Effect of spraying with Moringa leaf extract and inoculation with biofertilizers on some chemical and qualitative traits of spinach

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Abstract

This study aimed to evaluate the effects of foliar spraying with Moringa (*Moringa oleifera*) leaf extract and seed inoculation with biofertilizers on selected chemical and quality traits of spinach (*Spinacia oleracea*). The experiment was conducted at Station (A), College of Agricultural Engineering Sciences, University of Baghdad, during the autumn season of 2023–2024. The experiment included two factors: biofertilizer inoculation and Moringa leaf extract spraying. Biofertilizer treatments were control (0), mycorrhizal fungi (M1), Azotobacter bacteria (B1), and the combined inoculation (M1B1). Moringa leaf extract was applied at 0% (G0), 7.5% (G1), and 15% (G2). Treatments were arranged as a factorial experiment in a randomized complete block design (RCBD) with three replications. The results showed that the combined inoculation (M1B1) produced the highest values for calcium in leaves (1.70%), iron concentration (268.00 ppm), magnesium (0.69%), protein (10.72%), and ascorbic acid (97.89 mg 100 g^{-1} fresh weight), and reduced oxalic acid to 61.66 mg 100 g^{-1} fresh weight. Foliar application of Moringa leaf extract at 15% (G2) also improved calcium content (1.52%), iron concentration (202.25 ppm), magnesium (0.64%), protein (10.37%), and ascorbic acid (85.67 mg•100 g^{-1} fresh weight), while producing the lowest oxalic acid content (66.56 mg 100 g^{-1} fresh weight). The two-way interaction (M1B1G2) recorded the maximum calcium percentage (1.85%), iron concentration (298.00 ppm), magnesium (0.73%), protein (10.94%), and ascorbic acid (101.67 mg 100 g^{-1} fresh weight), with the lowest oxalic acid concentration (58.20 mg 100 g^{-1} fresh weight). Overall, under Iraqi conditions, spinach growth and productivity can be enhanced through environmentally friendly and sustainable fertilization practices, particularly by combining biofertilizer inoculation with Moringa leaf extract foliar spraying.

Keywords: Biofertilizers, Moringa leaf extract, Spinach.

Introduction

Spinach (*Spinacia oleracea* L.) is among most valuable leafy green vegetables in the Chenopodiaceae family. It is known for its high nutritional value and important involvement in many functions of the human organism. It is thought to have originated in Asia, particularly Persia, then traveled to China during 674 BCE and then reached to

Europe in the 12th century [1]. The deep green color of spinach is due to the presence of chlorophyll, which is known to be anti-inflammatory and has detoxification capacity. Spinach, however, is high in antioxidants such as vitamin C, carotenoids, and flavonoids, it also a good source of essential minerals, specifically iron, magnesium, potassium, and calcium [2].

