HortiS (2025) 42(2):80-87
http://doi.org/10.16882/HortiS.1716339

RESEARCH PAPER

HORTICULTURAL STUDIES

Synergistic Effects of Biofertilizers and Chemical Fertilizers on Yield and Nutritional Quality of Greenhouse-Grown Lettuce

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Article History
Received 18 March 2025
Accepted 05 June 2025
First Online 13 June 2025

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Keywords
Bacillus licheniformis
Calcium
Glomus intraradices
Nitrogen
Nutritional content

Abstract

This study investigates the impact of biofertilizers, chemical fertilizers, and their combined application on lettuce yield and nutrition. A randomized block design experiment was conducted in a greenhouse, comparing control, chemical fertilizer (CF), Bio Veria (BF_1), Bacillus Mix (BF_2), CF+BF_1, and CF+BF_2 treatments. Results showed that combined applications of CF+BF_1 and CF+BF_2 significantly increased lettuce fresh weight by 56% and 61%, respectively, and dry weight by 80% and 112% compared to the control. The CF+BF_2 treatment achieved the highest yield at 34.6 t ha^{-1}, a 61% improvement over the control and a 28% increase compared to CF alone. Furthermore, the mixture treatments demonstrated the highest nitrogen (N), phosphorus (P), and calcium (Ca) contents in lettuce leaves. Specifically, CF+BF_2 had the highest N content, a 29% improvement over the control and a 20% increase over CF. The CF+BF_1 treatment resulted in a 54% improvement in P content over the control and a 21% increase over CF. The CF+BF_2 treatment also produced a 54% improvement in Ca content over the control and a 25% increase over CF. The integrated application of biofertilizers and chemical fertilizers significantly improved lettuce yield and nutritional content, highlighting the potential of biofertilizers containing multi-species in optimizing lettuce production.

1. Introduction

Lettuce, a widely consumed vegetable known for its rich fiber content, folate, iron, and other health-promoting components (Kim et al., 2016), is typically consumed fresh. In greenhouse lettuce cultivation, achieving optimal growth and yield depends on several factors, including irrigation frequency, fertilization rates, incorporation of biological supplements, properties of growth medium, and controlled growth conditions (Liu et al., 2012; Vetrano et al., 2020; Wang and Xing, 2016). In Türkiye, lettuce holds significant agricultural importance, with a total greenhouse production of 154,000 tons in 2023 (TÜİK, 2023). To enhance growth and yield, inorganic mineral fertilizers are commonly applied to lettuce in greenhouses after transplanting (Vetrano et al., 2020; Trinh et al., 2018; Zhao et al., 2010). These fertilizers provide essential nutrients to plants, such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn), which are crucial for maximizing crop productivity (White and Brown, 2010). However, excessive application of chemical fertilizer may cause nutrient runoff, nitrogen pollution, and leaching losses, which can adversely affect the environment, biodiversity, and human health (Baweja et al., 2020; Zhao et al., 2010). The increasing challenges posed by climate change further threaten crop production and productivity, requiring innovative approaches to meet the rising demand for food and other agricultural products (Hamid et al., 2020; McLaughlin and Kinzelbach, 2015). Therefore, biofertilizer serves as an alternative to inorganic mineral fertilizers. Biofertilizers are biological products containing living microorganisms that when applied to seed, plant surfaces or soil stimulate growth through a variety of mechanisms including boosting the plant's ability to absorb nutrients, increasing the amount of biomass or root area, and increasing the supply of nutrients (Pylak et al., 2019). These formulations contain beneficial microorganisms that enhance nutrient availability through biological processes. As a result, they contribute to improved soil health and stimulate plant growth (Basu et al., 2021).

