

## Effect of biofertilizer inoculation and foliar application of Moringa leaf extract on vegetative growth and leaves nutrients of spinach

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### Abstract

The experiment was conducted at Station (A) of the College of Agricultural Engineering Sciences, University of Baghdad, during the 2023–2024 fall season to investigate the effect of foliar application of Moringa leaf extract and biofertilizer inoculation on the vegetative growth and some chemical traits of spinach. The study included two factors: the first factor was seed inoculation with biofertilizers, which consisted of four treatments: no inoculation (control) (0), inoculation with mycorrhizal fungi (M1), inoculation with Azotobacter bacteria (B1), and a combined treatment of mycorrhizal fungi and Azotobacter bacteria (M1B1). The second factor (G0, G1 and G2) was the foliar application of Moringa (*Moringa oleifera*) leaf extract at three concentrations 0%, 7.5% and 15%. The experiment was arranged in a randomized complete block design (RCBD) with a factorial structure. GenStat software was used for data analysis and the least significant difference (LSD) was calculated at 5% probability level. The results showed the best performance classified to the combined biofertilizer treatment (M1B1) compared to other treatments in the studied traits. Plants also showed an increase in height (49.08 cm), total leaf (21.29 leaves/plant), dry weight of the vegetative biomass (9.33 g) and leaf area (111.30 cm<sup>2</sup>/plant). Moreover, it considerably increased total chlorophyll content (234.67 mg/100 g fresh weight), nitrogen content in leaves (4.43%), phosphorus content (0.36%), and potassium content (3.31%). Similarly, foliar spraying with Moringa leaf extract at the highest concentration (G2) showed a significant improvement in the studied traits. This included plant height (48.00 cm), total number of leaves per plant (19.67 leaves/plant), dry weight of the vegetative biomass (7.78 g/plant), leaf area (89.28 cm<sup>2</sup>/plant), total chlorophyll content in leaves (219.79 mg/100 g fresh weight), nitrogen percentage in leaves (3.32%), phosphorus percentage in leaves (0.36%), and potassium percentage in leaves (3.74%). Moreover, the two-way interaction effect of bio-fertilizer application and foliar spraying with Moringa leaf extract ((M1B1G2) was significant enhancement for the traits studied. This was reflected in plant height (51.83 cm), total number of leaves per plant (25.20 leaves/plant), dry weight of vegetative biomass (10.00 g/plant), leaf area (118.07 cm<sup>2</sup>/plant), total chlorophyll content in leaves (238.40 mg/100 g fresh weight), nitrogen percentage in leaves (3.45%), phosphorus percentage in leaves (0.37 %), and potassium percentage in leaves (4.36 %). The potential interactions between biofertilizers and extract of Moringa leaf could be beneficial in improving spinach growth and chemical composition.

**Keywords:** Sustainability, Consumption and production, clean food, Azotobacter, Mycorrhizal fungi

## Introduction

Spinach [*Spinacia oleracea* L.] is one of the economically important leafy vegetable crops of Chenopodiaceae family. It is highly prized for its nutritional value and is an important therapeutic agent contributing to many physiological processes in the human body. Its native area in Asia, Possibly Persia, spread to China in 674 BCE and to Europe in the 12th century [1], a chlorophyll in spinach make it rich in energy pigment that gave its characteristic green and also has many beneficial properties, effects like anti-inflammatory and detoxification. Spinach is also a rich source of the antioxidant compounds vitamin C, carotenoids and flavonoids that could help explain some of its health benefits. It is also a source of some important nutrients and minerals such as: iron, magnesium, potassium, calcium [2.]

Biofertilizers promote vegetative growth by the supply of nutrients to plant thereby resulting in increased yield and quality of the crop [3]. Inoculation of okra plants with mycorrhizae including root fungi led to significant improvement of vegetative characteristics of okra plants such as plant height, total number of leaves and leaf area [4]. Moreover, AL-Samarai et al. [5] highlight the importance of mycorrhizae in the performance of local okra variety “Btera.”

