

ASSESSMENT OF COMBINATIONS OF BIO-ACTIVATED ROCK-PHOSPHATE AND DI-AMMONIUM PHOSPHATE ON PHOSPHORUS USE EFFICIENCY, GROWTH AND YIELD OF WHEAT (*Triticum aestivum* L.)

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Phosphorus (P) is the second most important nutrient after nitrogen (N) required to plants for optimum growth and development. For alkaline and calcareous soils, the phosphorus use efficiency ranges from 10 to 25%, as major portion of phosphatic fertilizers becomes precipitated in soil and results in decreased crop yield. Rock phosphate (RP) as P source particularly in compost form has captivated the attention being cost effective P-fertilizer. However, the solubility of RP is a major concern. The bio-augmentation and bio-stimulation i.e. addition of phosphate solubilizing bacteria (PSB) and provision of limiting nutrients (molasses and urea), along with the organic manures can alleviate the problem of the rock phosphate solubilization by producing organic and mineral acids, decreasing soil pH, releasing anions and covering soil aggregates surfaces. Hence, bio-activated rock-phosphate (B-RP) was prepared through composting process by using poultry and animal manures along with RP, bio-augmented with consortium of PSB i.e. group of three strains of bacteria including *Bacillus* (MN-54), *Enterobacter* (MN-17) and *Pseudomonas* (E-11) and bio-stimulated with molasses (5 %) and urea (10 %). Thereafter this bio-activated rock phosphate was used alone and in different combinations with di-ammonium phosphate (DAP) during field experiment. Treatment B-RP + DAP in equal proportion improved biological yield by 19.81 %. It also resulted in 34.19, 71.24 and 29.42 % increase in N, P, K contents of grains, respectively and 40.09, 64.90 and 25.21 % increase in N, P, K contents of straw, respectively as compared to sole application of DAP as P-source i.e. DAP (100 % P). It had also improved total P-uptake (100.71 %) and recovery efficiency of P (121.86 %) of wheat as compared to that of DAP (100 % P). Moreover, the leading treatment also improved 21% grain yield over sole application of DAP. So, It is concluded that integrated use of B-RP and DAP with equal proportion of recommended P may serve as a better management practice for improving quantity of grain produced.

Keywords: Rock-phosphate, phosphorus use efficiency, phosphate solubilizing bacteria (PSB), animal and poultry manures.

INTRODUCTION

Phosphorus (P) is the second most important nutrient after nitrogen (N) as it plays a vital role in energy transformation, photosynthesis, respiration and as component of hereditary materials e. g. DNA and RNA (Khan *et al.*, 2014). The major portions of soils in Pakistan (80-90 %) are regarded as phosphorus (P) deficient. The fertilizer use efficiency, more importantly, the fertilizer use efficiency of phosphorus is very low. Phosphorus use efficiency ranges from 5 to 25 % owing to the different management practices (Khan *et al.*, 2009). When it is applied in the form of fertilizers, it becomes unavailable through fixation and precipitation reactions with Ca^{2+} in calcareous soils, and Al^{3+} and Fe^{3+} in acidic soil conditions (Rathor *et al.*, 2018). The cost of chemical or synthetic fertilizers is very high and their nutrient use efficiency especially phosphorus is very low (Khan *et al.*, 2014). Pakistan has to import these fertilizers from other countries (Reddy *et al.*, 2002). It has become so

important to find new, innovative and cost-effective viable solutions to increase P efficiency either through increasing the recovery and solubility of applied P fertilizers and/or replacing the expensive chemical P fertilizers with cheaper and efficient P sources, such as indigenous rock phosphates (RPs).

Rock phosphate (RP), a naturally occurring mineral source of phosphate, could serve as an alternative source of phosphorus in developing countries. Hazara division (Pakistan) is estimated with approximately 6.9 million tons of RP-deposits (Matiullah and Sharif, 2012). However, crude rock phosphate has poor solubility when used as a fertilizer in soils. The composting of organic manures/agricultural wastes with rock phosphate results in increasing the solubility and availability of phosphorus (Agyarko *et al.*, 2016). The rock-phosphate can be made more soluble through partially acidulation and co-application of RP and organic fertilizer. The use of P-solubilizing bacteria (PSB) is a new generation technology

