



Article

Comparative Effects of Bio-Wastes in Combination with Plant Growth-Promoting Bacteria on Growth and Productivity of Okra

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Abstract: Plant growth-promoting rhizobacteria with multiple growth-promoting traits play a significant role in soil to improve soil health, crop growth and yield. Recent research studies have focused on the integration of organic amendments with plant growth-promoting rhizobacteria (PGPR) to enhance soil fertility and reduce the hazardous effects of chemical fertilizers. This study aims to evaluate the integrated application of biochar, compost, fruit and vegetable waste, and *Bacillus subtilis* (SMBL 1) to soil in sole application and in combined form. The study comprises eight treatments—four treatments without inoculation and four treatments with SMBL 1 inoculation in a completely randomized design (CRD), under factorial settings with four replications. The results indicate that the integrated treatments significantly improved okra growth and yield compared with sole applications. The integration of SMBL 1 with biochar showed significant improvements in plant height, root length, leaf chlorophyll a and b, leaf relative water content, fruit weight, diameter and length by 29, 29, 50, 53.3, 4.3, 44.7 and 40.4%, respectively, compared with control. Similarly, fruit N, P and K contents were improved by 33, 52.7 and 25.6% and Fe and Zn in shoot were 37.1 and 35.6%, respectively, compared with control. The results of this study reveal that the integration of SMBL 1 with organic amendments is an effective approach to the sustainable production of okra.

Keywords: *Bacillus subtilis*; biochar; compost; okra; organic amendments; sustainable

1. Introduction

Okra (*Abelmoschus esculentus* L.) belongs to the Malvaceae family and is one of the most famous vegetables. The main purpose of this crop is to consume its immature pods as boiled and fried vegetable or add them to soups, stews or salads [1]. Okra is extensively used as vegetable crop and provides important nutritional contents for the human diet, such as minerals, proteins, carbohydrates and vitamins [2]. Young okra pods contain 0.2% fat, 2.2% protein, 86% water, 10% carbohydrates and vitamins A, B, C [3]. During 2019, 121.5 thousand tons of okra were produced from an area of 15.7 thousand hectares with a major contribution of Punjab (68.8 thousand tons), followed by Sindh (20.3 thousand tones),

Khyber Pakhtunkhwa (17.7 thousand tons) and Baluchistan (14.8 million tons), showing a decrease compared with previous years due to harsh environmental conditions [4].

Climate change, harsh environmental conditions and other abiotic factors are responsible for lowering crop yields in arid and semiarid regions. Furthermore, extensive cultivation of and agricultural production obtained from the same piece of land are responsible for nutrient imbalance, decreased soil fertility, loss of biodiversity and expedite organic matter decomposition. Due to these reasons, okra yields are gradually declining not only in Pakistan but in all the developing countries. High cropping intensity and leaching, soil particle fixation and surface runoff are the major causes for the loss of nitrogen and other nutrients [5]. Excessive use of agrochemicals (fertilizers and pesticides) after the green revolution has posed negative effects on the environment, such as greenhouse gas emissions, nutrients leaching, increase in agrochemical residues in food and salinization of agricultural soils [6]. Tillage operations have expedited the organic matter decomposition in cultivated lands and have altered the pH, provoking a shift towards alkalinity, which is responsible for the depletion of the soil nutrient pool. Furthermore, the slow release of nutrients by organic amendments has caused a reduction in the application of organic amendments, which further deteriorated the soil conditions [7]. Soil organic carbon (SOC) performs a vital role in the development of soil biological and physiochemical characteristics for crop yield improvement via increasing the water holding capacity, carbon sequestering, reduced nutrient leaching, soil erosion, improved nutrient cycling and gaseous exchange in soil [8]. The use of organic amendments/biofertilizers of plant and animal origin increases soil organic carbon [9]. As organic matter is a key factor for controlling soil properties and enhancing crop yield, Ramesh [10] demonstrated that the application of microbes and organic amendments is the only method to improve organic carbon contents in soil. Organic farming, such as the repeated application of beneficial microbes and organic amendments, is gaining importance day by day in relation to enhancing microbial diversity and sustainable production of crops [11].

Biochar, an organic amendment, is a pyrolysis product of plant waste [12] and has ability to influence/improve chemical, physical and biological attributes of soil, making it favorable for crop production [13,14]. The application of biochar has proved to be a sustainable approach as it improves the yield, growth and quality of fruit, as well as their nutrient status and the water use efficiency of crops (WUE) [15]. Moreover, the improvement in soil chemical properties (cation exchange capacity (CEC), exchangeable sodium percentage (ESP) and soil pH) through biochar application under salt stress was found responsible for improving the nutritional status of sunflower [5]. Sanger et al. [16] suggested that the biochar application alone and in combination with other organic amendments, biofertilizers or mineral fertilizers to marginal lands provides sustainable crop production by not only improving physical (water holding capacity and high surface area) and chemical attributes (high CEC) of soil [17], but also enhancing its biological parameters, such as microbial populations and their activities [18–20]. Similarly, compost is also beneficial for improving plant growth by enhancing the water-holding capacity and organic carbon in soil [21]. The use of compost in soil conserves the environmental quality as a sustainable agriculture practice [22]. Compost is also an important organic amendment that can be applied to lessen the chemical fertilizers requirements, promote water-holding capacity, microbial enzyme activities and biological populations not only under normal soil conditions but also under stress environments [23,24].

