spraying with sprouted barley grain extract at a concentration of 100 g L^{-1} (Al-Khafaji & Al-jubouri, 2022).

The plants were sprayed twice, with the first spray applied the day after spraying with the amino acids.

Reparation of Sprouted Barley Grain Extract

One kg of barley grains (Iba 265 variety, obtained from the Ministry of Agriculture, Agricultural Research Department, Al-Suwaira Research Station) was washed and soaked in water for 24 h. After soaking, the grains were drained and spread in a single layer on trays lined with gauze in a dark place. They were sprayed with water as needed to maintain moisture. The grains germinated after 72 h (appearance of the radicle). The sprouted grains were blended in an electric blender until well mixed, then the mixture was filtered, and the volume was adjusted to 10 liters. The extract was sprayed on the plants early in the morning. To compare and calculate the nutritional availability, a water extract was also prepared from dormant barley grains. 100 g of dormant barley grains were washed, blended, and filtered, and the volume was adjusted to 1 L. Table (1) presents the chemical and physical properties and the nutritional availability coefficient of the aqueous extracts of both dormant and sprouted barley grains.

Experimental Design

The experiment was conducted using a factorial design (3*4*2) within a Randomized Complete Block Design (RCBD) arranged in a split-split plot system with three replicates. The biofertilization treatments were assigned to the main plots, amino acid treatments to the sub-plots, and sprouted barley grain extract treatments to the sub-sub plots (Al-Rawi & Khalaf Allah, 2000).

The means of the treatments were analyzed using the Least Significant Difference (L.S.D) test at a 0.05 probability level. The statistical analysis of the experimental data was performed using GenStat V.12 software. The field was divided into three blocks, with a 50 cm distance between blocks. Each block contained 24 experimental units, spaced 50 cm apart. Each experimental unit consisted of two rows, spaced 20 cm apart. One row was designated for vegetative measurements, and the other for seed and oil measurements. Each row contained 10 plants, spaced 20 cm apart, resulting in 20 plants per experimental unit. The Egyptian arugula seeds used in the experiment were sourced from a certified agricultural supplier. Seed germination was tested by randomly selecting 100 seeds, which were placed on water-saturated filter paper in

a dish and left at room temperature for 48 hours. The germination rate was calculated to be 98%. Traditional fertilizers (K, P, N) were applied to the soil at rates of 10, 100, and 100 kg ha^{-1}, respectively, as per the recommendations (Taherlou & Dursun, 2019). Soil samples were collected from three locations within the field at a depth of 30 cm and mixed before planting to conduct physical and chemical analyses. Table 2 presents the results of the soil analyses conducted at the Soil and Water Laboratory, Directorate of Agriculture, Al-Qadisiyah. The field was sown on October 1, 2023, for the first season and on January 15, 2024, for the second season. Three seeds were placed in each hole and covered with fine soil. Drip irrigation was applied immediately after planting. After the emergence of true leaves, thinning was conducted to leave one plant per hole. Manual weeding was performed 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

Table (2): Physical and Chemical Properties of the Field Soil.

Soil Texture			N%	P%	K ppm	Organic Matter	Electrical Conductivity 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 Measuring the Active Ingredients in the Oil

Fat Esterification: The sample was prepared according to the method adopted by the AOAC (1995) which relies on fat esterification by reacting it with methanolic potassium hydroxide. To prepare the solution, 11.2 grams of potassium hydroxide were dissolved in 100 ml of methanol. Then, 1 g of fat was taken, and 8 ml of methanolic potassium hydroxide, along with 5 ml of hexane, were added. The mixture was shaken quickly for 30 seconds and left to separate into two layers. The upper layer (hexane layer), which contains the esterified fat, was then taken and injected into the machine.

Chromatographic Analysis of the Sample: The analysis was conducted in the laboratories of the Ministry of Science and Technology / Environmental and Water Directorate. Fatty acid compounds were analyzed using a gas chromatography device (GC – 2010) made by Shimadzu (Japan). A flame ionization detector (FID) was used, and a capillary column of type SE-30 with dimensions (30 m * 0.25 mm) was used for separation (Zhang *et al*., 2015). 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	120-290 (10C /MIN)
Gas Flow Rate	100 Kpa

Results & Discussion

Table (4) illustrates the individual effects of the added treatments. Regarding the individual effect of the biofertilizer, *Trichoderma harzianum* had a positive effect on the increase in fatty acid content over both seasons, *Bacillus subtilis* also showed positive results compared to the control treatment. As for the amino acids and their effects on fatty acids, the amino acid *Arginine* resulted in the highest fatty acid content, followed by the amino acid *Phenylalanine*. Moreover, the extract of sprouted barley seeds showed a positive effect compared to the control treatment in both seasons.

