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Phosphate Solubilizing Bacteria and Arbuscular Mycorrhizal Fungi Impacts on Inorganic Phosphorus Fractions and Wheat Growth

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Abstract: Despite abundant amounts of phosphorus in parent material, the soil phosphors availability is limited for plant. Some soil micro-organisms enhance solubility of phosphate in calcareous soils. This study investigated the effects of phosphate solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) and their interactions on crop performance, changes in biological population and inorganic phosphorus fractions. The experimental design was split plot factorial with on a complete randomized block design. The treatments included four soil types (clay, clay loam, loam and sandy loam), three phosphorus fertilizer levels (0, 20 and 40 mg kg^{-1}) and four levels of phosphate solubilizing microorganisms (PSM). At time physiological maturity, dry matter weight (shoots or roots), plant height, spike length, grain spike number and grain yield in each spike were measured. The percentage of colonized roots, number of PSB and fungi spore and inorganic phosphorus fractions in the root zone were determined. Resulted indicated that the highest shoot dry matter was in clay loam soil (21.5 g pot^{-1}). Combined application of PSB and AMF increased shoot dry matter yield, seed grain spike number and grain yield by 52, 19 and 26%, respectively compared to the controls. Phosphorus application increased ΔOlsen-P, ΔCa_2-P and ΔCa_8-P% while biological fertilizers reduced the amount of ΔCa_2-P and Δca_8-P%.

Key words: Phosphate solubilizing bacteria · Arbuscular mycorrhizal · Wheat · Colonization · Spore

INTRODUCTION

Phosphorous (P) is an essential plant macronutrients required by plants [1]. In calcareous soil, P is precipitated and plants are unable to utilize the precipitated P [2]. Conversion of the insoluble forms of P to an accessible form by plants (ortho-phosphate) is an important trait of phosphate-solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF).

Application of biological fertilizers such as biological phosphate fertilizers improves soil fertility. Bacteria (such as PSB) and fungi (e.g. AMF) are usually effective on phosphate solubility due to different mechanisms such as production and secretion of organic acids [3]. Many bacteria produce enzymes which enhance releasing phosphate from organic P compounds. These bacteria also produce other biological materials such as auxin, gibberlic acid, vitamins and hormones that increase the dissolution of phosphate [4]. Strains of *Pseudomonas bacteria, Bacillus, Rhizobial*, *Enterobacter, Aspergillus* and *Penicillium* are the most efficient in solubilizing phosphorus [5].

AMF play a key role in nutrients cycling in ecosystem and also increasing plants resistance to different environmental stresses [6]. Soya bean was inoculated with AMF, the result of which was an increase concentration of both phosphorus and nitrogen in the plant biomass [7]. Application of both PSB and plant growth-promote bacteria (PGPR) increase P uptake efficiency by 50% [8]. Application of PSB increase yield of maize, legumes and potatoes [9]. The impact of PSB *Bacillus FS3* and *Aspergillus FS9* together with phosphorus application rates on strawberry (*Fragaria ananasa*) plants in soil low in phosphorus was studied, the results of which indicated a reduction of phosphorus application rate by bacteria of 149 and

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102 kg/ha, respectively. This also increased the nitrogen, potassium, calcium and iron in the plant leaves and fruits [10].

A synergistic relation between PSB and AMF had been observed [11]. These observations also showed that a combined application of *Glomus fasciculatum* and *Azotobacter* increases the concentration of P, K and N uptake by the mulberry (*Morus nigra*) leaf of 10, 16 and 5.8%, respectively. In another study the influence of three strains of *pseudomonas putida, pseudomonas fluorescens CHAO* and *Tabriz Pseudomonas fluorescens* on the rate of phosphorus release from iron hydroxides was investigated, the results of which showed the rate of P released by these strains of 51, 29 and 62%, respectively [12].

The purpose of this study was to investigate the effects of PSB and AMF and their interactions on crop performance, biological properties and inorganic phosphorus fractions of different soil types.

MATERIALS AND METHODS

Soils: Four soil samples (0-10 cm), low in phosphorus, were used for the pot experiments (Table 1). Subsamples were used to determine chemical properties and soil texture. Electrical conductivity of a saturated extract [13] and pH of a saturated paste were determined. Organic carbon was measured by wet oxidation [14]. Particle size distribution was determined by the pipette method [15]. Calcium carbonate equilibrium was determined by reverse titration [16]. Available P was extracted by Olsen [17]. Table 1 indicates physical and chemical properties of soils.

