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Integration Between Biochar and Plant Growth Promoting Bacteria Affecting Growth of Pepper (*Capsicum annum* L.) Plant

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Abstract: Biochar amendment of soil proved to improve soil quality and retain nutrients, thereby enhancing plant growth. A greenhouse experiment was carried out to investigate the combined effects of biochar and plant growth promoting endophytic bacteria (PGPE) (*Pseudomonas* spKY6489821.1 and *Klebsiella oxytoca* KY648983.1) or plant growth promoting rhizobacteria (PGPR) (*Paenibacilluspolymyxa* MG309677.1 and *Ochrobactrum intermedium*MG309678.1) on the growth and yield of pepper plants. Compared with individual application, the combination of biochar with PGPE exhibited significant increases in all predestined parameters (soil enzymes, growth parameters, some macro and microelements, chlorophyll, carotenoids, sugar, free amino acid, vitamin C, TSS and yield). Similarity, there were remarkable increases in the previous parameters in plants treated with biochar in the presence of PGPR followed by chemical fertilizers. The present study demonstrates that the beneficial effect of biochar not only improve the soil properties but also enhanced the performance of biofertilizers in promoting plant growth.

Key words: Biochar · Endophytic bacteria · PGPR · Soil amendment · Pepper yield

INTRODUCTION

Long-term use of chemical fertilizers resulted in degradation of the soil environment, including soil compaction, reduced fertility and biological activities, which also reduced crop yields. Fertilizer and pesticide residues in the soil seep into the ground water system result in contamination of drinking water and lakes indirectly [1]. As a result, some health and environmental risks have been caused [2]. Also, the high fertilizer costs and the harmful effects of long-term chemical fertilizer application led to the progressive application of alternative fertilizers, such as microbial and organic fertilizers.

Biofertilizers are a combination of living microorganisms (bacteria or fungi) that directly or indirectly affect crop growth and productivity through many mechanisms [3, 4].

Plant-growth promoting rhizobacteria (PGPR), areis very useful as biofertilizers [5]. Plant growth stimulation mechanisms vary among bacterial strains and depend on the different metabolites produced by these strains. For example, production gibberellin, auxins, cytokinin and ethylene varied among rhizobacteria strains [6, 7]. These hormones can change plant growth directly or indirectly with bacterial secondary metabolites in a concentration-dependent manner [8-10]. Endophytic bacteria (PGPE) are defined as bacteria that colonize the internal tissues of plants without causing infection or negative effects on their hosts and bacteria reside in apoplasm or symplasm [11]. They are often able of triggering physiological changes that promote plant growth and development and may stimulate host plant growth through several mechanisms such as nitrogen fixation, biological control, production of growth regulators, induction of systemic resistance to pathogens and enhancement of mineral nutrients and water uptake [12, 13].

Improving soil fertility, especially under low-input farming systems, requires the use of organic matter such as crop residues, green manure, animal manure and organic fertilizer [14]. Organic materials are vital because

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of their availability as sources of nutrients and improving soil properties [15].

Biochar (BC) is a soil enhancer that has been tested to improve the chemical and physical properties of soil [16, 17]. It is prepared by pyrolysis of organic waste materials, in addition as a carbon-rich material produced by heating a wide range of organic biomass (feedstocks) at low levels or in the absence of oxygen (pyrolysis or charring) [18]. Moreover, rice biochar contains carbon and nitrogen as 630 and 9 g Kg^{-1} and C / N ratio of 70 [19]. Biochar has been shown to reduce soil nutrient leaching such as nitrogen (N), phosphorus (P) and potassium (K) [20, 21].

This study was conducted to evaluate the integration between biochar as a soil enhancer and endophytic bacterial strains (*Pseudomonas* sp KY6489821.1 and *Klebsiella oxytoca* KY648983.1) and PGPR strains (*Paenibacillus polymyxa* MG309677.1and *Ochrobactrum intermedium* MG309678.1) for improving growth and yield of pepper.

MATERIALS AND METHODS

Biochar: The rice straw derived biochar (made at temperature 500°C) was obtained from College of Resources and Environment, Huazhong Agricultural University, Wuhan, China. The basic physio-chemical properties of biochar were as follow: pH, 10.28; EC, 1.8 dsm^{-1}; Cd, 0.03 mg^{-1} and Pb, 22 mg kg^{-1}.

