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EFFECT OF INOCULATION WITH VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI AND PHOSPHATE SOLUBILIZING BACTERIA ON GROWTH AND PHOSPHORUS UPTAKE OF WHEAT PLANTS

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ABSTRACT

A pot experiment was conducted to study the effect of inoculating wheat *(Triticum aestivum L.)* with arbuscular mycorrhizal (AM) fungi, *Glomus spp.* and phosphate solubilizing bacteria (PSB), *Bacillus megatherium* with different levels of P in a P-deficient calcareous soil.

Results showed that the highest percentage of mycorrhizal root infection and the maximum population of PSB in wheat rhizosphere soil were found in plants inoculated with dual inocula (AM fungi and PSB) at panicle initiation stage. Generally, mycorrhizal root infection and PSB population reduced at either very low or very high P levels. Inoculation of wheat plants with PSB singly or incombination with AM fungi resulted in significant higher concentration of available P in the rhizosphere soil of inoculated compared with the uninoculated plants. Concentrations of available P in wheat rhizosphere soil reached their peaks when plants inoculated with dual inoculants.

In general, shoots and roots dry weight; grain and straw yields as well as P-content of plant were significantly increased with increasing P levels. There was a significant increase in shoots and roots dry weight; grain and straw yields as well as P-content of plants inoculated with AM fungi and PSB singly or incombination compared with uninoculated plants, and the maximum values obtained with dual inoculation treatments. The interaction between P levels and inoculation treatments significantly affected shoots and roots dry weight; grain and straw yields as well as P-content of plant.

INTRODUCTION

Egyptian soils are normally alkaline in reaction, therefore, the low availability of P to plants is common specially in highly calcareous soils. This often lead to an excess application of P fertilizers to crops. This unmanaged excess of P application may be both an environmental and economic problem. The development of sustainable agricultural systems will require new techniques that help to minimize fertilizer application rates, while maintaining adequate crop yields. The application of biological resources to explore nutrients is currently an active field of research that may hold promise for the future (Jeffries and Barea,1994). Utilizing soil microorganisms to increase nutrient uptake by plants may represent another potential approach.

Supplying plants with P through biological means is a viable alternative. Arbuscular mycorrhizal fungi and phosphate solubilizing bacteria are considered as a biological fertilizers, which have an important role in P solubility and enhances its absorption by plants. Plant growth in the presence of arbuscular mycorrhizal (AM) fungi has been demonstrated Improvement in soil containing low available P. AM fungi enhances the uptake, translocation and transport of phosphate ions from the soil solution to the root cell (Clark, 2002 and Hussain *et al.*, 2001). Consequently, colonization of roots by AM fungi can increase plant growth. Many plants take up more phosphate and grow better when inoculated with AM fungi. This result was observed with onion, maize, wheat and other field crops (Ortas *et al.*, 2001 and Song *et al.*, 2002). On the other hand, phosphate solubilizing bacteria (PSB), solubilize insoluble P by producing various organic acids. This available P is taken up by plants (Rodriguez and Fraga, 1999). Dual inoculation of AM fungi and PSB stimulate plant growth better than inoculation with either organism alone (Jeffries and Barea, 1994 and Toro *et al.*, 1997).

This study was carried out to evaluate the effect of inoculation with AM fungi, PSB singly or incombination with different levels of P fertilizer on mycorrhizal colonization; PSB population; available P in the rhizosphere soil as well as plant growth and P uptake of wheat plants grown in a P- deficient calcareous loamy sand soil.

MATERIALS AND METHODS

1.Experimental design:-

The effect of inoculating wheat (*Triticum aestivum* L.) with arbuscular mycorrhizal (AM) fungi, *Glomus spp.* and phosphate solubilizing bacteria (PSB), *Bacillus megatherium* with different levels of phosphorus in a P-deficient calcareous loamy sand soil was studied. All treatments were arranged in randomized complete design with three replicates of each treatment.

2.Materials:-

2.1.Soil:-

The tested soil was collected from Nubaria region, Behera Governorate.The soil having the following properties :pH 7.9 (1:1 soil water ratio) after Richards (1954); available P was 3 mg kg^{-1} soil according to Olson et al .(1954); organic matter percent was 0.65 determined by oxidizing with cromic acid according to Walkley and Black, as described by Jackson (1973); calcium carbonate (15%) estimated using Collins calcimeter according to Piper(1950) and soil texture was loamy sand .

