INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 5, p. 349-356, 2019

RESEARCH PAPER OPEN ACCESS

Evaluation of phosphate solubilizing bacteria role with biochar on the growth of wheat

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Key words: Biochar, Maize, Phosphate solubilizing bacteria, Morphological attributes.

http://dx.doi.org/10.12692/ijb/14.5.349-356 Article published on May 10, 2019

Abstract

Deficiency of phosphorus (P)in calcareous soils significantly reduces plant growth and yield. High pH and more Ca availability are major hurdles in the way of phosphorus mobilization in thesoil. The ability of phosphate solubilizing bacteria (PSB) to solubilize fixed P for plant uptake is a documented fact. As far as the co-application of PSB and biochar (BioC) for P mobilization is concerned, there are yet few investigations. Therefore, it was hypothesized that the co-application of PSB and BioC might be more effective and novel approach to make P readily available for plants. Six PSB isolates (M1, M2, M3, M4, M5, M6)were isolated from the rhizosphere of *Zea mays*, *Sorghum bicolor* and *Oryza sativa* on Pikovskaya's medium. Freshly prepared inocula of the PSB and wheat straw based BioC were treated to soil and seeds were sown in axenic conditions. It was observed that co-application of PSB and BioC significantly enhanced the growth attributes in wheat seedlings. Treatments BioC+M3 and BioC+M5 performed best for the improvement in germination, and seedling fresh weight in P deficient soil. However, more P solubilization in soil and higher uptake in seedlings was recorded for M3+BioC and M5+BioC. It can be inferred from the results that the co-application of PSB and BioCis anefficient approach to enhance the bioavailability of P to wheat in P deficient, alkaline calcareous and low organic matter soils as compared to PSB inoculation or BioC application alone.

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Introduction

Phosphorus plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plants. A large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer become immobile rapidly and unavailable to plants (Goldstein, 1986). This immobilization and non-bioavailability of phosphorus directly affect the elongation of roots in most of thecrops that lead to poor plant growth (Srinivasan *et al.*, 2012).

To solubilize the inorganic immobile phosphorus in the soil, most ofthe scientists recommended the inoculation of phosphorus solubilizing bacteria (PSB) (Kloepper and Schroth, 1978) that have the ability to make phosphorus mobile and bioavailable to plants via rhizosphere acidification(Glick, 1995; Hussain *et al.*, 2017). Wide ranges of PSB such as *Azospirillum, Alcaligenes, Arthrobacter, Acinetobacter, Bacillus, Burkholderia, Pseudomonas, Enterobacter, Erwinia, Rhizobium, Flavobacterium*have been reported to transform soil immobile phosphorus to inorganic phosphorusand play an integral part in phosphorus cycle(Kloepper and Schroth, 1978, Rodríguez and Fraga, 1999).

Many scientists have noted that the soils wherePSB are in high density, fixation or adsorption of phosphorus become less(Khan and Joergensen, 2009). These bacteria adopt the mechanism of pH change to make phosphorus mobile by secretion of organic acids (gluconic, 2-ketogluconic, glyoxylic, citric, malic, lactic acids) in soil solution (He *et al.*, 2002). The organic acids also serve as a chelating agentthat increase the retention of mineral phosphorus and make it phyto-available (Kpomblekou and Tabatabai, 2003). In the presence of labile carbon, PSB serve as a sink and source for phosphorus as well (Bünemann *et al.*, 2004). Inoculation of PSB to plants or seeds can play a significant role in economizing the use of inorganic fertilizers without disturbing the productivity of thecrop.

Application of activated black carbon biochar (BioC) in thesoil remunerates the soil fertility by improving cation exchange capacity (CEC) and water holding capacity (WHC)(Danish *et al.*, 2014, Revell *et al.*, 2012). Soils amended with BioC mostly have higher contents of carbon that directly influenced the C: N ratio to facilitate rhizobacteria for increased proliferation (Pietikäinen *et al* 2000). BioC has a large surface area to retain mobile nutrients like nitrogen (N)and decrease its losses by leaching or volatilization (Novak *et al.*, 2010). Depending upon the feedstock used for pyrolysis, BioC serves as a source of fertilizer to supply potassium and phosphorus in soil(Chan *et al.*, 2007).

In cereals, *Triticum aestivum* L. is a major cash and staple cropthat directly influences the economy and food security of many countries, particularly in South East Asia. As a staple food,*Triticum aestivum* L. provides 55% carbohydrates and 20% of food requirement for theexponentially increasing population (Bos *et al.*, 2005).

