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Soil Phosphorus Distribution in Response to Chemical P and Phosphate Dissolving Fungi

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Abstract—*Phosphorus (P) deficiency is one of the important constraints in food production especially in the tropical soils of India. Biological extraction of P by phosphate dissolving fungi (PDF) holds a good promise in improving the release of chemically fixed P in soil under sustainable agriculture. The present study aimed to characterize the inorganic P fractions in mustard grown cultivated field managed under two rates of chemical P fertilization (recommended and 1/2 the recommended dose of single super phosphate) and PDF inoculation. Soil P forms including water soluble, sodium bicarbonate, sodium hydroxide, hydrochloric acid and residual P were sequentially extracted in samples drawn from 0-15 cm soil layer. Distribution of inorganic P pools varied with different rates of chemical P input as well as with crop growth. Sodium bicarbonate extractable inorganic P fraction accounted for 32.06 % of soil total extracted P. This fraction of P registered an increase of 30.43 % due to fungal inoculation when compared with its respective un-inoculated counterpart receiving 100 % recommended dose of chemical P. Sodium hydroxide extracted P ranged from 98-187.31 mg kg^{-1} soil. The acid extracted inorganic P was the predominant form and the values for this fraction ranged from 167.27 to 312mg kg^{-1} soil. Soil P fractionation approach is useful to point out the potentiality of mineralization of P pools. However, being a static tool, conclusion regarding the effect of soil management practices on P dynamics needs to be drawn with utmost care.*

Keywords*: Fractions, Phosphorus, Fertilizer, Fungal inoculation*

1. INTRODUCTION

Despite the high content of total phosphorus (P) in soil worldwide, its availability to plant is deficient. Low availability of P affects the crop productivity adversely. Improving soil and plant P nutrition is of utmost importance to provide food security to the growing population. The primary approach to achieve the desired target for crop yield is either by use of chemical P fertilizer or scavenging the native or fixed P using natural resources. The former approach though can provide soluble P at a much faster rate but the poor use efficiency of chemical P results in higher fixation of fertilizer-P added exogenously. Thus, to meet the nutritional needs of crop plants P has to be applied in quantities in excess of the crop requirement, thereby putting financial constraint on marginal farmers and affecting microbial diversity. The second approach using natural resources is environment friendly and relies on the use of phosphate dissolving microorganisms (PDM) that have the ability to release P from both organic and inorganic P sources. Among PDM, phosphate dissolving fungi (PDF) have higher potential to mediate the process of P release at low cost. Due to their non specificity for plant association, members of genera *Aspergillus* and *Penicillium* have widely been explored for range of plant production system (Mendes et al, 2014). *Penicillium* sp. exhibits a positive effect on phosphate solubilization and inhibits the transformation of soluble phosphate to insoluble phosphate (Salih et al., 1989). The soil P contains different P fractions and that may change with crop management practices (Satyavir 2014), soil environment, climate change and with phosphate dissolving microbial strain. Accurately characterizing P forms is important for evaluation of its availability status in soil. Fractionation of soil P is important to overcome the limited information that total P analysis provides. With this back ground we studied the interactive effects of co-application of P fertilizer and seed inoculation with *Penicillium* sp. on changes in soil inorganic P fractions under mustard crop.

2. MATERIALS AND METHODS

2.1 Qualitative evaluation of *Penicillium* sp. for phosphate solubilisation

In vitro phosphate solubilisation ability of *Penicillium sp.* was examined on Pikovskaya's agar medium (1948) with tri-calcium phosphate as the sole P source. A total of 10 µl of fungal broth was spotted on cited medium agar plates, and incubated at 28^{o}C for 5-6 days. The capacity to utilize P on agar medium was examined by the formation of distinct halo zone around the fungal colonies that indicated solubilisation of inorganic P. The colonies with halo zones were picked, sub cultured and maintained on potato dextrose agar (PDA) slants at 4^{o}C and used for further studies.

