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PHOSPHORUS SOLUBILIZING BACTERIA AND GROWTH AND PRODUCTIVITY OF MUNG BEAN (*VIGNA RADIATA*)

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Abstract

Phosphorous solubilizing bacteria (PSB) are known as beneficial bacteria that enhance the growth of the plants, when applied to the crops. An experiment was conducted to study the effect of two PSB spp. i.e., *Pseudomonas striata* and *Bacillus polymyxa* on the growth and development of mung bean. Seeds of mung bean i.e. 4 seeds per pot having 500 g loamy soil/pot were inoculated with broth of selected isolates and sown. Nutrition was provided through Hoagland nutrient solution. For control, sterilized flasks having 0.03 M $MgSO_4$ solution were used to dip the seeds. After forty days, mung bean plants were harvested and important growth parameters were recorded. Results revealed that inoculation with PSB, increased plant height (11.88-30.69%), branchs/plant (18.35-39.20%), leaves/plant (12.10-41.30%), pods/plant (26.84-57.54%), seeds/plant (6.901-23.43%), pod length (13.00-38.02%) and 1000seed weight (10.07-35.83%), number of effective nodules (7.0-18.0%), nodule density (12.0-21.0%), chlorophyll a and b (5.4-11.4% and 6.6-12.8%), carotenoids (11.4-43.5%), protein (0.05-0.15%) and proline (0.09-0.321%), respectively as compared to un-inoculated control. Moreover, PSB also enhanced the availability of P and N up to 13.56% and 8.56% respectively in soil and uptake of macronutrients i.e., N (0.35-1.21% and 0.51-1.56%), P (0.18-0.65% and 0.26-0.77%), K (1.01-2.89% and 1.21-3.11%), Ca (0.19-0.74% and 0.23-0.88%) and Mg (0.15-0.61 and 0.19-0.78%) in root and shoot of plant compared to control. Further PSB enhanced the soil microbial biomass i.e. MBC (35.4-48.6%), MBN (19.6-35.3%) and MBP (8.41-18.2%), and decreased the non-effective nodules ranging from 5.5-3.0%. The PSB had a convincingly positive impact on the growth, development and productivity of the mung bean and fruit quality, and P and N availability in the soil, and macronutrients uptake in plant without polluting the environment. Therefore, PSB can be used as an alternative and eco-friendly approach for sustainable crop production.

Key words: PSB, *Pseudomonas striata, Bacillus polymyxa,* nutreints, SMB, mung bean

Introduction

Different microorganisms present in soils, play an important role in the cycling of nutrients (Bautista-Cruz *et al*., 2015; Ahemad & Khan, 2013). Terrestrial plants rely on their roots to survive and proliferate, as a result, role of rhizosphere microbes, which colonize the roots become more vital (Sahran & Nehra, 2011). Among these rhizosphere microbes, phosphate solubilizing bacteria (PSB) are naturally occurring soil bacteria (Sharma *et al*., 2013). The PSB are a heterogeneous group of bacteria that profit the plants by providing nutrients (Sharma *et al*., 2013). Directly, PSB provide the growth promoting substances e.g. auxin and enhance the availability of the fixed nutrients e.g., P in the soil (Sharma *et al*., 2013; Alia *et al*., 2013; Wakelin *et al*., 2004).