Microbes utilized in the production of biofertilizers not only mobilize N and P but also contribute to the natural cultivation of crops. Arbuscular mycorrhizal fungi (AMF) are effective biofertilizers that enhance plant growth and nutrient absorption, particularly in lettuce cultivation (Molaei et al., 2024). This symbiosis significantly improves the uptake of insoluble and immobile phosphate ions from the soil. Arbuscular mycorrhizal fungi produce phosphatases to hydrolyze phosphate from organic sources, boosting crop yield in P-deficient conditions (Lee et al., 2014; Majewska et al., 2017). AMF inoculation has been shown to significantly boost lettuce yield, with studies reporting an average production increase of 186% (Epelde et al., 2020). Furthermore, AMF colonization in greenhouse lettuce can also increase the accumulation of secondary metabolites, vitamins, and minerals, enhancing the intake of beneficial compounds without raising lettuce consumption (Baslam et al., 2013). For instance, *Glomus intraradices*, a well-known arbuscular mycorrhizal fungus, significantly improves nutrient acquisition, water uptake efficiency, and overall biomass production in lettuce (Kohler et al., 2010). Similarly, plant growth-promoting rhizobacteria (PGPR) offer a sustainable alternative to inorganic mineral fertilizers by enhancing plant growth and production (Basu et al., 2021). *Bacillus spp*. are one of the most used strains in microbial biofertilizers. Bacillus species enhance plant development through mechanisms such as phytohormone production, nutrient solubilization, antibiotic synthesis, and promotion of systemic resistance to pathogens (Tejera et al., 2013). Vetrano et al. (2020) reported that applying *Bacillus spp*. to lettuce seedlings improved growth, yield, and nitrate content in greenhouse and field experiments, while maintaining the natural rhizosphere microbiome (Kröber et al., 2014).

Despite the well-documented advantages of biofertilizers in improving yield, biomass, water retention capacity, and nutrient availability (Cipriano et al., 2016), there is a lack of knowledge regarding the combined use of chemical and biofertilizers in lettuce cultivation. Addressing this gap is essential for optimizing nutrient management strategies that balance productivity and environmental sustainability. Accordingly, this study aimed to investigate the impact of biofertilizers, chemical fertilizer, and their combined application on the yield and nutritional content of lettuce, thereby contributing to a more sustainable and productive greenhouse cultivation system.

2. Materials and Methods

2.1. Study site

The experiment was conducted in the autumn of 2021 in a glass greenhouse located at the Batı Akdeniz Agricultural Research Institute (Serik, Antalya, Türkiye), on silty loam (61% sand, 21% clay, and 18%) soil characterized by slightly alkaline pH (7.8), nonesaline (EC: 1.1 dS m^{-1}), and low lime content (3.3%). The soil also contained 1.65% organic matter (Table 1). The region has a mean annual temperature of 18.9°C and an average annual precipitation of 1039.8 mm (Türkiye State Meteorological Service, 2024).

Table 1. The physical and chemical properties of the experimental soil.

Parameters	Values
pH (1:2.5)	7.80
Lime (%)	3.30
EC (dS m^{-1})	1.10
Sand (%)	61.00
Clay (%)	21.00
Silt (%)	18.00
Organic matter (%)	1.65
Phosphate (ppm)	70.00
Potassium (ppm)	365.00
Calcium (ppm)	3700.00
Magnesium (ppm)	285.00
Iron (ppm)	4.40
Manganese (ppm)	12.00
Zinc (ppm)	2.10
Copper (ppm)	8.00

Table 2. Ingredients of biofertilizers used in the experiment.

Bio veria	Bacillus Mix
Glomus intraradices (0.001 progagule g^{-1})	*Glomus intaradices* (0.01 progagule g^{-1})
Bacillus licheniformis (10^7 CFU ml^{-1})	*Bacillus licheniformis* (10^9 CFU ml^{-1})
	Bacillus megaterium (10^9 CFU ml^{-1})

2.2. Biofertilizer and plant material

The lettuce (*Lactuca sativa* L.), hybrid AG Tohum Caipira®, was used in the experiment. The commercial biofertilizers applied were Bio Veria® (liquid) and Bacillus Mix® (solid), both manufactured by ED&F MAN (UK). The composition of these biofertilizers is provided in Table 2.

