

Effect of Biofertilization with Two Types of Cyanobacteria (*Anabaena* and *Nostoc*) and Their Application Method on the Vegetative and Root Growth Characteristics of Spearmint (*Mentha spicata*)

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Abstract

This study aimed to isolate, purify, and morphologically identify local nitrogen-fixing cyanobacteria (blue-green algae) from various agricultural environments in the Al-Alam district, Salah al-Din Governorate. It also aimed to evaluate their bio-efficacy as fertilizers and extracts for foliar spraying and soil drenching to support vegetative and root growth traits, as well as the essential oil yield of spearmint (*Mentha spicata*), as a sustainable alternative to traditional chemical fertilizers. Materials and Methods: The experiment was conducted in the glasshouse of the College of Education for Women, University of Tikrit, during the 2025-2026 agricultural season. The experiment was designed according to a Randomized Complete Block Design (RCBD) as a factorial experiment involving two factors: the first factor was cyanobacteria of the genus *Nostoc*, and the second was of the genus *Anabaena*. Treatments included three application methods: (foliar spray with extract, soil drench with extract, and a control treatment with distilled water only). Several morphological and physiological indicators were measured at the end of the season, including: plant height, number of leaves, leaf area, fresh and dry weights of the vegetative and root systems, and root length. Results: The results showed a statistically significant superiority for all biofertilization treatments compared to the control treatment. The interaction treatment involving soil drench with both types together (*Anabaena*+ *Nostoc*) recorded the highest significant response in most of the studied traits; it achieved a plant height of 30.67 cm, a 64% improvement over the control, while the number of leaves reached 211 leaves/plant (with the peak for individual superiority of *Nostoc* drench reaching 217.33 leaves/plant). The dry weight of the vegetative shoot was 4.48 g, an increase of 103.55%, while the fresh weight of the shoot was 16.57 g, a 101.82% improvement. Regarding root length and fresh weight, the drench interaction achieved a record root elongation of 39.00 cm (a 129.41% increase) and a root fresh weight of 11.53 g (a 197.14% increase). For root dry weight and leaf area, the same treatment excelled with a root dry weight of 2.58 g (a 256.32% increase) and a leaf area of 2.54 cm².

1. Introduction

Recent decades have witnessed a growing challenge of achieving increased agricultural production while maintaining environmental integrity, which is a central concern of the scientific and international community (1). The escalating global demand for food has intensified agricultural production; however, this trend has been accompanied by notable negative environmental impacts on global ecosystems (2). The excessive use of chemical fertilizers is a common strategy to ensure high crop productivity and

achieve economic profitability for the agricultural sector (3). However, this practice brings serious environmental problems, including greenhouse gas emissions, deterioration of soil and water quality, and loss of biodiversity (4). Additionally, improper use of chemical fertilizers can have significant effects on human health (5). Among the various types of biofertilizers currently available, formulations based on photosynthetic organisms, including cyanobacteria, have garnered significant interest due to their

remarkable ability to improve soil fertility and enhance plant growth, Cyanobacteria are a diverse group of unicellular microorganisms that include a wide range of species, encompassing both prokaryotic cyanobacteria and eukaryotic microorganisms such as cyanobacteria and diatoms (Fernandez, 2020). Due to their photosynthetic capacity, cyanobacteria are considered promising cellular factories capable of efficiently converting carbon dioxide into biomass and a wide range of products, including nutrients, biofuels, and various high-value-added compounds (6). For these properties, cyanobacteria have found widespread application as a valuable biological resource across multiple industries, including the food industry, biofuel production, wastewater treatment, cosmetics, and pharmaceuticals, as well as in the agricultural field (6). Biofertilizers derived from cyanobacteria are rich in micro- and macronutrients, plant hormones, and biologically active compounds, leading to positive biochemical effects in the soil ecosystem through crop-microbiome interactions (5). Furthermore, cyanobacteria possess remarkable nutrient recovery capabilities for their biochemical processes, enabling them to grow and efficiently assimilate phosphorus and nitrogen, even in nutrient-limited environments (15). On the other hand, this remarkable group of organisms contributes to the enrichment of soil organic matter. Cyanobacteria release organic compounds and contribute biomass to the soil, improving its structure, moisture retention capacity, and overall fertility. Additionally, their ability to produce metabolites such as plant hormones, polysaccharides, and others can bring additional benefits to agricultural

Materials and Methods

3-1 Chemicals Used

3-1-1 Liquid BG-11 Medium:

