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EVALUATION OF RHIZOSPHERIC FUNGI FROM ACID SOILS OF JHARKHAND ON PHOSPHATE SOLUBILIZATION

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ABSTRACT
Phosphate solubilising fungi (PSF) were isolated from various pea, mango and cauliflower-rhizosphere soils of Jharkhand. Three efficient phosphate solubilising fungi were selected based on their ability to form clear zone on Pikovskaya's agar medium. Incubation experiment was conducted with 3 sources of inorganic phosphorus imposed on 3 isolated strains of native phosphate solubilizing fungi. Among the PSF, *Aspergillus niger* produced the highest phosphate solubilization zone and colony growth in all the sources of inorganic phosphorus compared to *Trichoderma viride* and *Penicillium chrysogenum*. *Aspergillus niger* attained the highest phosphorus solubilization efficiency and phosphorus solubilization index after 120h of incubation in tricalcium phosphate, 96h of incubation in both potassium dihydrogen phosphate and ferric phosphate source. *Trichoderma viride* and *Penicillium chrysogenum* attained highest peak of phosphorus solubilization efficiency and phosphorus solubilization index after 144 and 120h of incubation in tricalcium phosphate and potassium dihydrogen phosphate, respectively. In ferric phosphate source, *Trichoderma viride* and *Penicillium chrysogenum* attained highest peak of phosphorus solubilization efficiency and phosphorus solubilization index after 96 and 120h of incubation, respectively. *Aspergillus niger* was found to be the most efficient in solubilizing phosphate from the inorganic sources at the earliest followed by *Trichoderma viride* and *Penicillium chrysogenum*

INTRODUCTION

Phosphorus (P) is one of the major nutrient elements limiting agricultural production in the world. Phosphorus, however, plays an important role in N_2-fixation process, as the reduction of atmospheric nitrogen requires large amount of energy. On an average, soil contains 0.02 to 0.5% total P. It is added to the soil in the form of phosphatic fertilizers, a part of which (1%) is utilized by plants and the rest is rapidly converted into insoluble complexes, e.g., calcium phosphate, iron phosphate and aluminum phosphate in the soil (Gyaneshwar *et al*., 2002). This leads to the need of frequent application of phosphatic fertilizers, but its use on a regular basis has become a costly affair and environmentally undesirable.

Soil microorganisms play an important role in making the phosphorus available to plants by mineralizing the organic phosphorus in the soil (Richardson, 2001). These microorganisms have been isolated from a number of different soils in India (Vikram *et al*., 2007). Several varieties of phosphate-solubilizing microorganisms (PSMs) have been isolated from the rhizospheric soils of crops. Of these, 20%-40% are culturable soil microorganisms. A majority of the isolated organisms are bacterial organisms, although several fungi are also known to solubilize phosphates. These bacteria and fungi have the potential to be used as biofertilizers. Rhizospheric phosphate solubilizing bacteria and fungi are capable of solubilizing insoluble or inorganic phosphates into soluble organic forms (Chen *et al*., 2006; Pradhan and Sukla, 2005). Such PSMs are known to be abundant in the rhizospheric soils of various plants. They can be divided into 2 groups: phosphate solubilising bacteria (PSB) and phosphatesolubilizing fungi (PSF). Fungal diversity affects soil agglomeration, thereby increasing the soil quality and fertility; the health of the plant is thus affected directly. Some bacterial and fungal organisms have solubilizing potential for organic and inorganic phosphorus, respectively (Khiari and Parent, 2005).

Soil phosphates mainly the apatites and metabolites of phosphatic fertilizers are fixed in the form of calcium phosphates under alkaline conditions. Many of the calcium phosphates, including rock phosphate ores (fluoroapatite, francolite), are insoluble in soil with respect to the release of inorganic P (Pi) at rates necessary to support agronomic levels of plant growth (Goldstein, 2000). Phosphate solubilization is the result of combined effect of pH decrease and organic acids production (Fankem *et al.*, 2006). Microorganisms through secretion of different types of organic acids *e.g.* carboxylic acid (Deubel and Merbach, 2005) and rhizospheric pH lowering mechanisms (He and Zhu, 1988) dissociate the bound forms of phosphate like $Ca_3(PO_4)_2$. Aluminium phosphates and iron phosphate mostly dominates in acid soil and the secretion of carboxylic acids by PSF mainly solubilise Al-P and Fe-P (Henri *et al.*, 2008) through direct dissolution of mineral phosphate as a result of anion exchange of PO_4^{3-} by acid anion, or by chelation of both Fe and Al ions associated with phosphate (Omar, 1998).