2.3. Experimental design and setup

The experiment was arranged in a randomized block design with four replications. The following fertilizer treatments were applied: Control (no fertilization), chemical fertilizer (CF) at the recommended dose, Bio Veria (BF_1), Bacillus Mix (BF_2), Chemical fertilizer + Bio Veria ($CF+BF_1$), and Chemical fertilizer + Bacillus Mix ($CF+BF_2$). The chemical fertilizers used were urea (46% N), monoammonium phosphate (MAP) (12% N, 61% P_2O_5), and potassium nitrate (13% N, 45% K_2O). The application rates of chemical fertilizers were determined based on plant growth stages. Fertigation was applied on average every four days, with intervals adjusted based on soil moisture. The fertigation solution was maintained at a pH of 6.5-7.0 and an electrical conductivity (EC) of 1.5-2.0 dS m^{-1}, depending on the crop developmental stage.

A drip irrigation system was used, and chemical fertilizers were applied through this system. Bio Veria and Bacillus Mix applications were split into two doses: the first at sowing and the second 14 days after sowing. The total biofertilizer application per hectare was 10 liters for Bio Veria and 2500 grams for Bacillus Mix.

Planting was carried out on October 14, 2021. The experimental unit consisted of two rows, each 4.6 m in length, with 0.4 m row spacing and 0.5 m in-row spacing, accommodating 20 plants per plot. This resulted in a theoretical plant population density of 60,000 plants per hectare.

2.4. Plant sampling and analysis

Lettuce plants were harvested 52 days after planting, and four plants were collected from each plot to determine fresh and dry weights. The total yield per plot was measured, and the data were converted to tons per hectare (t ha^{-1}).

Leaf samples were dried at 65°C, ground, and prepared for chemical analysis. In the filtrates obtained from wet digestion using a nitric:perchloric acid mixture ($4HNO_3 + HClO_4$), the concentrations of P, K, Ca, Mg, Fe, Zn, Mn, and Cu were determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Nitrogen (N) content in lettuce leaves was analyzed using the modified Kjeldahl method (Kacar and İnal, 2008).

2.5. Statistical analysis

All statistical analyses were performed using RStudio® (RStudio Team, 2020) with R version 4.2.2. A randomized block design (RBD) with four replications was used to assess the effects of fertilizer treatments on plant growth and nutrient content. The data were subjected to analysis of variance (ANOVA) using the aov function from the base R package. Data visualization and summary statistics were performed using the ggplot2 (Wickham, 2016) and dplyr (Wickham et al., 2023) packages. The statistical model used for the analysis was:

$$Y_{ijk} = \mu + T_i + B_j + \varepsilon_{ijk}$$

where:

Y_{ijk} represents the observed response variable (e.g., fresh weight, dry weight, total yield, or nutrient content) for the i-th fertilizer treatment in the j -th block (replication),
μ is the overall mean,
T_i is the fixed effect of the i-th fertilizer treatment (i=1, 2,...,6),
B_j is the random effect of the j-th block (replication) (j=1, 2, 3, 4),
ε_{ijk} is the random error term, assumed to follow a normal distribution with mean 0 and variance σ^2 ($\varepsilon_{ijk} \sim N(0, \sigma^2)$).

Analysis of variance (ANOVA) was conducted to determine the statistical significance of treatment effects on all measured parameters. When significant differences were detected, Fisher's least significant difference (LSD) test was applied to compare treatment means at a 5% significance level ($p < 0.05$).