The microorganisms present in soil are the main source of nutrient recycling, such as fixation of nitrogen (N) and mineralization of SOC; in this way, they improve nutrient sustainability [25]. These microbes secrete different kinds of extracellular enzymes to capture carbon and other nutrients, converting complex molecules into simpler forms by degradation depending on the abundance and microbial diversity [26]. Therefore, plant growth-promoting rhizobacteria (PGPR) can improve the growth of plants by defending plants from stress conditions and soil-borne diseases and enhance plant growth via synthesizing extracellular enzymes and phytohormones, as well as promoting antifungal activity,

nitrogen fixation, nutrient availability, improving ACC-deaminase activity and phosphorus, potassium and zinc solubilization [27–29]. Hales and his coworkers [30] used biochar as an alternate PGPR carrier instead of peat and vermiculite and found promising results. Nadeem et al. [31] found increased growth and physiological attributes of cucumber and soil biological parameters (CFU = colony forming unit) through the synergistic use of *Pseudomonas fluorescens*, compost and biochar under water deficit conditions. Thus, in view of the above situation, this research work was conducted to assess the effects of the combined use of biochar, fruit and vegetable waste, and compost with the *Bacillus subtilis* SMBL 1 strain on the growth and yield of okra and soil microbial population. The hazardous impact of chemical fertilizers on the environment through conventional applications has posed serious threats to crop production [6] which needs to alter with sustainable approaches to enhance nutrient fortification in crops, especially vegetables.

2. Materials and Methods

2.1. Collection of Biochar, Fruit and Vegetable Waste, and Compost

Compost made of wheat straw and farmyard manure as raw material and biochar derived from wheat straw were taken from Environmental Microbiology Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad Pakistan. Fruit and vegetable wastes were collected from the local fruit and vegetable market of Bahawalpur, air dried in shade and then ground into powder with the help of a grinder.

2.2. Identification through 16S rRNA Gene Sequencing of Bacterial Strain (SMBL 1)

The pre-isolated P and Zn solubilizer, IAA producer and ACC-deaminase-producing strain (SMBL 1) was obtained from the Soil Microbiology and Biotechnology Laboratory, Department of Soil Science, The Islamia University of Bahawalpur. The identification of strain SMBL 1 was performed through partial gene sequences of 16S rRNA. Initially, DNA extraction was carried out from a SMBL 1 strain cell culture using an extraction buffer containing proteinase K [32]. DNA extraction was followed by a PCR reaction to amplify the 16S rRNA sequence using the 27F and 1492R universal primer pair. The reaction mixture contained 3.7 µL of crude DNA, 3 µL of 27F (10 µM), 3 µL of 1492R (10 µM) and 7.5 µL of H₂O. The conditions for thermal cycling were 95 °C, 1 cycle for 1 min, followed by 32 amplification cycles of 1 min at 94 °C and 20 s cycle of 55 °C hold. The PCR products (3 replicates) were sent to the commercial sequencing service Macrogen[®] Seoul, Korea for 16S rRNA sequencing. The obtained sequences of nucleotide were BLAST-compared with the available genome database of NCBI and identified as *Bacillus subtilis*. Following the Thompson et al. [33] method, a phylogenetic tree was constructed on the MEGA-X software (Figure 1). The processing of data was carried out by using an NJ Plot according to the neighbor joining method [34]. The partial sequence of nucleotides was submitted to the Gen Bank database of NCBI under the accession number of the (SMBL 1) strain, which was MN007187.

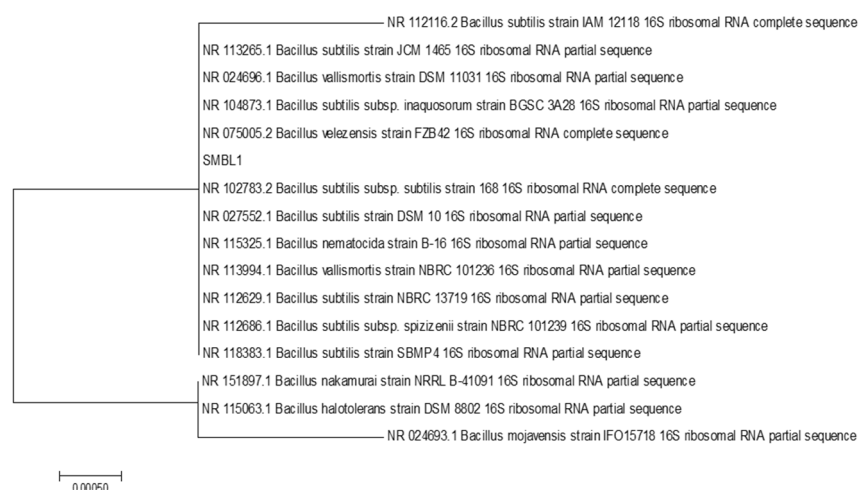


Figure 1. Phylogenetic tree of the bacterial strain *Bacillus subtilis* (SMBL 1) (accession #MN007187).

