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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(10): 823-827

www.thepharmajournal.com
Received: 07-08-2021
Accepted: 04-09-2021

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Release pattern of soil phosphorus as affected by phosphate solubilizing microorganisms in an Ultisol

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Abstract
The exploitation of the fixed P pool in soil is an urgent need since rock phosphate supplies, the raw material for phosphatic fertilizer manufacturing, are quickly depleting. Phosphate solubilizing microorganisms (PSMs) are a viable approach for solubilizing this fixed P pool in soil. An incubation study was carried out in the laboratory at ambient temperature to assess the release of P from highly weathered acid soil of Assam (order- Ultisol, pH= 4.23) as influenced by phosphate solubilizing microorganisms (PSMs) by applying pure cultures of either phosphate solubilizing fungi (PSF), *Aspergillus niger* or phosphate solubilizing bacteria (PSB), *Enterobacter* sp. to the soil. Although the soil had very low available P content (8.59 kg ha^{-1}), total P content of the soil was 526 kg ha^{-1}, indicating a high amount of P might be present in fixed form, which could be solubilized by PSMs. Results indicate that the PSF significantly increased the release of P from soil over control (0.10 to 0.14 mg kg^{-1}). However, the increase was maximum initially and the magnitude of increase decreased gradually with increasing days of incubation. On the other hand, PSB was ineffective in mediating the release of P from fixed pool in this soil.

Keywords: phosphate solubilizing microorganism (PSM), Ultisol, P release pattern, solubilization, fixed pool

Introduction
Phosphorus (P) is a necessary plant macronutrient, and a sufficient supply of P is required for optimal crop yield. Although most of the soils have a large total P stock, soil solution P concentrations are typically low, ranging from 0.05-0.30 g P mL^{-1}, which is insufficient for optimal crop yield (Bolan, 1991) [3]. The P shortage is projected to impair agricultural crop productivity on more than 40% of the world's cultivable area (Balemi and Negisho, 2012) [2]. As a result, a large quantity of water soluble phosphatic fertilizers is used in intensive agriculture to meet the crop demand. However, because of its high reactivity, the availability of this vital nutrient is often limited by its fixation in soil, particularly in acid soil due to the presence of considerable amounts of Fe and Al oxides and hydroxides (Sanyal and De Datta, 1991) [15]. As a result, the use efficiency of applied P rarely exceeds 20-22 percent, resulting in an around 80 percent loss of applied P (Srinivasarao *et al*., 2015) [19]. In highly weathered Ultisols, high concentrations of cations such as aluminium (Al), iron (Fe), and manganese (Mn) cause the precipitation of soluble P in the form of insoluble hydroxy-phosphate compounds, whereas the surfaces of insoluble oxides of Fe, Al, and Mn strongly adsorb P, rendering it unavailable to plants (Brady and Weil, 2002) [4]. Because of the high P fixation ability of these soils and the poor P use efficiency (PUE) of most of the crops, the surplus P input from fertilizers tends to accumulate in the fixed pool of soil. This fixation or accumulation of P eventually results in the wastage of expensive P fertilizers. On the other hand, rock phosphate stocks, which are used to make phosphatic fertilizers, are fast depleting, and global commercial rock phosphate reserves may no longer be available for use in 50-100 years (Sattari *et al*., 2012) [16]. As a result, the only alternative left is to improve the use efficiency of applied P while still exploiting the soil's native fixed P pool. In this context, Khan *et al.* (2007) [10] estimated that, if made available, the accumulated P in agricultural soils will be adequate to achieve maximum crop yields worldwide for a long time. However, rendering the accumulated P in soil available to the plant is not an easy task. Phosphate solubilizing microorganisms (PSMs) are well-known for their ability to convert complex inorganic and organic soil P to plant-available forms via diverse processes of solubilization and mineralization (Mohammadi, 2012) [11]. The PSM is a cost-effective and environmentally benign way to increase P availability for plants (Owen *et al*., 2015) [13].

The fixed or relatively unavailable fraction of P may be distributed to proportionally more labile fractions, which can be taken up by the plants, as a result of PSM application. In case of Ultisols of India, there is very little information on the pattern of P release into soil solution due to microbial solubilization of fixed soil P. Therefore, the present investigation was carried out to assess the release of P from fixed pool as mediated by phosphate solubilizing microorganisms (PSMs) in a highly weathered Ultisol of Assam, India.

