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Biofertilizers Improve the Plant Growth, Yield, and Mineral Concentration of Lettuce and Broccoli

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Abstract: Biofertilizers and organic fertilizers are eco-friendly treatments that reduce the consumption and problems associated with chemical fertilizers. The aim of this research was to investigate the effects of biofertilizers and organic fertilizers on reducing consumption and improving the effectiveness of chemical fertilizer treatments by comparing the growth parameters, yield, quality criteria, and nutrient concentration in lettuce and broccoli grown under greenhouse conditions. The biofertilizer (BM-MegaFlu®) comprised *Bacillus megaterium*, *Pseudomonas fluorescens*, and *Pantoea agglomerans* bacteria. The experiment consisted of six treatments comprising (1) biofertilizer (BF), (2) chemical fertilizer + biofertilizer (CF + BF), (3) chemical fertilizer (CF), (4) CF (1/2 dose) + BF, (5) CF (1/3 dose) + BF, and (6) organic fertilizer (OF + BF). BF did not adversely affect the head height and root collar diameter of lettuce; on the contrary, it showed non-significant differences with CF + BF, BF, CF (1/2) + BF, and CF (1/3) + BF treatments and CF alone. The highest total and marketable yields were obtained from CF + BF, CF, CF (1/2) + BF treatments in lettuce. The total yield was the highest in the CF + BF, CF, CF (1/2) + BF, and CF (1/3) + BF treatments in broccoli. In conclusion, the biofertilizer had a supportive effect on the use of chemical fertilizers in lettuce and broccoli production, especially the CF (1/2) + BF treatment in lettuce. The CF (1/2) + BF and CF (1/3) + BF treatments in broccoli showed similar yields to CF. In both crops, BF could provide 50% chemical fertilizer savings.

Keywords: fertilizer savings; microbial fertilizer; plant growth promoter; quality; yield

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1. Introduction

Cruciferous (*Brassicaceae*) vegetables are grown and used in different cultures around the world due to their good adaptability to environmental conditions [1]. Broccoli belonging to the *Brassicaceae* family is one of the most important vegetables in the world [2]. Broccoli is highly nutritious due to its high ascorbic acid, vitamins B1 and B2, calcium, and phosphorus minerals contents [3]. Lettuce belonging to the *Asteraceae* family is one of the annual leafy cool climate vegetables with exceptional demand in both national and international vegetable markets. Lettuce is consumed mainly as a salad and processed vegetable and has great importance in human nutrition due to its vitamin and mineral contents and antioxidant capacity. The advantage of eating raw lettuce is that it contains more nutrients than thermally processed lettuce [4,5].

The decrease in agricultural lands with population growth, urbanization, and industrialization forced growers to use excessive chemical fertilizer inputs for higher yields over the last 150 years, which has led to pollution, decreased soil fertility, and serious health and environmental problems. Biofertilizers can be used to reduce these problems [6]. Biofertilizers contain living microorganisms. The application of biofertilizers to the seed, the plant surfaces, or the rhizosphere increases the supply of mineral element concentrations to the crops, thus promoting growth [7]. Biofertilizers are alternative fertilizers used to increase the plant growth and yield and play a multifunctional role in terms of soil and plants [8]. When they are applied to the soil, they colonize the rhizosphere and increase the uptake of plant nutrients. Their use is more effective, as they are environmentally friendly and provide plants with access to nutrients according to chemical or organic fertilizers [9].

