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Effects of Phosphate Solubilizing Microorganisms on Wheat Yield and Phosphatase Activity

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PHOSPHATE solubilizing capacity of four strains, *Pseudomonas fluorescence*, *Bacillus megaterium*, *Serratia marcescens,* and *Bacillus subtilis* was assessed in liquid National Botanical Research Institute's phosphate medium containing rock phosphate (RP). A greenhouse pot experiment was conducted to evaluate the effects of inoculation with arbuscular mycorrhizal (AM) fungi alone or in combination with each strain with and without RP on wheat (*Triticum aestivum* Gemeza-9) growth, yield, nutrient uptake and the activity of alkaline phosphatase. The amounts of P released from RP by bacterial strains ranged from 0.22 to 80.8mg P L^{-1} and the pH values of the cultures were reduced from initial value of 7.3 to values varied between 4.04 and 6.62. The results indicated that *B. subtilis* was the most effective strain in solubilizing RP in liquid culture. The combined inoculation with bacterial strains and AM fungi led to a significant increase in soil P content and alkaline phosphatase activity compared with both the non-inoculated and the individually inoculated soil, and this increase was much higher after 69 days comparing with those after 130 days. In RP-amended soil, *B. subtilis* and *P. fluorescence* were more effective in increasing NPK uptake of wheat straw and grains compared with *S. marcescens* and *B. megaterium* when inoculated with AM fungi. This study is concluded that the combined inoculation plus RP gave better results for wheat grown in sandy soil. Further researches are required to estimate this study under field conditions and different soils to give reliable results.

Keywords: AM fungi, NPK, Phosphatase, Phosphate solubilizing bacteria, Wheat.

Introduction

Phosphorus is a critical element for food security in the future decades due to an increase in global food demand. Phosphate fertilization is a main agricultural research subject and is excessively used to improve soil fertility, crop production and for pest control in conventional Egyptian farming. These findings can lead to a contamination of deep-water reservoirs, produce significant direct hazards to the rural population, disrupt the local environment and reduce product quality. Rock phosphate (RP) has been direct used for acid soils as a valuable alternative for industrial P fertilizers. However, RP can be applied to neutral and alkaline soils, but some processes should be conducted before their application such as RP acidulation (Rahman et al., 2018), applied RP with organic manure or composts (Nishanth & Biswas, 2008), and applied RP with microbial inoculants (Hellal el al., 2019; Gurdeep & Reddy, 2015). There are many studies were concentrated on the application of RP with arbuscular mycorrhizal (AM) fungi and/or phosphate solubilizing bacteria (PSB) to enhance the availability of P through solubilization of fixed or insoluble phosphate and modification of root properties (Bücking & Shachar-Hill, 2005; Abd El-Azeem et al., 2007; Mahanta et al., 2018).

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In this regard, PSB play a vital role in transformation and accumulation of P to plant roots that re-translocated in seeds and fruits. These bacteria have ability to produce low molecular weight organic acids in the rhizosphere such as acetic, citric, gluconic, lactic, succinic, propionic and oxalic acids, that well-recognized and widely accepted approach as principal mechanism for P-solubilization (Trivedi & Sa, 2008; Khan et al., 2014; Billah et al., 2019). Also, PSB can facilitate growth and increased of different plants *via* changing the concentration of plant growth promoting like substances such as indoleacetic acid (IAA), synthesizing siderophores, asymbiotic N_2 fixation, biocontrol activity, produce antibiotics and cyanide, synthesizing 1-aminocyclopropane-1- carboxylate (ACC) deaminase that can modulate plant ethylene levels and helping in bioremediation process (Abd El-Azeem et al., 2007; Poonguzhali et al., 2008; Aarab et al., 2019). Several works have shown that the strains *Bacillus subtilis, Serratia marcescens* and *Pseudomonas fluorescens* used as bacterial inoculants to enhance the growth of plants through at least one mechanism; production of IAA, siderophores, antifungal activity, HCN and ACC deaminase (Singh et al., 2008; Selvakumar et al., 2008; Shaharoona et al., 2008). Similarly, arbuscular mycorrhizal (AM) fungi are recognized to promote the growth of host plant through increasing the nutrient uptake in soil and increasing the confrontation of host plants to biotic and abiotic stresses (Bücking & Shachar-Hill, 2005). The host plant that inoculated with AM fungi alone were more benefit in P nutrition due to their ability to increase the absorptive surface area of the roots and it is accumulate under high external supply and to remobilize this storage pool under P stress and to maintain a continuous flux of P (Singh & Kapoor, 1999; Bücking & Shachar-Hill, 2005).

Surprisingly, phosphate solubilizing microorganisms can interact with each other and gave the better results for plant and nutrient uptake when using as combined inoculations. Specifically, AM fungi and bacteria can interact synergistically to stimulate plant growth due to improve nutrient acquisition and inhibition of fungal plant pathogens. Additionally, mycorrhiza helper bacteria can stimulate mycelial growth of mycorrhizal fungi, stimulate spore germination and enhance mycorrhizal formation on the root (Toro et al., 1997; Artursson et al., 2006). On the contrary, AM fungi affect the chemical composition of root exudates that are major nutrient source for the bacteria in the rhizosphere (Artursson et al., 2006). Several studies have shown that the presence of PSB in the soil increases the positive effect of mycorrhizal interactions on P nutrition (Artursson et al., 2006). However, the beneficial traits of root-colonizing bacteria and fungi have been mainly studied separately. Moreover, several studies were conducted to study the effect of combined inoculation with AM fungi and PSB in sterilized soil where competition from indigenous microorganisms in the soil has been avoid.