Experimental Design: The experimental treatments were arranged in split plot factorial based on a complete randomized block design including four soil types (clay, clay loam, loam and sandy loam), three phosphorus fertilizer levels (0, 20 and 40 mg kg^{-1}P), four levels of phosphate solubilizing microorganisms (PSM). The PSM used in this study were:

Mixture of three phosphate solubilizing bacteria (PSM) including *Azotobacter chrocooccum* strain 5, *Pseudomonas fluorescens* 187 and *Pseudomonas fluorescens* 36, 2- mixture of arbuscular mycorrizal fungi (AMF) including *Glomus mossea* and *Glomus intraradices*, 3- mixture of PSB and AMF and 4- control. The experiment was replicated three times; total numbers of treatments were 144.

Pots with a diameter of 25 cm were used. Pots were filled with soils that passed through 2-mm sieve. 40 mg kg^{-1} nitrogen using urea fertilizer was added to each pot. Nitrogen fertilizer was top dressed in three portions, one third at the time of planting, one third before flowering and the remain at the time of grain filling. Bacteria were inoculated using seed inoculation method and fungi were inoculated with soil inoculation method. Ten g of fungi were placed 2 cm below the soil surface. Ten wheat seeds (verinak variety) were planted in each pot. Planting time was Nov. 10, 2009.

Plant Analysis: Five wheat plants were selected from each pot. Dry matter yield (shoots or roots), plant height, spike length, grain spike number and grain yield were measured.

The AMF colonization rate of roots was measured. The fresh root samples (0.2 g) were thoroughly washed in running tap water and cut into 1 cm long segments. The root segments were cleared in 10% (w/v) KOH (30 min, 90°C), acidified with lactic acid (10 min) and stained with 0.5% Try pan blue [18]. Fifty root fragments (approximately 1 cm) long were mounted on slides in a polyvinyl alcohol-lactic acid-glycerol solution [19] and examined at 100× magnification under microscope to obtain the percentage of root length colonized by AM fungi. The percentage of root length colonized by AM fungi was determined using the magnified line-intersect method of McGonigle *et al*. [20].

Spores of AMF were extracted from 50 ml of air-dried sub-samples of each soil sample by wet sieving followed by floatation centrifugation in 50% sucrose [21].

Table 1: Some physical and chemical properties of soils

	Clay	Silt	Sand	EC (dSm^{-1})	pH	total N	$CaCO_3$	OM	Olsen-P (mg kg^{-1})
	(%)					(g kg^{-1})			
Clay	43.0	25.5	31.5	1.1	7.6	0.8	350.0	9.2	6.2
Clay loam	15.0	27.5	57.5	1.3	7.3	0.7	290.0	5.7	3.3
Loam	23.5	39.0	37.5	1.5	7.5	0.6	270.0	4.5	3.1
Sandy loam	27.0	35.5	37.5	1.0	7.5	0.5	230.0	3.2	2.9

OM= Organic matter

The finest sieve used was 50μm. The spores were collected on a grid patterned (4×4) filter paper, washed three times with distilled water to spread them evenly over the entire grid and counted using microscope at 30 × magnification.

To measure the phosphate solubilizing bacteria (PSB) number, 90 ml of sterile water was added to 10 g soil and shacked 20 min (150 rpm). For decimal dilution to 10^{-7} was prepared in sterile water. Value of each dilution 0.1 ml in three replicates on sperber medium (containing 10g glucose, 0.5g yeast extract, 0.23g hydrated magnesium sulfate, 0.14g calcium chloride, 2.5g three calcium phosphate, 0.5g cyclohexemide and 25g agar per one liter sterile water) were separated. The number PSB was counted after two week of incubation at 28-30°C [22].

Insoluble Phosphorus (P_i) Fractions: The calcium phosphate is classified into di-calcium phosphate, octa-calcium phosphate and apatite types. Sequentially fractionated for inorganic phosphorus (P_i) fractions was performed; Ca_2P by $NaHCO_3$, Ca_8P by NH_4Ac, AL-P by NH_4F, Fe-P by $NaOH\text{-}Na_2CO_3$, occluded-P by $Na_3Cit\text{-}Na_2S_2O_4\text{-}NaOH$ and $Ca_{10}P$ by H_2SO_4 [23]. Percent of change in each Pi fractions ($\%\Delta P_i$) as follows:

$$\%\Delta p_i = [(P_{i2} - P_{i1})/P_{i1}] \times 100$$

Where Pi_2 is concentration of each fraction (mg kg^{-1}) in soil after of cutting and Pi_1 is concentration of each fraction (mg kg^{-1}) in soil before of planting.