2.2.Inocula used:-

2.2.1.Mycorrhizal inoculants:-

Mycorrhizal spores suspension (mixture of *Glomus mosseae, Glomus fusciulatum and Glomus clarum*) were multiplied in pot cultures with onion and maize grown for 4 months in 1:1:1 (v:v:v) vermiculite: perlite: peat (Badr El-Din *et al.*, 2000). Mycorrhizal inoculums consisted of coarsely chopped root fragments, spores, hyphae and growth media.

2.2.2.Phosphate solubilizing bacteria (PSB):-

Bacillus megatherium were used as inoculant and obtained from Department of Agricultural Microbiology, National Research Centre, Cairo, Egypt. Five days old culture of Bacillus on nutrient broth medium containing 10^8 cell ml^{-1} was used as liquid inoculant in pot experiments.

3.Experimental methods:-

3.1. Plant growth :-

Plastic pots of 10 kg-soil capacity were filled with soil sample. Supper-phosphate at levels of 0,10,20 and 30 kg P_2O_5 fed^{-1} was applied and mixed well with the whole soil of each pot. A basal dose of K fertilizer, equivalent to 50 kg K_2O fed^{-1}, was added as potassium sulphate and mixed thoroughly with the soil. Nitrogen in the form of ammonium nitrate was applied at the rate of 20 kg N fed^{-1} as basal dose before planting.After that 40 kg N fed^{-1} was applied 3 weeks after planting. At each level of applied P, there were four inoculation treatments, uninoculation (control), AM fungi inoculation, PSB inoculation and mixture of AM fungi and PSB.

Seeds of wheat (*variety Giza 164*) were sown, and after germination, plants were thinned to 12 plant per pot. Soil moisture content was kept near field capacity during the experimental period which extend to harvest stage.

3.2. Bacterial and Mycorrhizal inoculation:-

Bacterial inoculation was done by adding 10 ml of bacterial suspensions to each pot. Mycorrhizal inoculation was done by adding 10g of inoculum consisted of coarsely chopped root fragments, spores and hyphae to each pot by planting the seeds over a thin layer of the pot culture mycorrhizal inoculum.

4.Plant and soil analysis:-

Plant samples were collected at 50, 80 and 150 days after sowing (tillering, panicle initiation and maturity stage, respectively). Plant samples were up rooted as gently as possible without tearing of the root system and the shoots were separated. Dry weight of both shoots and roots at tillering and initiation stages was recorded and analysed for P- content . At maturity stage , grain and straw yield were recorded and analysed for P- content.

In soil samples, percentage of root infection by mycorrhizal fungi as well as counts of PSB in the rhizosphere soil of wheat plants were enumerated at tillering, panicle initiation and maturity stages. Available P in the rhizosphere soil at tillering and panicle initiation was also determined

5. Microbiology Methods:-

5.1. Root infection with AM fungi:-

The root systems of wheat plants were washed with tap water several times to remove adhering soil particles. The roots were cut into small segments and treated with 10% potassium hydroxide in test tubes and heated in water bath for 10 minutes at 80-90 ^{o}C. Thereafter, the root segments were washed with tap water followed by 10% HCl. The Trypan blue stain (0.05 percent) in lactoglycerol (875 ml lactic acid, 63 ml glycerol and 63 ml top distilled water) were added to the roots and heated at 80-90 ^{o}C for 5 minutes (Phillips and Hayman, 1970). The root segments were picked up and placed on glass slides, then a few drops of fresh lactic acid were added. Mycorrhizal infection was noted in each segment in order to calculate the percentage of the root infection. Mycorrhizal colonization was determined by the grid intersect method (Giovannetti and Mosse, 1980).

5.2.Determination of PSB population:-

For counts of PSB in the rhizosphere wheat plants, the technique described by Louw and Webley (1959) was followed. The serial dilution plate method was used for counting phosphate solubilizing bacteria on Bunt and Rovira (1955) medium modified by Louw and Webley (1959).