2.2 Quantitative estimation of soluble P

One hundred mL of Pikovskaya's broth with separately added TCP and RP as the sole P source was dispensed in 250 mL

Erlenmeyer's flasks, in triplicate. The flask contents after sterilization at 15 psi for 15 min were cooled and inoculated with 0.5 ml spore suspension of five-day-old broth culture of cited fungal strain, keeping un-inoculated controls as necessary checks and incubated at 30◦ C. At the end of 4, 8, 12, and 16 days of incubation, the contents were filtered through previously weighed Whatman No. 42 filter paper (GE Healthcare, Little Chalfont, UK). The filtrate was used for estimation of soluble P (Murphy and Riley 1962).

2.3 Assay of acid phosphatase activity

To evaluate the phosphate mineralization ability, the test strain was grown in sterilized Czepk's Dox broth for 16 days. Acid phosphatase activity of fungal strain was measured at 4d interval in a cell-free extract, using acetate buffer of pH 5.4, at 37◦C. The substrate used for enzymatic activity was *p*-nitrophenyl phosphate. A blank was also included for comparison. The intensity of yellow color formed because of production of *p*-nitro-phenol was measured at 400 nm (Tabatabai and Bremner, 1969).

2.4 Experimental site and details

The field experiment was conducted at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi, India, situated at an latitude of 28° 40' N and longitude of 77° 12' E, altitude of 228.6 meters above mean sea level. The soil of experimental field had available N-140 kg/ha, available P-15.64 kg/ha, available K- 283 kg/ha, organic carbon -0.56 % and pH -8.0. Mustard crop was grown on sub-plot of 12 m^2 each with three replicates, following randomized block design. The two rates of chemical P fertilizer included 100 % P (recommended dose of chemical P) and 50 % P (½ the recommended dose of chemical P). The different treatments were T1-un-inoculated control ($N_{120}P_{30}K_{60}$), T2- $N_{120}P_{30}K_{60}$ +fungal inoculums, T3- $N_{120}P_{60}K_{60}$ recommended dose of chemical fertilizers T4- $N_{120}P_{60}K_{60}$ +fungal inoculum. Urea, single super phosphate and muriate of potash were applied at 120 kg N, 60 and 30 kg P_2O_5 and 60 Kg K_2O ha^{-1}, respectively to meet the N, 100 % P and 50 % P and K needs of the test crops. The chemical fertilizers were evenly spread on the soil surface of each plot on the date of sowing. Mustard (var. Pusa M-30, seed rate- 4 kg ha^{-1}) seeds were treated with *Penicillium* sp. inoculum. All the agronomic practices including irrigation, weeding etc were maintained during crop growth.

2.5 Soil sampling and analysis

Finely ground and sieved soil samples drawn at 0-15 cm depth were oven dried prior to estimation of pH, and phosphorus fractions. Soil pH was measured using digital pH meter with soil water ratio of 1:5. The phosphorus fractions were estimated by the method of Hedley et al. (1982). All the determinations were performed in triplicate and results were expressed on dry weight basis.

3. RESULTS AND DISCUSSION

The fungal growth diameter as well as the halo zone diameter was much bigger with tri-calcium phosphate compared to rock phosphate (Fig.1)

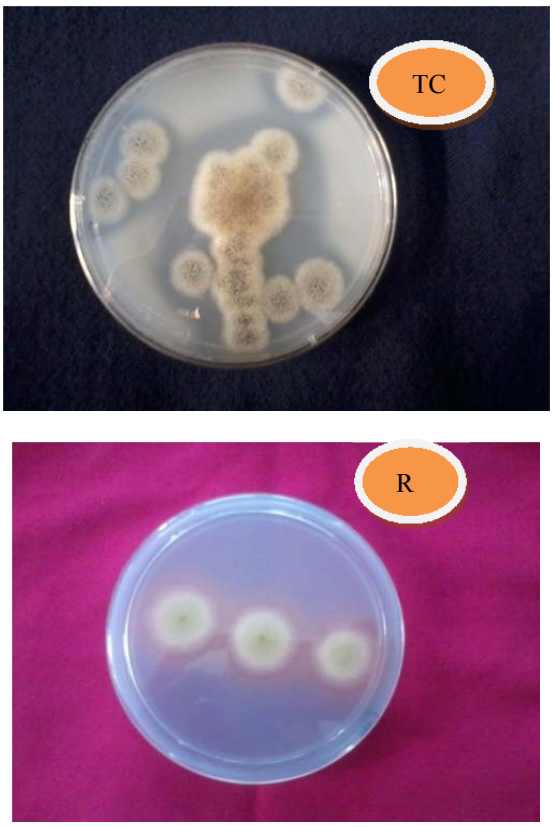

Fig. 1: Fungal solubilisation of tricalcium phosphate (TCP) and rock phosphate (RP