Microorganisms used as biofertilizers include bacteria that fix nitrogen (N), dissolve potassium (K), dissolve phosphorus (P), and mobilize phosphorus [10]. Researchers have focused especially on the *Pseudomonas* and *Bacillus* genera. The spore-forming properties of the *Bacillus* species are of increasing interest as biofertilizers or biopesticides [11]. *B. megaterium* is known for its dissolution of P and K in the soil [12] and promotes plant growth [13]. Regarding this, the *B. megaterium* strain BM18-2 increased chlorophyll synthesis, the root and shoot length, the biomass and dry weight, and the total nitrogen ratio. BM18-2 was found to be a cheap and effective product at the commercial level, as it is an efficient substitute for chemical fertilizers [14]. A biofertilizer containing *Azotobacter chroococcum*, *A. vinelandi*, *Derxia* sp., *B. megaterium*, *B. lichenformis,* and *Bacillus subtilis* bacteria increased the leaf length and width in cauliflower when used as a foliar application or in drip irrigation [15]. The addition of *B. megaterium* to conventional fertilization treatment increased the yield by 11.8–15.2%, and the reduction in P and K during conventional fertilization did not reduce the yields and quality of cucumber at the same time [16]. Civelek [17] determined that the application of *Bacillus subtilis* RC63 and *Pseudomonas fluorescens* RC77 in combination with different organic and chemical fertilizers in cauliflower plants contributed significantly to the yield, plant growth, and nutritional properties. Tanwar et al. [18] found that the *P. fluorescens* strain used had a potential role in the increasing broccoli growth, phosphatase activity, chlorophyll content, nutrients, uptake, and yield when combined with the recommended dose of fertilizer. *Paenibacillus* and *Pantoea* spp. are relatively new plant growth-promoting rhizobacteria (PGPR) with limited information available in the literature. *Pantoea agglomerans* is known as a bacterium that dissolves P and produces indoleacetic acid (IAA) [19,20]. Cocetta et al. [21] stated that the application of microbial inoculants during romaine lettuce cultivation contributed to the maintenance of the perceived quality attributes of leaves during the shelf life. Microorganisms significantly increased the weight of red lettuce and contributed to the high vitamin C concentration [22].

This research was carried out to determine the effects of biofertilizer applications on the yield, quality, and mineral concentrations in lettuce and broccoli in greenhouse conditions and whether it will reduce the use of chemical fertilizers.

2. Materials and Methods

2.1. The Experimental Area and Materials

The experiment was carried out in a greenhouse (53° N, 30°39′′ E) at Akdeniz University, Turkey in two different species of vegetables: Caipira curly lettuce (*Lactuca sativa* var. *crispa*) and Marathon F_1 broccoli (*Brassica oleraceae* var. *italica*) cultivars. The experiment included the BM-MegaFlu® commercial product biofertilizer, pH = 6–7, containing *B. megaterium*, *P. fluorescens*, and *Pantoea aglomerans* bacteria, with a total viable content of 1×10^7 cfu/mL. This biofertilizer is produced by the Biomarket Agricultural Biotechnological Products Marketing Company, Türkiye, and is certified for use in organic agriculture, with the number TR-OT-004. In the organic fertilization treatment, commercially marketed Biofarm® organic manure was used by mixing with the soil before the seedlings were planted. Biofarm® is an advanced technology fermented cattle manure produced by Camli Livestock Industry and Trade Company. Biofarm® had 50% organic matter, 2% N, 2% P_2O_5, 10% Humic + Fulvic acid, a 20% moisture content, an EC (dS m^{-1}) of 9.5, a C/N of 12.6, and a pH of 6.8–8.8.

Soil samples taken from 0–20 cm depths in the experimental area for each plot were analyzed for some physical and chemical properties (Table 1). The total $CaCO_3$, organic matter, pH, EC, texture, total N, available P, extractable K, Ca, Mg, and Na, and available Fe, Zn, Mn, and Cu were determined [23].

Table 1. Physical and chemical properties of the soil in the research greenhouse.

Parameters	Units	Value
Total $CaCO_3$	%	16.62
Organic matter	%	1.57
pH		7.58
EC	dS m^{-1}	0.45
Texture		Loamy
Total Nitrogen (N)	%	0.077
Available Phosphorus (P)	mg kg^{-1}	53.79
Extractable Potassium (K)	meq 100 g^{-1}	0.59
Extractable Calcium (Ca)	meq 100 g^{-1}	16.59
Extractable Magnesium (Mg)	meq 100 g^{-1}	2.24
Available Iron (Fe)	mg kg^{-1}	0.91
Available Zinc (Zn)	mg kg^{-1}	0.44
Available Manganese (Mn)	mg kg^{-1}	1.19
Available Copper (Cu)	mg kg^{-1}	0.29

2.2. Experimental Design and Fertilizer Treatments

The experiment was conducted between January and May. Lettuce was produced between 1 February 1 and 20 April, while broccoli was grown between 25 January and 10 May. Six treatments were included for both lettuce and broccoli in the study. The treatments were carried out with three replications according to a completely randomized design for each. Lettuce seedlings were planted with 50 cm row spacing and 30 cm intra-row spacing (plot size 1.2 m^2), while broccoli seedlings were planted with 70 cm row spacing and 40 cm intra-row spacing (plot size 2.2 m^2). Each plot contained 10 plants for both crop types. Sufficient space was left between the plots so that the applications did not affect each other. The plants were irrigated with a drip irrigation system in both crops. The treatments included in the study and the abbreviations of the treatment's names are given in Table 2.

