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Effects of PGPR Inoculation and Vermicompost on the Growth, Physiological Traits, Microbial Activity, and Yield of Lettuce (*Lactuca sativa* L.) Plants

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REDUCED soil productivity is one of the effects of climate change. This lowers agricultural output, and using inorganic fertilizers will further degrade the quality of the soil. Thus, using organic fertilizer is a good way to increase the number and quality of lettuce plants while using less chemical fertilizer. A randomized block arrangement pot experiment was conducted to examine the effects of plant growth promoting rhizobacteria (PGPR, *Azospirillum lipoferum* and *Pseudomonas koreneesis*) and vermicompost (VC, 10 tone ha^{-1}) on the vegetative growth, physiological traits, soil microbiological activity, chemical composition, and yield of lettuce plants (*Lactuca sativa* L. c.v. Alcapucci) in the 2021 and 2022 growing seasons. Following 35 days of transplanting, results revealed that T8 therapy (VC + *A. lipoferum* + *P. koreneesi*s inoculation) recorded the greatest values of total chlorophyll, carotenoids, and total soluble sugar in seasons 2021 and 2022, compared to control treatment (T1). Furthermore, it was discovered that the rhizosphere of lettuce plants cultivated under soil additives with VC and PGPR inoculation in both growing seasons had a significantly ($P < 0.05$) different microbial community, which comprises the total number of bacteria, *Azospirillum*, *Pseudomonas*, and DHA enzyme. For various soil amendment applications with VC and PGPR inoculation, the highest percentage of N, P, and K obtained T8 > T6 > T7 > T5, and for various PGPR inoculation alone, T4 > T3 > T2 > T1. Additionally, in comparison to the control treatment during the 2021 season, the T8 treatment (combination) results in records for Fe, Cu, Mn, and Zn (mg Kg^{-1}) in lettuce leaves under soil additives with VC of 92.34, 6.76, 41.76, and 52.84, respectively. Similar trends to those observed in the 2022 season may be seen in the production metrics of 70-day-old lettuce plants, including fresh and dry weight, plant height, leaf area, and number of leaves under different treatments of soil amendments (VC) and inoculation with PGPR. Thus, using PGPR + VC in combination with other safe and healthful methods greatly enhanced the growth dynamics of lettuce plants in the study.

Keywords: Lettuce, Biofertilizers, Physiological characteristics, Microbial community, Yield parameters.

1. Introduction

Horticultural crops make up a significant portion of human nourishment. The quality of vegetables that increase physical performance, decrease the risk of disease and extend life spans through effective and sustainable farming are in greater demand (Rashwan, and Elsaied, 2022; Çakmakçı, 2023). Dylag et al. (2023) state that lettuce (*Lactuca sativa* L.) is a low-calorie and low-fat (saturated). It is also rich in potassium, folic acid, as well as vitamins A, C, and E, and fiber. Lettuce is the predominant vegetable cultivated for the fresh market in various areas (USDA Foreign Agricultural Service, 2007). Research has shown that the utilization of organic fertilizers in vegetable farming can help reduce nitrate-N levels compared to crops treated with traditional chemical

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fertilizers (Zhang et al. 2024). However, depending on the source and rate of application of organic fertilizer, even organic fertilizers may result in elevated nitrate-N levels in vegetables (Hina 2024).

Plant growth-promoting rhizobacteria (PGPR) are commonly referred to as "biofertilizers" since they are living organisms that help the host plant's nutritional status through their ongoing positive connection (Omara et al. 2017; Afify et al. 2018; Ouf et al., 2023). Studies have demonstrated that rhizobacteria play a crucial role in enhancing plant growth by facilitating various mechanisms such as producing phytohormones to promote growth, fixing atmospheric N_2 for plants, and generating siderophores to aid in iron uptake by plant roots. The application of plant growth promoting rhizobacteria (PGPR) in integrated nutrient management systems has proven to be effective in reducing nutrient runoff from fertilized soils and curbing nutrient accumulation (Khoso et al. 2024). Nutrient runoff is often associated with problems related to human and environmental health (Juncal et al. 2023). It is simple to substitute vermicompost a naturally occurring product of specific earthworm species activity for chemical fertilizers. According to Wu et al. (2019) and Toor et al. (2024), this biological fertilizer is a substance that has high concentrations of vital nutrients, including nitrogen and P in both organic and inorganic forms, plant growth regulators, and advantageous microbes. Vermicomposting, according to Elbagory, (2018) and Oyewole et al. (2013), is a process that recycles organic waste materials under particular temperature and aeration conditions into a more digestible form with higher nutritional and mineral content and helpful bacteria. The soil's fertility can then be restored and crop development can be improved by using this organic matter as organic matter. Because vermicompost is rich in organic matter, micronutrients, and essential plant macronutrients, it can be used as a soil conditioner (Ingelmo et al. 2012). It has been discovered that using organic fertilizer made from vermicompost is a sustainable and eco-friendly way to enhance soil quality and encourage plant development. Soil fertility and productivity are largely dependent on the microbiological characteristics of the soil (Atiyeh et al. 2001; Rehman et al. 2023).