which could bring the biological solubilization of RP by using the natural microbes either by inoculation with effective microbes or with specific PSB (Naveed *et al.*, 2017). Manures and organic wastes can not only improve soil physical and chemical properties but also increase the plant growth and improve the yield by making the nutrients more available for plant uptake (Marschner, 1995). Among other different factors, the most important one is the soil pH which is affected the most by the incorporation of organic manures. Carbon dioxide (CO₂) concentration is increased as a result of decomposition of these organic sources, which leads to the formation of carbonic acid and resultantly increases the dissolution of unavailable or complex forms of P (Muhammad and Khattak, 2009). Animal and poultry manures are a widespread source of organic wastes available throughout the country. The problem associated with these, is the bulky nature of organic compounds and relatively low in nutrients as compared to the chemical or synthetic fertilizers. So, enrichment of these manures with rock phosphates as P source seems to be a viable option. During composting or decomposition process different organic acids are produced, of which oxalic and citric acids are produced in larger quantities. The most efficient regarding P-solubilization is citric acid (Kumari *et al.*, 2008). The process of composting can further be accelerated, and compost can be prepared in far lesser time by using the microbial ways i.e. the introduction of phosphate solubilizing bacteria (PSB), especially the genera of *Bacillus*, *Enterobacter* and *Pseudomonas* (Whitelaw, 2000; Joseph and Jisha, 2009; Khan *et al.*, 2009). However, the growth and multiplication of inoculated microorganisms depends upon the availability of readily available carbon and nitrogen sources. So, addition of molasses as readily available source of carbon and urea as nitrogen source is an effective way of decomposing the organic wastes or manures as the addition of these nutrients enhances the biodegradation or decomposition of organic compounds through accelerated multiplication and growth of PSB (Buscot, 2005) which subsequently enhances the P-solubilization from rock-phosphate. The integrated use of microorganisms, manures and rock-phosphate bio-stimulated with molasses and urea, increases microbial populations and improves soil structure, infiltration rate, soil aeration, water holding capacity and decreases soil pH and subsequently improves crop yield (Sharif *et al.*, 2013).

So, bio-activated rock-phosphate (B-RP) was prepared after composting of rock-phosphate, poultry and animal manures which were bio-augmented with consortium of three strains of phosphate solubilizing bacteria i.e. *Bacillus* MN-54, *Enterobacter* MN- 17 and *Pseudomonas* E-11(Sharma *et al.*, 2013; Yaseen *et al.*, 2016; Naveed *et al.*, 2017) and bio-stimulated with molasses (5 %) and urea (10 %). This B-RP was tested in different combinations with di-ammonium

phosphate (DAP) in a field experiment for enhancing growth, yield and phosphorus use efficiency of wheat.

MATERIALS AND METHODS

Bio-activated RP Preparation: Bio-activated RP was prepared using the poultry manure PM (25% P), animal manure AM (25% P), rock phosphate RP (50 %) along with the consortium of PSB containing all three strains, bio-stimulated with molasses (10 %) and urea (5 %). So, the insoluble rock phosphate was converted into the soluble or bio-activated rock-phosphate through the process of composting at faster rate.

Soil Sampling and Soil Analysis: A representative soil sample (composite sample out of 15 samples) was taken from the field at the Research Area of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad located in a semi-arid region. The ground and sieved soil (2mm sieve) was analyzed for its different characteristics (Table 1) with methods described in U.S. Salinity Lab. Staff, (1954), otherwise mentioned. Textural class was identified using Bouyoucos (1962) method.

Table 1. Soil Characteristics

Properties	Readings	Units
Sand	46	%
Silt	29	%
Clay	25	%
Extractable Potassium	120	mg kg ⁻¹ soil
Organic matter	0.68	%
Olsen Phosphorus	6.43	mg kg ⁻¹ soil
Total nitrogen	0.043	%
Saturation percentage	31.5	%
CEC	13.6	cmol _c kg ⁻¹
pHs	7.819	----
ECe	1.98	dS m ⁻¹
Texture	Sandy clay loam	

Soil was classified as sandy clay loam by using the textural triangle according to the United State Department of Agriculture Classification System. The saturation percentage was recorded as difference between wet/saturated soil and oven dried soil weight, divided by the oven dried soil weight. The pH and EC of soil paste were measured with pH and EC-meters, respectively. Jackson (1962) method was deployed for total nitrogen (N). The plant available/extractable soil potassium and cation exchange capacity (CEC) were estimated using flame photometer (Rhoades, 1982). Organic matter in soil was determined by the loss in weight after ignition in muffle furnace at 400-600 °C for 4-6 hours (Gallardo *et al.*, 1987). Sodium bicarbonate solution (0.5 M with 8.5 pH) was used as P-extractant for Olsen's P determination (Olsen *et al.*, 1954).

Table 2. Analysis of Organic Sources and Bio-Activated Rock Phosphate.

Organic sources	Animal manure	Poultry manure	Press mud	Bio-activated rock phosphate	Rock-phosphate
Ash (%)	34.49	30.07	27.95	62.10	98.7
Organic matter (%)	63.51	69.93	72.05	37.90	Traces
Carbon (%)	35.28	38.85	40.03	21.06	--
Nitrogen (%)	1.19	1.85	1.24	2.77	--
Phosphorus (%)	0.69	1.25	0.85	4.92	9.5
Potassium (%)	0.54	1.10	0.73	2.25	--
Carbon: Nitrogen (C:N)	29.64	21.00	32.28	7.60	--

Organic Sources and Bio-activated Rock-Phosphate

Analysis: The organic sources including animal manure (AM), poultry manure (PM) and bio-activated rock-phosphate (B-RP) were analysed for ash, organic matter, carbon, nitrogen, phosphorus, potassium and C/N ratio before applying and testing for field experiment (Table 2). Total organic matter and ash were determined directly by the loss in weight after ignition of the experimental materials in muffle furnace at 400-600 °C for 4-6 hours (Gallardo *et al.*, 1987). The total organic carbon was calculated by dividing the organic matter with 1.8 (Brake, 1992). Nitrogen was determined in all the organic sources according to the Kjeldhal's method (van Schouwenberg and Walinge, 1973). Wet digestion was carried out for the determination of phosphorus and potassium (Ryan *et al.*, 2001).

