

Impact of Adding some Biostimulants (Bacteria and Fungi), and Nitrogen Fertilizer, on the Growth and yield of Arugula Plant

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ABSTRACT

The experiment was carried out in Babylon Province, on one of the farms affiliated with the College of Agriculture - Al-Qasim Green. This experiment was conducted during autumn season 2022-2023 in one of the private fields in Al-Azawia in Al-Musayab district it is located 40 kilometers north of the center of Al-Hillah city in Babil Governorate, within specific longitude $44^{\circ} 25' 10.67''$ E and latitude $32^{\circ} 27' 49.21''$ N coordinates, to study the production of arugula organically comparing used nitrogenous chemical fertilizer (urea), within a randomized complete block design (RCBD) with three replicates. And with two factors:- The first factor: control treatment included (without addition) and carrier substances only (40% decomposed palm compost - 30% decomposed sheep waste - 20% poultry waste - 5% charcoal - 5% sawdust) adding the bio-fertilizer that It contains bacteria (50ml.liter^{-1}), Trichoderma (30g), Mycorrhiza (30g) and a combination of microorganisms (bacteria, Trichoderma and mycorrhizal fungi). The second factor: Nitrogen fertilizer (urea) control treatment included (without addition) and the addition of nitrogen fertilizer (urea) a quarter of the recommended (15kg/hectar) and half of the recommended (30kg/hectar). The results showed that: each of these traits increased significantly, such as Plant height, leaf count, leaf area, and dry matter percentage in the first harvest and quantity of yield in the second harvest, total chlorophyll content, and leaves content of soluble total carbohydrate increased upon the addition of combination of microorganisms and nitrogen fertilizer (urea). Carrier materials didn't affect all studied traits significantly.

Keywords: biostimulants, organic agriculture, leafy vegetables, nitrogen fertilizer and carrier materials, waste products.

INTRODUCTION

Arugula is a plant of the family Brassicaceae that grows in temperate areas year-round, except in very hot or cold seasons (1). Arugula is known for its medicinal and nutritional benefits. This plant is cultivated year-round in temperate areas. It grows in areas where it is not as hot or cold as other plants. Bio-fertilizers can improve soil health and nutrient availability, as well as improve plant performance. A bio-fertilizer is a modern agricultural technique that involves the use of products containing one or more species of

beneficial microorganisms, such as bacteria, fungi. These microorganisms can be added to seeds or plants, to soil, or all of them. The purpose of this technique is to use beneficial organisms to improve the chemical, physical, and biological properties of soil. In addition to improving soil health, a bio-fertilizer can also help maintain nutrient balance in agricultural lands. It can convert nutrients into readily available forms that can be used for plant nourishment. This can lead to increased

productivity and lower agricultural production costs (2).

Chemical fertilizers are widely used in agriculture as a main source to increase crop yield. They provide important soil nutrients such as nitrogen, phosphorus, and potassium in an easily digested form, leading to high crop yields. However, overuse of chemical fertilizers has a negative impact on land ecosystems, leading to surface runoff and nutrient loss, which negatively impacts plant growth and decreases microbial diversity (Fungi and Bacteria) (3). Therefore, the objective of this study was to investigate the effects of adding a mixture of microorganisms (Bacteria and Fungi) and nitrogen fertilizer, as well as their interactions, on various parameters of arugula growth and yield during the fall season.

MATERIALS AND METHODS

The experiment was conducted in a private field Al-Azawia in Al-Musayab district. Before the experiment, soil samples were taken from various areas of the field, ranging from 0-30 cm in the root zone. The samples were air-dried and then ground and sieved using a sieve with a diameter of 2 mm. The chemical and physical properties of the

