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Full Length Article

Effectiveness of Various Approaches to Use Rock Phosphate as a Potential Source of Plant Available P for Sustainable Wheat Production

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Abstract

Low bioavailability of P in soil from the applied phosphatic fertilizers due to fixation/precipitation is considered the most critical factor in limiting optimum crop yields Thus availability of alternate and cheap P resources is imperative for sustainable crop production and is a dire need of the hour. Rock phosphate (RP) is a cheap source of P but cannot be used directly as a soil amendment because of its very poor water solubility (0.1%). However, the bioavailability of RP-P can be enhanced by complexing it with compost and/or through the use of specific bioinoculants. A series of experiments were conducted to assess the effectiveness of various approaches to solubilize RP-P and the impact of bioavailable RP-P on growth and yield of wheat under wire house and field conditions. Soil was spiked with RP, RP + compost, RP-enriched compost (RP-EC) and RP-EC+PSB, to determine the release of bioavailable P from RP for 15 weeks. Results revealed that RP-EC + PSB combination resulted in maximum release of plant available P, followed by RP-EC and RP + compost in descending order. For pot and field trials on wheat, RP-EC containing 25% or 50% of the P requirement of the crop was used and balance amount of P was supplied through mineral P fertilizer. Specific bioinoculants (PSB and ACC-deaminase containing PGPR) were either added to RP-EC or used to inoculate wheat seeds to enhance P acquisition. Application of 50% RP-EC inoculated with both PSB and ACC-deaminase PGPR proved to be the best in promoting growth and yield of wheat in both pot and field trials. Similarly P contents of grain and straw in both pot and field trials were improved significantly. Other treatments also produced higher yield contributing traits than uninoculated NPK control but with relatively less proficiency. The results of these studies may imply that RP-EC inoculated with novel PGPR proved to be a viable approach to use low grade RP and organic waste for persistent crop production as well as for supporting healthier environment.

Keywords: PSB; ACC-deaminase; Rock phosphate; RP-EC; Wheat

Introduction

Phosphorus (P) is essentially required for biological growth and development.Its significance is apparent in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch and transportation of genetic traits (Wu *et al.*, 2005). After application of phosphatic fertilizers to soil, a significant amount of P is rapidly transformed into less available forms due to fixation/precipitation which results in limiting crop yields (Vance *et al*., 2003; Harris *et al.*, 2006).Costs of soluble P fertilizers in developing countries including Pakistan are increasing rapidly. Another serious problem is low recovery (10–30%) of applied phosphatic fertilizers (Nisar, 1985). Under such circumstances, it becomes imperative to search for alternative cheap phosphorus sources.

Rock phosphate (RP), a naturally occurring mineral source of insoluble phosphate is much less expensive than soluble phosphatic fertilizers. The problem with RP is its low solubility, particularly in non-acidic soils (Kennedy and Smith, 1995; Caravaca *et al.*2004). Extensive work has been conducted to use RP as P source by employing different strategies for solubilization of RP-P in soil. The most extensively applied approach has been the use of specific microorganisms capable of solubilizing insoluble form of P, commonly called as phosphate-solubilizing microorganisms (PSB). PSB can transform the insoluble P to soluble forms by acidification, chelation, exchange reactions, and polymeric substances formation (Delvasto *et al*., 2006). It has been reported that *Pseudomonas, Bacillus* and *Enterobacter* spp. possess the ability to bring insoluble soil phosphates into soluble forms by secreting acids such as formic, acetic, propionic, lactic, glucolic, fumaric and succinic (Puente *et al*., 2004; Parassana *et al*., 2011). These acids lower the pH (Rodriguez *et al.*, 2006) which increases the dissolution of RP. Numerous studies have documented the solubilization of insoluble mineral phosphates by PSB thereby promoting growth and yield of different crops (Canbolat *et al*., 2006; Gyaneshwar *et al*., 2006).