For optimization of *Triticum aestivum* L. growth and yield, it mostly required a large amount of nutrients which make it an exhausting crop regarding soil fertility point of view (Schulz *et al* 2013).Most of the soils in Pakistan are high in pH and calcareous so availability of phosphorus is limited in these soilstorestrict the growth and yield of thecrop. Therefore, keeping in mind the importance of wheat as a staple cereal and cash crop a laboratory experiment was conducted to examine the co-application of PSB and BioC on the bioavailability of P in calcareous and high pH soils.

Materials and methods

An experiment was conductedin Laboratory of Soil Microbiology and Biochemistry, Department of Soil Science to examine the effectiveness of co-application of BioC and PSB regarding improvement ingrowth attributes of *Triticum aestivum* L. cultivated in calcareous and less P available soils following the completely randomized (CRD) design in three replications.

Isolation and purification of phosphate solubilizing rhizobacteria

Isolation of rhizobacteria was done by taking the rhizosphere soilfrom maize, jawar and rice crops on Agricultural Research Farm of the Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University Multan. The plants along with rhizosphere soil were uprooted and brought into thelaboratory inairtight polythene bags. Root adhering soil was separated in sterilized Petri plates. Serial dilutions of soil samples were made ranging10^{-1} to 10^{-6} and 100 µL soil suspension was spread on sterilized (121 °C for 20 min) Pikovskaya'saga rmedium (Yeast extract = 0.5g/L, Dextrose = 10g/L, TriCalcium phosphate $Ca_3(PO_4)_2$ = 5 g/L, Ammonium phosphate $(NH_4)_2PO_4$ = 0.5 g/L, Potassium chloride (KCl) = 0.2 g/L, Magnesium sulphate ($MgSO_4.7H_2O$) = 0.2 g/L, Manganese sulphate ($MnSO_4.2H_2O$) = 0.002 g/L, Ferrous sulphate ($FeSO_4.7H_2O$) = 0.002 g/L and Agar = 15 g/L at pH 6.8) for the isolation of PSB under aseptic conditionsin laminar flow hood.Paraffin was used to airtight Petri dishesand incubated at room temperature (24 ± 3 °C). After 24h, PSB colonies appeared on Petri dishes which further purified by repeated (four times) streaking on freshly prepared Pikovskaya's medium. Pure PSB cultures obtained were preserved in test tube slantsin a refrigerator (4 °C) for future experiments.

Phosphorus solubilizing capacity and selection of PSB

Phosphorus solubilizing capacity (PSC) of sixteen purified PSB isolates from maize, jawar and rice rhizosphere was examined. Sterilized broth media containing Tri-Calcium phosphate was used for the analysis. Freshly prepared inocula of the PSB were inoculated in both media and incubated for 72h at room temperature with three replications. Uninoculated control was also maintained. Phosphorus in the medium was determined by following the malachite green method (Ohno and Zibilske, 1991). The difference in soluble phosphorus of broth culture before and after inoculation gives the capacity of PSB to solubilize phosphorus. Finally, three PSB isolates were selected on the basis of their phosphorus solubilizing capacity (PSC)which were cultured on Pikovskaya's medium.

Preparation of Inoculum

Broth culture was prepared using Pikovskaya's media (PVK) without agar. Three isolates picked from the slants while three PSB acquired from Ayub Agriculture Research Institute (AARI), Faisalabad inoculated in fresh sterilized PVK broth in 100 mL flasks. The flasks were shaken at 100 rpm for 72h at room temperature.

Inoculation of PSB and BioC application

Soil, sandy clay loam, alkaline calcareous (pH 8.2, $CaCO_3$ 5.4%), low in organic matter (0.69%) and containing Olsen's P (7.5 mg kg^{-1}), was collected from 0-15 cm surface layer of Agricultural Research Farm of the Bahauddin Zakariya University for the experiment. Soil (50 g) was poured in petri plates and autoclaved twice for sterilization. Biochar (BioC) prepared by wheat straw waste as described by Qayyum *et al.* (2014) in a specially designed pyrolyzer was applied at 1% (w/w) in respective Petri plates and mixed well.Nine seeds of wheat cv. Lasani-2008 were sterilized with 0.1% $HgCl_2$(Sadiq and Ali, 2013) and after washing sowed in the Petri plates. Fresh culture of selected six PSB isolates was poured in respective plates at 10 mL. The treatments established were as Uninoculated soil (control), M2 inoculation, M3 inoculation, M4 inoculation, M5 inoculation, M6inoculation, BioC, M2 + BioC, M3 + BioC, M4 + BioC, M5 + BioC and M6 + BioC.

