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Effects of Phosphate Solubilizing Fungi on Growth and Yield of Haricot Bean (*Phaseolus vulgaris* L.) Plants

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Abstract

Haricot bean (*Phaseolus vulgaris* L.) is one of the most important cash crops and export commodities besides its use in human food and soil fertility improvement. Phosphorus (P) is one of the major bio-elements that limits agricultural production. However, phosphate-solubilizing fungi play a noteworthy role in increasing the bioavailability of soil phosphates for plants. The purpose of this study was to evaluate the effects phosphate solubilizing fungi on the growth of haricot bean plants. Cultural and morphological features were used to tentatively identify the fungal isolates to genus level. Based *In vitro* phosphate solubilization efficieny conducted in both solid and liquid PVK medium following standard procedures, two best isolates were selected and evaluated under greenhouse for their performance on haricot bean. Under greenhouse experiment, shoot height (47.31 cm plant^{-1}), root length (41.01 cm plant^{-1}), nodule number (65.67 plant^{-1}), nodule dry weight (0.59 g plant^{-1}), shoot fresh weight (62.73 g plant^{-1}), shoot dry weight (14.33 g plant^{-1}), number of pod (12.89 plant^{-1}), 50-seed weight (35.89 g plant^{-1}), P content (0.59%) and N content (1.96%) were significantly increased by co-inoculation of two isolates (PSFAP) in the soil amended with rock phosphate (RP) compared to control. Moreover, the highest number of leaves (59.55 leaves plant^{-1}) and root fresh weight (14.19 g plant^{-1}) were recorded as a result of inoculation with isolate PSFP compared to control. The present study indicated the presence of potential plant associated fungi that possess phytobeneficial traits for extending their use as microbial biofertilizers after testing their suitability for the desired purpose.

Keywords: biofertilizers, phytobeneficial traits, rock phosphate, tricalcium phosphate

1. Introduction

Haricot bean (*Phaseolus vulgaris* L.) is the most important pulse crop predominantly grown in the central rift valley for cash crop and export commodity to generate foreign exchange in Ethiopia (Ferris & Kaganzi, 2008). The crop also plays an important role in improving the soil fertility by fixing atmospheric nitrogen with the association of *Rhizobium* species present in the root nodules (Thalooth et al., 2006).

Phosphorus and Nitrogen are the most critical bio-elements that limit Haricot bean production in Ethiopia (Taye, 2007; Girma, 2009; Gifole et al., 2011). Phosphorus (P) is one of the most indispensable macronutrients next to nitrogen for the growth and development of plants (Hameeda et al., 2008).

Although chemical fertilizers are being added to the soil to increase the availability of phosphorus for plants, a large portion of which is rapidly immobilized and becomes unavailable to plants and can lead to an overall reduction in soil fertility after application (Das et al., 2003). This leads to frequent application of chemical phosphatic fertilizers. However, a regular use of chemical fertilizers can cause severe environmental degradation in addition to their escalating costs. For instance, the repeated and injudicious applications of P fertilizers can lead to the loss of soil fertility by disturbing microbial diversity, and consequently reduces yield of crops (Gyaneshwar et al., 2002). Therefore, the necessity to develop economical and eco-friendly fertilizers is steadily increasing (Reddy et al., 2002; Chuang et al., 2007).

An increase in phosphorus availability to plants through the inoculation of phosphate-solubilizing microorganisms in rock phosphate (RP) amended soil has also been reported under greenhouse and field conditions (Whitelaw, 2000; Hameeda et al., 2008). Several authors reported enhanced growth and yield on

wheat (Xiao et al., 2009), soybean (Iman, 2008), maize (Richa et al., 2007; Bojinova et al., 2008) and pea (Kevin & Krista, 2001) through inoculation of P-solubilizing fungi (PSF) in rock phosphate (RP) amended soils. Emphasis is therefore, being placed on the possibility of utilization of rock phosphate, which may be made available to plants by microbiologically mediated processes in order to provide efficient and environmentally desirable approach compared to current technology for industrial P fertilizer production (Bojinova et al., 2008).

Among the rhizosphere microbes, the important genera of P-solubilizing bacteria include *Rhizobium*, *Bacillus* and *Pseudomonas* (Wani et al., 2007; Muleta, 2012). *Penicillium* and *Aspergillus* spp. are the dominant P solubilizing filamentous fungi found in rhizosphere (Wakelin et al., 2004). They are widely used as producers of organic acid. *Aspergillus niger* and some *Penicillium* species have been tested for solubilization of RP and other biotechnological importance such as biocontrol, biodegradation and phosphate mobilization (Chuang et al., 2007; Richa et al., 2007; Pandey et al., 2008).

In Ethiopia, few studies on effects of phosphate solubilizing microorganisms on different crops have been undertaken. Accordingly, effects of plant growth promoting rhizobacteria (PGPR) on growth and yield of tef was evaluated by Woyessa and Assefa (2011) and the effects of phosphate solubilizing rhizobacteria with different doses of poultry manure and RP on the growth and yield of tomato was also undertaken by Gebremeskel and Muleta (2011). Furthermore, the effect of phosphate solubilizing fungus on growth and yield of tef was studied by Hailemariam (1993). However, information on the effects of phosphate solubilizing fungi in RP amended soil on growth of crop plants is scanty in the country.

Hence, this study was to isolate phosphate solubilizing fungi and evaluate their effects on the growth, yield and nutrient content (N and P) of haricot bean plant grown in soil amended with RP under greenhouse condition.

2. Materials and Methods

2.1 Description of the Study Area

The study was carried out in Jimma University (JU), Jimma town, located at 353 km to the south-west of the capital, Addis Ababa. The microbial analysis was conducted in Postgraduate and Research Laboratory, Department of Biology. The greenhouse experiment and soil analysis were conducted at College of Agriculture and Veterinary Medicine, Soil Laboratory, JU. The total area of Jimma zone is 18415 km^2 and located between latitudes $7^o18'$N and $8^o56'$N and longitudes $35^o52'$E and $37^o37'$E.

2.2 Collection of Rhizosphere Soil Sample

A total of one hundred fifty rhizosphere soil samples were collected from 30 plant samples of cabbage (*Birassica interifolia*), faba bean (*Vicia faba* L.), haricot bean (*Phaseolus vulgaris* L.), sugar cane (*Saccharum officinarum* L.) and tomato (*Lycopersicon esculentum* Mill). The rhizosphere soil samples were collected from selected kebeles of Jimma town (Becho Bore, Ginjo Guduru and Awetu Mendera) and Mana district (Sombo Mana, Hunda Toli, Kemise Waraba, Buture and Gudeta Bula) of different farm land sites. The kebeles were purposively selected based on the preliminary survey made to identify potential growing areas for the crop. The samples were randomly collected from agricultural fields within 1 to 2 km interval between the sampling sites (Woyessa & Assefa, 2011). Roots with adhering soils of healthy plants were collected and transferred to sterile plastic bags and transported to the laboratory and stored at 4 °C for further analysis within 24 hrs.

2.3 Isolation of Phosphate Solubilizing Fungi

Collected rhizosphere soil samples were used for the isolation of phosphate solubilizing fungi on Pikovskaya's agar medium (PVK) as described by Pikovskaya (1948). The medium was autoclaved at 121 °C for 15 minutes. About 20 ml of the sterilized molten agar medium was poured into each petri plate and supplemented with 25 µg ml^{-1} chloramphenicol and allowed to solidify before inoculation.