Regarding the dual interactions of treatments, the interaction between A2B2 showed the highest significant value compared to other interactions. The interaction between the amino acids and the sprouted barley seed extract gave the best results for both seasons in the B4C2 treatment. As for the interaction between the biofertilizer and the extract, the combination of A2C2 showed the best positive effect compared to the control. Finally, for the three-way interactions of the treatments, Table 5 shows that the A2B3C2 interaction outperformed all other three-way combinations, giving the highest value for the fatty acid content in arugula seed oil

Table (4): shows the individual and dual effects of the treatments on the fatty acid content of arugula seed oil.

Treatment	Erucic%		Palmatic%		Stearic%		Oleic%		Lenolic%		Linolenic%	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
A1	36.06	29.83	6.14	4.43	3.00	1.83	13.30	9.98	18.32	14.05	1.67	1.34
A2	37.52	31.18	7.79	5.85	4.60	2.92	15.23	11.53	19.94	15.47	2.67	2.36
A3	36.95	30.82	7.16	5.52	3.98	2.63	14.46	11.15	19.33	15.13	2.22	2.09
L.S.D (A)	**0.016**	**0.027**	**0.015**	**0.070**	**0.015**	**0.031**	**0.016**	**0.017**	**0.013**	**0.007**	**0.015**	**0.018**
B1	36.10	29.95	6.24	4.62	3.06	1.88	13.39	10.16	18.43	14.23	1.68	1.41
B2	37.23	30.88	7.41	5.60	4.25	2.70	14.82	11.20	19.61	15.17	2.45	2.13
B3	36.90	30.52	7.09	5.11	3.92	2.42	14.39	10.74	19.25	14.76	2.22	1.87
B4	37.15	31.09	7.38	5.75	4.22	2.84	14.72	11.44	19.51	15.37	2.38	2.31
L.S.D (B)	**0.017**	**0.011**	**0.017**	**0.057**	**0.016**	**0.014**	**0.017**	**0.007**	**0.018**	**0.012**	**0.017**	**0.011**
C1	36.13	30.03	6.21	4.71	3.06	1.95	13.38	10.24	18.41	14.29	1.68	1.48
C2	37.56	31.19	7.85	5.83	4.66	2.97	15.28	11.53	19.99	15.48	2.69	2.38
L.S.D (C)	**0.012**	**0.006**	**0.012**	**0.040**	**0.012**	**0.008**	**0.012**	**0.007**	**0.012**	**0.006**	**0.012**	**0.008**
A1B1	35.51	29.49	5.57	4.15	2.41	1.48	12.66	9.61	17.77	13.70	1.33	1.01
A1B2	35.93	29.73	5.93	4.44	2.79	1.74	13.09	9.93	18.21	14.00	1.50	1.25
A1B3	36.08	29.67	6.24	4.00	3.11	1.78	13.40	9.67	18.35	13.82	1.73	1.28
A1B4	36.72	30.43	6.82	5.13	3.71	2.30	14.06	10.72	18.96	14.69	2.11	1.82
A2B1	36.55	30.33	6.76	4.99	3.55	2.18	13.96	10.60	18.94	14.63	1.96	1.72
A2B2	38.08	31.62	8.37	6.34	5.21	3.32	15.91	12.06	20.48	15.92	3.06	2.70
A2B3	37.70	31.33	7.92	6.04	4.73	3.06	15.42	11.67	20.13	15.61	2.79	2.44
A2B4	37.78	31.45	8.12	6.03	4.92	3.11	15.63	11.79	20.23	15.72	2.87	2.57
A3B1	36.26	30.04	6.39	4.71	3.21	1.97	13.55	10.26	18.58	14.36	1.77	1.51
A3B2	37.68	31.29	7.92	6.02	4.76	3.03	15.46	11.62	20.14	15.60	2.79	2.43
A3B3	36.93	30.57	7.12	5.28	3.91	2.43	14.35	10.89	19.27	14.86	2.15	1.88
A3B4	36.94	31.37	7.21	6.09	4.02	3.11	14.47	11.82	19.33	15.71	2.18	2.53
L.S.D (A.B)	**0.028**	**0.028**	**0.027**	**0.101**	**0.026**	**0.033**	**0.028**	**0.018**	**0.028**	**0.019**	**0.028**	**0.021**
B1C1	35.54	29.44	5.54	4.11	2.40	1.46	12.59	9.58	17.79	13.70	1.34	1.03
B1C2	36.67	30.47	6.93	5.12	3.72	2.30	14.19	10.73	19.07	14.76	2.03	1.79
B2C1	36.70	30.41	6.83	5.14	3.69	2.30	14.11	10.68	19.00	14.68	2.05	1.78
B2C2	37.76	31.35	7.99	6.06	4.82	3.10	15.53	11.73	20.22	15.65	2.86	2.47
B3C1	35.97	29.83	6.04	4.50	2.87	1.78	13.17	9.99	18.25	14.08	1.56	1.29
B3C2	37.83	31.22	8.15	5.72	4.96	3.07	15.61	11.49	20.26	15.45	2.89	2.44
B4C1	36.31	30.46	6.44	5.10	3.30	2.25	13.65	10.70	18.61	14.69	1.79	1.81
B4C2	37.98	31.71	8.33	6.40	5.14	3.43	15.79	12.18	20.41	16.06	2.98	2.80