Table 2. Fertilizer treatments and their abbreviations.

Treatments	Abbreviations
Biofertilizer (alone)	BF
Chemical Fertilizer + Biofertilizer	CF + BF
Chemical Fertilizer (alone)	CF
Chemical Fertilizer (1/2 dose) + Biofertilizer	CF (1/2) + BF
Chemical Fertilizer (1/3 dose) + Biofertilizer	CF (1/3) + BF
Organic Fertilizer + Biofertilizer	OF + BF

The chemical fertilizers applied to lettuce and broccoli plants were calculated based on doses using 140 kg ha^{-1} N, 100 kg ha^{-1} P, and 200 kg ha^{-1} K [24,25]. According to these recommendations, 16.8 g N—12 g P—24 g K per parcel was applied to lettuce, and 30.8 g N—22 g P—44 g K per parcel was applied to broccoli, respectively. It is used at a dose of 2500 kg ha^{-1} (300 g $parcel^{-1}$ for lettuce and 550 g $parcel^{-1}$ for broccoli) used by Biofarm® in organic fertilizer parcels. Organic fertilizer was mixed at a root depth height before planting lettuce and broccoli seedlings.

For the dipping of the seedlings, a solution was prepared by mixing 60 mL of biofertilizer in 100 L of water. The roots of broccoli and lettuce seedlings were dipped in this solution for 10 min, except for CF, and then planted in plots. Biofertilizer was applied to all treatments, except chemical fertilizer alone, 20 days after the lettuce and broccoli seedlings were planted. For biofertilizer applications, a solution was prepared by mixing 1

L of biofertilizer with 100 L of water. A total of 20 days after the seedlings were planted, this solution was applied to the root zone of the plants as 200 mL plant^{-1} for a week.

2.3. Examined Parameters

For lettuce, the parameters examined were the head height (cm), root collar diameter (mm), total chlorophyll content, lightness (L*), chroma (C*) and hue angle ($h°$) values of leaf color, and total and marketable yield (kg m^{-2}). For broccoli, the parameters examined were the head height (cm), head diameter (mm), leaf color values (L*, C*, $h°$), total chlorophyll content, and total yield (kg m^{-2}). Macro (N, P, K, Ca, Mg) and micro (Fe, Zn, Mn, Cu) nutrient concentrations were also analyzed in lettuce leaves and broccoli crowns [23,26].

The total chlorophyll content was detected using an SPAD 502 (Konica Minolta Sensing, Inc., Osaka, Japan) chlorophyll meter. The color values of L*, a*, and b* for both the lettuce and the broccoli leaves were measured by a Minolta CR400 Model (Konica Minolta Sensing, Inc., Osaka, Japan) color chroma meter. The measured a* and b* values were used to calculate the C* and $h°$ values by using the following formulae [27].

$$C^*\text{: } \sqrt{(a^2 + b^2)}$$

$$h^\circ\text{: } \tan^{-1}(b/a)$$

The L* (changes between 0 and 100) value indicates lightness, the a* (+a*: red, −a*: green) value indicates redness-greenness, and the b* (+b*: yellow, −b*: blue) value indicates yellowness-blueness. C* measures the color saturation or intensity. $h°$ determines the red, yellow, green, blue, purple, or intermediate colors between adjacent pairs of these basic colors [28].

2.4. Statistical Analysis

The data collected in two experiments were subjected to analysis of variance (ANOVA) by using the SAS (version 9.00; SAS Institute Inc., Cary, NC, USA) statistical software program, and the least significant difference test (LSD) at $p \leq 0.05$ was used for comparisons of the means.