Wheat is the most important cereal crop in the world, and Egypt vision 2030 suggested to cultivate more area with high productivity using the recommended cultural practices and eco-friendly approaches. Therefore, the objective of this study was to evaluate the effect of single and dual inoculations with PSB and/or AM fungi with and without RP on soil available P, wheat growth and yield as well as NPK uptake in natural P-deficient sandy soil. The effects of these inoculations on the activity of alkaline phosphatase in the rhizosphere were also investigated.

Materials and Methods

Microorganisms

Four bacterial strains, *Pseudomonas fluorescens*, *Serratia marcescens, Bacillus megaterium* and *Bacillus subtilis* SBMP4, were used in this study. The first three strains were obtained from Department of Microbiology, Soils, Water and Environmental Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt. *Bacillus subtilis* strain was obtained from Soil Microbiology lab, Faculty of Agriculture, Suez Canal University. This strain was isolated from the rhizospheric soil of faba bean grown in sandy soil in Ismailia, Egypt and was identified using 16S rRNA gene sequencing with similarity 99% based on BLASTn software (Al-Attar, 2017). Arbuscular mycorrhizal (AM) fungi mixture were obtained from Microbiological Resource Center (MIRCN), Ain Shams University. The AM spore fungal solution containing three different species (*Glomus intraradices, G. monosporum, G. etunicatum*) with a concentration of 50 spore ml^{-1}.

Rock phosphate solubilization in batch culture

The potential of tested strains to solubilize rock phosphate [$Ca_5(PO_4)_3(OH)$] was evaluated using National Botanical Research Institute's Phosphate (NBRIP) broth medium described by (Nautiyal, 1999) contained the following constituents (g L^{-1}): Glucose, 10; $Ca_3(PO_4)_2$, 5; $MgCl_2.6H_2O$, 5; $MgSO_4.7H_2O$, 0.25; KCl, 0.2; $(NH_4)_2SO_4$, 0.1. The experiment was conducted in 100ml conical flask containing 40ml of sterilized NBRIP broth medium. To each flask 0.5g RP (0.25-0.5mm) was added after separately sterilized by autoclaving for 20min at 121˚C. The initial pH was adjusted to 7.3 to ensure a minimum concentration of soluble phosphate. The tested strains were added by 0.5ml aliquots (approximately 10^7-10^8 colony forming units (CFU) ml^{-1}) of actively growing bacterial cultures to each flask. Uninoculated broth medium was used as control. Flasks were incubated at 30˚C and triplicate samples were taken after 0, 2, 3, 7, 10, 12, and 14 days' post-inoculation. The culture suspensions were centrifuged at 3000rpm for 10min and soluble phosphate in the supernatant was determined spectrophotometrically by the phosphomolybdate blue method (Jackson, 1973). The pH of the broth medium was also measured with a digital pH meter after regular intervals.

Preparation of the microbial inocula

The 100 ml Erlenmeyer flask containing 40ml of sterilized tryptic soy broth medium (Starr et al., 1981) contained the following constituents (g L^{-1}): Tryptone, 15.0; Soybean peptone, 5.0; NaCl, 5.0 was inoculated with the bacterial strains. The inoculated flasks were incubated at 30 ºC for 3 days. The viable cell in the bacterial suspensions were counted and usually ranged from 10^7 to 10^8 CFU ml^{-1}. For inoculation, wheat seeds (*Triticum aestivum* cv. Gemeza-9) were surface sterilized by dipping in 95% ethanol solution for 5 min and then washed thoroughly with sterilized water (Jacobson et al., 1994). The sterilized seeds were soaked in 40 ml of the cell suspension for 1hr for each bacterial strain and dried before cultivation, whereas, the AM fungal inoculum was added to the soil after three weeks from cultivation at a rate of 150-spores $plant^{-1}$ based on the treatments. For the uninoculated control, sterilized wheat seeds were soaked in 40ml of sterilized tryptic soy broth medium.

Greenhouse pot experiment

The experiment was conducted at the farm of the College of Agriculture, Suez Canal University, Ismailia, season of 2016. The experiment was aimed to evaluate the synergistic impact of AM fungi and four bacterial strains with and without rock phosphate on wheat growth and yield and nutrient uptake in a natural P-deficient unsterile sandy soil. The effect of combined inoculation with bacterial strains and AM fungi on the activity of alkaline phosphatase was also determined. The sandy soil was uniformly packed in plastic pots (30cm height, 18.6cm mean diameter) at a rate of 25.0kg pot^{-1}. The upper 10 kg of the soil in each pot was thoroughly mixed with 1% cattle manure as an organic fertilizer (equal 250g air-dried CM pot^{-1}). Some properties of the soil and CM used in this study were determined according to Gee & Bauder (1986) and Sparks et al. (1996) (Table 1).

The experiment was composed of 20 treatments and the experimental design consists of two blocks one with and the other without AM inoculation. Each block divided into ten different sections, five bacterial strains (non-inoculated control or inoculation with one of the four bacterial strains) and two fertilizer treatments (control soil and rock phosphate application). All treatments were replicated three times, giving a total of 60 experimental units that arranged in a randomized complete block (factorial) design. All pots received nitrogen and potassium fertilizers at rates of 120mg N Kg^{-1} soil (equivalent to 120kg N fed^{-1}) and 50mg K_2O kg^{-1} soil (equivalent to 50kg K_2O fed^{-1}) in the forms of ammonium sulfate (21.6% N) and potassium sulfate (50% K_2O). Potassium sulfate was applied to all pots at two equal splits after 45 and 70 days from sowing. Ammonium sulfate was applied in three levels (20, 30 and 50% of the total amounts) after 21, 45 and 70 days from sowing. Eight inoculated wheat seeds (*Triticum aestivum* cv. Gemeza-9) were sown in each pot and frequently irrigated to 50-70% water holding capacity with Ismailia Canal water (EC, 0.30dS m^{-1}) during the experiment. The seedlings were thinned to four uniform plants pot^{-1} after two weeks from sowing. The plants were harvested after 69 days (vegetative stage) and 130 days (ripeness stage) from sowing, dried at 65˚C and the dry weights of the shoot, straw and grains were recorded and analyzed for NPK. Soil samples were also collected at the two abovementioned growth stages and analyzed for available P, pH and measured alkaline phosphatase activity.