L.S.D (B.C)	**0.023**	**0.013**	**0.023**	**0.078**	**0.023**	**0.018**	**0.023**	**0.012**	**0.024**	**0.015**	**0.023**	**0.015**
A1C1	35.33	29.30	5.31	3.96	2.19	1.29	12.32	9.38	17.47	13.47	1.23	0.92
A1C2	36.79	30.35	6.97	4.90	3.82	2.36	14.28	10.59	19.17	14.63	2.10	1.76
A2C1	36.82	30.55	7.01	5.24	3.85	2.40	14.30	10.83	19.16	14.85	2.12	1.89
A2C2	38.23	31.81	8.57	6.46	5.35	3.44	16.16	12.22	20.73	16.09	3.22	2.83
A3C1	36.25	30.24	6.31	4.93	3.16	2.15	13.52	10.50	18.60	14.54	1.70	1.63
A3C2	37.66	31.39	8.01	6.12	4.79	3.12	15.40	11.79	20.07	15.72	2.74	2.54
L.S.D (A.C)	**0.019**	**0.027**	**0.019**	**0.075**	**0.019**	**0.030**	**0.019**	**0.017**	**0.018**	**0.009**	**0.019**	**0.018**

A2: *Trichoderma harzianum* 10^8, A3: *bacillus subtilis* 10^8, 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. S1: first season, S2: second season.

Table (5): shows the effect of the three-way interactions of the treatments on the fatty acid content of arugula seed oil.

Treatment	Erucic%		Palmatic%		Stearic%		Oleic%		Lenolic%		Linolenic%	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
A1	36.06	29.83	6.14	4.43	3.00	1.83	13.30	9.98	18.32	14.05	1.67	1.34
A2	37.52	31.18	7.79	5.85	4.60	2.92	15.23	11.53	19.94	15.47	2.67	2.36
A3	36.95	30.82	7.16	5.52	3.98	2.63	14.46	11.15	19.33	15.13	2.22	2.09
L.S.D (A)	**0.016**	**0.027**	**0.015**	**0.070**	**0.015**	**0.031**	**0.016**	**0.017**	**0.013**	**0.007**	**0.015**	**0.018**
B1	36.10	29.95	6.24	4.62	3.06	1.88	13.39	10.16	18.43	14.23	1.68	1.41
B2	37.23	30.88	7.41	5.60	4.25	2.70	14.82	11.20	19.61	15.17	2.45	2.13
B3	36.90	30.52	7.09	5.11	3.92	2.42	14.39	10.74	19.25	14.76	2.22	1.87
B4	37.15	31.09	7.38	5.75	4.22	2.84	14.72	11.44	19.51	15.37	2.38	2.31
L.S.D (B)	**0.017**	**0.011**	**0.017**	**0.057**	**0.016**	**0.014**	**0.017**	**0.007**	**0.018**	**0.012**	**0.017**	**0.011**
C1	36.13	30.03	6.21	4.71	3.06	1.95	13.38	10.24	18.41	14.29	1.68	1.48
C2	37.56	31.19	7.85	5.83	4.66	2.97	15.28	11.53	19.99	15.48	2.69	2.38
L.S.D (C)	**0.012**	**0.006**	**0.012**	**0.040**	**0.012**	**0.008**	**0.012**	**0.007**	**0.012**	**0.006**	**0.012**	**0.008**
A1B1	35.51	29.49	5.57	4.15	2.41	1.48	12.66	9.61	17.77	13.70	1.33	1.01
A1B2	35.93	29.73	5.93	4.44	2.79	1.74	13.09	9.93	18.21	14.00	1.50	1.25
A1B3	36.08	29.67	6.24	4.00	3.11	1.78	13.40	9.67	18.35	13.82	1.73	1.28
A1B4	36.72	30.43	6.82	5.13	3.71	2.30	14.06	10.72	18.96	14.69	2.11	1.82
A2B1	36.55	30.33	6.76	4.99	3.55	2.18	13.96	10.60	18.94	14.63	1.96	1.72
A2B2	38.08	31.62	8.37	6.34	5.21	3.32	15.91	12.06	20.48	15.92	3.06	2.70
A2B3	37.70	31.33	7.92	6.04	4.73	3.06	15.42	11.67	20.13	15.61	2.79	2.44