6. Statistical analysis :-

All data obtained from this study were statistically analyzed through analysis of variance (ANOVA) and least significant difference (LSD) at 0.05 probability level, and applied to make comparisons among treatment means according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

1-Effect of inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply on mycorrhizal root infection and population of phosphate solubilizing bacteria :-

1-1 Mycorrhizal root infection:-

Data presented in Table 1 indicate that the percentage of mycorrhizal root infection of wheat plants by the inoculation with AM fungi were relatively low at tillering stage and colonization of these plants reached a maximum at panicle initiation stage, then decreased at maturity stage. The limitations of root exudates during early stage of wheat plants may restrict flow of energy to the fungus and prevent extensive colonization. This result is in accordance with Fares(1997) who found that the mycorrhizal root infection in wheat plants was increased at the late growth stage compared with the vegetative stage.

In general, there was a significant increase in root colonization in the presence of AM fungi or a combination of AM fungi and PSB over the uninoculation treatment (control) at tillering, panical initiation and maturity stages. The maximum root colonization was observed in dual inoculation treatment. Low percentage of mycorrhizal root infection in the unionculated plants indicated that the native AM fungi are present in the soil was at low density. The significant increase in AM fungi colonization in dual inoculation treatment compared to inoculation with AM fungi alone may have been due to the production of phytohormones by these microorganisms which apparently stimulate mycorrhizal infection (Azcon *et al.*,1978).

Table 1: Percentage of mycorrhizal root infection as effectedby inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply on .

Phosphorus Levels (A)	Inoculated treatments (B)				
	Uninoculated (Control)	AM	PSB	AM+PSB	Mean
	Tillering stage				
P_0	15.333	41.333	15.667	37.667	27.500
P_1	23.000	68.000	24.000	71.000	46.500
P_2	21.333	61.333	23.667	66.333	43.167
P_3	16.333	59.000	17.667	66.000	39.750
Mean	19.000	57.417	20.250	60.250	
L.S.D (0.05) for Phosphorus levels (A)				= 1.7646	
L.S.D (0.05) for inoculation treatments (B)				= 1.7646	
L.S.D (0.05) for (A) x (B)				= 3.5250	
	Panicle initiation stage				
P_0	20.333	84.333	20.000	87.333	53.000
P_1	29.333	88.333	27.000	90.000	58.667
P_2	27.667	86.333	22.667	86.333	55.750
P_3	25.000	84.667	20.333	85.667	53.917
Mean	25.583	85.917	22.500	87.333	
L.S.D (0.05) for Phosphorus levels (A)				= 1.5914	
L.S.D (0.05) for inoculation treatments (B)				= 1.5914	
L.S.D (0.05) for (A) x (B)				= 3.1790	
	Maturity stage				
P_0	17.000	72.000	19.000	75.667	45.917
P_1	26.000	86.000	22.667	89.333	56.000
P_2	24.667	85.333	20.667	88.000	54.667
P_3	20.667	85.000	18.667	87.333	52.917
Mean	22.084	82.083	20.250	85.083	
L.S.D (0.05) for Phosphorus levels (A)				= 2.0552	
L.S.D (0.05) for inoculation treatments (B)				= 2.0552	
L.S.D (0.05) for (A) x (B)				= 4.1060	

Generally, P levels significantly affected AM fungi colonization.Data in Table 1 indicate that root colonization was significantly reduced at no P addition treatment P_0 and P_2 and P_3 treatments compared with P_1 treatment, both in mycorrhizal inoculated and uninoculated plants. Several workers have shown that the degree of mycorrhizal infection may be reduced at either very high or very low phosphorus availabilities (Arias *et al.*, 1991 and Koide, 1991). This effect appears may be due to changes in the P status of the plant rather than to changes in the P status of the soil. It has been proposed that an increase in the P status of the plant restricts the formation of AM fungi because it is associated with decrease in the concentrations of possible fungal metabolites such as soluble carbohydrates and free amino-nitrogen compounds in roots (Thomson *et al.*,1986) and in root exudates (Graham *et al.*,1981). The primary effect of these changes appears to be due to reduce in the growth reduction of external hyphae which in turn reduces the rate of spread of the mycorrhizal fungus by secondary infections(Schwab *et al.*, 1983). Sylvia and Schenck (1983) suggested that inhibitation by P of root colonization by AM fungi with high application of P may be due to reduction in membrane-mediated loss of root metabolites. On the other hand, arbuscule formation is sensitive to P supply and a low to moderate supply is required. If the P supply is very low, colonization appears to be inhibited (Bolan *et al.*, 1984). Without P fertilization Glomus *intraradices* formed only intercellular hyphae but not arbuscules in roots of tomato and the subsequent application of P triggered arbuscules formation (Ezawa *et al.*, 2002).