Materials and methods

Collection and characterization of the experimental soil

One bulk surface soil sample (0-15cm) was obtained for this study from the Negheriting tea estate in the Golaghat district of Assam, India (order- Ultisol, pH= 4.23). After that, the bulk sample was air-dried and crushed until it could pass through a 2-mm sieve. Standard procedures were used to analyze the processed soil sample for major physicochemical parameters (Table 1) (Page *et al*., 1982) [14]. The Bouyoucos hydrometer method was used to do mechanical analysis of soil, which included determining the relative quantities of sand, silt, and clay particles. The pH of the soil was determined using a digital pH meter with a 1: 2 soil-water solution, and the same supernatant was further utilized to determine the electrical conductivity (EC) of the soil using an Electrical conductivity meter. The soil sample's cation exchange capacity (CEC) was evaluated using the ammonium acetate method. The wet oxidation method was used to measure the percentage of organic carbon (SOC) in the soil, whereas the alkaline permanganate method was used to determine the amount of available nitrogen (N) in the soil. The soil sample's available potassium (K) was extracted using 1 *N* ammonium acetate solution with a pH of 7.0. The Bray-Kurtz P1 reagent (0.03 *N* NH_4F + 0.025 *N* HCl) was used to extract available P in soil (Bray and Kurtz, 1945) [5], and P content in the extract was measured using the ascorbic acid reduced phospho-molybdic blue colour technique at 730 nm using a UV-VIS spectrophotometer (Watanabe and Olsen, 1965) [22]. The soil belonged to the clay loam textural class with acidic pH and non-saline EC. Although, the soil had very low available P (8.59 kg ha^{-1}) but the total P content was quite high (526 kg ha^{-1}), indicating that majority of the P in this soil might be in fixed pool.

Maintenance of microbial population in pure culture

Pure culture of phosphate solubilizing bacterium (PSB), *Enterobacter* sp. and phosphate solubilizing fungus (PSF), *Aspergillus niger* was maintained in the respective media and stored at 4°C for further use. The composition of Pikovskaya medium used for PSB was as follows: yeast extract: 0.50 g L^{-1}, dextrose: 10.0 g L^{-1}, calcium phosphate: 5.00 g L^{-1}, ammonium sulphate: 0.50 g L^{-1}, potassium chloride: 0.20 g L^{-1}, magnesium sulphate: 0.10 g L^{-1}, agar: 15.0 g L^{-1} and pinch of manganese sulphate and ferrous sulphate. For preparation of potato dextrose agar medium used for PSF, 200g of sliced potatoes were boiled in 1 litre of distilled water for 1 hour. Then the content was filtered and 20 g D-glucose and 15 g agar was added to it. The volume was then increased to 1 litre. The content was steamed until agar was dissolved. The isolates in pure culture were counted using the dilution plate technique (Waksman and Fred, 1922) [20]. Cell counts were performed after 2 days for the fungus treated soil and 4 days for the bacteria treated soil in petri plates kept at 28 °C in an incubator. In pure culture, the number of PSB and PSF isolates was (13 $\times 10^{10}$) c.f.u. mL^{-1} and (59 $\times 10^{9}$) c.f.u. mL^{-1}, respectively.

Incubation study

An incubation study was conducted in the laboratory at ambient temperature (average temperature was 25±2 ºC throughout the incubation period) to assess the release of P from soil as influenced by PSMs. For this purpose, three levels of PSM were used: No PSM, PSB (*Enterobacter* sp.) and PSF (*Aspergillus niger*). A series of 50 g processed soil samples were taken in 500 mL wide mouth plastic reagent bottles with lids closed. Fifteen mL of liquid pure culture of either PSB or PSF was added to each bottle as per the treatment combinations. Another 15 mL of double-distilled water (DDW) was added to maintain field capacity moisture content inside the bottle. In case of control bottles, simply 30 mL DDW was added. Each treatment combination was replicated thrice in a completely randomized design using 81 bottles (3 PSM levels × 9 sampling times × 3 replicates). Four to five fine perforations were made in the lid of each bottle to facilitate air exchange and to prevent the development of anaerobic condition inside the bottle. Constant moisture content of the soil within the bottle was maintained by periodic water addition, after determination of water loss from each bottle after every 4 days. Periodic samplings were done at 7, 14, 21, 28, 35, 42, 49, 56 and 90 days after incubation (DAI). Whole amount of soil in each bottle was extracted by 100 mL 0.01 *M* $CaCl_2$ at a soil: solution ratio of 1:2 with 2 hours of shaking period. and P concentration was measured by ascorbic acid blue colour method as mentioned earlier (Watanabe and Olsen, 1965) [22]. The P in the extract was considered as water soluble P present in soil solution.

Statistical analyses

Analysis of variance method appropriate to the experimental design (factorial completely randomized design) with least significant difference at $P = 0.05$ was followed to study the impact of PSMs on P release pattern in soil (Snedecor and Cochran, 1967) [18].