A2B4	37.78	31.45	8.12	6.03	4.92	3.11	15.63	11.79	20.23	15.72	2.87	2.57
A3B1	36.26	30.04	6.39	4.71	3.21	1.97	13.55	10.26	18.58	14.36	1.77	1.51
A3B2	37.68	31.29	7.92	6.02	4.76	3.03	15.46	11.62	20.14	15.60	2.79	2.43
A3B3	36.93	30.57	7.12	5.28	3.91	2.43	14.35	10.89	19.27	14.86	2.15	1.88
A3B4	36.94	31.37	7.21	6.09	4.02	3.11	14.47	11.82	19.33	15.71	2.18	2.53
L.S.D (A.B)	**0.028**	**0.028**	**0.027**	**0.101**	**0.026**	**0.033**	**0.028**	**0.018**	**0.028**	**0.019**	**0.028**	**0.021**
B1C1	35.54	29.44	5.54	4.11	2.40	1.46	12.59	9.58	17.79	13.70	1.34	1.03
B1C2	36.67	30.47	6.93	5.12	3.72	2.30	14.19	10.73	19.07	14.76	2.03	1.79
B2C1	36.70	30.41	6.83	5.14	3.69	2.30	14.11	10.68	19.00	14.68	2.05	1.78
B2C2	37.76	31.35	7.99	6.06	4.82	3.10	15.53	11.73	20.22	15.65	2.86	2.47
B3C1	35.97	29.83	6.04	4.50	2.87	1.78	13.17	9.99	18.25	14.08	1.56	1.29
B3C2	37.83	31.22	8.15	5.72	4.96	3.07	15.61	11.49	20.26	15.45	2.89	2.44
B4C1	36.31	30.46	6.44	5.10	3.30	2.25	13.65	10.70	18.61	14.69	1.79	1.81
B4C2	37.98	31.71	8.33	6.40	5.14	3.43	15.79	12.18	20.41	16.06	2.98	2.80
L.S.D (B.C)	**0.023**	**0.013**	**0.023**	**0.078**	**0.023**	**0.018**	**0.023**	**0.012**	**0.024**	**0.015**	**0.023**	**0.015**
A1C1	35.33	29.30	5.31	3.96	2.19	1.29	12.32	9.38	17.47	13.47	1.23	0.92
A1C2	36.79	30.35	6.97	4.90	3.82	2.36	14.28	10.59	19.17	14.63	2.10	1.76
A2C1	36.82	30.55	7.01	5.24	3.85	2.40	14.30	10.83	19.16	14.85	2.12	1.89
A2C2	38.23	31.81	8.57	6.46	5.35	3.44	16.16	12.22	20.73	16.09	3.22	2.83
A3C1	36.25	30.24	6.31	4.93	3.16	2.15	13.52	10.50	18.60	14.54	1.70	1.63
A3C2	37.66	31.39	8.01	6.12	4.79	3.12	15.40	11.79	20.07	15.72	2.74	2.54
L.S.D (A.C)	**0.019**	**0.027**	**0.019**	**0.075**	**0.019**	**0.030**	**0.019**	**0.017**	**0.018**	**0.009**	**0.019**	**0.018**

A2: Trichoderma harzianum 10^8, A3: bacillus subtilis 10^8, 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. S1: first season, S2: second season.

The results in the table showed the superiority of the individual factors of the study and their interactions on the active compounds content in the oil. This superiority can be attributed to the role of biofertilizers through microbial processes performed by soil organisms that mobilize unavailable nutrients in the soil and enhance plant growth, making them essential for sustainable agriculture (Jacob & Paranthaman, 2023; Al-Maamori, 2024). Bacillus species act as bio-stimulants by producing plant hormones such as auxins and cytokinins, which contribute to plant growth and development (Zubair *et al*., 2019; Mostafavian *et al*., 2008). By working together as a harmonious team, they comprehensively activate plant growth and stimulate the formation of active compounds and oil. The combined stimulation of auxins and cytokinins can lead to improved lateral and adventitious root branching and an increase in the absorption of nutrients, thereby activating metabolic processes (Sosnowski *et al*., 2023).