Table 1. Some properties of the soil and cattle manure (CM) used in this study.

Properties	Soil	CM
Particle size distribution (%)		
Sand	94.60	-
Silt	3.50	-
Clay	1.90	-
Textural class	Sand	-
pH	8.02†	7.45‡
EC_e (dS m^{-1})§	1.41	12.8
Soluble cations (meq L^{-1}) §		
Ca^{2+}	3.25	42.0
Mg^{2+}	1.65	51.0
Na^{+}	6.60	29.5
K^{+}	3.11	14.5
Soluble anions (meq L^{-1}) §		
HCO_3^-	2.11	46.5
Cl^-	6.20	77.5
SO_4^{2-}	6.30	135
Organic C (g kg^{-1})	1.55	139.5
Total N (g kg^{-1})	0.17	12.2
Available N (mg kg^{-1})	4.68	127.0
Available P (mg kg^{-1})	4.10	116.0

†In soil-water suspension (1:2.5), ‡In CM-water suspension (1:5), §In CM and soil saturated extracts.

Soil and plant analyses

The available soil P was determined using Olsen method (0.5 M $NaHCO_3$-soil extract) (Kuo, 1996). The pH values were also measured in soil-water suspensions (1:2.5) using pH meter. The activity of alkaline phosphatases (mg *p*-nitrophenol liberated kg^{-1} soil h^{-1}) was estimated through the incubation of soil *p*-nitrophenyl phosphate (Tabatabai, 1994). Total N in plant was determined by the Kjeldahl method (Bremner, 1996), while the P and K contents were determined after wet digestion using sulfuric (H_2SO_4) and hydrogen perioxide (H_2O_2). The P was measured spectrophotometrically with the molybdenum-blue method (Jackson, 1973) and the potassium was measured using the flamephotometer.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using Costat statistical software, Version 6.311, 1990 (Cohort Program). The least significant difference test (LSD) was applied to make comparison between the means ($P<0.05$). Pearson correlation between soluble P and pH values was also analyzed using SPSS program.

Results and Discussion

Solubilization of rock phosphate in liquid culture

The results in this experiment indicated that the level of P released from RP increased with longer incubation periods and the amount of P released into the medium was dependent on the type of strain (Fig. 1). The highest values of soluble P were observed at 14 days for all tested bacterial strains. The amounts of P solubilized was ranged from 0.22 to 80.8mg P L^{-1} and the pH values of the cultures were reduced from initial value of 7.3 to values varied between 4.05 and 6.62. The amount of P released from RP was 80.8mg P L^{-1}, 14 days after an inoculation with *Bacillus subtilis* and the pH value of the medium were reduced from initial value of 7.3 to 4.95 (Fig. 1). This result indicated that *Bacillus subtilis* was most efficient strain in solubilizing RP in liquid culture. In contrary, the lowest soluble P was observed when inoculated with *Bacillus megaterium* and the amount of P-solubilized was 13.7mg P L^{-1}. After 14-day from incubation. The amount of P-solubilized by *Serratia marcescens* and *Pseudomonas fluorescence* was 57.7 and 44.6 and mg P L^{-1} and the pH values were decreased from 7.3 to 4.32

and 4.20, respectively. These findings indicated that the tested bacterial strains had a wide range of variation in P-solubilization efficiency. In this regard, Qian et al. (2010) clarified that the efficiency of P-solubilization is primarily dependent on the vital of bacterial strains, production of organic acids and phosphatase and the composition of medium.

A highly significant negative ($r = -0.713^{***}$) correlation was observed between the amount of solubilized P and pH values. The decrease pH values clearly indicate the production of organic acids by bacterial strains during the metabolism of composition of medium. It has suggested that the bacterial strain that decreased the medium pH during growth was efficient P solubilizers. Hence, the decrease pH values in partial indicate the production of acids is the main mechanism responsible for P solubilization (Abd El-Azeem et. al., 2007; Wei et. al., 2018; Liu et. al., 2019). This finding agrees with a study of Cherchali et al. (2019) showing that the solubilization of insoluble phosphate was related to decrease pH of the NBRIP medium and they found that a high negative correlation (r = - 0.939) between soluble P released and pH. The relationship between P-solubilization and pH is power function (Fig. 2), indicating that the response of P-solubilization is proportional to the explanatory pH raised to a power.