Results and Discussion

Effect of phosphate solubilizing microorganisms on release of P into soil solution

An incubation study was conducted to assess the impact of PSMs on the release of P from soil and the results are presented in table 2. Results of the incubation study indicate significant positive effect of PSMs on 0.01*M* $CaCl_2$ extractable P. Significant positive effect of PSM application on 0.01*M* $CaCl_2$ extractable P in soil as obtained in the present investigation is in agreement with the findings of few other researchers (Illmer *et al*., 1995; Wang *et al*., 2014; Alam *et al*., 2020) [1, 7, 21]. The PSMs can solubilize complexed or insoluble forms of inorganic P through various mechanisms like acidification, chelation and exchange reactions (Singh and Reddy, 2011; Wang *et al*., 2014) [17, 21]. The fungus used in the present study, *A. niger* is capable of producing a large amount of organic acids (predominantly citrate) and is an efficient solubilizer of $AlPO_4$ (Illmer *et al*., 1995) [7]. Hydroxyl and carboxyl groups of such organic acids chelate cations like Al^{3+}, Fe^{3+} or Ca^{2+} and thereby release P associated with these cations to the soil solution (Illmer *et al*., 1995) [7]. Also, these organic anions (ligands) compete with phosphate for adsorption sites in the soil reducing P fixation

(Mohammadi, 2012) [11]. Moreover, PSMs release H^+ ions in the soil, which lowers the pH of surrounding soil (Illmer and Schinner, 1992) [8]. This low pH helps in solubilization of native soil P. In the present study, on an average, the PSF (*A. niger*) significantly increased the release of P from soil over control (0.10 to 0.14 mg kg^{-1}) (Fig. 1). On the other hand, the PSB (*Enterobacter* sp.) was ineffective in mediating P release into the soil solution (Fig. 1).

Release pattern of phosphorus in soil as mediated by phosphate solubilizing microorganisms

In PSF treated soil, there was a gradually decreasing trend in the amount of P released. On 7th day of incubation, the value of released P was 0.20 mg kg^{-1} in PSF treated soil, which decreased gradually and became equal with that of control (0.10 mg kg^{-1}) on 90 days of incubation (Fig. 2). Notably, the increase in the release of P into soil solution due to PSF inoculation was highest initially but the magnitude of increase decreased gradually with increasing days of incubation (Fig. 2). In general, a rapidly declining trend in the density of artificially introduced PSF is commonly seen upon incubation in soils (Khan *et al*., 2010) [9]. With increasing days of incubation, there was a gradual increase in the concentration of soluble P in soil solution (by means of solubilization of fixed P), which inhibited further phosphate solubilization (Narsian *et al*., 1995) [12], ultimately leading to the characteristic P release pattern. Another school of thought suggests that an organo-P compound is formed, induced by the different organic metabolites that are released into the solution by the fungal culture and this reduces the amount of P in solution subsequently (Illmer and Schinner, 1992; Goenadi and Sugiarto, 2000) [6, 8]. On the other hand, very low pH of the soil might have inhibited the PSB to establish and solubilize the fixed P pool in this highly weathered acid soil.

Table 1: Physical and chemical properties of the experimental soil

Property	Value
pH	4.23
Electrical conductivity (dS m^{-1})	0.13
Organic carbon (%)	0.58
Cation exchange capacity [cmol (p^+) kg^{-1}]	8.87
Textural class	Clay loam
Sand (%)	36.1
Silt (%)	35.6
Clay (%)	28.3
Major nutrients (kg ha^{-1}):	
Available N	209
Available P	8.59
Available K	111
Total P	526

Table 2: Effect of phosphate solubilizing microorganisms on release of phosphorus (mg/kg) from soil with time

Days of incubation (D)	PSM (M)			Mean
	Control	PSB	PSF	
7	0.10	0.11	0.20	0.14
14	0.11	0.15	0.19	0.15
21	0.11	0.13	0.17	0.13
28	0.10	0.11	0.15	0.12
35	0.09	0.10	0.14	0.11
42	0.09	0.09	0.14	0.10
49	0.10	0.10	0.11	0.11
56	0.09	0.09	0.09	0.09
90	0.10	0.10	0.10	0.10
Mean	0.10	0.10	0.14	
LSD (P= 0.05)	D = 0.01 M = 0.01 D × M = 0.03			

PSM= Phosphate solubilizing microorganism; PSB= Phosphate solubilizing bacteria; PSF= Phosphate solubilizing fungi; LSD= Least significant difference

