Effect of biofertilization and foliar application of moringa extract on growth and chemical characteristics of two eggplant cultivars

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Abstract

The experiment was conducted at the Research Station of the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Diyala, during the 2025 growing season to investigate the effect of biofertilizer application and foliar spraying with aqueous moringa extract on the growth and yield of two eggplant hybrids grown under protected cultivation. The experiment included 12 treatments: two eggplant hybrids (Nasr and Wissam) as the first factor, application of *Bacillus mucilaginosus* at 10 mL compared with the control as the second factor, and foliar spraying with aqueous moringa extract at concentrations of 0, 20, and 40% as the third factor. The experiment was arranged as a factorial trial using a randomized complete block design (RCBD) with a split-split plot system. Results showed that hybrid V1 significantly excelled in plant height (80.60 cm) and anthocyanin content (95.83 mg 100 g^{-1}), while hybrid V2 recorded a higher number of lateral branches (5.033 branch $plant^{-1}$). No significant differences were observed between hybrids in total leaf area and fiber percentage. The biofertilizer treatment significantly improved plant height (81.07 cm), number of lateral branches (5.311 branch $plant^{-1}$), total leaf area (411.6 dm^2 $plant^{-1}$), fiber content (2.881%), and anthocyanin content (103.1 mg 100 g^{-1}). Foliar application of moringa extract showed superior performance, achieving the highest values in plant height (85.63 cm), number of lateral branches (5.766 branch $plant^{-1}$), total leaf area (502.1 dm^2 $plant^{-1}$), anthocyanin content (114.6 mg 100 g^{-1}), and fiber percentage (2.957%).

Keywords: Plant growth-promoting,, *Bacillus mucilaginosus*, foliar nutrition, phytochemical content

Introduction

Eggplant (*Solanum melongena* L.) is an important vegetable crop belonging to the family Solanaceae,

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which comprises about 90 genera and nearly 2000 species. The crop is characterized by a relatively long growing season and is considered a heavy feeder, requiring substantial amounts of soil nutrients. Eggplant fruits are widely consumed in developing countries due to their high nutritional and medicinal value, as they are rich in potassium, iron, and several vitamins including A, B_1 , B_2 , and B_5 (1). Crop is planted in tropical and temperate zones and is planted in summer as an open field crop and in winter as a covered cultivated crop that has grown substantially to meet year round market demand (2). Iraq is estimated to have a cultivated area of 51, 000 dunums and produces about 215,744 tons (3).

Cultivar selection is considered to be one of the greatest ways of determining the productivity and quality of the crops since genetic make-up is considered as the determinant that separates the possible level of crop yields and the characteristics of the fruits (4). The current research in horticulture is therefore being focused more on improvement of yielding by use of high yielding cultivars and hybrids with fine consumer characteristics (5). Higher requirements of the horticultural production of the world with the low chemical loading were more urgent during the recent years, which prompts the necessity to use the more environment-friendly alternatives in the form of plant extracts. Among them, leaf extract of *Moringa oleifera* has gained much attention as a natural plant growth regulator since it contains a large amount of not only the auxins but also phytohormones (zeatin [cytokinin]) in addition to gibberellins, vitamins, organic acids, minerals and antioxidants, which can individually promote plant growth, productivity and resistance to environmental stresses (6).

Moreover, the application of biofertilizers (beneficial microorganisms which enhance the provision of nutrients in the soil) can be used to enhance plant growth and yield .These microorganisms also aid in decomposition of nutrients not accessible to all plants especially those that are essential say nitrogen, phosphorus, potassium and sulfur . One of the potential substitutes is biofertilizers, which are reportedly sustainable and environmentally friendly; therefore, they are gaining popularity in most regions of the world. Some typical types of microorganisms are bacteria, fungi and cyanobacteria that are utilized to increase the potential of soil and the tolerance of plants to environmental problems (7).Following the modern tendency on sustainable and environment-friendly agricultural systems, the study in question was meant to determine the reaction of two hybrids of eggplants stored in unheated plastic houses to biofertilizer application and moringa aqueous extract application as foliar sprays, and to determine the influence on their vegetative growth, yield and fruit quality.