According to the literature currently available, vermicomposting in combination with the inoculation of effective microorganisms can increase the amount of nutrients such as phosphorus and total nitrogen, decrease the time it takes for the soil to decompose, increase the humic substances, and stabilize the organic fraction. Raising the carbon content also helps the soil's microbial structure (Pereira et al. 2022; Poornima et al. 2024). Additionally, it can boost soil biodiversity, contribute to the synthesis and release of phytohormones that affect plant metabolism, and inhibit pathogens in soil phytopathogens and organic residues (Khoso et al. 2024; Toor et al. 2024). In addition, the study examined the impacts of various combinations of chemical, and bioorganic treatments on the growth of the mustard plant (*Brassica campestris*), results showed that the vermicompost-applied parcels produced the best results and that the combination of vermicompost and 25% reduced chemical fertilizer produced favorable results in many parameters in agroclimatic conditions when compared to the control group (Mondal et al. 2015). On the other hand, because of the different PGPR strains and vermicompost applications, it has been discovered that PGPR with VC are most applied way to increase the yield, and nutrient content of lettuce plants when compared to PGPR applications alone. Also, the combination treatment (bacteria + vermicompost) stood out and significantly improved the yield components by affecting the lettuce plant's head weight, number of leaves, stem length and diameter, and levels of elements (Çelik 2023).

Our study focused on these specific elements and used a pot experiment to investigate the joint effects of PGPR inoculation (*Azospirillum lipoferum* and *Pseudomonas koreneesis*) and bio-organic fertilizer (VC, 10 ton ha^{-1}) on the vegetative growth, physiological traits, soil microbiological activity, chemical composition, and yield of lettuce plants (*Lactuca sativa* L. c.v. Alcapucci) in the 2021 and 2022 growing seasons.

2. Materials and Methods

2.1 Vermicompost used and their characterization

Vermicompost (VC) was brought from the Agricultural Research Center, Giza, Egypt. VC had an OM content of 43%, EC, 3.5 dS m^{-1}, total N, P and K, 2.4, 7.1 and 0.8% respectively, pH 7.1, and polyphenol 8.5%; WHC 140; and C 18.1 %.

2.2. PGPR and growth conditions

The Bacteriology Lab., SWERI, ARC, Egypt provided two bacterial strains, *Azospirillum lipoferum* SP2 and *Pseudomonas koreneesis* MG209738. *A. lipoferum* was grown in semi - solid malate (SSM) medium as per Döbereiner and Day (1976), while *P. koreneesis* was cultured in King's B broth medium following the method described by King et al. (1954). Previous studies have shown that these bacteria exhibit plant growth promoting (PGP) abilities including the production of IAA and P solubilization (Hafez et al. 2019) as well as the production of siderophores (Ghazy and El Nahrawy 2021).

2.3. Pots trial

To investigate the combined effects of bio-organic fertilizer (VC) and PGPR inoculation on the vegetative growth, physiological characteristics, soil microbiological activity, chemical composition, and yield of lettuce plants (*Lactuca sativa* L. c.v. Alcapucci), a pot experiment was carried out on January 5 and 10, 2021 and 2022, at the Microbiology Lab. greenhouse in Kafr El-Sheikh Governorate, Egypt. The clay soil's physical and chemical characteristics are displayed in Table 1. The seedlings of lettuce were bought from a private nursery, Baltim city, Egypt.

Before transplanting, one healthy 20-day-old lettuce plant seedling, each pot (30 cm (diameter) by 35 cm (height) polyethylene bag) was filled with 6 kg pot^{-1}. The soil was thoroughly mixed with VC at a rate of 10.0 ton ha^{-1} according to Hafez et al. (2021). Each pot was irrigated before the transplanting, and the moisture content reached to 40%. Ammonium sulphate (20.6% N), a mineral fertilizer containing nitrogen was administered at a rate of 238 kg ha^{-1} in two equal amounts at 21 and 45 days following transplanting. Potassium sulphate (48% K_2O) was applied at a rate of 119 kg ha^{-1} in two equal split administrations, i.e., four and six weeks after transplanting, while calcium superphosphate (15.5% P_2O_5) was applied at a rate of 476 kg ha-1 prior to transplanting. 64 lettuce plants were used in the experiment. They were split into 8 treatments, each with 8 duplicates, and placed in the following randomized block arrangement: T1: Control (without VC), T2: Inoculation with 3 ml of *A. lipoferum* (without VC, 1x10^{8} CFU ml^{-1}), T3: Inoculation with 3 ml of *P. koreneesis* (without VC, 1x10^{8} CFU ml^{-1}), T4: combination (T2+T3); T5: Control (with VC), T6: Inoculation with 3 ml of *A. lipoferum* (with VC, 1x10^{8} CFU ml^{-1}), T7: Inoculation with 3 ml of *P. koreneesis* (with VC, 1x10^{8} CFU ml^{-1}), and T8: combination (T6+T7).

TABLE 1. Physical and chemical analysis of the soil used.

Season	Mechanical analysis (%)			Texture	pH (1:2.5)	EC (dS m^{-1})	OM (g Kg^{-1})	Available elements (mg Kg^{-1})		
	Sand	Silt	Clay					N	P	K
2021	21.14	24.69	54.17	Clayey	7.67	2.69	16.15	8.56	8.02	381.30
2022	20.33	26.02	53.65	Clayey	7.72	2.85	17.32	9.12	8.46	350.07

2.4. Traits Measurements

Physiological Characteristics

Photosynthetic pigments

To calculate total chlorophylls and carotenoids, 0.1 g of four samples (leaf) were crushed, extracted in acetone (5 ml, 80%), following Lichtenthaler's (1987) instructions. Following a 10-minute centrifugation at 13,000 xg, the supernatant's absorbance was measured at 663, 645, and 470 nm. The amounts of carotenoids and chlorophylls were determined as mg g^{-1} FW and µg g^{-1} FW, respectively.

Total soluble sugar

Utilizing the Hendrix (1993) methodology. In short, 0.5 g of four samples (leaf) were homogenized in ethanol (5 ml, 80%), then deposited in a water bath at 80 °C for 30 minutes. After centrifuging at 10,000 ×g for 10 min, the supernatants were measured at 620 nm wavelength using a UV Spectrophotometer (Model 6705). The results were reported as µg g^{-1} FW based on a glucose standard curve.