1-2- Population of phosphate solubilizing bacteria (PSB)

Table 2 show that the number of indigenous PSB in the rhizosphere soil of wheat plants was very low (0.051×10^4 cell g^{-1} dry soil). The limitation of root exudates during the initial phase of bacteria development may restrict the flow of energy to the bacteria and decline the proliferated of bacteria, thereafter, prevent reached an extensive cells. These limitations of root exudates may be a result of the slow root growth rate of plants growing in the nutrient-deficient soils (Lesica and Antibus, 1986).

P fertilization, generally, increased population of PSB in all treatments, both inoculated and non-inoculated, reaching their peak almost on the panicle initiation stage, then declined at maturity stage. Such differences may be due to the changes in multiplication rate of PSB as a result of qualitative changes in nature of root exudates of the plants during the different growth stages (Abdel-Ati *et al.*, 1996).

The introduced PSB increased its population in treatments inoculated with PSB singly or incombination with AM fungi. However, the population of PSB in the AM fungi and uninoculation treatments did not exceed that in the inoculation treatments with PSB singly or in combination with AM fungi.

The population of PSB in the rhizosphere soil of wheat plants was larger than in the treatments which were inoculated with the AM fungi and PSB. This may have been due to high metabolic activities of PSB for a longer period in the rhizosphere of these plants due to inoculation with AM fungi (Singh and Singh, 1993). In the present study, AM fungi increased population of PSB. Substances such as polysaccharides and amino acids, for example, are good sources of carbon or nitrogen, which could released by AM fungi and could stimulation population density of PSB (Vancura *et al.*, 1989).

2- Effect of inoculation with arbuscular mycorrhizal(AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply on available phosphorus in rhizosphere soil.

Data listed in Table 3 indicate that, available P in rhizosphere soil increased with increasing P levels at both tillering and panicle initiation stages. As shown in Table 3, there were significantly higher concentrations of available P in the rhizosphere soil of plants inoculated with PSB singly or in combination with AM fungi compared with uninoculated plants. Amounts of available P reached their peaks when the soil was inoculated with dual inoculants. These findings are in line with those reported by Kim *et al.*, (1998) and Zaghloul (1999).

Table 2: Population of PSB ($x10^4$) cell g^{-1} dry soil as effected by inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply.

Phosphorus Levels (A)	Inoculated treatments (B)				
	Uninoculated (Control)	AM fungi	PSB	AM+PSB	Mean
	Tillering Stage				
P_0	0.051	0.713	42.667	3136.667	795.025
P_1	0.203	3.090	90.000	3956.667	1012.49
P_2	0.550	7.190	69.667	6633.333	1677.685
P_3	0.710	8.750	81.500	5043.333	1283.573
Mean	0.379	4.936	70.959	4692.500	
L.S.D (0.05) for Phosphorus levels (A)				= 273.540	
L.S.D (0.05) for inoculation treatments (B)				= 273.540	
L.S.D (0.05) for (A) x (B)				= 546.479	
	Panicle initiation stage				
P_0	1.810	21.967	1083.333	7800.000	2226.778
P_1	2.270	28.467	1656.667	10100.000	2946.851
P_2	2.103	20.000	7450.000	8000.000	3868.851
P_3	1.045	19.033	5516.667	9056.667	3648.353
Mean	1.807	22.367	3926.667	8739.167	
L.S.D (0.05) for Phosphorus levels (A)				= 247.700	
L.S.D (0.05) for inoculation treatments (B)				= 247.700	
L.S.D (0.05) for (A) x (B)				= 494.843	
	Maturity Stage				
P_0	0.390	7.300	60.667	396.667	11.256
P_1	0.583	11.100	69.667	623.333	176.171
P_2	1.190	9.087	99.333	203.333	78.236
P_3	1.807	8.000	49.000	105.000	40.952
Mean	0.993	8.872	69.667	332.083	
L.S.D (0.05) for Phosphorus levels (A)				= 22.037	
L.S.D (0.05) for inoculation treatments (B)				= 22.037	
L.S.D (0.05) for (A) x (B)				= 44.024	

Higher values of soil available P were obtained at tillering stage and decreased to lower values at panicle initiation stage. This is related to the different uptake rates by plants during the different growth stages.