Statistical Analysis: Three replicates per treatment were established. Three factor analyses of variance (ANOVA) and Duncan multiple range test (test at 1 and 5% level of probability) were used to partition the variance into the main effects and the interaction between soil type, phosphorus and biological fertilizers. Statistical analysis was performed using SPSS statistical package 18.

RESULTS AND DISCUSSION

Soil Properties: Table 1 shows selected physical and chemical characteristics of soils. Clay, silt and sand of soils ranged from 15-43, 25.5-39 and 31.5-57.5%, respectively. Soils are medium to fine texture. Soil acidity varied from 7.3-7.6 and electrical conductivity of soils ranged from 1.0 -1.5 dS $m^{-1.}$ The nitrogen concentration of soils is low and the total nitrogen ranged from 0.5 to 0.8g kg^{-1}. Soils are calcareous and $CaCO_3$ from 230 to 350 kg^{-1}. Soils are low in organic matter (3.2 to 9.2 g kg^{-}) and phosphorus content ranged from 2.9 to 6.2 mg kg^{-1}.

The results in Table 2 show the concentration of different phosphorus species. The concentration of Ca10 –P, O-P, Fe-P, Al-P, Ca8 –P, Ca2 -P and total P ranged from; 255 to 432, 11 to 22, 14 to 20, 10 to 19,113 to 152, 4.6 to 7.6 and 262 to 697 mg kg^{-1} soil, respectively.

Crop Performance: The effects of soil type (S), phosphorus (P) and biological (B) fertilizers on SWD of wheat were significant ($P \le 0.01$). The interactive effect between S and B was significant ($P \le 0.05$) (Table 3).

Table 2: The concentration of inorganic phosphorus fraction (mg kg^{-1}) of different soil types

Soil type	Ca_{10}-P	O-P	Fe-P	Al-P	Ca_8-P	Ca_2-P	Total P
Clay	432	22	20	18	152	7.6	952
Clay loam	392	16	17	19	142	4.9	822
Loam	401	17	16	10	130	6.2	789
Sandy loam	255	11	14	15	113	4.6	510

Table 3: Analysis of variance of measured parameters of crop performance and biological properties

Variable	df	Shoot dry weight	Root dry weight	Plant height	Spike length	Grain spike number	Grain yield	Colon percent	Spore number	PSB number
Replication ®	2	0.8	0.3	2.7	19.8	5.2	0.4	5.3	10.8	1.1×10^{9}
Soil type (S)	3	5.3**	0.1	25.7	21.4	5.1	0.5	51.5	429.3**	7.4×10^{9}**
Phosphorus fertilizer (P)	2	7.8**	0.3	0.9	18.9	10.9	0.1	7671.0**	1300.5**	10.0×10^{9}**
S × P	6	0.2	0.2	21.4	19.6	7.3	0.1	136.0	26.4	1.2×10^{9}
Biological fertilizer (B)	3	14.7**	0.7**	1.0	21.7	45.2**	16.9**	9089.0**	662.3**	3.4×10^{10}**
S× B	9	1.3*	0.2	23.0	21.1	7.3	1.6**	27.7	97.9**	8.1×10^{8}
P× B	6	0.9	0.4	14.0	21.7	9.1	3.6**	2245.0**	429.4**	3.9×10^{8}
S× P ×B	18	1.1	0.1	24.2	20.9	2.9	0.5	12.8	15.4	8.1×10^{8}
Error	93	0.6	0.1	18.6	0.4	17.8	0.6	94.9	17.8	8.2×10^{8}
CV	-	20.4	19.4	8.0	9.3	23.6	8.9	47.7	23.6	32.3