The liquid BG-11 medium was prepared by weighing the chemical compounds

production (Guo, 2020). Cyanobacteria can also be considered biological control agents or biopesticides, due to their ability to effectively control the growth of pathogens, including fungi, bacteria, and nematodes. This is achieved through the production of biocidal compounds, such as benzoic acid, majusculonic acid, and lytic enzymes (7). This study aims to isolate and morphologically identify local cyanobacterial species, measure their nitrogen-fixing capabilities, and evaluate their vital impact on the growth and productivity of spearmint. Despite the potential of cyanobacteria in the agricultural field, sporadic knowledge and a lack of understanding regarding the effects and mechanisms of cyanobacteria on soil and plants under diverse conditions continue to hinder their widespread use in agricultural environments (8).

1-2. Study Objectives:

1. Isolation and purification of different types of cyanobacteria from local agricultural environments.
2. Isolation and identification of atmospheric nitrogen-fixing species.
3. Selection of the most efficient species in nitrogen fixation for use in experiments.
4. Testing and evaluating the efficiency of these species in supporting the growth of spearmint (*Mentha spicata*).
5. Preparation of cyanobacterial extracts for foliar spraying and as a soil biofertilizer.
6. Analysis of changes in growth and quality traits (oil quantity and quality) using Gas Chromatography (GC) or GC-MS.
7. Conducting a preliminary economic and technical analysis for the potential application of the product as a biofertilizer.

mentioned previously in Table (3-1) using a sensitive balance, according to the

concentrations also mentioned in the same table. They were then dissolved in an Erlenmeyer flask containing one liter of distilled water, and 1 ml of the stock solution indicated in Table (3-1) was added. The mixture was stirred using a magnetic stirrer with a hot plate to ensure complete dissolution of the medium components. The pH of the medium was adjusted to a range between 7.3-7.4 using sodium hydroxide (NaOH) or dilute hydrochloric acid (HCl) via a pH meter; the final pH reading was recorded. The

3-2 Solid BG-11 Medium:

The solid medium was prepared by adding agar at a concentration of 10.0 g to the same chemical compounds used for the liquid medium mentioned above, at the same concentrations, in an Erlenmeyer flask containing 1 liter of distilled water. 1 ml of the previously mentioned stock solution was added, and the mixture was stirred using a magnetic stirrer to ensure complete dissolution and mixing of the materials. The pH was also adjusted using

3-3 Sample Collection:

Study samples were collected from different environments within Salah al-Din Governorate / Al-Alam District during the months of October and November. They were transported in sterile bottles. The

3-4 Cyanobacteria Cultivation and Isolation:

The previously collected samples were inoculated by spreading water droplets, submerged soil particles, or rock fragments onto the surface of the agar in Petri dishes (considered the concentrated sample). Then, diluted concentrations were prepared for each sample at ratios of 10, 100, and 1000. The first dilution (10%) was made by adding 1 ml of the sample to 9 ml of distilled water and plating on Petri dishes. Then, 1 ml from the first diluted solution was added to 9 ml to represent the second dilution (100), and 1 ml from the second dilution was added to 9 ml to represent the third dilution (1000). After the initial plating process, the dishes were

medium was then distributed into 250 ml flasks, with 70 ml per flask. The flask openings were plugged with medical cotton and gauze, then covered with cellophane. The flasks were placed in an autoclave for sterilization of the culture media at a temperature of 121°C and a pressure of 1.5 pounds/inch² for 30 minutes. After the sterilization process was completed, the culture media were left in the laboratory at room temperature to cool and become ready for use.

the same method. Afterward, the flask opening was plugged with medical cotton, gauze, and cellophane, and sterilized in the autoclave at 121°C and 1.5 pounds/inch² pressure. After sterilization, the medium was left to cool to 40-50°C, then poured into Petri dishes under sterile conditions near a Bunsen burner. The dishes were left to cool and solidify, thus becoming ready for inoculation.

samples included rocks with algal growth taken from the edge of the Tigris River, a water sample, a clay sample from the river, and a soil sample from cultivated land.