In last century, tremendous increase in the crop production and yield in agriculture sector has been achieved due to the use of chemical fertilizers (Ibiene *et al*., 2012). This gives an impetus and expands the use of chemical fertilizers around the globe (Ibiene *et al*., 2012). Farmers even started to think that crop production is literally impossible without chemical fertilizers (Ibiene *et al*., 2012). From 2011 to 2014-15, it has been estimated that the world's fertilizer demand is increased at the rate of 2.0 percent per annum (Current world fertilizer trends and outlook to 2015). The global total demand of the consumption of nitrogenous fertilizers increased up to 7 percent in 2014 and highly likely to increase up to 10 percent in 2015 (Current world fertilizer trends and outlook to 2015). Nevertheless, excess and intensive use of chemical fertilizers started to exhibit their drastic effects (Ibiene *et al*., 2012); for example, causing death of microorganisms and other beneficial insects, aggravating the vulnerability of crops to insects and diseases, effecting the soil fertility and overall irreparable damage to system (Ibiene *et al*., 2012). Therefore, search of other reliable sources, which have the potential to replace chemical or synthetic fertilizers completely or in partial is direly needed. The inoculation of agricultural crops with PSB's attracted the considerable attention of the microbiologist (Glick, 2012; Hussain *et al*., 2016). Therefore, it is essential to look for diverse and proficient strains of PSB's and conduct experiments in order to test their positive effects on the increase of crop production on sustainable basis. This will also enable us to curtail the world's dependency on the synthetic fertilizers.

Therefore the present study was designed and conducted with the objectives: (1) isolation and selection of important PSB strains (2) to see the potential of PSB as an alternative and eco-friendly source in enhancing the soil health, and to assess effect of its inoculation on the growth and development of mung bean (*Vigna radiata* L.) plant.

Materials and Methods

The soil was collected with the help of an augur at the depth of 0-5 cm. The samples were air-dried and passed through 2-mm sieve. Some physico-chemical properties of soil used in this study are given in Table 1.

Table 1. Basic properties of soil.

Soil characteristics	Value	Unit
Sand	55	%
Silt	26	%
clay	19	%
Class	Sandy loam	-
Soil pH	7.45	-
Soil EC	0.41	(dS m^{-1})
Available P	3.56	mg kg^{-1}
Available N	3.41	mg kg^{-1}
Soil organic matter	2.1	g kg^{-1}

Rhizosphere soils were collected for the isolation of bacterial strains from the maize roots. Dilution plate technique was used for the isolation of rhizobacteria. For phosphorus solubilizing activity National Botanical Research Institute's Phosphate (NBRIP) growth medium was used. Rhizobacterial strains were cultured and a loop was placed on the plates (five per plate) and the plates were incubated at 28°C for a week. After a week region, clearing around the colonies considered as a positive for phosphate solubilization. However, phosphate solubilizing index (PSI) of these isolates was measured according to the formula of Premono *et al*., (1996).

$$\text{PSI} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

An incubation (green house) experiment was conducted in completely randomized design to study the effect of two PSB spp. i.e., *Pseudomonas striata* and *Bacillus polymyxa* on the growth and development of mung bean. Seeds of mung bean (4 seeds) were inoculated with broth of selected isolates and planted in each pot having 500 g loamy soil/pot. Hoagland nutrient solution was source of nutrition. For control treatments, the mung bean seeds were dipped in sterilizes flasks having 0.03 M $MgSO_4$ solution. After 40 days of germination, mung bean plants were cropped and important growth parameters were recorded. For nodulation density, number of clusters was counted, and to determine the effective and non-effective nodules, nodules were sliced opened. The nodules having pink color were counted as effective nodules, where nodules having white, brown or green color were counted as non-effective nodules. Soil microbial biomass (SMB) i.e. microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and microbial biomass phosphorous (MBP) was determined by the chloroform fumigation-extraction (Hassan *et al*., 2013).

The available P and N in soil were determined calorimetrically by using the method of page *et al*. (1982). The macronutrients (N, P, K, Ca and Mg) and micronutrients (Zn, Cu, Fe and Mn) in shoot and root of the plant was determined by the method of Hassan *et al*., 2014. Chlorophyll and carotenoid contents were determined by using the method of acetone extract (80% v/v) and measured in spectrophotometer at 663, 645 and 480 nm for chlorophyll a, b and carotenoid, respectively (Hassan *et al*., 2015). The concentration of protein contents was determined by using bovine serum albumin as a standard (Hassan *et al*., 2016). The contents of the proline were measured spectrophotometrically at 520 nm wavelength (Bates *et al*., 1973). Statistix 8.1 statistical package (Statistix, USA) was used for statistical analysis. Least significant difference (LSD) test at $p<0.05$ was used to determine the significance level.