In a study by Abdul-Alhussein [6] it has been found that the level of the evaluated characteristics were higher in okra treated with mycorrhizal fungi. In a similar way, the beneficial influence of mycorrhizal inoculation on studied characters of potato plants was mentioned [7]. According to Al-Khafaji and Al-Jubouri [8], mycorrhizae had a great impact on lettuce plant characteristics. Moreover, Al-Rukabi and Al-Jebory [9]

determined that the biofertilizers, such as the natural nitrogen-fixing bacteria : *Azotobacter*, were very important in stimulating plants measuring more length, leaf number and dry vegetative weight of the green bean crops (*Phaseolus vulgaris*). Moreover, *Azotobacter* bacteria have strong effects on the vegetative characteristics and yield of eggplant [10]. Additionally, Al-Dolaimy et al. [11] observed the positive effect of *Azotobacter* bacteria and mycorrhizal fungi on the nitrogen, phosphorus, and potassium (NPK) content in the leaves of cauliflower (*Brassica oleracea* var. botrytis). Similarly, Kazem and Abed Mutar [12] highlighted the significant role of *Azotobacter* in increasing the NPK content in the leaves of spinach.

Foliar nutrition had a significant effect on vegetative growth and yield of lettuce plants [13]. Similarly, all characteristics studied were extremely affected by foliar spraying in sweet corn [14]. The applied foliar in the studied traits caused considerable increase of onion plants [15]. According to Al-Ubaidy and Mohammed [16], the foliar spraying of organic nutrients was significantly effective on all traits of red cabbage (*Brassica oleracea* var. capitata f. rubra). Similarly, Al-Mharib et al. [17] deepened by the confirmed findings that the growth indicators, yield, and qualitative and quantitative traits of spinach were significantly improved with the percentage increase after the spraying of organic nutrients. One of the most commonly used natural products in the agricultural production is *Moringa* (*Moringa oleifera*) leaf extract, which has been shown to greatly increase the vegetative growth, productivity, quality, and nutritional value of several crops around the globe [18, 19]. As reported by Mohammed and Majeed [20], foliar

application of different extracts, particularly Moringa (*Moringa oleifera*) leaf extract, significantly increased plant height, number of leaves and total yield in strawberry (*Fragaria × ananassa*). Likewise, Al-Tamimi [21] noted the beneficial effect of separating leaf plants on application PL-AE, indicating the potential of these plant extracts as natural biostimulants in improving leaf yield production. Evaluation of the effects of Moringa leaf extract on growth and productivity in cabbage was studied [22], the foliar spray significantly increased most of the evaluated traits ( $P <$

0.05), such as plant height, number of leaves, leaf length, total yield, and marketable yield, when compared to the control treatment. Moringa extract sprayed on leaves also increased nutrient uptake and N, P, and K concentrations in cabbage heads.

According to the previous results, Therefore, the study aimed to investigate the extent of the impact of the study factors, represented by the addition of bio-fertilizers and spraying with Moringa leaf extract, and the interaction between them, on nutrient concentration in the leaves and vegetative growth of spinach .

## Materials and Methods

The experiment was carried out at Station (A) of the College of Agricultural Engineering Sciences, University of Baghdad during autumn season 2023–2024. The factorial trial followed a Randomized Complete Block Design (R.C.B.D.) in the conduction of the experiment. The study included two factors:

.1 Seed inoculation with biofertilizers , which consisted of four treatments:

- No inoculation (control, labeled as 0,(
- Inoculation with mycorrhizal fungi (labeled as M1,(
- Inoculation with Azotobacter bacteria (labeled as B1,(
- Combined treatment of mycorrhizal fungi and Azotobacter bacteria (labeled as M1B1.(

.2 Foliar spraying with Moringa leaf extract, applied at three concentrations (0%, 7.5%, and 15%), labeled as G0, G1, and G2, respectively.

The treatment was replicated three times, resulting in 12 treatment combinations ( $3 \times 4$ ). Treatments were randomly distributed across the replicates, yielding a total of 36 experimental units. Each experimental unit contained 180 plants. Seeds of a local spinach variety were directly sown in the field on October 23, 2023. All standard soil management practices were carried out, and a drip irrigation system was used. Plants were fertilized with 25% of the recommended amount of chemical fertilizers.