The principal aim of this investigation was to isolate the

phosphate solubilizing fungi from the rhizospheric soils of horticultural plants grown in acid soils of Jharkhand, India. The study further aimed to detect the phosphorus solubilising efficiency of rhizospheric fungi with 3 different sources of phosphates: tricalcium phosphate (TCP), potassium dihydrogen phosphate (KHP) and ferric phosphate (FP).

MATERIALS AND METHODS

Isolation of fungal species from rhizospheric soil

Different fungal species namely *Aspergillus niger*, *Penicillium chrysogenum* and *Trichoderma viride* were obtained from the rhizosphere soils (acid soils, pH 5.5) of Pea, Mango and Cauliflower, respectively from plandu, Jharkhand, India and screened for their ability to solubilize inorganic phosphate in Pikovskaya's medium (Pikovskaya, 1948). The cultures were maintained on potato dextrose agar slants.

Rhizosphere soil samples from pea, mango and cauliflower were separated and the pH of the soil samples was determined and air dried at room temperature (30 ± 2^oC). Samples of 10g of the soil were dispersed in 90mL of sterile water in 250mL conical flasks. The flasks were incubated at 28 ± 2^oC at 200 rpm in an incubator cum shaker (Scigenics Orbitech, India). The supernatant was serially diluted in sterile water with dilution to 10^{-4} and plated in 10-cm petridishes containing Pikovskaya's agar medium: (g L^{-1}), glucose–10; $(NH_4)_2SO_4$ - 0.5; NaCl-0.3; $MgSO_4.7H_20$-0.1; K_2SO_4-0.2; yeast extract-0.5; $FeSO_4 \cdot 7H_2O$-0.03; $Ca_3(PO_4)_2$- 5.0; $MnSO_4$. $7H_20$-0.02: Agar-20 (Pikovskaya, 1948) by spread plate technique. The pH of the medium was adjusted to 6.8-7.0 before sterilization. The fungal discs were incubated at 28 ± 2^oC for 5-7 days in an incubator.

Screening of the fungal species for Phosphate solubilisation

The fungal colonies showing a clear zone of solubilization and their growth were sub-cultured on potato dextrose agar slants. The fungal species were morphologically characterized after staining with lactophenol cotton blue (Himedia, India) under microscopic observation. Clear zones around the colonies indicated the capacity of phosphate solubilization (Gaur, 1990).

Qualitative estimation of phosphate solubilization

Qualitative estimation of phosphate solubilization was tested on Pikovskaya's agar medium. The Pikovskaya's agar medium, amended with 5 gL^{-1} each of tricalcium phosphate (17% P), potassium dihydrogen phosphate (23%P) and ferric phosphate (18% P) separately in three different set. Fungal discs from four day old potato dextrose agar cultures each of *Aspergillus*, *Penicillium* and *Trichoderma* (4 mm dia.) were placed on all the above media plates containing Pikovskaya's agar with TCP, KHP and FP separately. Each inoculation was replicated 5 times. The plates were incubated at 30ºC for 1-6 days (144h). The halo formation was observed after 24h onwards. Colonies forming a clear halo around them, indicating P solubilization, were counted and further used to determine the P-solubilization index and P-solubilization efficiency. Solubilization index (PSI) was measured using following formula (Premono *et al*., 1996):

PSI = (Colony diameter + Halozone diameter) / Colony diameter

P-solubilization efficiency was measured using the following formula (Ponmurugan and Gopi, 2006):

PSE = (Z-C)/C

Where, Z is Solubilization Zone and C is Colony diameter.

