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Residual effect of rock-phosphate and PSB on rice yield and soil properties

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ABSTRACT

Use efficiency of direct application of phosphorus (P) hardly exceeds 15-20% because of fixation to soils. The fixed-P could be utilized subsequently if it is brought to plant usable form. An experiment was conducted at Division of Soil Science and Agricultural Chemistry, ICAR-Indian Agricultural Research Institute, New Delhi during 2017–18 to investigate the residual effects of different P sources like di-ammonium phosphate (DAP), rock phosphate (RP), RP+ phosphorus solubilizing bacteria (PSB) and DAP+RP+PSB on rice yield, P uptake, P dynamics and enzyme activities in an Inceptisol. Results indicated that application of DAP_{30}+RP_{60}+PSB had 20.7, 24.3,19.6, 21.0, 28.1, 33, 15.2, 14.4 and 29.4% higher rice grain and straw yields, grain and straw P uptakes, available and saloid P, dehydrogenase, acid phosphatase and alkaline phosphatase activities respectively, as compared to DAP treatment. Thus, it can be inferred that application of DAP+RP+PSB can reduce fertilizer P consumption by 50%, and support the residual (second) crop better than conventional P-fertilizers, and improve soil available and saloid P status as well as enzymatic activities.

Keywords: Phosphorus, Phosphate solubilizing bacteria, Residual effect, Rice, Rock phosphate.

Low phosphorus (P) use efficiency (PUE) of conventional fertilizers has become a challenge in the era of intensive agriculture. Inadequate reserve of high-grade rock phosphate (HGRP, >30% P_2O_5) is forcing India to utilize low-grade rock phosphate (LGRP, <20% P_2O_5) as P source to crops. Earlier researches showed that LGRP could be utilized by partial acidulation (Biswas and Narayanasamy1995); organic acid loaded RP formulations (Roy *et al.* 2018) and developing polymer coated P-fertilizers (Sarkar *et al.* 2015, 2018, 2020). But, high cost in synthesis of new RP-formulations made us to think in a different way. Zhou *et al*. (1992) reported that only 0.1% of total P in soil is present in bioavailable form during residual fertility assessment. Thus, there is an opportunity to utilize the fixed-P by subsequent crops after converting them into plant usable forms. Here, application of phosphate solubilizing bacteria (PSB) can play a vital role as they may continue to solubilize P for the subsequent crops after the main crop. Use of PSB like *Pseudomonas* spp., *Bacillus* spp., *etc*. has the potentiality to solubilize fixed-P in soil and significantly increase available P in soil by producing organic acids (Roy *et al.* 2019, Sarkar *et al.* 2021). However, very scarce information is available on the residual effects of PSB and RP application on soil total P, inorganic P (IP), organic P (OP), microbial biomass P (MBP), and different fractions of inorganic P like saloid P (Sal-P), aluminium P (Al-P), iron P (Fe-P), calcium P (Ca-P) and reductant soluble P (Res-P). For this, we aimed to study the residual effect of LGRP and PSB on rice yield and P uptake; and to assess their influence on P dynamics among different soil P fractions and enzyme activities. We hypothesized that PSB will continue to solubilize P from RP and provide better residual effect as P source as compared to conventional fertilizer P.

MATERIAL AND METHODS

Bulk soil sample (0–15 cm) was collected from the experimental farm, ICAR-IARI, New Delhi (2017–18). Part of soil sample was processed and analyzed for physicochemical parameters. The other portion of soil was passed through 5-mm sieve and used for pot-culture experiment. Mechanical analysis was done following the procedure of Bouyoucos (1962). Soil pH and electrical conductivity was determined using soil: water ratio of 1:2.5 (Jackson 1973). Oxidizable organic C was determined by rapid titration method (Walkley and Black 1934). Available P was extracted with 0.5 M $NaHCO_3$ (*p*H 8.5) and determined spectrophotometrically using ascorbic acid blue colour method (Watanabe and Olsen 1965). Total P, IP and OP were estimated following the method outlined