3. Results

3.1. Head Height and Root Collar Diameter of Lettuce

The head height (cm) and root collar diameter (mm) growth of curly lettuce were significantly affected by different fertilizer treatments (Table 3). The highest head heights were obtained from the CF + BF, CF, BF, and CF (1/2) + BF treatments. The lowest head height values were found in the CF (1/3) + BF and OF + BF treatments. The OF + BF treatment had the lowest value, while the other treatments had higher values in terms of the root collar diameter.

Table 3. Effects of different fertilizer treatments on the head height and root collar diameter of lettuce.

Treatments	Lettuce	
	Head Height	**Root Collar Diameter**
BF	18.73 ab	23.20 ab
CF + BF	19.27 a	23.65 a
CF	18.73 ab	22.75 ab
CF (1/2) + BF	18.53 ab	22.82 ab
CF (1/3) + BF	18.07 b	23.23 ab
OF + BF	18.13 b	20.77 b
LSD	0.8865	2.5591

Means followed by different letters in each column differ significantly according to the least significant difference test at the $p \leq 0.05$ level. LSD, least significant difference; ns, not significant; BF, biofertilizer; CF, chemical fertilizer; OF, organic fertilizer.

3.2. Color Measurements and Total Chlorophyll Content of Lettuce

Different fertilizer treatments had significant effects on the color (L*, C*) and total chlorophyll content, except for the $h°$ of lettuce (Table 4).

Table 4. Effects of different fertilizer treatments on the color (light, L*; chroma, C*; and Hue, $h°$) and total chlorophyll contents of lettuce leaves.

Treatments	Lettuce			
	L*	***C****	***h°***	**Total Chlorophyll Content**
BF	57.83 b	37.17 ab	116.02	22.03 ab
CF + BF	60.13 a	37.44 ab	115.64	23.37 a
CF	59.74 ab	37.79 ab	115.92	21.40 bc
CF (1/2) + BF	59.18 ab	37.94 ab	115.96	21.60 bc
CF (1/3) + BF	59.75 ab	36.87 b	116.08	22.20 ab
OF + BF	58.28 ab	38.45 a	115.94	20.57 c
LSD	2.2279	1.5586	ns	1.412

Means followed by different letters in each column differ significantly according to the least significant difference test at the $p \leq 0.05$ level. LSD, least significant difference; ns, not significant; BF, biofertilizer; CF, chemical fertilizer; OF, organic fertilizer.

The highest L* values of the lettuce leaves were in the CF + BF, CF (1/3) + BF, CF, and CF (1/2) + BF treatments, and there was no statistical difference between these treatments. The lowest L* value was measured in BF. While the OF + BF, CF (1/2) + BF, CF, CF + BF, and BF treatments had the highest C* values, the lowest value was detected in CF (1/3) + BF. The total chlorophyll content of lettuce leaves was the highest in CF + BF, CF (1/3) + BF, BF. The lowest total chlorophyll content was recorded in the OF + BF treatment.

3.3. Total and Marketable Yield of Lettuce

Different fertilizer treatments had significant effects on the total and marketable yield of lettuce (Table 5). The total yield values varied in the range of 6.50–8.08 kg m^{-2}, with marketable yield values between 5.48 and 6.83 kg m^{-2}. Combinations of CF, CF + BF, and CF (1/2) + BF provided increases in the total and marketable yield values in lettuce.

Table 5. Effects of different fertilizer treatments on the total and marketable yield of lettuce.

Treatments	Lettuce	
	Total Yield (kg m^{-2})	**Marketable Yield (kg m^{-2})**
BF	6.61 c	5.64 bc
CF + BF	8.08 a	6.77 a
CF	7.65 ab	6.83 a
CF (1/2) + BF	7.54 ab	6.48 ab
CF (1/3) + BF	6.82 bc	5.70 bc
OF + BF	6.50 c	5.48 c
LSD	0.8376	0.9003

Means followed by different letters in each column differ significantly according to the least significant difference test at the $p \leq 0.05$ level. LSD, least significant difference; BF, biofertilizer; CF, chemical fertilizer; OF, organic fertilizer.

3.4. Mineral Concentrations of Lettuce

Macroelement (P, K, and Mg) concentrations and microelement (Fe, Zn, and Mn) concentrations were positively affected by the different fertilizer treatments (Table 6).

Table 6. Effects of different fertilizer treatments on mineral concentrations (DW: dry weight) of lettuce.