soil were then analyzed. Table 1 shows some of the physicochemical and chemical characteristics of the field soil: Plowing, harrowing, and leveling were used to prepare the experimental field. The field was divided into 3 replicates with 18 experimental units per replicate. Seeds were planted on trays with 5 rows per experimental unit, spaced 50 cm apart and plants within a row were spaced 15 cm apart. The composition of the experiment included: Control Treatment (No addition) Carrier Substances (Decomposed Palm Waste 40%, Decomposed Sheep Waste 30%, Decomposed Poultry Waste 20%, charcoal/wood shavings 5%, Bacteria 50 ml/liter, Trichoderma 30g, Mycorrhiza 30 g. Nitrogen fertilizer (UREA) (without addition) and the addition of nitrogen fertilizer (urea) a quarter of the recommended (15 kg/hectar) and half of the recommended (30 kg/hectar) (4). Following the experiment, three replicates were used for the experiment and two factors. The design of the experiment was a randomized block-based design (R.C.B.D) with three replicates per unit and two factors. The mean comparison was made using the L.S.D test (5% significance level) (5) had used. The statistical analysis was done with the GenStat software.

Table 1 shows some physical and chemical characteristics of field soil before planting.

Values	Units	Traits
2.6	$^3\text{m/ds}$	The degree of electrical conductivity EC
7.3	---	Ph
10.2	g.kg^{-1}	organic matter
13.8	mg.kg^{-1}	available nitrogen
5.6	mg.kg^{-1}	available phosphorous
184	mg.kg^{-1}	available Potassium
1.13	g.cm^3	bulk density
600	g.kg^{-1}	Sand
245	g.kg^{-1}	Silt
155	g.kg^{-1}	Clay

***The field soil was analyzed at the Babylon Agriculture Directorate / Soil Analysis Laboratory.**

Studied traits

Plant height (cm)

The plant height (cm) was determined by taking five randomly selected plants from each unit. From the soil contact area to the highest point of the plant two months after planting, the height was measured using metric tape. The average height was then calculated.

Number of Leaves (Leaves. Plant⁻¹)

Five randomly selected leaves were taken from each unit, and the average of the leaves was calculated.

Leaves area (cm²)

The area was determined using Corkbores on a dry weight method (6). 10 leaves were taken from 5 randomly chosen plants in each test

unit. 1 cm diameter discs were punched out of each leaf to yield 20 leaf discs. The discs were dried in the electric oven for 72 hours until they reached a constant weight. The leaf area was determined by dividing the dry weight of 10 leaves by the area of 20 leaf discs. This area was then divided by 10 to yield the area per leaf. The area per plant was calculated by multiplying the area per plant by its average number of leaves.

Dry matter percentage (g.100 g fresh weight⁻¹)

The dry matter percentage was calculated by weighing the leaves while fresh and drying them in the electric oven at a constant temperature for 72 hours. The total vegetative mass percentage was calculated as (Dry weight of plant) / (Fresh weight of plant) × 100 (7)

Yield per experimental unit (kg / 6 m²)

Cutting of plants at the ground level of each experimental unit Direct weighing of plants in the field.

Total chlorophyll content of Leaves (mg. 100g fresh weight⁻¹)

The chlorophyll in the leaves was estimated by taking five random samples from the leaves for each experimental unit according to the method of (8), and washed with a water well to remove suspended dust, then 1 gm was taken from each sample, and 20 ml of acetone at a concentration of 85% was added to it. The leaf sample was crushed with a ceramic mortar, then a solution was isolated. Dyes on plant tissue with filter paper, then take the filtrate leave the sediment, and complete the volume to 20 ml of acetone, then the dyes were estimated using a spectrophotometer to measure the optical absorption of the total chlorophyll dye after adjusting the device at two wavelengths (663) and 645) nm, and then according to the dye concentration according to the following equation:

$$\text{Total chlorophyll} = 20.2 * D(645) + 8.02 * D(663) (V/W * 1000) * 100$$

: Since

(663) D = optical absorption reading at a wavelength of 663 nm

(645) D = optical absorption reading at a wavelength of 645 nm

V = final volume of extract (20 mL)

W = tissue weight (1g)

Leaves content of total carbohydrates (mg.gm⁻¹ dry weight)