incubated in an incubator at 25°C under continuous illumination with an intensity of 2500 lux. After three to four weeks, cyanobacterial colonies were observed growing in various colors such as green, bluish-green, and blackish-brown. A light microscope was used for the initial identification of the growing colonies. To isolate the cyanobacteria, solid BG-11 media were prepared using the same method mentioned previously. After preliminary identification of the growing colonies, specific colonies were selected for the study. The colonies were transferred using a loop to Petri dishes under sterile conditions near a Bunsen

burner and also incubated at 25°C under continuous illumination at 2500 lux. After a shorter period, the colonies were examined microscopically to confirm their purity. Subsequently, an appropriate amount of the pure cyanobacterial culture from the Petri dish was transferred using a loop into 250 ml Erlenmeyer flasks containing 100 ml of liquid BG-11 medium under sterile conditions and

3-5 Propagation of Cyanobacterial Isolates:

Glass flasks with a capacity of 5 liters were used. 5000 ml of sterile culture medium was added to each flask, and each flask was inoculated with 500 ml of the

3-6 Algal Extract:

The algal extract was prepared according to . The culture medium was filtered using filter paper to obtain the biomass for both genera. The biomass was then divided into two halves: one half for soil drench (applied once) and the other half for foliar

3-7 Experiment Location and Execution:

The research plan was executed in the glasshouse of the scientific departments of the College of Education for Women / University of Tikrit for the 2025-2026

3-8 Soil Preparation and Planting Method:

This experiment was conducted in the glasshouse using plastic pots for cultivation with a height of 25 cm, a diameter of 25 cm, and a capacity of 10 kg of sandy river soil (Zmeej). The number of treatments was 9 interactive treatments,

3-9 Experimental Design:

The experiment was conducted using a Randomized Complete Block Design (RCBD) as a factorial experiment consisting of two factors. Three weeks after planting, once the plants were

3-10 Study Factors:

The experiment included two factors.

3-10-1 First Factor: Nitrogen-fixing cyanobacteria of the genus Nostoc

(Nostoc0: distilled water only; Nostoc w: plant drench with cyanobacterial extract; Nostoc f: plant spray with cyanobacterial extract). The drenching process was carried out once after the plants were

incubated at 25°C with continuous light at 2500 lux until adequate growth was obtained. After obtaining sufficient growth, a larger quantity of liquid medium was prepared for multiplication and to obtain the appropriate amount; liquid medium at five times the initial concentrations was prepared in 5 liters of distilled water using a 5-liter glass flask.

algal sample to be propagated to obtain cultures for the experiments. These flasks were incubated under suitable conditions of pressure and temperature for 30 days .

spray (applied in four applications). The portion used for foliar spraying on the shoot system was placed in distilled water and disrupted using an Ultrasonic Homogenizer for 30 minutes to make it ready for spraying.

agricultural season, from 9/3/2025 to 5/9/2025, to study the effect of two types of nitrogen-fixing cyanobacteria on the growth of spearmint.

with 27 replicates, averaging three replicates per treatment. Spearmint plants were planted in these pots after being rooted for one week in distilled water. Irrigation treatments were carried out according to the plant's needs.

established in the soil, the initial treatments were applied inside the glasshouse. When temperatures rose, the plants were moved outside, and the remaining treatments were completed.

established in the soil on 30/3/2025, using 300 ml per treatment. The spraying process was carried out in four applications: the first on 31/3/2025 using 600 ml per treatment, the second on

7/4/2025, the third on 14/4/2025, and the fourth on 21/4/2025, with a one-week

interval between sprays.

3-10-2 Second Factor: Nitrogen-fixing cyanobacteria of the genus Anabaena

(Anabaena0: distilled water only; Anabaena w: drench; Anabaena f: plant spray with cyanobacterial extract). This was carried out using the same measurements and

dates as the first factor. Afterward, the plants were left to grow further until visual differences in the vegetative growth were observed, and measurements were taken.

3-11 Studied Traits of the Experiment:

3-11-1 Vegetative Growth Traits:

3-11-1-1 Plant Height (cm):

Measured from the point where the stem meets the soil to the growing tip using a

measuring tape at the end of the growing season.

3-11-1-2 Number of Leaves:

This trait was measured by counting the number of leaves at the end of the season.

3-11-1-3 Leaf Area:

Leaf area was measured for 10 leaves per treatment from different replicates. Each

leaf was punched with a cork borer of known diameter.

3-11-1-4 Fresh Weight of the Vegetative Shoot (g):

Measured using a sensitive balance.

3-11-1-5 Fresh Weight of the Root (g):

Measured using a sensitive balance.

3-11-1-6 Dry Weight of the Vegetative Shoot (g):

Performed after drying the sample in a drying oven for two days at 65°C, then measured using a sensitive balance.

3-11-1-7 Dry Weight of the Root (g):

Performed using the same method as for the vegetative shoot.

