

Received 20th April 2026,
Accepted 19th June 2026

Cite this: NJBM. 2026, 2(2), 39-48

Keywords: Azotobacter chroococcum; Foliar feeding; Molybdenum; Copper; Eggplant; Solanum melongena; Biofertilizer; Micronutrients; Sustainable agriculture

Role of Azotobacter chroococcum and Foliar Feeding with Molybdenum and Copper in Improving Growth and Productivity of Eggplant (*Solanum melongena* L.)

Ali Mohsen Essa¹

¹ Department of Chemistry, College of Education, University of Al-Qadisiyah, Al-Diwaniyah, Iraq

Correspondence author email: Email: ali.mohsen.essa@qu.edu.iq

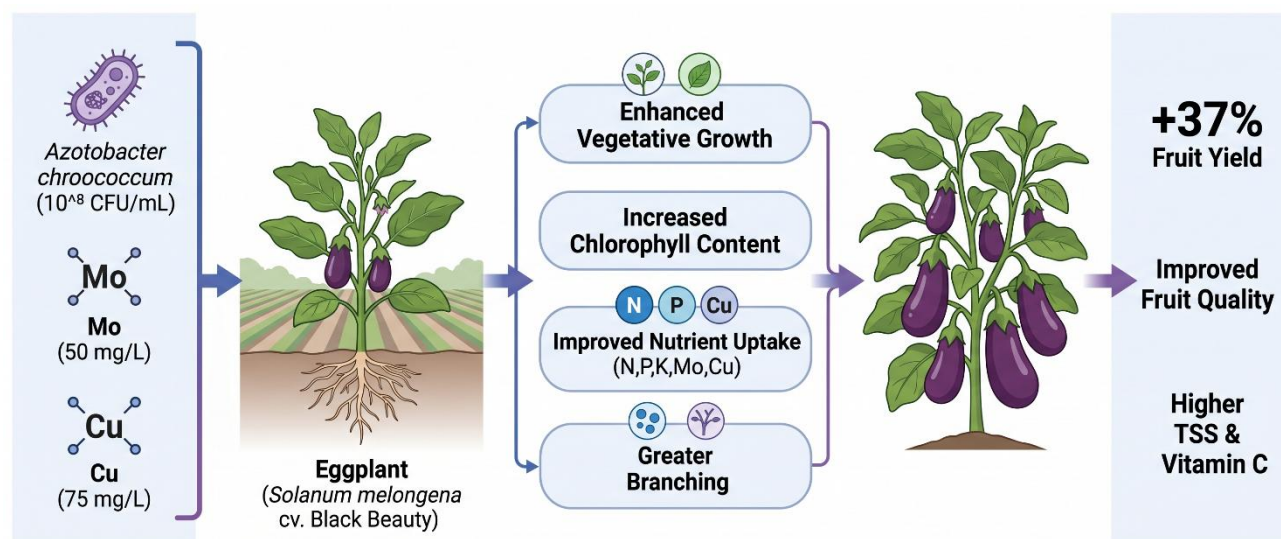
ABSTRACT

The present study was conducted during the two successive spring-summer seasons of 2023 and 2024 at the experimental farm of the College of Agriculture, University of Al-Qadisiyah, Iraq. The aim was to investigate the individual and combined effects of Azotobacter chroococcum inoculation and foliar application of molybdenum (Mo) and copper (Cu) on the vegetative growth, fruit yield and nutrient status of aubergine (*Solanum melongena* L., cv. Black Beauty). The experiment was arranged in a randomised complete block design (RCBD) with three replications. Treatments consisted of two levels of biofertilizer (with and without A. chroococcum) and four foliar feeding levels (control, Mo at 50 mg L⁻¹, Cu at 75 mg L⁻¹ and Mo + Cu). It was found that the tallest plants, number of branches per plant, leaf area, chlorophyll content and individual fruit weight were observed in the plants treated with A. chroococcum + foliar spray of Mo + Cu. Moreover, total fruit yield per hectare was significantly raised with the combination treatment in comparison with the control by 34.6% and 37.3% in the first and second seasons, respectively. The combined application of A. chroococcum and micronutrient foliar feeding caused a significant increase in the uptake of N, P, K, Mo and Cu in leaves. These results imply that the combined application of biofertilizer inoculation with foliar nutrition of micronutrients is a potential green approach to increase aubergine productivity under the semi-arid conditions.

Highlights

- Azotobacter chroococcum inoculation significantly enhanced vegetative growth parameters and fruit yield of eggplant.
- Foliar spraying with Mo (50 mg L⁻¹) and Cu (75 mg L⁻¹) improved chlorophyll content, nutrient uptake, and fruit quality.
- The combined application of biofertilizer and micronutrient foliar spray outperformed individual treatments across both seasons.
- Biofertilizer–micronutrient integration may reduce dependence on synthetic fertilizers in semi-arid horticultural systems.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Eggplant (*Solanum melongena* L.) is one of the most frequently grown Solanaceous vegetable crops in the world with a worldwide production of more than 56 million tonnes yearly and mostly planted in China, India and Mediterranean countries [1,21]. Eggplant fruits are rich sources of vitamins, phenolic acids, flavonoids and anthocyanins associated with different health advantages [22]. But, maintaining high yields under semi-arid field conditions is a big issue and is mainly due to nutrient deficits and dependence on chemical fertilisers which erode soil health over time [16].

Use of biofertilizers is recently getting more attention as environment friendly approach to improve crop nutrition. *Azotobacter chroococcum* a free living diazotrophic bacterium has shown great potential to enhance plant growth through biological nitrogen fixation, production of phytohormones (auxins, cytokinins, gibberellins), phosphate solubilisation and suppression of soilborne pathogens [2,3,4]. *Azotobacter* inoculation has been known to increase crop yields by 10–40% depending on crop species and soil conditions [3,24]. Recent field experiments have similarly confirmed that *A. chroococcum* inoculation enhances growth, nitrogen uptake and yield in vegetable and legume crops while permitting a reduction in mineral fertilizer inputs [41,44,45]. In Solanaceous crops such as tomato and pepper, inoculation with *A. chroococcum* has been observed to considerably improve plant height, fruit output and total soluble solids [1,31].