Materials and Methods

A field experiment was conducted during the 2025–2026 growing season in a plastic greenhouse at the Research Station of the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Diyala, to evaluate the effect of biofertilizer (*Bacillus mucilaginosus*) and foliar spraying with *Moringa oleifera* aqueous extract on the growth and yield of two eggplant hybrids grown under protected cultivation.

The soil was prepared by plowing, harrowing, and leveling. Well-decomposed farmyard manure was incorporated into the soil at a rate of 20 m^3 $dunum^{-1}$. The greenhouse area was 207 m^2 (23 × 9 m) and divided into three blocks. Eight ridges were prepared with 75 cm spacing between ridges and a 1 m central walkway. A drip irrigation system (GR type) with 40 cm emitter spacing was used. Before manure application and planting, soil samples were collected from a depth of 0–30 cm and analyzed for selected physical and chemical properties in a specialized laboratory, as shown in Table 1.

Table 1. Selected physical and chemical properties of the greenhouse soil

Measured trait	Value	Unit
PH	7.57	-
EC	7.75	Ds m^{-1}
Available Nitrogen	18.4	mg kg^{-1}
Available Phosphorus	56.65	mg kg^{-1}
Available Potassium	85.05	mg kg^{-1}
Organic matter	1.3	%
Calcium carbonate ($CaCO_3$)	132	g kg^{-1}

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Soluble ions		
Na	1.69	(mmol L^{-1})
K	0.59	
Mg	6.08	
Cl	1.62	
Sand	41.00	g kg^{-1}
Clay	17.00	
Silt	42.00	
Soil texture	Sandy loam	-

Experimental Treatments

The study was conducted in a plastic greenhouse under protected cultivation to evaluate the effects of three factors on two eggplant hybrids:

1. **Cultivar (Factor 1):** Two eggplant hybrids were used, which were registered, Nasr (V1) and Wissam (V2). The seeds were acquired in the Vegetable Research Station, the Ministry of Agriculture.
2. **Application of biofertilizer (Factor 2):** 10 mL L^{-1} *Bacillus mucilaginosus* was used and a control (B0) was used . The Microorganism was chosen, and it was cultured in the Microbiology Laboratory of University of Diyala Department of Soil Science, in a nutrient mixture that contained glucose (5 g L^{-1}), $MgSO_4$ (0.5 g L^{-1}), $CaCO_3$ (0.1 g L^{-1}), $FeCl_3$ (0.005 g L^{-1}) and calcium phosphate (2 g L^{-1}) The pH was adjusted to 7.2, sterilized at 121°C in 20min and left to cool. Root inoculation (10 mL/per plant) was performed in the case of transplantation (16/10/2025). Bacteria were added two weeks after transplanting
3. **Moringa leaf extract (Factor 3):** *Moringa oleifera* weigh powdered the leaf was extracted in the aqueous to make an extract . It was done in three concentrations (0% (M0), 20% (M1), and 40% (M2)). The powder (100 g) was first wetened in 1 L water in 24 hours then filtered and diluted to the desired concentrations .

Spraying of foliars commenced 1/11/2025 and sprayed after every two weeks until the expiry of the growing season, number of sprays reached seven during the growing season. The experiment was a factorial design of a randomized

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complete block design (RCBD) and split plots split split.

