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Original Research Article

Phosphorus solubilizing bacteria and biochar improve maize growth in different textured soils

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Abstract

Phosphorus is an important macronutrient which directly affects the growth and yield attributes of maize. Besides its presence in soil, it is highly immobilized which decreased its phytoavailability. This problem can be overcome by inoculation of phosphate solubilizing bacteria (PSB) and biochar. Inoculation of PSB can mobilize phosphorus in soil and increase the root elongation for better uptake of nutrients. Biochar is another allied amendment which can decrease the leaching and runoff losses of phosphorus fertilizers, especially in sandy texture soils. That is why the current study was conducted to examine the role of biochar and PSB on maize growth under different soil textures. There were two PSB *Achromobacter xylosoxidans* (BS1) and *Alcaligenes faecalis* (BS15) applied with and without biochar in clay loam and sandy clay loam textures. Results showed that PSB (*Alcaligenes faecalis*) and biochar collectively showed better results from PSB (*Achromobacter xylosoxidans*) with biochar. It is probably because of the reason that they have better abilities to colonize around root surface and improved K contents were noticed in coarse textured soil.

Keywords: Nutrients, Organic amendment, Rhizobacteria, Solubilization

INTRODUCATION

Maize ranks 3rd in the list of most important cereal crops grown in Pakistan (GOP, 2018). Besides being used as food and fodder, a large number of products like soaps and paints have also been manufactured from maize on an industrial scale (Kumar and Jhariya, 2013). Mineral fertilizers are to be applied in a huge quantity to meet the nutritional requirement of the crop, even at the cost of environmental safety. It is, therefore, the need of the time that the scientists around the globe should focus on environmental friendly techniques to minimize the dependence on mineral fertilizers.

Phosphorus (P) is a vital element for all the crops (Osava, 2007) and improves grain yield and grain and crop quality and helps in the development of roots (Zhang et al., 2018). Phosphorus is thought to be playing an important role in certain physiological processes like cell division, plant respiration, storage of energy and photosynthesis in addition to several other key metabolic activities. The plant suffers a lot in terms of growth and yield with the limited availability of P. The P availability to plants from soil has always been a problem (Shenoy and Kalagudi, 2005). Soil insoluble phosphates restrict the P uptake by the plants. Phosphatic fertilizers are applied regularly to provide the plants with P, but a major part of the applied P is fixed in soils as insoluble phosphates.

The techniques to solubilize the fixed P may be helpful in this context. Use of microorganisms is an environmental friendly option to solubilize the unavailable P (Harinathan at el., 2016). For sustainable production of crops, plant and microbial associations in the rhizosphere, play an essential role in multiple processes like mobilization, transformation as well as solubilizing the nutrients out of a confined nutrient bank thus supplying the nutrients essential to plants. Plant growth promoting rhizobacteria (PGPR) are known and established well to be playing their role in improvement of plant growth with multiple mechanisms. Some PGPR can produce a dynamic enzyme, 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase) that plays a pivotal role in lowering the level of ethylene in roots which in response, promotes the growth as well as the length of roots (Penrose and Glick, 2001). Currently their use in combination with decreased quantity of chemical fertilizers directs towards a way to reduce the dependence on chemical fertilizers in the days to come (Richardson and Simpson, 2011). Certain PGPR have potential to solubilize soil phosphates which are insoluble. These are generally referred as phosphate solubilizing bacteria (PSB). Bacillus spp., *Serratia marcescens*, *Pseudomonas fluorescens* are included in this category. They have been reported to help in plant growth through smooth provision of P to the plants (Dey et al., 2004, Sahin et al., 2004, Hameeda et al., 2008).

On the other hand, a carbon-rich stable product of pyrolysis, biochar, has gained much attention of the scientists these days. Biochar is generally synthesized from animal or plant origin biomass. Biochar has been observed to be a good soil conditioner, as it is rich in carbon. It reportedly improves soil physicochemical and biological properties, so could help to promote crops growth and productivity. Additionally, biochar has a large enough surface area because of its porous structure (Lehmann et al., 2006; Lehmann, 2007).

Whenever phosphorus is added to the soil, it faces certain reactions like precipitation, adsorption and diffusion which restrict the provision of P to the plants (Almeida et al., 2003). The direct effect of soil texture has been noticed on the uptake of P by the plants (Machado and Souza, 2012). Coarse textured soils serve as a conducive medium to provide the plants with the externally added P as compared to the fine-textured soil. Fine texture soils exhibit much more P adsorption capacity relatively. A positive correlation has been observed between the clay contents and P adsorption capacity of the soil (Falcão and Silva, 2004). Thus in fine texture soils, P adsorption is higher comparatively, while coarse-textured soils render more fraction of added P, available for plant uptake (Machado and Souza, 2012). A pot study was designed with the hypothesis that wood husk biochar and PSB strains would behave differently whether applied alone or in combinations in different textured soils in terms of maize growth.