Inoculum Preparation: Endophytic bacteria and PGPR strains were obtained from the Microbiology Department, Faculty of Agriculture, Benha University, Qalyubia Governorate, Egypt. The activities of the strains are presented in Table (1).

The consortium of endophytic strains of 10^8 cfu/ml (*Pseudomonas* sp KY6489821.1 & *Klebsiellaoxytoca* KY648983.1) and PGPR strains of 10^7 cfu/ml (*Paenibacilluspolymyxa* MG309677.1 & *Ochrobactrum intermedium* MG309678.1) were prepared separately in LB medium for 2 days at 30°C. The mixed inoculum was prepared by mixing equal volumes of the desired cell suspension.

Pot Experiment: A greenhouse experiment was conducted at the Faculty of Agriculture, Benha University, Qalyubia Governorate, from March to May 2019. Fiveweek-old pepper seedlings (obtained from Kaha City nursery) were soaked for 30 min. in a bacterial suspension of either PGPE or PGPR mixture before cultivation and Arabic gum (20%) was added as an adhesive agent. Suspension of the mixed culture was added to the pots three times during growing season at a rate of 100 mL/pot. In un-inoculated treatments, seedlings were treated with un-inoculated media. Pepper seedlings were planted into 30 cm width, 30 cm height plastic pots, containing 10 kg of clay loam soil (clay, 44.14%; silt, 25.22%; sand, 24.04%; pH, 7.5, organic matter, 1.52%; total nitrogen, 0.23%; CaCO3, 0.55%; total K,0.27; total P,0.12%) and arranged in a randomized complete block design with three replicates.

The full doses of chemical fertilizers; 148.26 kg (N), 61.78 kg (P2O5) and 111.20 kg (K2O)/ ha were used in the control treatment as ammonium sulfate, calcium superphosphate and potassium sulfate, respectively. While the other treatments received half doses of chemical fertilizers. Chemical fertilizer was used twice in equal doses at vegetative and flowering stages of plants. The soil was utterly mixed after adding the biochar at a rate of 205.83 kg/ ha. All pots were irrigated with equal amounts of water. The experiment contained six treatments included the full dose of chemical fertilizers, PGPR mixture, PGPE mixture, biochar, biochar combined with PGPR and biochar combined with PGPE.

Microbiological Activities: Dehydrogenase, phosphatase and nitrogenase activities were estimated at the flowering stage after 30 days of transplanting, according to Hardy *et al.* [24]; Tabatabai [25] and Silvester [26], respectively.

Determinations: Plant height, leaves number, branches number, flowers number and plant dry weight (shoots & roots) were determined at flowering stage after 30 days of transplanting. While at harvest, after 60 days of transplanting, number of fruits, the weight of fruit and plant yield (Kg) were recorded.

Table 1: Beneficial activities of the PGPE and PGPR strains.

	Strains	Activities	References
PGPE	*Pseudomonas* sp. KY6489821.1	P solubilization, K solubilization siderophores and IAA production	Ashry, Noha [22]
	Klebsiella oxytoca KY648983.1	N_2- fixation, K solubilization, HCN, siderophores, IAA and ammonia production	
PGPR	*Paenibacillus polymyxa* MG309677.1	N_2fixation, Ksolubilization P solubilization, ammonia, siderophores and IAA production	Morsi, Hoda [23]
	Ochrobactrum intermedium MG309678.1	HCN, IAA production and K solubilization	

Plant samples were taken after 30 days of transplanting to measure the contents of macroelements (nitrogen, phosphorus and potassium) according to the methods described by A.O.A.C [27]; A.P.H.A [28] and Dewis and Freitas [29], respectively and microelements (Fe and Zn) contents using spectrophotometer according to Mgbeze and Omodamwen [30]. Whereas, rhizosphere soil samples were taken after 30 days of transplanting to determine available nitrogen, phosphorus and potassium contents according to the method described by Black *et al.* [31]. Also, photosynthetic pigments (chlorophyll A & B and carotenoids) were spectrophotometrically determined, according to Nornal [32] and calculated as mg/g fresh weight of leaves.

Total sugars were calorimetrically determined according to Thomas and Dutcher [33] while, free amino acids were determined according to the method described by Rosein [34]. The total soluble solids were determined in the filtrate by carl Zeiss refractometer; also, vitamin C was assayed in the juice of pepper fruits using 2, 6-dichlorophenol indophenol dye method A.O.A.C [27].

Statistical Analysis: Costatic program was used for statistical analysis of data. The differences between the mean values of different treatments were compared by Duncan's multiple range test [35].