Quantitative estimation of soluble P in cell free extract of tri calcium (TCP) and rock phosphate (RP) as P source also confirmed the qualitative observation. The maximum amount of soluble P from both the P sources was recorded on 8th day of incubation followed by a decline with subsequent incubation (Fig 2).

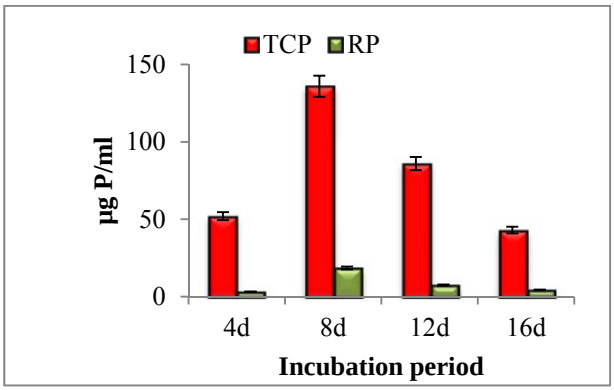

Fig. 2: Fungal mediated release of P from TCP and RP

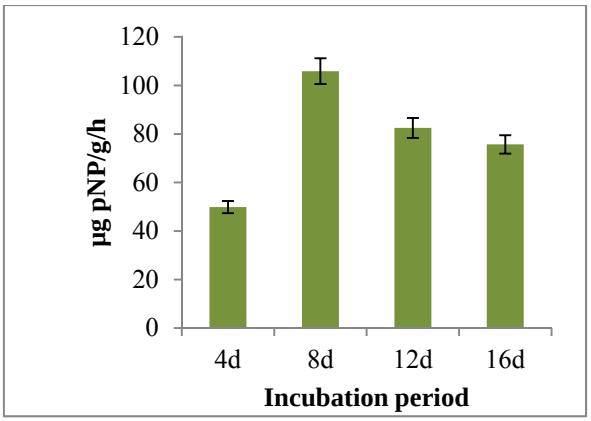

Fig. 3: Acid phosphatase activity of *Penicillium* sp.

The fungal strain exhibited the maximum release of extra-cellular acid phosphatise activity on 8th day of incubation followed by a decline with incubation. The high potential of test fungus for extracellular phosphatase may be attributed to high permeability of its cell membrane.

3.1 Changes in soil pH

Analysis of experimental field soil for pH estimation revealed that the values for all the treatments were higher at 60 d interval and decreased with crop growth. Soil pH in T1 (½ the recommended dose of P) after 120d of crop growth registered a decline of 0.6 units compared to soil samples drawn at 60 d of crop growth. Recommended dose of chemical P (100 %) in T3 resulted in relatively lower pH value at 60 d sampling of soil compared with its T1 counterpart that received ½ the recommended dose of chemical P. Integrated use of fungal inoculums and 100 % chemical P resulted in maximum decrease of soil pH. It is the production of organic acids by the test organism that is responsible for reduction in soil pH, resulting in dissociation of insoluble phosphates and metal ions in the soil. The minimum pH recorded was 7.5 in T4. (Fig.4). Soil pH directly affects the microbial activity, soil physical properties especially clay dispersion and aggregation. A positive correlation between soil final pH and soluble phosphate was observed.