Treatments	Macroelement (%)					Microelement (mg kg^{-1})			
	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
BF	2.27	0.29 ab	3.48 ab	1.01	0.16 ab	38.7 ab	18.7 b	22.2 ab	3.1
CF + BF	2.58	0.24 ab	2.93 b	1.05	0.18 a	30.8 bc	16.1 bc	19.9 b	3.0
CF	2.42	0.23 b	3.11 b	0.92	0.14 b	23.3 c	14.8 c	18.6 b	2.6
CF (1/2) + BF	2.29	0.27 ab	3.04 b	0.97	0.16 ab	34.3 bc	17.8 bc	20.5 ab	3.3
CF (1/3) + BF	2.34	0.25 ab	3.47 ab	1.07	0.17 ab	21.7 c	17.1 bc	22.2 ab	2.8
OF + BF	2.32	0.30 a	4.01 a	1.11	0.16 ab	53.3 a	22.4 a	25.1 a	3.1
LSD	ns	0.068	0.832	ns	0.025	15.203	3.636	5.107	ns

Means followed by different letters in each column differ significantly according to the least significant difference test at the $p \leq 0.05$ level. LSD, least significant difference; ns, not significant; BF, biofertilizer; CF, chemical fertilizer; OF, organic fertilizer.

Different fertilizer treatments had no significant effects on the N, Ca, and Cu concentrations of the lettuce, which varied between 2.27 and 2.58%, 0.92 and 1.11%, and 2.60 and 3.30%, respectively. Biofertilizer generally increased the P, K, and Mg concentrations of the lettuce as compared to chemical fertilizer alone. In macroelement concentrations, the increase in P, K, and Mg varied between 4.3 and 30.4%, 18.4 and 36.9%, and 14.3 and 28.6%, respectively. In terms of microelement concentrations, the OF + BF and BF treatments had the highest Fe concentrations in lettuce plants, while the highest Zn concentration was in OF + BF. Treatments other than CF and CF + BF made a significant contribution to the increase in the Mn concentration.

3.5. Color Measurement and Total Chlorophyll Content of Broccoli

The L^* parameter reached maximum values in the CF, CF + BF, and OF + BF treatments (Table 7). While the L^* value increased proportionally with the chemical fertilizer doses, the minimum value was obtained in BF alone. The highest C^* value was noted in the CF + BF, CF, BF, CF (1/2) + BF, and OF + BF treatments, while the lowest C^* value was calculated in CF (1/3) + BF. The highest $h°$ values were in the CF + BF, CF, BF, CF (1/2) + BF, and CF (1/3) + BF treatments while the lowest $h°$ value was in OF + BF. The highest total chlorophyll content of broccoli was measured in the CF, CF (1/3) + BF, and CF (1/2) + BF treatments, while the lowest was in BF.

Table 7. Effects of different fertilizer treatments on the color (light, L^*; chroma, C^*; and Hue, $h°$) and total chlorophyll content of broccoli leaves.

Treatments	Broccoli			
	L^*	C^*	$h°$	Total Chlorophyll Content
BF	48.25 d	29.08 ab	115.96 ab	65.25 c
CF + BF	50.27 ab	30.31 a	117.93 a	68.25 abc
CF	50.81 a	29.63 ab	117.37 a	70.85 a
CF (1/2) + BF	50.06 b	28.88 ab	117.31 a	69.15 ab
CF (1/3) + BF	49.35 c	28.42 b	116.30 ab	69.30 ab
OF + BF	50.24 ab	28.61 ab	114.52 b	66.15 bc
LSD	0.6857	1.7911	2.1268	3.376

Means followed by different letters in each column differ significantly according to the least significant difference test at the $p \leq 0.05$ level. LSD, least significant difference; BF, biofertilization; CF, chemical fertilization; OF, organic fertilization.

3.6. Head Diameter, Head Height, and Total Yield of Broccoli

Different fertilizer treatments impacted the head diameter, head height, and total yield of broccoli (Table 8). It was noted that biofertilizer combined with low doses of chemical fertilizers provided approximately the same results as CF alone. Except for the BF and OF + BF applications, applications containing CF and BF showed significant effects on the head diameter in broccoli, while better results were obtained in applications containing CF and BF in terms of head height compared to OF + BF. The CF and CF + BF combinations had an increasing effect on the total yield values of broccoli, and a 63–98% yield increase was achieved compared to the minimum value.