## Results and Discussion

### Vegetative growth Traits

#### Plant Height (cm)

The results in Table (1) indicate that the addition of biofertilizers significantly increased plant height. The treatment M1B1 outperformed all other treatments, achieving the highest value of 49.08 cm. This was not significantly different from the treatment M0B1. In contrast, the control treatment M0B0 (no inoculation) recorded the lowest value at 37.36 cm.

Similarly, the results shows that foliar spraying with Moringa leaf extract significantly increased plant height. The treatment G2 surpassed all other treatments, achieving the highest value of 48.00 cm, while the control treatment G0 recorded the lowest value at 40.17 cm.

Furthermore, the interaction between biofertilizer application and Moringa leaf extract spraying significantly influenced plant height. The treatment M1B1G2 achieved the highest value of 51.83 cm, which was not significantly different from the treatments M1B1G1, M0B1G2, and M0B1G1. On the other hand, the M0B0G0 treatment recorded the least value at 28.58 cm.

#### Leaves Total Number (Leaves/Plant)

The data proved a very noticeable effect of biofertilizers on total number of leaves per spinach plant (Table 1). The highest value was for the treatment M1B1 (21,29 leaf plant-1), which was the outperforming treatment. However, M0B0 (12.16 leaf plant-1), the control treatment, had the lowest value. Moringa leaf extract spray also significantly increased the total number of leaves. The G2 (19.67 leaf plant-1) treatment performed best out all treatments, while the G0 (15.35 leaf

plant-1) control treatment performed the lowest. In addition, the evaluated interaction between the application of biofertilizer and spraying with Moringa leaf extract had a significant effect on the total number of leaves.

The highest value achieved by M1B1G2 (25.20 leaf plant-1) and the lowest value to M0B0G0 (7.73 leaf plant-1). These results emphasize the combined effect of Moringa leaf extract and biofertilizer treatments on increasing leaf yield in spinach.

#### Vegetative Biomass Dry Weight (g)

The results displayed in table (1) showed that the application of biofertilizers had a significant effect on the dry weight of vegetative biomass in spinach. The M1B1 treatment had the highest value (9.33 g) and the M0B0 treatment had the lowest (5.76 g). In the same context, foliar application of Moringa leaf extract greatly increased the dry biomass. Maximum dry weight (7.78 g) was recorded in G2 (15%) and minimum (6.80 g) was recorded in G0 (0%).

This effect was even more pronounced when biofertilizer was applied in conjunction with Moringa leaf extract. Dry weight (10.000 g) was highest in the M1B1G2 treatment which did not differ significant from M1B1G1, whereas the lowest was recorded (5.26 g) in the M0B0G0 treatment.

#### Plant Leaf Area (cm<sup>2</sup> plant-1)

The results indicate that the addition of biofertilizers significantly increased the leaf area per plant (Table 1). The treatment M1B1 outperformed all other treatments, achieving the highest value of 111.30 cm<sup>2</sup> plant-1, while the control treatment M0B0 recorded the lowest value at 59.13 cm<sup>2</sup> plant-1.

Similarly, foliar spraying with Moringa leaf extract significantly increased the leaf area per plant. The treatment G2 surpassed all other treatments, achieving the highest value of 89.28 cm<sup>2</sup> plant<sup>-1</sup>, while the control treatment G0 recorded the lowest value at 72.10 cm<sup>2</sup> plant<sup>-1</sup>.

Furthermore, the interaction between biofertilizer application and Moringa leaf extract spraying significantly influenced the leaf area per plant. The treatment M1B1G2 achieved the highest value of 118.07 cm<sup>2</sup> plant<sup>-1</sup>, which was not significantly different from M1B1G1. Conversely, the treatment M0B0G0 recorded the lowest value at 37.80 cm<sup>2</sup> plant<sup>-1</sup>.