2.3. Analysis of Biochar, Compost, and Fruit and Vegetable Waste

The chemical analyses of biochar, compost and fruit and vegetable waste were performed at the Department of Soil Science, Soil Microbiology and Biotechnology Lab, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur Pakistan, following the standard method [35]. The electrical conductivity and the pH of fruit and vegetable waste, compost and biochar with a mass to volume ratio of 1:20 in distilled or deionized water were calculated with an EC- and a pH-meter, respectively. With the help of the method found in [36], the C contents were measured in fruit and vegetable waste, biochar and compost. A Kjeldahl digestion and distillation apparatus was used to determine N contents [37]. For potassium and phosphorus determination, fruit and vegetable waste, compost and biochar samples were digested through H_2SO_4 (sulfuric acid) and H_2O_2 (hydrogen peroxide) [38]. A flame photometer was used to determine potassium contents in a digested sample (Model BWB-XP, BWP Technologies, UK). Phosphorus determination in a digested filtrate was performed by a color development in the spectrophotometer (Model G6860A, Cary 60 UV-Vis Agilent Technologies, Australia). The observed chemical characteristics of biochar, compost, and fruit and vegetable waste are given in (Table 1).

Table 1. Physical-chemical parameters of organic amendments and soil used for pot trial.

Characteristics	Unit	Values
Biochar		
EC	($dS\ m^{-1}$)	1.69 ± 0.0053
pH	-	7.8 ± 0.017
Nitrogen	%	0.20 ± 0.0039
Phosphorus	%	0.31 ± 0.0053
Potassium	%	1.98 ± 0.0053
Carbon	%	36.07 ± 0.048
Compost		
Organic matter	%	45.25 ± 0.39
pH	-	6.55 ± 0.035
Carbon	%	26.77 ± 0.22
Nitrogen	%	1.13 ± 0.013
Potassium	%	1.02 ± 0.0085
Phosphorus	%	1.88 ± 0.039

Table 1. Cont.

Characteristics	Unit	Values
Fruit and vegetable waste		
Organic matter	%	46.5 ± 0.24
pH	-	6.8 ± 0.013
Carbon	%	27 ± 0.068
Nitrogen	%	1.15 ± 0.003
Potassium	%	1.035 ± 0.005
Phosphorus	%	1.95 ± 0.053
Soil		
Clay	%	15.4 ± 0.045
Silt	%	38.5 ± 0.026
Sand	%	46.1 ± 0.057
Textural Class	-	Sandy Loam
EC _e	(dS m ⁻¹)	1.4 ± 0.027
pH _s	-	8.1 ± 0.030
Saturation percentage	%	40 ± 0.45
Phosphorus	mg kg ⁻¹	5.4 ± 0.018
Potassium	mg kg ⁻¹	145 ± 0.26
Nitrogen	%	0.021 ± 0.0006
Organic matter	%	0.58 ± 0.0030

Values are means of four replications ± standard error.

2.4. Inoculation of Seed and Management of Experiment

Okra seeds were purchased from a local market (variety: Pusa Sawani) and sterilized by dipping for 2 min in ethanol (95%) followed by HgCl₂ (0.2%) for 4 minutes then washed with autoclaved distilled water for 5–6 times. Afterwards, the seeds were soaked in a 48 h old broth culture of the SMBL 1 strain for 30 min prior to sowing for inoculated treatments, while the seeds of un-inoculated treatments were dipped in a sterilized growth medium (Luria-Bertani or LB medium) [39–41]. The treatment of this study comprised two sets, i.e., the un-inoculated set (T0 = control, T1 = compost, T2 = biochar and T3 = fruit and vegetable waste) and the inoculated set (T0 = SMBL 1, T1 = compost + SMBL 1, T2 = biochar + SMBL 1 and T3 = fruit and vegetable waste + SMBL 1), replicated thrice.

The pot experiment was carried out in the wire house of the Department of Soil Science, Faculty of Agriculture and Environment of Islamia University of Bahawalpur, Pakistan. Soil physical-chemical characteristics were examined prior to use in the experiment (Table 1). Each pot (length, 18 inch; width, 12 inch) was filled with approximately 10 kg of air dried, clean (free from leaves, stems and stones) and sieved soil. Un-inoculated and inoculated seeds (10 seeds per pot) were sown in different pots and a homogeneous plant population (3 plants per pot) were maintained after germination. Organic amendments with biochar (0.25% ratio), compost (600 kg ha⁻¹), fruit and vegetable waste (600 kg ha⁻¹) and, N:P:K recommended doses (150:112:75 kg ha⁻¹) were applied as urea, DAP (di-ammonium phosphate) and MOP (muriate of potash). Recommended agronomic practices (thinning, irrigation, fertilizer application and weeding) were adopted where and when needed. DAP and MOP were applied at sowing time, while the application of urea was performed in three splits; the 1st dose was applied at sowing time and the remaining splits were applied after a fifteen-day interval. Good quality water fulfilling the fitness criteria as purposed by Ayers and Westcot [42] was used to irrigate the pots. Data regarding physiological attributes were recorded at physiological maturity while the data about growth factors (weight and length of root and shoot) and parameters of yield were noted at harvest.

2.5. Plant and Fruit Analysis

After 60 days of germination, okra leaves samples were taken and evaluated for RWC (relative water contents) by using the formula proposed by Mayak et al. [43]. A

spectrophotometer was used for chlorophyll 'a' and 'b' contents and their values were calculated by following method as described by Arnon [44].

At the time of fruit maturity, fruits were harvested and parameters such as number of fruits per plant, fruit fresh and dry biomass and fruit average diameter were recorded. The shoot and fruit samples from each pot were oven dried and ground separately. Samples were digested using the wet digestion procedure as proposed by Wolf [45]. Nutrient concentration in okra shoots and fruit samples were determined using four replicates of each treatment. The Kjeldhal method was used for nitrogen determination in shoot and fruit samples [35]. Phosphorus was measured by the vanadomolybdophosphoric acid yellow color method [37], while the potassium (K) in shoot and fruit samples was measured with a flame photometer [35]. Digested plant samples were examined for Zn and Fe contents using atomic absorption spectrophotometry (Model 240FS AA, Agilent Technologies Australia, Mulgrave, VIC, Australia).