Results

Data indicated that inoculation of mung bean seedlings with PSB spp. i.e., *Pseudomonas striata* and *Bacillus polymyxa*, increased plant height significantly ($p<0.05$) compared to control (Fig. 1). The increase in the plant height ranged between 11.88 to 30.69%. The effectual trend of PSB spp. on the plant height was in the order of *Bacillus polymyxa* > *Pseudomonas striata.* The increase in the number of branchs/plant and number of leaves/plant was ranged from 18.35 to 39.20% and 12.10 to 41.30%, respectively (Fig. 2). The *Bacillus polymyxa* caused an increase of 2.21-fold, whereas, PSB *Pseudomonas striata* caused an increase of 1.98-fold compared to control. Similarly, inoculation with PSBs increased the number of pods/plant and pod length, which ranged from 26.84 to 57.54% and 13.10 to 38.02% correspondingly compared to control (Fig. 3). Comparing the effect of two PSB spp., it was observed that *Bacillus polymyxa* has slightly higher positive effect than the *Pseudomonas striata*. The increase caused by the *Bacillus polymyxa in the* number of pods/plant and pod length was 1.05-fold higher than the *Pseudomonas striata.* Inoculation with PSBs also enhanced the number of seeds/plant and 1000-seed weight that ranged from 6.911 to 23.43% and 10.07 to 35.83% respectively (Fig. 4). Similarly inoculation with PSBs enhanced the number of effective nodules (7.0-18.0%), and nodule density (12.0-21.0%), whereas decreased the number of non-effective nodules (5.0-3.0%), compared to control (Figs. 5-6). Increasing trend has been also observed in the plant chlorophyll a (5.4-11.4%) and chlorophyll b (6.6-12.8%) and carotenoids (11.4-43.5%) compared to control (Fig. 7). Further an increase in the protein (0.05-0.15%) and proline (0.09-0.321%) contents respectively has also been observed upon inoculation with PSBs as compared to un-inoculated control (Fig. 8). Moreover, PSB also enhanced the availability of P and N up to 13.56% and 8.56% respectively (Fig. 9) and uptake of macronutrients i.e. N (0.35-1.21% and 0.51-1.56%), P (0.18-0.65% and 0.26-0.77%), K (1.01-2.89% and 1.21-3.11%), Ca (0.19-0.74% and 0.23-0.88%) and Mg (0.15-0.61 and 0.19-0.78%) in root and shoot of plant compared to control (Figs. 10 and 11). Further PSB enhanced the soil microbial biomass (Fig. 12) i.e. MBC (35.4-48.6%), MBN (19.6-35.3%) and MBP (8.41-18.2%). Comparing the efficacy of two PSB spp., it was noticed that *Bacillus polymyxa* showed higher positive influence than *Pseudomonas striata.* The overall effectual trend of PSBs was in the order *Bacillus polymyxa* > *Pseudomonas striata*

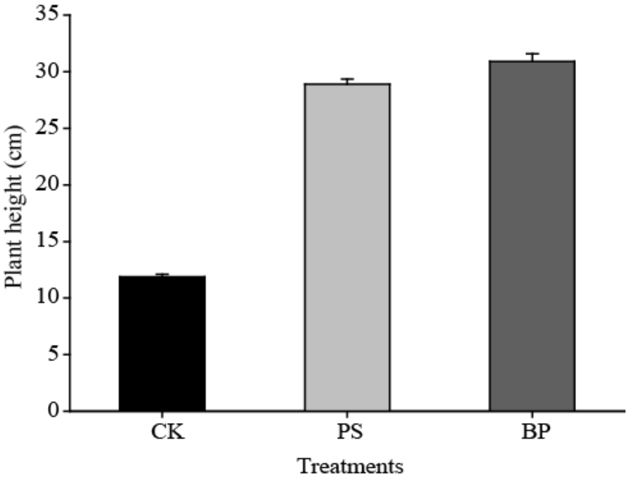

Fig. 1. Effect of phosphorous solubilizing bacteria on the plant height of mung bean.
CK, control; PS, *Pseudomonas striata*; BP, *Bacillus polymyxa*.
The *error bars* represent standard error of the means