Morphological growth attributes

After 5 days of germination of first, the rate of germination (%) was examined. At20^{th}daystarting from sowingthe seedlings were harvested to examine seedling height and fresh weight of shoot on analytical grade balance. The shoots of wheat seedlings were oven dried at 65 °C for 24h and seedling dry weight was noted on analytical grade balance. The uptake of phosphorus in seedling was examined by following yellow color method (Jones *et al.*, 1991) by digesting seedlings with di-acid(Chapman and Pratt, 1961) and taking

absorbance at 420nm on spectrophotometer (HITACHI U-2000).

Pre and post characterization of Soil

The pH of saturated soil paste (pH_s) was measured with pH meter (Jenwey-3510), standardized at 4.0, 7.0 and 9.2 pH buffer solution (Shaaban *et al*., 2013).Phosphorous was determined by dissolving the extracts of 30 µL by making volume 1mL using blue color Olsen's methodology (Olsen and Sommers, 1982). The color intensity was measured on spectrophotometer (HITACHI U-2000)at 880 nm wavelength.

Results

Effect of PSB with and without BioCon germination percentage, seedling height, seedlings fresh and dry weight

The interaction of BioC and PSB inoculation remained no significant for germination percentage, seedling height, seedling fresh and dry weight among each other therefore main effects have been described (Table 1). Mainly the application of BioC to soil significantly improved the germination percentage, seedling height, seedling fresh and dry weight as compared to control without BioC (Table 1).

Table 1. Effect of PSB and Biochar on germination, seedling height, seedling fresh weight and seedling dry weight of wheat

PSB	Various levels of Biochar					
	0% BC	1% BC	Mean	0% BC	1% BC	Mean
	Germination (%)			Seedling Height (cm)		
Soil	46.7	63.3	55.0 b	9.30	13.6	11.4 a
M2	50.0	60.0	55.0 b	10.2	11.2	10.7 a
M3	56.7	90.0	73.3 a	10.4	14.9	12.6 a
M4	40.0	63.3	51.7 b	8.00	12.0	10.0 a
M5	66.7	76.7	71.7 a	11.0	12.7	11.9 a
M6	40.0	50.0	45.0 b	4.80	13.6	9.20 a
Mean	50.0 b	67.2 a		9.00 b	13.0 a	
	Seedling Fresh Weight (g)			Seedling Dry Weight (g)		
Soil	0.41	0.58	0.50 bc	0.07	0.09	0.08 bc
M2	0.36	0.59	0.48 cd	0.04	0.09	0.07 c
M3	0.59	0.79	0.69 a	0.09	0.12	0.11 ab
M4	0.28	0.44	0.36 d	0.07	0.10	0.09 a-c
M5	0.68	0.76	0.72 a	0.10	0.13	0.12 a
M6	0.64	0.61	0.63 ab	0.08	0.09	0.09 a-c
Mean	0.49 b	0.63 a		0.08 b	0.11 a	

Means sharing similar letters are statistically at par to each other at 5% probability. Non-significant effects did not have any letter.

Inoculation with isolate M3 and M5 mainly showed a significant increase in germination percentage and seedling fresh weight as compared to uninoculated control. However, seedling height remained unchanged due to inoculation over uninoculated control. But the seedling dry weight was significantly increased by the inoculation of M5 in contrast to uninoculated control. Maximum increase of 0.30-fold in germination was noted in the treatment M3+BioC as compared to control (No PSB). As far as seedlings fresh and dry weight is concerned, maximum increase of 0.44 and 0.5-fold was recorded, respectively, due to treatment M5+BioC as compared to control (No PSB).

Effect of PSB on soil pH and solubilization of soil phosphorus

The PSB isolates M2, M3, M4 and M5 significantly

reduced the pH of PVK media over uninoculated control, however, the strain M3 and M5 remained most efficient acidifiers and were statistically similar to each other (Figure 1). The maximumdecreaseup to 0.38-fold in the pH of Pikovskaya's media was noted due to isolate M3 inoculation.

Availability of soil P was increased by the application of BioC or PSB, whereas, the combination of both further increased it (Figure 2). Treatments M3+BioC, M5 and M5+BioC performed best among the treatments and gave up to 65 µg g^{-1}available soil P.

Without BioC maximum increase of 1.33-fold was noted in M5 as compared to control (No PSB + No BioC). However, as compared to control BioC (No PSB) maximum increase of 0.60-fold in the soil available P was recorded in M3+BioC.