At both tillering and panicle initiation stages, available P concentrations of inoculated plants with PSB alone or in combination with AM fungi were significantly higher than of uninoculated plants at all P levels. The interaction effect of P levels and AM fungi treatments in terms of available P was found to be insignificant (Table 3).

3-Effect of inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply on the growth of wheat plants:-

Shoots and roots dry weights and grain and straw yield were determined for plants of each treatment as an indication for plant growth.

Table 3: Means of available phosphorus (ppm) in rhizosphers as effect by inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply.

Phosphorus Levels (A)	Inoculated treatments (B)				
	Uninoculated (Control)	AM fungi	PSB	AM+PSB	Mean
	Tillering stage				
P_0	2.287	2.741	2. 870	3.760	2.915
P_1	4.927	5.674	6.880	7.100	6.145
P_2	7.195	7.914	9.770	10.480	8.840
P_3	9.803	8.972	10.570	12.050	10.349
Mean	6.053	6.325	7.523	8.348	
L.S.D (0.05) for Phosphorus levels (A)				= 0.3943	
L.S.D (0.05) for inoculation treatments (B)				= 0.3943	
L.S.D (0.05) for (A) x (B)				= 0.7897	
	Panicle initiation stage				
P_0	0.827	1.250	1.400	2.420	1.474
P_1	2.577	2.945	5.183	4.370	3.769
P_2	4.363	4.830	5.900	6.643	5.434
P_3	6.930	6.530	7.400	8.123	7.246
Mean	3.674	3.889	4.971	5.389	
L.S.D (0.05) for Phosphorus levels (A)				= 0.2738	
L.S.D (0.05) for inoculation treatments (B)				= 0.2738	
L.S.D (0.05) for (A) x (B)				= 0.5480	

3-1-Shoots and roots dry weights:

Phosphorus effects on plant growth expressed as shoots and roots dry weight (g/pot) in inoculated and uninoculated wheat plants are presented in Tables (4 and 5). The applied P significantly increased shoot and root dry weight of plants as a result of enhancing P nutrition for plants. Generally, inoculated plants had greater shoot and root dry weight compared to uninoculated plants (control) both at tillering and panicle initiation stages. Inoculation with AM fungi and PSB in combination resulted in higher shoot and root dry weight than when these organisms were used alone. The shoot dry weight increases resulted from inoculation of plants with AM fungi, PSB and the combination of AM fungi and PSB were 39 , 24 and 44 % respectively at tillering stage, while at panicle initiation stage it were 31 , 20 and 39 % compared with uninoculated plants, the corresponding increases in root dry weight were 26 , 16 and 31 % at tillering stage and 23 , 16 and 30 % at panicle initiation stage. Mycorrhizal inoculation was more effective in increasing plant dry weight than inoculation with PSB. This indicates that AM fungi was most efficient in increasing P uptake and other nutrients from the soil than were PSB. These results are in agreement with those obtained by Al-Karaki and Al-Raddad (1997); Mikhaeel *et al.,* (1997) and Saad and Hammad (1998).

Table 4: Means of shoot dry weight (g/pot) of wheat plants as effected by inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply.

Phosphorus Levels (A)	Inoculated treatments (B)				
	Uninoculated (Control)	AM fungi	PSB	AM+PSB	Mean
	Tillering stage				
P_0	1.435	3.461	1.607	3.468	2.493
P_1	3.018	4.652	4.532	5.146	4.337
P_2	4.520	6.242	5.944	6.495	5.800
P_3	6.143	6.693	6.690	6.699	6.556
Mean	3.779	5.262	4.693	5.452	
L.S.D (0.05) for Phosphorus levels (A)				= 0.3085	
L.S.D (0.05) for inoculation treatments (B)				= 0.3085	
L.S.D (0.05) for (A) x (B)				= 0.6180	
	Panicle initiation stage				
P_0	1.557	5.431	2.649	5.934	3.893
P_1	5.451	7.367	7.268	8.052	7.035
P_2	8.721	10.818	10.690	11.173	10.351
P_3	11.415	11.925	11.949	12.540	11.957
Mean	6.786	8.885	8.139	9.425	
L.S.D (0.05) for Phosphorus levels (A)				= 0.9001	
L.S.D (0.05) for inoculation treatments (B)				= 0.9001	
L.S.D (0.05) for (A) x (B)				= 1.8030	