MATERIALS AND METHODS

A maize experiment was carried out in pots in the experimental area of the Faculty of Agricultural Sciences and Technology, BZ University, Multan. The experiment was laid out following the completely randomized design (CRD), consisting of six treatments in two different soil textures i.e., fine and coarse. Three replications were made for every treatment. The soils were obtained from at the depth of 0-15cm of the upper surface.

Phosphorus solubilizing bacterial (PSB) strains collection

Two phosphorus solubilizing bacterial (PSB) strains i.e., *Achromobacter xylosoxidans* (BS1) and *Alcaligenes faecalis* (BS15) were obtained from the Laboratory of Soil and Environmental Microbiology, Department of Soil Science, BZ University, Multan.

Biochar

Wood husk was taken from a local wood-mill and subjected to the pyrolysis at very low O_2 at the temperature of 550°C (Harris, 1999). The pyrolyzed material was passed through a 0.2 mm sieve to get a final product, referred to as biochar (BC).

Treatments

Factor 1

Six treatments were applied as follows; Control, BS1, BS15, BC, BC+BS1 and BC+BS15.

Factor 2

Two different textured soils that are TS1 (clay loamy) and TS2 (sandy clay loam) were taken for the trial.

The pots were filled with 7 kg of soil in each pot. In respective pots, 0.50% (33.50 g) biochar was added as per treatment plan. The sieved soil and biochar were mixed well in the respective pots.

Seed Coating and Sowing

For seed inoculation, respective broths of the strains were mixed with clay and peat along with 20% sugar solution in a ratio of 1:1:1. Five seeds were sown in each pot. Later on, after 15 days of sowing, only two seed were left to grow. Recommended NPK fertilizers were applied as urea, SSP and MOP at the rate of 120:90:60 $kgha^{-1}$. The nitrogenous fertilizer was applied thrice i.e., at sowing time and then after 20 and 35 days of sowing. Plants were watered as per needed.

Agronomic parameters

Stove weight i.e., yield was noted using weight balance. Similarly, root length was measured by using the standard scale.

Chlorophyll a and b

Chlorophyll a and b content were assessed in the maize plants. Initially, 0.50 g leaf samples were taken and put into 10 ml of acetone solution of 80% concentration. The solution was filtered followed by determining the optical density using a spectrophotometer at a wavelength of 645 and 663 nm. Finally, the chlorophylla and b content were computed (Arnon, 1949; Ravelo-perez et al., 2008).

Organic matter

Soil organic matter was recorded using the Walkley-Black method (Walkley, 1947). For nitrogen determination following equation was used
Soil Nitrogen (%) = Organic Matter/20

Statistical analyses

Statistical analyses were completed using the software, 'Statistix' (Version8.1) was. Least significant difference (LSD) test was applied at 5% probability (Steel et al., 1997).

RESULTS

Yield

Maximum and significantly increased stove yield was noticed in BC+BS15 (64% as compared to control) application followed by BC+BS1 and BS15 (53% higher than control) in coarse-textured soil. Effect of PSB and biochar on stove yield of maize was evaluated under different textured soils BS15 and BS1 in fine-textured soil were statistically non-significant to each other but significantly different with other treatments. Minimum yield was observed in control in both soil textures.

Root length

Effect of PSB and biochar on root length of maize in different textured soils were statistically analyzed. Highest and significantly improved root lengths were noticed in BC+BS15 and BC treatments (55% as compared to control) followed by BC+BS1 in coarse-textured soil. BC and BS15 in fine-textured soil depicted similar results with each other while differed with their respective control. While the treatments of BS1 and BS15 in coarse-textured soil and BC+BS1 in fine-textured soil also proved statistically similar to each other while different from other treatments. Overall coarse-textured soil treatments imparted better result over fine-textured soil treatments

Organic Matter

The effect of PSB along with 0% and 0.5% biochar on organic matter in different textured soils were statistically analyzed. Significant differences ($p \leq 0.05$) was recorded for soil organic matter. The BC+BS1 imparted highest organic matter (56%) than control and alone biochar application in fine-textured soil. The treatments BC+BS1, BC+BS15 (77%) in coarse-textured soil while BS1 and BS15 (52%) in fine-textured soil revealed similar differences with one another while different from other treatments. Lowest organic matter was noticed in control in both soils. Biochar and bacterial strains enhanced the organic matter in fine textured soil as compared to coarse textured soil.