For each sample, the loosely adherent soils were removed by agitating the roots strongly; the root samples with their adhering soil were cut in to pieces (1-2 cm) by sterile scissors and used for isolation. Ten grams of each plant root fragment with adhered soil was aseptically weighed and transferred to 250 ml Erlenmeyer flask containing 90 ml of 0.85% saline solution. The suspension was shaken on 110 rpm for 25 minutes on a rotary shaker and then allowed to settle for 10 min. Aliquots of 1 mL of the supernatant from the sample was transferred to 9 ml of sterile physiological saline solution in test tubes and serially diluted from 10^{-1}-10^{-6}. From appropriate serially diluted soil suspension, 0.1 ml aliquots were transferred and spread plated on Pikovskaya's agar plates and incubated at 25 °C-28 °C for 5-7 days. Fungal isolates that showed clear zones around the colonies were further purified by transferring into Pikovskaya's agar medium. The pure cultures were then preserved on Potato Dextrose Agar (PDA) slant at 4 °C for further investigation.

2.4 Screening of Fungi for Phosphate Solubilization

The Fungal isolates obtained from rhizospheric soils were evaluated on agar plates and liquid culture containing sparingly soluble phosphates for their activity in mobilizing phosphate from insoluble sources (tricalcium and rock Phosphate media).

2.5 Determination of Solubilization Index on Solid Medium

All the isolates were screened under *In vitro* condition for their phosphate solubilization activity following the method described by Iman (2008) on Pikovskaya agar medium. A spot inoculation of fungal isolates was made on to the plates in triplicate under aseptic condition and incubated at 25-28 °C for 7 days. Uninoculated PVK agar plate served as control. Comparative solubilization index measurement was carried out on day seven of incubation by measuring clear zone and colony diameters in centimeter. Phosphate solubilization index was determined by using: ratio of the total diameter (colony + halo zone) and the colony diameter (Edi-Premono et al., 1996).

$$\text{Solubilization Index (SI)} = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}} \quad (1)$$

2.6 Identification and Characterization of Phosphate Solubilizing Fungi

The characteristics of fresh cultures with best *In vitro* P solubilization efficiency were further characterized with mycological identification keys and taxonomic description (Cheesbrough, 2000) to identify the isolated fungi to the genus level. Identification was based on colony characteristics and microscopic features. Among the colonial characteristics such as surface appearance, texture and colour of the colonies both from upper and lower sides were considered. In addition, conidia, conidiophores, arrangement of spores and vegetative structures were determined with microscopy. The identified fungi were maintained on Potato Dextrose Agar (PDA) slant at (4 °C) for further investigation.

2.7 Selection of Potential Bioinoculants

Based on qualitative solubilization index (SI) on solid medium and quantitative solubilization efficiency using PVK broth containing TCP & RP, two isolates designated as PSFA and PSFP were selected to evaluate their performance as individual inoculum and as co-inoculants (PSFAP) on haricot bean under greenhouse condition.

2.8 Greenhouse Experiment

A greenhouse experiment was conducted to evaluate the effectiveness of PSF in improving the availability of P levels and the growth of haricot bean plants in rock phosphate amended soil conducted at College of Agriculture and Veterinary Medicine (JUCAVM).

2.8.1 Physicochemical Analysis of Soil and RP

The physico-chemical properties of soil and Rock Phosphate samples used for pot experiment were initially characterized. The soil was obtained from uncultivated land of Jimma University, College of Agricultural and Veterinary Medicine (JUCAVM). Composite samples were taken using sterilized polyethylene bag across the field from a depths of 0-30 cm and bulked for laboratory analysis and immediately transported to Jimma University College of Agriculture and Veterinary Medicine, Soil Testing Laboratory. The soil samples were then air-dried, crushed using a mortar and pestle, and sieved through a 2 mm mesh. Bikilal Rock Phosphate was crashed, grounded with pestle and mortar and then sieved to 2 mm mesh size. The sieved samples were stored in new polythene bags for laboratory chemical and physical analyses.

All laboratory analyses of soil and RP samples were done following the procedures as outlined by Sahlemedhin and Taye (2000) manual. The soil samples were air-dried and ground to pass a 2 mm sieve and 0.5 mm sieve (for total N) before analysis. Soil texture was determined by Bouyoucos hydrometer method (Black et al., 1965). The pH and electrical conductivity of the soils were measured in water (1:2.5 soil:water ratio). Organic carbon content of the soil was determined following the wet combustion method of Walkley and Black (Black et al., 1965). Total nitrogen content of the soil was determined by wet-oxidation (wet digestion) procedure of Kjeldahl method (Sahlemedhin & Taye, 2000). The available phosphorus content of the soil was determined by Bray II method. The available potassium was determined by Morgan's extraction solution (Bray & Kurtz, 1945).

2.9 Preparation of Fungal Inoculum and Seed Inoculation

2.9.1 Fungal Inoculum

The inocula of the two selected fungal isolates were prepared according to Zaidi and Khan (2006). Fungal

spores from 10-day old cultures were transferred to 100 ml of sterilized potato dextrose broth medium and incubated on orbital shaker at 120 rpm at 25-28 °C for seven days. The cultures broth were filtered through Whatman No. 42 filter paper into a sterile glass bottle and washed with sterilized distilled water under aseptic condition. The pelleted cells were re-suspended with sterilized distilled water. Then the suspensions were adjusted to approximately 10^6 spore cells ml^{-1} with sterilized distilled water by using a haemocytometer for seed inoculation.

2.9.2 Seed Inoculation

For inoculation, a haricot bean seed, Awash Melka variety was kindly obtained from Institute of Jimma Agricultural Research Center, Ethiopia. The healthy seeds were briefly surface sterilized in 0.1% sodium hypochlorite for 2 minutes then washed repeatedly five times with sterile distilled water (Siddiqui & Akhtar, 2007). A total of 90 seeds out of the 120 haricot bean seeds were inoculated by soaking seeds into each suspension of potato dextrose liquid culture medium of the isolates PSFA, PSFP and mixed suspension of PSFA and PSFP with 10% gum Arabic as adhesive for 2 hrs to deliver inoculants of equal volumes of the suspensions about 10^6 cells ml^{-1} from each fungal isolates per seed. For combined inoculations, the liquid cultures of each isolates were mixed in equal proportion (half from each) per seed according to Zaidi and Khan (2006). The inoculated seeds were coated with 5 g $CaCO_3$ and shaken well till fine coating appeared on seeds in polythene bag, and the spore suspension was drained off and seeds were air dried over night aseptically in laminar air flow. The rest 30 seeds were soaked in distilled water amended with $CaCO_3$ (spore free solution) served as control treatment for comparison (Zaidi & Khan, 2006).

2.9.3 Treatments and the Experimental Design

Experiments were conducted in plastic pots having 17 cm diameter and 25 cm deep that had been sterilized with 20% sodium hypochlorite solution, filled with 4.0 kg of non-sterile soil. Rock phosphate of Bikilal was added as phosphatic P (22.5 mg kg^{-1}) and mixed well with the soil before seeding (Zaidi & Khan, 2006). The inoculated seeds were sown at 2 cm depth soil in each plastic pot (5 seeds pot^{-1}) and thinned down to three plants per pot after 5 days of emergence. The experiments were arranged into 8 treatments: soil only (Uninoculated), soil with isolate PSFA, soil with isolate PSFP, soil with both isolates PSFA and PSFP, soil amended with RP, RP amended soil with isolate PSFA, RP amended soil with isolate PSFP and RP amended soil with both isolate PSFA and isolate PSFP. The pots with different treatments were arranged in a randomized complete block design (RCBD) with three replications of each treatment. The plants were watered using tap water every three days or regularly depending on moisture contents in the pots. The plants' seedlings were allowed to grow for 80 days in the greenhouse under natural conditions (12 hrs photoperiod, temperatures of 16-28 °C, and relative humidity of 65%).