Another strategy could be the use of plant growth promoting rhizobacteria (PGPR), which help in greater

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proliferation of root growth with greater surface area to explore more soil volume for better uptake of less mobile nutrient like P (Dey *et al*. 2004). Several studies have shown tremendous increase in root growth in response to inoculation with PGPR containing ACC- deaminase activity (Shaharoona *et al*., 2006; 2007; Saleem *et al*., 2007; Shahzad *et al*., 2010; Zabihi *et al*., 2010) most likely through decrease in ACC-dependent ethylene synthesis in plant roots (Glick *et al*., 1998). Co-inoculation of PSB and ACC-deaminase PGPR may have additive impact on plant growth through solubilization of insoluble P in soil by the former and efficient uptake of P due to prolific root growth by the latter bioinoculants. Third strategy could be the use of compost which can help in solubilization of insoluble P such as RP-P. Compost as a soil amendment not only improves the physical and chemical conditionsof the soil but also have a positive effect on the nutrient status of the plant (Ahmad *et al*., 2006). Additionally the solubilization of RP-P by organic acids released from compost is another major advantage (Singh and Amberger, 1991). A lot of work on the combined use of RP and PSB has been reported in existing literature, however, very little or no work has been done on the simultaneous application of RP + PSB + compost to supply P to plants in desired amount. Similarly preparation of RP-enriched compost (RP added during composting process) as well as RP-enriched compost inoculated with PSB has not been undertaken. Thus, this study was planned to compare the effectiveness of various approaches including use of specific bioinoculants (PSB alone or in co-inoculation with ACC-deaminase PGPR), compost, compost enriched with RP (RP-EC) or RP-EC inoculated with bioinoculants for promoting release of plant available RP-P and to assess the impact of these approaches on growth and yield of wheat in the presence of RP.

Materials and Methods

A series of laboratory, wire house and field experiments were conducted at Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad (UAF), Pakistan to assess the effectiveness of different approaches for the solubilization of phosphorus from RP for improving growth and yield of wheat.

Selection of Rhizobacteria and Inocula Preparation

Pre-isolated and identified strains of rhizobacteria containing ACC-deaminase (*Pseudomonas putida* biotype A*)* and P solubilizing traits (*Bacillus subtilis*) obtained from Soil Microbiology and Biochemistry Lab, UAF were used for lab, pot and field trials. The inocula of *P. putida* biotype A and *B. subtilis* were prepared by inoculating the sterilized broth with specific isolates in the 250 mL conical flasks having DF minimal salt medium[KH_2PO_4, 4.0 g; Na_2HPO_4, 6.0 g; $MgSO_4.7H_2O$, 0.2 g; glucose, 2.0 g; gluconic acid, 2.0 g; citric acid, 2.0 g; $(NH_4)_2SO_4$, 0.67 g and micronutrient solution 2 ml L^{-1}] (Dworkin and foster, 1958) and NBRI-P medium [glucose, 10.0 g: $Ca_3(PO4)_2$, 5.0 g; $MgCl_2.6H_2O$, 5.0 g; $MgSO_4.7H_2O$, 0.25 g; KCl, 0.2 g and $(NH_4)_2SO_4$, 0.1 g L^{-1}] (Nautiyal, 1999), respectively. The flasks were incubated at 28±1ºC for 48 h in the orbital shaking incubator (Model OSI-503 LD, Firstek Scientific, Japan) at 100 rev min^{-1}. Optical density (0.5) of broth containing PGPR was maintained at 535 nm by dilution to maintain uniform cell density (10^8–10^9 cfu mL^{-1}) for seed inoculation. Inoculated seeds were kept overnight for drying.

Preparation of RP-enriched Compost (RP-EC)

Organic materials containing fruit and vegetable waste were collected from the fruit market of Faisalabad, sorted out to remove unwanted substances (plastic bags, stones, glass materials, cork etc.) air dried, and then oven dried at 70°C for 24 h. Oven dried material was ground in an electrical grinder and transferred to a locally fabricated composting unit (500 kg capacity). Composting process was carried out for seven days under controlled temperature (ranging from 30 to 70°C and then 30°C), moisture (40% w/v) and aeration (shaking at 50 rev min^{-1}). The temperature of processing unit ranged between 30 and 70°C during 3rd and 4th day and decreased gradually to 30°C after fourth day of processing. Batches of compost were prepared with and without enrichment (@ 25 and 50% of the crop requirement of P (90 kg P_2O_5 ha^{-1}) with suitable amounts of RP (28% P_2O_5) during the composting period. After seven days of incubation, composted material was passed through a granulator. The air dried granulated compost was packed in gunny bags prior to use in pot and field trials. Compost was applied @ 500 kg ha^{-1}. Analysis of 25 and 50% RP enriched compost is given in Table 1. The concentration of N, P and K present in the compost was taken into the account for the application of chemical fertilizers as a source of N, P and K.