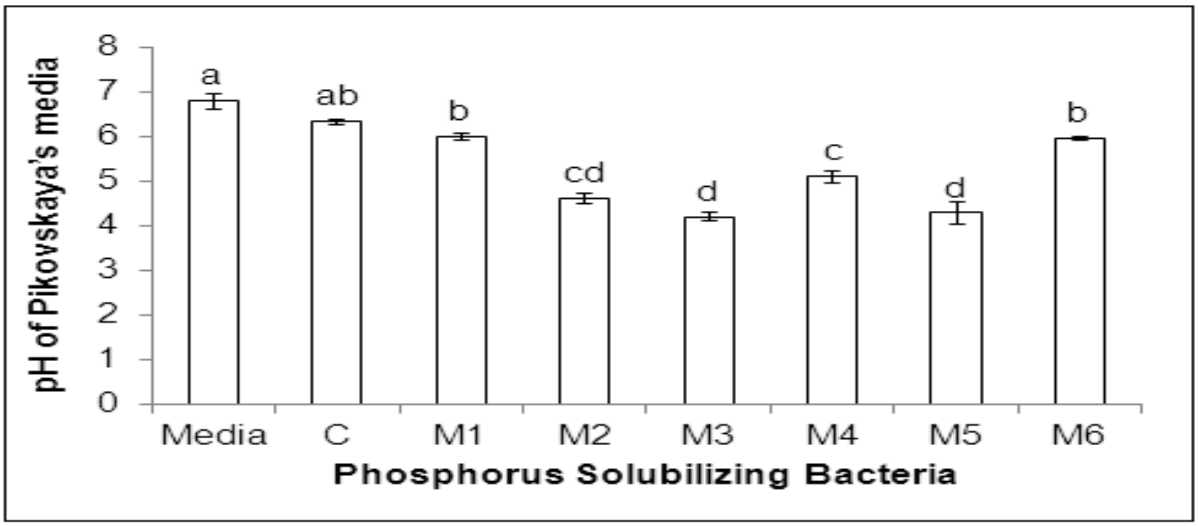

Fig. 1. Effect of phosphorus solubilizing bacteria (PSB) on pH of Pikovskaya's media (PVK).

Effect of PSB with and without BioC on number of seedlings and P concentration

Inoculation with PSB without BioC could not show a significant change in the number of seedlings per plate as compared to uninoculated control (Figure 3).

Whereas, M3+BioC, M4+BioC and M5+BioC significantly increased the number of seedlings as compared to control without inoculation and without BioC. However, only M3+BioC could produce significantly higher seedlings as compared to uninoculated control with BioC.

Seedling P concentration was significantly increased due to M5+BioC, M3+BioC, M4+BioC and M5 as compared to uninoculated control with or without BioC (Figure 4). Maximum increase (0.91 and 0.11-fold) in the number of seedlings and seedlings P concentration was noted in M3+BioC and M5+BioC,respectively,as compared to control (No PSB + No BioC).

Discussion

In the present study, results showed that inoculation with PSB and application of BioC (1%) as a single amendment significantly enhanced the germination of wheat seeds, seedlings fresh and dry weight as compared to control (No PSB and No BioC). However, the seedlings height did not differ significantly by PSB and BioC. Richardson *et al.*(2009) suggested that the better uptake of P not only promote the root length but also enhanced the fresh and dry weight of plants. BioC in soil provides habitat to PSB which facilitates their colonization in the rhizosphere(Siddiqui *et al.*, 2016). In this study, the inoculation ofM3 and M5 significantly decreased pH of Pikovskaya's media as compared to all other strains. This decrease in pH signified role of M3 and M5 for improvement in the availability of immobilized P in soil. The production of organic acids by PSB might have played its pivotal role in the mobilization of inorganic fixed soil P (Duarah *et al.*, 2011, Gyaneshwar *et al.*, 2002, Khan *et al.*, 2009).

The secretion of acid phosphatases enzyme is another important factor that would have enhanced the mineralization of fixed soil P (Dodor and Tabatabai 2003). BioC application in the soil also enhanced the P availability to the plants due to the presence of P in their structure but the mechanism of P release is slow(Wang *et al.*, 2015).