Biofertilizers play a fundamental role in maintaining soil fertility, as they are considered a renewable source of essential nutrients required for healthy plant growth. The microorganisms present in biofertilizers facilitate the transformation of soil nutrients from complex or unavailable forms into soluble, plant-available forms [3]. Biofertilizers [mycorrhiza and Azotobacter] provides different vitamins to the plant and improves its crop yield and quality [4]. The primary role of mycorrhiza, an environmentally friendly biofertilizer, lies in its contribution to supplying essential nutrients to plants. It enhances biodiversity, supports environmental protection, accelerates growth, and increases crop yields. Mycorrhizal fungi also play a crucial role in facilitating nutrient uptake from the soil and their translocation, improving carbon assimilation rates, and mitigating abiotic stresses. The mechanism by which arbuscular mycorrhizal fungi function is influenced by several factors, including increased root surface area, enhanced nutrient transport, hyphal penetration into soil micropores inaccessible to roots, stimulation of root exudation, modification of root morphology, and activation of plant defense mechanisms [5]. Mycorrhizal inoculation positively affected the qualitative traits of potato plants reported by Al-Zaidi and [6]. Similarly, AL-Dolaimy et al. [7] stated that Mycorrhizal fungi could be beneficial to plants by providing essential nutrients. Several researchers have indicated that one of the most widely used and important microbial species in biofertilization is *Azotobacter chroococcum*, a free-living nitrogen-fixing bacterium. This species is capable of converting atmospheric nitrogen into forms readily available for plant uptake. It has been extensively utilized as a biofertilizer under the name *Azotobactrine* and is known to associate with a wide range of crops. In addition to nitrogen fixation, *A. chroococcum* secretes enzymes, hormones, vitamins, and growth regulators, all of which play a significant role in plant nutrition. Its contribution is not limited to nitrogen provision; it also enhances phosphorus availability and the solubilization of other nutrients through the decomposition of organic matter, as confirmed by Abd El-Gawad *et al.*, [8]. However, use of mycorrhizal fungi can enhance nutrient uptake and the overall growth performance of plants [9]. Carrot plant characterization at different mycorrhizal fungi usage Moreover, according to Al-Amiri [10], the application of Azotobacter bacteria increased leaf nutrient content and decreased oxalate accumulation. Azotobacter inoculation of cucumber increases growth, yield, and nutrient concentration in leaves [11]. Similarly, AL-Dolaimy et al. [7] noted the beneficial role of Azotobacter in enhancing nutrient absorption in cauliflower plants. Kazem and Abed Mutar [12] highlighted also that Azotobacter played an important role in enhancing the nutrients contents of spinach leaves. In addition, Al-Khafaji and Al-Jubouri [13] emphasized the optimal role of Azotobacter in enhancing plant nutrition and yield performance. Providing micronutrients to lettuce in foliar form is a common practice. Foliar application played a major role in various traits of pea plants as reported by Mohammed and Al-Ubaidy [14]. The results are also in agreement with Omrani and Al-Ubaidy [15] who reported that foliar application led to a significant increase in the tested traits of onion plants.

Foliar spray of Moringa leaf extract is also reported to increase vitamin C content in strawberry plants through enhanced nutrient uptake [16]. Moreover, Sura and Al-Hilfy [17] reviewed the presence of macro- and micronutrients such as nitrogen, calcium, and magnesium, as well as key vitamins found in Moringa leaf extract, which improve photosynthetic efficiency. These nutrients bring about increased rates of photosynthesis and vegetative growth, enhancing chlorophyll synthesis, which are superior to control treatment facts for Moringa foliar application. Furthermore, Al-Tamimi [18] demonstrated the positive response of sugar beet plants to foliar application of plant extracts in general, emphasizing their role as natural biostimulants that enhance leaf yield. Sardar *et al.* [19] reported the potential of foliar application of moringa leaf extract in enhancing growth and increasing the mineral content of stevia leaves, including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), zinc (Zn), iron (Fe), and magnesium (Mg), compared to the control treatment.

Based on the aforementioned findings, this study aimed to investigate the effect of foliar spraying with Moringa leaf extract and inoculation with biofertilizers on certain chemical and qualitative traits of spinach.

Materials and methods

The experiment was conducted at Station (A) of the College of Agricultural Engineering Sciences, University of Baghdad, during the 2023–2024 autumn season. This experiment was carried out as a factorial experiment by a randomized complete block design (RCBD) with three replications, included two factors as seed inoculation with biofertilizers and foliar spraying with Moringa leaf extract. Biofertilizer treatment levels were control (0), inoculated with mycorrhizal fungi (M1), inoculated with Azotobacter bacteria (B1), and inoculated with mycorrhizal fungi and Azotobacter (M1B1). Three concentrations (0% (G0), 7.5% (G1), and 15% (G2)) of Moringa leaf extract were used for foliar application. Each experimental unit containing 180 plants. Seeds of a local spinach cultivar were directly sown in the field on October 23, 2023, with all necessary soil management practices applied, including drip irrigation and the application of 25% of the recommended chemical fertilizer dose. Nitrogen was applied in the form of urea at a rate of 125 kg N ha^{-1}, while phosphorus was applied as phosphate fertilizer at 135 kg P_2O_5 ha^{-1} [20].

The soil used in this experiment was analyzed before planting to determine its main physicochemical properties (e.g., pH, EC, organic matter, and available N, P, and K).

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The results of the soil analysis are presented in Table (1).