Micronutrient management using foliar application has been identified as a successful supplementary method together with biofertilization. Molybdenum (Mo) is an essential co-factor of the nitrogenase enzyme in nitrogen-fixing bacteria and the nitrate reductase in plants, directly relating the availability of Mo to the effectiveness of nitrogen assimilation [5,7,35]. Copper (Cu) is another vital mineral that serves as a cofactor for

critical enzymes such as polyphenol oxidase, Cu/Zn-superoxide dismutase, plastocyanin and laccase involved in photosynthesis, respiration, lignin production and antioxidant defence [8,26]. The functions of Mo and Cu in plant physiology have been thoroughly characterised while their combined foliar application with biofertilizer inoculation has not been adequately investigated in aubergine. Hence, the present study was conducted to assess the individual and interactive impacts of soil inoculation with *A. chroococcum* and foliar application of Mo and Cu on the growth, yield and nutritional quality of aubergine under semi-arid Iraqi conditions.

2. MATERIALS AND METHODS

2.1. Experimental site and soil characteristics

The field experiment was conducted at the experimental farm of the College of Agriculture, University of Al-Qadisiyah, Al-Diwaniyah, Iraq ($31^{\circ}58'N$, $44^{\circ}55'E$; 18 m a.s.l.) during the spring–summer growing seasons of 2023 and 2024. The area is characterized by a hot semi-arid climate with mean temperatures ranging between 22 and 45 °C during the experimental period, and limited rainfall. Prior to transplanting, composite soil samples were collected from the upper 0–30 cm layer and analysed following standard methods [28]. The physical and chemical properties of the experimental soil are presented in Table 1.

Table 1. Physical and chemical properties of the experimental soil (0–30 cm depth) before planting.

Property	2023 Season	2024 Season
Texture class	Silty clay loam	Silty clay loam
Sand (%)	18.4	18.4
Silt (%)	46.2	46.2

Clay (%)	35.4	35.4
pH (1:1 soil:water)	7.58	7.62
EC (dS m ⁻¹)	2.84	2.91
Organic matter (%)	1.12	1.08
CaCO ₃ (%)	24.3	23.8
Available N (mg kg ⁻¹)	38.5	40.2
Available P (mg kg ⁻¹)	8.7	9.1
Available K (mg kg ⁻¹)	165	172
Available Mo (mg kg ⁻¹)	0.08	0.09
Available Cu (mg kg ⁻¹)	1.25	1.30

2.2. Plant material and seedling preparation

Seeds of eggplant cv. Black Beauty seedlings were sowed in plastic trays containing peat moss and perlite (2:1 v/v) and grown in a nursery with natural air. The four-true-leaf-stage seedlings (about 40 days after sowing) were transplanted in the experimental plots with a spacing of 60 cm between plants and 80 cm between rows, thereby resulting in a plant density of approximately 20,833 plants ha⁻¹.

2.3. Experimental design and treatments

The experiment was conducted in randomised complete block design (RCBD) with two factors and three replications. The first factor was biofertilizer inoculation with two levels: (i) without *A. chroococcum* (B₀) and (ii) with *A. chroococcum* inoculation (B₁). The second factor was foliar feeding with four levels: (F₀) distilled water (control); (F₁) Mo at 50 mg L⁻¹ as sodium molybdate (Na₂MoO₄·2H₂O); (F₂) Cu at 75 mg L⁻¹ as copper sulphate (CuSO₄·5H₂O); and (F₃) Mo at 50 mg L⁻¹ + Cu at 75 mg L⁻¹. This yielded a total of 8 treatment combinations replicated three times, giving 24 experimental units. Each plot measured 3.2 m × 4.0 m, containing four rows with 20 plants per plot.

Table 2. Description of the eight treatment combinations used in the experiment.

Code	Biofertilizer	Foliar treatment	Concentration
B ₀ F ₀	Without <i>A. chroococcum</i>	Distilled water	—
B ₀ F ₁	Without <i>A. chroococcum</i>	Molybdenum (Mo)	50 mg L ⁻¹
B ₀ F ₂	Without <i>A. chroococcum</i>	Copper (Cu)	75 mg L ⁻¹
B ₀ F ₃	Without <i>A. chroococcum</i>	Mo + Cu	50 + 75 mg L ⁻¹

B ₁ F ₀	With <i>A. chroococcum</i>	Distilled water	—
B ₁ F ₁	With <i>A. chroococcum</i>	Molybdenum (Mo)	50 mg L ⁻¹
B ₁ F ₂	With <i>A. chroococcum</i>	Copper (Cu)	75 mg L ⁻¹
B ₁ F ₃	With <i>A. chroococcum</i>	Mo + Cu	50 + 75 mg L ⁻¹

2.4. Preparation and application of *Azotobacter chroococcum* inoculum

A. chroococcum strain (locally isolated and identified, deposited in the Department of Biology, College of Science, University of Al-Qadisiyah) was cultured on Ashby's nitrogen-free mannitol agar at 28 ± 1 °C for 72 h. Liquid inoculum was prepared by transferring colonies into Ashby's broth and shaking at 150 rpm for 48 h until the cell density reached approximately 10⁸ colony-forming units (CFU) mL⁻¹ [2]. The inoculum was applied by dipping seedling roots in the bacterial suspension for 30 min immediately before transplanting, and a soil drench (50 mL plant⁻¹) was applied around the root zone at 15 and 30 days after transplanting (DAT).

2.5. Foliar feeding

Foliar sprays were applied thrice at 30, 50 and 70 DAT using a hand operated backpack sprayer calibrated to apply about 500 L ha⁻¹. Solutions of sodium molybdate and copper sulphate were made up at the specific concentrations in distilled water, and 0.1% (v/v) Surfactant Tween-20 was added to increase leaf adherence and uptake. Spraying was undertaken in the early morning hours to prevent quick evaporation and leaf burn.