* Significant at P $\le$0.05

** Significant at P $\le$ 0.01

Table 4: Mean comparisons of the main effects on Wheat growth properties

Treatment	Shoot dry weight	Root dry weight	Grain yield	Plant height	Spike length	Grain spike number
	-------------------(g pot^{-1})--------------------			---------------------(cm)--------------------		
P level						
P0	16.4^{c}	20.9^{a}	6.5^{a}	53.6^{a}	6.8^{a}	37.4^{a}
P20	17.3^{bc}	21.6^{a}	7.1^{b}	54.0^{a}	7.1^{a}	38.0^{a}
P40	20.3^{a}	21.3^{a}	8.4^{c}	54.2^{a}	7.1^{a}	38.4^{a}
Soil kind						
Clay	19.7^{ab}	17.0^{a}	7.3^{a}	54.5^{a}	7.0^{a}	37.9^{a}
Clay loam	21.5^{a}	18.2^{a}	7.4^{a}	54.1^{a}	6.8^{a}	38.0^{a}
Loam	18.1^{b}	16.3^{a}	7.5^{a}	53.9^{a}	7.0^{a}	37.8^{a}
Sandy loam	16.9^{bc}	17.2^{a}	7.4^{a}	52.6^{a}	6.6^{a}	37.1^{a}
Biological fertilizer						
Blank	15.6^{c}	15.1^{b}	6.5^{b}	54.6^{a}	6.8^{a}	$.7^{c}$
PSB	18.8^{b}	16.8^{a}	7.7^{b}	54.4^{a}	6.9^{a}	37.5^{b}
AMF	17.9^{b}	16.9^{a}	7.2^{b}	52.9^{a}	7.0^{a}	37.3^{b}
PSB and AMF	23.7^{a}	17.2^{a}	8.2^{a}	53.9^{a}	7.0^{a}	41.3^{a}

Means with different superscript letter(s) are significantly different at $P \leq 0.01$ according to Duncan test