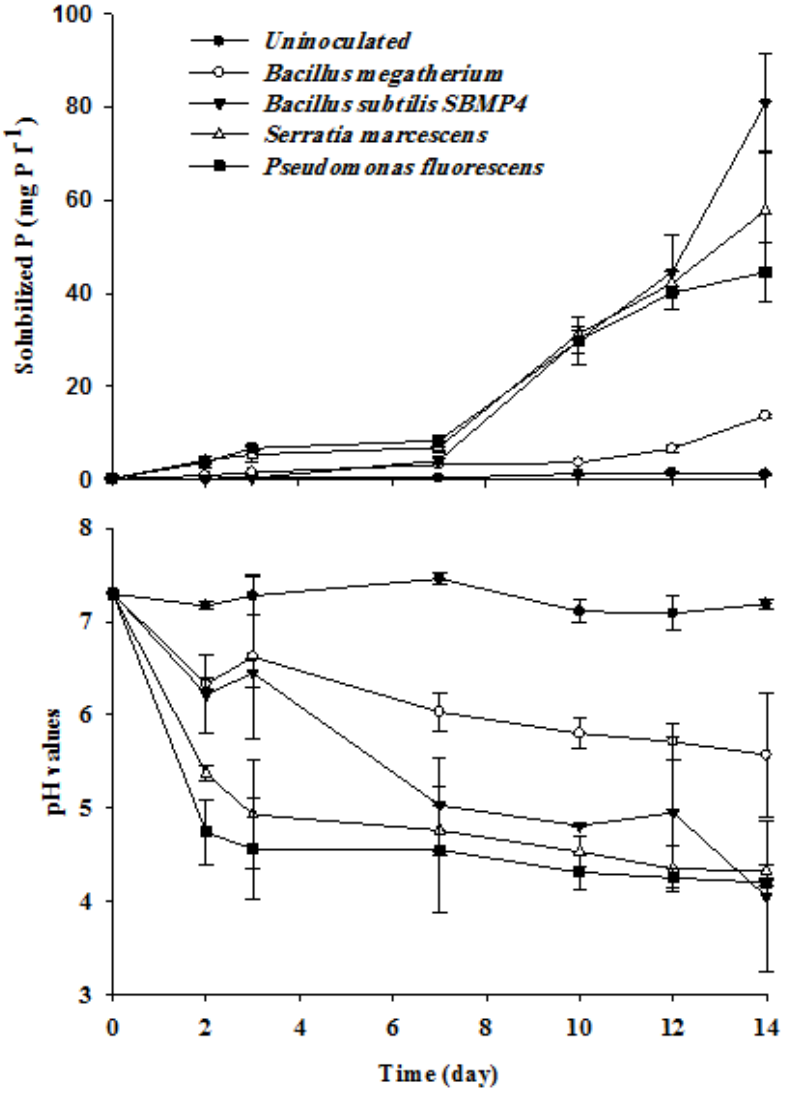

Fig. 1. Changes in solubilized P (a) and pH (b) in the liquid medium during the experiment [The values are the average ± SD from triplicate experiment].

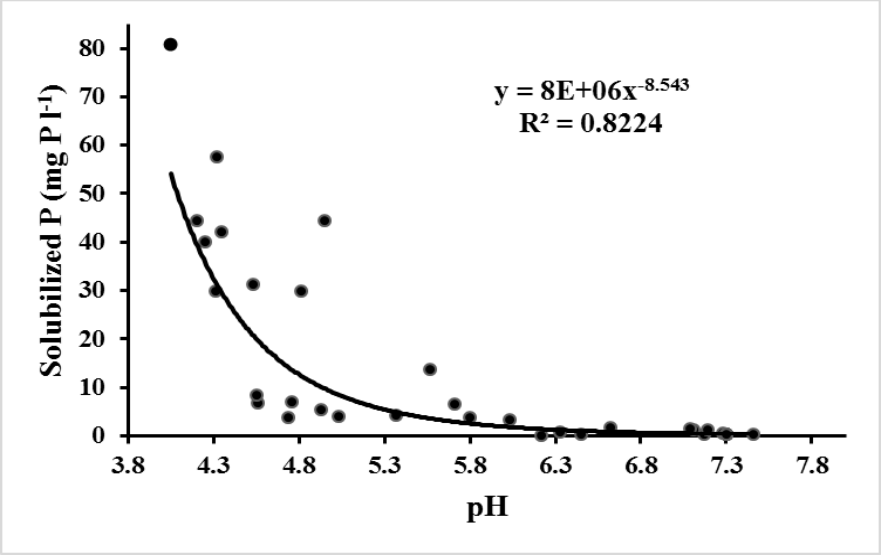

Fig. 2. Regression between P-solubilization and pH on the liquid NBRIP medium during experiment periods.

Single and interaction effects of bacterial strains and AM fungi with and without application of RP

The results indicated that the inoculation with bacterial strains, AM fungi and application of RP significantly affected on wheat growth, yield and nutrient uptake. Regarding the main effects, Table 2 shows that the inoculation with bacterial strains, AM fungi and application of RP were significantly influenced of all parameters that measured in this experiment except soil pH values in case inoculation with AM fungi or not. Concerning the main effect of seed inoculation with bacterial strains on all parameters, Table 2 also indicates that inoculation with tested bacterial strains caused significantly increased for all measured parameters as compared to control. No significant difference was observed between bacterial strains in most cases.

Soil available phosphorus and pH

Soil available P was significantly increased by inoculating the soil with AM fungi and bacterial strains comparing with non-inoculated or the individually inoculated (Table 3). Applying RP at rate of 31.0kg P_2O_5 fed^{-1} to AM fungi and bacterial strains resulted in a significant increase in soil available P compared with the RP-untreated soil. Soil available P content was higher after 69 than after 130 days from wheat sowing. The highest P values were found between 10.19 -11.10mg kg^{-1} while the lowest values were ranged between 9.14 -10.00mg kg^{-1} the soil receiving RP and inoculated with AM fungi and *Bacillus megaterium* or *Pseudomonas fluorescence* after 130 and 69 days from wheat sowing, respectively. The significant increase in the availability of P in the soil cultivated with AM fungi- and/or bacterial strains inoculated plants compared to the uninoculated control could be partially attributed to the essential roles of these microbes in increasing the counts of total bacteria in the rhizosphere of wheat plants, thereby increasing the activity of phosphatases and production of organic acids which led to significant reductions in soil pH values (Al-Attar, 2017). Additionally, bacteria and fungi in the rhizosphere produce hormones that influence root architecture, development of root hairs and affinity of roots for phosphate, thus indirectly affecting the P uptake by plants (Deubel & Merbach, 2005). *Bacillus* spp. were the most efficient phosphate dissolvers by producing organic acids such as acetic, glycolic, isovaleric, isobutyric, malonic and succinic acids.