RESULTS AND DISCUSSION

Soil Enzyme Activities of Pepper Rhizosphere: Soil enzyme activities studies provide information on the biochemical processes occurring in soil. DHA, P- ase and N_2-ase activities of the different treatments are shown in Fig (1). There was a high trend of enzyme activities in soils inoculated with bacterial inocula (with or without biochar addition) compared to that in soils treated with both inorganic fertilizers and biochar alone, this could be attributed to the use of PGPE or PGPR mixture. This result is compatible with Schoebitz *et al.* [36] and Sahin *et al.* [37] who reported that the microbial consortia was superior to inoculation with individual strains. Moreover, higher improvement in case of consortia inoculation is due to the combined interaction of their

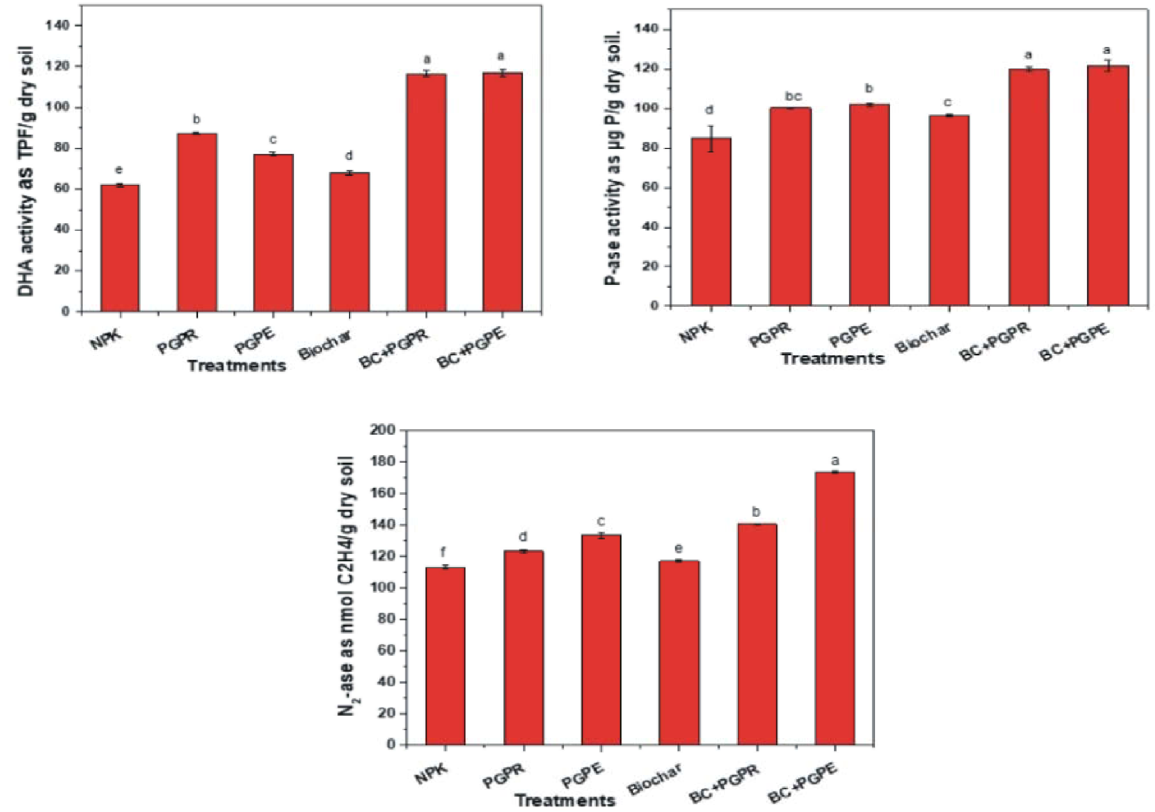

Fig. 1: Effects of different treatments on DHA, P-ase and N_2- ase activities of pepper rhizosphere soils at flowering stage. Small letters indicate significant differences ($P \leq 0.05$) between treatments.

Table 2: Effects of different treatments on growth characteristics.