3-11-1-8 Root Length (cm):

Measured using a measuring tape.

3-11-1-9 Number of Branches:

The number of branches was counted after the plant completed its growth.

3-11-1-10 Percentage of Chlorophyll A and B in Leaves (%):

1 g of fresh leaves was taken and ground in a mortar, and 50 ml of 96% methanol was added. The extract was separated using a centrifuge at 100 rpm, then filtered and separated again at 2500 rpm. It was

measured using a spectrophotometer at wavelengths of 666 nm for chlorophyll a and 653 nm for chlorophyll b, according to .

3-12 Statistical Analysis:

The data were statistically analyzed using a Randomized Complete Block Design (R.C.B.D). Significant differences between means were compared using Duncan's

Multiple Range Test at a significance level of 0.05. The SAS program was used for statistical analysis.

4. Results and Discussion

Vegetative Growth Traits

1 Plant Height Trait (cm)

Table (4-1) shows the effect of drench and foliar spray treatments with *Anabaena* and *Nostoc* on the plant height trait of spearmint. It is clear that the drench

treatments recorded (30.67a, 26.67b) cm for both genera, respectively, while the spray treatments recorded (28.67ab) cm for both treatments.

Table (1) Plant Height Trait (cm)

U0: without addition, Uw: drench treatment, Uf: spray treatment, and similarly for Sa...

<i>Anabaena</i> <i>Nostoc</i>	0	Spray	Drench	Mean of Factor U
0	18.67b (0)%	26.00ab (39.36%)	28.33a	25.22b
Foliar Spray	27.33a (46.40%)	27.67cd (48.19%)	27.33a (46.40%)	26.78a
Soil Drench	26.35ab (42.00%)	26.33ab (41.05%)	30.68a (64.26%)	27.67b
U Factor Mean	24.33a	27.44b	28.11a	

Means that are followed by the same letters indicate no statistically significant differences between them according to Duncan's Multiple Range Test at a probability level of ($p \leq 0.05$).

Plant Height Trait (cm) (Continued)

The results shown in Table (1) demonstrate the effect of spray and drench treatments with both types of cyanobacteria, *Anabaena* and *Nostoc*, on the height trait of spearmint plants. While the highest average plant height in the control treatment was 18.67 cm, the results showed varying positive responses depending on the treatment type. The spray treatment with *Anabaena* extract recorded 26 cm, and the spray with *Nostoc* extract recorded 27.33 cm. No clear difference was observed in the interaction between the two treatments on plant height, as the spray treatment with both *Anabaena* and *Nostoc* recorded 27.67 cm, representing a 48% improvement in mint height compared to the control value. Meanwhile, the drench treatment with *Anabaena* alone outperformed the spray treatments with both types, reaching a

plant height of 28.33 cm, a 51% improvement over the control. The highest recorded mint height was 30.67 cm, achieved under the interaction of the drench treatments with both *Anabaena* and *Nostoc*, representing a 64% improvement in spearmint plant height over the control value. This superiority under the influence of foliar spray and drench treatments with the nitrogen-fixing cyanobacteria *Anabaena* and *Nostoc* can be explained by their role in supporting the plant's need for essential nutrients, especially nitrogen, which is a key element in building amino acids, proteins, and nucleic acids necessary for cell division and elongation. Furthermore, these two species are capable of producing various plant growth regulators such as auxins, gibberellins, and cytokinins, which stimulate cell division and elongation, directly reflected in

increased plant height (28). These results are consistent with those of (9), who indicated that some cyanobacterial species contribute to increased plant growth through nitrogen fixation and production of growth regulators. They also agree with (11) regarding the importance of nitrogen nutrition in enhancing vegetative growth and plant height. (10) also pointed out that biofertilization improves vegetative growth by enhancing nutrient availability and increasing the efficiency of physiological processes within the plant. This improvement in shoot height under the foliar spray and drench treatments with

2 Number of Leaves Trait

The results in Table (4-2) show the effect of spray and drench treatments with both types of cyanobacteria, *Anabaena* and *Nostoc*, on the number of leaves trait of spearmint plants. The average number of leaves in the control treatment was 130.67. The treatments showed varying positive responses depending on the treatment type. The average number of leaves under the spray treatment with *Anabaena* extract alone reached 185 leaves/plant, while the interaction treatment via drench with both *Anabaena* and *Nostoc* together achieved a significant superiority, reaching 211.00 leaves/plant, a 62% improvement compared to the control treatment. However, the peak of significant superiority appeared in the drench. The increase in leaf number and total leaf area enhances the plant's efficiency in capturing light, which positively reflects on photosynthesis rates and dry matter accumulation. Conversely, the low leaf number in the control treatment is directly due to limited nitrogen availability, which suppressed meristematic activity and cell