Similarly, Kohler et al. (2007) observed a synergistic interaction between phosphate solubilizing bacterium *B. subtilis* and the AM fungus *G. intraradices* resulted in high phosphatase activities which in turn enhanced available P in the soil. A highly significant negative correlation was observed between soil pH values and levels of soil available P in soil samples at 69 and 130 days from wheat sowing.

TABLE 2. The individual effect of rock phosphate application, phosphate solubilizers and mycorrhizae inoculations on the study variables after 69 and 130 days from wheat sowing.

Treatments	Available P mg kg^{-1}		pH		Phosphatase		Shoot DW	Grain yield	Straw yield	Shoot			Straw			Grains		
										N	P	K	N	P	K	N	P	K
	69	130	69	130	69	130	69	130		69			130					
Rock phosphate (RP)																		
RP0	6.66	5.81	7.63	7.44	54.9	47.4	3.87	8.04	10.7	102	7.25	32.7	153	7.25	70.1	174	18.8	79.5
RP1	8.63	7.73	7.37	7.24	69.9	60.6	4.66	12.6	15.2	131	12.9	68.6	251	12.9	111	312	61.3	138
$LSD_{0.05}$	**0.841**	**0.841**	**0.171**	**0.060**	**2.14**	**5.58**	**0.302**	**2.05**	**2.05**	**3.08**	**0.68**	**2.70**	**28.4**	**0.902**	**14.7**	**66.7**	**19.2**	**31.2**
Phosphate solubilizing bacteria																		
Control	6.56	5.68	7.61	7.44	54.6	45.1	4.12	8.18	10.8	112	7.17	42.25	169	7.17	75.14	194	32.7	86.1
P. fluorescence	7.70	6.83	7.44	7.28	67.0	52.3	4.79	9.89	12.5	132	11.0	57.9	187	10.9	87.5	223	33.9	104
B. megaterium	8.06	7.19	7.51	7.35	65.0	58.4	3.84	11.1	13.7	103	12.3	42.1	212	12.3	99.9	259	33.8	121
S. marcescens	7.62	6.74	7.45	7.29	63.3	56.6	4.24	10.7	13.3	115	10.7	56.5	209	10.6	87.7	252	53.8	106
B. subtilis	8.28	7.40	7.50	7.32	62.0	57.5	4.35	11.7	14.3	119	9.28	54.3	232	9.29	102	287	46.0	126
$LSD_{0.05}$	**0.495**	**0.495**	**0.126**	**0.109**	**2.71**	**3.83**	**0.229**	**0.659**	**0.659**	**8.53**	**2.10**	**4.76**	**15.9**	**2.08**	**5.11**	**21.9**	**8.82**	**7.25**
Arbuscular mycorrhizal (AM) fungi																		
- AM	6.63	5.78	7.56	7.38	56.9	48.5	4.03	9.23	11.8	108	8.19	43.5	177	8.11	80.5	209	209	94.8
+ AM	8.66	7.75	7.44	7.29	67.9	59.5	4.50	11.39	13.1	125	12.0	57.7	227	12.0	100	278	278	123
$LSD_{0.05}$	**0.265**	**0.265**	**0.160**	**0.095**	**2.73**	**2.47**	**0.227**	**0.429**	**0.429**	**8.88**	**0.516**	**4.63**	**6.56**	**0.517**	**4.31**	**12.8**	**12.8**	**7.29**

- Rock Phosphate (R0, without and RP1, application of RP at rate of 31.0kg P_2O_5 fed $^{-1}$).
- AM fungi: A mixture of *G. intraradices*, *G. monosporum* and *G. eutnicatum*.
- Alkaline phosphatase (µg *p*NP g^{-1} soil h^{-1}).
- Straw and grain yields (g pot^{-1}).
- NPK uptake mg (pot^{-1}).

TABLE 3. Effects of rock phosphate (RP), bacterial strains and AM fungi on soil available P and pH after 69 and 130 days from wheat sowing.

Treatments			Available P (mg kg^{-1})		pH	
RP	**AM fungi**	**Bacterial strains**	**69 days**	**130 days**	**69 days**	**130 days**
0	Non-inoculated	Non-inoculated	5.04	4.24	7.77	7.57
		Pseudomonas fluorescence	5.48	4.68	7.63	7.43
		Bacillus megaterium	6.56	5.76	7.61	7.41
		Serratia marcescens	6.30	5.50	7.70	7.50
		Bacillus subtilis	6.56	5.76	7.69	7.49
	Inoculated	Non-inoculated	6.81	5.91	7.69	7.49
		Pseudomonas fluorescence	7.94	7.04	7.39	7.24
		Bacillus megaterium	7.41	6.51	7.56	7.36
		Serratia marcescens	6.57	5.67	7.64	7.44
		Bacillus subtilis	7.88	6.98	7.64	7.44
31.0kg P_2O_5 fed^{-1}	Non-inoculated	Non-inoculated	6.68	5.78	7.63	7.43
		Pseudomonas fluorescence	7.35	6.45	7.50	7.30
		Bacillus megaterium	7.19	6.29	7.56	7.36
		Serratia marcescens	7.44	6.54	7.13	7.13
		Bacillus subtilis	7.69	6.79	7.36	7.21
	Inoculated	Non-inoculated	7.69	6.79	7.35	7.28
		Pseudomonas fluorescence	10.0	9.14	7.24	7.18
		Bacillus megaterium	11.1	10.19	7.32	7.28
		Serratia marcescens	10.2	9.26	7.33	7.10
		Bacillus subtilis	11.0	10.1	7.28	7.15
$LSD_{0.05}$			**0.968**	**0.960**	**0.263**	**0.208**