3. Results and Discussion

3.1. Effects of fertilizers on plant growth

The effects of fertilizers on growth parameters, including fresh weight (FW), dry weight (DW), and total yield, are shown in Figure 1. The application of BF individually and combined with CF significantly

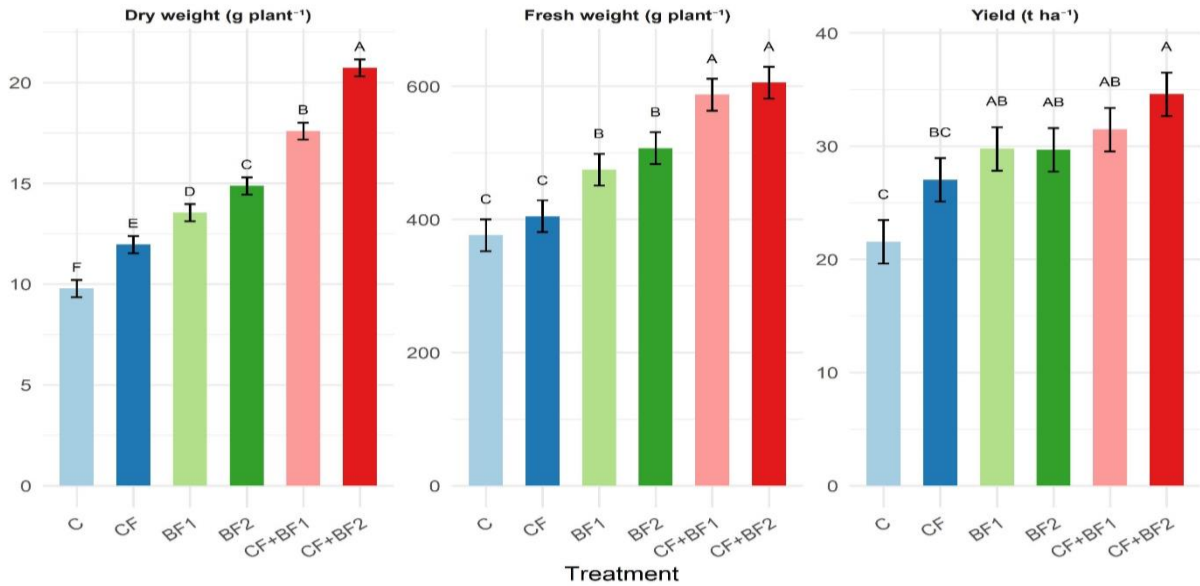

Figure 1. Effect of biofertilizer and chemical fertilizer treatments on dry and fresh weights and yield (Values are the means of four replicates and error bars represent the standard errors, n=4. Significant differences from Fisher's LSD test at $p<0.05$ are shown as different letters. C: Control, CF: Chemical Fertilizer, BF1: Bio veria, BF2: Bacillus mix, CF+BF1: Chemical Fertilizer+ Bio veria, CF+BF2: Chemical Fertilizer+Bacillus mix.

increased the FW of lettuce compared to control plants. The dual application of CF+BF_1 and CF+BF_2 resulted in the highest values, reaching 587.6 g plant^{-1} and 605.7 g plant^{-1}, respectively. Compared with the control, CF+BF_1 and CF+BF_2 increased FW by 56% and 61%, respectively. Moreover, the combination of BFs and CF significantly enhanced lettuce FW compared to single applications of CF and BF, while no significant differences were observed between CF+BF_1 and CF+BF_2. The results obtained are consistent with Stoll et al. (2018) and they found a notable increase in the FW when bacterial strains were inoculated into lettuce seedlings, resulting in up to 30% more weight compared to the control.

Regarding lettuce DW, the obtained results revealed that all treatments significantly increased DW compared to control plants (Figure 1). The highest increases were observed in plants treated with CF+BF_1 and CF+BF_2, which improved the DW by 80% and 112%, respectively, compared to the control. These dual applications also resulted in significantly higher DW values than single fertilizer applications. Similar findings were reported by Sánchez et al. (2014) who assessed consortia of bacterial strains in lettuce (*L. sativa*) plants and found that bacterial consortium treatment increased DW by 102% compared to CF alone, likely due to improved root growth and nutrient uptake.