Microbial community and soil enzymes estimations

Thirty-five days after transplanting, ten g of soil samples (rhizosphere) were combined with ninety milliliters of distilled water that had been sterilized, well mixed, and agitated for thirty minutes at 150 rpm. Soil extract agar medium was used to quantify the total count of bacteria (Allen, 1959). Furthermore, the most probable number (MPN) approach

(Cochrane, 1950) and SSM medium (Döbereiner et al., 1976) were used to calculate the total count of *Azospirillum*. However, King et al. (1954) reported that the total count of *Pseudomonas* by King's B medium. All microbial counts were calculated as (log CFU 10 g^{-1} dry soil). Also, by converting 2, 3, 5, triphenylotetrazolium chloride to triphenyl formazon (red-colored), soil samples were submitted to spectrophotometric to determine dehydrogenase activity which calculated as mg TPF g^{-1} soil day^{-1} (Casida et al. 1964).

Macro- and Micro-Nutrients

0.5 g of crushed leaf sample were digested using 30% H_2O_2 and concentrated sulfuric acid on a hot plate 70 days after transplanting, following the procedures described by Jones et al. (1991). According to Peters et al. (2003), micro-Kjeldahl was used to determine the nitrogen (%). Using Page et al. (1982) spectrophotometric methods, the phosphorus (%) was calculated. Using a Flame photometer and Cottenie et al.'s (1982) methodology, the potassium (%) was determined. Furthermore, micronutrients including Zn, Mn, Fe, and Cu were assessed in milligrams per kilogram using atomic adsorption spectrophotometry (Perkin Elmer 3300) as per the methods outlined by Cottenie et al. (1982).

Yield parameters

Four healthy plants to each treatment were removed at 70 days after transplanting, which measurements were made of their fresh and dry weights (g $plan^{-1}$), height (cm $plant^{-1}$), leaf area (cm^2), and leaf count.

2.5 Statistical analyses

Results were statistically analyzed according to the analysis of variance (ANOVA) approach by CoStat software, and the differences were assessed at $p < 0.05$ using DMRT (Duncan, 1955). Data are presented as means ± SD.

3. Results

3.1. Physiological Characteristics

After 35 days of transplanting, there were notable variations ($P < 0.05$) in the amounts of total chlorophyll, carotenoids, and total soluble sugar in lettuce leaves based on the vermicompost applied and the PGPR inoculation (Fig. 1). In seasons 2021 and 2022, data showed that the highest total chlorophyll values of 2.71 and 2.78, 2.87 and 2.47 mg g^{-1} FW for T8 treatment (VC + inoculation with *A. lipoferum* + inoculation with *P. koreneesis*), and T6 treatment (VC + inoculation with *A. lipoferum*) over control treatment (T1), respectively.

Conversely, as compared to the same treatments but without VC additives (T1, T2, T3, and T4), VC treatments (T5, T6, T7, and T8) displayed the greatest values for carotenoids and TSS under additives. For example, in comparison to other examined treatments, T5, T6, T7, and T8 treatments recorded 0.75, 0.94, 0.91, and 0.96 for carotenoids (μg g^{-1} FW) and 5.78, 6.03, 5.71, and 6.96 for TSS (μg g^{-1} FW) in season 2021 (Fig. 1B, C). In the 2022 season, the same pattern was observed. According to the previously indicated data, T8 > T6 > T7 > T5 for various applications of VC soil amendments and PGPR inoculation.

3.2. Soil microbiological activity

After 35 days of transplanting, the microbial community, which includes the total number of bacteria, *Azospirillum*, *Pseudomonas*, and DHA enzyme, was found to be significantly ($P < 0.05$) different in the rhizosphere of lettuce plants grown under soil additives with VC and PGPR inoculation in both the 2021 and 2022 growing seasons (Fig. 2). Overall, the findings show that the microbial community varied depending on the microbial inoculation. In comparison to the other treatments, the T8 treatment (combination) demonstrated the highest population of total counts of bacteria (7.66 and 7.92 CFU log10 g^{-1}; Fig. 2A); *Azospirillum* (4.59 and 4.82 CFU log10 g^{-1}; Fig. 2B), *Pseudomonas* (3.07 and 3.29 CFU log10 g^{-1}; Fig. 2C); and DHA enzyme (268.00 and 276.40 mg TPF g^{-1} soil day^{-1}, Fig. 2D) during the first (2021) and second (2022) growing seasons, respectively.