Treatments	Plant height(cm)	Leaves number	Branches number	Flowers number	Shoot dry weight(g) / plant	Root dry weight (g) / plant
Chemical fertilizers	71^{a}	55^{b}	5.00^{a}	27^{b}	26^{a}	18^{ab}
PGPR	55^{c}	42^{d}	3.00^{bc}	19^{c}	21^{c}	16^{b}
PGPE	54^{c}	44^{c}	4.00^{ab}	20^{c}	24^{b}	15^{b}
Biochar	60^{b}	44^{c}	4.00^{ab}	19^{c}	21c	13^{c}
Biochar + PGPR	70^{a}	54^{b}	5.00^{a}	26^{b}	25^{ab}	19^{a}
Biochar + PGPE	70^{a}	57^{a}	5.00^{a}	30^{a}	26^{a}	18^{ab}

Small letters indicate significant differences ($P \leq 0.05$) between treatments.

supplementary features that might have promoted their colonization and growth [38].

Biochar addition with PGPE or PGPR improved the enzyme activities more than individual application because of synergistic effects, possibly due to the activities of most soil enzymes associated with the nutrient content and soil microorganisms' load. These results are in harmony with those obtained by Tao *et al*. [39] who reported that the addition of bacteria and biochar together improved the activities of some enzymes in soil.

No significant differences in DHA and P-ase activities were recorded in plants inoculated with biochar and PGPE or biochar and PGPR but soils inoculated with biochar and PGPE had significantly higherN_2-ase activity than those treated with biochar and PGPR. The endophytic bacteria have an environmental advantage over the rhizobacteria as they are preserved from harmful external environmental conditions such as those associated with temperature, osmotic potentials and ultraviolet radiation, which are significant factors limiting long-term bacterial survival [40].Contrariwise, the control treatment without any amendment had the lowest records of all estimated parameters.

Growth Characteristics: The growth parameters of pepper were measured at the flowering stage (Table 2) and directly reflect plant growth in the different treatments. Concerning the impact of chemical fertilization, no significant increases in plant height and branches number were observed in plants treated with chemical fertilizers and plant treated with biochar combined with either PGPE or PGPR. Moreover, pepper plants treated with biochar and PGPE had the highest leaves number and flowers number than those of the other treatments while, the highest significant records of root dry weights were observed when pepper plants were treated with biochar and PGPR, possibly due to endophytes production of growth stimulants as well as biochar role in soil improvement as agreed with Dawwam *et al*. [41] who revealed that the increased parameters of peppers in the greenhouse experiments are due to positive effects of biochar and biofertilizers on plant growth, especially endophytic bacteria.

These findings affirm those of previous studies that have shown that application of biochar boosts plant growth by increasing population densities of PGPR and soil WHC by serving as a nutrient source [42, 43]. Alongside, inoculation with endophytic bacterial strains also had a good effect on root growth and morphology, presumably because of the production of phytohormone (indole acetic acid, organic acid, gibberellins and cytokinins, etc.) and consequently, improved nutrient and water uptake [44]. Additionally, the estimated growth parameters did not show significant differences between the application of NPK or biochar and PGPE.

Available NPK in Soil: Available N, P and K soil contents at different treatments are shown in Fig (2). Data illustrated that the significant maximum values of available nutrients (N, P and K) were found in soil treated with biochar combined with PGPE, this may be attributed to fact that biochar can release soluble P in the soil environment, reduces the leaching of $NH4^{+}$ and nitrate and has a high ability to retain nutrients, i.e., nutrients are not easily lost from biochar-treated soil [45-47]. In this context, endophytes are known to play an essential role in increasing the availability of nitrogen£¬phosphorus and potassium [48].

Based on the estimates, there were no differences in soil treated with either NPK or biochar and PGPR. The endophytic bacteria may be more efficient than rhizospheric bacteria in promoting plant growth as they do not compete with rhizosphere microorganisms and achieve strong contact with the plant tissues [49].

Macro and Microelements Uptake in Pepper Leaves: Data presented in Table (3) showed that, nutrient uptake was significantly higher in plants inoculated with PGPE alone than plants inoculated with PGPR and the individual biochar. Zn was markedly higher in endophyte-treated plants compared to other treatments [50].