Within inoculation treatments, P levels significantly affected shoot and root dry weight Tables (4 and 5). At no P addition treatment (P_0) and lower P level treatments (P_1 and P_2), inoculation of plants with AM fungi or PSB or the combination of AM fungi and PSB resulted in significant increase in shoot and root dry weight compared with uninoculated plants, while no significant difference was observed at the highest P level (P_3) at both tillering and panicle initiation stages Tables (4 and 5). Similar findings were observed by Mosse (1973) who found that increased shoot and root dry weight in the presence of AM fungi has been demonstrated in soil containing little available P.

4- Effect of inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply on grain and straw yield of wheat plants:

Fertilization with P resulted in a significant increase in the grain and straw yield, also, inoculation with AM fungi or PSB singly or in combination significantly increased grain and straw yield compared with uninoculation treatment (control) Table (6). The maximum grain and straw yield was observed in the inoculation treatments with AM fungi and PSB. The mean magnitude of increase in grain yield as a result of inoculation plants with AM fungi, PSB and the combination of AM fungi and PSB were 19 , 9 and 33 % respectively, the corresponding values in the straw yield were 21 , 9 and 28 % in the same order. These results are in agreement with those obtained by Saad and Hammad (1998) and Singh and Kapoor (1999).

Table 5: Means of root dry weight (g/pot) of wheat plants as effected by inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply.

Phosphorus Levels (A)	Inoculated treatments (B)				
	Uninoculated (Control)	AM fungi	PSB	AM+PSB	Mean
	Tillering stage				
P_0	0.914	1.633	1.014	1.650	1.303
P_1	1.509	2.054	2.042	2.308	1.978
P_2	2.131	2.514	2.451	2.523	2.405
P_3	2.339	2.518	2.506	2.528	2.473
Mean	1.723	2.180	2.003	2.252	
L.S.D (0.05) for Phosphorus levels (A)				= 0.160	
L.S.D (0.05) for inoculation treatments (B)				= 0.160	
L.S.D (0.05) for (A) x (B)				= 0.320	
	Panicle initiation stage				
P_0	0.927	1.990	1.323	2.232	1.618
P_1	2.162	2.676	2.710	2.779	2.582
P_2	2.915	3.410	3.407	3.535	3.317
P_3	3.496	3.613	3.590	3.759	3.615
Mean	2.375	2.922	2.758	3.076	
L.S.D (0.05) for Phosphorus levels (A)				= 0.2373	
L.S.D (0.05) for inoculation treatments (B)				= 0.2373	
L.S.D (0.05) for (A) x (B)				= 0.4750	

The results in Table 6 indicate that the inoculation with PSB alone at no P addition treatment (P_0) resulted in a significant decrease in both grain and straw yield compared with the other inoculation treatments. This may be due to the insufficient pool of readly metabolisable carbon energy such as glucose and sucrose which promote PSB population (Banik,1983).The soil used in the present study was poor in organic carbon and had inadequate level of P which may have also contributed to the decrease in PSB inoculant population. This is consistent with an increase in PSB numbers with increasing P levels.

At no P addition treatment (P_0) and low P level (P_1), grain and straw yield of inoculated plants were significantly higher than those of uninoculated plants. While at P_2 and P_3 treatments, no significantly differences in grain and straw yield were observed between inoculated and uninoculated plants. The positive response to inoculation with AM fungi and PSB in terms of plant growth has generally been observed in soils of low P availability (Toro *et al.*, 1996); this effect was not observed in soils with a good P supply (Domey,1996).

Table 6: Grain and straw yield (g/pot) of wheat plants as effected of inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply.