2.6. Cultural practices

All plots were uniformly fertilised with 120 kilogram N ha⁻¹ as urea, 80 kg P 2 O 5 ha⁻¹ as triple superphosphate and 100 kg K 2 O ha⁻¹ as potassium sulphate according to the local recommendations for eggplant growing. Nitrogen was applied in three equal splits during transplanting, at the commencement of flowering and at fruit setting. Drip irrigation system was used for irrigation. During the growing period regular pest and disease management procedures were followed.

2.7. Growth and yield measurements

At 90 DAA, five plants were randomly tagged from each plot for the measurement of the following growth parameters: plant height (cm), number of branches per plant, leaf area per plant (dm²) measured by the disc method, and stem diameter (mm). Average fruit weight (g), fruit length (cm) and fruit diameter (cm) were recorded at each harvest fruit number per plant. Total fruit yield per plot (kg) was totalled across repeated pickings and converted to tonnes per hectare (t ha⁻¹).

2.8. Physiological and biochemical measurements

Total chlorophyll content (SPAD units) was measured on completely grown leaves at 75 DAT using a portable chlorophyll meter (SPAD-502 Plus, Konica Minolta, Japan). Leaf samples for mineral analysis were oven-dried at 70 °C to constant weight, crushed and digested with concentrated H₂SO₄ and H₂O₂. Nitrogen concentration was evaluated by the micro-Kjeldahl method, phosphorus by the vanadomolybdate colorimetric approach, potassium by flame photometry and molybdenum and copper by atomic absorption spectrophotometry [28,29].

2.9. Statistical analysis

Data from both seasons were analysed using GenStat (12th edition) following the factorial RCBD model using analysis of variance (ANOVA). Each of the eight treatment combinations was replicated three times in three randomised blocks, giving 24 experimental units per season; the five plants tagged in every plot (Section 2.7) were averaged so that each plot contributed a single mean value, and the sample size entered into the ANOVA was therefore three replicates (n = 3) per treatment. Before the analysis, the normality of the residuals was confirmed using the Shapiro–Wilk test and the homogeneity of variances was checked using Bartlett’s test, and both assumptions were satisfied. Means were compared using Duncan’s multiple range test at the 5% level of significance [30]. When the homogeneity of variance was established by Bartlett’s test, the analysis of both seasons was combined.

3. RESULTS AND DISCUSSION

3.1. Vegetative growth parameters

The results obtained in Table 3 and Figure 1 showed that biofertilizer inoculation and foliar micronutrient feeding had a substantial effect on vegetative development features of aubergine in both seasons. Plants inoculated with *A. chroococcum* (B1) showed significantly taller plants, more branches, wider stem diameter and higher leaf area compared to non-inoculated controls (B0). In particular, inoculation with *A. chroococcum* enhanced the plant height by 14.2% and 12.8% in the first and second seasons, respectively compared to uninoculated treatment. This improvement could be attributable to the ability of the bacteria to perform biological nitrogen fixation, and to synthesise growth promoting hormones, including as indole-3-acetic acid (IAA), gibberellins and cytokinins, which promote cell division and elongation in the shoot apical meristem [2,3]. These results are in agreement with the results of Sharma et al. [1] who observed that *A. chroococcum* greatly enhanced vegetative growth of greenhouse grown eggplants when paired with reduced chemical fertilisation. Azotobacter-based biofertilizers can promote crop development up to 40% in some species due to their various plant-growth encouraging features [3]. Aasfar et al. [3] have reported the same. Regarding foliar treatments, the maximum values of plant height, number of branches, stem diameter and leaf area were obtained by the combination of Mo + Cu (F₃) followed by Mo alone (F₁) then Cu alone (F₂) and at last

the control with water spraying (F₀). The synergistic effect of Mo and Cu on vegetative development may be explained by the complementarity of the physiological roles of these two minerals. Molybdenum as a component of the nitrate reductase enzyme increases nitrogen uptake in plant tissues and causes stimulation of protein synthesis and vegetative growth [5,7]. On the other hand, copper is involved in photosynthesis via plastocyanin and cell wall lignification via laccase, both required for strong vegetative development [8,26]. These results are in agreement with the results of Shahid et al. [14] who reported that multi-micronutrient foliar fertilisation greatly improved leaf area index and branching of aubergine.

A substantial interaction was observed between *A. chroococcum* and Mo + Cu foliar spray (B₁F₃) which gave the overall highest values for all vegetative growth characters in both seasons. This interaction likely reflects a positive feedback loop wherein Azotobacter-mediated nitrogen fixation and phytohormone release create a more vigorous root system that absorbs Mo and Cu more efficiently, while Mo simultaneously promotes the activity of nitrogenase in the rhizosphere bacteria, amplifying the nitrogen-fixing capacity of *A. chroococcum* [35].

Table 3. Effect of *A. chroococcum* inoculation and foliar application of Mo and Cu on vegetative growth parameters of eggplant during 2023 and 2024 seasons.

Treat.	PH (cm)		Branches		SD (mm)		LA (dm ²)	
	2023	2024	2023	2024	2023	2024	2023	2024
B ₀ F ₀	52.3 g	54.1 g	5.2 f	5.5 f	11.2 g	11.5 g	42.6 g	44.1 g
B ₀ F ₁	58.7 d	60.2 d	6.1 d	6.4 d	12.6 d	12.9 d	48.3 d	49.8 d
B ₀ F ₂	56.4 e	57.8 e	5.8 e	6.1 e	12.1 e	12.4 e	46.1 e	47.5 e
B ₀ F ₃	63.5 c	65.1 c	6.9 c	7.2 c	13.8 c	14.2 c	53.7 c	55.2 c
B ₁ F ₀	60.8 d	62.3 d	6.3 d	6.7 d	13.1 d	13.5 d	50.4 d	52.1 d
B ₁ F ₁	67.2 b	68.9 b	7.4 b	7.8 b	14.5 b	14.9 b	56.8 b	58.5 b
B ₁ F ₂	64.6 c	66.4 c	7.0 c	7.3 c	13.9 c	14.3 c	54.2 c	55.9 c
B ₁ F ₃	73.4 a	75.8 a	8.5 a	8.9 a	16.2 a	16.7 a	63.5 a	65.7 a
LSD 0.05	3.2	3.0	0.5	0.4	0.6	0.5	2.4	2.2

PH = Plant height; SD = Stem diameter; LA = Leaf area. Means within columns followed by different letters differ significantly (P ≤ 0.05) by Duncan’s test.