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by Page *et al*. (1982). In sequence, further fractionation of IP namely, Sal-P, Al-P, Fe-P, Ca-P and Res-P was done following the modified P fractionation scheme of Kuo (1996). Phosphatase enzyme activity (PA), viz. acid PA and alkaline PA were determined by *p*-nitrophenol method (Tabatabai and Bremner 1969). Dehydrogenase activity (DHA) was determined following the protocol of Klein *et al.* (1971). Microbial biomass phosphorus (MBP) was determined using the procedure of Brookes *et al*. (1982).

The experimental soil was non-saline (EC 0.18 dS/m), alkaline (*p*H 8.4); and sandy-loam in texture (59.8% sand, 24.9% silt and 15.3% clay). Soil had organic C 4.1 g/kg; available P, total P, IP, OP, Sal-P, Al-P, Fe-P, Ca-P and Res-P were 9.5, 501,397,104, 9.12, 15.5, 16.8, 249.8 and 85.5 mg/kg, respectively; DHA 9.1 µg TPF/g soil/h, acid PA 67.2 µg PNP/g soil/h; and alkaline PA 134 µg PNP/g soil/h. Udaipur RP (100-mesh size), an indigenous LGRP, was used in this experiment. It contained 7.95% total P; 0.004% water-soluble P; 1.58% citrate-soluble P and 6.38% citrate insoluble P. Wheat was grown as main crop with five treatments namely, T_1 = Control; T_2= 60 mg P_2O_5 /kg soil from DAP (DAP_{60}); T_3= 120 mg P_2O_5/kg soil from RP (RP_{120}); T_4= 120 mg P_2O_5/kg soil from RP+ PSB (RP_{120}+PSB) and T_5= 30 mg P_2O_5 from DAP+ 60 mg P_2O_5/kg soil from RP+ PSB (DAP_{30}+RP_{60}+PSB) using completely randomized design.

Approximately 5 mL liquid PSB culture (*Pseudomonas striata,* ~10^8 cfu/mL) was used per pot (5 kg soil). Uniform doses of nitrogen (100 mg N) and potassium (60 mg K_2O) through urea and KCl were applied to each pot. After wheat, rice (*var* PB 1401) was grown on the same pots to assess the residual fertility. Continuous submergence was maintained during rice. Before rice, T_1, T_2, T_3, T_4 and T_5 correspondingly had 492, 525, 610, 603, 554 mg/kg total P; 399, 431,518, 521, 474 mg/kg IP; 92.8, 93.6, 91.9, 82.3, 79.7 mg/kg OP and 8.92, 13.5, 9.3, 11, 14.6 mg/kg available-P. From each treatment, soil was sampled at 40 days after transplanting (DAT), 70 DAT and maturity stage and analyzed for available-P. Soil samples collected after rice maturity was also analyzed for different soil P fractions and enzyme activities. After rice harvesting, grain and straw yields were recorded. Samples (grain and straw) were oven dried (60±5°C for 48 h), digested with di-acid (HNO_3:$HClO_4$ in 9:4) and analyzed P content in the acid extract spectrophotometrically using vanadomolybdo-phosphate yellow colour method (Jackson 1973).