This directly increases the quality of the arugula seed oil. Plant Growth-Promoting Rhizobacteria (PGPR) play an important role in enhancing plant growth through a variety of mechanisms, including the production of siderophores (molecules that help plants absorb iron) (Choudhary *et al*., 2011; García-Fraile *et al*., 2015). Siderophores are cofactors for enzymes involved in converting Acetyl-Coenzyme A (Acetyl-CoA) to Malonyl-Coenzyme A (Malonyl-CoA), which is a key step in the fatty acid biosynthesis pathway, as proven by Ye *et al*. (2020) regarding the role of Acetyl-CoA in fatty acid synthesis in plants. Additionally, the effectiveness of *Trichoderma harzianum* in promoting plant growth through disease reduction (Yousif & Hassan, 2023). by colonizing the roots and competing with harmful pathogens, has been demonstrated. Therefore, the use of *Trichoderma harzianum* contributed to improving the health of arugula, enhanced nutrient absorption, and consequently increased both its growth and yield, both in quantity and quality (Matas-Baca *et al*., 2023). Regarding the application of amino acid spraying on plants, its significant effect was evident in the table, indicating the effect of arginine, which is considered a precursor to polyamine synthesis (Bagni & Tassoni, 2003; Illingworth *et al*., 2001). Polyamine synthesis in *Arabidopsis* begins with arginine, which is converted by the enzyme arginine decarboxylase to agmatine. This process is important for the balance of nitrogen (N) and carbon (C) within the cell, and this balance is crucial for fatty acid formation (Gupta *et al*., 2016). The increase in fatty acid content in the oil due to tryptophan is attributed to its nitrogen content, which the plant absorbs directly when sprayed on the leaves. This, in turn, activates the root system, including potassium and phosphorus, thereby increasing carbon fixation and improving nutrient absorption efficiency. Since amino acids are precursors for the synthesis of hormones and growth regulators, as well as for the formation of secondary metabolites in plants, they play a key role in stimulating growth and encouraging plant development under unfavorable climatic conditions (Taher *et al*., 2017). In some cases, plants can convert amino acids into Acetyl-Coenzyme A through deamination, which might play a role in increasing fatty acid content. The effect of arginine and phenylalanine may be due to their role in the synthesis of proteins and enzymes needed for the formation of bioactive compounds within the plant (Reham *et al*., 2016). Additionally, phenylalanine is considered one of the aromatic amino acids, which is presumed to be the precursor for the

synthesis of active compounds in the plant (Tzin & Galili, 2010; Perkowski & Warpeha, 2019). It is one of the glucogenic and ketogenic amino acids, which are important for the preparation of the Krebs cycle with carbon. Three carbon atoms from phenylalanine are converted into fumarate, while the remaining part is converted into isoleucine, which enhances the Krebs cycle with three-carbon compounds that serve as a source for generating other active compounds within the plant (Hyun *et al*., 2011; Al-Yasiry & Majwel, 2024; Alpresem *et al*.,2025).

Regarding the superiority of barley sprout extract spray, its effect is attributed to its good content of gibberellin, which is known for promoting cell division and elongation, leading to an increase in the size of tissues that contain oils, such as seeds. This increase in biomass may contribute to higher oil production. production (Castro-Camba *et al*.,2022). Additionally, it stimulates metabolic processes, including fatty acid and oil synthesis pathways. It also enhances the activity of enzymes responsible for forming fatty acids, the primary component of oils (Essa, 1992). Furthermore, the extract contains easily soluble and bioavailable nutrients, including nitrogen in the form of amino acids and small peptides, and phosphorus in the form of soluble phosphates (Table 1), which makes them easily absorbed by the plant's leaves, thus increasing the biochemical activities based on that. The results of the first season were better than those of the second season, which may be due to the favorable environmental conditions for biological processes, especially photosynthesis, leading to an increase in compounds (Ugur *et al*., 2010). In his study on arugula, he found variations in fatty acids due to different planting periods

Conclusion

The fertilization program followed in this study showed promising results that encourage the use of environmentally friendly resources in the direction of clean agriculture and cost reduction. The program recorded satisfactory results in the agricultural aspect (especially in fertilization), with significant differences observed for all the treatments used. This opens new horizons for sustainable agriculture in Iraq.

Acknowledgements

We extend our sincere gratitude to the College of Agriculture, Al-Qasim Green University, and to everyone who contributed to the completion of this research

Contributions of authors

H.H.M: Conducting field and laboratory work, writing the original draft, and editing the research.

A.K.M: Preparing the research plan, monitoring the work, and conducting statistical analysis.

ORCID

H.H.M: https://orcid.org/0009-0001-8209-9328.
A.K.M: https://orcid.org/0000-0002-7846-5356.

Conflicts of interest

The authors declare that they have no conflict of interests.

References

AOAC (1995). Association of Official Analytical Chemists). Official Methods of Analysis, 16th Edition. AOAC International, Gaithersburg, MD.