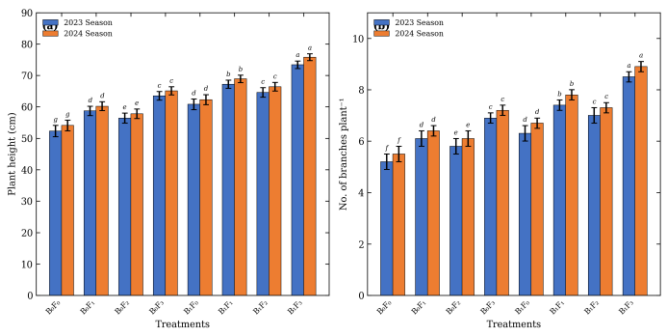


Figure 1. Comparison of (a) plant height and (b) number of branches per plant across biofertilizer and foliar feeding treatments in eggplant during the 2023 and 2024 seasons. Error bars represent $\pm$ SE. Different letters indicate significant differences at $P \leq 0.05$ by Duncan's test.

3.2. Chlorophyll content

Total chlorophyll content as measured by SPAD readings was significantly affected by both experimental factors (Table 4 and Figure 2). The inoculation with *A. chroococcum* increased SPAD values by approximately 11.5% and 10.8% compared with the uninoculated control in the 2023 and 2024 seasons, respectively. This could be due to the improved nitrogen availability mediated by *Azotobacter*, given that nitrogen is a central constituent of the chlorophyll molecule [12]. Among the foliar treatments, the Mo + Cu spray (F₃) gave the highest chlorophyll readings, which was statistically on par with Mo alone (F₁) in the second season. Molybdenum boosts nitrate reductase activity, so more nitrogen is directed towards amino acid and chlorophyll synthesis, while copper is engaged in the electron transport chain of photosystem I via plastocyanin [5,26,38]. This is consistent with recent reports showing that foliar molybdenum raises leaf nitrogen concentration and enhances yield through increased nitrate reductase activity [46]. These present findings are in agreement with recent work of Villora et al. [5] who showed that Mo distribution in eggplant leaves was directly related to nitrogen metabolism and nitrate reductase activity.

The interaction B1 x F3 gave the maximum SPAD values in both seasons which indicates the synergistic effect of combining biological nitrogen fixation with enhanced Mo-dependent enzymatic nitrogen assimilation. The discovery further reinforces the functional relationship between Mo and nitrogenase, where sufficient availability of Mo is needed for the cofactor FeMoCo at the active site of the enzyme [35].

Table 4. Effect of *A. chroococcum* inoculation and foliar Mo and Cu on chlorophyll content (SPAD value) of eggplant.

Treatment	SPAD 2023	SPAD 2024
B ₀ F ₀	38.2 g	39.1 g
B ₀ F ₁	43.5 d	44.2 d
B ₀ F ₂	41.8 e	42.5 e

B ₀ F ₃	46.3 c	47.1 c
B ₁ F ₀	43.1 d	44.0 d
B ₁ F ₁	48.6 b	49.4 b
B ₁ F ₂	46.7 c	47.6 c
B ₁ F ₃	52.4 a	53.5 a
LSD 0.05	1.8	1.7

Means within columns followed by different letters differ significantly ($P \leq 0.05$) by Duncan's test.

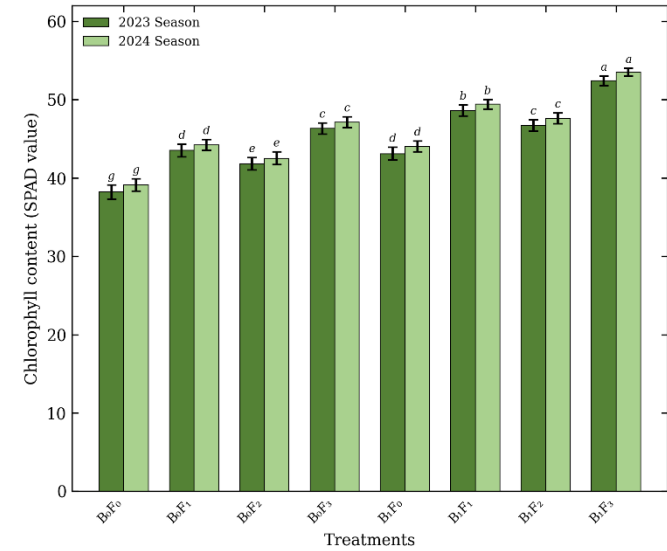


Figure 2. Chlorophyll content (SPAD units) in eggplant leaves as influenced by *A. chroococcum* inoculation and foliar Mo and Cu application in the 2023 and 2024 seasons. Bars bearing different letters differ significantly ($P \leq 0.05$).

3.3. Yield and yield components

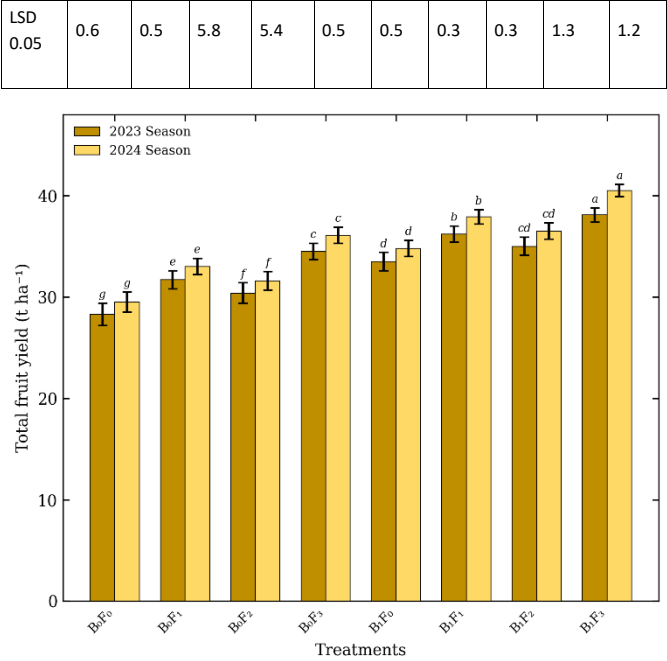
The data pertaining to yield and its components are presented in Table 5 and Figure 3. Biofertilizer inoculation with *A. chroococcum* significantly increased fruit number per plant, average fruit weight, fruit length, fruit diameter, and total yield ($t\ ha^{-1}$) over the uninoculated treatment in both seasons. The yield advantage from *Azotobacter* inoculation ranged between 18.3% and 21.6% across the two years. These results are consistent with the report of Sharma et al. [1] who showed that combined bio-inoculation with *Azotobacter* and mycorrhizal fungi under reduced chemical fertilization improved eggplant yield without any significant quality loss. The mechanism underlying this enhancement likely involves the combined effects of improved nitrogen nutrition, phytohormone-mediated increase in flowering and fruit set, and enhanced phosphate solubilization that supports energy metabolism during reproductive growth [3,16]. Among foliar treatments, F₃ (Mo + Cu) consistently produced the highest yield and yield components across seasons, followed by F₁ (Mo alone). The positive effect of combined Mo