Data generated from pot experiments were assessed through Analysis of Variance (ANOVA) for completely randomized design using SAS program. The least significance difference between the treatment means was calculated at P≤0.05 (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Application of RP (T_3) had non-significant impact on rice grain and straw yields as compared to unfertilized control (T_1) (Table 1). This is obvious because of low solubility of RP particularly in neutral to alkaline soil as source of P (Russell 1973). It was observed that rice grain and straw yields with RP+PSB treatment (T_4) were ~23.8 and 33.3% higher over those received from RP treatment (T3). Application of DAP+RP+PSB (T_5) recorded ~20.7 and 24.3% higher grain and straw yields, respectively over DAP treatment. The P uptakes from T_5 treatment by rice grain and straw was correspondingly ~19.6 and 21% higher over those obtained from T_2. Because applied P from fertilizer gets fixed to plant unavailable forms (Roy *et al.* 2018). On the other hand, inoculation of PSB resulted in continuous solubilization of P from RP and soil-fixed-P, simultaneously PSB also inhibited P-fixation. Thus, P availability increased to the crops compared to DAP. Ghosal *et al.* (2013) also reported that residual effect of Morocco RP was better over DAP to increase rice yield and P uptake.

At maturity stage (MAS), application of DAP_{30}+RP_{60}+PSB resulted ~28.1% higher soil available-P as compared to DAP_{60} treatment (Table 1). Heterotopic PSB produced organic acids like citric acid, oxalic acid etc. (Sarkar *et al.* 2021).These organic acids solubilized P from RP and soil by chelation, complexation of cations (Al^{3+}, Fe^{3+} and Ca^{2+}), ligand exchange of phosphate ions by organic anions (Khan *et al*. 2009) and increased soil available P. At 40 DAT, available P was moderate, but it was minimum at 70 DAT, which may be due to maximum crop

Table 1 Residual effect of different forms of applied P and phosphate solubilizing bacteria on yield, P uptake by rice and available P in soil

Treatment	Yield (g/pot)		P uptake (mg/pot)		Available P in soil (mg/kg soil)		
	Grain	Straw	Grain	Straw	40 DAT	70 DAT	Maturity stage
T_1	5.39^D	7.17^D	11.2^D	4.76^D	6.60^C	5.44^A	7.01^C
T_2	8.70^B	11.1^B	22.4^B	10.0^B	7.98^A	5.98^A	8.51^B
T_3	5.75^D	7.23^D	10.7^D	4.74^D	7.13^{BC}	6.01^A	7.12^C
T_4	7.12^C	9.64^C	16.1^C	7.96^C	7.58^{AB}	5.68^A	8.65^B
T_5	10.5^A	13.8^A	26.8^A	12.1^A	7.54^{AB}	5.66^A	10.9^A

T_1 = Control; T_2 = DAP @ 60 mg P_2O_5/kg soil (DAP_{60}); T_3= RP @ 120 mg P_2O_5/kg soil (RP_{120}); T_4 = RP @ 120 mg P_2O_5/kg soil + PSB (RP_{120}+PSB); and T_5 = DAP @ 30 mg P_2O_5 + RP @ 60 mg P_2O_5/kg soil + PSB (DAP_{30}+RP_{60}+PSB, DAT= Days after transplanting. Means having no common letter within each column are significantly different as per DMRT at P<0.05.

Table 2 Residual effect of different forms of applied P and phosphate solubilizing bacteria on different soil P fractions after rice

Treatment	Different forms of soil P (mg/kg soil)				Fractionations of inorganic P (mg/kg soil)				
	TP	IP	OP	MBP	Sal-P	Al-P	Fe-P	Ca-P	Res-P
T_1	461.6^{B}	370.6^{C}	91.1^{A}	2.50^{C}	6.38^{D}	11.0^{B}	15.6^{D}	225.6^{D}	63.5^{B}
T_2	470.1^{B}	380.2^{C}	89.9^{A}	2.81^{B}	8.74^{C}	13.8^{A}	22.0^{BC}	238.4^{CD}	68.0^{AB}
T_3	576.7^{A}	488.5^{A}	88.2^{A}	2.77^{BC}	6.75^{D}	11.3^{B}	20.7^{C}	275.6^{AB}	71.4^{A}
T_4	561.8^{A}	482.8^{A}	79.0^{B}	3.16^{A}	9.96^{B}	13.0^{A}	23.5^{AB}	278.8^{A}	70.0^{A}
T_5	499.4^{B}	422.9^{B}	76.5^{B}	3.14^{A}	11.62^{A}	13.5^{A}	24.7^{A}	252.2^{BC}	71.0^{A}

*Treatment details are given in the foot note of Table 1. Means having no common letter within each column are significantly different as per DMRT at $P<0.05$. TP=Total P, IP=Inorganic P, OP=Organic P, MBP=Microbial biomass P, Sal-P=Saloid P, Al-P=Aluminium P, Fe-P=Iron P, Ca-P=Calcium P, Res-P=Reductant soluble P.