2.9.4 Measurement of Growth Parameters and Yield Components

Growth parameters and yield components of haricot bean plant were measured following the technique of Zaidi and Khan (2006). At 45 days of plant age from each pot, 3 plants per pot (9 plants per treatment) were chosen to measure some morphological characteristics such as length of the shoot, root, and number of leaves. At 80 days of harvest, the whole plants were carefully uprooted from the pots, washed gently under running tap water to remove the adhering soil particles. Plants were pulled carefully without damaging to the shoot and roots. Shoot and root height was measured and the mean was calculated and expressed in centimeter. Shoot and root fresh and dry weights, number of pods/plant, number of nodules and 50-seed weight (g) were recorded. The shoots and roots from each growth unit were placed in paper bags and dried at 70 °C for 48 hours and their dry weight mean was expressed in g $plant^{-1}$. The nodules were collected, counted and their dry weight was determined in the same way as the shoots and roots.

2.9.5 Nitrogen and Phosphorous Analysis

Oven dried leaves were grounded and digested in 15 ml $HClO_4$ and 5 ml HNO_3 then analyzed for the major nutrients using the standard procedures. The total Phosphorus content was determined by triple acid digestion followed by subsequent estimation by Vanadomolybdate phosphoric yellow colour method and total nitrogen content of the shoots was determined by modified "Wet" Kjeldahl method according to Sahlemedhin and Taye (2000).

2.9.6 Nitrogen Analysis

Ground shoot sample (0.3 g) was transferred into digestion tube; 2.5 ml of the digestion mixture was added to each digestion tube, swirled carefully to moisten the ground shoot samples and allowed to stand for 2 hours. The tubes were placed on heating block and heated at 100 °C for 2 hours. After two hours, the tubes were removed

from the block and allowed to cool. Three 1ml of 30% H_2O_2 was added successively into each digestion tube and mixed thoroughly. The digestion tubes were again placed on the preheated block and heated at 300 °C until the digest turned to colorless or light yellow. The tubes were removed from the block, cooled to room temperature and 48.3 ml of distilled water was added to each tube, mixed and then allowed to stand overnight. On the next day, the content of each digestion tube was mixed again by shaking, filtered on a 100 ml volumetric flask and brought to the volume with distilled water. Each 100 ml of the acid digest was transferred into a macro - Kjeldahl tube and 20 mL of boric acid solution was measured from a dispenser flask into 250 mL Erlenmeyer flask corresponding to the number of samples.

Two drops of mixed indicator solution were added to each 20 ml of 2% boric acid solution, mixed thoroughly and placed under the condenser. After adding 75 ml of 40% NaOH solution to each digestion tube containing the digest, it was fitted to the corresponding holder and distillation was started. When the distillation was completed, that is, when about 80 ml of the distillate had been collected to boric acid, the flask was removed and distillation process of another sample was continued. Titration was then performed by using 0.1 N H_2SO_4 until the colour of the distillate turned from green to pink at the end point and the utilized H_2SO_4 for titration was recorded volumetrically. Finally the percent of N_2 content of the samples were calculated after correcting for the blank as described by Sahlemedhin and Taye (2000).

2.9.7 Phosphorous Analysis

The total phosphorus content of the shoots was determined by Vanadomolybdate phosphoric yellow colour method of Jackson as outlined by Sahlemedhin and Taye (2000). Five hundred mg of leaf sample were taken in a 250 mL capacity conical flask and were added with 2.5 mL concentrated HNO_3. The flasks were swirled to moisten the entire sample and placed on a hot plate at 180 °C to 200 °C. Five ml of tri-acid mixture (conc. HNO_3, conc. H_2SO_4 and 60% $HClO_4$ in the ratio of 10:1:4) were added to predigested sample and further digestion was carried out at 180 °C to 200 °C on a digestion mantle until the content in the flask became clear white.

The contents of the flasks were cooled and 10 to 15 mL of 6 N HCl added and stirred well. The acid digest was transferred to 50 ml volumetric flask and the volume was made up to 50 mL with distilled water. From this wet oxidized digested sample, five milliliter of aliquot was mixed in 10 mL of Barton reagents and total volume was made as 50 mL. The Barton reagent was prepared by Ammonium molybdate (25 g) was dissolved in 300 mL of distilled water and Ammonium metavenadate (1.25 g) was dissolved in boiling water (300 mL), and then cooled and 250 mL of concentrated HNO_3 was added and cooled at room temperature. Both solutions were mixed and volume was made up to 1 L with distilled water. The samples were kept for half an hour and phosphorus was determined by spectrophotometer using standard curve using various concentrations of standard 10 ppm KH_2PO_4 solution.

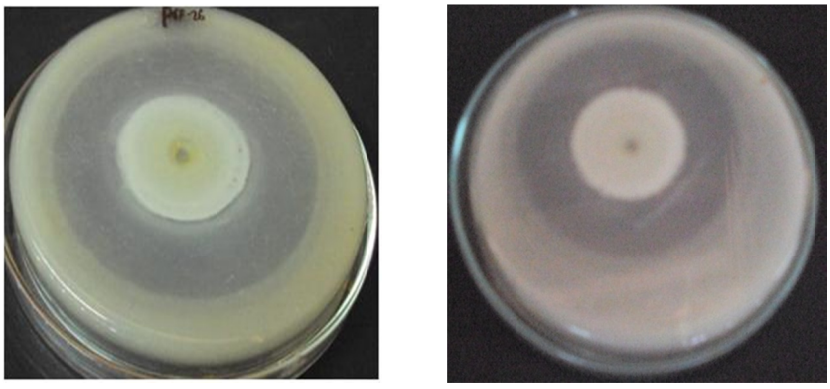
2.10 Data Analysis

All data were subjected to one-way analysis of variance (ANOVA) using the (SAS) Statistical Analysis System software Program (Version 9.1). Treatment means were compared using Tukey's test and differences were accepted as significant when $p < 0.05$.

3. Results

3.1 Isolation, Characterization and Selection of Efficient Isolates for Greenhouse Evaluation

Nine fungal isolates that showed larger Solubilization index (SI) and were preliminarily selected as better phosphate solubilizers to evaluate their efficiency on PKV broth using TCP and RP as inorganic phosphate sources. Of the fungal isolates which showed better SI, two of the isolates coded as PSFA and PSFP were found to show the highest (2.85 and 2.39) SI, respectively.



A. PSFA B. PSFP

Figure 1. Insoluble phosphate solubilization studies on Pikovskaya's agar plate (PVK): (A) and (B) two efficient superior phosphate solubilizing isolates (large haloes)

3.2 Physical and Chemical Analysis of Soil Samples and Rock Phosphate (RP)

From the physico-chemical characteristics of the soil and rock phosphate (RP) used for pot experiments, it was confirmed that both the soil and RP were slightly acidic (Table 1).

Table 1. Chemical and physical characteristics of soil and RP used in greenhouse study

Samples	pH-H_2O	EC (dS m)	OC (%)	TN (%)	AVP (ppm)	AVK (ppm)	Sand (%)	Silt (%)	Clay (%)
Soil	6.34	0.097	3.14	0.27	10.27	151	28	44	28
RP	6.52	-	-	-	189.19	100	-	-	-

Note. EC = electrical conductivity, OC = Organic carbon, TN = Total nitrogen, AVP = available Phosphorus, AVK = available Potassium.