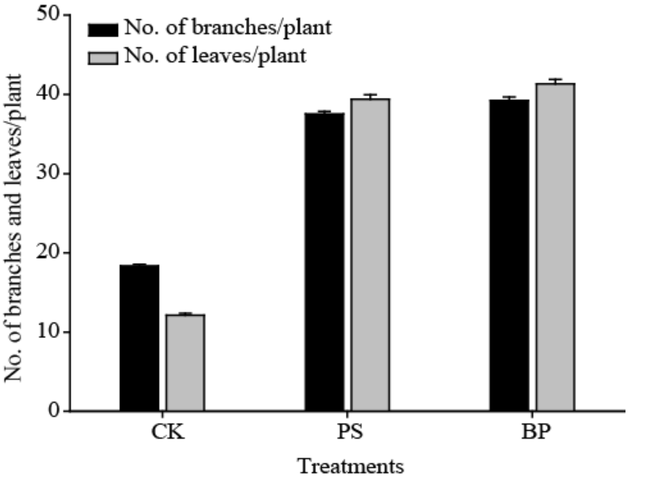

Fig. 2. Effect of phosphorous solubilizing bacteria on the number of branches and leaves/plant of mung bean.
CK, control; PS, *Pseudomonas striata*; BP, *Bacillus polymyxa*
The *error bars* represent standard error of the means

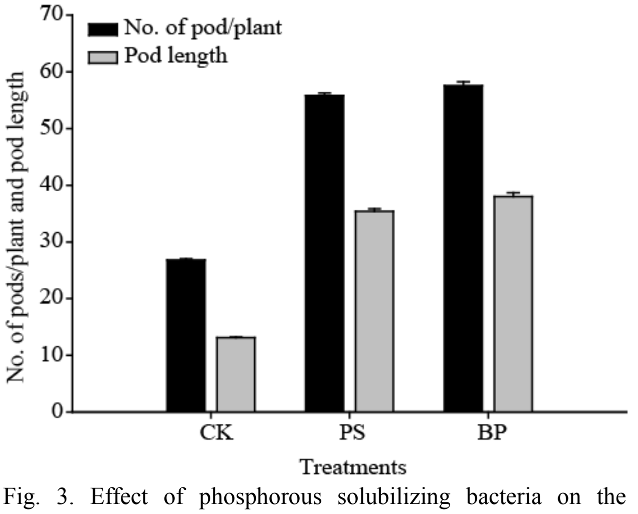

Fig. 3. Effect of phosphorous solubilizing bacteria on the number of pods/plant and pod length of mung bean.
CK, control; PS, *Pseudomonas striata*; BP, *Bacillus polymyxa*
Unit of pod length: cm
The *error bars* represent standard error of the means