For phosphorus spectrophotometer was used to record the absorbance at 430 nm wavelength (Anderson and Ingram, 1993). Phosphorus was also determined in rock phosphate. Potassium was determined in wet digested samples using Sherwood-410 Flame Photometer (Winkleman *et al.*, 1986). The C/N ratio was calculated by dividing the carbon with nitrogen.

Treatments Description: The bio-activated rock phosphate (B-RP) was prepared, analyzed and applied on the P-equivalent basis to the soil alone and in different combinations with DAP to evaluate their effect on wheat P-use efficiency and yield. Mixing rates of B-RP and DAP were applied as B-RP: DAP 00:100, 25:75, 50:50, 75:25 and 100:00. All the fertilizers and manures were applied at the recommended rate i.e. N: P: K at the rate of 120:90:60. Urea was used as nitrogen source in three splits; 1/3rd at sowing time while remaining fertilizer was applied after 30 and 45 days of sowing. Rock-phosphate (RP), bio-activated rock phosphate (B-RP), animal manures (AM), poultry manure (PM) and di-ammonium phosphate (DAP) were used as phosphorus source. Muriate of potash (MOP) was used as potassium source. Both P and K-sources were applied at sowing. Nitrogen and potassium were applied to all the plots including control where there was no application of phosphatic fertilizer. Wheat variety 'Galaxy' was sown by drill-seeded and fertilized through broadcast method at recommended rate and irrigated by canal water. All other agronomic practices were same and there was no difference

in all the treatments except that of phosphorus source. Nine treatments consisted of control 00 %P (T₁), RP 100 %P (T₂), AM 100 %P (T₃), PM 100 %P (T₄), B-RP 100 %P (T₅), B-RP 75 %P+DAP 25 %P (T₆), B-RP 50 %P+DAP 50 %P (T₇), B-RP 25 %P+DAP 75 %P (T₈) and DAP 100 %P (T₉) were applied at the time of sowing in the field. The experiment was conducted in randomized complete block design (RCBD) with three replications.

Plant Sampling and Sample Analysis: Data regarding different agronomic parameters including spike length, plant height, fertile tillers, grain weight (1000 grains) and biological, grain and straw yields were measured for wheat plants. The spike length and plant height at maturity was recorded. Fertile tillers were calculated after harvesting 1 m² area of the field at maturity just before the harvesting of crop. The grain weight (1000 grains) was recorded on an electrical balance. Biological and grain yields were calculated in kilogram (kg). Chemical parameters (N, P and K in grains and straw) were also estimated as percent. Total phosphorus up take and phosphorus use efficiency (recovery efficiency) was calculated by the formulae given below;

$$\text{Total P-uptake} = \text{Grains P-uptake} + \text{Straw P-uptake}$$

and;

$$\text{PU} = \frac{\text{Oven dried grain or straw weight/yield}}{100} \times \text{P} (\%)$$

Where, PU= Phosphorus Uptake

$$\text{RE} = \frac{\text{Total P uptake by FP} - \text{Total P uptake by UFP}}{\text{Amount of fertilizer applied}} \times 100$$

Where, RE = Recovery Efficiency, FP = Fertilized Plant, UFP = Unfertilized Plant

Statistical Analysis: In this field experiment treatments were arranged according to RCBD with three replications. Treatment means were compared by using honestly significance difference (HSD) at 5% significance (Steel *et al.*, 1997).

RESULTS

Effect of Combinations of B-RP and DAP on Agronomic Parameters of Wheat: Although the sole application of B-RP gave better results regarding different growth parameters over that of control, RP, AM and PM yet maximum results

were observed with the combined application of B-RP and DAP sharing equal proportion of P from each source. As maximum plant height (120.3 cm) was observed in treatment B-RP (50 % P) + DAP (50 % P) which was at par (113.0 cm) with treatment B-RP (at 25 % of recommended P) + DAP (at 75 % of recommended P). These two treatments increased plant height by 13.2 and 6.3 %, respectively over the treatment DAP (at 100 % of recommended P).