Total Chlorophyll Content in Leaves (mg per 100 g fresh weight)

Results in Table 1 indicate that the biofertilizer application has increased the total

chlorophyll content of spinach leaves. The M1B1 treatment recorded the highest Chlorophyll Content (234.67 mg per 100 g of fresh weight) while the M0B0 treatment recorded the lowest (178.90 mg per 100 g of fresh weight). Similarly, foliar application with the Moringa leaf extract improved chlorophyll content. The highest value (219.79 mg per 100 g of fresh weight) was recorded in G2, while the lowest (201.06 mg per 100 g of fresh weight) was observed in G0 (0%).

The interaction between biofertilizer application and Moringa leaf extract further increased chlorophyll content. M1B1G2 treatment had the highest value (238.40 mg per 100 g fresh weight) of chlorophyll content, not significant from M1B1G1, M0B1G2, and M0B1G1. However, the minimum content (162.43 mg/100 g fresh weight) was recorded in M0B0G0 treatment.

**Table 1: Effect of biofertilizers, foliar application of Moringa leaf extract, and their interaction on the vegetative traits of spinach.**

Treatments	Plant Height (cm)	Total Number (leaves)	Leaf per	Vegetative dry weight (g)	Leaf Area (cm <sup>2</sup> plant <sup>-1</sup> )	Total Chlorophyll Content (mg)
M0B0	37.36	12.16		5.76	59.13	178.90
M0B1	48.72	19.41		7.46	82.37	230.15
M1B0	42.19	17.83		6.64	73.30	199.58
M1B1	49.08	21.29		9.33	111.30	234.67
<b>L.S.D 0.05</b>	<b>1.99</b>	<b>1.34</b>		<b>0.50</b>	<b>4.74</b>	<b>3.72</b>
G0	40.17	15.35		6.80	72.10	201.06
G1	44.86	18.00		7.31	83.19	211.62
G2	48.00	19.67		7.78	89.28	219.79
<b>L.S.D 0.05</b>	<b>1.72</b>	<b>1.19</b>		<b>0.43</b>	<b>4.10</b>	<b>3.22</b>
M0B0G0	28.58	7.73		5.26	37.80	162.43
M0B0G1	39.50	13.87		5.76	67.27	184.93
M0B0G2	44.00	14.87		6.26	72.33	189.33
M0B1G0	45.58	18.73		7.03	79.73	222.10
M0B1G1	50.17	19.53		7.53	82.23	232.83
M0B1G2	50.42	19.97		7.83	85.13	235.53
M1B0G0	41.67	16.33		6.33	66.57	191.33
M1B0G1	39.17	18.53		6.56	71.73	191.50
M1B0G2	45.75	18.63		7.03	81.60	215.90
M1B1G0	44.83	18.60		8.60	104.30	228.37
M1B1G1	50.58	20.07		9.40	111.53	237.23
M1B1G2	51.83	25.20		10.00	118.07	238.40
<b>L.S.D 0.05</b>	<b>3.45</b>	<b>2.39</b>		<b>0.87</b>	<b>8.21</b>	<b>6.44</b>

#### Chemical Traits

##### Nitrogen Percentage in Leaves (%)

The addition of biofertilizers significantly increased the nitrogen composition in spinach leaves, as shown in Table (2). Treatment M1B1 recorded the highest nitrogen content (4.43%), while treatment M0B0, the control treatment, recorded the lowest (3.03%).

The foliar application of Moringa leaf extract markedly increased the percentage of nitrogen in spinach leaves. The highest value (3.32%) was recorded in G2 (15%), while the lowest (3.10%) was observed in G0 (0%).

Furthermore, the interaction between biofertilizer application and Moringa leaf extract spraying significantly influenced the nitrogen percentage. The treatment M1B1G2 achieved the highest value of 3.45%, which

was not significantly different from M1B1G1. Conversely, the treatment M0B0G0 recorded the lowest value at 2.73%.

##### Percentage of Phosphorus in Leaves(%)

Results show that the use of biofertilizers caused a considerable increase in phosphorus content of spinach leaves (Tab. The highest phosphorus content (0.36%) was recorded in treatment M1B1, while the lowest (0.28%) was recorded in treatment M0B0, the control treatment.