2.6. Bacterial Population

Soil samples were collected after okra harvesting and stored at 4 °C in a polythene bag until analyzed for CFU. The serial dilution plate technique was used to find the CFU in soil as described by Alexander [46].

2.7. Statistical Analysis

An analysis of variance (ANOVA) was used to assess the data by the use of a factorial design (up to two-way interaction); we compared the means by LSD (least significant difference) tests at a probability level of 5% [47].

3. Results

3.1. Growth Parameters

The results revealed that the application of *Bacillus subtilis* (SMBL 1) with biochar, fruit and vegetable waste, and compost promoted plant height, as well as shoot fresh and dry biomass, while sole biochar, fruit and vegetable waste, and compost application also showed prominent results as compared to control (Table 2). However, root length and root fresh and dry biomass of okra was only improved when these organic amendments were applied with SMBL 1 (Table 3). Notably, the highest increase in plant height, root length and biomass were recorded in the biochar + SMBL 1 treatment.

Table 2. Effect of compost, biochar, fruit and vegetable waste (F and V waste) and *Bacillus subtilis* SMBL 1 on plant height and shoot fresh and dry biomass of okra.

Treatments	Plant Height (cm)		Shoot Fresh Biomass (g plant ⁻¹)		Shoot Dry Biomass (g plant ⁻¹)	
	Un-Inoculated	Inoculated	Un-Inoculated	Inoculated	Un-Inoculated	Inoculated
Control	53.9 h	60.4 f	131.3 g	140.5 e	57.1 g	60.9 f
Compost	57.3 g	66.5 d	136.8 f	146.8 cd	63.6 e	67.8 cd
Biochar	70.0 c	77.9 a	148.0 c	156.0 a	69.9 c	75.8 a
F and V waste	63.1 e	72.9 b	143.8 d	151.5 b	67.0 d	72.6 b
LSD ($p \leq 0.05$)	2.1113		3.1584		2.3259	

Means sharing different letters are statistically significant in respect to each other at a 5% level of probability ($n = 4$).

Table 3. Effect of compost, biochar, fruit and vegetable waste (F and V waste) and *Bacillus subtilis* SMBL 1 on root length, and root fresh and dry biomass of okra.

Treatments	Root Length (cm)		Root Fresh Biomass (g plant ⁻¹)		Root Dry Biomass (g plant ⁻¹)	
	Un-Inoculated	Inoculated	Un-Inoculated	Inoculated	Un-Inoculated	Inoculated
Control	15.4 g	17.8 ef	54.3 g	57.8 f	20.9 g	24.5 de
Compost	16.9 f	20.6 c	61.0 ef	65.5 cd	22.0 fg	25.8 cd
Biochar	19 d	23 a	67.0 bc	74.8 a	27.0 bc	31.5 a
F and V waste	18.1 e	22 b	63.5 de	70.0 b	23.1 ef	28.8 b
LSD ($p \leq 0.05$)	0.8589		3.3449		2.1911	

Means sharing different letters are statistically significant in respect to each other at a 5% level of probability ($n = 4$).

3.2. N, P, K Contents in Shoot and Fruit

Inoculation of seed with SMBL 1 and soil amended with organic amendments (biochar, fruit and vegetable waste, and compost) showed significant increase in NPK concentration in okra shoot and fruit over control (Tables 4 and 5). Integrated use of SMBL 1 with biochar improves the N contents up to 21.3%, P contents up to 41.8% and K contents up to 26% in okra shoot, while NPK in fruit increased up to 33%, 52.7% and 25.6%, compared with control. Fruit and vegetable waste and compost also increased the NPK concentration in shoot and fruit. In the sole application of organic amendments, biochar also showed better NPK concentration in shoot up to 16.9%, 37.8% and 17.6%, respectively, while in fruit N improved by 24%, P improved by 33.7% and K improved by 28.4% compared with control.

Table 4. Effect of compost, biochar, fruit and vegetable waste (F and V waste) and *Bacillus subtilis* SMBL 1 on N, P, K in shoot of okra.

Treatments	Nitrogen in Shoot %		Phosphorus in Shoot %		Potassium in Shoot %	
	Un-Inoculated	Inoculated	Un-Inoculated	Inoculated	Un-Inoculated	Inoculated
Control	1.93 f	2.35 cd	0.25 f	0.31 d	1.28 f	1.64 cd
Compost	2.08 ef	2.58 bc	0.27 ef	0.36 c	1.38 ef	1.80 bc
Biochar	2.25 de	2.85 a	0.34 c	0.43 a	1.50 de	2.06 a
F and V waste	2.18 de	2.65 ab	0.30 de	0.39 b	1.43 ef	1.86 b
LSD ($p \leq 0.05$)	0.2452		0.0310		0.1867	

Means sharing different letters are statistically significant in respect to each other at 5% level of probability ($n = 4$).

Table 5. Effect of compost, biochar, fruit and vegetable waste (F and V waste) and *Bacillus subtilis* SMBL 1 on N, P, K in fruit of okra.