All the data were analyzed by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Microorganisms capable of producing a clear zone due to P

Table 1: Phosphate solubilisation of different phosphates by fungal species in PVK agar (supplemented with different phosphates)

Incubation time (hr)	Solubilization zone of inhibition (mm)		
	Tri-Calcium phosphate	Potassium di-hydrogen phosphate	Ferric phosphate
Aspergillus niger			
24	7.1 ± 0.23	9.1 ± 0.58	8.2 ± 0.70
48	21.6 ± 1.16	26.4 ± 1.99	25.7 ± 1.16
72	40.3 ± 2.31	52.3 ± 1.92	54.6 ± 1.73
96	60.6 ± 2.89	88.0 ± 1.16	88.3 ± 1.2
120	88.0 ± 1.16	-	-
144	-	-	-
Penicillium chrysogenum			
24	6.8 ± 0.23	6.9 ± 0.53	6.8 ± 0.49
48	12.6 ± 1.16	15.3 ± 1.34	14.3 ± 1.35
72	25.6 ± 1.73	30.2 ± 1.86	31.3 ± 1.88
96	40.3 ± 1.53	50.2 ± 1.87	51.3 ± 1.92
120	60.3 ± 1.92	88 ± 1.16	87.6 ± 1.2
144	88.5 ± 1.28	-	-
Trichoderma viride			
24	6.3 ± 0.58	7.2 ± 0.58	6.4 ± 0.58
48	13.4 ± 1.36	17.4 ± 1.36	25.6 ± 1.16
72	30.3 ± 1.88	34.3 ± 1.3	58.6 ± 2.10
96	45.2 ± 1.87	51.6 ± 2.08	88.1 ± 1.57
120	60.3 ± 1.92	87.6 ± 0.88	-
144	89.6 ± 1.02	-	-

± Standard error

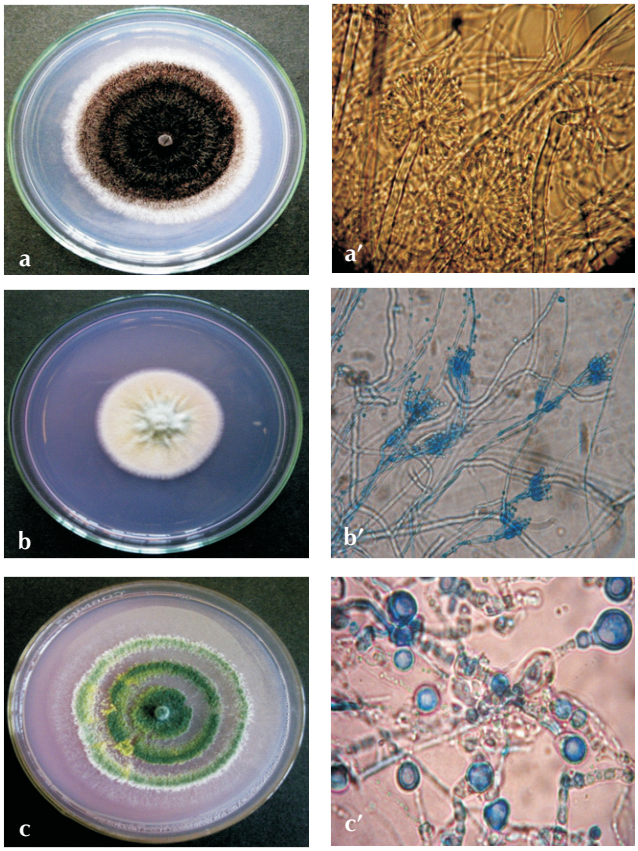


Figure 1: Phosphate solubilising fungi isolated from the soil cleared the red colour of PVK medium, a. Colony and a′. Micrograph of *Aspergillus niger* showing conidiophores bearing radiating phialides and phialospores, b. Colony and b′. Micrograph of *Penicillium chrysogenum* bearing penicillate phialides and phialospores, c. Colony and c′. Micrograph of *Trichoderma viride* having profuse chlamydospores