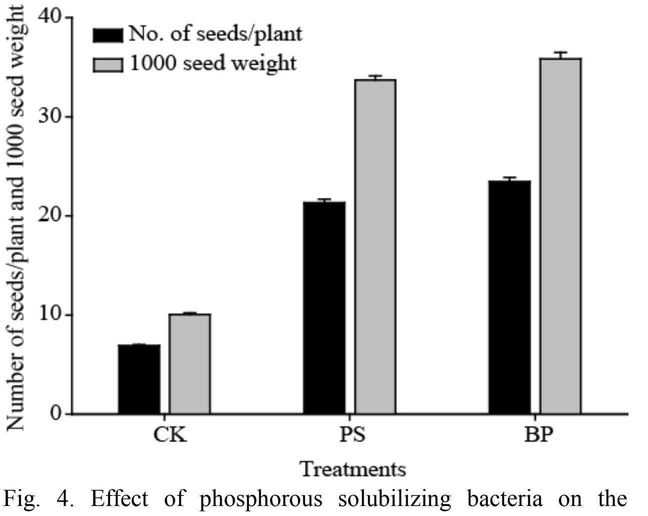

Fig. 4. Effect of phosphorous solubilizing bacteria on the number of seeds/plant and 1000 seed weight of mung bean.
CK, control; PS, *Pseudomonas striata*; BP, *Bacillus polymyxa*
Unit of seed weight: g
The *error bars* represent standard error of the means

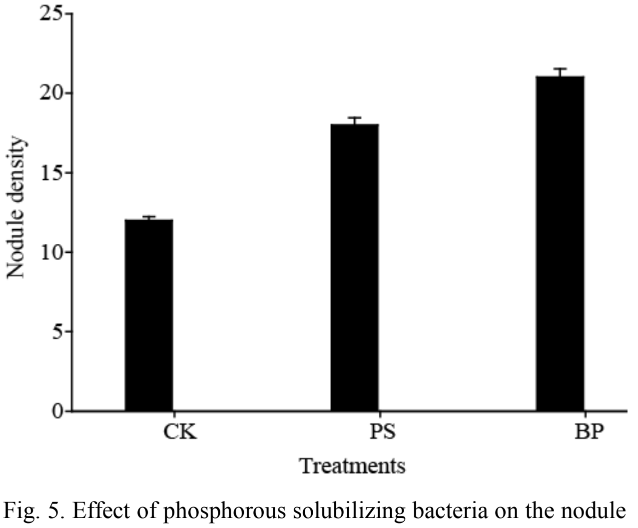

Fig. 5. Effect of phosphorous solubilizing bacteria on the nodule density of mung bean.
CK, control; PS, *Pseudomonas striata*;
BP, *Bacillus polymyxa*
The *error bars* represent standard error of the means

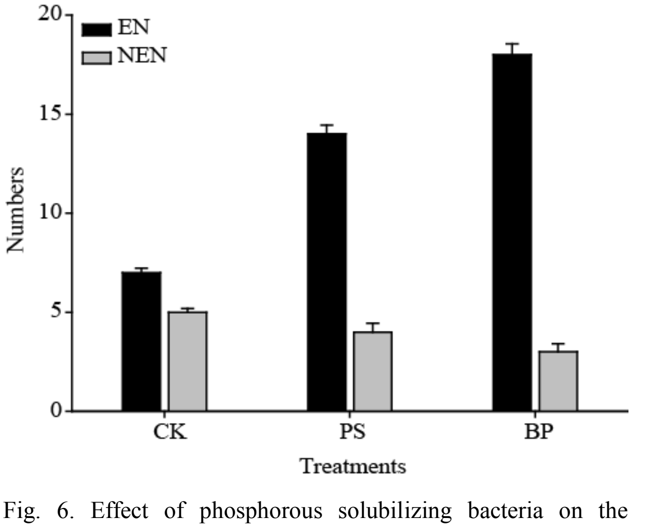

Fig. 6. Effect of phosphorous solubilizing bacteria on the effective and non-effective nodules of mung bean.
CK, control; EN, effective nodules; NEN, non effective nodules; PS, *Pseudomonas striata*; BP, *Bacillus polymyxa*
The *error bars* represent standard error of the means