Solubilization for RP-P in Amended Soil

A laboratory study was conducted to check the release of plant available P from different combinations of RP in soil. Sandy clay loam soil having pHs 7.9, ECe 2.2dS m^{-1}, organic matter 5.01 g kg^{-1}, soil available P 3.76 mg kg^{-1} and K 92.0 mg kg^{-1} was used. Soil (500 g soil, passed through 2 mm sieve) was taken in plastic beakers and spiked with different treatments containing 100 g RP-P kg^{-1}soil, the treatments include RP only, RP + compost (100:112), RP-EC (100:112), RP + PSB and RP-EC + PSB, to determine the release of bioavailable P from RP.Ten mL inoculum of PSB (*B. subtilis*) was applied in RP + PSB and RP-EC + PSB treatments. Control without any spiking was kept for comparison. Soil was maintained at 50% water holding capacity throughout the experiment. Beakers were incubated at 28±1°C. Moist soil samples were drawn from each beaker after every week up to 15 weeks and analyzed for 0.5 M $NaHCO_3$ (pH 8.5) extractable P. For P determination 5 g soil was taken in 250 mL Erlenmeyer flask and added

100 mL of 0.5 M $NaHCO_3$ solution and placed on a shaker at 300rpm for 30 min. After filtering the solution through Whattman No. 40 filter paper, 10 mL of clear filtrate was pipetted into a 50 mL volumetric flask followed by addition of 8 mL color developing reagent carefully to prevent loss of sample due to excessive foaming and made the volume up to 50 mL with deionized water. After 10 min. Color intensity was measured on spectrophotometer (Beckman photometer 1211) at 880 nm (Ryan *et al*. 2001). The trial was laid out in CRD with four replications.

Seed Inoculation

Seeds of wheat (Sehar-2006) and maize (DK-919) were surface sterilized by dipping in 95% ethanol solution for five min., 0.2% $HgCl_2$ solution for 3 min., and washed thoroughly with sterilized water. Then seeds were inoculated with peat based slurry inoculated with respective PGPR. The peat based slurry for inoculation of 100 g seed consisted of 50 g sterilized peat, 50 g sterilized clay (50:50), 10 mL sugar solution (15%) and 10 mL inoculum. The seeds were shaken well for fine coating. Control was treated with sterilized peat plus clay containing sterilized broth and sugar solution. Inoculated seeds were placed overnight for drying under lab conditions.

Pot Trial

Pot experiments were conducted to evaluate the effect of plant growth promoting rhizobacteria and RP enriched compost on growth, yield and P nutrition of wheat (*Triticum aestivum* L.cv. Sehar-2006). Surface disinfected seeds were inoculated with selected isolates of PGPR. There were three replications for each treatment. Six seeds were sown in pots containing 12 kg of soil, which were thinned to three plants pot^{-1} after germination. Recommended doses of N, P and K @ 120, 90 and 60 kg ha^{-1} respectively were applied. All P and K were applied as basal dose in all treatments, while, half N was applied at the time of sowing and remaining half N was applied at first irrigation. P was applied @ 90 kg ha^{-1} at sowing time from various P sources (SSP, RP-EC) with the following treatment plan.

T_1 = NPK [all as chemical fertilizer (CF)]
T_4 = NPK + PSB
T_7 = NPK + PSB + ACC-deaminase
T_2 = NP (25% RP-EC + 75% CF) K*
T_3 = NP (50% RP-EC + 50% CF) K*
T_5 = NP (25% RP-EC + 75% CF) K + PSB
T_6 = NP (50% RP-EC + 50% CF) K + PSB
T_8 = NP (25% RP-EC + 75% CF) K+ PSB + ACC-deaminase
T_9 = NP (50% RP-EC + 50% CF) K + PSB + ACC-deaminase

* 25% and 50 % RP-EC was prepared in accordance with 90 kg P_2O_5 ha^{-1} requirement for wheat.

* All the treatments were adjusted to receive same amount of NPK which was applied in T_1.

Data regarding growth and yield parameters i.e. plant height, number of tillers , number of grains per spike, 100-grain weight, biomass, straw and grain yield were recorded pot^{-1} at maturity, while P-contents in grain and straw, available P in soil were determined at harvest.