and Cu foliar spray on yield could be due to the crucial functions played by these elements during the reproductive phase. Molybdenum is associated with the nitrogen metabolism, which provides enough amino acids for protein buildup in the growing fruits [7,35]. For example, copper, as a cofactor for polyphenol oxidase and superoxide dismutase, improves the antioxidant defence and reduces oxidative stress during fruit development, hence reducing fruit abortion and cracking [8, 10]. Further, Cu-dependent.laccase activity contributes to cell wall reinforcement in the pedicel and fruit skin, supporting heavier fruit loads per plant [26].

The relationship of azotobacter inoculation and foliar micronutrient administration was important for total yield per acre. The maximum overall yield was seen in B₁F₃ treatment which was around 34.6 % and 37.3 % higher than the control (B₀F₀) in the first and second season correspondingly. Importantly, the combined treatment boosted simultaneously individual fruit weight and number of fruits per plant, showing that the yield improvement was due to both fruit size and fruit number, not to a single component alone. This is an interesting discovery from an agronomic point of view since it shows that the yield gain could not be only related to vigour or partitioning alone but rather to a general improvement in the source–sink balance.

Table 5. Effect of *A. chroococcum* inoculation and foliar Mo and Cu on yield components and total fruit yield of eggplant.

Treat.	Fruits		Fruit		Fruit		Fruit		Yield	
	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024
B ₀ F ₀	8.3 g	8.7 g	162.4 g	168.2 g	13.2 f	13.5 f	5.8 f	6.0 f	28.3 g	29.5 g
B ₀ F ₁	9.8 e	10.2 e	178.6 e	184.3 e	14.6 d	15.0 d	6.4 d	6.6 d	31.7 e	33.0 e
B ₀ F ₂	9.4 f	9.8 f	172.3 f	177.8 f	14.1 e	14.4 e	6.2 e	6.4 e	30.4 f	31.6 f
B ₀ F ₃	11.2 c	11.6 c	191.5 c	197.4 c	15.8 b	16.2 b	6.9 b	7.1 b	34.5 c	36.1 c
B ₁ F ₀	10.1 d	10.5 d	183.7 d	189.5 d	14.8 cd	15.2 cd	6.5 cd	6.7 cd	33.5 d	34.8 d
B ₁ F ₁	11.8 b	12.3 b	198.2 b	204.6 b	16.1 b	16.5 b	7.1 b	7.3 b	36.2 b	37.9 b
B ₁ F ₂	11.3 c	11.7 c	192.8 c	198.7 c	15.5 c	15.9 c	6.8 c	7.0 c	35.0 cd	36.5 c
B ₁ F ₃	13.5 a	14.1 a	215.3 a	222.1 a	17.4 a	17.9 a	7.6 a	7.9 a	38.1 a	40.5 a



Fruit L = Fruit length; Fruit D = Fruit diameter. Means within columns followed by different letters differ significantly ($P \leq 0.05$).

Figure 3. Total fruit yield (t ha^{-1}) of eggplant as affected by *A. chroococcum* inoculation and foliar Mo and Cu application during 2023 and 2024 seasons. Means followed by different letters are significantly different ($P \leq 0.05$).

3.4. Fruit quality characteristics

Biofertilizer inoculation and foliar application of micronutrients had a significant influence on fruit quality parameters such as total soluble solids (TSS), dry matter content, vitamin C and fruit firmness (Table 6). *A. chroococcum* treated plants displayed greater TSS and vitamin C concentrations compared to uninoculated plants, possibly owing to the better nitrogen-to-carbon partitioning caused by biological nitrogen fixation [18]. The foliar application of Mo + Cu further promoted the accumulation of TSS and vitamin C, probably due to the fact that adequate supply of Cu enhances ascorbate metabolism through the ascorbate oxidase pathway, while Mo benefits the formation of abscisic acid, a phytohormone that triggers the accumulation of soluble solids during fruit ripening [12,26]. These quality improvements are relevant for fresh market value and consumer health, since aubergine is valued as a source of nutritional antioxidants [22].

Table 6. Effect of *A. chroococcum* inoculation and foliar Mo and Cu on fruit quality of eggplant.

Treat.	TSS (°Brix)	DM (%)	Vit C (mg/100g)	Firmness (N)
	2023	2024	2023	2024
B ₀ F ₀	4.1 g	4.2 g	6.8 g	7.0 g
B ₀ F ₁	4.5 e	4.6 e	7.4 e	7.6 e
B ₀ F ₂	4.3 f	4.4 f	7.1 f	7.3 f
B ₀ F ₃	4.8 c	4.9 c	7.9 c	8.1 c
B ₁ F ₀	4.5 e	4.6 e	7.5 de	7.7 de
B ₁ F ₁	5.0 b	5.1 b	8.2 b	8.4 b
B ₁ F ₂	4.8 c	4.9 c	7.8 cd	8.0 cd
B ₁ F ₃	5.4 a	5.5 a	8.8 a	9.1 a
LSD 0.05	0.2	0.2	0.3	0.3

TSS = Total soluble solids; DM = Dry matter; Vit C = Vitamin C. Means within columns followed by different letters differ significantly ($P \leq 0.05$).