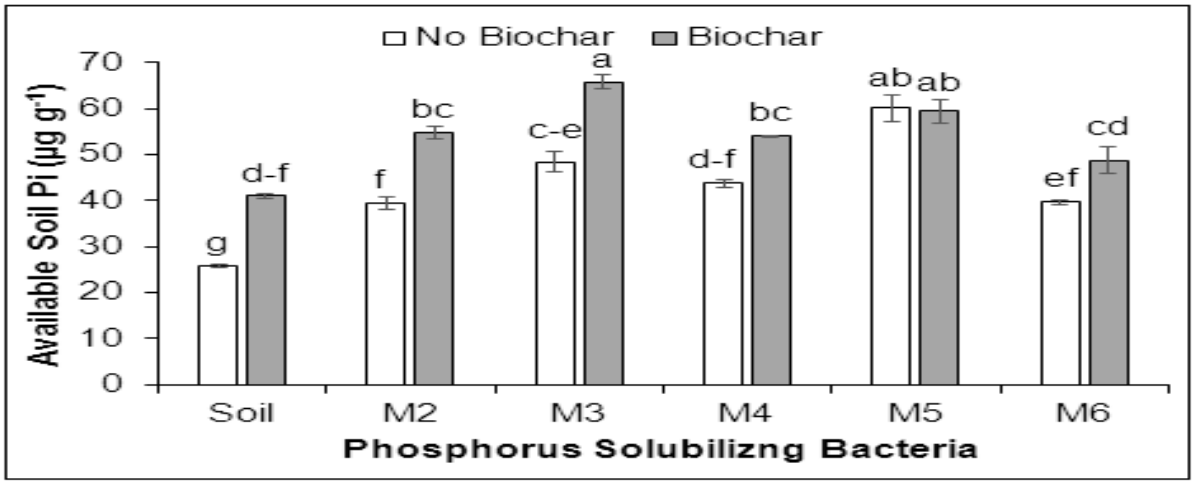

Fig. 2. Effect of phosphorus solubilizing bacteria (PSB) and wheat straw biochar on available soil phosphorus.

It was noted that the co-application of PSB and BioC promoted the number of seedlings to grow as compared to control (No PSB and No BioC). The concentration of P in seedlings was significantly better in M3+BioC and M5+BioC that confirm the ability of M3 and M5 isolates to show a synergistic effect with wheat straw BioC to enhance the P uptake. According to the Siddiqui *et al.*(2016), the presence of micro and macronutrients in the ash content of applied BioC promote the biological activities when applied in combined form. However, the extent of P solubilization by PSB and BioC depends upon the type of BioC feedstock and PSB species (Bandara *et al.*, 2017, Richardson, 2001).

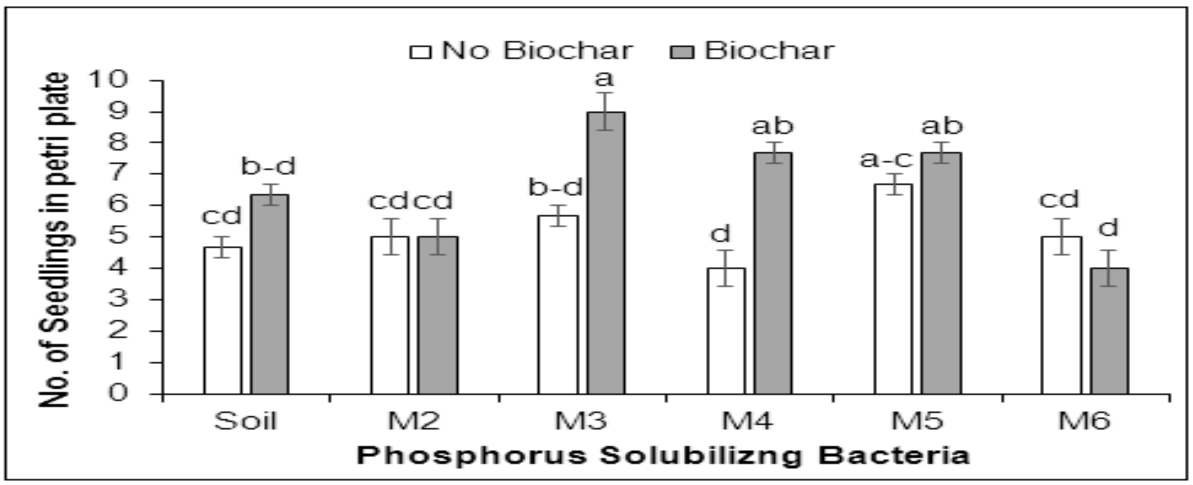

Fig. 3. Effect of phosphorus solubilizing bacteria (PSB) and wheat straw biochar on number of seedlings in petri plate.

Conclusion

It is concluded that the application of PSB can decrease the soil pH that helps in the solubilization of immobilized soil phosphates. However, the co-application of wheat straw BioC at 1% and PSB (M3 and M5) is a more effective amendment to mitigate the phosphorus deficiency in wheat. However, more investigation is needed to introduce the M3 and M5

as PSB and their synergism with wheat straw BioCfor the cultivation of wheat.

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