Field Trial

The treatments plan used in pot trial was also applied in the field to further confirm the results of pot trial. Data regarding growth and yield parameters was recorded at maturity. The plant and soil samples collected at harvest were analyzed for P content in plants and available P status in soil.

Soil and Plant Analysis

Available P in soil was extracted with 0.5 M $NaHCO_3$. Phosphorus in the extract was determined by spectrophotometer (Watanabe and Olsen, 1965). NH_4OA_C extractable K was determined by the flame photometer PFP-7 using the (Method 6.1.3) described by Rashid *et al*. (2001).

For plant analysis, sulfuric acid and hydrogen peroxide method was used for digestion of plant material as described by Wolf (1982). For P determination procedure was used as mentioned by Ryan *et al*. (2001).

Compost Analysis

Carbon in RP-EC was estimated by loss-on-ignition method defined by Nelson and Sommers (1999) and Ryan *et al*. (2001). Total N in enriched compost was determined by Kjeldahl distillation method described by Jackson (1962).For P determination, 1.0 g of dried and ground compost sample was digested in 25 mL of concentrated HNO_3 followed by 20 mL of 60% $HClO_4$ and total P content in the acid digest was determined by spectrophotometer (Beckman photometer 1211) after developing the vanadomolybdo-phosphoric yellow color complex in nitric acid medium (Jackson, 1973). K content in the filtrate was determined by Jenway PFP-7 flame photometer described by (Table 1).

Statistical Analysis

Statistical analysis was run according to CRD factorial design in axenic and pot experiments. While statistical analysis of field experiments was performed by using RCBD factorial design. The data was analyzed statistically by using computer software Statistix 8.1. Least significant difference (LSD) test was employed to separate the treatment means ($P<0.05$).

Results

Solubilization of RP-P in Amended Soil

The results of laboratory incubation study revealed thatdifferent soil amendments had variable effects on solubility of RP-P. In general release of plant available P from RP increased up to 13 weeks of incubation and afterwards a slight decrease was noticed (Fig. 1). Among the treatments, RP-EC+PSB proved to be the most effective and released a maximum of 36.25 mg kg^{-1} P from RP at the end of 13^{th}week. Next to it was RP-EC (26.6 mg kg^{-1}) followed by RP + compost (19.2 mg kg^{-1}) and RP + PSB (12.7 mg kg^{-1}) in descending order. Release of P in soil was hardly recorded in case of spiking with RP only. At the 13^{th} week of incubation, soil amendment with RP-EC+PSB enhanced the release of P from RP by 6 folds over that where only RP was added to soil. Similarly spiking with RP-EC resulted in 4.4 folds higher plant available P from RP compared to that recorded in case of RP spiking only. Soil amendment with RP + compost and RP + PSB also substantially increased bioavailable P in soil compared to that observed in case of soil spiking with RP only.

Effect of PGPR and Rock phosphate enriched Compost on Growth and Yield of Wheat

In response to the results of laboratory incubation study different combinations of treatments were tested in pot and field trials on wheat to further confirm the validity of enhanced availability of P from RP-EC in the presence or absence of PGPR. Impact of these treatments on different growth parameters of wheat grown in pots and field conditions is evident from the data summarized in Tables 2, 3 and 4.

Statistical analysis showed significant differences in the growth parameters as influenced by various treatments. Seed inoculation with PSB or PSB + ACC-deaminase PGPR promoted plant height compared to uninoculated NPK control. Substitution of 25% of phosphatic fertilizer with RP-EC slightly improved the plant height, while 50% substitution reduced the plant height over NPK control. However, when these RP-EC substitutes were combined with seed inoculation either with PSB or PSB + ACC-deaminase PGPR, plant height further improved but were statistically at par with NPK control and RP-EC only. Maximum improvement in plant height was recorded with 50% RP-EC+ PSB + ACC-deaminase. Similar results were recorded in field trial as well (Table 2 and 3).

Co-inoculation of PSB + ACC-deaminase PGPR markedly promoted grains per spike of wheat compared to uninoculated NPK control. Replacement of 25% of phosphatic fertilizer with RP-EC further improved, while 50% replacement decreased grains per spike over NPK control. However, substitution with RP-EC in combination with bioinoculants (PSB or PSB +ACC-deaminase PGPR) resulted in significant increase in grains per spikeas compared to NPK control and RP-EC only. Highest grains per spike of wheat grown in pots were recorded with the application of 50% RP-EC+ PSB + ACC-deaminase. Similar and highly promising results regarding grains per spike were also observed under field conditions (Table 2, 3).