P_0, P_{20} and P_{40} = 0, 20 and 40 mg kg^{-1}P respectively

PSB: phosphate solubilizing bacteria, AMF: arbuscular mycorrhizal fungi

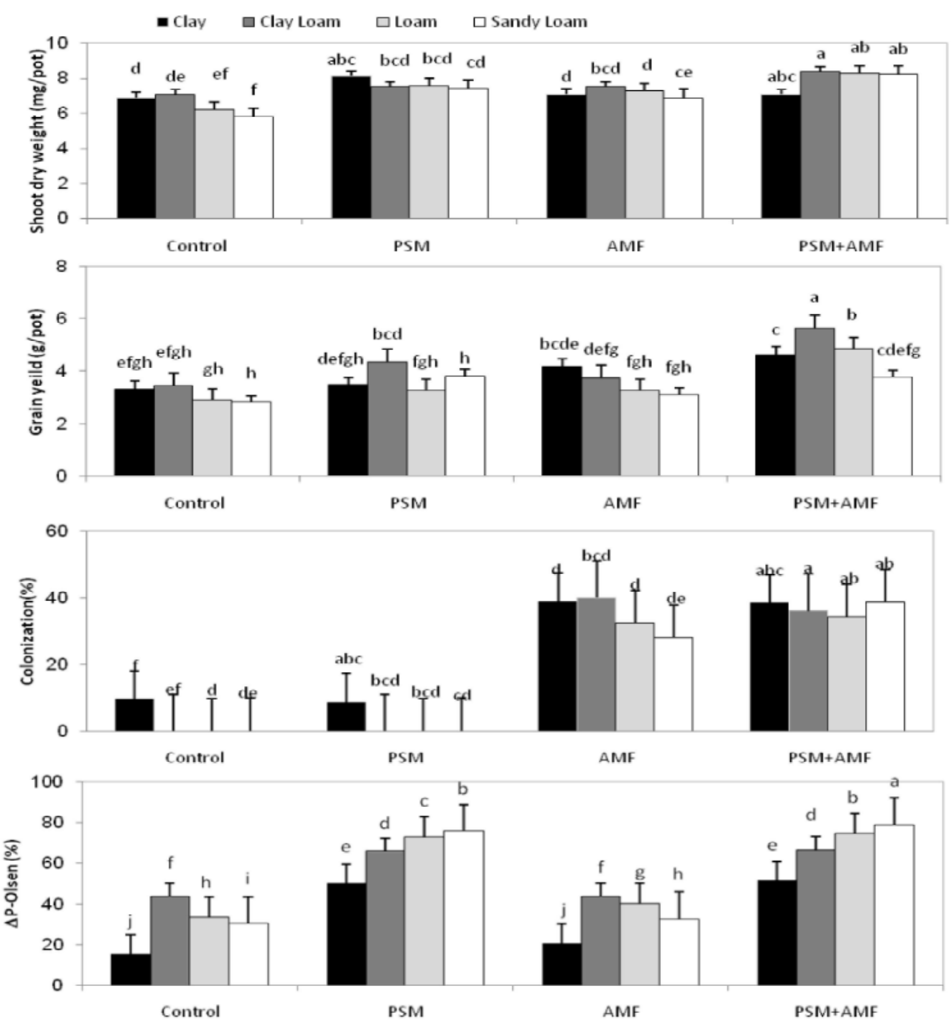

Fig. 1: Interaction effects Soil kind and Biological fertilizer on shoot dry weight (a), grain yield (b), colonization(c) and ΔP-Olsen (d). (PSB: phosphate solubilizing bacteria, AMF: arbuscular mycorrhizal fungi)

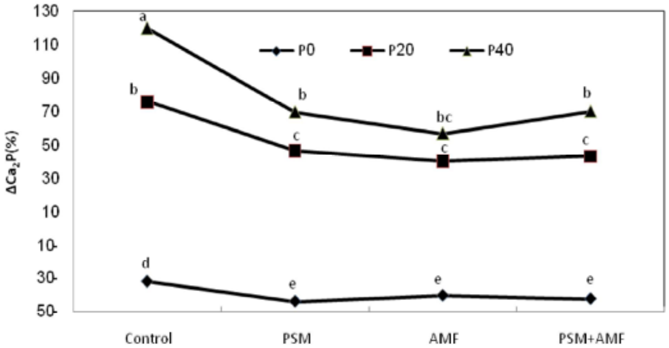

Fig. 2: Interaction affects Soil kinds and phosphorus level (P0=0, P20=20 and P40=40 mg kg^{-1}P) on ΔCa2-P percentage (PSB: phosphate solubilizing bacteria, AMF: arbuscular mycorrhizal fungi)

Application of phosphate fertilizer increased the average shoot dry weight (SDW). The highest SDW was with 40 mg kg^{-1} P application (20.3 g pot^{-1}). The SDW was different among soil textures. The highest SDW was in clay loam soils followed by clay and loam and the lowest SDW was is sandy loam. This might be due to the differences in soil fertility and water holding capacity (Table 4). Biological treatments increased the SDW compared to control in all soil types. The lowest SWD of 2.82 g pot^{-1} was obtained in sandy loam soil without soil biological fertilizer addition. In contrast the highest yield of 5.65 g pot^{-1} was obtained in clay loam soil with addition of the mix PSB and AMF (Fig. 1a).

The PSB and AMF treatments led to an increase in SWD due to development of the root length and phosphorus uptake by roots [24]. This was confirmed by another study in the green house environment which showed as increase in PSB of tomato (*Solanum lycopersicum*) plant dry matter compared to the control experiment [25]. The same trend was also reported for wheat [26]. However, the biological bacterial fertilizer application was not reported to change the dry matter yield of soybean [27].

Root dry weight (RDW) was only significantly affected by biological fertilizers (Table 3). The highest RWD was in the combined PSB and AMF treatment (17.2 g pot^{-1}) compared to control (Table 4).

The statistical analysis of data (Table 3) revealed that none of the treatments, e.g. soil type, phosphorus fertilizer and biological factors and their interactions had significant effects on plant height and spike length. It was found that the changes in plant height and spike length are possibly a genetic trait than any other factors [28].

The biological factor significantly influenced the grain spike number (Table 3). The PSB, AMF and PSB+AMF treatments increased the number of grains per spike compared to control treatment. The highest number (41.3) of grains per spike obtained using PSB+AMF and the least number (34.7) was of control treatment (Table 4). It was found that application of seven kinds of PSB, with and without calcium phosphate fertilizer, resulted in a significant increase in the number of wheat grains seed than control sample. The importance being that this was arrived at without the application of phosphate fertilizer [26]. The same trend was also observed to be true for the corn crop [29]. In another study the effect of AMF on bean seeds per plant was investigated, the results of which showed that no significant effect on grain number [30].