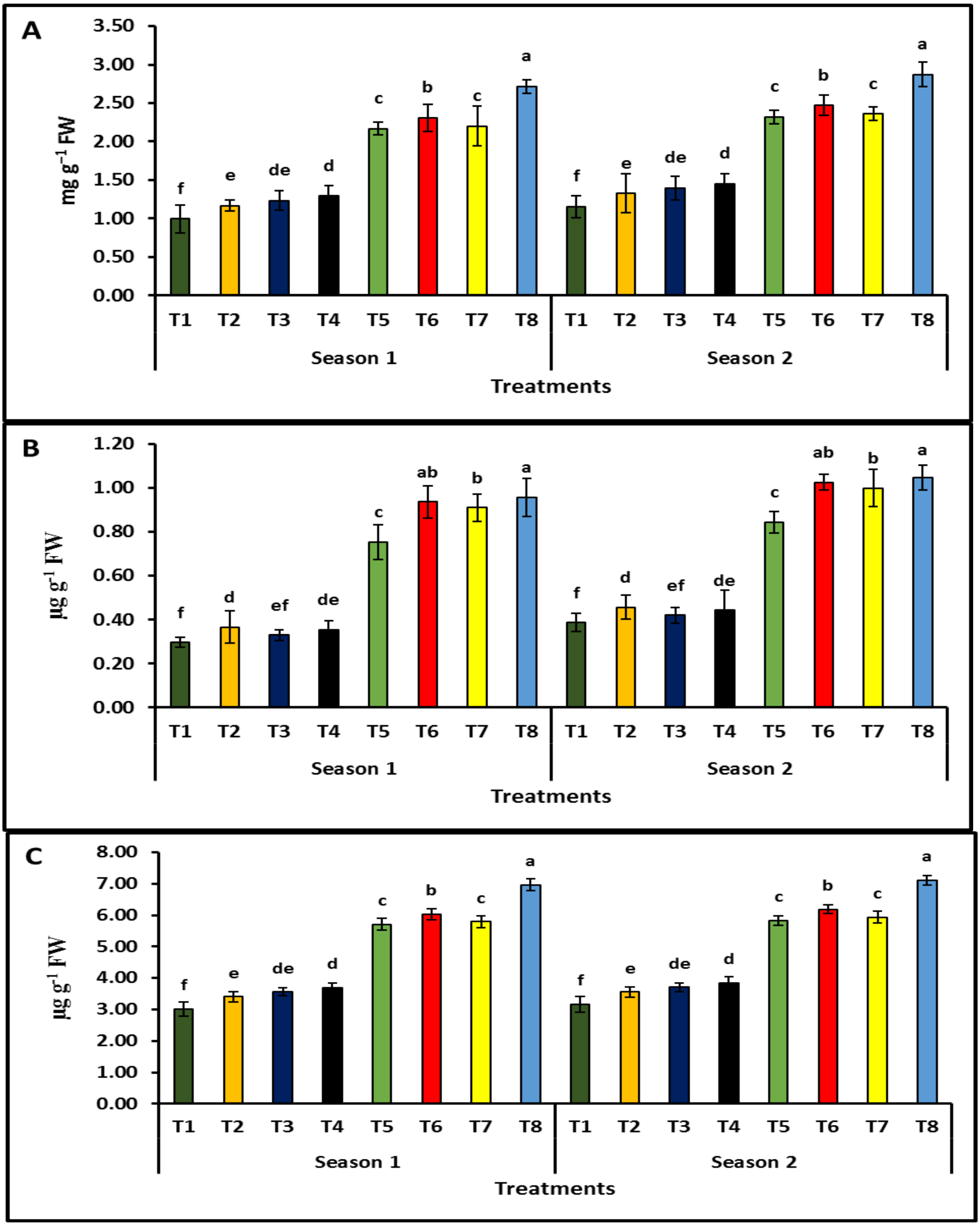
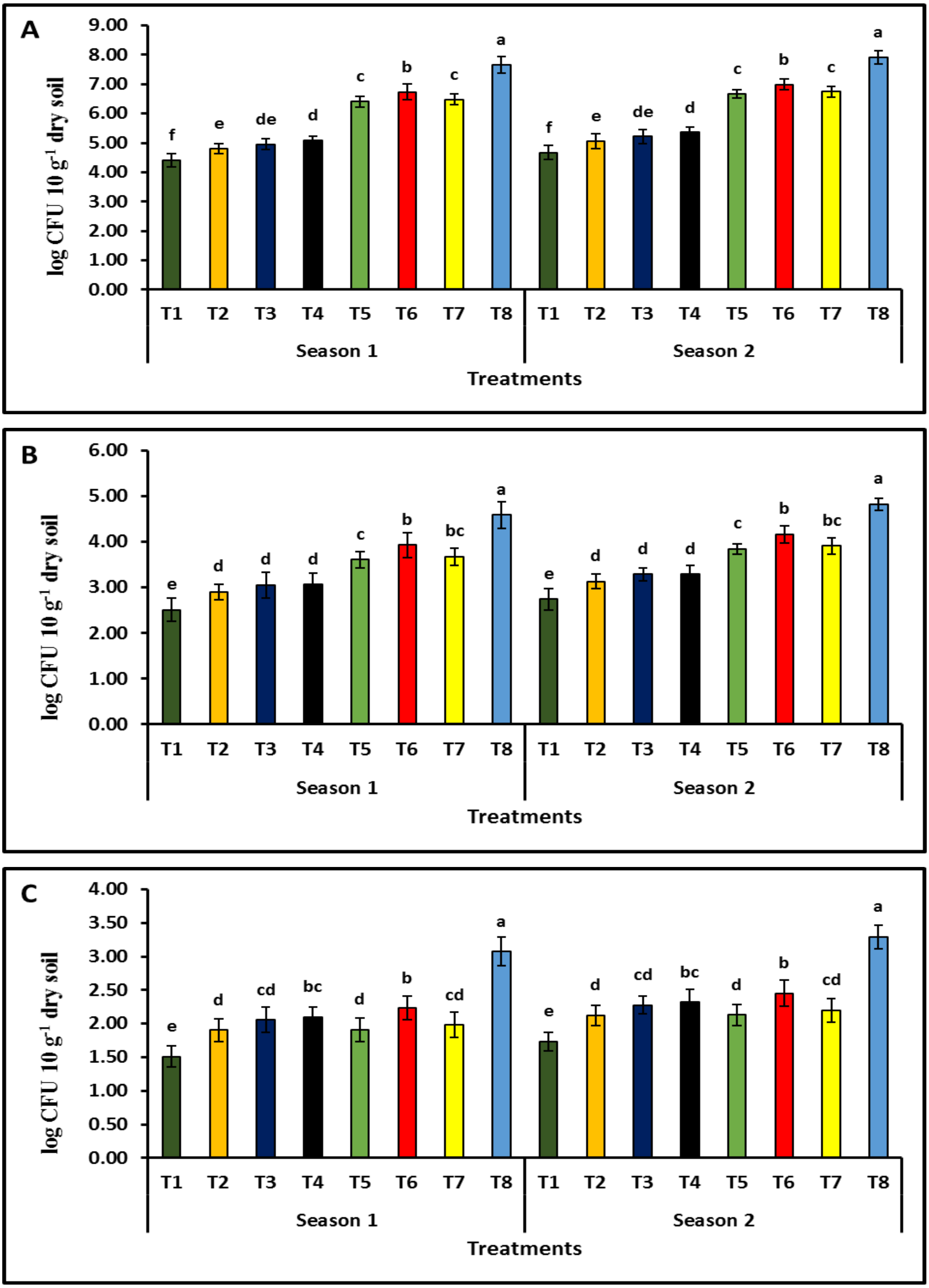