3.5. Leaf mineral content

Mineral analysis of aubergine leaves indicated that inoculation with *A. chroococcum* considerably increased amounts of N, P and K in leaf tissue compared to the non-inoculated control (Table 7 and Figures 4-5). The increase in leaf N is due directly to biological nitrogen fixation by *A. chroococcum* which may fix ~15–20 kg N ha⁻¹ year⁻¹ under favourable conditions [3,24]. The high P content is due to the phosphate solubilising activity of *Azotobacter* that secretes organic acids that mobilise inaccessible soil phosphorus [2,25]. The increase in K levels in inoculated plants could be an indirect effect of increased root development and, consequently, increased absorption of K from the soil [3,31].

Leaf tissue concentrations of Mo and Cu were greatly enhanced by foliar sprays of Mo and Cu, respectively. The F₃ (Mo + Cu) treatment increased Mo and Cu leaf contents by about 3.8- and 2.6-fold, respectively, compared with the control (F₀) over the seasons. It is worth noting that foliar uptake of micronutrients can be more efficient than soil application because the nutrients bypass soil fixation reactions and are directly absorbed through the leaf cuticle and stomatal pores [9,39]. Additionally, foliar-applied Mo was associated with higher N content in leaves, corroborating the well-established functional relationship between Mo and nitrate reductase in nitrogen assimilation [5,6,7]. The interaction B₁ × F₃ produced the highest mineral concentrations overall, suggesting a synergistic mechanism wherein *Azotobacter*-enhanced root growth improves basipetal nutrient transport while foliar feeding supplies nutrients acropetally, collectively maximizing whole-plant nutritional status [36].

Table 7. Effect of *A. chroococcum* inoculation and foliar Mo and Cu on leaf mineral content of eggplant.

Treat.	N (%)		P (%)		K (%)		Mo (mg/kg)		Cu (mg/kg)	
	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024
B ₀ F ₀	2.18 g	2.24 g	0.28 f	0.29 f	2.85 f	2.92 f	0.42 e	0.45 e	6.8 e	7.1 e
B ₀ F ₁	2.56 d	2.63 d	0.31 cd	0.32 cd	3.04 cd	3.12 cd	1.38 b	1.45 b	7.4 de	7.7 de
B ₀ F ₂	2.35 f	2.41 f	0.30 de	0.31 de	2.96 de	3.03 de	0.48 de	0.51 de	14.2 b	14.8 b
B ₀ F ₃	2.78 c	2.85 c	0.34 b	0.35 b	3.18 bc	3.26 bc	1.52 b	1.59 b	15.6 b	16.3 b
B ₁ F ₀	2.65 d	2.72 d	0.33 c	0.34 c	3.12 cd	3.20 cd	0.56 d	0.59 d	7.8 de	8.1 de
B ₁ F ₁	3.04 b	3.12 b	0.37 ab	0.38 ab	3.35 ab	3.44 ab	1.65 ab	1.73 ab	8.5 cd	8.8 cd
B ₁ F ₂	2.83 c	2.91 c	0.35 bc	0.36 bc	3.24 bc	3.32 bc	0.62 cd	0.65 cd	16.4 ab	17.1 ab
B ₁ F ₃	3.35 a	3.44 a	0.41 a	0.42 a	3.58 a	3.67 a	1.82 a	1.91 a	18.2 a	19.0 a
LSD 0.05	0.12	0.11	0.02	0.02	0.14	0.13	0.15	0.14	1.4	1.3

Means within columns followed by different letters differ significantly ($P \leq 0.05$) by Duncan's test.

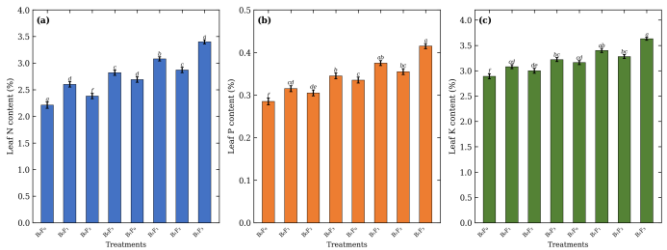


Figure 4. Leaf (a) N, (b) P, and (c) K concentrations (%) in eggplant as affected by *A. chroococcum* inoculation and foliar Mo and Cu application. Combined data of 2023 and 2024 seasons. Means ± SE; different letters indicate significance at $P \leq 0.05$.

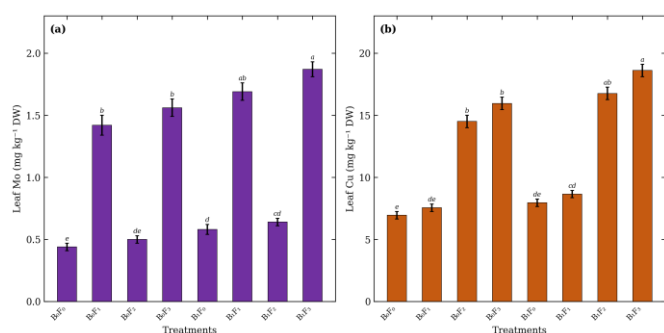


Figure 5. Leaf (a) Mo and (b) Cu concentrations (mg kg⁻¹ DW) in eggplant as influenced by foliar micronutrient application with and without *A. chroococcum* inoculation. Combined data of 2023 and 2024 seasons.

3.6. General discussion and practical implications

Taken together, the results of this study demonstrate that the integration of *A. chroococcum* bio-inoculation with foliar application of Mo and Cu offers a practical approach to improving eggplant productivity under semi-arid conditions. The synergy between biological nitrogen fixation and micronutrient supplementation addresses two of the most common nutritional constraints in calcareous Iraqi soils — nitrogen deficiency and low micronutrient bioavailability [15,27]. From an economic viewpoint, biofertilizer application can reduce the requirement for synthetic nitrogen fertilizers by up to 25–50%, which lowers input costs and minimizes the environmental footprint of intensive vegetable production [16,3]. These benefits are corroborated by recent studies in which PGPR-based biofertilizers improved nutrient uptake, yield and quality while reducing mineral fertilizer requirements, including under arid and semi-arid conditions [42,43,45]. The cost of micronutrient foliar sprays is relatively modest, and the returns in terms of yield and quality gains make this strategy highly cost-effective for smallholder farmers [14,39]. Future research should focus on the residual effect of *Azotobacter* inoculation on subsequent crops, the long-term effect on soil microbial diversity, and the optimisation of Mo and Cu spray doses under different agro-climatic conditions.