Anabaena and *Nostoc* can also be attributed to the improvement in root system growth and length, as shown in Table (4-5). Improved root growth, in turn, increases its efficiency in absorbing water and nutrients, leading to improved plant nutritional status and enhanced physiological processes, particularly photosynthesis, respiration, and the synthesis of organic compounds necessary for growth. Additionally, the adequate moisture resulting from drenching helped increase the solubility of nutrients in the root zone and improved their translocation to the aerial parts (28).

treatment with *Nostoc* alone, reaching 217.33 leaves/plant, a 67% improvement over the control. This superiority is attributed to the ability of cyanobacteria to improve plant nutrition with atmospherically fixed nitrogen. Nitrogen is the fundamental building block for the synthesis of amino acids, structural proteins, and chlorophyll, which are essential for producing new vegetative growth. This continuous nitrogen supply stimulates meristematic activity in axillary and lateral buds. Cyanobacteria also play a vital role in secreting exogenous cytokinins, which break apical dominance and activate cell division to form new leaves (27).

division. These results are consistent with the study by (10) and are supported by a recent study by (12), who indicated that fertilization with nitrogen-fixing cyanobacteria increases leaf density in aromatic plants through enhancing endogenous hormones and improving nutritional status.

Table (2) Number of Leaves Trait U0: without treatment, Uw: drench treatment, Uf: spray treatment, and similarly for Sa...

	0	Spray	Drench	Mean of Factor U
0	130.67b (0.15%)	185.00ab (42.3%)	192.67a (48.2i%)	167.44a
Foliar Spray	181.33ab (39.49%)	194.00a (49.24%)	191.33a (47.18%)	189.33a
Soil Drench	217.33a (67.18%)	189.00a (45.38%)	211.00a (62.31%)	198.33a
U Factor Mean	169.44b	188.89ab	205.78a	

3 Dry Weight of the Vegetative Shoot Trait (g)

The results presented in Table (4-3) show a clear significant superiority of the biofertilization treatments in increasing the dry weight of the vegetative shoot of spearmint plants. The control treatment recorded a dry weight value of 2.20 g. Meanwhile, spraying with *Anabaena* extract alone raised the weight to 3.32 g, representing a 50% improvement over the control plants. The superiority peaked with the interaction treatment via drenching with both types together (*Anabaena** and *Nostoc*), which achieved the highest dry weight of 4.48 g, representing a 103% increase compared to the value recorded under the control treatment. The direct physiological interpretation of this increase is closely linked to the efficiency of biological nitrogen fixation due to the direct biological activity of both **Anabaena** and *Nostoc* in the root zone.

This sustainable supply of nitrogen supports the requirements for building chlorophyll molecules and key enzymes such as RuBisCo, responsible for carbon fixation in the Calvin cycle, thereby enhancing photosynthetic efficiency (13). Consequently, the rate of synthesis of carbohydrates and proteins increases, leading to greater accumulation of dry matter in vegetative tissues. Furthermore, the release of organic acids by cyanobacteria contributes to dissolving nutrients and facilitating their uptake (14). These results are consistent with (15) and align with what indicated in their study on medicinal plants, that algal biofertilizers definitively increase the dry weight of the vegetative shoot of plants by improving nitrogen and carbon assimilation efficiency and delaying cellular senescence.

Table (3) Dry Weight of the Vegetative Shoot Trait (g)

	0	Spray	Drench	Mean of Factor U
Foliar Spray	2.20c (0.18%)	3.32b (50.94%)	3.26b (48.18%)	2.89b
Soil Drench	2.96bc (34.69%)	2.94bc (33.85%)	2.64bc (19.78%)	3.12ab
U Factor Mean	3.50b (59.08%)	3.08bc (40.29%)	4.48a (103.55%)	3.46b
	2.93b	2.85b	3.69a	