Fig. 1. Combined effects of soil amendments with VC and inoculation with PGPR on total chlorophyll (A), carotenoids (B) and TSS (C) in lettuce leaves at 35 days from transplanting during 2021 and 2022 seasons. Duncan's test, means with different letters after them denote significant differences ($P < 0.05$) between the treatments. SD: means ± standard deviation which derived from three replicates. T1: Control (without VC), T2: Inoculation with *A. lipoferum* (without VC), T3: Inoculation with *P. koreneesis* (without VC), T4: combination (T2+T3); T5: Control (with VC), T6: Inoculation with *A. lipoferum* **(with VC), T7: Inoculation with** *P. koreneesis* **(with VC), and T8: combination (T6+T7).**


A
B
C
log CFU 10 g-1 dry soil
T1 T2 T3 T4 T5 T6 T7 T8
Season 1
Season 2
Treatments

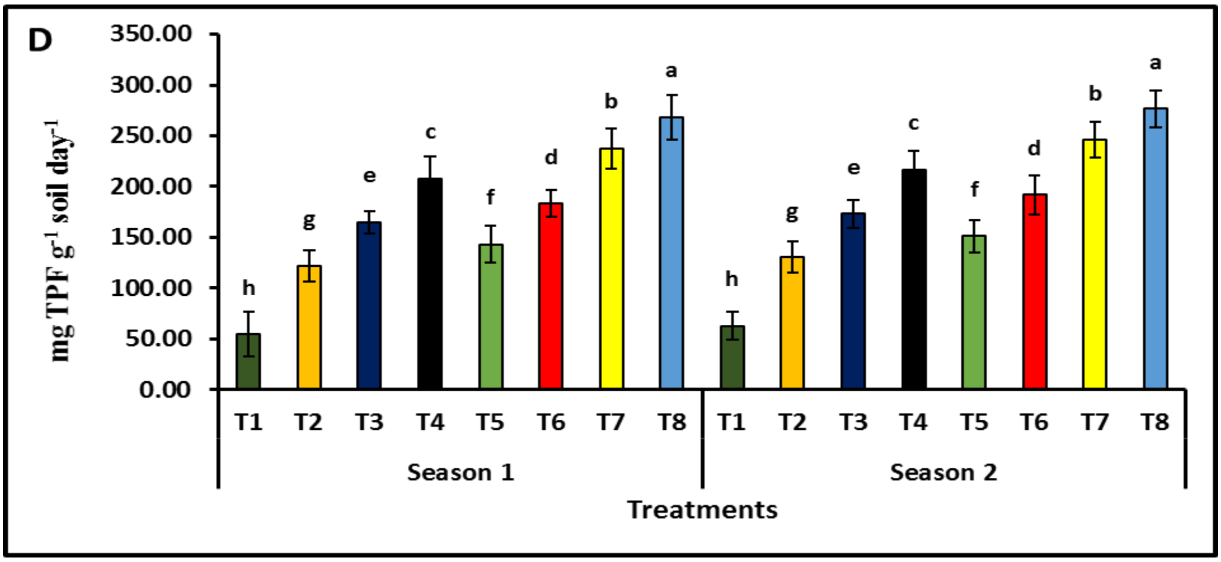


Fig. 2. Combined effects of soil amendments with VC and inoculation with PGPR on total bacteria (A), *Azospirillum* (B), *Pseudomonas* (C) and DHA enzyme (D) in lettuce leaves at 35 days from transplanting during 2021 and 2022 seasons. Duncan's test, means with different letters after them denote significant differences (P < 0.05) between the treatments. SD: means ± standard deviation which derived from three replicates. T1-T8: See footnote Figure 1.

3.3. Macroelements in lettuce leaves

The effects of PGPR inoculation and soil additions with VC on the percentages of N, P, and K in lettuce plant leaves throughout both growth seasons were statistically significant (P < 0.05) (Table 2). In seasons 2021 and 2022, respectively, T4 treatment yielded the highest percentage of N attained 2.1 and 2.04% under inoculation with *A. lipoferum* + *P. koreneesis* without soil additives with VC, followed by T3 treatment (inoculation with *P. koreneesis*), attained 1.97 and 2.03%, compared to T1 treatment (control), 1.42 and 1.50%. However, under inoculation with *A. lipoferum*, + *P. koreneesis* with soil additives with VC, T8 treatment gave the high percent of N attained 2.98 and 3.02 % followed by T6 treatment (inoculation with *P. koreneesis*), attained 2.14 and 2.21 %, compared to T5 treatment (control with VC), 1.89 and 1.97 %, in seasons 2021 and 2022, respectively (Table 2). Conversely, in comparison to the other treatments, the T8 treatment (combination + VC) exhibited the highest values of P and K%, with values of 0.90 and 0.91 % and 2.63 and 2.64 % during the, 2021 and 2022 seasons, respectively. Based on the previously indicated results, T8 > T6 > T7 > T5 for various soil amendment applications with VC and PGPR inoculation, and T4 > T3 > T2 > T1 for various PGPR inoculation alone (Table 2).