The amount of total soluble carbohydrates in the leaves was estimated according to the method of. (9), where 250 mg was taken by

dry weight and ground from each experimental unit, then 10 ml of distilled water was added to it, then it was discarded in a centrifuge and the filtrate was taken, and the volume was added to 10 ml of distilled water. 1 ml of the filtrate was added to 1 ml of phenol reagent with a concentration of 5% and 5 ml of sulfuric acid with a concentration of 80%, then the samples were left for 25 minutes at room temperature, after which the intensity of the optical absorption was measured at a wavelength of 490 nm, and Blank was prepared in the same way as before, but without adding the sample, the standard curve was prepared using glucose to calibrate the readings

Statistical analysis

The results of the experiment were analyzed statistically to estimate the effect of each of the factors of the study represented by the addition of biological and nitrogen fertilizer and the interaction between them and for all characteristics according to the method of analysis of variance (ANOVA) and the design of (RCBD). The statistical analysis program Genstat V12.1 (10) was used.

RESULTS AND DISCUSSION**Plant height (cm)**

Table 2 shows significant differences between the bio-fertilizers and chemical fertilizers (nitrogen) for the plant height trait: Bio-fertilizers treatment B6 showed statistically significant superiority compared to the other experiment treatments for the average plant height (66.84 cm) compared to control treatment B1 (28.73 cm). Nitrogen fertilizer treatment A3 significantly increased the plant height (49.26 cm), compared to control treatment A1 (43.82 cm). In addition, the interaction effect (bio-fertilizer + nitrogen fertilizer) showed a significant effect on plant height (70.70 cm) compared with control treatment A1 B1 (25.70 cm).

Table 2: Effect of Adding Bio-fertilizer and Nitrogen Fertilizer, and Their Interaction, on Plant Height (cm) of Arugula Plants for the Fall Season 2022-2023

Average Nitrogen fertilizers	Bio fertilizers						Nitrogen fertilizers
	B6	B5	B4	B3	B2	B1	
43.82	63.10	38.20	54.10	47.10	34.70	25.70	A1
46.88	66.73	41.30	55.77	51.00	37.77	28.73	A2
49.26	70.70	43.07	58.63	51.43	39.97	31.77	A3
	66.84	40.07	56.17	49.84	37.48	28.73	Average Bio fertilizers
B		A*B		A		L.S.D(0.05)	
1.049		1.942		1.211			

Number of Leaves (Leave.Plant⁻¹)

Table 3 shows how bio-fertilizers and chemical fertilizers (nitrogen) interact to affect the leaves per plant. Bio-fertilizer treated with B6 produced the highest average leaves per plant at 28.50 (Leave. Plant⁻¹), significantly higher than control treatment B1 at 12.54(Leave. Plant⁻¹) Nitrogen fertilizer

treatment also had a significant effect on leaves per plant. Treatment with nitrogen fertilizer addition (A3) produced the highest average (Leave. Plant⁻¹) at 20.68, significantly higher than the control treatment (A1B1) at 17.98. The interaction treatment (A3B6) produced the highest at 30.00, significantly higher than A1B1 at 11.63(Leave. Plant⁻¹).

Table 3: Effect of Adding Bio-fertilizer and Nitrogen Fertilizer, and Their Interaction, on the Leaves Count (Leave. Plant⁻¹) of Arugula Plants for the Fall Season 2022-2023

Average Nitrogen fertilizers	Bio fertilizers						Nitrogen fertilizers
	B6	B5	B4	B3	B2	B1	
17.98	26.93	14.60	21.86	18.46	14.43	11.63	A1
19.33	28.56	16.70	23.70	19.30	15.26	12.46	A2
20.68	30.00	17.53	24.96	20.56	17.50	13.53	A3
	28.50	16.27	23.51	19.44	15.73	12.54	Average Bio fertilizers
B		A*B		A)0.05(L.S.D	
0.320		0.553		0.308			

Leaf Area (cm²/plant⁻¹)