Table 1: Analysis of rock phosphate enriched compost (RP-EC)

Parameters	25% RP-EC	50% RP-EC
Carbon %	27.50	25.50
Nitrogen %	1.75	1.70
Phosphorus %	1.36-1.81	1.71-2.28
Potassium %	1.80	1.78
C:N ratio	15.71	15.00
C:P ratio	15.19-20.22	11.18-14.91
C:K ratio	15.26	14.32
pH	4.56	4.46

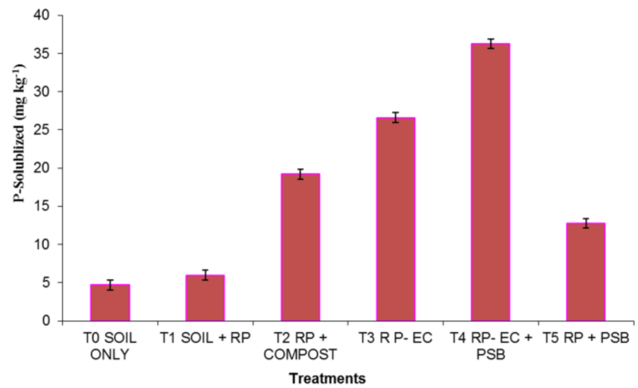

Fig. 1: Solubilization of RP-P in soil after 13 weeks
RP = Rock phosphate, RP-EC= Rock phosphate enriched compost, PSB= *B. subtilis*

Data regarding 100/1000-grain weight, biomass and grain weight/yield (Table 2 and 3) illustrates statistical significance of integrated application of bioinoculants with RP-EC substitutes over NPK control in both pot and field trials of wheat. Maximum increase in all the parameters was recorded in 50% RP-EC+PSB+ACC-deaminase treatment.

PSB or PSB+ACC-deaminase inoculation significantly improved straw weight of wheat over uninoculated NPK control. Substitution of 25% P fertilizer with RP-EC improved the straw weight over NPK control while 50% substitution resulted in significant decrease over control. However combined application of RP-EC and inoculation with PSB+ACC-deaminase PGPR produced significantly higher straw weight per potas compared to NPK control and RP-EC alone (Table 2). In case of field trial, straw yield in combined application of RP-EC and inoculation with PSB + ACC-deaminase PGPR though improved as compared to NPK control but results were statistically non-significant (Table 3).

Effectiveness of various treatments on root growth (root length and root dry weight) of wheat grown in pots is also summarized in Table 3. Statistically significant results were obtained regarding various treatments. Seed inoculation with PSB+ ACC-deaminase significantly improved root length and root dry weight of wheat over uninoculated NPK control.Addition of 25%RP-EC as P fertilizer increased root length and enhanced root dry weight, while 50% RP-EC reduced the root growth as

Table 2: Effect of PGPR and rock phosphate enriched compost (RP-EC) on plant height, grains per spike, 100-grain weight, biomass, grain weight, straw weight, root length, root dry weight of wheat grown in pots

Treatments	Plant height (cm)	Grains spike^{-1}	100-grain weight (g)	Tillers (pot^{-1})	Biomass (g pot^{-1})	Grain weight (g pot^{-1})	Straw weight (g pot^{-1})	Root length (cm)	Root dry weight (g)
T 1	73.5	46.4de	4.07d	15.0ef	40.7fg	17.0cd	23.6ef	22.3fg	4.76cd
T 2	76.3	49.3cd	4.48bcd	16.3de	43.0def	17.5bcd	25.5cde	24.3eve	5.33c
T 3	77.2	53.0bc	4.63bc	17.3bcd	45.1cde	17.6bc	27.5bcd	26.9cd	6.59b
T 4	75.1	48.6cd	4.31cd	16.7cde	42.4d	17.4bcd	24.9def	24.0eve	5.30cd
T 5	73.8	43.5e	3.53e	14.3f	38.5g	16.4d	22.1f	19.5g	4.57d
T 6	77.5	54.6ab	4.74bc	18.0bcd	46.2bcd	18.0bc	28.2abc	29.7cd	6.53b
T 7	79.6	56.3ab	4.94ab	18.7ab	48.4abc	18.3b	30.1ab	33.9ab	6.63b
T 8	80.3	57.3ab	4.94ab	18.3abc	48.9ab	18.5ab	30.4a	31.7bc	6.86ab
T 9	84.2ns	59.0a	5.25a	19.6a	50.3a	19.6a	30.7a	35.5a	7.40a