Measured Traits

Measurements were taken from random samples consisting of five plants per experimental unit for each replicate. The studied traits included the following:

1. **Plant height (cm)** Plant height was measured using a measuring tape at the end of the growing season, from the point of stem attachment to the soil up to the highest growing tip, and the average was calculated.
2. **Number of lateral branches (branch plant^{-1})** The number of lateral branches formed on the main stem was counted at the end of the growing season, and the final average was recorded.
3. **Total leaf area (dm^2 plant^{-1})** Leaf area was estimated using the gravimetric method based on fresh weight. Five fully expanded leaves were sampled and weighed using a sensitive balance. Discs of known area were then cut from these leaves using a circular punch (1 cm diameter) and weighed. Leaf area was calculated using the following equations:

Leaf area per leaf = (Fresh weight of leaves × Area of cut discs) / Fresh weight of cut discs ÷ 5

Total leaf area (dm^2) = Leaf area per leaf × Number of leaves per plant

4. **Anthocyanin content in fruit peel (mg 100 g^{-1})** Total anthocyanin content in fruit peels was determined using the pH differential method as described by (8). Absorbance was measured using a UV–Visible spectrophotometer at wavelengths of 520 and 700 nm. The concentration was calculated based on the molar absorptivity of cyanidin-3-glucoside and expressed as mg kg^{-1} dry weight.
5. **Percentage of dietary fiber (%)** Crude fiber content in fruits was determined according to the standard method described by (9) using the sequential acid and alkaline digestion method (Crude Fiber Method). Results were expressed as a percentage (%) of dry weight

Results and Discussion

Plant height

The results presented in Table (2) revealed a significant superiority of

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hybrid V1, biofertilizer application, and foliar spraying with aqueous moringa extract on plant height. Hybrid V1 recorded a significantly higher plant height (80.60 cm) compared to hybrid V2, which showed the lowest value (76.40 cm).Similarly, the application of *Bacillus mucilaginosus* at 10 mL significantly increased plant height (81.07 cm) compared to the control treatment (75.92 cm). Foliar spraying with moringa extract at 40% concentration resulted in the highest plant height (85.63 cm), whereas the control treatment recorded the lowest value (73.83 cm).

The interaction between hybrid V1 and biofertilizer (10 mL) showed a significant increase in plant height (83.55 cm) compared to V1 without biofertilizer, which recorded the lowest value (77.64 cm). The interaction between hybrid V1 and moringa extract at 40% produced the highest value (89.06 cm), while the interaction between hybrid V2 and 0% moringa extract recorded the lowest value (72.03 cm).Furthermore, the interaction between biofertilizer (10 mL) and moringa extract at 40% showed a significant superiority, reaching 87.53 cm compared to the control treatment (70.80 cm). The three-way interaction also showed a significant effect, where the combination of hybrid V1, biofertilizer at 10 mL, and moringa extract at 40% recorded the highest plant height (90.86 cm), compared to the control treatment with hybrid V2, which gave the lowest value (70.33 cm).

Table 1. Effect of cultivar, Biofertilizer and Moringa extract and interaction of Plant height
(cm)

Hybrid (Cultivar)	Biofertilizer mL L⁻ ¹	Moringa extract			Hybrid * Biofertilizer
		0 %	%20	%40	
V1	0	71.26 hi	74.40 g	87.26 b	77.64 c
	10	78.20 f	81.60 d	90.86 a	83.55 a
V2	0	70.33 i	72.06 h	80.20 e	74.20 d
	10	73.73	77.86 f	84.20 c	78.60 b
Hybrid * Moringa extract					Hybrid average
V1		74.73 d	78.00 c	89.06 a	80.60 A

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V2	72.03 e	74.96 d	82.20 b	76.40 B
Biofertilizer * Moringa extract				Biofertilizer average
0	70.80 f	73.23 e	83.73 b	75.92 B
10	75.96 d	79.73 c	87.53 a	81.07 A
Moringa Average	73.83 C	76.48 B	85.63 A	

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

Number of lateral branches

Results in Table (3) showed a significant superiority of hybrid V2, biofertilizer application, and foliar spraying with aqueous moringa extract on the number of lateral branches. Hybrid V2 recorded the highest value (5.033 branch plant^{-1}) compared to V1 (4.611 branch plant^{-1}).

