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# INFLUENCE OF BIOCHEMICAL TREATMENTS ON CONSORTIUM OF RHIZOBACTERIA AND SOIL FERTILITY

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**Abstract**

Soil was treated with different biochemicals *i.e.* NPK, compost, biochar, humic acid and their combination. New approach was designed to assess the impact of applied biochemicals on the activities of whole bacterial community in spite of pure isolates. The results showed that these biochemically mediated plant beneficial bacteria taken together efficiently solubilized the tri-calcium phosphate, when supplemented in Pikovskaya's broth (PKV). Phosphorus solubilization ranged from 0.231 to 0.605 µg/ml. The isolated consortium of rhizobacteria was positive for IAA production both with LB only and LB + tryptophan medium. The amount of IAA was increased by 0.25 - 1.5 folds with the addition of tryptophan to LB medium. These biochemical treatments were further tested for their effects on soil fertility improvement. Soil available phosphorus was found to improve significantly available phosphorus which ranged from 6.33 to 8.733 mg/kg. Soil pH, moisture content and organic matter were found to increase remarkably by the application of these treatments.

## Introduction

During late 19th and beginning of 20th centuries, major essential compounds containing macronutrients nitrogen, potassium and phosphorus (NPK) were industrially synthesized and commercially used as fertilizers. The harmful impacts and disadvantages of pesticides and fertilizers became dreadful for agricultural processes, as land turns infertile, plants resistance to pest lost and they become more susceptible to diseases, also are causing many health hazards to the human beings and environment. Chemical fertilizers increase the soil pH and degrade ecosystem by causing soil erosion (Goulding 2016). By the start of 21st century, scientists accentuated much on the potential of plant beneficial bacteria (Nakkeeran *et al.* 2005). Different bacterial species i.e. *Azotobacter*, *Pseudomonas*, *Azospirillum*, *Klebsiella*, *Enterobacter*, *Arthrobacter*, *Alcaligenes*, *Bacillus* and *Burkholderia* have been reported to improve the plant growth (Ma *et al.* 2011).

Plant growth promoting rhizobacteria (PGPR) improve growth of plant by indirect or direct processes. The direct mechanisms of growth promotion include solubilization of phosphorus and other minerals, nitrogen fixation, plant hormone production e.g. abscisic acid (ABA), IAA and the indirect mechanisms take place when rhizobacteria reduce or stop the negative impacts of pathogens on plants (Hayat *et al.* 2010). The growth and population dynamics of plant beneficial bacteria depends on many factors specifically on characteristics of soils and upon the changing behavior on different species of plants (Tilak *et al.* 2005). Agriculture development and production can be sustained by accentuating the appropriate and judicious use of PGPR as bio-fertilizer. Currently world population is about 7.2 billion, and continuously increasing so that by

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the end of 2020 it is estimated to reach up to 8 billion which strikes an alarm keeping in consideration the global food security. There is a need to keep continuing the increase in agriculture yield. PGPRs have been found to increase the agriculture productivity as a supplement by reducing the overall cost of fertilizers (Vessey 2003). The first commercialized PGPR was rhizobium that was used in 1895. Since then, many different genera of PGPR have been introduced to seeds or soils and many of them are sold commercially (Herrmann and Lesueur 2013).

Unlike nitrogen, phosphorus (P) is a major growth-limiting nutrient; there is no large atmospheric source present that can be made biologically available. Root growth, stability of stalk and stem, flower development and formation of seed, crop production and quality of crop, N-fixation and disease resistance are the characteristics related with phosphorus nutrition. A little information is available on P solubilization as compared to nitrogen fixation. Rhizobacteria play a vital role in soil P dynamics and ultimately make P available to plants. Heterotrophic microorganisms release organic acids which dissolve phosphatic minerals directly and solubilize inorganic forms of P into solution. Strong evidences are available in the literature which corroborate with the fact that soil bacteria change soil P to the form that is easily accessible to plant (Khan and Joergensen 2009). Phosphorus solubilizing bacteria (PSB) function as a sink for phosphorus and immediately immobilize P in the presence of labile carbon even in low phosphorus soils. Consequently, PSBs become a major source of P for plants upon its release from cells. Plant beneficial bacteria could reduce 50% use of chemical P fertilizer without compromising the yield and productivity of the crop (Yazdani *et al.* 2009). Various bacterial species of genera *Pseudomonas, Bacillus* and *Rhizobium* have been confirmed to be the most dominant P solubilizing bacteria (Vessey 2003). Most of the early historic formation describes that acidification dissolves calcium phosphates. Therefore, those microbes which acidify their external medium reveal some sort of P solubilizing activity (Pradhan and Sukla 2006).