‡ AM fungi: A mixture of *G. intraradices*, *G. monosporum* and *G. eutnicatum.*

Regarding soil pH, the results indicated that soil pH values significantly decreased by increasing RP application rate from 0 to 31 P_2O_5 kg/fedden at both 69 and 130 days. The decrease in soil pH was obviously noticed after 130 days compared with those after 69 days. The same trends in decreasing soil pH values were observed by the individually bacterial strains and AM fungi inoculations compared with the non-inoculated soil. Soil pH values for the bacterial strain inoculated- and non-inoculated soil were ranged between 7.44-7.61 and 7.28-7.44 at 69- and 130-day intervals, respectively. Whereas, these values were ranged between 7.44-7.56 and 7.29-7.38 for the AM fungi inoculated- and non-inoculated soil at the same intervals. Regarding to bacterial strains, the pH values were ranged between 7.44-7.51 and 7.28-7.35 for the soil inoculated with *Pseudomonas fluorescence* and *Bacillus megaterium* after 69 and 130 days, respectively. Reduction in soil pH values may be attributed to produce organic acids and/or increased partial pressure of CO_2 of the soil atmosphere due to increased activity of the native and applied microorganisms (Altomare & Tringovska, 2011).

Activity of alkaline phosphatase

A significant increase in alkaline phosphatase activity in the soil rhizosphere was observed by the application of RP compared to control (Table 4). The highest values of alkaline phosphatase activity in rhizospheric soil were found in all treatments after 69 days compared with those after 130 days from wheat sowing. The alkaline phosphatase activity was significantly increased by inoculating soil with AM fungi and bacterial strains comparing with the non-inoculated and the individually inoculated soil. Applying RP to AM fungi and bacterial strains inoculated-soil resulted in a significant increase in alkaline phosphatase activity compared with the RP-untreated soil and non-inoculated. Activity of the alkaline phosphatase reached its maximum levels in the two soils samples under the treatments AM fungi

+ *Pseudomonas fluorescence* + RP and AM fungi + *Bacillus subtilis* + RP, respectively. Phosphatase activity values were ranged between 75.5-79.9µg *p*NP g^{-1} soil h^{-1} for *Bacillus subtilis* and *Pseudomonas fluorescence*, respectively, after 69 days and they were ranged between 60.1-70.4µg *p*NP g^{-1} soil hr^{-1} for *Pseudomonas fluorescence* and *Bacillus subtilis*, respectively, after 130 days for the soil receiving rock phosphate and inoculated with AM fungi (Table 4).

There are highly significant negative correlations between soil pH and soil available P content and alkaline phosphatase activity in two soil samples (69 and 130 days from wheat sowing) (Table 5). The inoculation with bacterial strains alone or in combination with AM fungi and RP are responsible for increase in available P and alkaline phosphatase activity in soil (Tables 3 and 4). Al-Attar (2017) and Kohler et al. (2007) reported similar results of the synergistic interaction between *B. subtilis* as phosphate solubilizing bacterium and *G. intraradices* resulted in high phosphatase activity in the soil. Czarnes et al. (1999) found that the combined inoculation with AM fungi and PSB improved soil quality by increasing extracellular enzyme activities of phosphatases, urease and dehydrogenase. The phosphate solubilizing microorganisms have ability to produce phosphatase that able to mineralize organic P to mineral P and consequently utilized by plant. Phosphatase activity affected by soil carbon, nitrogen and organic matter content, and thus it may specify soil fertility status (Nannipieri et al., 2011).

TABLE 4. Effects of rock phosphate (RP), bacterial strains and AM fungi on the activity of alkaline phosphatase in wheat rhizosphere after 69 and 130 days from sowing.

Treatments			Alkaline phosphatase (µg *p*NP g^{-1} soil hr^{-1})	
RP	**AM fungi**	**Bacterial strains**	**69 days**	**130 days**
0	Non-inoculated	Non-inoculated	43.2	32.8
		Pseudomonas fluorescence	55.3	36.2
		Bacillus megaterium	56.4	49.7
		Serratia marcescens	47.8	46.6
		Bacillus subtilis	44.2	35.8
	Inoculated	Non-inoculated	49.8	42.7
		Pseudomonas fluorescence	63.5	54.9
		Bacillus megaterium	61.2	57.9
		Serratia marcescens	62.9	54.9
		Bacillus subtilis	64.8	62.1
31.0kg P_2O_5 fed^{-1}	Non-inoculated	Non-inoculated	59.6	47.8
		Pseudomonas fluorescence	69.4	58.1
		Bacillus megaterium	63.5	56.7
		Serratia marcescens	66.4	59.9
		Bacillus subtilis	63.3	61.5
	Inoculated	Non-inoculated	65.9	56.9
		Pseudomonas fluorescence	79.9	60.1
		Bacillus megaterium	78.8	69.4
		Serratia marcescens	76.1	65.1
		Bacillus subtilis	75.5	70.4
$LSD_{0.05}$			**5.41**	**7.47**

‡ AM fungi: A mixture of *G. intraradices*, *G. monosporum* and *G. eutnicatum.*

TABLE 5. Correlation coefficients (r) between soil pH values, available P and alkaline phosphatase in the rhizosphere of wheat plants after 69 and 130 days from wheat sowing.