3.3 Effects of PSF on the Shoot and Yield Parameters of Haricot Bean Plants

All the test fungi significantly ($p < 0.05$) promoted the plant height compared to the control except PSFA (Table 2 and Figure 2). Among the treatments, the effect of co-inoculation of fungal isolates PSFA (*Aspergillus* sp.) and PSFP (*Penicillium* sp.) produced the highest shoot length (47.31 cm $plant^{-1}$) followed by *Aspergillus* sp. PSFA inoculation which gave a shoot length of 45.48 cm $plant^{-1}$in the RP amended soil (Figure 2 and Table 2).

All inoculation with fungal isolates either alone or in co-inoculation had significantly ($p < 0.05$) higher shoot fresh weight than the control (Figure 2 and Table 2).

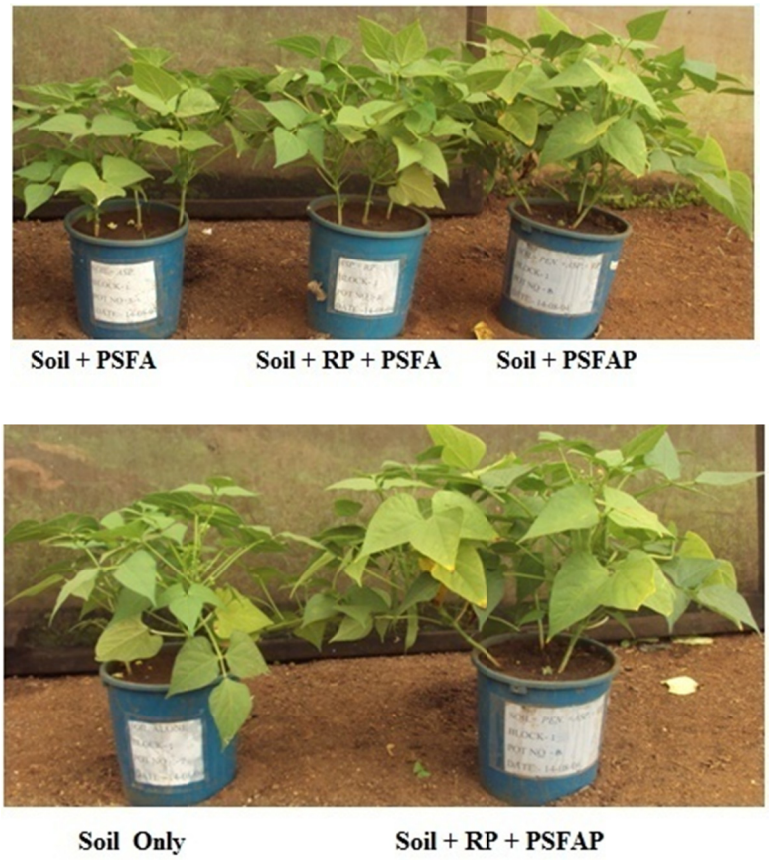


Figure 2. Phosphate solubilizing fungal isolates for improving growth of haricot bean grown in pots amended with rock phosphate after 45 days of sowing in the greenhouse

Note. RP: rock phosphate.

Among the treatments, PSFA (*Aspergillus* sp.) along with RP amended soil and PSFP (*Penicillium* sp.) without RP showed a maximum shoot fresh weight of 58.78 and 56.52 (g/plant), respectively compared to the control. However, the effect was more pronounced (62.73 g/plant) in case of co-inoculation with PSFA (*Aspergillus* sp.) and PSFP (*Penicillium* sp.) in the RP amended soil (Table 2). Furthermore, the treatment showed a significant ($p < 0.05$) difference in shoot dry weight, number of leaves and pods plant^{-1} as well as weight of 50-seeds (Table 2). The plant inoculated with fungal isolates designated as PSFAP which are *Aspergillus* sp. and *Penicillum* sp. in the presence of RP gave the maximum shoot dry weight (14.33 g/plant) followed by inoculation of the same combination (PSFAP) without RP (12.63 g/plant) and single inoculation of *Aspergillus* sp. (PSFA) in the presence of RP (12.58 g/plant) compared to the control (Table 2).

Table 2. Effect of PSF on shoot growth and yield of haricot bean in the rock phosphate amended soil in the greenhouse

Treatments	SH (cm)	SFW (g plant^{-1})	SDW (g plant^{-1})	LN plant^{-1}	PN plant^{-1}	50-seed Weight (g)
Soil only (control)	40.43^{e}	46.34^{d}	7.67^{e}	42.44^{c}	11.36^{c}	34.44^{cd}
Soil + RP	41.53^{d}	46.39^{d}	8.09^{e}	42.44^{c}	11.38b^{c}	34.57^{cd}
Soil + PSFA	40.42^{e}	50.94^{c}	9.54^{d}	43.89^{c}	11.93^{c}	34.79^{bcd}
Soil + PSFP	43.66^{c}	56.52^{b}	11.19^{c}	57.89^{a}	11.36^{c}	34.44^{cd}
Soil + PSFAP	44.96^{b}	51.95^{c}	12.63^{b}	58.00^{a}	11.71^{c}	34.83^{bcd}
Soil +RP + PSFA	45.48^{b}	58.78^{b}	12.58^{b}	52.00^{ab}	12.51^{ab}	35.50^{ab}
Soil + RP + PSFP	43.70^{c}	51.11^{c}	11.67^{c}	49.78^{b}	12.32^{abc}	35.27^{abc}
Soil+ RP+ PSFAP	47.31^{a}	62.73^{a}	14.33^{a}	58.00^{a}	12.89^{a}	35.87^{a}
LSD	0.64	4.13	0.96	3.8	0.6	0.87
CV	0.51	3.13	3.14	2.67	1.76	0.88
P-value	*	*	*	*	*	*

Note. SH = shoot height, SFW = shoot fresh weight, SDW = shoot dry weight, LN = leaves number, PN = pod number and RP = rock phosphate; Mean values followed by the same superscripts within a column are not significantly different at $p < 0.05$. LSD = Least significant difference, CV = Coefficient of variation.

Maximum number (58.00) of leaves plant^{-1} were observed in plants treated with (PSFAP) both in the presence and absence of RP followed by sole inoculation of PSFA without RP (57.89 number of leaves plant^{-1}). The non-inoculated treatments showed the lowest number (42.44) of leaves plant^{-1}.

Increase in number of pods/plant was recorded with the application of single or dual inoculation of the test PSF isolates and accordingly, co-inoculation of (PSFAP), resulted in the largest number (12.89) of pods plant^{-1} when the soil enriched with RP compared to the control. The least number of pods 11.36 plant^{-1} was obtained from uninoculated treatment (Table 2). Similarly, the highest mean weight of 50-seeds 35.87 and 35.50 g plant^{-1} was obtained from co-inoculation of (PSFAP) and single inoculation with PSFA in the rock phosphate amended soil, respectively (Table 2). The least mean value 34.44 g plant^{-1}was obtained from control.

3.4 Effects of PSF Isolates on the Root Growth and Nodulation of Haricot Bean Plants

There were significant variation ($p < 0.05$) among the treatments regarding root length, root fresh and dry weight, nodule number and nodule dry weight as depicted in Table 3 and Figure 3. The highest root length (41.06 cm) plant^{-1} was recorded in case of RP amended soil with dual inoculation of the fungal isolates *Aspergillus* sp. plus *Penicillium* sp. (PSFAP) followed by single inoculation of *Penicillium* sp. (PSFP) and *Aspergillus* sp (PSFA) in the soil amended with RP (36.56 and 36.50 cm plant^{-1}), respectively.

The root fresh weight was significantly greater in treatments with inoculum both with single and co-inoculation than in control except with inoculation of *Penicillium* sp. (PSFP) without RP (Table 3).