3.4. Microelements in lettuce leaves

Lettuce plants were exposed to soil additives (VC) and inoculation with PGPR (*A. lipoferum* and *P. koreneesis*) showed significant (P < 0.05) higher plant microelements i.e. Fe, Cu, Mn and Zn (Table 3). By day 35 after transplanting, the combination treatment of T8 resulted in significantly higher levels of Fe, Cu, Mn, and Zn (mg Kg^{-1}) in lettuce leaves, reaching record values of 92.34, 6.76, 41.76, and 52.84, respectively, compared to the control treatment when soil additives with VC were applied during the 2021 season. This trend was also evident during the 2022 season (Table 3). As a result, compared to other treatments under study, the T8 treatment (combination) demonstrated the highest uptake of microelements in lettuce plants. The decreasing order of the treatments was T8 (*A. lipoferum* + *P. koreneesis* + VC) > T6 (*A. lipoferum* + VC) > T7 (*P. koreneesis* + VC) > T5 (VC, control) according to Table 3. After being exposed to soil additions (VC) and PGPR (*A. lipoferum* and *P. koreneesis*) inoculation, lettuce plants displayed significantly (P < 0.05) greater levels of plant microelements, such as Fe, Cu, Mn, and Zn . When compared to the control treatment, the T8 treatment (combination) results in records for Fe, Cu, Mn, and Zn in lettuce leaves at 35 days after transplanting, which are 6.76, 41.76, 52.84, and 92.34, respectively, under soil additives with VC throughout the 2021 season. A comparable pattern was noted in the season of 2022 (Table 3). As a result, compared to other treatments under study, the T8 treatment (combination) demonstrated the highest uptake of microelements in lettuce plants. The decreasing order of the treatments was T8 (*A. lipoferum* + *P. koreneesis* + VC) > T6 (*A. lipoferum* + VC) > T7 (*P. koreneesis* + VC) > T5 (VC, control) (Table 3).

TABLE 2. Combined effects of soil amendments with VC and inoculation with PGPR on N, P and K % in lettuce leaves at 35 days from transplanting during 2021 and 2022 seasons.

Treatments	N (%)	P (%)	K (%)
		First season (2021)	
T1	1.42 ± 0.10 e	0.24 ± 0.02 f	1.07 ± 0.10 e
T2	1.81 ± 0.13 d	0.31 ± 0.02 d	1.46 ± 0.13 d
T3	1.97 ± 0.06 cd	0.27 ± 0.03 ef	1.62 ± 0.06 cd
T4	2.01 ± 0.12 bc	0.29 ± 0.02 de	1.66 ± 0.12 bc
T5	1.82 ± 0.08 d	0.69 ± 0.04 c	1.47 ± 0.08 d
T6	2.14 ± 0.08 b	0.88 ± 0.02 ab	1.79 ± 0.08 b
T7	1.89 ± 0.07 cd	0.85 ± 0.01 b	1.54 ± 0.07 cd
T8	2.98 ± 0.11 a	0.90 ± 0.02 a	2.63 ± 0.11 a
LSD 0.5%	0.16	0.03	0.14
		Second season (2022)	
T1	1.50 ± 0.11 e	0.27 ± 0.01 f	1.11 ± 0.14 e
T2	1.88 ± 0.15 d	0.33 ± 0.03 d	1.50 ± 0.17 d
T3	2.03 ± 0.09 cd	0.30 ± 0.05 ef	1.64 ± 0.09 cd
T4	2.04 ± 0.11 bc	0.32 ± 0.01 de	1.71 ± 0.18 bc
T5	1.91 ± 0.07 d	0.71 ± 0.05 c	1.51 ± 0.09 d
T6	2.21 ± 0.04 b	0.90 ± 0.06 ab	1.81 ± 0.05 b
T7	1.97 ± 0.03 cd	0.85 ± 0.04 b	1.63 ± 0.03 cd
T8	3.02 ± 0.10 a	0.91 ± 0.08 a	2.64 ± 0.15 a
LSD 0.5%	0.19	0.07	0.16

Duncan's test, means with different letters after them denote significant differences ($P < 0.05$) between the treatments. SD: means ± standard deviation which derived from three replicates. T1-T8: See footnote Figure 1.

TABLE 3. Combined effects of soil amendments with VC and inoculation with PGPR on microelements (Fe, Cu, Mn and Zn mg Kg^{-1}), in lettuce leaves at 35 days from transplanting during 2021 and 2022 seasons.