solubilization in the surrounding medium (Gaur, 1990) were selected as potential phosphate solubilizers and a total of 3 fungal species were resulted from the primary screening (Fig. 1). Inorganic phosphate solubilization by three isolated native PSF was studied on three sources of inorganic P. The result showed that the solubilization zone gradually increased with the increasing hour of incubation irrespective of sources of phosphorus in all the PSF (Table 1). In case of *Aspergillus niger*, maximum solubilization zone (88mm) was achieved after 120h of incubation with TCP supplemented PVK agar media. However, the maximum solubilization zone (88mm) was achieved after 96h of incubation in both KHP and FP. In *Penicillium chrysogenum*, the maximum solubilization zone (88mm) was recorded after 144h of incubation in PVK agar supplemented with TCP, while the maximum zone was achieved in 120h of incubation in both inorganic sources of P *i.e.,* KHP and FP. In case of *Trichoderma viride*, maximum solubilization zone was achieved after 144h of incubation in TCP supplemented PVK agar, while the maximum zone in KHP and FP supplemented PVK agar media was recorded after 120 and 96h of incubation, respectively. Among the different sources of phosphates supplemented in PVK agar, the solubilization zone caused by different PSF was recorded early in FP and KHP supplemented PVK agar. Among the PSF, *Aspergillus niger* was most effective in creating solubilization zone in all the inorganic sources of P followed by *Trichoderma viride* and *Penicillium chrysogenum*. Padmavthi and Usha (2012) reported that *Aspergillus* sp. recorded the highest solubilization zone of inhibition compared to other fungal strains *in vitro* when liquid medium was supplemented with both tricalcium phosphate, potassium dihydrogen phosphate and rock phosphate separately. In a similar study, it was reported that isolates of *Aspergillus* and *Penicillium* isolated from agricultural soil showed maximum level of phosphate solubilization activity *in vitro* when liquid medium was supplemented with both tricalcium phosphate and rock phosphate separately (Pradhan and Sukla, 2005).

Table 2: Colony diameter of fungal species in PVK agar (supplemented with different phosphates)

Incubation time (hr)	Colony diameter (mm) Tri-Calcium phosphate	Potassium di-hydrogen phosphate	Ferric phosphate
Aspergillus niger			
24	6.3 ± 0.19	8.3 ± 0.58	7.3 ± 0.77
48	18.6 ± 1.16	24.0 ± 1.16	20.6 ± 1.16
72	26.0 ± 1.73	33.0 ± 1.45	34.0 ± 1.5
96	36.0 ± 1.45	44.0 ± 1.0	44.2 ± 0.74
120	44.0 ± 0.58	-	-
144	-	-	-
Penicillium chrysogenum			
24	6.6 ± 0.29	6.6 ± 0.47	6.6 ± 0.39
48	12.0 ± 1.16	14.3 ± 1.35	12.0 ± 0.69
72	21.3 ± 1.35	20.3 ± 1.35	20.0 ± 1.33
96	27.6 ± 1.54	30.0 ± 1.73	30.0 ± 1.5
120	36.3 ± 1.35	44.3 ± 0.67	43.8 ± 0.64
144	43.6 ± 0.88	-	-
Trichoderma viride			
24	6.0 ± 0.58	7.0 ± 0.58	6.0 ± 0.46
48	10.3 ± 0.77	13.6 ± 0.97	19.0 ± 0.98
72	21.0 ± 1.16	22.6 ± 1.54	36.0 ± 1.04
96	27.6 ± 1.54	29.0 ± 1.45	44.0 ± 0.95
120	32.3 ± 1.35	44.0 ± 1.0	-
144	44.0 ± 1.0	-	-