The treatment B-RP (at 75 % of recommended P) + DAP (at 25 % of recommended P) with plant height 104.0 cm was at par to the DAP (at 100 % of recommended P). The incorporation of B-RP and DAP sharing equal amounts of recommended P, resulted in maximum spike length (19.7 cm) which proved to be non-significant to the treatment applied with 25 % P from B-RP and 75 % of P from DAP giving 17.3 cm spike length while the treatment using 100 % from sole DAP i.e. DAP (100 % P) was observed with 15.7 cm spike length. On comparison of spike length of these two leading treatment with DAP (100 % P), these improved the spike length by 25.5 and 10.2% increase over DAP (at 100 % of P). The spike lengths, recorded in all the treatments with B-RP (alone and/or in combination with DAP), were at par to the spike length of the DAP (at 100 % of

recommended P). Maximum fertile tiller m^{-2} (480) in treatment B-RP (50 % P) + DAP (50 % P) was followed by (425) the treatments B-RP (25 % P) + DAP (75 % P). These resulted 25.6 and 11.3 % more fertile tillers, respectively as compared to that produced by sole application of DAP. The treatment B-RP (at 75 % of recommended P) + DAP (at 25 % of recommended P) was also at par to the DAP (at 100 % of recommended P). Flag leaf length ranged from 12.7 to 29.7 cm. It was the highest (29.7 cm) in the treatment B-RP + DAP (in 50:50 P) and followed by the treatments B-RP + DAP (25:75 P) and DAP (100 % P) as these treatments produced 27.7 and 23.3 cm flag leaf lengths, respectively. The outstanding treatment B-RP + DAP (in 50:50 P) produced estimated increases of 27.2, 53.5 and 58.9 % in flag leaf length over sole application of DAP, PM (100 % P) and AM (100 % P), respectively. The application of combined B-RP and DAP at equal rates resulted in maximum biological (11495 $kg\ ha^{-1}$), straw (6365 $kg\ ha^{-1}$) and grain (5130 $kg\ ha^{-1}$) yields and yielded 19.8, 21.0 and 18.3 % more biological, straw and grain yields, respectively over the sole application of DAP. The treatment using B-RP (at 25 % of recommended P) + DAP (at 75 % of recommended P) also improved biological, straw and grain

Table 3(a). Effect of Combinations of B-RP and DAP on agronomic parameters of wheat.

Treatments	Plant height (cm)	Spike length (cm)	Number of fertile tillers m^{-2}	Flag leaf length (cm)	1000 grain weight (g)	Biological yield (kg ha^{-1})	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})
Control (00%P)	68.7f	9.0e	175h	12.7e	27.8d	5931g	2414f	3518h
RP (100%P)	72.7f	10.7de	196gh	15.3de	29.4d	6297g	2605f	3692gh
AM (100%P)	82.3e	12.3c-e	241fg	18.7cd	33.2d	7308f	3226e	4083fg
PM (100%P)	90.3de	12.7cd	274ef	19.3cd	34.3d	7594f	3282e	4311ef
B-RP (100%P)	96.7cd	14.3bc	312de	20.7c	41.8c	8662e	3941d	4721de
B-RP (75%P)+DAP (25%P)	104.0bc	15.3bc	352cd	22.3c	45.8bc	9187d	4172cd	5014cd
B-RP (50%P)+DAP (50%P)	120.3a	19.7a	480a	29.7a	55.7a	11495a	5130a	6365a
B-RP (25%P)+DAP (75%P)	113.0ab	17.3ab	425b	27.7ab	50.2ab	10529b	4658b	5871b
DAP (100%P)	106.3b	15.7bc	382bc	23.3bc	48.0bc	9594c	4336bc	5258c
HSD	9.60	3.36	49.96	4.94	7.21	367.9	394.4	422.7

Table 3(b). Effect of combinations of B-RP and DAP on NPK contents of wheat grain and straw.

Treatments	Grain N (%)	Grain P (%)	Grain K (%)	Straw N (%)	Straw P (%)	Straw K (%)	Total P-uptake (kg ha^{-1})	PUE / RE (%)
Control (00 % P)	1.083e	0.068f	0.769g	0.750f	0.057e	1.207e	3.65f	0.0f
RP 100 % P)	1.170e	0.076f	0.780g	0.800f	0.077e	1.223e	4.84f	1.3f
AM (100 % P)	1.393d	0.152e	0.934f	1.117e	0.133d	1.467d	10.32e	7.4e
PM (100 % P)	1.570d	0.163de	1.005e	1.240de	0.146d	1.508d	11.64e	8.9e
B-RP (100 % P)	1.767c	0.191d	1.226d	1.333c-e	0.162d	1.907c	16.66d	14.4d
B-RP (75%P)+DAP (25%P)	1.997b	0.228c	1.326c	1.560bc	0.209c	2.120bc	18.93cd	17.0cd
B-RP (50%P)+DAP (50%P)	2.500a	0.399a	1.597a	1.957a	0.343a	2.583a	42.29a	42.9a
B-RP (25%P)+DAP (75%P)	2.357a	0.359b	1.390b	1.727ab	0.287b	2.302b	33.57b	33.2b
DAP (100 % P)	1.863bc	0.233c	1.234d	1.397cd	0.208c	2.063c	21.07c	19.3c
HSD	0.191	0.032	0.039	0.272	0.036	0.234	2.478	2.754

yields by 9.8, 11.7 and 7.4 %, respectively over the sole application of DAP. The application of B-RP, DAP and their combinations also resulted in significant gain in 1000-grains weight of wheat over control, sole application of rock-P, AM and PM. The treatments B-RP+DAP (in equal proportion) and B-RP+DAP (in 25:75 proportion) and DAP (100 % P) were the best treatments with 55.7 and 50.2 g of 1000 grains weight and showed 16.0 and 4.6 % increase, respectively over that of DAP (100 % P).