Treatments	Nitrogen in Fruit %		Phosphorus in Fruit %		Potassium in Fruit %	
	Un-Inoculated	Inoculated	Un-Inoculated	Inoculated	Un-Inoculated	Inoculated
Control	1.88 f	2.35 cd	0.25 f	0.28 def	1.28 f	1.56 cd
Compost	2.08 ef	2.58 bc	0.27 ef	0.33 cd	1.38 ef	1.80 b
Biochar	2.33 d	3.13 a	0.33 bc	0.43 a	1.64 c	1.96 a
F and V waste	2.20 de	2.80 b	0.30 cde	0.37 b	1.43 de	1.86 ab
LSD ($p \leq 0.05$)	0.2261		0.0461		0.1383	

Means sharing different letters are statistically significant in respect to each other at 5% level of probability ($n = 4$).

3.3. Yield Parameters

In the yield-contributing parameters of okra, the numbers of fruit plant⁻¹ were promoted significantly by the application of the SMBL 1 strain with biochar, fruit and vegetable waste, and compost (Table 6). The highest number of fruit plant⁻¹ (18.0) was obtained by the application of biochar + SMBL 1, followed by fruit and vegetable waste + SMBL 1

(16.0) and compost + SMBL 1 (11.0). While, in sole application of organic amendments, the highest number of fruit plant⁻¹ (14.0) was identified using biochar, compared with control.

Table 6. Effect of compost, biochar, fruit and vegetable waste (F and V waste) and *Bacillus subtilis* SMBL 1 on number of fruit plant⁻¹ and fruit fresh and dry biomass of okra.

Treatments	Number of Fruit Plant ⁻¹		Fruit Fresh Biomass (g plant ⁻¹)		Fruit Dry Biomass (g plant ⁻¹)	
	Un-Inoculated	Inoculated	Un-Inoculated	Inoculated	Un-Inoculated	Inoculated
Control	8.0 g	9.0 fg	59.3 g	72.8 e	11.0 g	12.0 fg
Compost	10.0 ef	11.0 de	67.5 f	82.0 c	13.0 ef	15.0 cd
Biochar	14.0 c	18.0 a	78.5 d	105.3 a	16.0 bc	18.50 a
F and V waste	12.0 d	16.0 b	74.5 e	96.3 b	14.0 de	17.0 b
LSD ($p \leq 0.05$)	0.2261		0.0461		0.1383	

Means sharing different letters are statistically significant in respect to each other at 5% level of probability ($n = 4$).

Data regarding the effect of PGPR with organic amendments on fruit fresh and dry biomass and fruit length and diameter, revealed that the biomass of fruit was improved due to these amendments (Tables 6 and 7). The highest increases in fruit fresh and dry biomass, fruit length and diameter were observed in SMBL 1 + biochar, that is, 44.7, 54.2, 40.4 and 27.4%, respectively. Fruit and vegetable waste and compost with the SMBL 1 strain also showed significant results, while, in sole application of organic amendments, biochar showed prominent results compared with control.

Table 7. Effect of compost, biochar, fruit and vegetable waste (F and V waste) and *Bacillus subtilis* SMBL 1 on fruit length and diameter in okra.

Treatments	Fruit Length (cm)		Average Fruit Diameter (cm)	
	Un-Inoculated	Inoculated	Un-Inoculated	Inoculated
Control	8.36 g	10.8 e	1.43 f	1.55 ef
Compost	9.65 f	12.6 c	1.60 de	1.70 b–d
Biochar	11.4 d	15.1 a	1.75 dc	1.98 a
F and V waste	10.8 e	14.0 b	1.68 c–e	1.83 b
LSD ($p \leq 0.05$)	0.4200		0.1390	

Means sharing different letters are statistically significant in respect to each other at 5% level of probability ($n = 4$).

3.4. Fe and Zn in Shoot

The results showed that the inoculation of seed with the SMBL 1 strain in the presence of biochar, fruit and vegetable waste, and compost significantly improved Fe and Zn contents in shoot of okra (Figure 2). Biochar + SMBL 1 improved Fe and Zn contents up to 37.1% and 35.6%, respectively, while, in the absence of the strain SMBL 1, organic amendments also showed better results than control.