Phosphorus Levels (A)	Inoculated treatments (B)				
	Uninoculated (Control)	AM fungi	PSB	AM+PSB	Mean
	Grain yield				
P_0	0.143	2.340	1.407	4.458	2.087
P_1	4.692	7.449	5.583	8.871	6.649
P_2	10.307	10.521	10.600	10.840	10.567
P_3	13.722	14.212	13.904	14.250	14.022
Mean	7.216	8.631	7.874	9.605	
L.S.D (0.05) for Phosphorus levels (A)				= 0.2682	
L.S.D (0.05) for inoculation treatments (B)				= 0.2682	
L.S.D (0.05) for (A) x (B)				= 0.5370	
	Straw yield				
P_0	2.752	7.939	4.255	7.970	5.729
P_1	7.557	9.031	8.988	10.757	9.083
P_2	11.344	11.754	11.666	11.861	11.656
P_3	14.120	14.656	14.310	15.448	14.634
Mean	8.943	10.845	9.805	11.509	
L.S.D (0.05) for Phosphorus levels (A)				= 0.6986	
L.S.D (0.05) for inoculation treatments (B)				= 0.6986	
L.S.D (0.05) for (A) x (B)				= 1.3990	

5- Effect of inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply on P content of wheat plants

5-1- Shoot P content:

At tillering and panicle initiation stages, shoot P content were significantly greater in AM fungi, PSB and dual inoculation treatments compared with the uninoculation treatment (control). The greatest increase in P-content occured in dual inoculated plants, (Table 7). The increase in P-content of plants inoculated with AM fungi was due to an increase in the number of uptake sites per unit area of roots and greater ability of these roots to exploite the soil for nutrient (Hayman and Mosse,1972).

Another factor which may lead to improved P uptake is that plants infected by mycorrhizae had elevated concentrations of CO_2 in the root. The increase in CO_2 concentration was highly correlated with total solution P. Total P uptake of inoculated plants was significantly higher than that of non-inoculated plants (knight *et al.*, 1989).

The increase in the uptake of P by plants in the treatment receiving PSB may be due to the solubilization of insoluble phosphate by PSB, the products of which were made available to plants (Toro *et al.*, 1996). The greater P uptake in plants inoculated with AM fungi and PSB can be attributed to transport of P by the AM fungus which was solubilized by PSB (Jeffries and Barea,1994 and Toro *et al.,* 1997).

At no P addition treatment (P_0) and lower P treatments (P_1 and P_2), shoot P content of inoculated plants were higher than that of uninoculated plants (control), while at the highest P treatment (P_3), no significant differences were observed between inoculated and uninoculated plants.

Table 7: Shoot phosphorus content (mg/pot) of wheat plants as effected of Inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply.

Phosphorus	Inoculated treatments (B)				
Levels (A)	Uninoculated (Control)	AM fungi	PSB	AM+PSB	Mean
	Tillering stage				
P_0	3.930	9.691	6.900	11.583	8.026
P_1	8.028	16.282	16.950	17.342	14.651
P_2	12.792	23.720	22.349	23.967	20.707
P_3	21.501	23.426	23.415	24.116	23.115
Mean	11.563	18.280	17.404	19.252	
L.S.D (0.05) for Phosphorus levels (A)				= 1.3847	
L.S.D (0.05) for inoculation treatments (B)				= 1.3847	
L.S.D (0.05) for (A) x (B)				= 2.7700	
	Panicle initiation stage				
P_0	4.344	15.913	13.682	21.125	13.766
P_1	18.533	28.658	28.050	28.987	26.057
P_2	31.221	43.164	40.622	40.335	38.836
P_3	39.382	46.508	46.601	45.896	44.597
Mean	23.370	33.561	32.239	34.086	
L.S.D (0.05) for Phosphorus levels (A)				= 4.4418	
L.S.D (0.05) for inoculation treatments (B)				= 4.4418	
L.S.D (0.05) for (A) x (B)				= 8.8970	

5-2- Grain and straw P content:

The data obtained for grain and straw P-content are recorded in Table (8). It was observed that P-content of grain and straw was gradually increased as P levels increased. A significant increase in grain and straw P-contents were observed in plants inoculated with AM fungi or PSB or its combinations compared to the uninoculated control. Inoculation with both AM fungi and PSB further improved P content as compared to inoculation with either AM fungi or PSB. Similar results were obtained by Saad and Hammad (1998) and Singh and Kapoor(1999).

Interaction was found between P levels and inoculation treatments Table (8). At no P addition treatment (P0) and low P level treatment (P1), grain and straw P-content of inoculated plants with AM fungi, PSB and the combination of AM fungi and PSB were significantly higher than that of uninoculated plants. Increasing P levels above P1 treatment not resulted in significant increase in grain and straw P content between inoculated and uninoculated plants.