Table 3: Effect of PGPR and rock phosphate enriched compost (RP-EC) on plant height, grains per spike, 1000-grain weight, biomass, grain yield, straw yield of wheat under field conditions

Treatments	Plant height (cm)	Grains spike^{-1}	1000-grain weight (g)	Tillers (m^{-2})	Biomass (t ha^{-1})	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
T 1	91.1	47.3d	39.0de	310de	16.5ef	5.3d	11.2
T 2	94.2	51.0cd	43.9cd	341cd	17.5cde	5.8cd	11.7
T 3	96.8	53.3c	46.0bc	378bc	18.4bcd	6.2bc	12.2
T 4	93.2	50.7cd	44.5c	341cd	17.3de	5.6d	11.7
T 5	91.2	47.2d	37.7e	278e	15.5e	4.7e	10.7
T 6	96.6	54.6bc	47.7bcd	389bc	18.6bc	6.2bc	12.4
T 7	98.7	59.4a	49.8ab	407b	19.3ab	6.4b	12.9
T 8	98.2	59.1ab	48.3abc	421b	19.2ab	6.6ab	12.6
T 9	100.8NS	62.2a	52.0a	484a	20.0a	7.0a	13.0NS

Table 4: Effect of PGPR and rock phosphate enriched compost (RP-EC) on P-uptake in wheat grain, straw and soil available P after harvest in pot and field trials of wheat

Treatments	P-uptake pot (mg pot^{-1})		P- uptake field (kg ha^{-1})		Soil available P (mg kg^{-1})	
	grain	straw	Grain	straw	Pot	Field
T 1	58.0 fg	43.8 f	21.1e	24.4 fg	8.20	8.25
T 2	73.4 de	58.5 e	24.9 cd	26.9 eve	8.42	8.52
T 3	77.5 cd	71.9 cd	27.2 c	30.6 de	8.45	8.53
T 4	66.2 ef	55.5 e	23.3 de	27.0 ef	8.32	8.32
T 5	50.6 g	39.1f	15.7 f	20.5 g	8.20	8.12
T 6	79.4 bcd	70.2 cd	27.9 c	32.3 cd	8.73	8.80
T 7	84.5 bc	80.2 bc	31.5 b	36.0 bc	8.53	8.62
T 8	87.1 b	84.0 b	34.4 b	36.5 ab	9.10	8.98
T 9	99.9 a	95.3 a	38.6 a	40.2 a	8.62NS	8.88NS

T_1 = NPK [all as chemical fertilizer (CF)]; T2 = NPK + PSB;T_3 = NPK + PSB+ ACC-deaminase; T_4= NP (25%RP-EC+ 75% CF) K; T_5 = NP (50% RP-EC+ 50% CF) K; T_6 = NP (25% RP-EC+ 75% CF) K + PSB; T_7 = NP (50%RP-EC+ 50% CF) K+ PSB; T_8 = NP (25%RP-EC+ 75% CF) K+ PSB+ACC-deaminase; T_9 = NP (50% RP-EC+ 50% CF) K+ PSB+ ACC-deaminase; PSB = *Bacillus subtilis;* ACC-deaminase = *Pseudomonas putida* biotype A, Means sharing the same letter(s) in a column do not differ significantly according to LSD test (p<0.05)

compared to NPK control. Integrated application of RP-EC and bioinoculants (PSB + ACC-deaminase PGPR) significantly improved the root growth parameters over NPK control as well as over RP-EC only. Maximum increase in root length and root dry weight was recorded in 50% RP-EC+PSB+ACC-deaminase inoculation followed by 50% RP-EC+ PSB and 25% RP-EC+ PSB + ACC-deaminase, respectively.