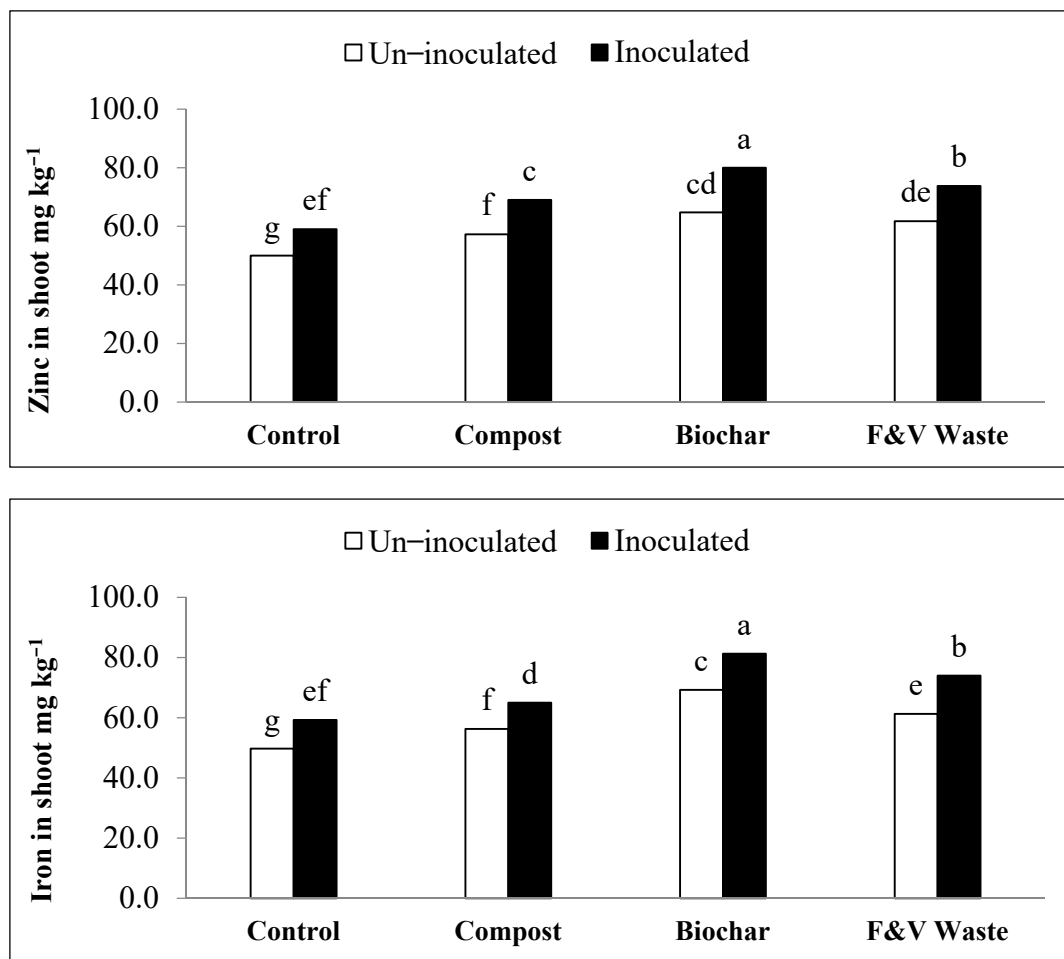


Figure 2. Effect of biochar, fruit and vegetable waste, compost and *Bacillus subtilis* SMBL 1 on Fe and Zn contents in okra with un-inoculated treatment set (Control = no bacterial inoculation) and inoculated treatment set (Control = SMBL 1). Different letters indicate significant differences according to least significant difference test at ($p \leq 0.05$).

3.5. Physiological Parameters of Okra

Inoculation with the SMBL 1 strain in the presence of organic amendments significantly improved the RWC (relative water content) and chlorophyll 'a' and 'b' contents (Figure 3). Compared with control, biochar and inoculation of SMBL 1 strain application showed the highest increase in RWC and chlorophyll 'a' and 'b', that is, 4.3, 50 and 53.3%, respectively. The sole use of organic amendments physiological attributes of okra showed better results than control.