Table 4 shows that the studied factors (bio-fertilizer addition, nitrogen fertilizer addition, and interaction) had a significant effect on leaf area per plant. B6 had the highest average leaf

area of 4538 (cm² / plant⁻¹), compared to control treatment B1 2487 (cm²/ plant⁻¹). The addition of nitrogen fertilizer had a significant effect as well. Treatment with nitrogen fertilizer addition (A3) showed the highest

average ($3912 \text{ cm}^2/\text{plant}^{-1}$) compared to control treatment A1 ($3536 \text{ cm}^2/\text{plant}^{-1}$) and Table 4 also shows that the interaction effect (bio-fertilizer + nitrogen fertilizer) was

significant for leaf area. The interaction treatment (A3B6) showed the highest leaf area ($4738 \text{ cm}^2/\text{plant}^{-1}$), which exceeded the control treatment (A1B1) ($2141 \text{ cm}^2/\text{plant}^{-1}$).

Table 4: Effect of Adding Bio-fertilizer and Nitrogen Fertilizer, and Their Interaction, on Leaf Area ($\text{cm}^2/\text{plant}^{-1}$) of Arugula Plants for the Fall Season 2022-2023

Average Nitrogen fertilizers	Bio fertilizers						Nitrogen fertilizers
	B6	B5	B4	B3	B2	B1	
3556	4346	3239	4237	4240	3133	2141	A1
3773	4530	3331	4455	4454	3294	2574	A2
3912	4738	3345	4646	4644	3354	2744	A3
	4538	3305	4446	4446	3260	2487	Average Bio fertilizers
B 3.737		A*B 7.226		A 5.492)0.05(L.S.D	

Percentage of Dry Matter for the First Harvest (g. 100 g fresh weight⁻¹)

Table 5 shows significant differences in dry matter percentage for the initial harvest. B6 had the highest average dry matter percentage (15.48%) compared to B1 (10.56%). Adding

nitrogen fertilizer had a significant effect, with A3's average percentage (13.77%) being the highest, compared to A1's average (13.05%) and A2's (13.54%). The same table shows significant interaction effects, with A3 having the highest average (15.82%) and A1 B1 having the lowest (10.24%).

Table 5: Effect of Adding Bio-fertilizer and Nitrogen Fertilizer, and Their Interaction, on the Percentage of Dry Matter for the First Harvest (g. 100 g fresh weight⁻¹) of Arugula Plants for the Fall Season 2022-2023

Average Nitrogen fertilizers	Bio fertilizers						Nitrogen fertilizers
	B6	B5	B4	B3	B2	B1	
13.05	15.17	13.25	14.24	14.21	11.21	10.24	A1
13.54	15.44	13.43	14.87	14.52	12.35	10.63	A2
13.77	15.82	13.74	14.84	14.81	12.57	10.83	A3
	15.48	13.54	14.65	14.51	12.04	10.56	Average Bio fertilizers
B 0.131		A*B 0.237		A 0.157)0.05(L.S.D	

Yield of Experimental Unit from Leaves for the Second Harvest (kg / 6 m²)

Table 6 shows significant differences in the experimental unit yield from leaves for the 2nd harvest. The two factors under study, bio-fertilizers and nitrogen fertilizers, and their interactions, have shown significant effects. Bio-fertilizer treatment B6 produced the highest average yield from the experimental unit from the 2nd harvest at 7.61 kg/6 m².

compared to control treatment B1 at 2.58 kg/ 6 m². The addition of nitrogen fertilizers also produced a significant effect. Nitrogen fertilizer treatment A3 achieved the highest average yield 5.20 kg/6 m² compared to control treatment A1 at 4.63/6 m². The table also shows significant interaction effects between bio-fertilizers and nitrogen fertilizers for the second harvest at 7.95kg/6m² for interaction A3B6 vs. control A1B1 at 2.25kg/6

Table 6: Effect of Adding Bio-fertilizer and Nitrogen Fertilizer, and Their Interaction, on the Yield of Experimental Unit from Leaves for the Second Harvest (kg / 6 m²) of Arugula Plants for the Fall Season 2022-2023