Table 1: chemical and physical indicators of the field soil

Measured trait	Value	Unit
Ph	7.6	
Ec	2.35	dS m-1
Available nitrogen (N)	31	mg kg-1
Available phosphorus (P)	8.2	mg kg-1
Available potassium (K)	154	mg kg-1
Organic matter	9.4	g kg-1
Calcium (Ca2+)	26.2	meq L-1
Magnesium (Mg2+)	17.3	meq L-1
Sodium (Na+)	22.1	meq L-1
Chloride (Cl−)	31.2	meq L-1
Sulfate (SO4^2−)	38.6	meq L-1
Sand	337	g kg-1
Silt	298	g kg-1
Clay	365	g kg-1
Soil texture	Clay loam	

Results

Chemical Traits

Calcium Percentage in Leaves (%)

Biofertilizers application increased the calcium percentage in spinach leaves significantly (Table 2a). The highest calcium content was found in the M1B1 treatment at 1.70%, while the value was lowest in the control treatment (M0B0) at 1.25%. Foliar application of Moringa leaf extract resulted in significant increment of calcium contents where the highest amount (1.52%) and the lowest amount (1.33%) was recorded with G2 and G0 , respectively. And also, the interaction of applying biofertilizers by spraying Moringa extract significantly influenced the calcium percentage, where the greatest calcium percentage (1.85%) was obtained from M1B1G2 combination in biofertilizer and Moringa extract field application, and the lowest value (1.11%) was observed in M0B0G0 treatment.

Iron Content in Leaves (ppm)

As illustrated in Table 2a, the application of biofertilizers considerably elevated the iron concentration in spinach leaves. M1B1 (combined inoculation with mycorrhizal fungi and Azotobacter) produced the highest iron content, at 268.00 ppm, and the lowest value, at 88.11 ppm, was recorded in the control treatment (M0B0). Foliar spraying was also effective in increasing iron content during the experiment; the highest (202.25 ppm) and the lowest (162.00 ppm)

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iron content was recorded under G2 treatment (15% Moringa extract) and G0 treatment (0% Moringa extract) respectively. Additionally, the application of Rhizobium biofertilizer along with the spraying of Moringa extract revealed a significant effect with M1B1G2 treatment producing maximum iron concentration (298.00 ppm) compared to M0B0G0 treatment showing minimum concentration (62.00 ppm).

Magnesium Percentage in Leaves (%)

The results presented in Table 2a show that the use of bio-fertilizers markedly increased the magnesium percentage in the leaves of spinach. The M1B1 treatment had the highest magnesium content (0.69%), while the lowest value (0.54%) was indicated in the control treatment (M0B0). Similarly, magnesium was also improved significantly through foliar spraying with Moringa leaf extract, with the treatment of G2 showing the highest magnesium content (0.64%), while the treatment of G0 showed the lowest magnesium content (0.59%). Also, the interaction of biofertilizer and spray of Moringa extract had a significant effect and the highest magnesium percentage (0.73%) was between M1B1G2, which didn't have a significant difference from (M1B1G1) treatment, but the lowest value was at M0B0G0 treatment (0.51%).

Table 2a. Main effects of biofertilizer and Moringa leaf extract on leaf mineral content.

Treatments	Magnesium (%)	Iron (ppm)	Calcium (%)
M0B0	0.54	88.1	1.25
M0B1	0.61	198.4	1.43
M1B0	0.62	162.8	1.31
M1B1	0.69	268.0	1.70
LSD (0.05)	**0.01**	**5.60**	**0.01**
G0	0.59	158.5	1.33
G1	0.62	177.2	1.43
G2	0.62	202.2	1.52
LSD (0.05)	**0.01**	**4.85**	**0.01**

Table 2b. Interaction effect (biofertilizer × Moringa leaf extract) on leaf mineral content.