Table 3. Effect of phosphate solubilizing fungi inoculation on root growth and nodulation of haricot bean in the rock phosphate amended soil in the greenhouse

Treatments	RL (cm plant^{-1})	RFW (g plant^{-1})	RDW (g plant^{-1})	NN (plant^{-1})	NDW (g^{-1} plant)
Soil only	25.24^{e}	7.60^{d}	2.66^{c}	30.33^{f}	0.20^{d}
Soil + RP	27.39^{d}	9.30^{c}	2.66^{c}	30.00^{f}	0.22^{cd}
Soil + PSFA	27.59^{d}	9.97^{c}	2.67^{c}	38.33^{d}	0.27^{c}
Soil + PSFP	27.59^{d}	7.65^{d}	2.66^{c}	32.33b^{ef}	0.25^{cd}
Soil+ PSFAP	33.06^{c}	11.97^{b}	2.71^{bc}	46.00b^{c}	0.55^{a}
Soil +RP + PSFA	36.50^{b}	14.19^{a}	3.13^{a}	57.67^{b}	0.55^{a}
Soil + RP + PSFP	36.56^{b}	13.78^{a}	2.95^{ab}	37.33^{de}	0.40^{b}
Soil +RP + PSFAP	41.06^{a}	14.09^{a}	2.96^{ab}	65.67^{a}	0.59^{a}
LSD	1.99	0.83	0.26	5.72	0.04
CV	2.17	2.92	3.17	4.74	3.38
P-value	*	*	*	*	*

Note. RL = root length, RFW = root fresh weight, RDW = root dry weight, NN = nodule number, NDW = nodule dry weight and RP = rock phosphate; Mean values are followed by the same superscripts within a column are not significantly different at (Tukey, $p < 0.05$). LSD = Least significant significance difference, CV = Coefficient of variation.

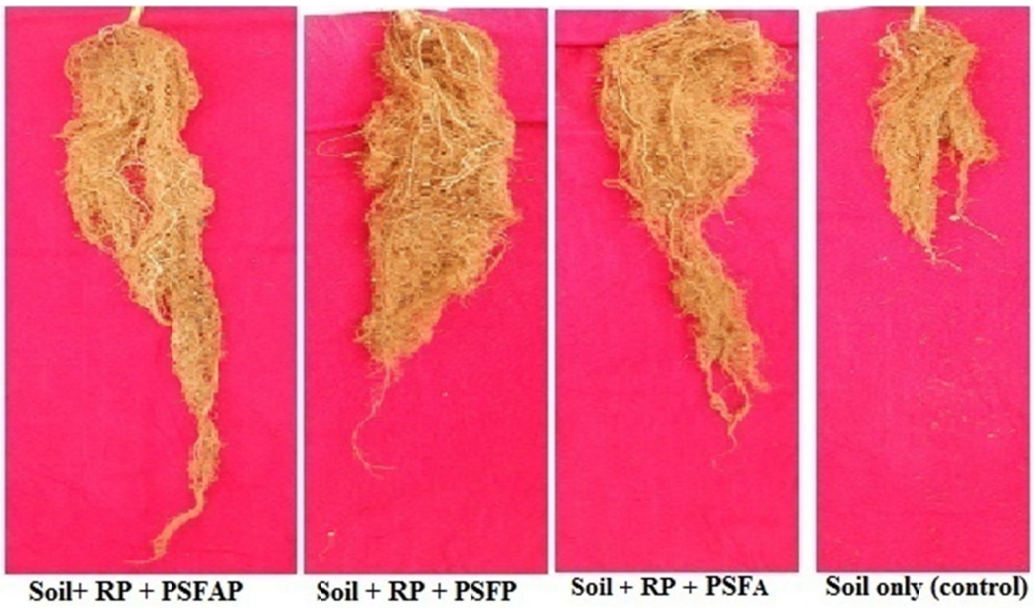
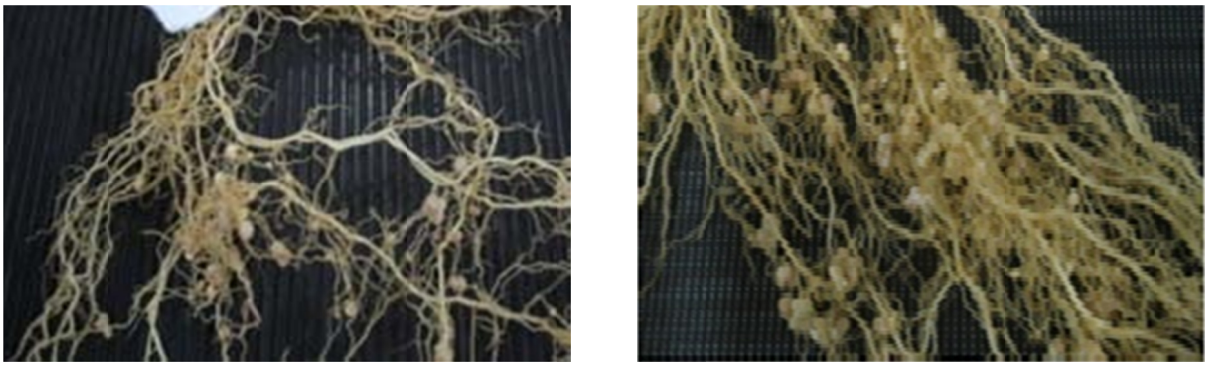


Figure 3. Effect of phosphate solubilizing fungal isolates on root growth of haricot bean grown in pots amended with rock phosphate in the greenhouse

Note. RP: rock phosphate.

The PSF isolates either in single or mixed inoculation promoted root length with a concomitant increase in the root dry weight of haricot bean plant compared to the control (Table 3). The single inoculation of *Aspergillus* sp. along with RP showed the highest increase in root dry weight (3.13 g plant^{-1}) followed by co-inoculation of *Aspergillus* sp. together with *Penicillum* sp. and *Penicillum* sp. along with RP - amended soil, resulting in 2.96 and 2.95 g plant^{-1}, respectively.

On the other hand, the maximum numbers of nodules (65.67) plant^{-1} was counted upon inoculation with *Aspergillus* sp. plus *Penicillium* sp. (PSFAP) followed by single inoculation of PSFA with 57.67 nodules plant^{-1} in the RP amended soil (Table 3 and Figure 4).



A. Soil +RP + PSFAP B. Soil only (control)

Figure 4. Effect of phosphate solubilizing fungal isolates on nodulation status of haricot bean grown in soil amended with rock phosphate: A) Inoculated; B). Control Soil only

Note. RP: rock phosphate.

Similarly, the highest nodule dry weight (0.59 g plant^{-1}) was recorded in response to co-inoculation (PSFAP) in RP amended soil. Sole inoculation of PSFA in soil amended with rock phosphate and co-inoculation of (PSFAP) without RP also resulted in significantly higher (0.55 g plant^{-1}/plant) compared to uninoculated control (Table 3).

3.5 Effects of PSF on Phosphorus and Nitrogen Content of Haricot Bean Plants

The treatments showed significantly variable results regarding the contents phosphorus and nitrogen contents in the leaves of haricot bean (Table 4). Accordingly, maximum content of phosphorus in haricot bean leaves was observed in plants treated with rock phosphate either single or co-inoculation of fungal isolates. The total phosphorus content of plant was significantly ($p < 0.05$) increased when treated with a mixed (PSFAP) fungal isolates in RP amended soil with P content in the leaves 0.58% followed by sole inoculation with PSFA and PSFP with RP enriched soil with P content of 0.54% and 0.52%, respectively. Similarly, the highest (1.92%) percentage of nitrogen content in the haricot bean leaves contained with coinoculation of the fungal isolates (PSFAP) followed by the isolates PSFA and PSFP in the presence of RP enriched soil each with (1.63%) nitrogen content in the leaves (Table 4). On the other hand, the lowest percentage (0.89%) of nitrogen was obtained from the control.