Influence of different treatments on P-uptake of wheat grain and straw and soil available P of wheat at harvest under pot and field conditions is summarized in Table 4. Similar to previously discussed parameters P-uptake in grain and straw improved with bioinoculants application over NPK control. With the substitution of 25% RP-EC as P source along with phosphatic fertilizer, P-uptake improved while 50% RP-EC produced non-significant results regarding P-uptake over NPK control. However, when these RP-EC substitutes were combined with bioinoculants the impact on P-uptake (grain and straw) was much stronger and statistically significant over NPK control and RP-EC only. Maximum P-uptake in grain and straw of wheat grown in pots was recorded when 50% RP-EC + PSB + ACC-deaminase PGPR were collectively applied. Identical and extremely promising results regarding P-uptake (grain and straw) were also observed under field conditions. Regarding soil available P at harvest, available P increased with bioinoculants application in combination with RP-EC but the interaction was statistically non-significant in pot trials. Similar trend was documented under field conditions.

Discussion

In order to assess the release of RP-P in soil, incubation study was conducted for 15 weeks. Soil was spiked with RP, RP + PSB (*B. subtilis*) RP + compost, RP-EC and RP-EC+PSB to determine available P in soil for 15 weeks. Addition of RP alone did not have any effect on bioavailable P contents of soil. However, bioaugmentation of soil with PSB resulted in release of bioavailable P from RP throughout the incubation period. This might be due to release of organic acids by PSB, which reduced the pH and increased the dissolution of RP and indigenous insoluble P. The results are in line with the findings of Banik and Dey (1981) that when RP was applied along with inocula of *Bacillus* and *Penicillinm sp*. P availability in soil was improved.

RP and compost improved the release of RP-P and/or indigenous insoluble P. It is very likely that compost served as a rich source of C for the indigenous microbes as well as PSB present in soil and decomposition of compost produced organic acids which enhanced the release of available P from RP and indigenous insoluble P. The compost also supported greater population of PSB, which might have also acted more efficiently. In this regard, Pramanik *et al.* (2009) reported that combined application of RP and vermicompost improved P-content as compared to sole application of either RP or vermicompost in soil. In our trial, soil spiking with RP enriched compost (RP-EC) further enhanced the dissolution of RP and soil indigenous insoluble P and improved the P release in the soil for plant use, which corroborates the findings of Imran *et al*. (2011). However, the maximum release of RP-P in soil was observed in case of soil amendment with RP-EC + PSB which might be due to additive effect of RP-EC and PSB; both simultaneously released organic acids during composting process as well as during soil incubation, which released maximum bioavailable P in soil. Biswas and Narayanasamy (2006) reported that RP-EC inoculated with *A. awamori* solubilized higher amount of available P in soil as compared to uninoculated RP-EC throughout the incubation period. In our incubation study, P release increased up to 13 weeks, this might be due to greater production of organic acids, which slowed down afterwards and solubilized P started to be converted into insoluble form.

Pot and field trials on wheat were conducted to assess impact of various organic amendments and inoculation with PSB and ACC-deaminase PGPR on P bioavailability from RP applied as partial substitution of soluble P as SSP. Replacement of soluble phosphatic fertilizer up to 25% with RP-EC proved better strategy than NPK control in both pot and field trials in improving growth and yield of wheat. This may imply that 25% RP-EC enhanced the supply of available P for plant uptake by acting as a source of available P but also by reducing the fixation and insolubilization of P (75%) added as commercial soluble phosphatic fertilizer. Moreover, 75% soluble phosphatic fertilizer fulfilled the initial requirements of the crop and addition of 25% RP-EC ensured the increased supply of P gradually during the growth period. However, 50% substitution of soluble phosphatic fertilizer with RP-EC was relatively less effective than 100% soluble phosphatic fertilizer (NPK control). This might be due to less bioavailable P from RP in the beginning and plant growth was slightly suppressed from less supply of P as only 50% P was added as soluble phosphatic fertilizer. Comparison of 25% and 50% RP-EC indicated that former treatment is more effective than the later one most likely due to more addition of soluble P in former case. In case of 50% RP-EC substitution, relatively less growth might be due to the addition of less soluble P or less P released from RP-EC for plant uptake and/or more assimilation of P by soil microbes.