Treatments	Fe	Cu	Mn	Zn
		First season (2021)		
T1	61.44 ± 0.75 f	3.33 ± 0.21 e	18.07 ± 0.57 f	21.94 ± 0.75 f
T2	64.50 ± 0.99 e	4.19 ± 0.28 d	20.42 ± 0.76 e	25.00 ± 0.99 e
T3	65.70 ± 0.45 de	4.52 ± 0.13 cd	21.34 ± 0.35 de	26.20 ± 0.45 de
T4	66.78 ± 0.68 d	4.61 ± 0.27 bc	22.17 ± 0.52 d	27.28 ± 0.68 d
T5	83.10 ± 0.54 c	4.36 ± 0.15 cd	34.27 ± 0.50 c	43.06 ± 0.65 c
T6	85.08 ± 0.62 b	4.91 ± 0.18 b	36.20 ± 0.48 b	45.58 ± 0.62 b
T7	82.56 ± 0.65 c	4.20 ± 0.18 d	34.68 ± 0.41 c	43.60 ± 0.54 c
T8	92.34 ± 1.02 a	6.76 ± 0.24 a	41.76 ± 0.78 a	52.84 ± 1.02 a
LSD 0.5%	1.27	0.36	0.97	1.24
		Second season (2022)		
T1	63.65 ± 0.79 f	3.53 ± 0.29 e	19.26 ± 0.51 f	22.13 ± 0.70 f
T2	68.71 ± 0.91 e	4.44 ± 0.20 d	22.63 ± 0.70 e	27.19 ± 0.88 e
T3	69.91 ± 0.41 de	4.71 ± 0.10 cd	23.51 ± 0.30 de	26.39 ± 0.33 de
T4	69.99 ± 0.60 d	4.80 ± 0.21 bc	24.33 ± 0.51 d	27.47 ± 0.59 d
T5	84.77 ± 0.55 c	4.41 ± 0.14 d	33.92 ± 0.45 c	45.79 ± 0.45 c
T6	87.29 ± 0.69 b	5.11 ± 0.12 b	38.45 ± 0.49 b	47.77 ± 0.36 b
T7	87.31 ± 0.50 c	4.55 ± 0.10 cd	36.42 ± 0.55 c	46.25 ± 0.87 c
T8	96.55 ± 1.09 a	6.99 ± 0.22 a	43.96 ± 0.71 a	55.03 ± 0.89 a
LSD 0.5%	1.29	0.33	0.93	1.27

Duncan's test, means with different letters after them denote significant differences ($P < 0.05$) between the treatments. SD: means ± standard deviation which derived from three replicates. T1-T8: See footnote Figure 1.

3.5. Yield parameters

Significant differences in the yield characteristics of lettuce plants under different treatments of soil amendments (VC) and PGPR inoculation were noted in the two growing seasons. Table 4 lists these parameters: height, leaf area, number of leaves, and fresh and dry weight. When it came to yield characteristics, the combination treatment (T8) performed better than the control treatment overall. Table 4 demonstrates that treated lettuce plants with T8 (VC + PGPR) greatly raised the dry weight (g plant^{-1}), 45.56 in 2021 and 48.66 in 2022, while also significantly increasing the fresh weight (g plant^{-1}), 512.00 in 2021 and 523.00 in 2022. Similar to the other treatments under study, in season 2021, the application of VC soil amendments and PGPR inoculation resulted in increased plant height, leaf area, and number of leaves, reaching 43.03 cm plant-1, 13.41 cm^2 and 46.04 for T8 treatment and 36.17 cm plant^{-1}, 11.31 cm^2 and 38.78 for T6 treatment, respectively. The 2022 season showed the same pattern (Table 4).

TABLE 4. Combined effects of soil amendments with VC and inoculation with PGPR on fresh weight, dry weight, height, leaf area and number of leaves in lettuce plant at 70 days from transplanting during 2020 and 2021 seasons.

Treatments	FW (g plant^{-1})	DW (g plant^{-1})	PH (cm plant^{-1})	LA (cm^2)	No. L plant^{-1}
			First season (2021)		
T1	297.67 ± 6.80 h	24.85 ± 0.61 h	12.84 ± 0.70 f	4.46 ± 0.21 f	15.12 ± 0.73 f
T2	407.67 ± 6.10 e	35.05 ± 0.54 e	15.73 ± 0.93 e	5.35 ± 0.28 e	18.18 ± 0.95 e
T3	365.00 ± 3.60 g	31.28 ± 0.32 g	16.87 ± 0.42 de	5.69 ± 0.12 de	19.38 ± 0.41 de
T4	451.00 ± 4.35 c	39.10 ± 0.39 c	17.89 ± 0.63 d	6.01 ± 0.19 d	20.46 ± 0.64 d
T5	387.00 ± 5.29 f	33.88 ± 0.48 f	33.79 ± 0.61 c	10.58 ± 0.19 c	36.26 ± 0.65 c
T6	481.33 ± 5.13 b	42.59 ± 0.56 b	36.17 ± 0.59 b	11.31 ± 0.18 b	38.78 ± 0.62 b
T7	427.33 ± 4.51 d	37.75 ± 0.41 d	34.30 ± 0.51 c	10.74 ± 0.16 c	36.80 ± 0.54 c
T8	512.00 ± 6.24 a	45.56 ± 0.57 a	43.03 ± 0.97 a	13.41 ± 0.30 a	46.04 ± 1.02 a
LSD 0.5%	9.26	0.86	1.20	0.36	1.27
			Second season (2022)		
T1	309.67 ± 6.11 h	28.95 ± 0.23 h	16.14 ± 0.45 f	4.57 ± 0.12 f	16.74 ± 0.71 f
T2	419.67 ± 6.22 e	39.15 ± 0.13 e	19.03 ± 0.87 e	5.46 ± 0.25 e	19.80 ± 0.82 e
T3	377.00 ± 3.67 g	35.38 ± 0.39 g	20.17 ± 0.69 de	5.80 ± 0.19 de	21.00 ± 0.19 de
T4	463.00 ± 4.44 c	43.20 ± 0.30 c	21.19 ± 0.46 d	6.12 ± 0.16 d	22.08 ± 0.55 d
T5	438.33 ± 4.71 f	36.98 ± 0.39 f	36.09 ± 0.29 c	10.67 ± 0.11 c	37.86 ± 0.78 c
T6	492.33 ± 5.23 b	45.69 ± 0.76 b	38.47 ± 0.37 b	11.40 ± 0.10 b	40.38 ± 0.71 b
T7	398.00 ± 5.46 d	40.85 ± 0.97 d	36.60 ± 0.78 c	10.83 ± 0.26 c	38.40 ± 0.42 c
T8	523.00 ± 6.20 a	48.66 ± 0.18 a	45.33 ± 0.39 a	13.50 ± 0.36 a	47.64 ± 0.99 a
LSD 0.5%	9.13	0.79	1.13	0.38	1.31

Duncan's test, means with different letters after them denote significant differences ($P < 0.05$) between the treatments. SD: means ± standard deviation which derived from three replicates. T1-T8: See footnote Figure 1. FW: fresh weight; DW: dry weight; PH: plant height; LA: Leave area; No.L: number of leaves.