Since late 19th century, it is very well reported that PSB release P from both inorganic and organic phosphorus containing resources through the process of solubilization and mineralization (Khan and Joergensen 2009). Phosphorus solubilizing bacteria enhance availability of P in soils through phosphorus release from adsorbed and insoluble forms, and also prevent P from adsorption. They convert soil P in a form which plant can easily take up from the rhizospheric region (Khan and Joergensen 2009). Mechanisms of P solubilization are influenced by the nitrogen, phosphorus and carbon sources. There are many causal factors of P solubilizing ability of bacteria which include the availability of N, C and metal ions; at where types of nitrogen source influence the capability of PSB and low levels of $Ca^{2+}$ and ethylenediamine tetraacetic acid (EDTA) increases the ability of PSB. A large number of microbiota is associated with the rhizospheric regions due to larger presence of carbon concentration. Carbon compounds are recycled in the soil by microbes that decompose plant residues. Most abundant chemical polymeric components (cellulose, hemicelluloses and lignin) of the plants are degraded by the microorganisms to provide the carbon sources in the soil (Richardson 2001).

**Materials and Methods**

Soil samples were collected from pots comprising mungbean plant (*Vigna radiata*) after harvest, conducted at Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi and stored in cold cabinet at 4°C to maintain the moisture content. The pot experiment contains following eight treatments i.e., T1 = Control, T2 = NPK, T3 = Compost, T4 = Biochar, T5 = Humic acid, T6 = Biochar + humic acid + compost, T7 = Biochar + compost + biochar + humic acid, T8 = Biochar + humic acid. All the treatments had three replicates.

Soil pH was determined in 1 : 1 ratio of soil and distilled water through the combined electrodes as described by Thomas ( 1996). Soil samples were air dried and 25 g was weighed for each sample and transferred in a 100 ml beaker. Distilled water (25 ml) was added and with the help of a rod, thoroughly stirred for 2 - 3 min. and placed on a shaker at 120 rpm for 2 hrs. Suspension in the beaker was allowed to settle down for 30 min. pH meter was calibrated with two buffer solutions of known pH one alkaline and other acidic. The pH of each sample was monitored by inserting the pH electrode in the supernatant solution. Soil water content was determined by taking 100 g air-dried soil samples (< 2 mm) in metal cans with lid and oven dried at 105°C for 24 hrs. The samples were removed from the oven, placed in desiccator and weighed after 30 min (Robinson *et al.* 1999).

Soil moisture contents = (Mass of wet soil – mass of dry soil) / mass of dry soil.

Bacterial strains were isolated by taking 10 g rhizospheric soil in 250 ml flask with 90 ml sterile distilled water in it. It was rotated at 120 rpm on rotary shaker for 10 min. One ml of each sample was serially diluted up to $10^{-7}$. The 0.1 ml of each diluted sample was spread on the surface of plate containing sterile tryptone soya agar (TSA) medium and placed at 28°C for 3 days. Morphologically appearing similar colonies were observed under the microscope and were tested for their synergism and then their consortium was made to do assessment of their activities. Quantification of phosphate solubilization of bacterial consortium was carried out in liquid medium (Nautiyal 1999). The 5 ml tryptone soya broth (TSB) was prepared in each test tube for each sample to prepare bacterial culture. Test tubes were shaked well for 24 hrs. From each test tube, 200 µl bacterial culture was added in 50 ml autoclaved nautiyal media (pH 7) and placed them in shaker for 7 days. After one week of continuous shaking, the bacterial cultures were centrifuged in 50 ml centrifuged tubes at 3000 rpm for 15 min. The supernatant was collected in 25 ml centrifuge tube. Supernatant was measured for P contents calorimetrically at 700 nm. The standard curve of $KH_2PO_4$ was used to detect the amount of soluble phosphorus. Quantitative determination of IAA activity of rhizobacterial consortium with and without tryptophan was performed by following the protocol described by Gorden and Paleg (1957). TSB (5 ml) was poured in each test tube for each sample to prepare bacterial culture and shaked the test tube for 24 hrs. Two sets of test tubes with LB medium 5 ml in each were prepared. In one set, tryptophan was added while the other set was without tryptophan (Tryptophan = 500 µg/ml). Then bacterial cultures (100 µl) from tryptic soya broth (TSB) to both sets of test tubes with LB were added and shaked for 48 hrs. The extract was taken from each test tube after centrifugation at 3000 rpm for 10 min and then the supernatant was collected in the separate tubes. Sokolowski reagent (4 ml) along with 0.1 ml orthophosphoric acid reagent were added to make 6 ml for each sample. All the samples were kept in dark for half an hour. Pink color development was measured for IAA content calorimetrically at 535 nm spectrophotometer. The concentration of IAA was detected from the standard curve of IAA. Analysis of variance was performed to analyze the data according to completely randomized design (CRD) in pot. Data were represented as mean ± standard error means for each sampling interval. Least significant difference (LSD) at $p \leq 0.05$ was carried out to compare the means (Steel *et al*. 1997).