Treatments	Magnesium (%)	Iron (ppm)	Calcium (%)
M0B0G0	0.51	62.0	1.11
M0B0G1	0.55	88.0	1.29
M0B0G2	0.56	114.3	1.35
M0B1G0	0.59	186.7	1.38
M0B1G1	0.62	193.7	1.42
M0B1G2	0.63	215.0	1.49
M1B0G0	0.61	146.3	1.24
M1B0G1	0.61	160.3	1.30
M1B0G2	0.65	181.7	1.38

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M1B1G0	0.64	239.0	1.58
M1B1G1	0.70	267.0	1.69
M1B1G2	0.73	298.0	1.85
LSD (0.05)	**0.02**	**9.7**	**0.01**

Protein Percentage in Leaves (%)

The results indicated that the application of biofertilizers had a significant effect on the percentage of protein in leaves of spinach (Table 2). The highest protein content of 10.72% was recorded in M1B1 (combined inoculation of mycorrhizal fungi and Azotobacter) and the lowest was found in M0B0 (control) 9.49%. Foliar spray with Moringa leaf extract had a comparable positive impact on protein content, where the G2 treatment (Moringa 15%) showed the most significant increase (10.37%) when compared to the G0 treatment (0% Moringa extract) which showed the least amount of protein (9.70%). Moreover, the interaction effects of biofertilizer application in conjunction with Moringa extract spraying were significant: The highest protein percentage (10.94%) was recorded in M1B1G2, which was not statistically different from M1B1G1, while the least value (8.52%) was for M0B0G0.

Ascorbic Acid Ascorbic acid content (mg·100 g^{-1} Fresh Weight)

The results showed that these treatments of biofertilizers influenced the ascorbic acid content in spinach leaves (Table 2). M1B1 was found to have the highest concentration of ascorbic acid (97.89 mg·100 g^{-1} fresh weight), however the lowest ascorbic acid concentration was recorded from M0B0 (control) with a value of 61.00 mg·100 g^{-1} fresh weight. The foliar application of Moringa leaf extract also increased the concentration of ascorbic acid, in which the G2 (Moringa 15%) treatment caused the highest level (85.67 mg·100 g^{-1} fresh weight) compared to the G0 treatment (0% Moringa extract), with the lowest value (72.84 mg·100 g^{-1} fresh weight). Additionally, the interaction effects of biofertilizer application with Moringa extract spraying were significant: The highest ascorbic acid content (101.67 mg·100 g^{-1} fresh weight) was recorded in M1B1G2, which did not significantly differ from M1B1G1, while the lowest content (49.67 mg·100 g^{-1} fresh weight) was found in M0B0G0.

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Table 3. Effect of biofertilizers, foliar spraying with moringa leaf extract, and their interaction on the qualitative traits of spinach.

Main effects

Treatments	Oxalic acid (mg·100 g^{-1} FW)	Ascorbic acid (mg·100 g^{-1} FW)	Protein (%)
Biofertilizer inoculation			
M0B0	87.39	61.00	9.49
M0B1	64.88	84.22	10.38
M1B0	78.11	72.78	9.82
M1B1	61.66	97.89	10.72
L.S.D. (0.05)	**2.94**	**1.29**	**0.11**
Moringa leaf extract (%)			
G0	83.84	72.84	9.70
G1	68.62	78.42	10.23
G2	66.56	85.67	10.37
L.S.D. (0.05)	**2.55**	**1.11**	**0.09**

Interaction (biofertilizer × Moringa leaf extract)

Treatments	Oxalic acid (mg·100 g^{-1} FW)	Ascorbic acid (mg·100 g^{-1} FW)	Protein (%)
M0B0G0	106.90	49.67	8.52
M0B0G1	80.13	61.00	9.92
M0B0G2	75.13	72.33	10.02
M0B1G0	70.60	81.67	10.24
M0B1G1	60.50	82.33	10.38
M0B1G2	63.53	88.67	10.51
M1B0G0	90.97	66.00	9.60
M1B0G1	74.00	72.33	9.85
M1B0G2	69.37	80.00	10.01
M1B1G0	66.90	94.00	10.45
M1B1G1	59.87	98.00	10.77
M1B1G2	58.20	101.67	10.94
L.S.D. (0.05)	**5.10**	**2.23**	**0.19**

Oxalic Acid Content (mg·100 g^{-1} Fresh Weight)