Table 4. Nitrogen and Phosphorus content of haricot bean plant leaves grown in the greenhouse

Treatments	Nitrogen %	Phosphorus %
Soil only	0.89^{d}	0.43^{e}
Soil + RP	0.89^{d}	0.46^{cd}
Soil + PSFA	0.90^{d}	0.44^{de}
Soil + PSFP	0.91^{d}	0.46^{cd}
Soil + PSFAP	1.07^{c}	0.48^{c}
Soil +RP + PSFA	1.63^{b}	0.54^{b}
Soil + RP + PSFP	1.63^{b}	0.52^{b}
Soil +RP+ PSFAP	1.92^{a}	0.58^{a}
LSD	0.07	0.03
CV	2.05	1.88
P-value ($p < 0.05$)	*	*

Note. RP = rock phosphate, Mean values are followed by the same superscripts within a column are not significantly different at (Tukey, $p < 0.05$). LSD = List significant significance difference, CV = Coefficient of variation.

4. Discussion

The present study showed the occurrence of *Aspergillus*, *Penicillium* and *Fusarium* species that were capable of

solubilizing sparingly soluble phosphorus from the rhizosphere of different plants collected from farm lands. Similarly, Chuang et al. (2007) isolated P-solubilizing fungi *Aspergillus niger* and *Penicillium* spp. from various subtropical and tropical rhizospheric soil samples of cabbage and maize. In the current study *Aspergillus* spp. and *penicillum* spp. were commonly isolated from the rhizosphere soils of faba beans (*Vicia faba* L.) and haricot bean plants (*Phaseolus vulgais* L.). This result is supported by the findings of Abdul Wahid and Mehana (2002) who had isolated *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillum pinophilum* from the rhizosphere of faba beans (*Vicia faba* L.), kidney bean (*Phaseolus vulgais* L.), and peas (*Pisum sativum* L.).

Phosphate solubilizing fungi belonging to the genera *Aspergillus* and *Penicillium* were also isolated from the rhizospheric soils of sugar cane. Mahamuni et al. (2012) also isolated several phosphate solubilizing fungal groups including *Aspergillus* spp., *Alternaria* spp., *Curvularia* spp., *Penicillium* spp. and *Trichoderma* spp. from the rhizosphere of sugarcane and sugar beet plants. Other studies on the PSF associated with rhizosphere of sugar cane roots showed the occurrence of phosphate solubilizing *Penicillium citrinum* Thom (Yadav, 2010).

The PSF isolates, PSFA and PSFP significantly increased the shoot height of haricot bean plant compared to uninoculated plant. The highest shoot height was observed in case of soils amended with rock phosphate and with dual inoculation of phosphate solubilizing isolates. The increase in plant shoot height due to PSF inoculation along with RP application could be the result of increased transformation of P into available forms as indicated by the increased P in plants by the PSF isolates PSFAand PSFP. Similar results of increase in shoot height due to PSF inoculation has been reported on groundnut plant (Malviya et al., 2011), wheat (Xiao et al., 2009), chickpea (Yadav et al., 2011) and soy bean (Iman, 2008).

The application of *Aspergillus* sp. (PSFA) and *Penicillum* sp. (PSFP) either individually or in combination led to increase in number of leaves compared to control. The effect was more pronounced with dual inoculation of PSFAP in the presence of RP and other treatments. This could be attributed to the highest values of available P and enhanced P uptake compared to other treatments due to the synergistic effect of fungal inoculants. These results are in harmony with those obtained by Patil et al. (2012) who reported that PSF both singly or in combination gave maximum number of leaves on maize plants due to the activity of P solubilization and released growth-promoting substances. In addition, El-Yazeid and Abou-Aly (2011) found that the integrated treatment of P-solubilizers and application of rock-P significantly led to large number and area of leaves as well as the photosynthetic pigments in tomato plant.

The shoot fresh weight was significantly increased by the inoculation of PSF compared to control and treatment receiving only rock phosphate. The highest shoot fresh and dry weight values obtained as a result of applying combined phosphate solubilizing fungal isolates (PSFAP) in rock phosphate amended soil may be due to the role of the two test fungi in solubilization and mineralization efficiency of phosphate pool in plant rhizosphere, which in return increased the level of available nutritional elements required.

Thus, increased transformation of P into available forms as indicated, increased content of P in plants by inoculation of PSFAP which consequently enhanced cell elongation, and multiplication and overall shoot growth of haricot bean plant. These results are in line with the findings of numerous workers (Chuang et al., 2007; Kapri & Tewari, 2010; Panhwar et al., 2011; Patil et al., 2012), who have reported increase in plant growth and fresh matter of different crop plants due to inoculation of phosphate solubilizing fungi along with phosphate sources. The increase in the dry weight of shoot may also be due to greater solubilization of P by the rhizosphere microorganisms that would lead to better symbiotic N_2-fixation by the legumes and the latter one was found to have maximum contribution in increasing dry matter production. These findings are similar to the earlier reports of Saber et al. (2009) where rock phosphate coupled with phosphate solubilizing *A. niger* and *Penicillium* sp. gave more significant dry biomass compared to uninoculated control.

The increase in root parameters such as highest root length, fresh and dry root weights could be attributed to high P-solubilizing ability of the fungal inoculants through which they might have contributed to the enhanced root growth thereby creating more root surface area for uptake of nutrients from the soil. Sharma et al. (2012) reported that one of the advantages of supplying of the plants with phosphorus along with PSF is to create deeper and more abundant roots. In addition, PSF can also release plant growth-promoting substances (Nenwani et al., 2010) that might be another probable means for enhanced root growth in haricot bean plant. Similar evidence on the increased root length with inoculation of PSF (*Aspergillus niger* and *Penicillium* sp.) along with RP has been reported by several workers (Mittala et al., 2008; Yadav et al., 2011; Malviya et al., 2011).

In the present study, application of PSF showed remarkable difference in nodule count and dry weight. The better nodulation in the case of co-inoculation could be due to the favorable synergistic effects of the isolates in mobilizing more P to make available to the plants, which eventually promoted root development that might also

have provided rhizobia more infection sites than others. The higher availability of P in the rhizosphere of plants inoculated with PSF probably induced good proliferation of root, providing enough number of sites for rhizobia to form more number of nodules. Rudresh et al. (2005) reported that increase in root growth provides more access to nodulating rhizobia. In another study, Saber et al. (2009) demonstrated that increase in the number and weight of nodule in mung bean with inoculation of *A. niger* and *Penicillium* sp. in RP amended soil.

The highest pod number and seed weight were recorded with inoculation of mixed isolates along with RP application. These highest values in the current study could be due to improved nutrients particularly P both its availability and up take since the availability of nutrients in the soil in sufficient quantities greatly determines the growth and yield of plants. For instance, Agasimani et al. (2002) observed significant increase in number of pods plant^{-1} in groundnut due to inoculation of P-solubilizing fungus (*Aspergillus awamori*). Rudresh et al. (2005) also reported that increase in pod number and seed weight of chickpea grown in phosphate-deficient soil amended with insoluble rock phosphate due to *Trichoderma* inoculation under both glasshouse and field conditions. Mittala et al. (2008) have reported the effect of six phosphate-solubilizing fungi (two strains of *Aspergillus awamoria* and four of *Penicillium citrinum*) isolated from rhizosphere of various crops caused increased growth, number of pods and increased seed production of chickpea plants in pot experiments.