4. CONCLUSION

The present study provides evidence that soil inoculation with *Azotobacter chroococcum* along with foliar application of molybdenum (50 mg L⁻¹) and copper (75 mg L⁻¹) significantly improved the vegetative growth, fruit yield, quality attributes and leaf mineral composition of eggplant (*Solanum melongena* L., cv. Black Beauty) under semi-arid field conditions over two consecutive seasons. The combined application (B₄F₃) was consistently superior to either the biofertilizer or the micronutrient spray applied alone, clearly indicating a synergistic interaction that optimised nitrogen fixation, nutrient assimilation and overall plant vigour. The interaction between foliar-applied Mo and the phytohormones produced by *Azotobacter* appeared to promote nitrogenase activity and nitrate reductase-mediated nitrogen metabolism in the plant

tissues. Meanwhile, Cu supplementation maintained antioxidant defence and photosynthetic efficiency during the reproductive period. Overall, this integrated approach offers a sustainable alternative to the sole reliance on synthetic fertilisers, with the potential to reduce production costs and environmental impact in semi-arid horticultural systems. It is recommended that farmers in similar agro-ecological zones adopt this biofertilizer–micronutrient strategy to enhance eggplant productivity, though further studies are needed to fine-tune application rates and timings for different cultivars and soil types.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL. Not applicable.

FUNDING. Not applicable.

REFERENCES

- [1] Sharma M, Delta AK, Brar NS, Yadav A, Dhanda PS, Baslam M, Kaushik P. *Rhizophagus irregularis* and *Azotobacter chroococcum* uphold eggplant production and quality under low fertilization. *Int J Plant Biol.* 2022;13(4):601–612.
- [2] Sumbul A, Ansari RA, Rizvi R, Mahmood I. *Azotobacter*: A potential bio-fertilizer for soil and plant health management. *Saudi J Biol Sci.* 2020;27(12):3634–3640.
- [3] Aasfar A, Bargaz A, Yaakoubi K, Hilali A, Bennis I, Zeroual Y, Meftah Kadmiri I. Nitrogen fixing *Azotobacter* species as potential soil biological enhancers for crop nutrition and yield stability. *Front Microbiol.* 2021;12:628379.
- [4] Wani SA, Chand S, Ali T. Potential use of *Azotobacter chroococcum* in crop production: An overview. *Curr Agric Res J.* 2013;1(1):35–38.
- [5] Villora G, Moreno DA, Romero L. Response of eggplant to nitrogen supply: Molybdenum-nitrate relationships. *Biol Plant.* 2002;45(4):621–623.
- [6] Villora G, Moreno DA, Romero L. Potassium supply influences molybdenum, nitrate, and nitrate reductase activity in eggplant. *J Plant Nutr.* 2003;26(3):661–672.
- [7] Steiner F, Zoz T, Zuffo AM, Machado PP, Zoz J, Zoz A. Foliar application of molybdenum enhanced quality and yield of crisphead lettuce (*Lactuca sativa* L., cv. Grand Rapids). *Acta Agron.* 2018;67(1):73–78.
- [8] Chen J, Shafi M, Li S, Wang Y, Wu J, Ye Z, Peng D, Yan W, Liu D. Physiological and molecular mechanisms of plant responses to copper stress. *Int J Mol Sci.* 2022;23(21):12950.
- [9] Shahid M, Dumat C, Khalid S, Schreck E, Xiong T, Niazi NK. Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake. *J Hazard Mater.* 2017;325:36–58.
- [10] Hamid B, Zaman M, Farooq S, Fatima S, Sayyed RZ, Baba ZA, Sheikh TA, Reddy MS, El Enshasy H, Gafur A, Suriani NL. Copper mitigates salinity stress by regulating water status, photosynthetic