Table 8. Effects of different fertilizer treatments on the head diameter, head height, and total yield of broccoli.

Treatments	Head Diameter (cm)	Head Height (cm)	Total Yield (kg m^{-2})
BF	11.89 bc	11.66 ab	1.14 c
CF + BF	14.13 a	12.66 a	2.26 a
CF	13.75 ab	11.94 ab	2.18 a
CF (1/2) + BF	13.55 ab	12.16 ab	2.14 a
CF (1/3) + BF	13.39 abc	12.78 a	1.86 ab
OF + BF	11.25 c	10.83 b	1.44 bc
LSD	2.1546	1.4737	0.6902

Means followed by different letters in each column differ significantly according to the least significant difference test at the $p \leq 0.05$ level. LSD, least significant difference; BF, biofertilization; CF, chemical fertilization; OF, organic fertilization.

3.7. Mineral Concentrations of Broccoli

In broccoli, the application of BF alone in P and Mg concentrations was not successful in increasing the element concentrations, and in the N concentration, applications other than the BF and CF (1/2) + BF applications provided significant increases. The treatments had no effect on K, Fe, Mn, and Zn concentrations. The combination of BF and OF resulted in a significant increase in the Ca concentration in broccoli. The BF and CF (1/2) + BF treatments had limited effects on the Zn concentration of broccoli (Table 9).

Table 9. Effects of different fertilizer treatments on the mineral concentrations (DW: dry weight) of broccoli.

Treatments	Macroelement (%)					Microelement (mg kg^{-1})			
	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
BF	4.66 bc	0.43 b	1.85	0.47 b	0.11 b	16.7	21.5 b	10.5	2.7
CF + BF	5.13 a	0.49 a	1.88	0.37 c	0.12 ab	16.8	24.6 ab	10.2	4.4
CF	5.09 a	0.47 ab	1.86	0.46 c	0.12 ab	19.5	22.6 ab	11.5	3.0
CF (1/2) + BF	4.51 c	0.48 ab	1.90	0.49 b	0.12 ab	16.3	21.8 b	10.3	3.9
CF (1/3) + BF	4.98 ab	0.51 a	1.95	0.48 b	0.13 a	21.8	23.3 ab	10.3	5.7
OF + BF	5.12 a	0.49 a	1.84	0.64 a	0.13 a	22.0	26.4 a	13.6	6.3
LSD	0.405	0.049	ns	0.095	0.013	ns	4.314	ns	ns

Means followed by different letters in each column differ significantly according to the least significant difference test at the $p \leq 0.05$ level. LSD, least significant difference; ns, not significant; BF, biofertilization; CF, chemical fertilization; OF, organic fertilization.

4. Discussion

The results obtained in this study revealed that the different fertilizer treatments affected the growth parameters of lettuce. The effects of biofertilizers were better with chemical fertilizers. In addition, the CF + BF, BF, CF (1/2) + BF, and CF (1/3) + BF treatments showed similar results to CF alone. The supporting effect of biofertilizers may be due to the fact that microorganisms increase the root activity in the rhizosphere, initiate hormonal activity, and, thus, increase the uptake of plant nutrients [8,9,14]. Several studies reported the supportive effects of biofertilizers on plant growth. Seif Sahandi et al. [29] indicated that the plant height of mint increased with the synergistic effect of *Pseudomonas putida* and *P. agglomerans* bacteria applied with 50 kg ha^{-1} P. *T. harzianum* increased the plant height by 7% in arugula and by 4% in garden cress [30]. Yildirim et al. [31] applied PGPR to lettuce plants under salt-stress conditions and determined that *Kocuria erythromyxa* increased the head length by 30% compared to the control.

The color values (*L**, *C**) of lettuce leaves were significantly affected by different fertilizer treatments. The L* values in lettuce showed that the leaf color was lighter in other treatments compared to BF, which had the lowest value. The best treatment was CF (1/3) + BF, with the lowest value in lettuce at the *C** value, which expresses the saturation of the leaf color. The broccoli leaves were brighter in the CF + BF and CF treatments. The *C** value was lowest in the CF (1/3) + BF application, so the color saturation was the best in CF (1/3) + BF. The lowest *h*° value was in the OF + BF treatment. The *h*° values of broccoli showed that the darkest green leaves were obtained in all treatments except for OF + BF. Üçok et al. [32] observed that the color values of curly lettuce (*L**: 56.23–59.43; *C**: 37.19–38.82; *h*°: 115.56–117.61) varied according to different organic fertilizers. Sönmez et al. [33] found that there was no change in color values when different organic fertilizers were used in lettuce production.