Effect of Combinations of B-RP and DAP on Chemical Parameters of Wheat: The statistical analysis of data on chemical parameters of wheat revealed that all the treatments performed better as compared to the treatments of control and sole application of rock-P as P-source. Wheat grains N concentration ranged from 1.083 to 2.500 %, having maximum N (2.500 %) with the treatment B-RP+DAP (P in equal proportion). This maximum N concentration was equivalent to N concentration (2.357 %) of B-RP+DAP (P in 25:75 proportion) and followed by the treatment B-RP (at 75 % of recommended P) + DAP (at 25 % of recommended P) with 1.997 % N. The sole application of B-RP with 1.767 % N was statistically at par to the N content of the DAP (at 100 % of recommended P). Significant differences were recorded in nitrogen (N) concentration of wheat straw in response to different treatments. The treatment B-RP (50% P) + DAP (50% P) increased 40.1 % straw-N as compared to the N-concentration in the plants of plot treated with DAP (at 100 % of recommended P). It was also noted that all the treatments using combination of B-RP and DAP were found superior to the sole application of the B-RP and DAP treatments. All the treatments showed a positive improvement in P concentration of wheat grain over control and sole application of rock-P. It ranged as 0.068 to 0.399 %, indicating maximum value of P (0.399 %) in the treatment B-RP + DAP (each at 50 % P) and minimum value of P (0.068 %) in control. This maximum and minimum values of P were closed to 0.359 % P in the treatment B-RP + DAP (with 25:75% P) and 0.076 % P in the treatment RP (100 % P), respectively. The two combinations i.e. B-RP (at 50 % of recommended P) + DAP (at 50 % of recommended P) and B-RP (at 25 % of recommended P) + DAP (at 75 % of recommended P) increased 71.2 and 54.1 % more P in grain than in the grain treated with sole DAP (at 100 % of recommended P). Overall results indicated that combined application of B-RP and DAP was found superior not only to the sole application of B-RP but also to the sole application of DAP. Similarly, maximum wheat straw P-concentration (0.343 %) was found in the treatment B-RP + DAP (each at 50 % P). The treatment B-RP (at 25 % of recommended P) + DAP (at 75 % of recommended P) showed 0.287 % P concentration in wheat grain. These two treatments 64.9 and 38.0 % increases over treatment of DAP (at 100 % of recommended P), respectively. All the four treatments

loaded with B-RP (alone and/or in combination with DAP) resulted in improved P concentration in straw as compared to AM (at 100 % of recommended P) and PM (at 100 % of recommended P). Maximum K concentration (1.597 %) in wheat grains was recorded in the treatment B-RP + DAP (each at 50 % P). This treatment was followed by the treatment B-RP (at 25 % of recommended P) + DAP (at 75 % of recommended P) with 1.390 % K concentration and minimum (0.769 and 0.780 %) was in control and sole RP, respectively. The treatment B-RP (at 75 % of recommended P) + DAP (at 25 % of recommended P) with 1.326 % K concentration was found statistically better treatment than that of DAP (at 100 % of recommended P). The treatment B-RP (at 100 % of recommended P) with K concentration (1.226 %) was also at par to that of DAP (at 100 % of recommended P). The combined use of B-RP and DAP was come out as a superior approach not only to the sole application of B-RP but also to the sole application of DAP. As the treatments B-RP (at 75 % of recommended P) + DAP (at 25 % of recommended P), B-RP (at 25 % of recommended P) + DAP (at 75 % of recommended P) and B-RP (at 50 % of recommended P) + DAP (at 50 % of recommended P) exhibited 7.5 and 8.2 %, 12.6 and 13.4 %, 29.4 and 30.3 % increase over that of DAP (at 100 % of recommended P) and B-RP (at 100 % of recommended P), respectively. Similarly in wheat straw, same trend was followed with maximum K concentration (2.583 %) was observed in the treatment B-RP + DAP (P in equal proportion) and B-RP + DAP (P in 25:75 ratio) and B-RP + DAP (P in 75:25 ratio) with 2.302 and 2.120 % K concentration, respectively. The application of DAP (at 100 % of recommended P) was found at par to the application of B-RP (at 100 % of recommended P). The treatments of B-RP and DAP i.e. B-RP (at 75 % of recommended P) + DAP (at 25 % of recommended P), B-RP (at 25 % of recommended P) + DAP (at 75 % of recommended P) and B-RP (at 50 % of recommended P) + DAP (at 50 % of recommended P) gave 2.3, 11.6 and 25.2 % increase in straw over that of DAP (at 100 % of recommended P), respectively. Total P-uptake was the sum of P-uptake by grain and straw. Total P-uptake of wheat plants ranged from 3.65 to 42.29 kg ha⁻¹. The treatment B-RP + DAP (in 50:50 P) gave maximum P-uptake (42.29 kg ha⁻¹) and B-RP + DAP (25:75 % of recommended P) with 33.57 kg ha⁻¹. These increased P uptake of wheat by 100.7 and 59.3 %, respectively over DAP (at 100 % of recommended P). B-RP + DAP (50:50 P), B-RP + DAP (25:75 P) and B-RP + DAP (75: 25 % P) gave 153.8, 101.5 and 13.6 % increases in P uptake, respectively over sole B-RP (100 % P). Phosphorus recovery efficiency was taken/calculated as zero (as it was calculated from the formula which employed the difference from control) in the treatment using no P-fertilizer (control) and it was found to be non-significant to RP (at 100 % of recommended P) with 1.3 % P recovery efficiency. The