± Standard error

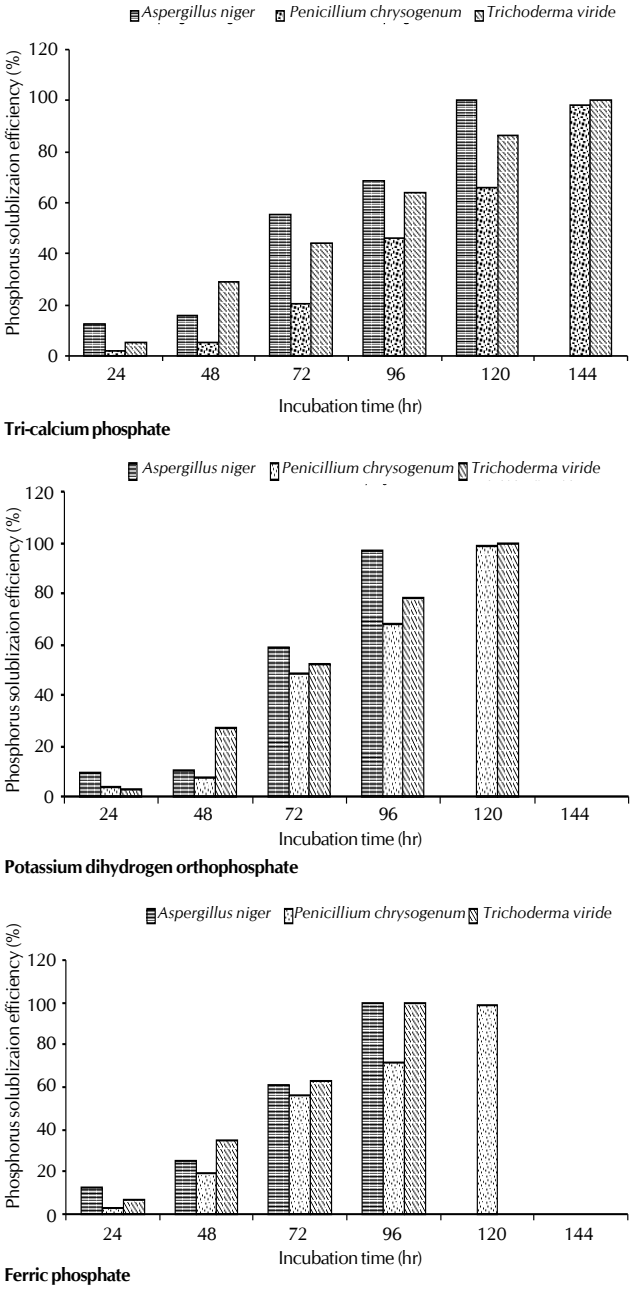
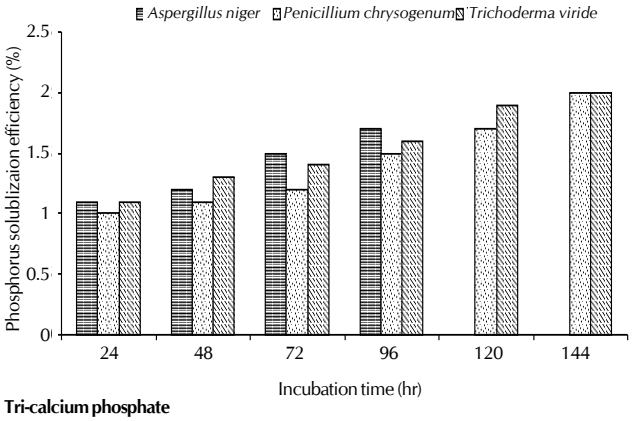


Figure 2: Phosphorus solubilization efficiency (%) of fungal species in different inorganic P source. Bars are ± standard error

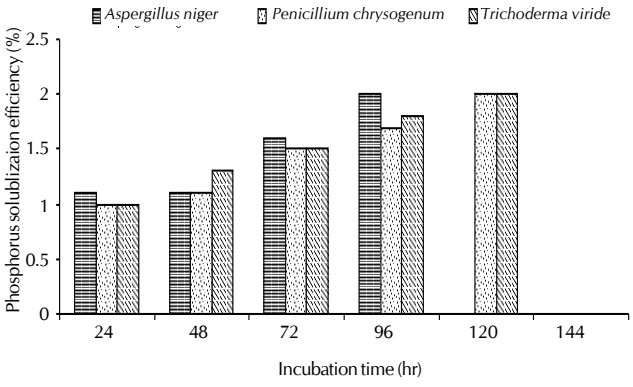
The colony diameter of different PSF gradually increased with the increasing days of incubation, irrespective of sources of phosphates (Table 2). In case of *Aspergillus niger*, the colony diameter was recorded highest of 44 mm after 120h of incubation in TCP supplemented PVK agar media. However, the maximum colony diameter (44 mm) was achieved after 96h of incubation in both KHP and FP supplemented PVK agar media. In *Penicillium chrysogenum*, the maximum colony diameter (44 mm) was recorded after 144h of incubation in TCP supplemented PVK agar, while the maximum colony diameter (44 mm) in KHP and FP supplemented PVK agar media was recorded after 120h of incubation. The maximum colony diameter (44mm) of *Trichoderma viride* was recorded after 144 hour of incubation in TCP supplemented PVK agar, while the maximum colony diameter in KHP and FP supplemented PVK agar media was recorded after 120 and 96h of incubation, respectively. Among the different sources of phosphates supplemented in PVK agar, the colony diameter of different PSF was recorded early in FP and KHP supplemented PVK agar. Among the PSF, *Aspergillus niger* was found to be most efficient in forming colony at the earliest followed by *Trichoderma viride* and *Penicillium chrysogenum*.