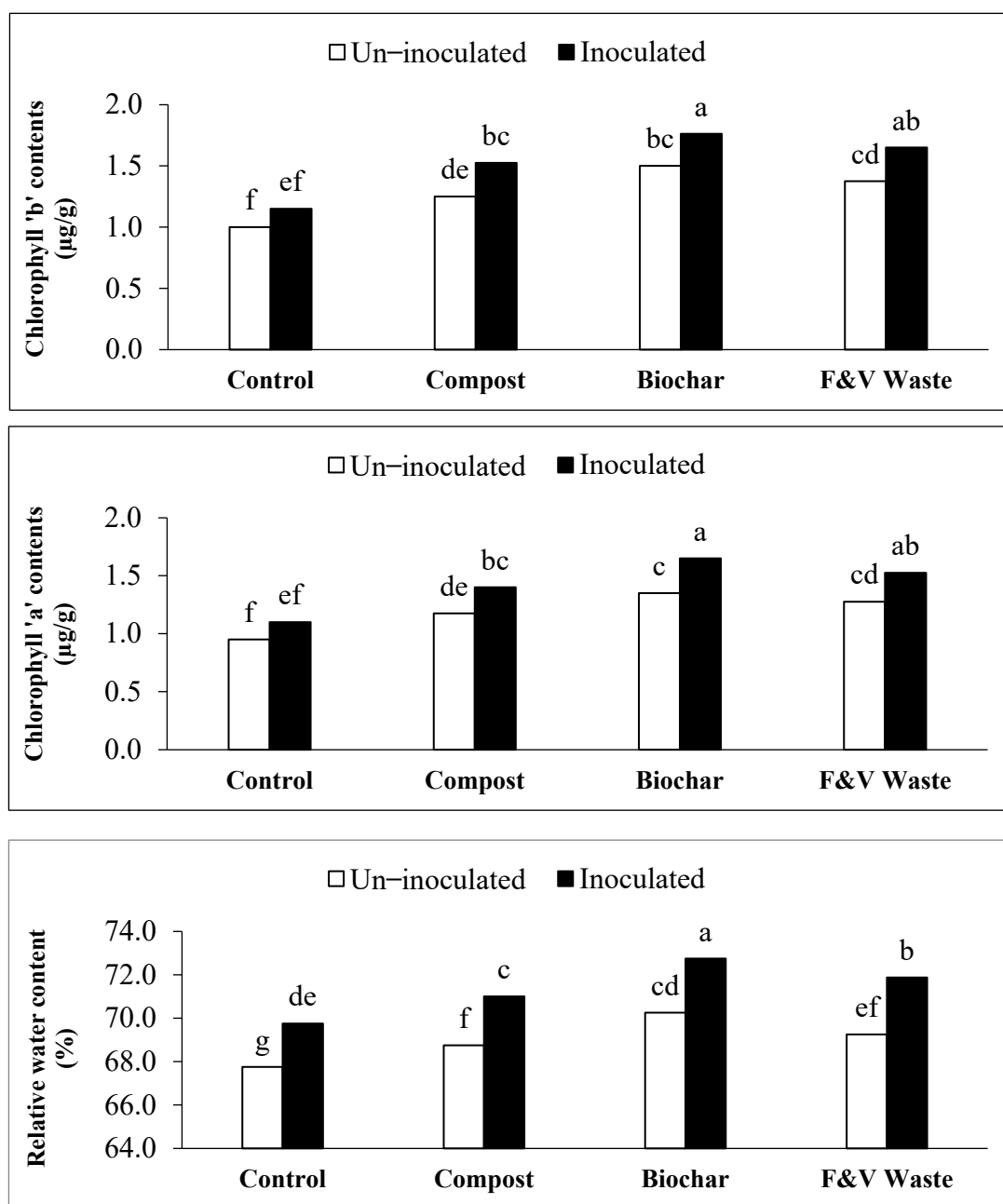


Figure 3. Effect of biochar, fruit and vegetable waste, compost and *Bacillus subtilis* SMBL 1 on relative water content, chlorophyll 'a' and 'b' in okra with un-inoculated treatment set (Control = no bacterial inoculation) and inoculated treatment set (Control = SMBL 1 only). Different letters indicate significant differences according to least significant difference test at ($p \leq 0.05$).

3.6. Microbial Population in Soil

As shown in Figure 4, the inoculation of the SMBL 1 strain significantly improved the effect of each organic amendment applied individually or in combined form on soil bacterial population or CFU. In general, the SMBL 1 strain showed the capability to increase the effectiveness of soil organic amendments (i.e., soil water-holding capacity and decomposition ability of soil). This strain showed maximum results with biochar that was 30.8%, followed by fruit and vegetable waste (20.3%) and compost (9.8%) treatments, compared to control, while, in the sole application of organic amendments, bacterial populations also improved (but not as much as with inoculated treatments) compared with control.

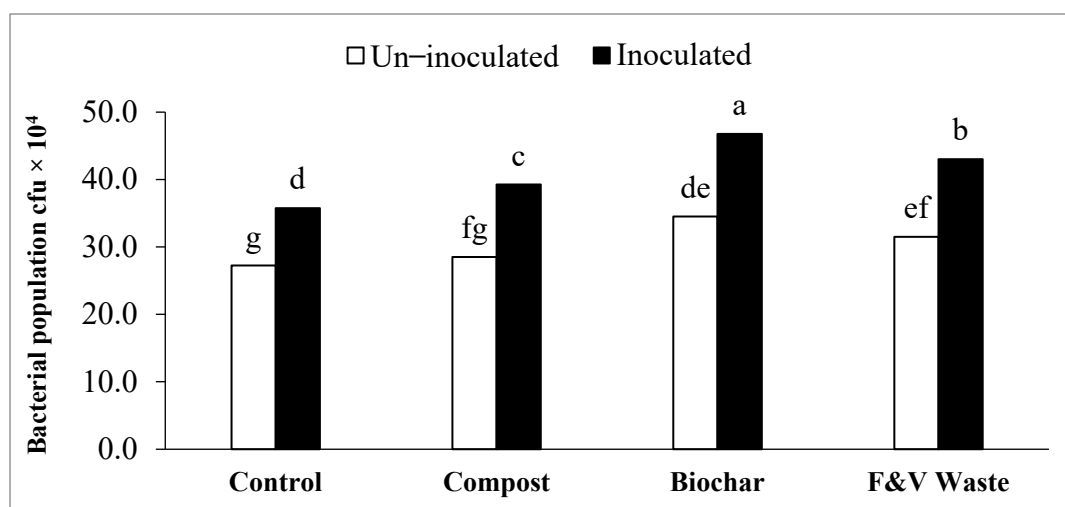


Figure 4. Effect of biochar, fruit and vegetable waste, compost and *Bacillus subtilis* SMBL 1 on bacterial CFU of soil with un-inoculated treatment set (Control = no bacterial inoculation) and inoculated treatment set (Control = SMBL 1 only). Different letters indicate significant differences according to least significant difference test at ($p \leq 0.05$).

4. Discussion

Different kinds of bacterial species are present in rhizospheric soil that colonize the roots of plants [48] and have various traits that are useful for sustainable agricultural production. PGPR are an important group of bacteria that increase the growth of plants by improving nutrient availability [49], resulting in better crop production. Thus, PGPR have potential for utilization as biofertilizers to overcome reduced nutrient uptake issues in plants [50]. The results of this study clearly confirm that the use of PGPR considerably improved growth and yield parameters in okra. Particularly, inoculation of seed with PGPR significantly increases the physiological, yield and growth parameters. Our findings are in line with the those in the literature, as it was found that the inoculation of the *Azotobacter*, *Pseudomonas* and *Azospirillum* strains in different crops also expressed a similar improvement in plant growth and yield [51].