4 Fresh Weight of the Vegetative Shoot Trait (g)

The results shown in Table (4) demonstrate the variation in the fresh weight of the vegetative shoot trait of spearmint plants when treated with drenching and spraying using extracts of the two mentioned species. A clear response in the fresh weight of the vegetative shoot is observed depending on the different bio-treatments. The control treatment recorded a value of 8.21 g. Meanwhile, the fresh weight increased with the spray treatment using *Anabaena* extract to 11.9 g, representing a 45% increase over the fresh weight value of the control treatment. The fresh weight of the vegetative shoot under the foliar spray treatment with *Nostoc* extract reached 12.72 g, with approximately a 55% improvement. The highest recorded value for fresh vegetative weight was achieved under the interaction treatment of drenching with both types together, reaching 16.57 g, achieving an improvement rate of 101.82% compared to the control treatment. This notable improvement from the effect of the combined drenching bio-treatment on the fresh weight of the vegetative shoot, which

was reflected in the total vegetative mass, is attributed to the sustainable supply of biological nitrogen that the plant received during the experiment. Biologically fixed nitrogen works to increase the content of chlorophyll, proteins, and enzymatic activities, thereby enhancing photosynthetic efficiency (13). The increased production of solutes within cells as a result of optimal nitrogen nutrition raises the cellular osmotic potential, which increases the cells' ability to draw in and retain water within the vacuoles, and this is reflected in increased fresh weight (16). Furthermore, the cyanobacterial promotion of root growth and elongation (Table 4-5) allowed the plant to explore larger areas of soil and absorb greater amounts of water and dissolved nutrients. These results are consistent with what was recently that inoculation of soil with living cyanobacteria improves the plant's water relations and increases the fresh weight of the vegetative shoot due to structural improvement of the rhizosphere and the secretion of polysaccharides that conserve soil moisture around the root.

Table (4) Fresh Weight of the Vegetative Shoot Trait (g)

	0	Spray	Drench	Mean of Factor U
0	8.21c (0.03%)	11.906 (44.89%)	12.98ab (58.10%)	11.61b
Foliar Spray	12.72ab (54.97%)	12.73ab (55.11%)	13.41ab (63.20%)	12.29ab
Soil Drench	13.90ab (69.30%)	12.26b (49.32%)	16.57a (101.80%)	14.32a
U Factor Mean	11.036	11.95ab	14.42a	

5 Root Length Trait (cm)

The results shown in Table (5) indicate highly significant differences between the interaction treatments in their effect on the

root length trait of spearmint plants. The root length under the control treatment was 17.00 cm. Meanwhile, root length

increased with the spray treatment using *Anabaena* extract to reach 24.33 cm, while the drench treatment reached 29.33 cm, representing a 72% increase. For the spray treatment with *Nostoc* extract, root length reached 22.33 cm, and the drench treatment reached 26.33 cm, with a 54% improvement. The maximum root length was achieved under the interaction treatment of drenching with both types together (*Anabaena* and *Nostoc*), reaching 39.00 cm, with a high improvement rate of 129.41% compared to the value recorded in the control treatment. This improvement in root growth, development, and elongation in spearmint plants can be explained by the direct function and biofertilization activity of the living cyanobacteria in the rhizosphere zone near the root system. Both *Anabaena* and *Nostoc* secrete considerable amounts of

endogenous auxins (especially indole-3-acetic acid, IAA), which directly stimulate the division and elongation of root cells and the formation of lateral roots and root hairs (17). The active localized fixation of nitrogen in the soil provides a nutrient-rich environment that drives the root to elongate and explore vertically and horizontally to absorb water and nutrients. The increased root length and branching increase the absorbing surface area, which positively reflects on the growth of the entire shoot system, as indicated by (16). These results also agree with recent research, such as the study by (18), which confirmed that cyanobacterial inoculation alters the morphological structure of roots and significantly increases their length and density as a result of regulating the gene expression related to auxin receptors in the plant.

Table (4-5) Root Length Trait (cm)

	0	Spray	Drench	Mean of Factor U
0	17.00b (0.00%)	24.33b (43.15%)	29.33ab (72.55%)	22.33b
Foliar Spray	22.33b (31.37%)	25.33b (49.02%)	26.33b (54.90%)	24.00b
Soil Drench	26.33b (49.02%)	22.33b (31.37%)	39.00a (129.41%)	31.56a
U Factor Mean	23.56a	25.44a	28.89a	