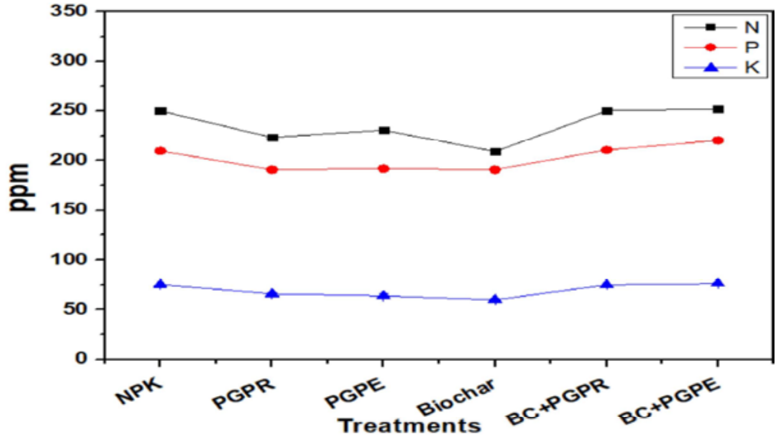

Fig. 2: Available N, P and K in rhizosphere soil of pepper plants

Table 3: Nitrogen, phosphor and potassium uptake in pepper leaves of the different treatments

Treatments	N	P	K	Fe	Zn
	---mg / l---			---%---	
Chemical fertilizers	5.00^{ab}	0.20^{a}	1.84^{a}	0.0051^{ab}	0.0051^{b}
PGPR	4.60^{b}	0.18^{ab}	1.50^{ab}	0.0037^{cd}	0.0039^{d}
PGPE	4.90^{ab}	0.18^{ab}	1.60^{ab}	0.0040^{c}	0.0043^{c}
Biochar	4.60^{b}	0.17^{ab}	1.30^{b}	0.0032^{d}	0.0039^{d}
Biochar + PGPR	5.00^{ab}	0.22^{a}	1.93^{a}	0.0057^{ab}	0.0058^{a}
Biochar + PGPE	5.60^{a}	0.23^{a}	2.00^{a}	0.0061^{a}	0.0059^{a}

Letters indicate significant differences ($P \leq 0.05$) between treatments.

Table 4: Effects of different treatments on chlorophyll a, chlorophyll b and carotenoids of pepper.

Treatments	Chl.a	Chl.b	Carotenoids
	---mg g^{-1} leaves fresh weight---		
Chemical fertilizers	1.33^{a}	0.59^{c}	0.73^{c}
PGPR	1.00^{c}	0.55^{d}	0.62^{e}
PGPE	1.36^{a}	0.69^{b}	0.79^{b}
Biochar	0.81^{d}	0.49^{e}	0.59^{f}
Biochar + PGPR	1.20^{b}	0.56^{d}	0.68^{d}
Biochar + PGPE	1.39^{a}	0.75^{a}	0.81^{a}

Letters indicate significant differences ($P \leq 0.05$) between treatments.

Overall, the highest amounts of these nutrients were observed with biochar combined with PGPE followed by biochar with PGPR and NPK treatments. These results are in proper line with those obtained by Hammer *et al*. [51] who showed that endophytes increase the ability of host plants to uptake macro- and micro-nutrients, particularly P, K, S and Ca. To further investigate the influence of biochar with PGPB, most studies have shown that biochar contains significant amounts of macro- and micro-nutrients, with the amount depending on the raw materials source, which leading to variation in the extent to which soil nutrient status is improved upon, the ability of a plant-associated endophytes to enhance nutrient transfer from biochar to the host plant [52].

Pigments contents of pepper leaves

Chlorophyll is the main pigment in plants that is involved in photosynthesis. Also, it is a reliable indicator of the photosynthetic capacity and health status of plants [53]. The chlorophyll content in the leaves of pepper plants from the different treatments is shown in Table (4).

Chlorophyll a, b and carotenoids scored significant levels in leaves of plants inoculated by PGPE alone or combined with biochar. This has been explained by Hunt *et al*. [54] who found a relationship between the endophyte load and total chlorophyll. Some endophyte species can increase the total chlorophyll and influence the rate of photosynthesis [55].

These results can be explained by the fact that photosynthesis is mainly regulated by the stimulation of endogenous signals and the environment, for instance,

Table 5: Contents of sugars, free amino acids, vitamin C and TSS in pepper plants under different treatments

Treatments	Sugars(%)	Free amino acid (mg/100g)	Vitamin C (mg/100g)	TSS (%)
Chemical fertilizers	0.38^{ab}	98^{c}	196.20^{c}	11.87^{c}
PGPR	0.29^{b}	93^{e}	194.20^{d}	10.18^{e}
PGPE	0.29^{b}	95^{d}	192.70^{e}	10.82^{d}
Biochar	0.25^{c}	88^{f}	150.90^{f}	9.10^{f}
Biochar + PGPR	0.39^{a}	101^{b}	203.11^{b}	15.60^{a}
Biochar + PGPE	0.40^{a}	103^{a}	210.31^{a}	15.30^{b}

Letters indicate significant differences ($P \leq 0.05$) between treatments.