3.2 Soil -P fractions

The highest availability of water soluble P at harvest was recorded in inoculated treatment (T4) compared to un-inoculated control and other treatments. This indicated that inoculation improved the P availability, though, a part of the easily available water soluble P must have been assimilated by fungal population for their cell growth. The lower content of water soluble P in treatment receiving recommended dose of chemical P (100 % P) compared to its inoculated counterpart showed that higher the amount of soluble form of P added through chemical P fertilizer, more is its chemical fixation in soil. At 120 d sampling, most available fraction of sodium bicarbonate (SB)-P was recorded in T4- 100 % P+ fungal inoculum. The values were much higher than 50 % chemical P + fungal inoculation. The treatment receiving fungal formulation + 100 % P ha^{-1} recorded 187.31mg P kg^{-1} soil, compared to 143.7 mg P kg^{-1} soil in its respective un-inoculated counterpart, thereby registering an increase of 30.43 % due to inoculation. Application of 100 % chemical P resulted in proportionately less availability of SB-P compared to ½ the recommended dose of chemical P, emphasizing the role of phosphate dissolving inoculants in improving the availability of chemically fixed soil P.

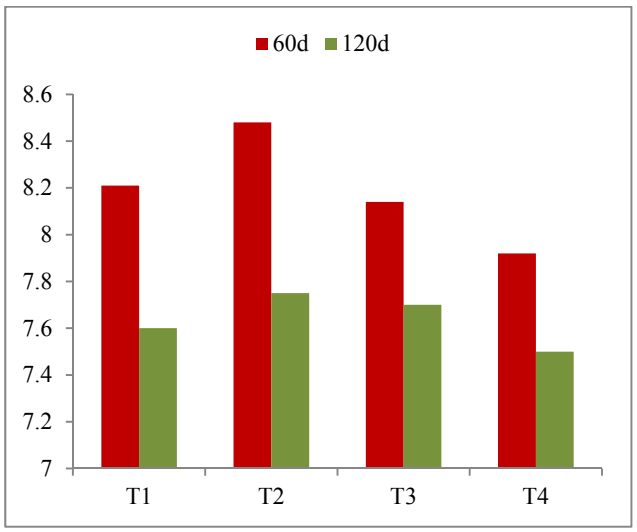

Fig. 4: Changes in pH of mustard grown soil

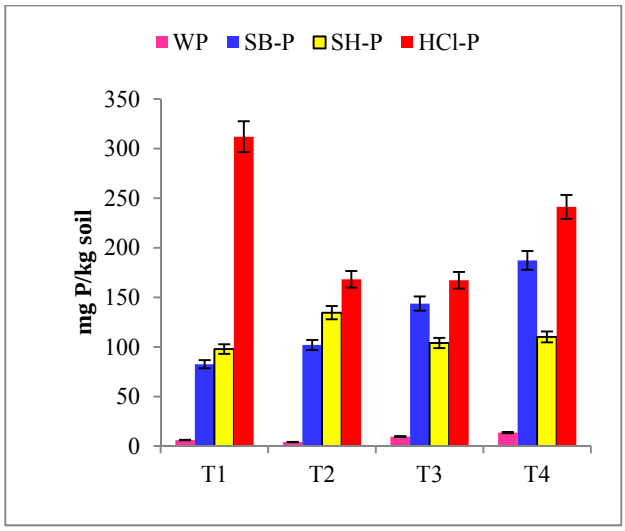

Fig. 5: Distribution of P in different fractions

T1- No seed inoculation + 50% P ha^{-1}, T2 - PSF seed inoculation + 50% P ha^{-1}, T3 - 100% P ha^{-1}, T4 - PSF seed inoculation @10.604 g/kg seed+ 100% P ha^{-1},

WP- water soluble-P, SB-P -sodium bicarbonate extractable-P, SH-P -sodium hydroxide extractable-P, HCl-P- acid extractable-P

The decline in P with crop growth reflected the P removal by plants (data not shown). More sodium hydroxide (SH)-P was extractable in treatments receiving 50 % recommended dose of P + fungal inoculums compared to its uninoculated control. No effect of inoculation was observed for SH-P in treatments receiving 100 % chemical P. The relationship between SH-P and pH showed that former was negatively related with pH of soil. The values for HCl-P ranged between 167 mg kg^{-1} to 312 mg kg^{-1} soil. The high values of HCl-P indicated the presence of Ca-containing minerals.

4. CONCLUSIONS

Phosphate dissolving fungi through their mechanism of action can improve soil P availability. By separating soil P into different fractions characterized by their mode of extraction, it was possible to identify the soil P fractions that get altered by crop management practices. Under mustard grown soil, the recommended dose of P and fungal inoculation could improve the P availability by >30 % over un-inoculated control.

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