6 Fresh Weight of the Root Trait (g)

Table (6) presents the results of the fresh weight of the root of spearmint plants after foliar spray treatment with extracts of both *Anabaena* and *Nostoc* and drenching with them in their living form. Significant differences are observed depending on the treatment type, with an increase in the fresh weight of the roots. The control treatment recorded a value of 3.88 g. Spraying with *Anabaena* extract increased the fresh root weight to 6.26 g, while spraying with *Nostoc* extract resulted in 5.71 g, representing percentage increases of 61% and 47%, respectively. The

interaction treatment via drenching with both types together recorded the highest fresh root weight, reaching 11.53 g, with a record percentage increase of 197% compared to the control value. This significant superiority of the drenching interaction treatment is attributed to the active positive role of both cyanobacterial species, *Anabaena* and *Nostoc*, in improving the physiological efficiency of the roots. Biofertilization through drenching places these atmospheric nitrogen-fixing microorganisms in direct contact with the root tissues, allowing for

an abundant supply of fixed nitrogen that accelerates meristematic cell divisions, formation of new cells, and accumulation of organic matter within root tissues (Taiz et al., 2015). Undoubtedly, the increased branching and elongation of roots increase their content of absorbed and stored water in the vascular tissues (xylem and

phloem). This result is consistent with what (19) mentioned and aligns with the study (20), who demonstrated that biofertilization with cyanobacteria increases the wet biomass of roots as a result of integrated nitrogen nutrition and improved rhizosphere capacity to retain water around living plant tissues.

Table (6) Fresh Weight of the Root Trait (g)

	0	Spray	Drench	Mean of Factor U
0	3.88C (0%)	6.26bc (61.35%)	7.05b (81.82%)	5.66b
Foliar Spray	5.71bc (47.07%)	6.30bc (62.33%)	7.206 (85.65%)	8.59a
Soil Drench	7.40b (90.68%)	5.22bc (34.47%)	11.53a (197.14%)	5.93b
U Factor Mean	5.73b	6.40c	8.05a	

4-1-1-7 Dry Weight of the Root Trait (g)

The results presented in Table (7) show a clear positive significant effect of the bio-treatments on the dry weight of spearmint roots. The control treatment recorded a root dry weight of 0.73 g. Meanwhile, the foliar spray treatment with Nostoc extract alone raised the dry weight to 1.40 g. The foliar spray treatment with Anabaena recorded 1.67 g, while the spray and drench treatments with Anabaena recorded (1.31, 1.58) g, respectively. This indicates that the highest value recorded for the individual effect was under the drench treatment, with improvement rates in root mass increase of 130% for Nostoc and 116% for Anabaena compared to the control treatment. The foliar spray treatment with both types recorded 1.55 g, a 114% improvement, while the drench treatment under the interaction of both types recorded 2.58 g, representing an improvement rate of 256% compared to the control value. This record increase in root dry weight is associated with increased flow and accumulation of carbohydrates and organic materials

synthesized in the leaves, transported through the phloem to the root system, as a result of the enhanced photosynthetic efficiency in the vegetative shoot supported by fixed nitrogen. The abundant nitrogen provided by the cyanobacterial species Anabaena and Nostoc enabled the plant to produce structural proteins, cellulose, and lignin, helping to form denser and more robust root tissues with a sharply higher percentage of dry matter. The low root dry weight in the control treatment is likely due to weak metabolic activity and limited nitrogen availability, especially since the soil used was washed soil, which restricts carbohydrate transport to the roots. These results are consistent with the work of (21) and are supported by a recent study by (22), which indicated that biofertilization with cyanobacteria creates an ideal nutritional balance (source-sink relationship) that ensures the direction of photosynthetic products toward building a strong and dry root system that enhances plant sustainability.

Table (7) Dry Weight of the Root Trait (g)

	0	Spray	Drench	Mean of Factor U
0	0.73c 0%	1.31bc (80.82%)	1.58b (116.67%)	1.26c
Foliar Spray	1.40b (92.81%)	1.55b (114.07%)	1.47b (102.57%)	1.33b
Soil Drench	1.676 (130.87%)	1.12bc (54.58%)	2.58a (256.32%)	1.78a
U Factor Mean	1.20b	1.47ab	1.79a	