CF, BF, and CF + BF combinations contributed positively to the total chlorophyll content in lettuce and were more effective than OF + BF. The contribution of the OF + BF application to the chlorophyll content was limited. The CF and reduced CF + BF treatments made significant contributions to the increase in the chlorophyll content in broccoli. Although the results of the research varied according to the different fertilizer experiments, it was shown that the addition of BF to the CF had supportive effects. In particular, with the reduced CF treatments, similar results to those of CF treatment alone were almost achieved. Previous studies showed that some PGPRs affect the chlorophyll content. Han and Lee [34] reported that the total chlorophyll content of lettuce increased with the application of Serratia spp. and Rhizobium spp. Seif Sahandi et al. [29] stated that the total chlorophyll content of mint increased due to the synergistic effect of Pseudomonas putida and *P. agglomerans* microorganisms. Ozbay et al. [30] reported that *T. harzianum* increased the chlorophyll content by 10% in arugula and by 7% in cress. Yildirim et al. [31] found the best results in terms of chlorophyll measurements in lettuce cultivation in B. subtilis and *Staphylococcus kloosii* bacteria.

The yield is the most important criterion in vegetable cultivation, as in other cultivated plants. In our research, the CF + BF, CF, and CF (1/2) + BF treatments provided the best result regarding the total and marketable yield in lettuce. For broccoli, the best results in terms of the total yield were obtained from the CF + BF, CF, CF (1/2) +BF, and CF (1/3) + BF treatments. It is a well-known practice to increase the yield with chemical fertilization, but the effective use of biofertilizers and reduced chemical fertilizer application may make it necessary to update fertilization practices. With this result in the yield parameter, it will be possible to reduce the use of chemical fertilizers and provide environmental and economic gains. It can be stated that this situation is caused by the activity of microorganisms in the soil. Several studies reported an increase in productivity and the reduced usage of chemical fertilizers through biofertilizers. Panda [35] and Berg [36] reported that biofertilizers are effective in the range of 35–65% on the yield. Some researchers also determined that the use of NPK can be reduced with the use of microbial fertilizers. A study conducted on *P. agglomenas*, *P. polymyxa*, and *Funneliformis mossea* bacteria showed that the highest fruit yield in French beans was obtained from *P. agglomenas*, *P. polymyxa* + *P. agglomenas*, and *F. mossea* experiments. The highest N and P contents were obtained from the mixture of *P. polymyxa* + *P. agglomenas* + *F. mossea*. As an alternative approach, the NPK fertilizers consumption in French bean cultivation was reduced by 25% with microbial inoculation [37]. Yildirim et al. [31] found that some PGPR applications in lettuce increased the head weight by 40%

compared to the control. Altuntaş [38] stated that the highest total head yield was obtained by using *B. subtilis* inoculations in broccoli.

In the study examining the effects of organic manure (Control) and *Bacillus cereus* (BC), *Rhizobium rubi* (RR), and *Brevibacillus reuszeri* (BR) inoculations on broccoli, the lowest yield per plant was obtained from the control when compared to the mineral fertilization and bacterial combinations. The BC, BR, and RR inoculations with manure treatment increased the yield per plant by 17%, 20%, and 24% and the plant weight by 17%, 17%, and 29% compared to the control. Notably, RR increased the yield per plant by 3.95% compared to the mineral treatment [39]. Young et al. [40] determined that PGPR (mixture of *Bacillus* sp., *B. subtilis*, *B. erythropolis*, *B. pumilus*, and *P. rubiacearum*) and 50% chemical fertilizer (1/2 CF + biofertilizer) increased the lettuce yield by 25%. Haque et al. [41] found that the average fruit weight was most effective in 50% N + 50% BioF applications, which reduced NPK doses by 50% with biofertilizer containing *Trichoderma harzianum* in tomato.