Application of 20 mg kg^{-1} P and 40 mg kg^{-1} P in all treatments increased grain yield compared to the control (7.1 and 8.4 g pot^{-1} respectively). The PSB, AMF and PSB+AMF treatments increased grain yield compared to control treatment (Table 4). Fig. 1b shows that the highest grain yield was in clay loam soil with the combined application of PSB and AMF (8.38 g pot^{-1}) and lowest grain yield was in control treatment of sandy loam soil (5.80 g pot^{-1}). The effects of PSB and various levels of P application rates on sugarcane yield was investigated where it was found that the 75% and 100% application of phosphorus fertilizer did not result in higher sugarcane (Saccharum officinarum) yield [31]. However, it was observed that the application of seven types of phosphate solubilizing bacteria with and without phosphate fertilizer had a significant increase in the wheat yield [26].

Biological Properties: The application of P fertilizer reduced the colonization rate of plant roots. Application of 20 and 40 mg kg^{-1} P reduced the colonization rate by 10.0% (from 28 to 18%) and 16%, respectively (Table 5).

Table 5: Mean comparisons of the main effects on Biological properties.

Treatment	Colon (%)	Spore Number ($10g^{-1}$soil)	PSB Number (g^{-1}soil)
P levels			
P_0	28.2[a]	21.6[a]	79531.0[c]
P_{20}	18.2[b]	17.9[b]	89221.0[b]
P_{40}	12.7[c]	15.3[c]	97187.0[a]
Soil kinds			
Clay	21.5[a]	22.6[a]	108875.0[a]
Clay loam	21.5[a]	18.2[b]	89094.0[b]
Loam	19.5[a]	16.8[b]	82344.0[bc]
Sandy loam	19.2[a]	13.8[c]	83125[c]
Biological fertilizers			
Blank	5.5[b]	2.5[b]	56656.0[b]
PSB	6.2[b]	2.1[b]	114969.0[a]
AMF	34.1[a]	35.4[a]	63125.0[b]
PSB and AMF	35.9[a]	33.4[a]	118688.0[a]

Means with different superscript letter(s) are significantly different at $P \leq 0.01$ according to Duncan test

P_0, P_{20} and $P_{40 = 0,}$ 20 and 40 mg kg^{-1}P respectively

PSB: phosphate solubilizing bacteria, AMF: arbuscular mycorrhiza fungi

Table 6: Analysis of variance of measured parameters of Insoluble phosphorus (P_i) fractions in soil.

Variable	(ΔP-Olsen)	(ΔCa_{10}-P)	(ΔO-P)	(ΔFe-P)	(ΔCa_8-P)	(ΔCa_2-P)	TotalΔ P
Replication ®	6.2	5.7	4.8	5.5	84.0	345.0	6.6
Soil type (S)	30.3**	14.3	12.3	8.5	340.0**	10675.0**	9.5
Phosphorus fertilizer (P)	414.0**	1.2	2.2	1.8	5068.0**	447612.0**	2.2
S × P	23.0	5.2	5.6	6.6	130.4	620.0	4.9
Biological fertilizer (B)	12359.0**	10.5	8.8	10.0	286.0**	8627.0**	9.1
S× B	267.0**	6.3	5.3	6.2	101.0	271.0	5.5
P× B	78.0	7.4	8.1	7.8	41.0	4332**	8.2
S× P ×B	1.0	5.7	6.3	5.2	49.0	541.0	6.3
Error	33.1	5.8	6.4	7.0	39.1	403.6	7.2
CV	35.2	23.6	20.6	22.6	70.1	65.2	27.1

** Significant at $P \leq 0.01$

Table 7: Mean comparisons of the main effects on Insoluble phosphorus (Pi) fractions in soil

	(ΔP-Olsen)	(ΔCa_2-P)	(ΔCa_8-P)
Treatment	(%)		
P levels			
P_0	19.1[a]	-39.6[c]	-6.3[c]
P_{20}	19.8[a]	42.4[b]	1.1[b]
P_{40}	20.2[a]	78.7[a]	6.3[a]
Soil kinds			
Clay	35.5[b]	4.6[b]	-0.3[b]
Clay loam	55.0[a]	5.2[b]	-1.7[b]
Loam	55.3[a]	29.7[a]	-0.4[b]
Sandy loam	54.5[a]	37.9[a]	3.3[a]
Biological fertilizers			
Blank	31.8[b]	44.0[a]	4.1[a]
PSB	66.2[a]	12.7[b]	-0.7[b]
AMF	34.4[b]	8.3[b]	-0.3[b]
PSB and AMF	67.9[a]	13.4[b]	-2.1[b]