4. Discussion

The use of biofertilizers (VC and PGPR) enhanced biological activity, the availability of nutrients, and subsequent plant development. The phosphate solubilizing activity and auxin and siderophore-producing capacities of *Azospirillum* and *Pseudomonas*, which were employed in this study, were characteristics that promoted plant growth. Furthermore, VC is abundant in nutrients, plant growth regulators, and advantageous microbes that facilitate the conversion of nutrients into forms that are accessible to plants and enhance plant growth.

The significance of investigating strain compatibility for plant co-inoculation under soil additions with organic fertilizers (VC) is underscored by our findings. This is supported by the fact that other researchers have used various strains of *Pseudomonas* and *Azospirillum*, most of which had not been evaluated for co-inoculation compatibility, with varying degrees of success. In the case of lettuce, for instance, reports have indicated that specific strain combinations yield better results (Mangmang et al. 2015; Aponte et al. 2017; Kishore et al. 2017; Çelik, 2023; Díaz et al. 2023; Toor et al. 2024). The application of VC and PGPR inoculation had the biggest influence on chlorophyll content in terms of physiological properties. Numerous studies have shown that these applications can improve plant growth and chlorophyll content (Khalid et al. 2017). Vermicompost's high nutrient content, which includes essential nutrients that the plants can access, is responsible for this improvement. According to Hussain et al. (2017), beneficial bacteria present in VC also improve soil quality, increase nutrient availability, and shield plants from illness. Additionally, vermicomposting increases the organic matter content, water-holding capacity, and soil fertility all of which support the growth of plants, levels of chlorophyll, carotenoids, and TSS (Toor et al. 2024).

Furthermore, Huang et al. (2017) demonstrated that organic matter and microorganisms in vermicompost, as well as enhanced nutrient availability and better soil structure, were responsible for the beneficial impacts. Consistent with the current study's findings, a prior investigation conducted by Liu et al. (2019) and Pereira et al. (2022) discovered that the microbial community and soil enzymes were most significantly affected by the use of VC and PGPR inoculation. Moreover, this is in line with a prior work by Wu et al. (2019), which found that applying VC and PGPR increased the C content of the microbial biomass. Nonetheless, increased DHA suggests that VC in conjunction with PGPR treatment benefits soil microbial activity and soil enzyme activity, including dehydrogenase activity (Wu et al. 2019; Toor et al. 2024).

Simultaneously, our research emphasizes the importance of combining PGPR and VC to enhance lettuce's nutrient absorption. This improvement in plant nutrition can be attributed to PGPR's ability to increase the absorption, solubility, and bioavailability of essential minerals, including P, K, Zn, and Fe. Moreover, the ability to physiologically fix atmospheric N_2 contributes to improved nutritional intake (Acurio Vasconez et al. 2020; Basu et al. 2021). Furthermore, PGPR's ability to stimulate the production of phytohormones including IAA and cytokinins, as well as gibberellins which is closely linked to increase the yield. These phytohormones are necessary to enhance overall plant growth and development (Khatoon et al. 2020; Ikiz et al. 2024). Furthermore, PGPRs can promote the synthesis and excretion of many chemical compounds. Lyu et al. (2023) list these as siderophores, organic molecules (volatile) and hydrolytic enzymes, including chitinases, proteases, cellulases, and pectinases.

Our findings are consistent with the context that PGPR and VC additive inoculation has been demonstrated to enhance the growth and development of lettuce plants, as demonstrated by improvements in key plant growth parameters such as leaf area, height, shoot weight, and dry matter (Acurio Vasconez et al. 2020; Rostaminia, 2021; Ikiz et al. 2024). This is relevant for improving the yield of lettuce plants and growth parameters. A dynamic interaction is revealed by the symbiotic link between bacteria and roots. Especially, shows the capacity to generate phytohormones, especially IAA, which plays a critical role in fostering the vegetative development of plants (Vetrano et al. 2022). Furthermore, the beneficial impacts of PGPR and VC cover significant processes such as atmospheric N_2 fixation and solubilization of potassium and phosphate in the nutritional solution (Reid et al. 2021). Together, these processes support the general growth of lettuce plants. Acurio Vasconez et al. (2020) provided evidence in support of this claim by demonstrating a significant rise in a number of growth indices following bacterial strain inoculation of lettuce plants as compared to non-inoculated treatment. These indicators included plant height, plant dry matter, plant thickness, root weight, and root dry matter. This observable beneficial effect demonstrates the proactive function that PGPR with VC plays in improving plant nutrition, encouraging greater root growth, and eventually improving the growth and development of lettuce plants as a whole.

5. Conclusion

Statistically significant differences were identified in the impact of various PGPR extra vermicompost applications on the yield criteria of lettuce plants throughout the 2021 and 2022 seasons in the pot experiment investigation. Consequently, the vegetative growth, physiological traits, soil microbiological activity, chemical composition, and yield of lettuce plants were improved by the combined application of bioorganic fertilizer (VC) and PGPR (*A. lipoferum* and *P. koreneesis*).

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