Biofertilizers may enhance root penetration and water retention capacity leading to increased plant growth (Bhardwaj et al., 2014). The remarkable 112% increases in DW observed in the CF+BF_2 treatment may be attributed to improved soil conditions, which facilitate better moisture and nutrient absorption by plant roots. The significant increases in FW and DW, particularly in the CF+BF_2 treatment, could be closely linked to the beneficial properties of PGPR and AMF, including nutrient solubilization and enhanced nutrient uptake. PGPR are well known for their ability to solubilize essential nutrients, particularly P, a key nutrient often limited in bioavailability in soils. The substantial increase FW (605.7 g plant^{-1}) and DW (20.7 g plant^{-1}) in the CF+BF_2 treatment likely resulted from improved nutrient uptake facilitated by PGPR. Aini et al. (2019) found that AMF and PGPR increase root surface area and improve nutrient uptake, especially P, leading to increased biomass.

3.2. Total yield response to fertilizer treatments

The total yield across all treatments showed significant improvements compared to the control (Figure 1). The control plants had the lowest yield (21.5 t ha^{-1}). The application of biofertilizers alone or in combination with chemical fertilizers significantly increased yield. BF_1 resulted in a yield of 29.8 t ha^{-1}, outperforming both the control and CF treatments, while BF_2 produced similar results to BF_1. The highest yields were obtained from combined applications, with CF+BF_1 yielding 31.5 t ha^{-1} and CF+BF_2 yielding 34.6 t ha^{-1}. CF+BF_2 exhibited the highest yield, representing a 61% increase over the control and a 28% increase compared to CF alone.

These results underscore the significant impact of integrated nutrient management strategies on crop productivity. Our findings align with literature emphasizing the benefits of combining chemical fertilizers with biofertilizers to enhance agricultural yields. Chatzistathis et al. (2024) conducted a greenhouse experiment to investigate the effects of PGPR and AMF on growth, nutrient uptake, and physiological performance of Batavia lettuce (*Lactuca sativa* L. var. longifolia) and found that

PGPR and AMF applications with inorganic NPK fertilizer resulted in higher total biomass compared to the inorganic fertilizer treatment. Other studies have similarly demonstrated that the integration of chemical and biofertilizers enhances crop yields across diverse agricultural systems. Oktaviani and Patiung (2024) highlighted the role of biofertilizers in increasing nutrient uptake and improving soil fertility, showing that biofertilizers can partially replace chemical fertilizers without compromising yield. Our results support this, as both biofertilizer treatments (BF_1 and BF_2) increased yields by 38.1% and 37.7%, respectively, compared to the control.

3.3. Nutrient content in lettuce leaves

In addition to yield improvements, nutrient concentrations in leaves were significantly influenced by fertilizer treatments (Figures 2 and 3). N content ranged from 3.52% to 4.55% among treatments, with the highest values recorded in the $CF+BF_1$ (4.22%) and $CF+BF_2$ (4.55%) treatments. The $CF+BF_2$ treatment resulted in a 29% increase over the control and a 20% increase compared to CF alone. P content varied between 0.40% and 0.61%, with $CF+BF_1$ (0.61%) and $CF+BF_2$ (0.59%) showing the highest levels. The $CF+BF_1$ treatment