The application of *Bacillus mucilaginosus* at 10 mL significantly increased lateral branches (5.311 branch plant^{-1}) relative to the control (4.333 branch plant^{-1}). Foliar spraying with moringa extract at 40% produced the highest value (5.766 branch plant^{-1}), while the control recorded the lowest (3.983 branch plant^{-1}).

For two-way interactions, the highest values were 5.488 branch plant^{-1} for the interaction between hybrid V2 and biofertilizer, and 6.100 branch plant^{-1} for the interaction between hybrid V2 and moringa extract at 40%. The lowest values were 4.088 and 3.900 branch plant^{-1} for V1 without biofertilizer and V1 with control moringa treatment, respectively. The interaction between biofertilizer at 10 mL and moringa extract at 40% gave the highest value (6.533 branch plant^{-1}), whereas the control recorded the lowest (3.600 branch plant^{-1}). The three-way interaction showed that the combination of hybrid V2, biofertilizer at 10 mL, and moringa extract at 40% produced the highest number of lateral branches (6.866 branch plant^{-1}), whereas the control treatment with hybrid V1 recorded the lowest value (3.533 branch plant^{-1})

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Table 2. Effect of cultivar, Biofertilizer and Moringa extract and interaction of Number of lateral branches (branch plant^{-1}).

Hybrid (Cultivar)	Biofertilizer mL L^{-1}	Moringa extract			Hybrid * Biofertilizer
		0 %	%20	%40	
V1	0	3.533 j	4.066	4.666	4.088 c
	10	4.266 gh	4.933 cde	6.200 b	5.133 a
V2	0	3.666 ij	4.733 def	5.333 c	4.577 b
	10	4.466 fgh	5.133 cd	6.866 a	5.488 a
Hybrid * Moringa extract					Hybrid average
V1		3.900 e	4.500 d	5.433 b	4.611 B
V2		4.066 e	4.933 c	6.100 a	5.033 A
Biofertilizer * Moringa extract					Biofertilizer average
0		3.600 d	4.400 c	5.000 b	4.333 B
10		4.366 c	5.033 b	6.533 a	5.311 A
Moringa Average		3.983	4.716	5.766	

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

Total leaf area

Table (4) indicated a significant effect of biofertilizer at 10 mL and foliar spraying with moringa extract at 40%, while no significant differences were observed between hybrids. The biofertilizer treatment recorded 411.6 dm^2 plant^{-1} compared to 353.9 dm^2 plant^{-1} in the control, whereas foliar spraying with 40% moringa extract gave the highest value (502.1 dm^2 plant^{-1}) compared to the control (304.5 dm^2 plant^{-1}).

For two-way interactions, the highest total leaf area was 424.4 dm^2 plant^{-1} for the interaction between hybrid V1 and biofertilizer at 10 mL, and 540.9 dm^2 plant^{-1} for hybrid V2 with 40% moringa extract. The lowest values were 325.4 and 295.0 dm^2

plant^{-1} for V1 with control biofertilizer and V1 with control moringa extract, respectively. The interaction between biofertilizer at 10 mL and 40% moringa extract reached 512.3 dm² plant^{-1}, whereas the control recorded 243.2 dm² plant^{-1}.The three-way interaction showed that the combination of hybrid V2, biofertilizer at 0 mL, and moringa extract at 40% produced the highest total leaf area (565.7 dm² plant^{-1}), while the lowest value (232.8 dm² plant^{-1}) was recorded for the control treatment with hybrid V1

Table 3. Effect of cultivar, Biofertilizer and Moringa extract and interaction of total leaf area (dm² plant^{-1}) .