**Results and Discussion**

Soil exposed to different biochemical treatments was investigated after the harvest of the mungbean crop. Basic soil properties i.e. pH, moisture content, organic matter and available soil phosphorus were determined. Effect of these treatments on the potential of PGPRs in soil was observed by monitoring the phosphorus solubilization and Indole acetic acid tests.

The pH of the soil treated with different inorganic and organic fertilizers was conspicuously different from each other ($p < 0.05$). The means were separated by the value (0.46) of LSD from

each other and the control which supported to compare values of individual treatment either they are different from each other or not. Fig. 1 showed that all the treatments were statistically dissimilar from each other which means the biochemical treatments had remarkable effect on the pH of the soil at where variable value of pH was observed in all differently treated soils. Data presented in Fig. 1 showed that almost all the soil samples with different treatments had acidic pH value except humic acid treated soil which shows slightly alkaline pH. The soil samples exhibited variations in pH which ranged from 4.9 to 7.16. Soil treated with biochar, humic acid and compost shows more acid value (4.9) whereas all other treated soils were found to be less acidic. Soil treated with NPK, biochar, biochar with compost-biochar-humic acid had smaller variation among the repeats of individual treatments whereas error bar of the soil treated with biochar-compost-humic acid, compost and humic acid showed that replicates were slightly variable from each other. The findings of present experiment corroborate with the previous work (Adnan *et al.* 2017) at where P-solubilizers were observed with the ability to reduce the soil pH (7.0 to 4.9). Possible reason of decrease of the soil pH is through the production of fumaric, succinic, gluconic, tartaric, citric, malic, transconitic, oxalicacids, citrate, lactate, fumarate, acetate, succinate, propionate, oxalate, pyruvate, tartarate, maleate, malonate and trans-aconite acids by PSB to solubilize the inorganic P, respectively ( Park *et al.* 2011).

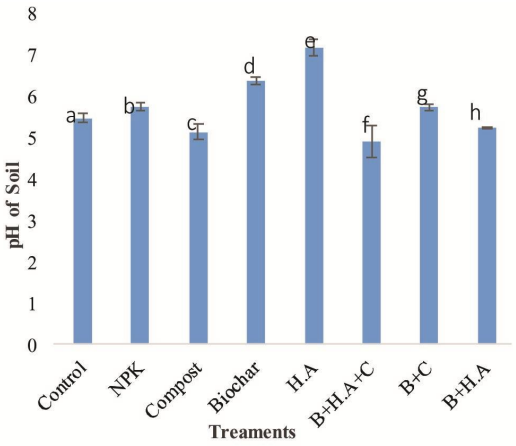


Fig. 1. Effect of different biochemical treatments on pH of soil LSD (0.46).

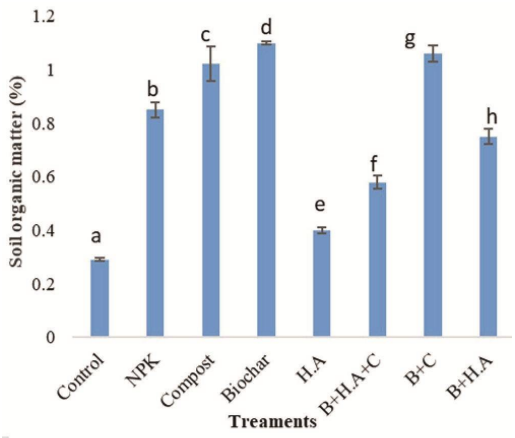


Fig. 2. Effect of different biochemical treatments on organic matter of soil LSD (0.09).