P uptake at this stage and at maturity stage, it attained the maximum value, because different forms of soil P maintain a complex and dynamic equilibrium (Roy *et al*. 2018). In post-harvest soils, P removal by the crop stopped and the P in soil system approaches to the equilibrium again, this phenomena may transform P from other forms to available P. Left-over roots also contributes available P because after maturity phloem transport moves back some of the plant nutrients from shoot to root, and to some extent to the soil (Mengel and Kirkby 1987), this might be another reason for increased available P in post-harvest rice soils.

There was non-significant impact of DAP_{60} as compared to control in terms of total P (Table 2). However, application of RP in T_3 and T_4 significantly increased soil total P, IP, Ca-P and Res-P over soil treated with DAP_{60}. Roy *et al.* (2018) reported that P in RP is mostly Ca-bound P, therefore, RP application had significantly increased soil total P, IP and Ca-P over DAP_{60} application and unfertilized control. The PSB resulted in higher quantity of MBP. In contrary, PSB application decreased soil organic P as compared to DAP_{60}.The treatments RP_{120}+PSB and DAP_{30}+RP_{60}+PSB treatments correspondingly had ~13.8 and 17.5% lower organic P, and 12.5 and 11.7% higher MBP as compared to DAP_{60} treatment. The PSB inoculation increased soil microbial population, which may immobilize soil P in their body and increase MBP. Overall, PSB inoculation increased