Hybrid (Cultivar)	Biofertilizer mL L^{-1}	Moringa extract 0 %	%20	%40	Hybrid * Biofertilizer
V1	0	232.8 f	325.3	418.2	325.4 b
	10	357.2 bcd	407.4 bc	508.6 a	424.4 a
V2	0	253.7 ef	327.7 cde	565.7 a	382.3 ab
	10	374.5	306.2	516.1	398.9 a
Hybrid * Moringa extract					Hybrid average
V1		295.0 d	366.3 c	463.4 b	374.9 A
V2		314.1 cd	316.9 cd	540.9 a	390.6 A
Biofertilizer * Moringa extract					Biofertilizer average
0		243.2 c	326.5 b	491.9	353.9 B
10		365.8 b	356.8 b	512.3	411.6 A
Moringa Average		304.5 C	341.6 B	502.1	

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

Anthocyanin content in fruit peel

Results in Table (5) indicated a significant superiority of hybrid V1, biofertilizer at 10 mL, and foliar spraying with moringa extract at 40%

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on anthocyanin content. Hybrid V1 recorded the highest value (95.83 mg 100 g^{-1}) compared to V2 (91.88 mg 100 g^{-1}). The biofertilizer treatment at 10 mL produced 103.1 mg 100 g^{-1}, whereas the control recorded 84.61 mg 100 g^{-1}. Foliar application of moringa extract at 40% resulted in the highest anthocyanin content (114.6 mg 100 g^{-1}) compared to 73.08 mg 100 g^{-1} in the control.

For two-way interactions, the highest anthocyanin values were 106.0 mg 100 g^{-1} for the interaction between hybrid V1 and biofertilizer, 117.6 mg 100 g^{-1} for V1 with 40% moringa extract, and 125.0 mg 100 g^{-1} for the interaction between biofertilizer at 10 mL and moringa extract at 40%, while the lowest values ranged from 62.33 to 83.55 mg 100 g^{-1}.The three-way interaction showed that the combination of hybrid V1, biofertilizer at 10 mL, and moringa extract at 40% produced the highest anthocyanin content (129.0 mg 100 g^{-1}), whereas the control treatment with hybrid V2 gave the lowest value (62.33 mg 100 g^{-1})

Table 4. Effect of cultivar, Biofertilizer and Moringa extract and interaction of Anthocyanin content in fruit peel (mg 100 g^{-1}) .

Hybrid (Cultivar)	Biofertilizer mL L^{-1}	Moringa extract			Hybrid * Biofertilizer
		0 %	%20	%40	
V1	0	62.33 g	88.33 e	106.3 c	85.66 c
	10	85.33 ef	103.6 c	129.0 a	106.0 a
V2	0	62.33 g	86.00 ef	102.3 c	83.55 d
	10	82.33 f	97.33 d	121.0 b	100.2 b
Hybrid * Moringa extract					Hybrid average
V1		73.83 e	96.00 c	117.6 a	95.83 A
V2		72.33 e	91.66 d	111.6 b	91.88 B
Biofertilizer * Moringa extract					Biofertilizer average
0		62.33 f	87.16 d	104.3 b	84.61 B
10		83.83 e	100.5 c	125.0 a	103.1 A
Moringa Average		73.08 C	93.83 B	114.6 A	

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

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Percentage of dietary fiber

Results in Table (6) indicated a significant effect of biofertilizer at 10 mL and foliar spraying with moringa extract on dietary fiber content, while no significant differences were observed between hybrids. The biofertilizer treatment recorded 2.881% compared to 2.825% in the control, whereas foliar spraying with 40% moringa extract produced the highest value (2.957%) compared to the control (2.750%).

For two-way interactions, the highest values were 2.923% for hybrid V1 with biofertilizer, 2.986% for hybrid V1 with 40% moringa extract, and 3.001% for the interaction between biofertilizer at 10 mL and moringa extract at 40%. The lowest values ranged from 2.736% to 2.822% in the respective control treatments.The three-way interaction showed that the combination of hybrid V1, biofertilizer at 10 mL, and moringa extract at 40% produced the highest dietary fiber content (3.060%), while the control treatment with hybrid V2 gave the lowest value (2.733%)

Table 5. Effect of cultivar, Biofertilizer and Moringa extract and interaction of Percentage of dietary fiber (%) .