Similarly, foliar application of Moringa leaves extract showed more increase in spinach leaves phosphorus percentage. On the other hand, for phosphorus, G2 treatment was higher than other concentrations with 0.36%, and G0 (no spraying) was the lowest with 0.28%.

The phosphorus percentage was increased more due to interaction of biofertilizer application and Moringa leaf extract spraying. In terms of phosphorus content, the M1B1G2 treatment reached the highest concentration of 0.37% (not significantly different from the other treatments). However, M0B0G0 treatment was found to have the lowest total phosphorus content of 0.21%. Therefore, the synergistic effect of type of biofertilizers and Moringa leaf extracts significantly promoted phosphorus uptakes and physiology of spinach plants. This may be due to enhanced root development and higher effectiveness of nutrient uptake assisted by biofertilizers along with the growth enhancing activity of Moringa leaf extract.

#### Potassium Percentage in Leaves(%)

Results of the study is shown in Table 2 revealed that the potassium percentage of spinach leaves enhanced significantly due to the biofertilizer application. Highest potassium (3.31%) was reported in M1B1 treatment while minimum (3.02%) was from M0B0 treatment. In spinach leaves, the percentage of Potassium was significantly greater after the Foliar Presentation of Moringa leaf extract. Maximum (3.74%) and minimum (3.39%) were recorded in G2 (15%) and G0, respectively.

Compared to K application alone, the combination of biofertilizer and Moringa leaf extract was even more effective in increasing potassium content in leaves. The highest potassium percentage (4.36%) was observed in M1B1G2, whereas the lowest (2.87%) was recorded in M0B0G0.

**Table 2: Effect of Biofertilizers, Foliar Application of Moringa Leaf Extract, and Their Interaction on Some Chemical Traits of Spinach.**

<b>Treatments</b>	<b>Nitrogen Percentage in Leaves (%)</b>	<b>Phosphorus Percentage in Leaves (%)</b>	<b>Potassium Percentage in Leaves (%)</b>
<b>M0B0</b>	3.03	0.28	3.02
<b>M0B1</b>	3.32	0.35	3.57
<b>M1B0</b>	3.14	0.30	3.42
<b>M1B1</b>	4.43	0.36	3.31
<b>L.S.D 0.05</b>	<b>0.03</b>	<b>0.01</b>	<b>0.01</b>
<b>G0</b>	3.10	0.28	3.39
<b>G1</b>	3.27	0.33	3.62
<b>G2</b>	3.32	0.36	3.74
<b>L.S.D 0.05</b>	<b>0.03</b>	<b>0.01</b>	<b>0.02</b>
<b>M0B0G0</b>	2.73	0.21	2.87
<b>M0B0G1</b>	3.17	0.32	2.10
<b>M0B0G2</b>	3.21	0.33	3.11
<b>M0B1G0</b>	3.28	0.35	3.34
<b>M0B1G1</b>	3.32	0.35	3.63
<b>M0B1G2</b>	3.36	0.36	3.74
<b>M1B0G0</b>	3.07	0.25	3.39
<b>M1B0G1</b>	3.15	0.30	3.40
<b>M1B0G2</b>	3.20	0.35	3.49
<b>M1B1G0</b>	3.34	0.32	3.96
<b>M1B1G1</b>	3.45	0.37	3.36
<b>M1B1G2</b>	3.50	0.40	4.62
<b>L.S.D 0.05</b>	<b>0.06</b>	<b>0.02</b>	<b>0.01</b>

## Discussion

The results in Tables 1 and 2 demonstrated that the , M1B1G2 treatment had the highest vegetative growth traits, such as plant height, total number of leaves, dry weight of vegetative biomass, leaf area, and total chlorophyll in leaves as compared to the other treatments. Likewise the higher levels of nutrient content (N, P, K) in spinach leaves were attained under this treatment. The synergistic effect of mycorrhizal fungi with Azotobacter and foliar application of Moringa leaf extract improved vegetative growth and some chemical properties of spinach. The increase in plant growth and nutrients could be

attributed to higher availability of nutrients in the soil as biofertilizers help to improve the chemical, physical and biological properties of soil leading to more nutrient uptake and enhanced plant growth and productivity [23, 24]. Therefore, the synergistic effect of type of biofertilizers and Moringa leaf extracts significantly promoted phosphorus uptakes and physiology of spinach plants. This may be due to enhanced root development and higher effectiveness of nutrient uptake assisted by biofertilizers along with the growth enhancing activity of Moringa leaf extract.