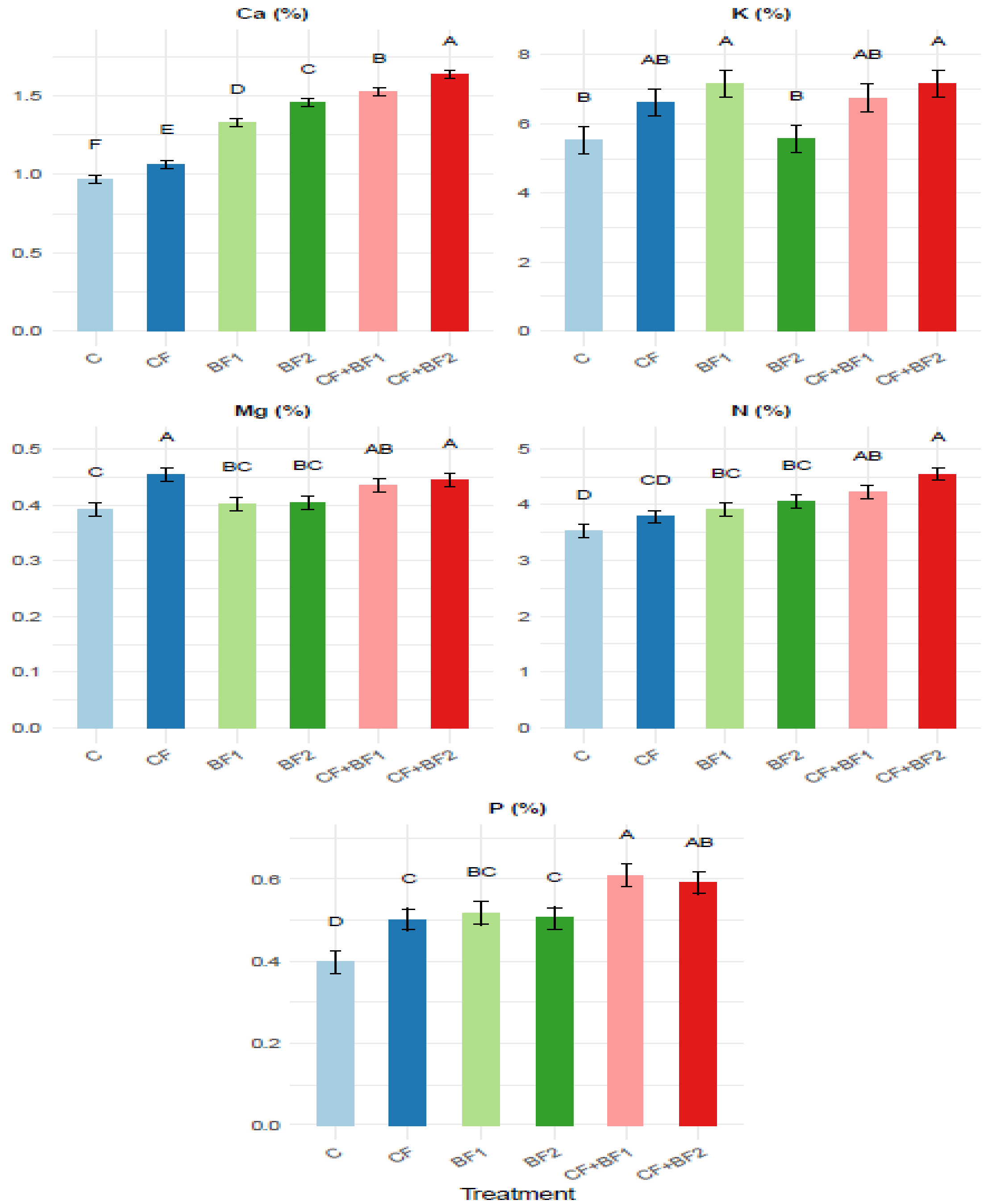

Figure 2. Effect of biofertilizer and chemical fertilizer treatments on macronutrients of lettuce leaves (Values are the means of four replicates and error bars represent the standard errors, n=4. Significant differences from Fisher's LSD test at p<0.05 are shown as different letters). C: Control, CF: Chemical Fertilizer, BF_1: Bio veria, BF_2: Bacillus mix, $CF+BF_1$: Chemical Fertilizer+ Bio veria, $CF+BF_2$: Chemical Fertilizer+Bacillus mix.