Average Nitrogen fertilizers	Bio fertilizers						Nitrogen fertilizers
	B6	B5	B4	B3	B2	B1	
4.63	7.24	3.33	5.23	6.45	3.32	2.25	A1
4.92	7.64	3.56	5.53	6.63	3.55	2.65	A2
5.20	7.95	3.85	5.84	6.93	3.83	2.84	A3
	7.61	3.58	5.53	6.67	3.56	2.58	Average Bio fertilizers
B 0.032		A*B 0.059		A 0.040)0.05(L.S.D	

Total Chlorophyll Content of Leaves (mg. 100g Fresh Weight⁻¹)

Table 7 shows the significant effects of factors under study (bio-fertilizer + nitrogen fertilizer + control treatment) on total chlorophyll (mg.100g new weight⁻¹): Bio-fertilizer Treatment B6 had the highest average value (52.49 mg.100g new weight⁻¹) compared to control treatment B1 (26.47 mg.100g new Weight⁻¹) compared to A1B1 (25.03 mg. 100g Fresh Weight⁻¹)

weight⁻¹). Nitrogen Fertilizer Treatment A3 (Nitrogen fertilizer + control treatment A1) had the highest average (42.46 mg. 100g fresh weight⁻¹), compared to control treatment A1 (39.87 mg. 100g new weight⁻¹) Table 7 shows significant interaction effect between bio-fertilizers and nitrogen fertilizer: Interaction Treatment A3B6 had the highest value (53.61 mg.100g Fresh

Table 7: Effect of Adding Bio-fertilizer and Nitrogen Fertilizer on the Total Chlorophyll Content of Leaves (mg. 100g Fresh Weight⁻¹) of Arugula Plants for the Fall Season 2022-2023

Average Nitrogen fertilizers	Bio fertilizers						Nitrogen fertilizers
	B6	B5	B4	B3	B2	B1	
39.87	51.28	40.77	44.51	42.91	34.73	25.03	A1
41.35	52.58	41.77	45.71	45.28	36.06	26.73	A2
42.46	53.61	42.75	47.03	46.21	37.54	27.66	A3
	52.49	41.76	45.75	44.80	36.11	26.47	Average Bio fertilizers
B 0.321		A*B 0.681		A 0.518)0.05(L.S.D	

Leaves content of total carbohydrates (mg.gm⁻¹ dry weight)

Table 8 shows the significant effects of factors under study (bio-fertilizers, nitrogen fertilizers, and their interactions) on total carbohydrate (mg. g⁻¹ dry weight) in leaves: Bio-fertilizers: Treatment B6 had the most significant effect (17.52 mg. g⁻¹ dry weight),

compared with the control treatment (6.77 mg. g⁻¹ dry weight). Nitrogen Fertilizers: Nutrient fertilizer treatment (A3) had the largest effect (13.56 mg. g⁻¹ dry weight), compared with control treatment (A1) (11.82 mg. g⁻¹ dry weight). Interactions: Table (A3B6) shows the most significant interaction effect (18.45 mg. g⁻¹ dry weight), compared with control A1B1 (6.18 mg. g⁻¹ dry weight).

Table 8: Effect of Adding Bio-fertilizer and Nitrogen Fertilizer on the Total Soluble Carbohydrate Content in Leaves (mg. g⁻¹ dry weight) of Arugula Plants for the Fall Season 2022-2023

Average Nitrogen fertilizers	Bio fertilizers						Nitrogen fertilizers
	B6	B5	B4	B3	B2	B1	
11.82	16.43	11.23	14.44	14.20	8.43	6.18	A1
12.77	17.67	12.39	15.62	15.17	9.47	6.68	A2
13.56	18.45	13.44	15.84	15.70	10.48	7.44	A3
	17.52	12.35	15.18	15.02	9.46	6.77	Average Bio fertilizers
B 0.079		A*B 0.135		A 0.069)0.05(L.S.D	

Through Tables (2-8), it can be observed the addition of microorganisms and nitrogen fertilizer had a significant positive impact on arugula's growth characteristics, including leaf formation and elongation. This was attributed to organic fertilizers increasing soil nutrient concentrations, particularly nitrogen, which played a crucial role in DNA construction, protein production, cell division stimulation, and auxin production, resulting in increased plant height and leaf count (11). Adequate nitrogen and phosphorus in the soil result in taller and healthier plants (12). This aligns with what Khalil et al. (13) mentioned about the essential role of nitrogen for numerous vital elements and organic compounds, including chlorophyll formation, protoplasm development, new cell generation, and elongation. Nitrogen deficiency, on the other hand, leads to weak growth and small leaves.