- pigments and ion homeostasis and increases the yield of eggplant (*Solanum melongena*). *Sci Hortic*. 2024;336:113439.
- [11] Barker AV, Pilbeam DJ. *Handbook of Plant Nutrition*. 2nd ed. Boca Raton: CRC Press; 2015.
- [12] Marschner H. *Marschner's Mineral Nutrition of Higher Plants*. 3rd ed. London: Academic Press; 2012.
- [13] Pramanik P, Goswami AJ, Ghosh S, Kalita C. An indigenous strain of potassium-solubilizing bacteria *Bacillus pseudomycoides* enhanced potassium uptake in tea plants. *J Appl Microbiol*. 2019;126(1):215–222.
- [14] Shahid MA, Balal RM, Khan N, Zotarelli L, Liu GD, Ghazanfar MU, Riaz A, Javed U, Hayat F, Mubeen M. Multi-micronutrient foliar fertilization in eggplant under diverse fertility scenarios. *Sci Hortic*. 2022;296:110903.
- [15] Alloway BJ. *Micronutrient Deficiencies in Global Crop Production*. Dordrecht: Springer; 2008.
- [16] Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microb Cell Fact*. 2014;13:66.
- [17] Pandey C, Gupta M. Selenium and auxin mitigate arsenic stress in rice (*Oryza sativa* L.) by combining the role of stress indicators, modulators and genotoxicity assay. *J Hazard Mater*. 2015;287:384–391.
- [18] Rouphael Y, Colla G. Editorial: Biostimulants in agriculture. *Front Plant Sci*. 2020;11:40.
- [19] Abd El-Mageed TA, Semida WM, Mohamed GF, Rady MM. Combined effect of foliar-applied salicylic acid and deficit irrigation on physiological–anatomical responses, and yield of squash plants under saline soil. *S Afr J Bot*. 2016;106:8–16.
- [20] Hussain A, Shah M, Hamayun M, Iqbal A, Qadir M, Alataway A. Growing *Azotobacter chroococcum* and sweet pepper under deficit irrigation in greenhouse conditions. *Sustainability*. 2022;14(9):5653.
- [21] FAOSTAT. Food and Agriculture Organization of the United Nations Statistics Division. Production quantities of eggplant. Rome: FAO; 2023.
- [22] Gürbüz N, Uluişik S, Frary A, Frary A, Doğanlar S. Health benefits and bioactive compounds of eggplant. *Food Chem*. 2018;268:602–610.
- [23] Hindersah R, Kalay M, Talahaturuson A, Lakburlawal Y. Nitrogen fixing bacteria *Azotobacter* as biofertilizer and biocontrol in long bean. *Agric*. 2018;30(1):25–32.
- [24] Jaga PK, Singh V. Effect of biofertilizer, nitrogen and sulphur on sorghum-mustard cropping system. *Proc Natl Seminar Agrotechnology*. 2010;1:69–71.
- [25] Kumar V, Narula N. Solubilization of inorganic phosphates and growth emergence of wheat as affected by *Azotobacter chroococcum* mutants. *Biol Fertil Soils*. 1999;28(3):301–305.
- [26] Yruela I. Copper in plants: acquisition, transport and interactions. *Funct Plant Biol*. 2009;36(5):409–430.
- [27] Shukla AK, Behera SK, Pakhre A, Chaudhari SK. Micronutrients in soils, plants, animals and humans. *Indian J Fertil*. 2018;14(2):30–54.
- [28] Page AL, Miller RH, Keeney DR. *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*. 2nd ed. Madison: ASA-SSSA; 1982.
- [29] AOAC. *Official Methods of Analysis*. 18th ed. Arlington: Association of Official Analytical Chemists; 2005.
- [30] Gómez KA, Gómez AA. *Statistical Procedures for Agricultural Research*. 2nd ed. New York: John Wiley & Sons; 1984.
- [31] Wani SA, Chand S, Wani MA, Ramzan M, Hakeem KR. *Azotobacter chroococcum* – A potential biofertilizer in agriculture: An overview. In: Hakeem KR, et al., editors. *Soil Science: Agricultural and Environmental Perspectives*. Cham: Springer; 2016. p. 333–348.
- [32] Ponmurugan KA, Sankaranarayanan C, Al-Dharbi NA. Biological activities of plant growth promoting *Azotobacter* sp. isolated from vegetable crops rhizosphere soils. *J Pure Appl Microbiol*. 2012;6(4):147–154.
- [33] El-Lattief EA. Use of *Azospirillum* and *Azobacter* bacteria as biofertilizers in cereal crops: A review. *Int J Res Eng Appl Sci*. 2016;6(7):36–44.
- [34] Nardi S, Pizzeghello D, Muscolo A, Vianello A. Physiological effects of humic substances on higher plants. *Soil Biol Biochem*. 2002;34(11):1527–1536.
- [35] Pramanik P, Safique S, Jahan A, Islam MA. Effect of molybdenum on nodulation, plant yield and nitrogen uptake in hairy vetch (*Vicia villosa* Roth). *Soil Sci Plant Nutr*. 2015;61(4):664–675.
- [36] Kumar M, Giri VP, Pandey S, Gupta A, Patel MK, Bajpai AB, Jenkins S, Siddique KHM. Plant-growth-promoting rhizobacteria emerging as an effective bioinoculant to improve the growth, production, and stress tolerance of vegetable crops. *Int J Mol Sci*. 2021;22(22):12245.
- [37] Nazir F, Hussain A, Fariduddin Q. Hydrogen peroxide modulate photosynthesis and antioxidant systems in tomato (*Solanum lycopersicum* L.) plants accessed by drought stress. *Dose-Response*. 2019;17(3):1559325819896515.
- [38] Pilon M, Abdel-Ghany SE, Cohu CM, Gogolin KA, Ye H. Copper cofactor delivery in plant cells. *Curr Opin Plant Biol*. 2006;9(3):256–263.
- [39] Fageria NK, Filho MPB, Moreira A, Guimarães CM. Foliar fertilization of crop plants. *J Plant Nutr*. 2009;32(6):1044–1064.
- [40] Rengel Z. Availability of Mn, Zn and Fe in the rhizosphere. *J Soil Sci Plant Nutr*. 2015;15(2):397–409.
- [41] Ali WM, Sultan SME, Ali AM, Al-Sayed HM, Ismail HG, Teiba II, et al. Organic fertilizers and *Azotobacter*: effects on onion growth, yield, metabolites, and soil fertility. *AMB Express*. 2025;15. DOI: 10.1186/s13568-025-01895-5.
- [42] Nabati J, Nezami A, Yousefi A, et al. Biofertilizers containing plant growth promoting rhizobacteria enhance nutrient uptake and improve the growth and yield of chickpea plants in an arid environment. *Sci Rep*. 2025;15:8331. DOI: 10.1038/s41598-025-93070-w.
- [43] Dasgan HY, Aldiyab A, Elgudayem F, Ikiz B, Gruda NS. Utilizing the power of plant growth promoting rhizobacteria on reducing mineral fertilizer, improved yield, and nutritional quality of Batavia lettuce in a floating culture. *Sci Rep*. 2024;14. DOI: 10.1038/s41598-024-51818-w.
- [44] Hindersah R, Yusuf AM, Adhiningtyas DI, Herdiyantoro D, Mulyani O, Suryatmana P. *Azotobacter* and chemical fertilizer for improving groundnut yield in field experiment. *J Agrotek Tropika*. 2024;12(4). DOI: 10.23960/jat.v12i4.8395.

- [45] Nadeem M, et al. Biofertilizers in sustainable agriculture: mechanisms, applications, and future prospects. *Discov Agric*. 2025;3(1):224. DOI: 10.1007/s44279-025-00318-0.
- [46] Delfim J, Moreira A, Moraes LAC. Foliar application of molybdenum combined with cobalt affects leaf nitrogen concentration, grain yield, and yield components of soybean. *Agron J*. 2025. DOI: 10.1002/agj2.70160.