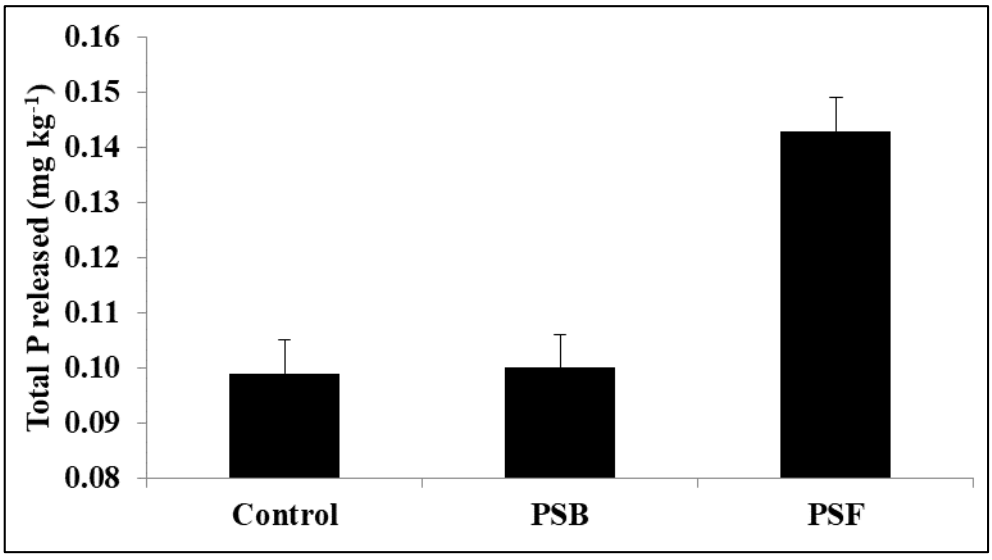

Fig 1: Impact of phosphate solubilizing microorganisms on total phosphorus released (mg/kg) in soil Error bars denote the least significant difference (LSD, P=0.05) between the two treatments

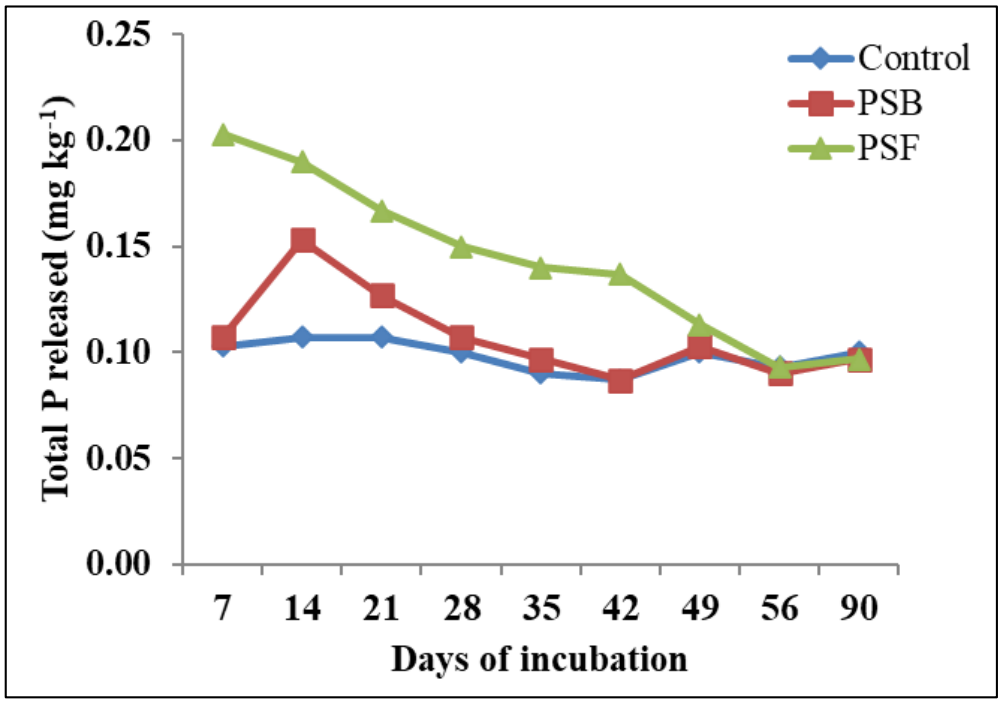

Fig 2: Release pattern of phosphorus (mg/kg) in soil as mediated by phosphate solubilizing microorganisms

Conclusions

The present study demonstrated that only the PSF, *Aspergillus niger* was effective in mediating P release from fixed P pool of this highly weathered Ultisol. The PSB *Enterobacter* sp., on the other hand, proved unsuccessful at solubilizing fixed P and hence facilitating P release. These findings are critical for sustainable and cost-effective P management, which is one of the most persistent concerns for global food security. However, further thorough field experimentation is needed to determine the efficacy of this microbial strain in P nutrition of crop plants.

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