According to Al-Silmawy and Abdul-Ratha [25], mycorrhizal fungi and Azotobacter-



inoculated treatments improved essential nutrient uptake, predominantly phosphorus and nitrogen. Utilization of mycorrhizal fungi as inoculums has significant impact in improving growth and yield of plants and reduction of chemical fertilizers.

Mycorrhizal fungi play a crucial role in the mobilization and increased solubility and uptake efficiency of phosphorus. This is particularly relevant with regard to Iraqi soils, since phosphorus is often in short supply because it is fixed in a complex of insoluble forms. Mycorrhizal fungi are capable of solubilizing various unavailable phosphate sources, thereby increasing phosphorus availability for plants [26]. Azotobacter bacteria can also facilitate plant growth from different mechanisms, such as nitrogen fixation, phosphate solubilization, and converting unavailable phosphorus to the plant's absorbable forms have been proven by research and studies. Azotobacter also produces plant growth regulators, which facilitate vegetative growth. In addition to such direct effects, Azotobacter plays a role in enhancing soil physical characteristics, inhibiting plant pathogens, and deactivating toxic elements. As a result of these positive characteristics, it is recognized as one of the best bio-stimulants and microbial inoculants for increase crop production ([27].

This advancement could be ascribed to the foliar nutrition that provides the plants

## Conclusion

The results from the current study demonstrated that increased both vegetative growth and chemical characters for spinach by using biofertilizers ( treated with mycorrhizal fungi and Azotobacter bacteria) + foliar spray of Moringa leaf extract. M1B1G2 (inoculating

with fundamental nutrients that are required for better vegetative growth [28]. Similarly, Al-Ubaidy et al. [15] found increases in nutrient content in plant leaves due to foliar application. Moreover, Sura and Al-Hilfy [29] reported that the extract of the Moringa leaf is known to be rich composition of macro- and micronutrients such as nitrogen, calcium and magnesium as well as vitamins. These nutrients participate directly in photosynthesis during the formation of chlorophyll molecules; they increase photosynthetic efficiency. Therefore, the vegetative growth in Moringa extracted foliar was superior to the Control treatment. Ahmed et al. [30], stated that the Moringa leaf extract could be a natural, safe, and effective biostimulant that would be used in organic farming; Their findings showed that Moringa extract foliar application significantly enhanced different growth traits: vegetative and reproductive, as well as yield factors such as plant height, leaf area, total leaf number, dry biomass, and total yield.

Additionally, Moringa extract application enhanced antioxidant levels and increased the concentration of plant growth hormones, particularly auxins, gibberellins, and cytokinins, in plant leaves. These factors contributed to improved growth performance and overall crop productivity.

with mycorrhizal fungi and Azotobacter bacteria plus 15% Moringa leaf extract) outperformed with the highest values of plant height, total leaf number, dry weight, leaf area, chlorophyll content, and nutrients (N, P, K) compared to all other treatments. These

improvements can be attributed to the synergistic effects of biofertilizers in enhancing nutrient availability and soil health combined with the growth-promoting effects of Moringa leaf extract

According to the study findings and conclusion, it is possible to achieve higher growth, nutrient uptake and yield of spinach (*Spinacia oleracea*) using the combined application of biofertilizer (mycorrhizal fungi

and *Azotobacter*) along with foliar application of Moringa leaf extract (15%). It can be a good alternative for traditional mineral fertilizers that enhance soil health, the quality of crops and minimize environmental impact. Further studies are suggested to explore the long-term effects and applicability of this approach in different soil conditions and crop varieties.

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