The results revealed that biofertilizers have an effective impact on spinach leaves oxalic acid content (Table 2). The highest oxalic acid concentration of 87.39 mg·100 g^{-1} fresh weight was recorded in M0B0 (control) whereas the lowest concentration was found in M1B1 (combined inoculation of mycorrhizal fungi and Azotobacter), which recorded 61.66 mg·100 g^{-1} µg·100 g^{-1} fresh weight. Moringa foliar concentration had also significant effect; the highest oxalic acid contents (83.84 mg ·100 g^{-1} fresh weight) belonged to G0 (0% Moringa extract) and the lowest reached to G2 (66.56 mg ·100 g^{-1}

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fresh weight). Additionally, the interaction of biofertilizer application and Moringa extract spraying had a significant effect on oxalic acid content, the highest value was recorded in M0B0G0 (106.90 mg·100 g^{-1} fresh weight) whereas the lowest oxalic acid concentration (58.20 mg·100 g^{-1} fresh weight) was observed in M1B1G2.

Discussion

The present results show that the combined treatment M1B1G2 produced the best performance for the chemical and quality traits reported in Tables 2 and 3. This response is attributed to the complementary effects of biofertilizer inoculation and Moringa leaf extract, which together enhance nutrient availability and uptake and, consequently, improve leaf nutritional quality. Biofertilizers (nitrogen-fixing and phosphate-solubilizing microorganisms) can increase the supply of available nutrients and improve soil biological activity, which is reflected in better plant performance [21, 22]. Mycorrhizal fungi, in particular, can stimulate root development and increase the absorbing surface area through their contribution to phytohormone-related activity and root hair formation [23]. They also enhance the acquisition of key minerals, including N, P, Ca, Fe, Zn, and others, especially under low nutrient availability. These mechanisms are consistent with the higher mineral contents observed under combined inoculation.

Heil [24] reported that mycorrhizal hyphae may contribute substantially to plant nutrient supply, including phosphorus and several micronutrients. Accordingly, mycorrhizal inoculation can reduce fertilizer requirements and improve water-use efficiency, while enhancing tolerance to drought and environmental stresses [25]. Azotobacter further supports plant growth through biological nitrogen fixation, phosphorus solubilization, and the production of growth regulators, in addition to improving soil physical conditions and suppressing certain pathogens [26]. Moreover, Azotobacter spp. can release siderophores that chelate iron and facilitate its availability to plants, which may explain the higher leaf Fe concentration recorded in inoculated treatments [27]. The superior response of M1B1 compared with single inoculations suggests a synergistic interaction between mycorrhiza and Azotobacter, rather than a purely additive effect.

Foliar nutrition can also contribute to higher nutrient status and improved quality because it delivers nutrients and bioactive compounds directly to the photosynthetic tissue, thereby supporting growth and yield [28]. Al-Ubaidy et al. [29] similarly reported that foliar spraying increased leaf nutrient content. Moringa leaf extract is widely used as a natural biostimulant and has been reported to improve vegetative growth, productivity, and quality in several crops [30, 31]. It contains minerals and amino acids and is a source of antioxidant compounds such as vitamin C, carotenoids, tocopherols, and polyphenols [17, 23, 32]. In the current study, increasing the Moringa concentration, particularly G2, was associated with improved mineral contents and quality traits, including higher protein and ascorbic acid and lower oxalic acid. The reduction in oxalic acid is agronomically important because oxalates can reduce the bioavailability of divalent minerals in edible tissues. Overall, combining biofertilizer inoculation with Moringa foliar spraying represents a practical and environmentally friendly approach to enhance spinach nutritional quality under Iraqi conditions [17, 34].

Conclusion

It could be concluded, according to the results of this research, the importance of used biofertilizer and foliar application of Moringa leaf extract in spinach chemical and qualitative traits. The combined treatment of mycorrhizal fungi and Azotobacter inoculation (15% Moringa extract M1B1G2) provided the highest content of nutrients (calcium, magnesium, iron, protein, and ascorbic acid) and reduced oxalic acid accumulation. Integration of biofertilizers and Moringa leaf extract proved to be a sustainable strategy and can enhance nutrient availability, plant growth promotion and increasing spinach quality. Long-term studies are needed to explore how this combination impacts soil health, find optimal application rates for different crops, and its performance in varying environmental conditions, so that this combination can be used for sustainable agriculture.

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