Means with different superscript letter(s) are significantly different at $P \leq 0.01$ according to Duncan test

P_0, P_{20} and P40 = 0, 20 and 40 mg kg^{-1}P respectively

PSB: phosphate solubilizing bacteria, AMF: arbuscular mycorrhizal fungi

Small amount of phosphorus at the beginning of crop growth is essential to create symbiosis between plant roots and fungi [32]. In presence excess P in the root zone the rate of symbiosis and thus the colonization rates are reduced. The highest colonization rates were obtained in PSB+AMF and AMF treatments without P application (51 and 49%) (Fig. 1c). The greenhouse studies revealed that high levels of phosphorus fertilizer application reduced the percentage of colonization [33].

Soil type, phosphorus fertilizer, biological fertilizer has significance effect on the number of phosphate solubilizing bacteria (PSB) (Table 3). The highest (108875) and lowest (83125) number of PSB were in clay soil and sandy loam soils, possibly due to leaching of the root zone. The P application increased the number of PSB after the harvest. The biological fertilizers also changed the PSB number. The PSB and PSB +AMF treatments -increased the PSB number by103 and 109% (from 56650 to 118688) compared to control treatment (Table 5). Another study indicated an increased in the number of phosphate solubilizing bacteria by the phosphorus fertilizer [34]. The study further indicated that the soil inoculation with the bacteria increases more than inoculation with seed.

Insoluble Phosphorus (P_i) Fractions in Soil: The change in percent different soil P_i fractions (%ÄP_i) were affected by Phosphorus fertilizer, biological fertilizer, soil type and their interaction (Table 6).

Addition of P to the soils increased the contents of all P_i fractions in soil. Much of the added P was recovered as P_i forms from the soils after the harvest. Addition of 20 and 40 mg kg^{-1} P to soil increased the%ΔP-Olsen (19.8 and 20.2%) compared to control. Adding AMF had no effect on%ΔP-Olsen while PSB and PSB+AMF treatments increased%ΔP-Olsen (Table 7). The highest amounts of%ΔP-Olsen were obtained in sandy loam treated with PSB+AMF (78.8%) and the lowest amount obtained of clay soil without biological fertilizer application (19.5%). (Fig. 1d).

The minimum (4.6%) and maximum (37.9%) of%ΔCa_2-P obtained for clay and sandy loam soil, respectively. A significant difference in the quantity of%ΔCa_2-P in soils was observed by phosphorus solubilizing microorganisms. The highest concentration of%ΔCa_2-P was obtained in 40mg kg^{-1}P application without biological fertilizer application (119.9%) and the least concentration was obtained in control with PSM (-63.8%) (Fig. 5).

Addition of 20 and 40 mg kg^{-1}P to soil increased the%ΔCa_8-P (1.1% and 6.3%) compared to control. In sandy loam soil the%ΔCa_8-P was 3.3% but in clay, clay loam and loam soils the amounts of%ΔCa8-P were decreased by 0.3, 1.7 and 0.4%, respectively. Adding PSB, AMF and Mix of PSB and AMF decreased%ΔCa_8-P compared to control (-0.7, -0.3 and -2.1, respectively) while% Δca_8-P increased in control treatment (4.1%) (Table 6).

Phosphorus in calcareous soils can be quickly converted to insoluble compounds [4]. A research conducted on calcareous soils showed that P application of 60 mg kg^{-1} increases the concentration of Ca_{10}-P by 18.8% [35]. It was also observed that the biological fertilizer application releases certain chemical compounds which in turn increases the phosphorus solubility. In another investigation it was found that the application of phosphorus fertilizers increases the amount of apatite in soil which after 4 to 5 years reaches more than 100% of initial concentration [36].

CONCLUSIONS

The present study demonstrated the benefits of arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing bacteria (PSB) for enhancing the growth of wheat. Application of biological fertilizers reduced%ΔCa_2-P and%ΔCa8-P and increased%ΔP-Olsen. Microorganisms can increase the solubility of inorganic P by releasing protons, H^+or CO2 and organic acid anions such as citrate, malate and oxalate. AMF no effect on%ΔP-Olsen and PSB number whereas increased colonization percentage and spore number. Application phosphorus fertilizer increased%ΔCa_2-P and%ΔCa_8-P and no effect on%ΔP-Olsen whereas reduced colonization percentage, spore and PSB number.

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