Statistical analyses revealed that different organic and inorganic applications had significant influence on the soil organic matter ($p < 0.05$). The soil sample showed variations in organic matter content of soil treated with different applications which ranged from 0.29 to 1.1%. Highest level of organic matter was observed in the soil treated with biochar alone and with biochar and compost, followed by compost only and NPK treated soils. Lower organic matter was observed in the soil treated with humic acid. Least significant difference value (0.09) separated the means of the treatments and showed that all the treatments means were different from each other statistically. From the Fig. 2, it is evident that there exists little variation among the different repeats of the soil treated with biochar, humic acid, biochar with humic acid which validated the reliability of the present data. Nevertheless, values of the repeats were found to be variable from each other in the soil treated with compost. An increase in level of soil organic carbon in fertilized soil and bio-inoculated as compared to control has been observed in previous studies too, as phosphate solubilizing bacteria release inorganic and organic acids (Stevenson 2005).

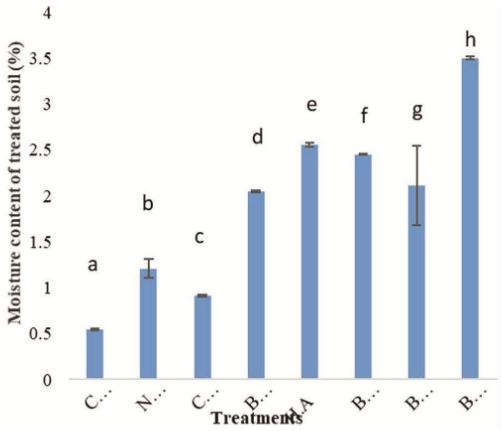


Fig. 3. Effect of different biochemical treatments on moisture content of soil LSD (0.47).

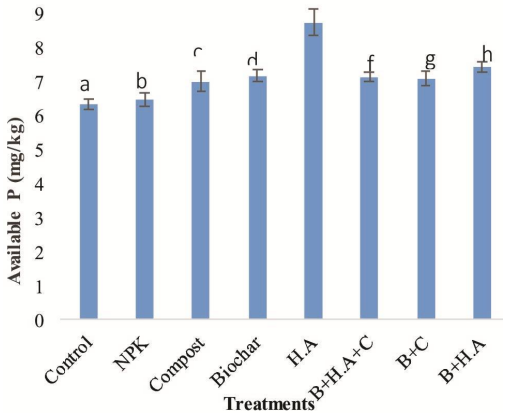


Fig. 4. Effect of different biochemical treatments on available phosphorus in soil LSD (0.68).

ANOVA results showed that different biochemical treatments had remarkable effect on soil moisture contents ($p < 0.05$). The soil sample showed variations in moisture content of soil which ranged from 0.55 to 3.5%. Fig. 3 shows that all the treatment applied to the soil had affected the soils differently as was evidenced from the results of LSD value (0.47) which separated means of all the treatments. Highest value of moisture content (3.5%) was found in the soil treated with combination of biochar with humic acid, followed by - with humic acid only (2.55%). Results presented in Fig. 3 reveals little variation among the values of repeats of treatments which strengthens the reliability of the present data except in the soil treated with biochar with compost where larger variations were noticed among the replicates of the treatment. Possible reason of increase in moisture content might be due to the amendments in the soil. The changes in soil helped soil to contain much water after enhancing the soil porosity which ultimately lead to enhance the soil - water retention capacity and subsequently it increases the nutrient holding capacity of soils (Chan *et al.* 2008).

Statistical analyses revealed the significant differential effect of various inorganic and organic treatments on available phosphorus of the soil ($p < 0.05$). As is obvious in Fig. 4, mean values of all the treatments were separated by LSD (0.68) and it shows that effect of different treatments on available soil P was statistically dissimilar in all the treatments. The samples exhibited variations in available P which ranged from 6.33 to 8.733 ppm where highest value of available P was found in soil which was treated with humic acid and lowest in the soil treated with NPK, followed by the control value of soil P. A smaller variation was observed among the replicates of each individual treatment which strengthen the reproducibility of the present data except in the soil treated with humic acid as is obvious from the error bars based on standard errors presented in Fig. 4.