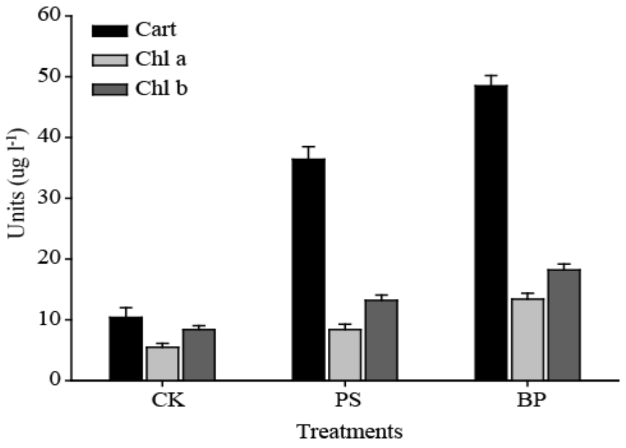

Fig. 7. Effect of phosphorous solubilizing bacteria on the chlorophyll a, b and carotenoids of mung bean.
CK, control; Cart, carotenoids; Chl, chlorophyll; PS, *Pseudomonas striata*; BP, *Bacillus polymyxa*
The *error bars* represent standard error of the means

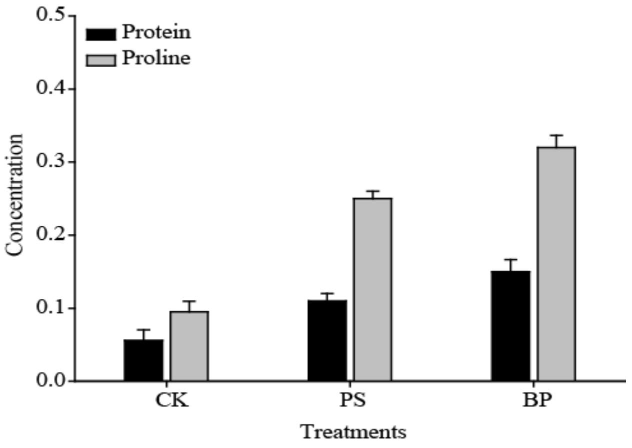

Fig. 8. Effect of phosphorous solubilizing bacteria on protein and proline contents of mung bean.
CK, control; PS, *Pseudomonas striata*; BP, *Bacillus polymyxa*
Unit: protein = mg g^{-1}; Proline = µmol g^{-1}
The *error bars* represent standard error of the means

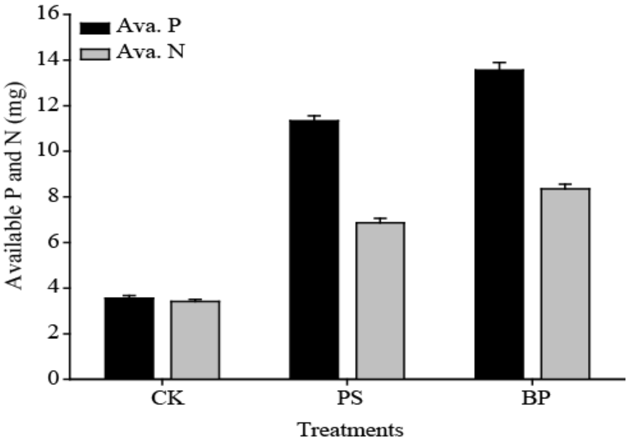

Fig. 9. Effect of phosphorous solubilizing bacteria on the available P and N in soil.
CK, control; PS, *Pseudomonas striata*; BP, *Bacillus polymyxa*; P, phouphorous; N, nitrogen, Unit: mg/Kg
The *error bars* represent standard error of the means