Inoculation with PSB had positive impact under all the fertilizer combinations (NPK control, 25 and 50% RP-EC) which can be inferred from yield and associated traits. This positive impact of PSB inoculation could be attributed to relatively less insolubilization/precipitation of the applied soluble P and/or due to solubilization of indigenous insoluble P fraction. In their studies, Kumar *et al*. (2001), Chen *et al*. (2006) and Adesemoye and Kloepper (2009), found that PSB bioinoculants could be used as cost-effective input to increase crop production with soluble phosphatic fertilizers through reduction in phosphate fixation in calcareous soils which in turn increase the efficiency of applied P (Ekin, 2010). Interestingly, PSB inoculation proved more effective when 25% or 50% of the phosphatic fertilizer was replaced with RP-EC. This appears to be due to greater activity of PSB as supplemented by the compost which resulted in more release of P for plant uptake from the added or indigenous P. Moreover, in the presence of compost, the applied soluble NPK might have remained available gradually for plant uptake over a long period.

It has been reported that RP enriched manures maintain higher levels of P in soil solution for a longer period than the fertilizer alone. The higher yield with enriched compost treatments might be due to improvement in microbial activities, better supply of secondary and micronutrients, which were not provided by 100% chemical fertilizer (Cheuk *et al*., 2003; Singh *et al*. 2004). Secondly, high response of PSM inoculation to crops was observed in previous studies, which indicated that inoculation with PSM can increase the efficacy of fertilizer even at low rates (Ekin, 2010; Zabihi *et al*., 2010). Enhanced P bioavailability might be due to production of organic acids which lower the pH and also chelate cations attached with phosphate rendering P available for plant use, which exerted its influence on plant growth as well as availability of soluble P. Likewise, Afzal *et al.* (2005) documented that PSM in combination with phosphorus fertilizer and organic manure significantly improved seed phosphorus content, tillers per unit area, grain and biological yield of wheat. The reason in yield promotion using PSB is the release of nutrients from compounds in soil through organic acid and phosphatase activity resulting in greater availability of nutrients to plants (Jutur and Reddy, 2007). Inoculation with PSB was even more effective in case when 50% P was applied as RP-EC. This might be due to slow and gradual release of RP-P for plant uptake over a longer period than 25% P added as RP-EC. Patil *et al*. (2011) reported that higher dose of rock phosphate with PSB applied along with compost might have resulted in higher availability of P due to better mineralization of nutrients. The increased yield attributing characters and yield in higher levels of rock phosphate with PSB and their interaction with organic manures were attributed to the an increased availability of P thus effecting yield attributes of chick pea (Kushwaha, 2007). These results substantiate the cost effectiveness of RP and PGPR (Zabihi *et al*., 2010).

Additive positive effect of dual inoculation of PSB and ACC-deaminase PGPR was observed in both pot and field trials on growth and yield parameters of wheat compared to single PSB inoculation under all the fertilizer treatments. This improvement in growth and yield parameters in dual inoculation treatments might be due to enhanced nutrients availability, due to increased root biomass and P uptake by plant. Shaharoona *et al*. (2008) found that efficiency of nutrients was improved with inoculation of *P. fluorescens* (ACC-deaminase PGPR) due to enhanced root growth and resulted in effective uptake of nutrients by plants.

Soil available P after harvest under pot and field trials showed an increasing trend in response to RP-EC and bioinoculants compared to NPK control. This might be due to relatively less insolubilization/precipitation of applied soluble P in the presence of compost, PSB and ACC-deaminase PGPR. Sharma and Prasad (2003) also reported increased availability of soil P with the inoculation of PSB which was further improved with the addition of crop residues. Biswas (2011) documented an increase in soil available P after harvest with the use of enriched and inoculated compost plus inorganic fertilizer over 100% chemical fertilizer. Available P in soil was low with fertilizer P, demonstrating quick fixation of soluble fertilizer P and somewhat less availability of fixed P as compared with P in RP-EC. Application of organic waste in the form of enriched compost also maintained higher level of available P in soil in addition to improving soil health.

In conclusion, results of all trials showed a consistent trend. Data suggest successful practical utilization of low grade RP as a partial supplement of soluble phosphatic fertilizer without compromising crop yields. To make P of RP available to plants, the simultaneous use of compost, PSB and ACC-deaminase PGPR has been found a useful strategy for obtaining multiple benefits regarding growth and productivity of wheat on sustainable basis. RP enriched organic fertilizer could not only support plant nutrition, in integration with chemical fertilizer, but also can improve soil health as well as enhances microbial populations effectively.This technology can partially reduce the use of expensive commercial phosphatic fertilizer and thus utilizing the nation’s reserves of low grade RP.

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