Phosphorus solubilization efficiency (PSE) was recorded on the basis of zone of solubilization as well as colony diameter (Fig. 2). Among the PSF, *Aspergillus niger* was most efficient phosphate solubilizer as the maximum PSE (100%) was achieved after 120h of incubation compared to *Penicillium chrysogenum* and *Trichoderma viride* in which, the maximum PSE was achieved after 144h of incubation in TCP amended PVK agar. However, *Apergillus niger* showed the maximum efficiency after 96h of incubation in both KHP and FP amended PVK agar. *Aspergillus niger* showed the following trend in solubilizing the P from different source as, FP e" KHP > TCP. The *Penicillium chrysogenum* culture under different sources of inorganic P showed the maximum efficiency after 144h of incubation in TCP supplemented PVK agar, 120h in both KHP and FP supplemented PVK agar media. The *Penicillium chrysogenum* showed the following trend in solubilizing P as, KHP = FP > TCP. The *Trichoderma viride* showed the maximum efficiency of 100 % after 144h of incubation in TCP supplemented PVK agar, while the maximum PSE in KHP and FP supplemented PVK agar was achieved after 120 and 96h, respectively. The *Trichoderma viride* showed the following trend in solubilizing P from different source as, FP > KHP > TCP. Among all the sources of phosphates supplemented in PVK agar, the PSE increased by different PSF and recorded early in FP and KHP supplemented PVK agar. Further, among the PSF, *Aspergillus niger* was most efficient followed by *Trichoderma viride* and *Penicillium chrysogenum* in solubilizing P from different source. Phosphate solubilization takes place through various microbial processes/mechanisms including organic acid production and proton extrusion (Surange, 1995; Dutton and Evans, 1996; Nahas, 1996). Tarafdar *et al*. (2003) reported that *Trichoderma* sp. was found to be most effective organic phosphorus mobilizers as compared to other fungi.

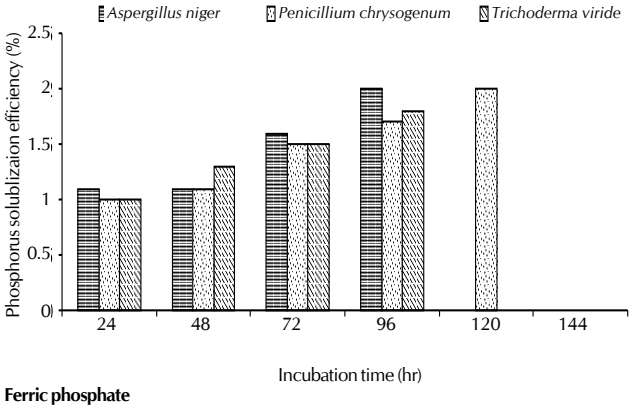
The result showed that the P-solubilizing index (PSI) gradually increased with the increasing hour of incubation irrespective of sources of phosphates in all the PSF (Fig. 3). Among the different PSF, *Aspergillus niger* showed maximum solubilizing index of 2.0 after 120 hour of incubation whereas *Penicillium chrysogenum* and *Trichoderma viride* showed the maximum PSI after 144h of incubation in TCP amended PVK agar. Further, the *Aspergillus niger* attained the maximum PSI after 96h of incubation while *Penicillium chrysogenum* and *Trichoderma viride* attained maximum PSI after 120h of incubation in KHP supplemented PVK agar. In FP supplemented PVK agar, both *Aspergillus niger* and *Trichoderma viride* attained maximum PSI after 96h of incubation, whereas *Penicillium chrysogenum* attained the maximum peak after 120h of incubation. Among all the



Tri-calcium phosphate



Potassium dihydrogen orthophosphate



Ferric phosphate

Figure 3: Phosphorus solubilization index of fungal species in different inorganic P source. Bars are ± standard error

sources of phosphates supplemented in PVK agar, the solubilization index increased by different PSF was recorded early in FP and KHP supplemented PVK agar. Among the different PSF, *Aspergillus niger* was found to be most prominent in increasing the PSI followed by *Trichoderma viride* and *Penicillium chrysogenum*. Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms (Sagoe *et al.*, 1998).

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