Alpresem, W. F., Al-Showily, A. K. N., & Alnajjar, M. A. (2025). Detection of medicinally Effective Compounds in Two Genera of Ornamental Palm Leaves and Roots (washingtonia filifera and Phoenix sp.). In IOP Conference Series: Earth and Environmental

Science (Vol. 1487, No. 1, p. 012047). IOP Publishing. https://iopscience.iop.org/article/10.1088/1755-1315/1487/1/012047/meta

Al-Khafaji, A. M. H. H., & Al-jubouri, k. D. H. (2022). Influence of aqueous extract of barley sprouts, trehalose, and calcium on growth, quality and yield of carrot. *Iraqi Journal of Agricultural Science* 53(1): 133-140. https://doi.org/10.36103/ijas.v53i1.1517.

Al-Maamori, H. A., (2024). Effects Bacterial Inoculation and organic Fertilization on Some Soil Properties and Growth of Potato Crop Role in Sustainable Agriculture. *Basrah Journal of Agricultural Sciences*, *37*(2), 264–275. https://doi.org//10.37077/25200860.2024.37.2.20

Al-Rawi, K. M., & Khalaf Allah, A. A. M. (2000). Design and Analysis of Agricultural Experiments, Dar Al-Kutub for Printing and Publishing, University of Mosul, Iraq. p. 488. https://scholar.google.com.eg/scholar?hl=ar&as_sdt=0,5&cluster=4976190406483417194

Al-Yasiry, Z. F. H., & Mijwel, A. K. (2024). Role of Bio- fertilizer and Amino acid spraying on growth and yield of two type Mint plant and its contents of some active constituent, *Euphrates Journal of Agriculture Science*,16 (2): 605-516.

Azza, S. M., & Yousef, R. S. (2015). Response of Basil Plant (*Ocimum sanctum* L.) to Foliar Spray with Amino Acids or Seaweed extract. *Journal of Horticultural, Science and Ornamental Plants*, 7 (3): 94-106.

Bagni, N., & Tassoni, A. (2001). Biosynthesis, oxidation and conjugation of aliphatic polyamines in higher plants, *Amino Acids* 20(3): 301–317. https://doi.org/10.1007/s007260170046.

Castro-Camba R., C. Sánchez, N. Vidal & Vielba, J. M. (2022). Plant Development and Crop Yield: The Role of Gibberellins. *Plants (Basel)*,11(19):2650. https://doi.org/10.3390/plants11192650. PMID: 36235516; PMCID: PMC9571322.

Choudhary, D. K., K. P. Sharma & Gaur, R. K. (2011). Biotechnological perspectives of microbes in agro-ecosystems. *Biotechnology Letters,* 33, 1905–1910. https://doi.org/10.1007/s10529-011-0662-0.

De Andrade L.A., C.H.B. Santos, E.T. Frezarin, L.R. Sales, & Rigobelo, E. C. (2023). Plant Growth-Promoting Rhizobacteria for Sustainable Agricultural Production. *Microorganisms*,11(4): 1088. https://doi.org/10.3390/microorganisms11041088.

Deng, Y., & Shanfa, L. (2017). Biosynthesis and Regulation of Phenylpropanoids in Plants. *Journal Critical Reviews in Plant Sciences* ,36(4):257-290. https://doi.org/10.1080/07352689.2017.1402852

Essa, Z. M. (1992). Physiological studies on some rose varieties. Ph. D. Thesis, Fac Agriculture. Ain-Shams.

Fuentes, E., M. Alarcon, M. Fuentes, G. Carrasco, & Palomo, I. (2014). A Novel Role of *Eruca sativa* Mill. (Rocket) Extract: Antiplatelet (NF-kB Inhibition) and Antithrombotic *Activities*, 6(12): 5839–5852. https://doi.org/10.3390/nu6125839.

García-Fraile, P., E. Menéndez, & Rivas, R. (2015). Role of bacterial biofertilizers in agriculture and forestry. *AIMS Bioengineerin,,* 2, 183–205. https://doi.org/10.3934/bioeng.2015.3.183

Gupta K, A. Sengupta, M. Chakraborty, & Gupta, B. (2016). Hydrogen Peroxide and Polyamines Act as Double-Edged Swords in Plant Abiotic Stress Responses. *Front Plant Science*,7:1343. https://doi.org/ 10.3389/fpls.2016.01343. PMID: 27672389; PMCID: PMC5018498.

Haran, M. S.., & Thaher, A. Z. T. (2019). Effect Bio-fertilizer of Bacillus, Azotobacter and Pseudomonas floresence in the Growth and Production of Corn Plant (*Zea mays* L.). *Basrah Journal of Agricultural Sciences*, *32*, 7–14.

https://doi.org/10.37077/25200860.2019.252

Hyun, M. Woo, Y. H. Yun, J. Y. Kim, & Kim, S. H. (2011). Fungal and Plant Phenylalanine Ammonia-lyase. *Mycobiology*, 39(4): 257-265. https://doi.org/ 10.5941/MYCO.2011.39.4.257.