Table 8: Grain and straw phosphorus content (mg/pot) of wheat plants as effect by inoculation with arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) under different levels of phosphorus supply.

Phosphorus Levels (A)	Inoculation treatments (B)				
	Uninoculated (Control)	AM fungi	PSB	AM+PSB	Mean
	Grain				
P_0	1.075	16.918	10.060	32.058	15.028
P_1	32.797	45.066	38.467	55.887	43.054
P_2	65.449	68.389	67.840	69.376	67.764
P_3	78.215	79.019	79.531	79.090	78.964
Mean	44.384	52.348	48.975	59.103	
L.S.D (0.05) for Phosphorus levels (A)				= 2.3708	
L.S.D (0.05) for inoculation treatments (B)				= 2.3708	
L.S.D (0.05) for (A) x (B)				= 4.7490	
	Straw				
P_0	4.266	10.797	7.343	12.194	8.650
P_1	10.202	14.990	13.372	15.060	13.406
P_2	15.042	17.749	16.332	18.000	16.781
P_3	21.697	22.277	23.325	24.433	22.933
Mean	12.802	16.453	15.093	17.422	
L.S.D (0.05) for Phosphorus levels (A)				= 1.4876	
L.S.D (0.05) for inoculation treatments (B)				= 1.4876	
L.S.D (0.05) for (A) x (B)				= 2.9800	

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تاثير التلقيح بفطر الميكوريزا و البكتريا المذيبة للفوسفات على النمو و امتصاص الفوسفور فى نباتات القمح.

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قيمت تجربة اصص لدراسة تأثير تلقيح نباتات القمح بفطريات الميكوريزا والبكتريا المذيبة للفوسفات تلقيحا منفردا من كل منها او تلقيح خليط منهما فى وجود مستويات مختلفة من الفوسفور وذلك فى تربة جيرية فقيرة فى محتواها من الفوسفور الميسر.

وقد أوضحت النتائج ان اعلى نسب لاصابة جذور القمح بفطريات الميكوريزا واقصى تعداد للبكتريا المذيبة للفوسفات فى منطقة نمو جذور القمح وجدت فى حالة النباتات التى لقحت بالخليط المزدوج من كل من فطريات الميكوريزا والبكتريا المذيبة للفوسفات وذلك عند مرحلة بداية تكوين السنابل. وبصفة عامة، فقد انخفضت نسب اصابة جذور القمح بفطريات الميكوريزا وكذلك انخفض تعداد البكتريا المذيبة للفوسفات عند كل من مستويات الفوسفور المنخفضة جداً والعالية جداً، وقد ادى تلقيح نباتات القمح بالبكتريا المذيبة للفوسفات منفرداً او فى خليط مع فطريات الميكوريزا الى زيادة تركيز الفوسفور الميسر فى منطقة نمو جذور القمحللنباتات الملقحه بالمقارنة بالنباتات غير الملقحة وقد وصلت تركيزات الفوسفور الميسر فى منطقة نمو الجذور الى اقصاها عندما لقحت نباتات القمح باللقاح المزدوج من فطريات الميكوريزا والبكتريا المذيبة للفوسفات.

عموما، ادت زيادة مستويات الفوسفور الى زيادة معنوية فى كل من الوزن الجاف لكل من الاجزاء الخضرية والجذور ومحصول الحبوب والقش وكذلك محتوى النبات من الفوسفور، كانت هناك زيادة معنوية فى الوزن الجاف لكل من الاجزاء الخضرية والجذور ومحصول الحبوب والقش وكذلك محتوى النبات من الفوسفور عند تلقيح النباتات بفطريات الميكوريزا والبكتريا المذيبة للفوسفات منفرداً او فى خليط منهما وذلك بالمقارنة بالنباتات غير الملقحة وكانت اعلى قيم متحصل عليها لهذه الصفات فى حالة التلقيح المزدوج لكل من فطريات الميكوريزا والبكتريا المذيبة للفوسفات، هذا ولقد وجد تأثير معنوى مشترك لكل من مستويات الفوسفور ومعاملات التلقيح على الوزن الجاف لكل من الاجزاء الخضرية والجذور ومحصول الحبوب والقش ومحتوى النبات من الفوسفور.