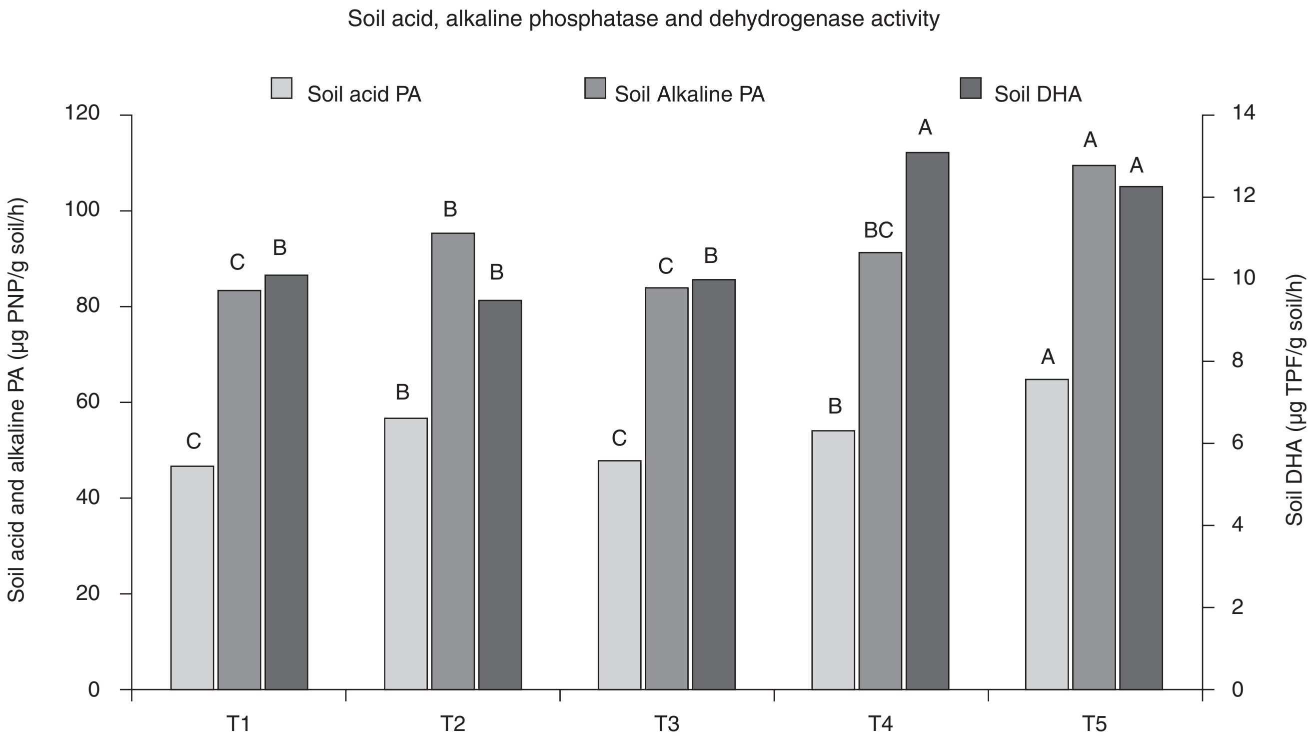

Fig 1 Residual effect of different forms of applied P and phosphate solubilizing bacteria on soil acid, alkaline phosphatase and dehydrogenase activity. (PA= Phosphatase activity, DHA= Dehydrogenase activity). Means having no common letter within each column are significantly different as per DMRT at $P<0.05$.
*Treatment details are given in the foot note of Table 1.

the inorganic P fractions (Table 2). Production of organic acids from PSB applied treatments may help in protonation and subsequently help to solubilize different inorganic but fixed P-fractions. The PSB produces phosphatase enzymes, which mineralizes organic P and converts it to plant usable mineral P (Roy *et al.* 2019, Sarkar *et al.* 2020), thus OP in PSB inoculated soils got reduced.

Solubilization of RP-P and mineralization of organic P by PSB in T_4 and T_5 treatments might have caused significant increase of Sal-P. Application of PSB significantly increased acid and alkaline PA (Fig 1). It was also found that RP_{120}+PSB and DAP_{30}+RP_{60}+PSB treatments, respectively had ~37.8 and 29.4% higher alkaline PA as compared to DAP_{60} treatment. This result aligned with the findings of Tarafdar and Jungk (1987), who had reported that PSB produce phosphatase enzymes effectively. This may be the reason behind increased acid and alkaline phosphatase activity under T_4 and T_5 treatments. Soil DHA also increased due to PSB application along with RP and DAP (Figure 1). DHA reflects the oxidative activity of all the soil micro-flora. The RP_{120}+PSB treated soil (T_4) had ~8.8% higher dehydrogenase activity as compared RP_{120} treated soil (T_3). Application of DAP_{30}+RP_{60}+PSB (T_5) had ~15.2% higher DHA as compared to DAP_{60} (T_2). Application of PSB in T_4 and T_5 treatments may have increased microbial population, which may increase DHA (Biswas *et al*. 2019).

From the above results and discussion, it is demonstrated that application of DAP_{30}+RP_{60}+PSB resulted in better residual effect to rice; hence, increased the rice yield and P uptake. Application of DAP_{30}+RP_{60}+PSB also increased soil available-P, saloid-P, microbial biomass P, soil dehydrogenase activity, and acid and alkaline phosphatase activity as compared to treatment receiving DAP alone. Overall, application of DAP_{30}+RP_{60}+PSB can reduce the rate of phosphatic fertilizers by 50% without hampering yield and nutrient uptake. Thus, it may be concluded that the low-grade rock phosphate along with phosphate solubilizing bacteria (PSB) can substitute chemical P fertilizer (DAP) by 50% for growing rice on residual fertility in Inceptisol.

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