Single and co-inoculation of the isolates (PSFA and PSFP) with or without RP amendment significantly improved the level of phosphate content in haricot bean plant compared to the control. Inoculants (mixed) which solubilized higher amount of phosphate enhanced the content of P over control and other treatments. The highest significant increase in the percentage of phosphate was observed in the co-inoculation of the fungal isolates (PSFAP) along with rock phosphate. This may be due to better utilization of soluble phosphorus from the pool of available phosphorus in the soil due to solubilization of native and added phosphorus by the actions of the PSF isolates. This result is in agreement with previous reports of Xiao et al. (2009) on wheat, Mittala et al. (2008) on chickpea, Richa et al. (2007) on maize and Agasimani et al. (2002) on groundnut plants.

Increased nitrogen content in the leaves of haricot bean plants was recorded by the inoculation of PSF isolates compared to the control. The increase in nitrogen content of haricot bean might be due to increased nodulation as well as the positive interaction between P-solubilizing fungal isolates and root nodulating bacteria. Malviya et al. (2011) demonstrated that the P-solubilizing fungi enhanced the N uptake from soil solution. The significant increase in phosphate and nitrogen uptake by haricot bean plants grown in soil inoculated with PSF proved that these isolates have not only the capability to solubilize RP *in vitro* but also that this phenomenon can be observed *in vivo* with a beneficial effect for plant growth.

Based on the above findings, the use of these PSF as bioinoculants may help to minimize the chemical fertilizer application, reduce environmental pollution and consequently may promote sustainable agriculture. This report suggests further screenings of phosphate-solubilizing microorganisms that requires extensive and consistent research activities to identify and characterize more rhizosphere competent phosphate-solubilizing fungi for their ultimate application as potential solubilizers of fixed soil phosphate to use in natural environment.

5. Conclusions

All the selected isolates were capable of mobilizing TCP and RP in PVK broth. The efficiency of phosphate solubilization is significantly higher in Pikovskaya medium containing TCP than in the medium containing RP. Both single as well as mixed inoculation treatments showed better plant shoot and root growth, number of pods 50-seed weight, and nutrients content compared to uninoculated control. Among the inoculation treatments, a combined inoculation (PSFAP) in the presence of rock phosphate was superior over the rest of the isolates and uninoculated control. Accordingly, from the present result, it can be concluded that the amendment of soil with RP along with the application of P-solubilizing fungi could alleviate soil fertility problem as a result of P fixation and contribute to environmental integrity by reducing artificially synthesized P fertilizers to promote sustainable agriculture.

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References

Agasimani, C. A., Mudalagiriyappa A., & Sreenivasa, M. N. (2002). *Studies on phosphate solubilizers in groundnut*. Nation. Sympo. Mineral Phosphate Solubilization, 14-16 Univ. Agril. Sci., Dharwad, India.

Bojinova, D., Velkova, R., & Ivanova, R. (2008). Solubilization of Morocco phosphorite by *Aspergillus niger*. *Biores. Technol., 99*, 7348-7353. http://dx.doi.org/10.1016/j.biortech.2007.08.047

Cheesbrough, M. (2000). *District Laboratory Practice in Tropical Countries Part 2* (pp. 47-54). Cambridge University Press, Cambridge.

Chuang, C. C., Kuo, Y. L., Chao C. C., & Chao, W. L. (2007). Solubilization of inorganic phosphates and plant growth promotion by *Aspergillus niger*. *Biol. Fert. Soils, 43*, 575-584. http://dx.doi.org/10.1007/s00374-006-0140-3

Das, K., Katiyar, V., & Goel, R. (2003). P solubilization potential of plant growth promoting *Pseudomonas* mutants at low temperature. *Microbiol. Res., 158*, 359-362. http://dx.doi.org/10.1078/0944-5013-00217

Edi-Premono, M., Moawad, M., & Vlek, G. (1996). Effect of phosphate solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indo. J. Crop Sci., 11*, 13-23.

El-Yazeid, & Abou, A. (2011). Enhancing growth, productivity and quality of tomato Plants using phosph ate solubilizing microorganisms. *Aust. J. Basic & Appl. Sci., 5*, 371-379.

Ferris, S., & Kaganzi, E. (2008). Evaluating marketing opportunities for haricot beans in Ethiopia. *IPMS (Improving Productivity and Market Success) of Ethiopian Farmers Project Working Paper 7* (p. 68). ILRI (International Livestock Research Institute), Nairobi, Kenya.

Gebremeskel, A., & Muleta, D. (2011). Evaluating the Effects of phosphate solubilizing rhizobacteria with different doses of poultry manure in Jimma town, Ethiopia. In A. Zaidi (Ed.), *Rhizobacteria and Vegetable Growth* (pp. 311-334). LAP Lambert Academic Publishing, New York, America.

Gifole, G., Sheleme, B., & Walelign, W. (2011). The Response of Haricot Bean (*Phaseolus vulgaris* L.) to Phosphorus Application on Ultisols at Areka, Southern Ethiopia. *J. Biol., Agric. and Healthcare, 1*, 38-49.

Girma, A. (2009). Effect of NP Fertilizer and Moisture Conservation on the Yield and Yield Components of Haricot Bean (*Phaseolus vulgaris* L.) in the Semi Arid Zones of the Central Rift Valley in Ethiopia. *Adv. Environ. Biol., 3*, 302-307.

Gyaneshwar, P., Kumar, G., Parekh, L., & Poole, P. (2002). Role of soil microorganisms in improving P nutrition of plants. *Plant Soil, 245*, 83-93. http://dx.doi.org/10.1023/A:1020663916259

Hailemariam, A. (1993). The effect of phosphate solubilizing fungus on the growth and yield of tef (*Eragrostis tef*) in phosphorous fixing soils. *Proceedings of the workshop on the third cycle local research grant, Eth. Sci. & Technol. Res. Report* (pp. 12-14).

Hameeda, B., Harini, G., Rupela, P., Wani, S. P., & Reddy, G. (2008). Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. *J. Microbiol. Res., 163*, 234-242. http://dx.doi.org/10.1016/j.micres.2006.05.009

Hinsinger, P. (2001). Bioavailability of soil inorganic P in the rhizosphere as affected by root induced chemical changes: A review. *Plant Soil, 237*, 173-195. http://dx.doi.org/10.1590/S1517-83822010005000001

Vessey, J. K., & Heisinger, K. G. (2001). Effect of *Penicillium bilaii* inoculation and phosphorus fertilization on root and shoot parameters of field-grown pea. *Can. J. Plant Sci., 80*, 361-366. http://dx.doi.org/10.4141/P00-083

Kim, Y. H., Bae, B., & Choung, Y. K. (2005). Optimization of biological phosphorus removal from contaminated sediments with phosphate solubilizing microorganisms. *J. Biosci. and Bioeng., 99*, 23-29. http://dx.doi.org/10.1263/jbb.99.23

Mahamuni, S. V., Wani, P. V., & Patil, A. S. (2012). Isolation of Phosphate Solubilizing Fungi from Rhizosphere of Sugarcane & Sugar Beet Using TCP & RP Solubilization. *Asian J. Bioche. and Pharmace. Rese., 2*, 237-244.

Malviya, J., Singh, K., & Joshi, V. (2011). Effect of Phosphate Solubilizing Fungi on Growth and Nutrient Uptake of Ground nut (*Arachis hypogaea*) Plants. *Adv. Biores., 2*, 110-113.

Mittala, V., Singha, O., Nayyarb, H., Kaura, J., & Tewari, R. (2008). Stimulatory effect of phosphate solubilizing fungal strains (*Aspergillus awamori* and *Penicillium citrinum*) on the yield of chickpea (*Cicer arietinum* L. cv. GPF2). *Soil Biol., and Bioche., 40*, 718-727. http://dx.doi.org/10.1016/j.soilbio.2007.10.008

Muleta, D. (2012). Legume responses to arbuscular mycorrhizal fungi inoculation in sustainable agriculture. In M. Khan (Ed.), *Microbes for Legume Improvement* (pp. 224-266). Springer Verlag Wien publishing house, New York, America.