Effect of all the experimental treatments was observed on soil bacterial community and also on the activities of soil bacterial community. Statistical results represent that various biochemical (organic and inorganic) treatments influenced remarkably ($p < 0.05$) on the capability of soil bacteria to solubilize insoluble phosphorus (Fig. 5). It has been observed that combination of biochar, compost and humic acid in the soil has much influenced the ability of soil bacterial community to solubilize the insoluble P and given highest value of phosphorus solubilization (0.605 µg/ml). This trend is followed up by the consortia of bacterial isolates in the soils which were treated with the combination of biochar and humic acid and with the combination of biochar and compost. It was further followed up by the remaining treated soil bacterial isolates and lowest

value was found in the soil treated with NPK - isolated consortia of bacterial strains (0.371 µg/ml) which significantly higher than control value (0.231 µg/ml). Error bars reveals smaller variation among the repeats of individual treatments which strengthens the reliability of the present data. The values of dissolved phosphate obtained from the bacterial community were convincingly revealing that the insoluble form was conspicuously converted into soluble form by bacterial consortia. Inferences drawn from the data of present study corroborate with the previous research studies (Park *et al.* 2011).

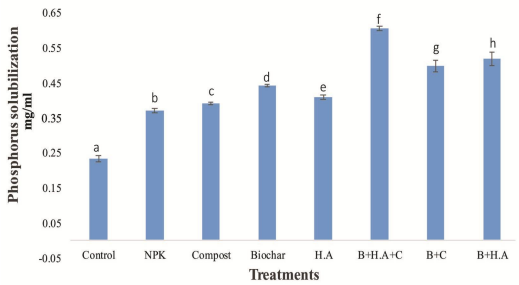


Fig. 5. Phosphorus solubilizing ability of plant beneficial bacteria (LSD 0.03).

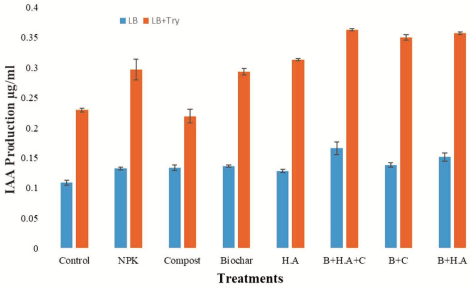


Fig. 6. IAA producing ability of plant beneficial bacteria in LB and LB tryptophan medium.

Ability of isolated bacterial consortia from soils treated with various applications to produce IAA was evaluated and compared with the control. Statistical analyses showed that various biochemical treatments applied in the soil had very significant effect on the ability of bacteria isolated consortia to produce IAA with and without tryptophan ($p < 0.05$). Result of ANOVA for IAA production in LB medium revealed that IAA production by bacterial consortia isolated from all the treated soil were significantly different from each other as is evident in the LSD values (0.016 without trytophan and 0.022 with tryptophan), which separated the mean values of all the treatments. There was one to one and half times increase in the value of IAA after adding tryptophan in it. Fig. 6 shows that maximum production of IAA by isolated bacterial consortia was in the soil treated with the combination of biochar with humic acid and compost (0.363 µg/ml) followed by combination of biochar with humic acid (0.357 µg/ml) and biochar with compost (0.350 µg/ml). Minimum production of IAA by bacteria was observed in soil which was only treated with compost (0.220 µg/ml). Error bars show smaller variations among the repeats of individual treatment which strengthens the reproducibility of the data except with NPK and compost. Previous research work has been found similar with the results of current study where rhizobial isolates were found to be capable to synthesize auxin without the addition of L-tryptophan, and IAA production was also observed to increase many folds after adding L-trytophan (Datta and Basu 2000, Tsavkelova *et al.* 2005, Etesami *et al.* 2009).

Plant beneficial bacteria were isolated from rhizospheric soil of mungbean plant which was treated with different biochemicals. This bacterial community showed significant phosphorus solubilization in tri calcium phosphate, supplemented in PKV liquid broth with the decrease in pH of culture medium. The aggregate community of rhizobacteria was positive for IAA production with and without tryptophan. In the absence of tryptophan, isolates produced IAA but with less concentration as compared to those supplemented with tryptophan medium. Soil physico-chemical properties were also significantly improved by the application of these biochemical treatments as compared to control. Taken together, it is concluded that biochemical applications showed a significant improvement in soil fertility. Soil moisture content, soil organic matter content and level of available phosphorus were also enhanced by the addition of these biochemicals. The

current findings add substantially to the understanding of impact of biochar, compost on soil health and on activities of plant beneficial rhizobacteria.