Cation Exchange Capacity

Organic matter enhances the cation exchange capacity of soil by adding nutrients into the soil as the study revealed about biochar and PSB application in different textured soils. Biochar and PSB had significant ($p \leq 0.05$) effect on CEC of soil. Maximum and significantly improved CEC was observed in the BC+BS1 treatment (23%) over other treatments in fine and coarse textured soil followed by BC+BS15 in fine textured soil as demonstrated. Moreover, BC treatments of both soils showed similar results with one another while different from other treatments. Similarly, BS1 and BS15 in coarse textured soil also produced similar results with each other. The control showed the lowest value for CEC. Overall, fine textured soil gave better response over coarse textured soil.

Chlorophyll a

Effect of PSB and different rates of biochar on maize

Table 1. Effect of PSB and biochar on yield and root length of maize in different textured soils

Treatments	Fine textured soil for yield (g)	Coarse texture soil for yield (g)	Fine texture soil for root length (cm)	Coarse texture soil for root length (cm)
Control	44.33 g	75.33 f	32.66 f	23.66 g
BS1	115.00 d	71.67 f	43.66 bcd	42.00 de
BS15	118.33 d	163.33 b	42.66 cde	40.66 de
BC	72.33 f	81.50 e	46.00 bc	53.00 a
BC+BS1	125.33 c	163.33 b	42.00 de	46.66 b
BC+BS15	82.33 e	215.00 a	39.00 e	51.00 a
Main Effect	**92.94 B**	**120.19 A**	**41.00 B**	**42.83 A**

Table 2. Effect of PSB and biochar on soil organic matter and cation exchange capacity

Treatments	Fine texture soil for organic matter (%)	Coarse texture soil for organic matter (%)	Fine texture soil for cation exchange capacity (cmol/kg)	Coarse texture soil for cation exchange capacity (cmol/kg)
Control	0.62 h	0.31 h	3.01 fg	2.88 g
BS1	1.31 bc	1.15 e	3.33 de	3.13ef
BS15	1.31 bc	1.05 f	3.75c	3.18ef
BC	1.2bcd	0.89 g	3.51 d	3.45d
BC+BS1	1.43 a	1.32 ab	4.01a	3.88 a
BC+BS15	1.24 d	1.37ab	3.97 ab	3.78 bc
Main Effect	1.23 A	1.01 B	3.60 A	3.38 B

Table 3. Effect of PSB and biochar on chlorophyll a and b (mg/g)

Treatments	Fine texture soil for chlorophyll a	Coarse texture soil for chlorophyll a	Fine texture soil for chlorophyll b	Coarse texture soil for chlorophyll b
Control	0.12 g	0.13 g	0.23h	0.31 g
BS1	0.43 d	0.37 de	0.54 de	0.49ef
BS15	0.50c	0.43 d	0.63 bc	0.58 cd
BC	0.35 e	0.25 f	0.45 f	0.37g
BC+BS1	0.66 a	0.42 d	0.79 a	0.62 bc
BC+BS15	0.59 b	0.54 bc	0.68 b	0.75a
Main Effect	**044 A**	**0.36 B**	**0.55 A**	**0.52 B**

Table 4. Effect of PSB and biochar on extractable soil P and exchangeable K

Treatments	Fine texture soil for extractable P	Coarse texture soil for extractable P	Fine texture soil for exchangeable K	Coarse texture soil for exchangeable K
Control	4.77 e	4.46 e	34.56 h	24.62
BS1	20.05b	21.89 ab	68.33 e	63.57ef
BS15	22.75 ab	21.45 ab	58.00 f	56.81 f
BC	8.96 d	9.70 d	42.11gh	43.30 g
BC+BS1	21.97 ab	21.54ab	91.78 c	110.06 a
BC+BS15	16.26 c	23.63 a	79.06 d	100.52 b
Main Effect	**15.97 B**	**17.11 A**	**62.30 B**	**66.48 A**

Table 5. Effect of PSB and biochar on total nitrogen and organic carbon

Treatment	Fine texture soil for N (mg/g)	Coarse texture soil for N (mg/g)	Fine texture soil for organic carbon (%)	Coarse texture soil for organic carbon (%)
Control	0.31 h	0.18i	5.62 e	2.13 f
BS1	0.65 bc	0.57 e	8.43cd	9.36c
BS15	0.66 bc	0.52f	6.80 de	7.76 cd
BC	0.64 cd	0.44 g	7.54cde	11.60 b
BC+BS1	0.71 a	0.67 bc	8.13cd	13.86 a
BC+BS15	0.61 d	0.68 b	11.60b	13.24 ab
Main Effect	**0.60 A**	**0.49 B**	**7.46 B**	**9.56 A**

production of chlorophyll a remained statistically different. Maximum chlorophyll a production was noticed in BC+BS1 (81%) in fine textured soil followed by BC+BS15 (79%) in both textured soils. After that, treatments BS1, BS15 and BC+BS15 in both fine and coarse textured soils were statistically similar with one another but different from other treatments. Overall, coarse textured soil depicted less chlorophyll a production than in fine textured soil.