Nenwani, V., Doshi, P., Saha, T., & Rajkumar, S. (2010). Isolation and characterization of a fungal isolate for phosphate solubilization and plant growth promoting activity. *J. Yeast and Fungal Rese., 1*, 9-14.

Pandey, A., Das, B., Kumar, K., Rinu, K., & Trivedi, P. (2008). Phosphate solubilization by *Penicillium* spp. isolated from soil samples of Indian Himalayan region. *World J. Microbiol. Biotechnol., 24*, 97-102. http://dx.doi.org/10.1007/s11274-007-9444-1

Panhwar, Q., Radziah, O., Zaharah, A., Sariah, M., & Mohd, R. (2011). Role of phosphate solubilizing bacteria on rock phosphate solubility and growth of aerobic rice. *J. Environ. Biol., 32*, 607-612.

Patil, R. M., Kuligod, V. B., Hebsur, N. S., Patil, C. R., & Kulkarni, G. N. (2012). Effect of phosphate solubilizing fungi and phosphorus levels on growth, yield and nutrient content in maize (*Zea mays*). *Karnataka J. Agric. Sci., 25*, 58-62.

Pikovskaya, I. (1948). Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiologia, 17*, 362-370.

Pradhan, N., & Sukla, B. (2005). Solubilization of inorganic phosphates by fungi isolated from Agricultural soil. *African J. of Biotec., 5*, 850-854.

Reddy, M., Kumar, S., Babita, K., & Reddy, M. S. (2002). Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis* and *Aspergillus niger*. *J. Biores. Technol., 84*, 187-189. http://dx.doi.org/10.1016/S0960-8524(02)00040-8

Richa, G. Khosla, B., & Reddy, M. (2007). Improvement of maize plant growth by phosphate solubilizing fungi in rock phosphate amended soils. *World J. Agric. Scie., 3*, 481-484.

Rudresh, D. L., Shivaprakash, M. K., & Prasad, R. D. (2005). Tricalcium phosphate solubilizing abilities of *Trichoderma* spp. In relation to P- uptake & growth yield parameters of chickpea (*Cicer arietinum* L.). *Canadian J. of Microbiol., 51*, 217-226. http://dx.doi.org/10.1139/w04-127

Saber, W. I. A., Ghanem, K. M., & El-Hersh, M. S. (2009). Rock Phosphate Solubilization by Two Isolates of *Aspergillus niger* and *Penicillium* sp. and Their Promotion to Mung Bean Plants. *Res. J. Microbiol., 4*, 235-250. http://dx.doi.org/10.3923/jm.2009.235.250

Sahlemedhin, S., & Taye, B. (2000). Procedures for Soil and Plant Analysis. National Soil Research Center, EARO, *Technical Paper No. 74*, Addis Ababa, Ethiopia.

Sanjotha, P., Mahantesh, P., & Patil, S. (2011). Isolation and screening of efficiency of Phosphate solubilizing Microbes. *Internati. J. Microbiol. Res., 3*, 56-58. http://dx.doi.org/10.9735/0975-5276.3.1.56-58

Selvi, B., Paul, A., Ravindran, A., & Vijaya, V. (2011). Quantitative estimation of insoluble inorganic phosphate solubilization. *International J. of Scien. and Natur., 2*, 292-295.

Sharma, A., Rawat, S., & Yadav, B. (2012). Influence of Phosphorus Levels and Phosphorus Solubilizing Fungi on Yield and Nutrient Uptake by Wheat under Sub-Humid Region of Rajasthan, India. *ISRN Agronomy, 2012*, 9. http://dx.doi.org/10.5402/2012/234656

Siddiqui, Z. A., & Akhtar, M. S. (2007). Biocontrol of a Chickpea ROOT-ROT Disease Complex with Phosphate-Solubilizing Microorganisms. *J. of Plant Pathol., 89*, 67-77.

Taye, B. (2007). *An overview of acid soils their management in Ethiopia*. Paper presented in the third International Workshop on Water Management (Wterman) Project, Haramaya, Ethiopia.

Thalooth, A. T., Tawfik, M. M., & Muhammad, M. H. (2006). A comparative study on the effect of foliar application of zinc, potassium and magnesium on growth, yield and some chemical constituents of Haricot bean plants growth under water stress conditions. *World J. Agric. Sci., 2*, 37-46.

Wahid, A., & Mehana, T. (2000). Impact of phosphate-solubilizing fungi on the yield and phosphorus-uptake by wheat and faba bean plants. *Microbiol Res., 155*, 221-227. http://dx.doi.org/10.1016/S0944-5013(00)80036-1

Wakelin, A., Warren, A., Harvey, R., & Ryder, H. (2004). Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. *J. Biol. Fert. Soils., 40*, 36-43. http://dx.doi.org/10.1007/s00374-004-0750-6

Wani, A., Khan, M., & Zaidi, A. (2007). Synergistic effects of the inoculation with nitrogen fi xing and phosphate solubilizing rhizobacteria on the performance of field grown wheat. *J. Plant Nutr. Soil Sci., 170*, 283-287. http://dx.doi.org/10.1002/jpln.200620602

Whitelaw, M. (2000). Growth promotion of plants inoculated with phosphate-solubilizing fungi. *J. Adv. Agron., 69*, 99-151. http://dx.doi.org/10.1016/S0065-2113(08)60948-7

Woyessa, D., & Assefa, F. (2011). Effects of Plant GrowthPromoting Rhizobaceria (PGPR) on Growth and Yield of Tef [*Eragrostis tef* (Zucc.) Trotter] under Greenhouse Condition. *Res. J. Microbiol., 6*, 343-355. http://dx.doi.org/10.3923/jm.2011.343.355

Wu, X., Wei, H., Sun, L., & Wang, Q. (2005). Phosphate availability alters lateral root anatomy and root architecture of *Fraxinus mandshurica* Rupr. seedlings. *J. Integr. Plant Bio., 47*, 292-301. http://dx.doi.org/10.1111/j.1744-7909.2005.00021.x

Xiao, C., Chi, R., He, H., Qiu, G., Wang, D., & Zhang, W. (2009). Isolation of Phosphate-solubilizing fungi from Phosphate mines and their effect on wheat seedling growth. *J. Appl. Biochem. Biotechnol., 159*, 330-342. http://dx.doi.org/10.1007/s12010-009-8590-3

Yadav, J., Verma, P., & Tiwari, N. (2011). Plant Growth Promoting Activities of Fungi and their effect on the chickpea plant growth. *Asian J. Biol. Sci., 4*, 291-302. http://dx.doi.org/10.3923/ajbs.2011.291.299

Yang, H., Fan, B., Gong, M., & Li, Q. (2008). Isolation and identification of a novel phosphate-dissolving strain P21. *Acta. Microbiol. Sin., 48*, 51-56.

Zaidi, A., & Khan, M. (2007). Stimulatory effects of dual inoculation with phosphate solubilizing microorganisms and *arbuscular mycorrhizal fungus* on chickpea. *Australian J. Experim. Agri., 47*, 1016-1022. http://dx.doi.org/10.1071/EA06046

Zaidi, A., & Khan, M. (2006). Co-inoculation Effects of Phosphate Solubilizing Microorganismsand *Glomus fasciculatum* on Green Gram-Bradyrhizobium Symbiosis. *Turk. J. Agric., 30*, 223-230.