	Time (day)	Available P	Alkaline phosphatase
pH values	69	-0.552***	-0.641***
	130	-0.592***	-0.587***
Available P	69	-	0.804***
	130	-	0.764***

Wheat growth and yield

Shoot dry weight significantly increased by inoculating the soil with AM fungi and bacterial strains when compared to non-inoculated or the individually inoculated soil (Table 6). Applying RP with AM fungi and bacterial strains led to a significant increase in shoot dry weight compared to RP-untreated soil at 69 days from sowing date. Table 5 also reveals that the highest shoot dry weight of the 69-day old plants was obtained in the soil received RP + AM fungi + *Bacillus subtilis* treatment. In the same way, the maximum grain yield was attained under the treatment AM fungi + *B. megaterium*+ RP followed by AM fungi + *Bacillus subtilis* + RP treatment. Similarly, the highest straw yield was also observed with the treatment AM fungi + *B. megaterium*+ RP followed by AM fungi + *Bacillus subtilis* + RP treatment.

Wheat seeds inoculated with bacterial strains and AM fungi showed overall better growth and yield and the single inoculation with PSB or AM fungi was less effective compared to dual inoculation, supporting hypothesis that these microbes positively act by enhancing nutrient (P and Zn) and water uptake (Saxena & Jha, 2014). These results are in accordance with those obtained by Zaidi & Khan (2005), Roesti el al. (2006), Mäder et al. (2011), Saxena & Jha (2014). The positive response of wheat yield to inoculation with bacterial strains could be partially explained on the basis that these strains possess some plant growth enhancing traits such as their ability to solubilize insoluble phosphates. Several studies reported that *Bacillus megaterium* and *Bacillus subtilis* having several mechanisms for promoting plant growth, indicating that these two strains give the highest wheat yield (Abd El-Azeem et al., 2008; Vurukonda et al., 2016).

Better wheat yield caused by AM fungi could be due to certain specific properties such as their phosphate solubilizing capability (Guinazu et al., 2010) and provide niches for native soil bacteria (Arora et al., 2010) that may contribute in enhancing plant growth. In this regard, Milleret et al. (2009) suggested that the inoculation with AM fungi might help in producing better root system, therefore more exudates would be released into rhizosphere. Root exudates could increase microbial count, contribute in the temporary decline in soil pH and improve soil aggregate stability. These possible changes could be reflected on crop production as the outcome in the end. These results are in concord with those obtained by Zaidi & Khan (2005) who studied the influence of AM fungi and phosphate solubilizing bacterial strains on growth and yield of wheat under a pot condition. They concluded that soil microorganisms have ability to positive interact in promoting plant growth as well as N and P uptake of wheat plants leading to improved yield.

As shown in Table 6, grain/straw ratio for all treatments varied from 0.677 in the non-inoculated treatment to 0.850 in AM fungi + *B. megaterium* + RP treatment with significant differences between the experimental treatments in most cases. This significant variation in ratio of grain/straw between the treatments may be attributed to the ability of some bacteria to fix nitrogen in the soil non-symbiotically, which encourages the vegetative growth and delays flowering of the crop and consequently decreases the grain/straw ratio. It was reported that the soil rich in nitrogen over the satisfactory range will tend to keep down the carbon/nitrogen ratio which delays flowering in nitronegative crops such as wheat (Martin et al., 1976). Similar results and conclusions were reported by Abd El-Azeem et al. (2008) and AlWerwary (2017).

Nutrients content in plant

The uptake of N, P and K in wheat plants were determined after 69 and 139 days from sowing date. Generally, the addition of RP in combination with AM fungi and/or bacterial strains significantly increased the uptake of NPK in shoot of wheat when compared to RP-untreated soil (Table 7). Table 6 also shows that the highest uptake of N and K by the 69-day old plants were

obtained under the treatment RP + *Pseudomonas fluorescence* + AM fungi, while the maximum uptake of P was observed in soil received RP and inoculation with AM fungi + *Bacillus megaterium*. These results indicate that bacterial strains *Pseudomonas fluorescence* and *Bacillus megaterium* were more effective in increasing the uptake of N, P and K of wheat shoot as compared with other bacterial strains in the RP-amended soil and inoculated with AM fungi. The highest uptake of N in straw was found in inoculated plants with *Bacillus subtilis* + AM fungi + RP when compared with other strains and control treatments. Likewise, the maximum P and K uptake in straw was observed in soil received RP and inoculated plants with *Bacillus megaterium* and AM fungi. The highest uptake of the N, P and K by straw was obtained in the soil received RP + AM fungi plus inoculation with *Bacillus subtilis*, *Serratia marcescens* and *Bacillus megaterium*, respectively.

TABLE 6. Effect of rock phosphate (RP), bacterial strains and AM fungi on plant growth and yield of wheat plants sampled at 69 and 130 days.

Treatments			69 days	130 days		
RP	**AMF**	**Bacterial strains**	**Shoot dry weight**	**Grain yield**	**Straw yield**	**grain/straw ratio**
			g pot^{-1}			
0	Non-inoculated	Non-inoculated	3.58	5.48	8.09	0.677
		Pseudomonas fluorescence	3.52	6.15	8.76	0.700
		Bacillus megaterium	3.59	6.68	9.29	0.716
		Serratia marcescens	3.30	6.5	9.11	0.713
		Bacillus subtilis	3.76	7.16	9.78	0.732
	Inoculated	Non-inoculated	4.18	6.85	9.46	0.723
		Pseudomonas fluorescence	4.53	9.78	12.4	0.789
		Bacillus megaterium	3.81	9.61	12.2	0.786
		Serratia marcescens	4.16	11.1	13.7	0.809
		Bacillus subtilis	4.25	11.2	13.8	0.811
31.0kg P_2O_5 fed $^{-1}$	Non-inoculated	Non-inoculated	4.18	9.75	12.4	0.789
		Pseudomonas fluorescence	5.36	11.4	14.0	0.811
		Bacillus megaterium	3.87	13.4	16.0	0.837
		Serratia marcescens	4.61	12.1	14.7	0.823
		Bacillus subtilis	4.50	13.6	16.3	0.838
	Inoculated	Non-inoculated	4.50	10.6	13.2	0.80
		Pseudomonas fluorescence	5.76	12.2	14.8	0.824
		Bacillus megaterium	4.08	14.8	17.4	0.850
		Serratia marcescens	4.87	13.1	15.7	0.834
		Bacillus subtilis	4.91	14.7	17.3	0.849
$LSD_{0.05}$			**0.462**	**1.43**	**1.43**	**0.028**

†RP: Rock phosphate (31.0 Kg P_2O_5 fed $^{-1}$).