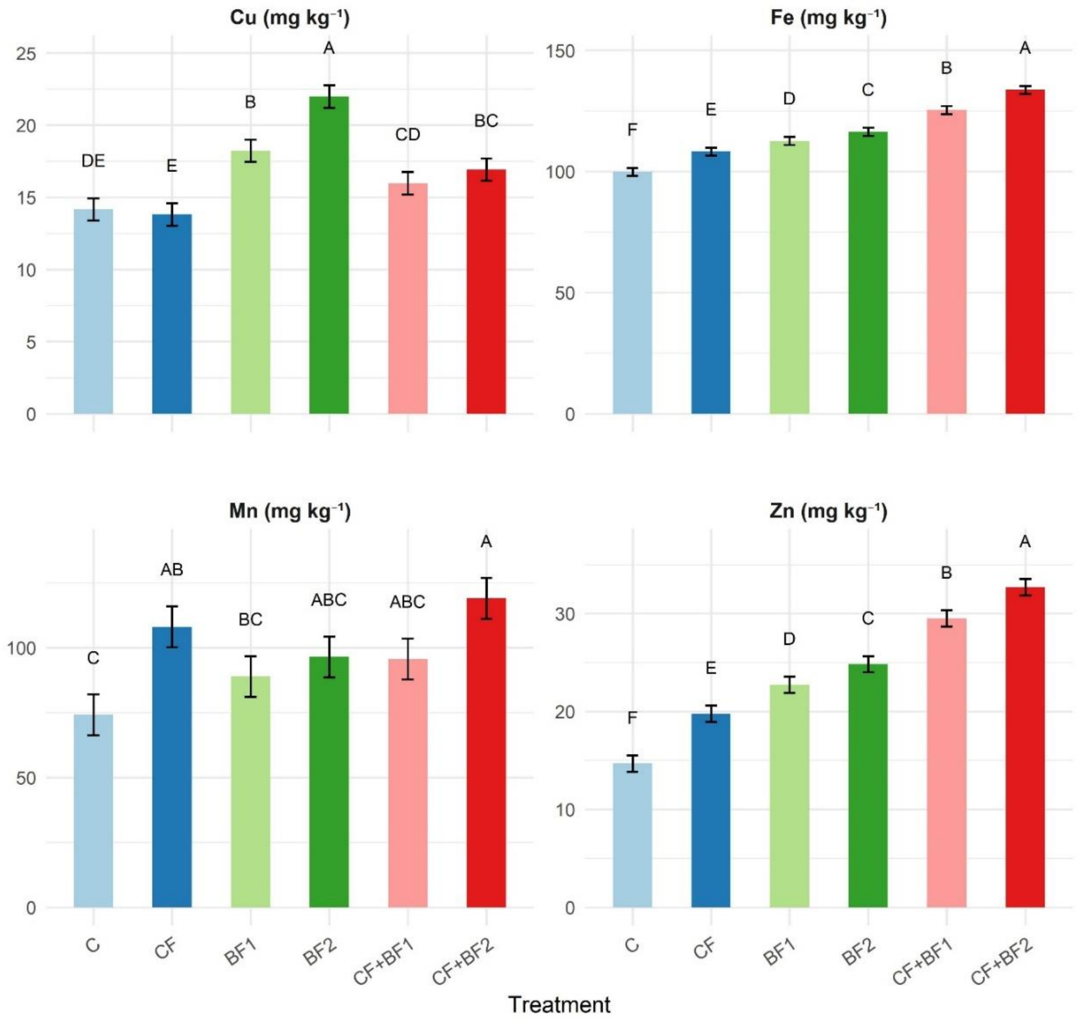

Figure 3. Effect of biofertilizer and chemical fertilizer treatments on micronutrients of lettuce leaves (Values are the means of four replicates and error bars represent the standard errors, n=4. Significant differences from Fisher's LSD test at p<0.05 are shown as different letters). C: Control, CF: Chemical Fertilizer, BF_1: Bio veria, BF_2: Bacillus mix, CF+BF_1: Chemical Fertilizer+ Bio veria, CF+BF_2: Chemical Fertilizer+Bacillus mix.

increased P content by 54% over the control and by 21% compared to CF. Potassium content ranged from 5.52% to 7.17%. The highest values were observed in CF+BF_1 (7.10%) and CF+BF_2 (7.15%), with CF+BF_2 increasing K content by 30% over the control. The Ca content of lettuce leaves ranged between 0.97% and 2.85% among treatments. The CF+BF_2 treatment produced a 54% improvement over the control and a 25% increase compared to CF. The Mg content of plant leaves varied from 0.39% to 0.46% between treatments. The dual applications did not significantly increase the Mg content in the leaves compared with the CF.