Hybrid (Cultivar)	Biofertilizer mL L⁻ ¹	Moringa extract			Hybrid * Biofertilizer
		0 %	%20	%40	
V1	0	2.743 g	2.830 d	2.913	2.828 b
	10	2.786 f	2.923 c	3.060	2.923 a
V2	0	2.740 g	2.813 e	2.913	2.822 b
	10	2.733 g	2.843 d	2.943	2.840 b
Hybrid * Moringa extract					Hybrid average
V1		2.765 e	2.876 c	2.986 a	2.876 A
V2		2.736 f	2.828 d	2.928 b	2.831 A
Biofertilizer * Moringa extract					Biofertilizer

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				average
0	2.741 f	2.821 d	2.913	2.825 B
10	2.760 e	2.883 c	3.001	2.881 A
Moringa Average	2.750 C	2.852 B	2.957	

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P ≤ 0.05).

The results presented above indicate that hybrid V1 outperformed in certain traits, such as plant height and anthocyanin content, whereas hybrid V2 showed superiority in the number of lateral branches. These differences are likely attributed to the distinct genetic compositions of the two hybrids, as each hybrid carries a set of genes that regulate growth patterns. Variation in these genes influences the physiological capacity of the plants to convert photosynthetic products into overall growth, highlighting the critical role of genetic factors in plant development, which is consistent with findings by (10).

Regarding *Bacillus mucilaginosus*, its application at 10 mL significantly enhanced most vegetative and biochemical traits, including plant height, number of lateral branches, total leaf area, anthocyanin content, and fruit dietary fiber percentage. This effect is attributed to the ability of biofertilizers to enrich soil with macro- and micronutrients, as well as plant growth regulators, while stimulating beneficial microbial populations. These microbes secrete compounds such as siderophores, antibiotics, enzymes, antifungal and antibacterial substances, and phytohormones, which collectively enhance plant growth, productivity, and stress resistance. Biofertilizers are classified into several types, including nitrogen-fixing, phosphorus-solubilizing, phosphorus-mobilizing, potassium-solubilizing, potassium-mobilizing, and sulfur-oxidizing microorganisms. The first practical use of a commercial biofertilizer, *Nitragin*, dates back to 1895, marking the initial experiments in this field. The production of biofertilizers requires large-scale cultivation of microorganisms under controlled conditions of pH, temperature, and cell density (11). Specifically, *Bacillus mucilaginosus* plays a crucial role in potassium solubilization, facilitating its uptake by plants. This bacterium is Gram-positive, produces spores and capsules that protect it

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from unfavorable environmental conditions, and is highly effective in the rhizosphere for releasing potassium from minerals into soluble forms available for plant absorption (12). Additionally, it can solubilize phosphate from insoluble mineral sources such as apatite, contributing to improved nutrient availability. *Bacillus mucilaginosus* also participates in nitrogen fixation, thereby supplying essential nutrients necessary for optimal plant growth (13).

Regarding the aqueous extract of *Moringa oleifera*, foliar application at a 40% concentration significantly enhanced all studied vegetative and biochemical traits, including plant height, number of lateral branches, total leaf area, anthocyanin content, and fruit dietary fiber percentage. This superior performance is attributed to the rich nutritional profile of *Moringa*, whose leaves contain ten times more vitamin A than carrots, seventeen times more calcium than milk, seven times more vitamin C than oranges, nine times more protein than milk, twenty-five times more iron than spinach, and fifteen times more potassium than bananas (14).

Furthermore, *Moringa* is a significant source of biologically active phenolic compounds, conferring multiple health benefits (15). The plant also provides essential proteins, carbohydrates, flavonoids, vitamins, beta-carotene, amino acids, and bioactive phytochemicals such as phytosterols, including a rich combination of sitosterol, zeatin, and quercetin (16). These compounds collectively contribute to its effectiveness in enhancing plant growth and improving biochemical attributes

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