Jacob, S. M., & Paranthaman, S. (2023). Biofertilizers: an advent for eco-friendly and

sustainable agriculture development, *Vegetos*, 36, p. 1141–1153. https://doi.org/10.1007/s42535-022-00550-9.

Illingworth, C., M. J. Mayer, K. Elliott, C. Hanfrey, A. J. Michael, & Walton, N. J. (2003). The diverse bacterial origins of the Arabidopsis polyamine biosynthetic pathway, *FEBS Letters*,549(1-3): 26–30. https://doi.org/ 10.1016/s0014-5793(03)00756-7.

Mahapatra, S. , R. Yadav, & Ramakrishna ,W. (2022). *Bacillus subtilis* impact on plant growth, soil health and environment: Dr. Jekyll and Mr. Hyde, *Journal of Applied Microbiology*, 132(5):3543-3562. https://doi.org/ 10.1111/jam.15480.

Matas-Baca M. A., M. A. Flores-Córdova, S. Pérez-Álvarez, M. J. Rodríguez-Roque, N. A. Salas-Salazar, M. C. Soto-Caballero, & Sánchez-Chávez, E. (2023). Trichoderma fungi as an agricultural biological control in Mexico, *Revista Chapingo Serie Horticultura*,Vol. 29(3):80-114. https://doi.org/10.5154/r.rchsh.2022.11.015

Mohammed, H.C., & Rafiq, A. (2009). Investigating possibility of using least desirable adible oil of *Eruca sativa* Mill. In bio diesel production, *Pakistan Journal. Botany*,41(1):481-487.

Mohamed, S. M., & Khalil, M. M. (1992). Effect of tryptophan and arginine on growth and flowering of some winter annuals. Egypt. *Journal Applied Science*., 7 (10):82-93.

Mostafavian, S. R., H. Pirdashti, M. R. Ramzanpour, A. A. Andarkhor, & Shahsavari, A. (2008). Effect of mycorrhizae, Thiobacillus and sulfur nutrition on the chemical composition of soybean *Glycine max* (L.) Merr. seed. *Pakistan Journal of Biological Science*,11(6): 826-835.https://doi.org/ 10.3923/pjbs.2008.826.835

Perkowski, M. C., & Warpeha, K M. (2019). Phenylalanine roles in the seed-to-seedling stage: Not just an amino acid, *Plant Science*,V. 289. https://doi.org/ 10.1016/j.plantsci.2019.110223.

Reham, M. S., M. E. Khattab, S. S. Ahmed, & Kandil, M. A. M. (2016). Influence of foliar spray with phenylalanine and nickel on growth, yield quality and chemical composition of genoveser basil plant. *African Journal Agriculture Research,*11(16): 1398-1410. https://doi.org/10.5897/AJAR2015.10699

Sosnowski J., M. Truba, & Vasileva, V. (2023). The Impact of Auxin and Cytokinin on the Growth and Development of Selected Crops, *Agriculture*, 13(3), 724. doi.org/10.3390/agriculture13030724

Sudhakar, M., & Balliah, R. (2015). Phytochemical determination of a polyherbal extract using FTIR and GC–MS analysis. *European Journal of Pharmaceutical and Medical Research,*2(7):173–178.

Suhag, M. (2016). Potential of bio fertilizers to replace chemical fertilizers. *International Advanced Research Journal in Science, Engineering and Technology*,3(5): 163-167. https://doi.org/10.17148/IARJSET.2016.3534

Taherlou, A., & Dursun, A. (2019). Evaluation of Leaf Yield of Rocket (*Eruca sativa* Mill.) for use as Salad Vegetable in Iran, *Journal of Horticultural Science & Ornamental Plants*, 11 (1): 01-06. https://doi.org/ 10.5829/idosi.jhsop.2019.01.06

Taher, M. S., & Al-ubaydi, R. M. (2017). Effect of Foliar spray of Tryptophan and Nitrogen fertilizer on Growth and yield of Rocket plant (*Eruca sativa* Mill.). *Euphrates Journal of Agriculture Science*, 9(4). https://www.iraqoaj.net/iasj/download/b3ceee59b8228e95

Taiz, L., E. Zeiger; I. Max Moller, & Murphy, A. (2014). Plant Physiology,and Development. sixth Edition Sinauer Assotiates, Inc., Publishers Sunderland, Massachusetts. pp: 761.

Tzin, V. & Galili, G. (2010). New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. *Moleculer Plant.* 3(6): 956-72.https://doi.org/ 10.1093/mp/ssq048.