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**References**

Adnan M, Shah Z, Fahad S, Arif M, Alam M, Khan IA, Mian IA, Basir A, Ullah H, Arshad M, Rahman I, Saud S, Ihsan MZ, Jamal Y, Ullah A, Hammad HM, and Nasim W 2017. Phosphate-solubilizing bacteria nullify the antagonistic effect of soil calcification on bioavailability of phosphorus in alkaline soils. Sci. Rep. **7**: 16131

Chan KY, Van-Zwieten L, Meszaros I, Downie A and Joseph S 2008. Using poultry litter biochars as soil amendments. Aust. J. Soil Res. **46**(5): 437-444.

Datta C and Basu PS 2000. Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub, *Cajanus cajan*. Microbiol. Res. **155**: 123-127.

Etesami H, Alikhani H and Akbari A 2009. Evaluation of plant growth hormones production (IAA) ability by Iranian soils rhizobial strains and effects of superior strains application on wheat growth. World Appl. Sci. J. **6**: 1576-1584.

Gorden SA and Paleg LG 1957. Observations on the quantitative measurement of indole acetic acid. Physiol. Plant **10**: 347-348.

Goulding KWT 2016. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. Soil Use Manage. **32**: 390-399.

Hayat R, Ali S, Amara U, Khalid R and Ahmed I 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. Ann. Microbiol. **60**: 579-598.

Herrmann L and Lesueur D 2013. Challenges of formulation and quality of biofertilizers for successful inoculation. Challenges of formulation and quality of biofertilizers for successful inoculation. Appl. Microbiol. Biotechnol. **97**: 8859-8873.

Khan KS and Joergensen RG 2009. Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. Biores. Technol. **100**: 303-309.

Ma Y, Rajkumar M, Luo YM and Freitas H 2011. Inoculation of endophytic bacteria on host and non-host plants-Effects on plant growth and Ni uptake. J. Hazard Mater. **195**: 230-237.

Nakkeeran S, Fernando WGD and Siddiqui ZA 2005. Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. PGPR: Biocontrol and Biofert. 257-296.

Nautiyal CS 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. Fems Microbiol Lett. **170**: 265-270.

Park JH, Bolan N, Megharaj M and Naidu R 2011. Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. J. Hazard Mater. 185: 829-836.

Pradhan N and Sukla LB 2006. Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afri. J. Biotechnol. **5**: 850-854.

Richardson, AE 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Funct. Plant Biol. **28**: 897-906.

Robinson DA, Gardner CMK and Cooper JD 1999. Measurement of relative permittivity in sandy soils using TDR, capacitance and theta probes: comparison, including the effects of bulk soil electrical conductivity. J. Hydrol. **223**: 198-211.

Rodriguez H and Fraga R 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. **17**: 319-339.

Steel RGD, Torrie JH and Dickey DA 1997. Principles and Procedures of Statistics: A Biometrical Approach, 3rd edition, pp. 400-428. McGraw Hill Book Co. Inc. New York.

Stevenson FJ and Cole MA 2005. Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur,Micronutrients. 2nd edition, pp. 448, Wiley.

Thomas GW 1996. Soil pH and soil acidity. *In:* Sparks, D.L. (Ed.), Methods of Soil Analysis, Part 3, Chemical Methods, SSSA and ASA, Madison 475-490.

Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal CS, Mittal S, Tripathi AK and Johri BN 2005. Diversity of plant growth and soil health supporting bacteria. Microbial Diversity **89**: 136-150.

Tsavkelova EA, Cherdyntseva TA and Netrusov AI 2005. Auxin production by bacteria associated with orchid roots. Microbiol. **74**: 46-53.

Vassilev N, Vassileva M and Nikolaeva I 2006. Simultaneous P-solubilizing and biocontrol activity of microorganisms: Potentials and future trends. Appl. Microbiol. Biotechnol. **71**: 137-144.

Vessey JK 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 255: 571-586.

Whitelaw MA 2000. Growth promotion of plants inoculated with phosphate-solubilizing fungi. Adv. Agro. **69**: 99-151.

Yazdani M, Bahmanyar MA, Pirdashti H and Esmaili MA 2009. Effect of phosphate solubilization microorganisms (psm) and plant growth promoting rhizobacteria (PGPR) on yield components of corn (*Zea mays* L.). Proc. World Acad. Sci. Eng. Technol. **25**: 90-92.

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