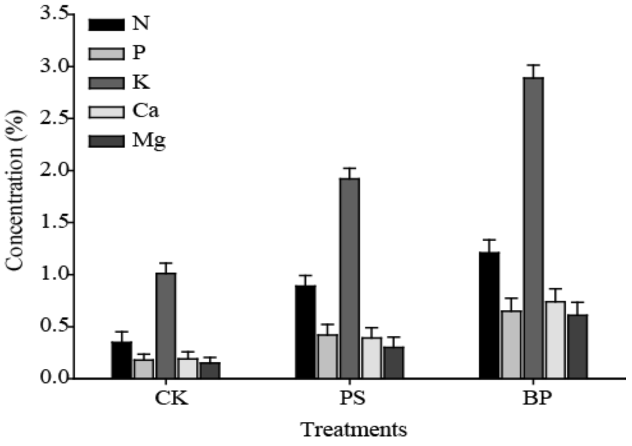

Fig. 10. Effect of phosphorous solubilizing bacteria on N, P, K, Ca and Mg uptake in mung bean root.
CK, control; PS, *Pseudomonas striata*; BP, *Bacillus polymyxa*; P, phouphorous; N, nitrogen; K, potassium; Ca, calcium; Mg, magnesium, Unit: mg/Kg
The *error bars* represent standard error of the means

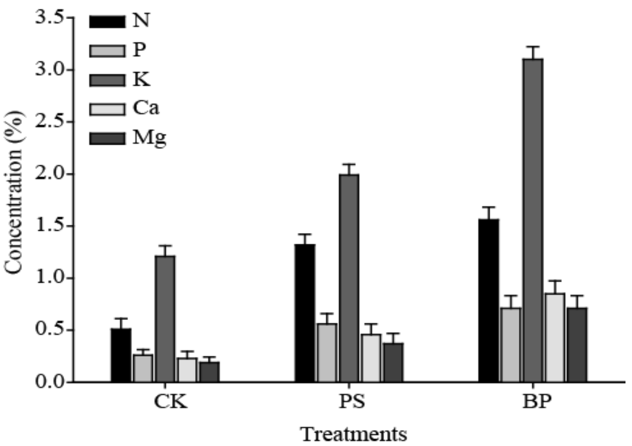

Fig. 11. Effect of phosphorous solubilizing bacteria on N, P, K, Ca and Mg uptake in mung bean shoot.
CK, control; PS, *Pseudomonas striata*; BP, *Bacillus polymyxa*; P, phouphorous; N, nitrogen; K, potassium; Ca, calcium; Mg, magnesium, Unit: mg/Kg
The *error bars* represent standard error of the means

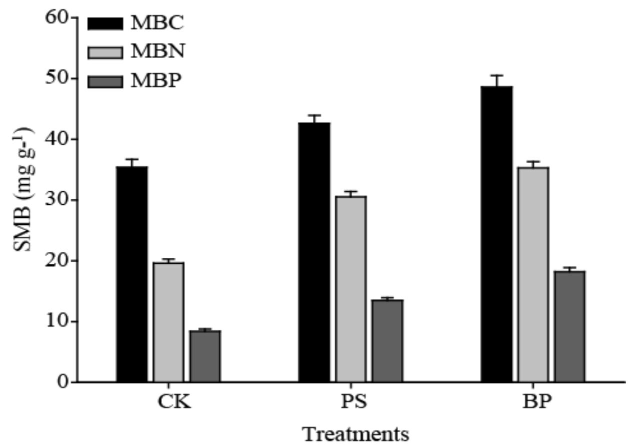

Fig. 12. Effect of phosphorous solubilizing bacteria on soil microbial biomass.
CK, control; PS, *Pseudomonas striata*; BP, *Bacillus polymyxa*; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorous, Unit: mg/Kg
The *error bars* represent standard error of the means

Discussion

Inoculation with PSB spp. *Bacillus polymyxa* and *Pseudomonas striata* significantly ($p<0.05$) increased the plant height, number of branchs/plant, number of leaves/plant, number of pods/plant and pod length than un-inoculated control (Figs. 1-3). The total increase in the mung bean plant height, number of branchs/plant, number of leaves/plant, number of pods/plant and pod length was 2.37-fold and 2.51-fold, 1.98-fold and 2.16-fold and 2.45-fold and 2.56-fold, respectively compared to the control. It has been investigated that PSB inoculation increased the height, growth and productivity i.e., number of branchs/plant, number of leaves/plant, and number of pods/plant and pod length of the plants e.g., walnut, maize and soybean (Xuan *et al*., 2011; Khan *et al*., 2010; Hameeda *et al*., 2008; Fernandez *et al*., 2007). Number of crops showed better performance e.g. wheat, maize and rice, which were treated with PSB (Young *et al*., 2003).