treatment B-RP + DAP (in equal proportion of P) was regarded as the best treatment with maximum P recovery efficiency (42.9 %) while the treatment B-RP + DAP (25:75 % P) was the second best treatment with 33.2 % P recovery efficiency. The treatments of B-RP (at 50 % of recommended P) + DAP (at 50 % of recommended P) and B-RP (at 25 % of recommended P) + DAP (at 75 % of recommended P) gave 121.9 and 71.8 % increases in P recovery efficiency, respectively over treatment DAP (at 100 % of recommended P). The combined use of B-RP and DAP was found better approach compared to the sole application of B-RP as there were 197.1, 130.0 and 17.5 % increases in P recovery efficiency of the treatments using B-RP (at 50 % P) + DAP (at 50 % P), B-RP (at 25 % P) + DAP (at 75 % P) and B-RP (at 75 % P) + DAP (at 25 % P), respectively over sole B-RP (at 100 % P).

DISCUSSION

This study was conducted to explore the effects of B-RP and its different combinations with DAP for improving physico-chemical parameters of wheat. Although the incorporation of sole B-RP found superior to control and sole untreated rock-P, yet it was inferior to integrated use of B-RP and DAP especially when these two sources were applied with equal proportion of P from each source.

The plots treated with 50 % P from B-RP and remaining 50 % P from DAP improved biological yield, plant height, spike length, number of fertile tillers, flag leaf length and 1000 grain weight by 19.8, 13.2, 25.5, 25.6, 27.2 and 16.0 %, respectively over sole DAP (100 % P). The incorporation of this treatment to the wheat crop enhanced yield by improving soil properties conducive for wheat growth. As this integrated approach contained organic and mineral sources of P-fertilizer and P-solubilizing bacteria, which might have resulted in improved soil physico-chemical properties which consequently resulted in enhanced moisture content, soil porosity, water holding capacity, organic matter content and nutrient cycling for improved turnover of nutrients. It ensured balanced and uninterrupted supply of water and nutrients to the crop to meet the crop nutritional requirements subsequently might have resulted in improved wheat crop yield (Haunge *et al.*, 2013). Yasmeen *et al.* (2018) explained that incorporation of organic materials along with microbes resulted in improved grain and straw yield of maize through improved soil organic status and soil physical health. This improved organic matter resulted in conservation of soil moisture, improvement in soil structure, enrichment of nutrients and reduction in nutrient losses (Morales-Corts *et al.*, 2018). The yield reduction in sole application of DAP as compared to the combined application of B-RP and DAP in equal proportions might have also been resulted due to the unavailability of nutrients especially P from soil. The decreasing trend in P-availability during later

stages might be due to the P-fixation with soil particles (Aziz *et al.*, 2018). At crucial stages, the inability of the DAP to supply adequate amounts of P to plants due to P-immobilization either by adsorption or chemical precipitation in soil, resulted in reduced wheat yield (Gyaneshwar *et al.*, 2002). The use of microbes/PSB to form B-RP might have resulted in nutrients availability and growth promoting hormones which might have increased crop yield (Yazdani *et al.*, 2009). Moreover, the increased release of P in soil, from insoluble P with the application of PSB, might be the consequence of acid productions, enzymes establishment, acidification, exchange reactions chelation and formation of polymeric substances, which resulted in enhanced plant growth by improving biological fixation (Delvasto *et al.*, 2006; Rathor *et al.*, 2018).