8 Single Leaf Area Trait (cm²)

The results presented in Table (4-8) show the leaf area trait of spearmint, and the significant differences in the effect of treatments on this important trait are observed. The control treatment recorded a leaf area value of 2.11 cm². Meanwhile, the spray treatment with Anabaena extract alone recorded 2.40 cm², with a comparable value for the drench treatment of 2.45 cm² for the same species. The spray treatment with Nostoc extract alone recorded 2.32 cm², also with a similar value for the drench treatment of 2.48 cm². The values recorded under the influence of foliar spray and drench treatments with both types were (2.52, 2.54) cm², which are close values and approximately 40% higher than the values recorded in the control treatment. The physiological explanation for the increase in leaf area can be attributed to the combined pivotal

role of cyanobacteria in supplying nitrogen and producing plant growth regulators. Nitrogen is the fundamental component of nitrogenous bases and amino acids necessary for cell division in the leaf blade. Additionally, the cytokinins and auxins secreted by Anabaena and Nostoc play a major role in stimulating cell elongation and expansion in leaves and delaying their senescence, giving the leaves a greater opportunity for expansion and enlargement (13). The increase in leaf area directly improves photosynthetic efficiency due to the increased light interception area and accumulation of organic materials (16). These results are consistent with recent studies such as that of (23), who confirmed that spraying with cyanobacteria increases leaf area and blade thickness through improving cellular water content and nitrogen availability.

Table (4-8) Single Leaf Area (cm²)

	0	Spray	Drench	Mean of Factor U
0	2.11g	2.40e	2.45d	2.31c
Foliar Spray	2.32f	2.52b	2.44d	2.49a
Soil Drench	2.48C	2.526	2.54a	2.48b
U Factor Mean	2.32c	2.43b	2.52a	

9 Number of Branches Trait (branch/plant)

The results in Table (9) for the number of branches of spearmint plants showed clear

significant differences between treatments. The control treatment recorded an average

number of branches of 5.33 branches/plant, with a rate of 0.06%. Meanwhile, the number of branches increased with the spray treatment using *Anabaena* extract to 7.33 branches/plant, with a rate of 37.59%, while the spray with *Nostoc* extract reached 6.33 with an 18.82% rate. The drench treatment for the same species reached 8.33 branches/plant with a 56.35% rate. The interaction treatment via drenching with both types together achieved the highest significant mean of 10.67 branches/plant, recording an increase rate of 100.18% (i.e., a doubling of the number of branches) compared to the control treatment. The physiological reason for the abundant branching is the disruption of the inhibitory hormonal balance, where the biologically secreted cytokinin reduces the effect of apical auxin that inhibits the growth of lateral buds, allowing them to develop into fully nutritionally equipped

independent branches. Cyanobacterial inoculation breaks apical dominance in Lamiaceae family plants and increases the number of oil-producing branches (24). Additionally, *Anabaena* and *Nostoc* cyanobacteria produce the cytokinin hormone, which is physiologically responsible for breaking the apical dominance imposed by apical auxin, leading to the stimulation and growth of lateral and axillary buds and the formation of new branches. The number of branches trait is of great importance in spearmint because it increases the total vegetative area and the number of vital glands responsible for synthesizing and secreting essential oils and active compounds. These results are consistent with the study of (25) and are supported by a recent study by (26), who indicated that cyanobacterial inoculation significantly enhances lateral branching in aromatic plants due to the biologically induced hormonal balance.

Table (9) Number of Branches Trait

	0	Spray	Drench	Mean of Factor U
0	5.33c (0.06%)	7.33bc (37.59%)	6.33cd (18.82%)	6.67a
Foliar Spray	6.33bc (18.82%)	6.33bc (18.82%)	(25.08%)	7.11a
Soil Drench	8.33ab (56.35%)	7.67bc (43.84%)	10.67a (100.13%)	7.89a
U Factor Mean	6.33b	6.44b	8.89a	

5-1 Conclusions

1. Biofertilization with cyanobacteria possesses high nutritional and physiological stimulatory capacity, which was positively reflected on the vegetative and root growth traits of spearmint plants. The soil drench application method (*Nostoc w*) was superior to the foliar spray method (*Nostoc f*) in most morphological and weight traits.

2. The interaction between soil drench application with cyanobacteria for both

factors (*Anabaena w* / *Nostoc w*) achieved the best significant response in vegetative and root growth traits, contributing to increased root length, number of leaves, fresh and dry weights, and improved leaf area. This was a result of stimulating biological activity in the root zone and increasing the efficiency of water and nutrient uptake.

5-2 Recommendations

1. Adopting the interaction treatment (Nostoc w / Anabaena w) as an effective agricultural strategy to improve vegetative and root growth and increase the dry matter and leaf yield of spearmint plants using cyanobacterial biofertilization.

2. Conducting future studies to evaluate the effect of cyanobacterial biofertilization on the physical and chemical properties of the soil, such as organic matter content, pH, and water holding capacity, as well as studying its effect on beneficial soil microorganisms.

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