Ugur, A. ipk, S. Sinem, & llkay, A. E. O. (2010). Variation in fatty acid composition of the seed of *eruca sativa* Mill. Causedby different sowing period and nitrogen*, pharmacognosy magazine*,6(24):305-308. https://doi.org/10.4103/0973-1296.71801

Yang, H. Q., & Gao, H. J. (2007). Physiological function of arginine and its metabolites in plants. *Journal of plant physiology and molecular biology*,33(1):1-8. https://pubmed.ncbi.nlm.nih.gov/17287563/

Ye, Y., K. Nikovics, A. To, L. Lepiniec, E. T. Fedosejevs, S. R. Van Doren, S. Baud, & Thelen, J. J. (2020). Docking of acetyl-CoA carboxylase to the plastid envelope membrane attenuates fatty acid production in plants, *Nature Commun*ications, 11(1): 6191. https://doi.org/10.1038/s41467-020-20014-5.

Yousif, A. A., & Hassan, W. A. (2023). Molecular Identification of Postharvest Moldy Core Pathogens on Apple and Application of Biocontrol Products of Essential Oils (EOs) and *Trichoderma harzianum. Basrah Journal of Agricultural Sciences*, *36*(1), 1–15. https://doi.org/10.37077/25200860.2023.36.1.01

Zhang, H., Z. Wang, & Liu, O. (2015). Development and validation of a GC–FID method for quantitative analysis of oleic acid and related fatty acids, *Journal of Pharmaceutical Analysis*, 5(4): 223230. https://doi.org/10.1016/j.jpha.2015.01.005.

Zubair, M., A. Hanif, A. Farzand,T.M.M. Sheikh, A. R. Khan, M. Suleman, M. Ayaz, & Gao, X. (2019). Genetic screening and expression analysis of psychrophilic *Bacillus sp*. reveal their potential to alleviate cold stress and modulate phytohormones in wheat, *Microorganisms*, 7(9): 337. https://doi.org/10.3390/microorganisms7090337

دور المخصبات الحيوية ومستخلص حبوب الشعير المستنبتة في تعزيز محتوى زيت بذور الجرجير من المواد الفعالة لتحقيق الإستدامة في الزراعة

حوراء حميد الجباوي[1] عباس خضير مجول[2]

[1]قسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة القاسم الخضراء، العراق

[2]قسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة القاسم الخضراء، العراق

المستخلص: يحتوي الجرجير على مواد ذات دور علاجي ووقائي ضد العديد من الامراض ويعزز من صحة الجسم فضلاً عن احتواء بذوره على زيت طبي واعد لدوره في تحسين الصحة العامة والوقاية من الامراض، ولتحسين كمية ونوعية الزيت في بذور النبات فقد نفذت تجربة عامليه خلال موسمين زراعيين (خريفي 2023–2024 وربيعي 2024) باستخدام تصميم القطاعات العشوائية الكاملة (RCBD) وفق نظام الالواح المنشقة – المنشقة في ثلاث مكررات وزع التسميد الحيوي (A) على الالواح الرئيسة (A) وشمل A_1 من دون إضافة للمقارنة A_2 المعاملة بفطر *Trichoderma harzianum* ، A_3 المعاملة ببكتريا *Bacillus subtilis* نظمت الأحماض الأمينية (B) ضمن الالواح الثانوية وتضمن B_1 المقارنة (الرش بالماء) B_2 Arginine, 150ملغم لتر$^{-1}$، B_3 Tryptophan 150 ملغم لتر$^{-1}$، B_4 Phenylalanine 150 ملغم لتر$^{-1}$. اما مستخلص حبوب الشعير المستنبتة (C) فقد تم تنفيذه في الالواح تحت الثانوية وشمل C_1 معاملة المقارنة، C_2: رش مستخلص بذور الشعير المستنبتة بتركيز 100غم لتر^{1}. حللت البيانات باستخدام البرنامج الاحصائي Genstat وقورت النتائج بالاعتماد على إختبار أقل فرق معنوي عند مستوى احتمال 0.05، وبينت النتائج ان هناك دور إيجابي ومعنوي لمعاملات التسميد الحيوي *Trichoderma harzianum* *Bacillus subtilis* والاحماض الامينية Arginine،Tryptophan،Phenylalanine ومستخلص حبوب الشعير المستنبتة وتداخلاتها في زيادة تركيز الاحماض الدهنية لزيت بذور الجرجير Palmatic Erucic، Stearic، Oleic ، Lenolic،Linolenic وهذا يحفز الاتجاه نحو الزراعة المستدامة لتعزير القيمة الصحية للزيت فضلاً عن الحفاظ على البيئة والنظام البيئي.

الكلمات المفتاحية: زيت بذور الجرجير، المخصبات الحيوية، الاحماض الامينية، الحبوب المستنبتة.