Chlorophyll b

Mean values for chlorophyll b production in maize leaves were statistically analyzed regarding the effect of PSB and biochar application in fine and coarse textured soils. The representing data were statistically significant. Maximum and significantly better chlorophyll b production was observed in BC+BS1 in fine textured soil and BC+BS15 in coarse textured soil as compared to other treatments. Data also revealed that BS15 and BC+BS15 in fine textured soil as well as BC+BS1 in coarse textured soil imparted similar results with each other but different than other treatments. On the contrary, control showed minimum chlorophyll b production in fine and coarse textured soils. Overall, fine textured soil exhibited higher response than coarse textured soil.

Extractable Phosphorous

Effect of PSB with and without biochar application on extractable soil P varied significantly in different textured soils. In coarse textured soil BC+BS15 application gave the maximum and significantly better extractable P (81%) than other treatments. The treatments, BS1, BS15 and BC+BS1 in coarse and fine textured soil produced statistically similar results while differed than other treatments. The BC treatment in both textured soils showed similar results with one another. Minimum and similar extractable P values were observed in control treatments in both types of soil.

Stove Nitrogen Concentration

Biochar and PSB imparted positive and significantly different ($p \leq 0.05$) effect on nitrogen content in maize stove. Maximum N concentration was analyzed for fine textured soil through BC+BS1 followed by BC+BS15 which was 98% and 95% respectively, more as compared to control. Whereas minimum N concentration was observed in both i.e. fine and coarse textured soils control treatments, which were also statistically similar. It can be observed that treatments BS1, BS15, BC and BC+BS15 (90-95%) of both soils behaved similarly with one another. Moreover, maize plants which were sown in fine textured soil showed more nitrogen than the plants grown in coarse textured soil.

DISCUSSION

The PGPR and biochar application enhanced plant growth, nutrients uptake and improved physic-chemical properties of soil. It is because of the addition of essential nutrients by PGPR into the soil from the rhizosphere. Biochar has high efficiency in nutrients availability (Mohan and Ayswarya, 2012, Hoberg et al., 2005). From the recent study, it is confirmed that biochar and PGPR have a positive impact on the growth of maize and nutrients uptake in plants as well as nutrients status in soil. Other research workers also reported similar results on other crops (Saxena et al., 2013, Ashrafi and Seiedi, 2011, Harinathan et al., 2016). Both bacterial strains in combination with and without biochar significantly increased stove yield in current findings; bacterial strains in sandy clay loam soil had a more significant effect on stove yield and root dry mass as compared to fine textured soil and the same was reported by Shanta et al. (2016) in their research of maize. Combination of biochar with PSB improved the plant height due to the availability of nutrients.

In this research, PSB with biochar (BC+BS1 and BC+BS15), enhanced the stove height. Biochar increased the root length of maize grown in pots. The same trend was observed by both types of soils. In clay loam, soil biochar provisionally influenced the root length, confirmed by Shanta et al. (2016). Maximum root length observed in coarse soil is due to the larger pores as they get easily penetrated in it. Photosynthesis rate in leaf was improved by high chlorophyll content which contributed to the biomass of plants. More chlorophyll contents were examined in treatments where individual biochar and combination with PSB were applied, these results were also confirmed by Danish et al. (2014). Fine textured soil has shown greater effects on chlorophyll content as compared to coarse textured soil. It is possibly due to its more resistance towards leaching nutrients and high organic matter contents.

More nitrogen and potassium concentrations in soil were noticed in combinations of PSB with biochar and a similar result was reported by (Richardson and Simpson, 2011) that PGPR and biochar impacted positively on N, K, and P contents in soil. While fine textured soil trapped more nitrogen and potassium. Biochar and PSB enhanced the availability of the nutrients in soil probably due to improved soil quality and physiochemical characteristics of soil (Danish et al., 2014). Biochar application maximizes the availability of N by increasing the organic N mineralization rate. In fine texture soils, high N concentration was possibly due to high organic matter, as OM has more retention of N (Lehmann et al., 2006; Lehmann, 2007). Data regarding the macro-nutrients (N, P, K) in maize plant revealed that maximum nutrients uptake was observed in PSB combination with biochar application in different textured soils. More N uptake was observed in fine textured soil, while high P and K contents were noticed in coarse textured soil (Richardson and Simpson, 2011).

CONCLUSION

The PSB and biochar enhanced the nutrients availability and uptake by PSB mediated essential nutrients, mineralization of P, siderophores and phosphatases production. Organic matter decomposition and more N retention in soil resulted in plant growth improvement. Overall, PSB (*Alcaligenes faecalis*) and biochar collectively showed better performance over PSB (*Achromobacter xylosoxidans*) with biochar, it is probably because of the reason that they have better abilities to colonize around root surface and higher K contents were noticed in the coarse textured soil.

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