Table 6: Interaction among biochar and PGPB on peeper yield.

Treatments	Fruits number/ plant	Fruits weight (g) /plant	Fruits yield (Kg) / pot
Chemical fertilizers	6.80^{c}	196.00^{e}	1.34^{c}
PGPR	5.50^{e}	209.00^{d}	1.19^{d}
PGPE	6.10^{d}	215.99^{c}	1.30^{c}
Biochar	5.10^{f}	195.90^{e}	0.95^{e}
Biochar + PGPR	8.20^{b}	224.00^{b}	1.83^{b}
Biochar + PGPE	9.10^{a}	250.00^{a}	2.03^{a}

Letters indicate significant differences ($P \leq 0.05$) between treatments.

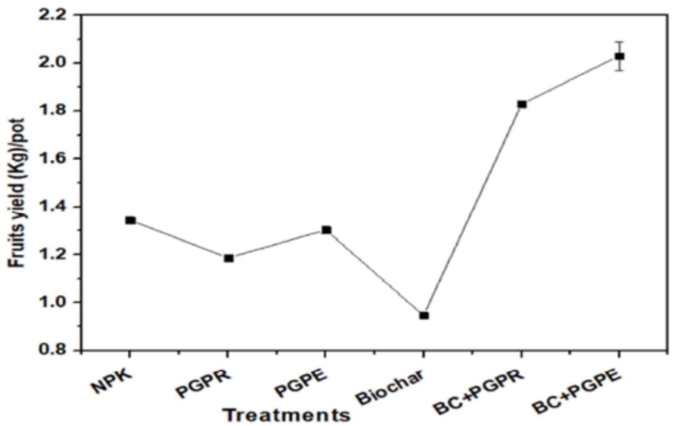

Fig. 3: Interaction between biochar and PGPB on peeper fruits yield.

plant growth promoting bacteria may regulate signal transduction pathways that are related to photosynthesis and biochar changes the soil environment, thereby stimulating the plant growth [56].The photosynthesis activity increased approximately 32% in plants treated with biofertilizers. This indicates that in addition to the stomatal conductance, the increased pigment content is one of the reasons for the increased photosynthetic speed of the plants inoculated with biofertilizers.

Yield Components of Pepper Plants: Contents of sugar, free amino acid, vitamin C and total soluble solids (TSS) in pepper plants are shown in Table (5). All estimated parameters were higher with pepper plants inoculated with PGPE or PGPR alone than those treated with biochar alone. While biochar with PGPE or PGPR application recorded the highest records of the estimated parameters, this could be explained by Elad *et al*. [47] who reported an increase in the soluble sugar content of plants and soil improvement due to addition of biochar. Besides, a combination of biochar and plant endophytes resulted in increased nutrient uptake and thus an increase in the accumulation of nutrients, such as sugar and amino acids, as reported with soybean plants [57]. Predominately, in our study, pepper plant growth was higher in response to treatment with a mixture of biochar with PGPE than all different treatments.

Yield of Pepper Plants: Data in Table (6) and illustrated by Figs (3) emphasized that the maximum significant values of yield were observed when pepper was inoculated with biochar in combination with PGPE or PGPR, followed by chemical fertilizers. The increase in yield might be related to the relationship between biochar and plant growth promoting. This synergistic effect improves the soil fertility. These results are in agreement with those obtained by Baldani *et al.* [58] who reported the benefit of biochar is due to its effect on the physical and chemical state of the soil.

The effect can be direct, through the provision of mineral nutrients, or indirect through the promotion of nutrient retention and thus its effect on the plant growth and productivity [59, 60]. Many of these effects may synergistically act to improve crop performance.

CONCLUSION

Agriculture plays a necessary role in the survival of peoples, so, maintaining their quantity and quality is very important for the nutrition of the population and economic exports. Eventually, the pepper plants treated by organic fertilizers especially biochar in combination with microbial inoculation, chiefly PGPE, resulted in positive effects on the growth and yield. The endophytic bacteria have an ecological trait over the PGPR in that they are protected from biotic and abiotic stresses, as well, organic fertilizers help to provide nutrient for growth and yield of pepper plant. In addition, we need to reduce the costs and prevent the health and environmental risks of chemical fertilizers. Therefore, the use of PGPB and organic fertilizers are less harmful to human health and the environment.

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