Among micronutrients, Fe content ranged from 99.87 mg kg^{-1} to 133.64 mg kg^{-1}, with CF+BF_2 achieving the highest value, 34% higher than the control. Manganese content varied from 74.16 mg kg^{-1} to 118.99 mg kg^{-1}, with CF+BF_2 showing the highest increase (60% over the control). Zinc concentrations ranged between 14.69 mg kg^{-1} and 32.69 mg kg^{-1}, with CF+BF_2 improving Zn content by 122% over the control and 20% over CF alone. The single application of biofertilizers significantly influenced the Cu concentration of the leaves, and the Cu content of the lettuce leaves varied from 13.82 mg kg^{-1} to 19.94 mg kg^{-1} between treatments.

3.4. Mechanisms behind improved nutrient uptake

The results demonstrate that combining BF with CF creates a synergistic effect, enhancing nutrient uptake. Various studies support this. Scuderi et al. (2011) found that inoculating lettuce plants with beneficial rhizosphere microorganisms significantly increased N content, likely due to improved root architecture and nutrient absorption. Hestrin et al., (2019) showed that synergistic interactions between AMF and soil microbial communities enhance nitrogen acquisition, leading to a tenfold increase in N uptake compared to non-mycorrhizal plants. Kalamulla and Yapa (2024) demonstrated that combining AMF with PGPR enhances nutrient acquisition, improving plant growth and productivity compared to single inoculants. Barea et al. (2002)

reported that the combination of AMF, phosphate-solubilizing bacteria, and nitrogen- fixing rhizobia significantly increased the availability of rock phosphate for legumes, a principle applicable to lettuce cultivation. Similar to our findings, the use of *Bacillus spp.* as a biofertilizer significantly increased the uptake of macronutrients (N, P, and K) and micronutrients (Fe, Zn, Cu, and B) in lettuce, when applied in combination with chemical fertilizers (Pagliarini et al., 2023).

The present study confirms the enhanced P uptake of plants through biofertilizers, containing particularly AMF and PGPR. The mechanisms involved in more P uptake of plants are producing organic acids and enzymes, solubilizing insoluble phosphate compounds in the soil by PGPR (Saxena et al., 2013; Widada et al., 2007) and extending root system through their hyphal network, allowing plants to access P from a larger soil volume by AMF (Santoyo et al., 2021; Xu et al., 2024). In addition, our results showed that Fe and Zn content in the leaves following the combination of biofertilizers and chemical fertilizers. This is likely due to siderophore-producing and solubilizing ability of PGPR and extension of the root system by AMF that enable better transportation and uptake of Fe and Zn (Nguyen et al., 2019; Widada et al., 2007).

These findings support the use of integrated nutrient management strategies to improve plant growth, yield, and nutrient uptake in lettuce cultivation.

4. Conclusion

This study provides strong evidence that integrating biofertilizers with chemical fertilizers significantly enhances both the yield and nutritional quality of lettuce. The combined use of Bio Veria® and Bacillus Mix® with chemical fertilizers demonstrated a synergistic effect, leading to substantial improvements in fresh and dry weight, and overall yield compared to individual fertilizer applications or the control. Among the tested treatments, CF+BF$_2$ demonstrated the greatest effectiveness the potential of multi-species containing biofertilizers in optimizing lettuce production since it outperforms other treatments by achieving the highest yield and nutrient uptake.

Moreover, the observed increases in macronutrient (N, P, K, Ca, and Mg) and micronutrient (Fe, Zn, Mn, and Cu) content in the leaves further highlight the benefits of this integrating biofertilizers with conventional fertilization strategies. The enhanced nutrient uptake can be attributed to the complementary mechanisms of PGPR and AMF, including nutrient solubilization and improved root architecture. However, future studies are needed to explore the long-term effects of this combined application on soil health and agro-ecosystems.

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