Our results demonstrated that the application of compost, biochar, fruit and vegetable waste and *Bacillus subtilis* (SMBL 1) inoculation led to a significant increase in the chlorophyll 'a' and 'b' contents and RWC of okra plant. This increase might be due to higher root lengths and biomass, which enhance the uptake of water from soil and RWC of plant. The results of our study are in agreement with the findings obtained by Ahmad et al. [52], who reported that co-inoculation of Rhizobia and PGPR can enhance root length and cause an increase in the uptake of water from deeper soil regions. Ullah et al. [53], Mumtaz et al. [54] and Fatawi et al. [55] argued that the use of rhizobacteria and organic amendments (compost and biochar) increases the root surface area, root length and stomatal conductance, that, in turn, improve the absorption of water from deeper regions and ultimately result in an increase in RWC. Co-inoculation of PGPR and *Rhizobium* possessing ACC-deaminase activity promotes roots elongation that aids the plant to absorb more water from soil [49]. The enhancement of chlorophyll contents in the present study might be due to the improvement in plant nutrient uptake, especially N, Fe and Mg (core components of chlorophyll molecules) by microbial inoculation, similar to the findings reported by Adesemoye and Kloepper [56]. Therefore, the improvement in chlorophyll contents expedites photosynthesis and the production of photosynthates, which is responsible for the increase in crop yield. The results of the present study clearly confirm that the application of organic amendments (biochar, compost, and fruit and vegetable waste) and *Bacillus subtilis* (SMBL 1) considerably enhanced plant height, shoot dry and fresh biomass and root length of okra. Parewa and Yadav [57] and Fatawi et al. [55] demonstrated the role of bacterial species such as *Arthrobacter*, *Azotobacter*, *Bacillus*, *Azospirillum*, *Enterobacter*, *Pseu-*

Pseudomonas and *Burkholderia* integrated with organic amendments to increase plant growth and yield attributes. Adesemoye and Ugoji [58] also observed similar results by evaluating the ability of *Pseudomonas* sp. to improve the growth of plants using three different crops (okra, tomato, African spinach). They found that the inoculation of *Pseudomonas* species is a promising strategy that can be used to improve crop growth. The microorganisms present in soil usually develop favorable conditions for suitable vegetative growth; for instance, they enhance nutrient uptake in plants and enhance plant height and root length of plant. Microorganisms used in biofertilizers play an important role in organic matter decomposition and the application of organic amendments with PGPR play a significant role in their decomposition and associate with soil health plant growth and nutrient cycling [59]. PGPR inoculation considerably increases the length of root, and dry and fresh biomass of root; similar findings were also recorded by Richardson [60] in terms of improvements in surface area and root biomass. The higher microbial population in the soil of SMBL1 inoculated plants was also found, as the microbial activity in soil is a function of root exudation in the rhizosphere and the availability of other C and nutrient sources, which have the ability to alter the soil biological and physiochemical properties; similar facts were also advocated by Nardi et al. [61]. Furthermore, these may improve the root surface area, which can hold more soil and alter the nutrient status and biological activity in the rhizosphere [62].

The inoculation of SMBL 1 and use of organic amendments (compost, biochar, and fruit and vegetable waste) considerably increased the uptake of nutrients in okra plant, resulting in improved crop yield. The results of our research are in agreement with Biari et al.'s [63], who described that the *Azospirillum* strain considerably improved the uptake of nutrients by plants such as N, P, K, Zn, Fe, Cu and Mn. The rise in the uptake of micro- and macro-nutrients capability of plants is due to the application of organic amendments and inoculation with PGPR that result in the formation and improvement of lateral roots and improve the root biomass. Similar findings were also presented in Ahmad et al. [48,52] and Nadeem et al. [64], who reported that the application of PGPR increased the root surface area through the propagation of roots and improved the nutrient availability. Organic amendments are rich in C, N, P, K in organic forms, while PGPR have ability to decompose the organic amendments and convert them into their respective inorganic/plant-available forms [65]. Bouain et al. [66] described that the micro- and macro-nutrients absorption by plants from the soil depends upon the release of organic acids such as lactic acid, citric acid, gluconic acid and 5-keto gluconic acid by rhizosphere microorganisms; thus, by lowering the microsite pH, these microorganisms increase the amount of nutrients available to the roots of the plant [67]. Similar reasons were also provided by Estrada et al. [68], Abaid-Ullah et al. [69] and Zhang and Kong [70], who explained that rhizo-bacteria have the capability of releasing acids such as oxalic, malic, lactic and gluconic acids for K, Zn, Fe and $(\text{PO}_4)^{-3}$ solubilization. Furthermore, the use of organic amendments improves the growth and yield of the plant by increasing the fertility status of soil. The yield improvements by inoculation might be linked with the increase in nutrient uptake through the increase in root biomass, enhancement of photosynthesis, growth-promoting substances by microorganisms and provision of favorable conditions by organic amendments [28].

5. Conclusions

The inoculation of *Bacillus subtilis* (SMBL 1) and use of organic amendments (biochar, fruit and vegetable waste, and compost) improved the soil properties and okra growth when applied individually and in combination with each other. Promising results were obtained by the integrated application of SMBL 1 and biochar, which caused an increase in RWC (4.3%), fruit N contents (33%), fruit P contents (52.7%), fruit K contents (25.6%), shoot iron contents (37.1%), shoot zinc contents (35.6%), rhizospheric microbial population (30.8%) and yield (44.7%) of okra, compared with control. Considering the promising results obtained by the integration of SMBL 1 and biochar, it can be concluded that the current approach has the potential to be developed into a sustainable technology for okra production after extensive evaluation in the field is carried out. The development of

biofertilizer for the sustainable production of vegetables is the need of the hour, not only to restore soil health but also to attain food security in Pakistan. Moreover, the entrapped nutrients in the organic amendments can be readily solubilized by SMBL 1, reduce chemical fertilizer application and improve the organic matter status and microbial diversity in soil for sustainable agricultural production.

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