This composite and integrated use of B-RP and DAP has also resulted in improved N, P and K contents of wheat grain and straw and phosphorus use efficiency of wheat as compared to control and sole application of DAP at recommended rate. An analogous effect of higher nutrients contents and nutrients use efficiency was documented by Nishanth and Biswas (2008) and Shrivastava (2011) with combined application DAP and B-RP (rock phosphate enriched compost) on wheat and mung bean, respectively. The combined effect of applied PSB and composted RP was found more efficient and effective approach in order to improve growth as well as nutrient uptakes due to additive effect of PSB and compost. Organic matter plays key role for sustained microbial population. Moreover, application of compost improves physico-chemical properties of soils creating suitable environment for optimum nutrient uptake (Behera *et al.*, 2017). Billah and Bano (2015) also narrated that wheat seeds inoculated with PSB and supplied with rock phosphate-enriched compost proved to enhance nutrients uptake and wheat grain yield through the process of compost using rock-P, poultry litter and phosphate solubilizing bacteria which could enhance the solubilization of insoluble phosphorus through the discharge of organic and mineral acids associated with lowering of pH and more availability of solubilized-P from rock-P (Kpombrekou-A and Tabatabai, 2003).

Conclusion: It can be concluded that this integrated approach of manipulating B-RP and DAP in combined application can improve nutrients uptake fetching enhanced grain yield. It can also add to maintain soil fertility and soil health through ameliorated soil physical and chemical properties. This eco-friendly approach manages organic wastes on one hand and the use of indigenous RP sources can save the huge amount being spent on importing DAP on the other hand.

REFERENCES

- Agyarko, K., A.A. Abunyewa, A.K. Asiedu and E. Heva. 2016. Dissolution of rock phosphate in animal manure soil amendment and lettuce growth. *Eurasian J. Soil Sci.* 5:84-88.
- Anderson, J.M. and J.S. Ingram. 1993. Colorimetric determination of ammonium and phosphorus. *Tropical Soil Biology and Fertility - A Hand Book of Methods.* C.A.B. International, Wallingford, U.K. Pp.70-80.
- Aziz, M.Z., M. Yaseen, M. Naveed and M. Shahid. 2018. Alginate-entrapped *Enterobacter* spp. MN17 coated diammonium phosphate improves growth, yield and phosphorus use efficiency of wheat. *Int. J. Agric. Biol.* 20: 2401-2407.
- Behera, B.C., H. Yadav, S.K. Singh, R.R. Mishra, B.K. Sethid, S.K. Dutta and H.N. Thatoi. 2017. Phosphate solubilization and acid phosphatase activity of *Serratia* sp. isolated from mangrove soil of Mahanadi river delta, Odisha, India. *J. Genet. Eng. Biotechnol.* 15:169-178.
- Billah, M. and A. Bano. 2015. Role of plant growth promoting rhizobacteria in modulating the efficiency of poultry litter composting with rock phosphate and its effect on growth and yield of wheat. *Waste Manag. Res.* 33:63-72.
- Bouyoucos, G. J. 1962. Hydrometer method improved for making particle size analysis of soils. *J. Agron.* 53:464-465.
- Brake, J. D. 1992. A practical guide for composting poultry litter. MAFES Bulletin 981. Department of Poultry Science, Mississippi State University, USA.
- Buscot, F. 2005. What are soils? *In: Microorganisms in Soils: Roles in Genesis and Functions*; Buscot, F. and S. Varma, Eds.; Springer: Heidelberg, Germany. 3:3-18.
- Delvasto, P., A. Valverde, A. Ballester, J.M. Igual, J.A. Muñoz, F. González, M.L. Blázquez and C. García. 2006. Characterization of brushite as a re-crystallization product formed during bacterial solubilization of hydroxyapatite in batch cultures. *Soil Biol. Biochem.* 38:2645-2654.
- Gallardo, J.E., J. Saavedra, T. Martin-Patino and A. Millan. 1987. Soil organic matter determination. *Comm. Soil Sci. Plant Anal.* 18:699-707.
- Gyaneshwar, P., K.G. Naresh, L.J. Parekh and P.S. Poole. 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant Soil* 245:83-93.
- Haunge, Q., S. Laixiang, X. Xinliang, C. Yaqing and B. Junfei. 2013. Effect of residue retention on paddy crop. *Soil Biol. Biochem.* 64:110-123.
- Jackson, M.L. 1962. Chemical composition of soil. *In: Bear, F.E. (Ed.), Chemistry of Soil*, Van Nostrand Reinhold Co. New York, USA, Pp.71-144.
- Joseph, S. and M.S. Jisha. 2009. Buffering reduces phosphate solubilizing ability of selected strains of bacteria. *World J. Agric. Sci.* 5:135-137.
- Khan, A., V. Jilani, M.S. Akhtar, S.M.S. Naqvi and M. Rasheed. 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. *J. Agric. Biol. Sci.* 1:48-58.
- Khan, M. S., A. Zaidi and E. Ahmad. 2014. Mechanism of phosphate solubilization and physiological functions of phosphate solubilizing microorganisms, *In: Phosphate Solubilizing Microorganisms: Principles and Application of Microphos Technology*, Springer International Publishing, Switzerland. 31-62, doi: 10.1007/978-3-319-08216-5_2, 201214.
- Kpomblekou-A, K. and M.A. Tabatabai. 2003. Effect of low-molecular weight organic acids on phosphorus release and phytoavailability of phosphorus in phosphate rocks added to soils. *Agric. Ecosyst. Environ.* 100:275-284.
- Kumari, A., K.K. Kapoor, B.S. Kundu and R.K. Mehta. 2008. Identification of organic acids produced during rice straw decomposition and their role in rock phosphate solubilization. *J. Plants Soil Envir.* 54:72-77.
- Marschner, H. 1995. *Mineral Nutrition of Higher Plants.* Academic Press International, San Diego, CA, USA.
- Matiullah, K. and M. Sharif. 2012. Solubility enhancement of phosphorus from rock phosphate through composting with poultry litter. *Sarhad J. Agric.* 28:415-420.
- Morales-Corts, M.R., R. Pérez-Sánchez and M.A. GómezSánchez. 2018. Efficiency of garden waste compost teas on tomato growth and its suppressiveness against soil borne pathogens. *Scientia Agricola.* 75: 400-409.
- Muhammad, D. and R.A. Khattak. 2009. Growth and nutrient concentration of maize in pressmud treated saline-sodic soils. *Soil Environ.* 28:145-155.
- Naveed, M., M.Z. Aziz and M. Yaseen. 2017. Perspectives of endophytic microbes for legume improvement. *In: A. Zaidi et al. (eds.), Microbes for Legume Improvement*, Springer, Switzerland. Pp. 277-299.
- Nishanth, D. and D.R. Biswas. 2008. Kinetics of phosphorus and potassium release from rock phosphate and waste mica enriched compost and their effect on yield and nutrient uptake by wheat (*Triticum aestivum* L.). *Bioresour. Technol.* 99:3342-3353.
- Olsen, R., C.V. Cole, F.S. Watanabe and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circular 939 United States Department of Agriculture, Washington DC.
- Rathor, G., S.K. Sharma, N. Chopra, K. Singh and G. Chourey. 2018. Management of Phosphorus Fertilizer in Maize Crop using PSB (phosphorus solubilising bacteria) in Vertisol. *Int. J. Pure App. Biosci.* 6:253-258.