Inoculation with PSBs enhanced the growth and quality parameters i.e. number of seeds/plant (2.56-fold), 1000-seed weight (2.13-fold), number of effective nodules (2.56-fold), nodule density (1.75-fold), chlorophyll a (2.02-fold) and chlorophyll b (1.93-fold), carotenoids (3.81-fold), protein (3.01-fold) and proline (3.56-fold) contents respectively as compared to un-inoculated control (Figs. 5-8). Moreover, inoculation of PSBs decreased the number of non-effective nodules, compared to control (Fig. 6). It has been found that treatment of phosphate solubilizing *Pseudomonas* and *Bacillus* species increased the yield and quality parameters e.g. seeds/plant and 1000-seed weight and chlorophyll, carotenoids, protein and proline etc in different crops e.g. wheat, walnut, maize and soybean (Xuan *et al*., 2011; Khan *et al*., 2010; Afzal & Bano, 2008; Afzal *et al*., 2005).

Moreover, PSBs also improved the availability of P (1.41-fold) and N (1.32-fold) in the soil and uptake of macronutrients i.e., N (3.45-fold and 3.05-fold), P (3.61-fold and 2.96-fold), K (2.86-fold and 2.57-fold), Ca (3.39-fold and 2.98-fold) and Mg (3.81-fold and 4.01-fold) in root and shoot of plant respectively compared to control (Figs. 9-11). The PSBs have the ability to change and make the nutrients available to the plants after transferring them from insoluble to soluble compounds (Pradhan & Sukla, 2005). A diverse group of PSBs have the potential to solubilize insoluble nutrients for easy absorption and uptake to the plants (Tripura *et al*., 2005). The PSBs have a tremendous potential in improving the P and N status of soil and making it available for the plants by means of nitrogen fixation, and enhancing the overall nutrients uptake (Sharma *et al*., 2013).

Inoculation with *Bacillus polymyxa* and *Pseudomonas striata* significantly ($p<0.05$) increased the soil microbial biomass i.e., MBC (1.37-fold), MBN (1.81-fold) and MBP (2.16-fold) compared to the un-inoculated control (Fig. 12). This increase in the SMB can be related to the availability of substrate for the microbes. Upon inoculation, PSBs enhance the availability of nutrients by solubilizing the insoluble nutrients, which serve as a substrate for the microbes. It has been observed that rhizobacteria favor the circulation of plant nutrients in the rhizosphere, as a result high microbial biomass and microbial community (Paul & Lade, 2014). Rhizobacteria are involved in a variety of biotic activities of the soil ecosystem to make it dynamic through mobilising nutrients in soils and making it sustainable for soil micro-flora and fauna (Diby *et al*., 2005). Soil microorganisms act as the primary driving agents of nutrient cycling, regulating the dynamics of soil organic matter and modifying soil physical structure and water regimes for the sustainability of soil ecosystems and making it a suitable niche for soil biota multiplication (Singh *et al*., 2011).

Conclusion

Keeping in mind the harmful effects of artificial fertilizers and their increasing prices, it is need of the day to find out and utilize environmental friendly and economical agro-technologies to improve crop production. In this regard, the use of PSB emerged as a potential strategy. The PSBs had a convincingly positive impact on the growth, development, productivity, nutrients uptake and quality of the mung bean. Moreover, PSBs also enhanced P and N availability in the soil, without polluting the environment. We recommend PSB as an alternative biotechnological solution for sustainable agriculture.

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