The enhancement in vegetative growth, including leaf area and leaf number, can be attributed to the application of bio-organic fertilizers. These fertilizers supply organic materials around the root zone, facilitating the absorption of organic matter and promoting increased vegetative growth by raising plant height and leaf area. This, in turn, enhances photosynthesis. Additionally, bio-organic fertilizers contribute to improved soil chemistry and physical properties, increased water retention capacity, and elevated levels of major nutrients in the soil. (14). Plants positively respond to bacterial inoculation in terms of seed germination, root development, leaf area, chlorophyll content, and root weight (15). (16) also indicated that dual bacterial and fungal inoculation of cress plants stimulates vegetative growth. These findings correlate with (17) on spinach and (18) on dill, where they highlighted the significant effect of bio-organic fertilizers in enhancing vegetative growth and plant height. (19) showed that the combined effect of bio-factors on basil led to significant growth enhancement in terms of plant height, branch count, fresh and dry weight, chlorophyll content, and

carbohydrates compared to no addition. (20) found that combining bio, organic, and mineral fertilizers had a significant positive impact on pea plant growth. The triple fertilization treatments with full or partial mineral fertilization outperformed dual fertilization treatments, as well as treatments with only bio, mineral, or organic fertilizers. These combined fertilization approaches led to significant improvements in both growth and yield attributes compared to the control treatment. As for the addition of nitrogen fertilizer, it positively affected all studied attributes. This is due to its high solubility, and when added appropriately, it increases the plant's mineral content. The significant increase in growth attributes can be attributed to the key role of organic fertilizers in increasing nutrient concentrations in the soil with a particular emphasis on nitrogen. Nitrogen plays a key role in the formation of leaves, leaf extension, DNA synthesis, and protein synthesis, confirming the centrality of nitrogen in the growth process (21,22). Moreover, the positive influence of bio-organic fertilizers has been established in terms of fostering enhanced vegetative growth. By providing essential organic materials in the rhizosphere area, these fertilizers contribute to increased nutrient absorption and subsequent vigorous growth, as evidenced by heightened plant height and leaf area (22). The intertwined effects of these fertilizers on soil properties, water retention capabilities, and nutrient enrichment further enhance their impact on plant growth and development. Bacterial inoculation has also emerged as a significant factor in promoting favorable outcomes in arugula cultivation. From improved germination to heightened root development, leaf area, and chlorophyll content, these positive effects demonstrate the potential of bacterial influences in optimizing plant growth (21,23). Furthermore, the inclusion of nitrogen fertilizer has proven to be a game-changing aspect. Its soluble nature and strategic application have led to a remarkable increase in plant mineral content,

aligning with an array of studies emphasizing its crucial role in promoting biochemical processes essential for robust vegetative growth (22,23). This comprehensive investigation has unveiled the intricate network of interactions within the plant-soil-microorganism continuum. It serves as a testament to the multifaceted role that bio-organic fertilizers, bacterial inoculation, and nitrogen fertilization play in shaping the growth and development of arugula. Such insights are invaluable for sustainable agricultural practices, highlighting the significance of harnessing these interactions to enhance crop yield, quality, and overall productivity (21,22,23,24).

CONCLUSION

Various aspects of vegetative growth are enhanced by the interaction of bio-organic elements and nitrogen. The central role nitrogen plays in driving crucial growth processes like leaf formation and protein production has been prominently highlighted. These fertilizers provide essential organic materials to the root area. As a result, they enhance nutrient absorption by plants, leading to vigorous growth. This is evident through a greater plant height and a more extensive leaf area. Fertilizers influence soil properties, water retention, and nutrient enrichment. Consequently, they play a vital role in improving plant growth and development.

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