- Reddy, M.S., S. Kumar and B. Khosla. 2002. Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis* and *Aspergillus*. *Bioresour. Technol.* 84:187-189.
- Rhoades, J.D. 1982. Cation exchange capacity. In A.L. Page *et al.* (ed.). *Methods of Soil Analysis part 2* (2nd ed.) Chemical and Microbiological Properties. Madison, Wisconsin USA. Pp. 152-153.
- Ryan, J., G. Estefan and A. Rashid. 2001. *Soil and Plant Analysis Lab Manual*. 2nd ed. International Centre for Agricultural Research in Dry Areas, Aleppo, Syria.
- Sharif, M., T. Burni, F. Wahid, F. Khan, S. Khan, A. Khan and A. Shah. 2013. Effect of rock phosphate composted with organic materials on yield and phosphorus uptake of wheat and mung bean crops. *Pak. J. Bot.* 45:1349-1356.
- Shrivastava, M., S.P. Kale and S.F. D'Souza. 2011. Rock phosphate enriched post-methanation bio-sludge from kitchen waste based biogas plant as P source for mung bean and its effect on rhizosphere phosphatase activity. *Eur. J. Soil Biol.* 47:205-212.
- Sharma, S.B., R.Z. Sayyed, M.H. Trivedi and T.A. Gobi. 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus.* 2:587.
- Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. *Principles and procedures of statistics*, 3rd Ed. McGraw Hill. Inc. Book Co. New York (USA). Pp. 352-358.
- U.S. Salinity Laboratory Staff. 1954. *Diagnosis and improvement of saline and alkali soils*. USDA Handbook No. 60. Washington D. C. USA.
- Van Schouwenberg, J. C. H. and I. Walinge 1973. *Methods of Analysis for Plant Material*. Agric. Univ., Wageningen, The Netherlands.
- Whitelaw, M.A. 2000. Growth promotion of plants inoculated with phosphate solubilizing fungi. *Adv. Agron.* 69:99-151.
- Winkelman, G.E., R. Amine, W.A. Rice and M.B. Tahir. 1986. Potassium in plant. In: *Methods Manual Soils Laboratory*. Barani Agric. Res. and Dev. Project. National Agricultural Research Centre, Islamabad, Pakistan.
- Yaseen, M., M.Z. Aziz and M. Naveed. 2016. Enhancing phosphorus use efficiency of wheat via controlled release of microbes by coating alginate loaded bacteria on diammonium phosphate. *International Conference on Research for Food Security, Natural Resource Management and Rural Development*, September, Tropentag, Vienna, Austria. Pp.18-21.
- Yasmeen, H., M. Yaseen, M.Z. Aziz, M. Naveed, M. Arfan-ul-Haq and T. Abbas, 2018. Wheat residue management improves soil fertility and productivity of maize. *Int. J. Agric. Biol.* 20:2181-2188.
- Yazdani M., M.A. Bahmanyar, H. Pirdashti and M. A. Esmaili. 2009. Effect of Phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of Corn (*Zea mays* L.). *Proc. World Acad. Science, Eng. Technol.* 37:90-92.

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