‡ AM fungi: A mixture of *G. intraradices*, *G. monosporum* and *G. eutnicatum.*

TABLE 7. Effect of rock phosphate (RP), bacterial strains and AM fungi on the uptake (mg pot^{-1}) of N, P and K in shoots, straw and grains of wheat plants after 69 and 130 days from sowing.

RP	AM fungi	Bacterial strains	Shoots			Straw			Grains		
			N	P	K	N	P	K	N	P	K
0	Non-inoculated	Non-inoculated	88.6	5.18	27.9	97.6	5.18	45.2	98.6	5.83	45.8
		Pseudomonas fluorescence	94.6	6.26	29.9	114	6.26	55.7	120	8.84	58.9
		Bacillus megaterium	91.7	7.66	29.1	132	7.66	64.1	142	9.97	68.8
		Serratia marcescens	84.2	5.21	26.1	139	5.21	51.0	148	16.9	54.5
		Bacillus subtilis	95.8	4.98	31.2	129	4.98	61.7	141	9.25	67.5
	Inoculated	Non-inoculated	111	6.11	36.2	149	6.10	67.8	164	17.9	74.1
		Pseudomonas fluorescence	122	9.43	39.2	179	9.43	85.4	210	20.1	101
		Bacillus megaterium	104	8.69	36.9	180	8.68	86.6	211	20.5	101
		Serratia marcescens	113	10.1	34.9	210	10.1	87.4	256	39.8	106
		Bacillus subtilis	116	8.94	35.5	202	8.94	95.9	246	38.4	116
31.0kg P_2O_5 fed^{-1}	Non-inoculated	Non-inoculated	116	7.56	37.1	199	7.56	89.4	235	48.4	105
		Pseudomonas fluorescence	148	13.2	63.9	205	13.1	101	253	37.7	123
		Bacillus megaterium	105	9.28	38.9	257	9.28	116	324	46.7	145
		Serratia marcescens	128	11.9	81.1	230	11.3	101	283	71.2	124
		Bacillus subtilis	125	10.6	69.8	267	10.5	121	337	54.2	152
	Inoculated	Non-inoculated	132	9.84	67.9	229	9.84	98.0	277	58.5	118
		Pseudomonas fluorescence	165	15.2	98.9	251	15.1	108	310	69.1	134
		Bacillus megaterium	112	23.5	63.6	280	23.5	133	358	58.1	169
		Serratia marcescens	137	15.7	83.8	255	15.7	112	318	87.3	140
		Bacillus subtilis	139	12.7	80.7	332	12.7	132	423	82.1	168
$LSD_{0.05}$			**16.9**	**3.86**	**9.39**	**31.1**	**3.83**	**11.0**	**60.6**	**18.8**	**24.8**

†RP: Rock Phosphate (31.0kg P_2O_5 fed^{-1}).

‡ AM fungi: A mixture of *G. intraradices*, *G. monosporum* and *G. eutnicatum.*

The increase in the N and P content in shoot, straw and grains of inoculated plants (Table 7) confirmed the effect of joining the efficient phosphate solubilizing bacterial strain and mycorrhizal fungi. Numerous studies have proved synergistic relations between PSB and AM fungi (Singh & Kapoor, 1999; Artursson et al., 2006; Mäder el al., 2011). For instance, Toro et al. (1997) studied the inoculation of plant with AM fungi and PSB alone or in combination under phosphate limited systems. Their study showed that the bacteria promoted mycorrhizal establishment whereas the mycorrhizal symbiosis increased the counts of PSB in the rhizosphere. The combined inoculation with AM fungi and PSB significantly increased plant biomass, N and P accumulation in plant tissues, compared with their controls which were not dually inoculated. Additionally, they reported that dually inoculated plants exhibited lower specific activities ($^{32}P/^{31}P$) than control plants using ^{32}P isotopic dilution technique, demonstrating that AM fungi and PSB interacted to make use of P sources otherwise inaccessible to plants. The results in this study agree with previous studies indicating that the combined inoculation of PSB and AM fungi with 50% recommended P as RP increased grain yield of soybean-wheat cropping system (Mahanta et al., 2018).

The use of AM fungi has been shown to possess the capacity to increase the nutrient uptake of plants through improving association with roots and also ease the P uptake by increasing root total length and the absorptive surface area of the mycorrhizal root system and extent the P-depletion zone away from the root surface (Zaida & Khan, 2005; Wu et al., 2014).

Conclusions

This study exposed that the capacity of bacterial strains was a wide variation for solubilizing rock phosphate in liquid culture. The results of this study also indicated that the double inoculation with phosphate solubilizing bacteria and AM fungi through insoluble rock phosphate enhanced plant vigor and nutrient uptake and caused a dramatic increase in plant growth and yield components of wheat crop as well as increase soil available P and activity of alkaline phosphatase. This study is recommending the use of bacterial inoculants in combination with mycorrhizae and rock phosphate for wheat grown in sandy soil. However, further research is required to estimate these bio-inoculants under field conditions and different soils before generalized as biofertilizers.

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