The highest head diameter in broccoli was achieved in the CF + BF treatment, while the highest head height was found in the CF + BF and CF (1/3) + BF treatments. Altuntaş [38] observed that the highest head diameter of broccoli (14.50 cm) was obtained with the *B. subtilis* strain QST 713 when compared with different biofertilizers.

Regarding macroelements, the different fertilizer treatments significantly affected the P and Mg concentrations in lettuce. In broccoli, the CF, OF + BF, and CF + BF combination treatments affected the N, P, and Mg concentrations. The combined application of the biofertilizer and organic fertilizer increased the uptake of macroelements in plants. The BF increases the availability of nitrogen and phosphorus, thus promoting the better utilization of nutrients by plants, encouraging greater root growth and development [3]. Some bacteria, such as *Bacillus* and *Azotobacter*, are capable of synthesizing organic acids and phosphates, which convert the non-absorbable form of phosphorus into an available form for plants [41]. Bacteria in the genera *Pseudomonas*, *Bacillus*, and *Rhizobium* are known as the strongest phosphate-dissolving bacteria [42]. Among the microorganisms involved in the dissolution of phosphorus, phosphate solvent bacteria have the potential to dissolve 1–50% of phosphorus [43], and phosphate-dissolving bacteria secrete phosphate organic acid metabolites containing hydroxyl and carboxyl group chelates and convert them into an available form by binding with cation bonds [44]. Rather et al. [45] revealed that *Azotobacter* and *Azospirillum* bacteria increase the amount of IAA and the root length, strengthening cytokinin formation and root branching, thus increasing the nutrient uptake from the soil and accelerating the growth of plants. It was reported that animal manure caused a significant increase in the macroelement contents of biofertilizer (*Azotobacter*) used together with NPK in broccoli cultivation [46]. Biofertilizer containing *Bacillus methylotrophicus* increased the nutrient concentration (K, Mg, Na, P, Fe, Zn, and N) in lettuce [47]. PGPR inoculation with *P. fluorescens* for lettuce plants significantly increased the shoot dry matter and shoot N, P, Ca, Mg, Mn, and Na uptake rates [48]. Macik et al. [49] stated that biofertilizers can enable the conversion of nutritionally important elements from unavailable forms to available forms through biological processes. Lal et al. [50] observed that *Azotobacter* and PSB (phosphate solubilizing bacteria) treatments increased the zinc content in broccoli, and this increase was equivalent to that of chemical fertilizer treatment. In another study, it was reported that biofertilizer-containing microorganisms increased microelements in lettuce plants [51]. According to Altuntaş [38], the *B. subtilis* strain QST 713 increased the Zn content in broccoli compared to the control. Biofertilizers can be examined in detail in order to increase the yield per unit area in agricultural production and to reduce the use of chemical fertilizers.

5. Conclusions

The biofertilizer treatments were evaluated separately for lettuce and broccoli. The findings obtained showed that a similar yield can be obtained by using less chemical fertilizer combined with biofertilizer as compared to the recommended full-dose chemical fertilizer application alone. Although the less chemical fertilizer combined with biofertilizer showed variations according to plant growth parameters, the best results were found in the CF + BF, CF, CF (1/2) + BF, and CF (1/3) + BF treatments in lettuce and broccoli. The highest total and marketable yields in lettuce were achieved in the CF + BF, CF, and CF (1/2) + BF treatments, which means that similar total and marketable yields can be achieved with the CF (1/2) + BF treatment, thereby using 50% less CF. In broccoli, it was noted that the highest total yield was obtained from the CF + BF, CF, and CF (1/2) + BF treatments. The CF (1/2) + BF treatment can yield as much as CF with 50% less usage of CF. These results are important due to the increase in chemical fertilizer input costs in vegetable cultivation and the harmful effects of chemical fertilizers on the environment and human health. In conclusion, the biofertilizer had supportive effects on the growth parameters, yield, quality, and mineral concentrations in both broccoli and lettuce. The CF (1/2) + BF treatment can be recommended in lettuce and broccoli cultivation, as it can save 50% CF with an almost similar yield to that of the full dose of CF. Moreover, when biofertilizers are used, it should be considered that the positive effects will continue to increase after 2–3 years depending on the beneficial microorganism activity in the soil.

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