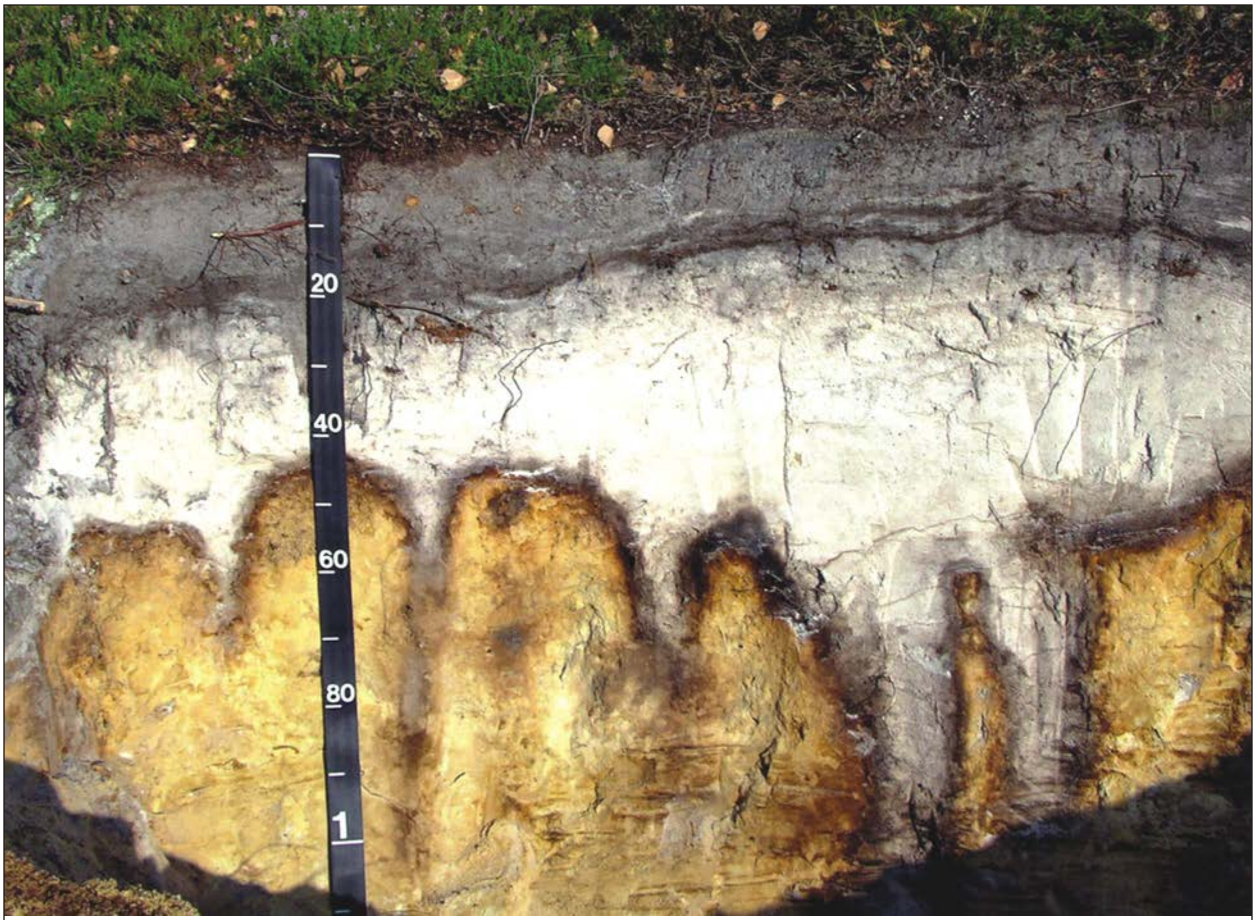

International Journal of

Soil Science

ISSN 1816-4978

Academic
Journals Inc.

www.academicjournals.com

International Journal of Soil Science 10 (2): 93-99, 2015
ISSN 1816-4978 / DOI: 10.3923/ijss.2015.93.99

Impact of Inoculation with Arbuscular Mycorrhizal, Phosphate Solubilizing Bacteria and Soil Yeast on Growth, Yield and Phosphorus Content of Onion Plants

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ABSTRACT

A factorial design experiment was conducted on field to investigate the effects of inoculation with AM fungi (*Glomus mosseae*), phosphate solubilizing bacteria strain (*Bacillus polymyxa*) and soil yeast strain (*Saccharomyces cerevisiae*) on growth, phosphorus content and yield of onion plants in calcareous soil under different levels of phosphorus fertilization (P_1: 50, P_2: 100 and P_3: 200 kg ha^{-1}). Results showed that inoculation with *G. mosseae* or *B. polymyxa* significantly (p = 0.05) increased plant height, shoot fresh and dry weights, root fresh and dry weights, average bulb diameter and total yield. The highest total yield and average bulb diameter were obtained from the inoculation treatment of AM fungus *G. mosseae*, recording 14.4 and 40.8% increases, respectively over the uninoculated control. Also, inoculation with AM fungus (*G. mosseae*) had a significant increase in all mineral content in onion plant compared with uninoculated control or other biofertilizers inoculation treatments (*B. polymyxa* or soil yeast strain *S. cerevisiae*). Only, inoculation with phosphate solubilizing bacteria *B. polymyxa* had significant increase in P content and non significant increases in N, K, Fe, Zn and Mn. Inoculation with soil yeast *S. cerevisiae* had a non-significant increase in all minerals content of onion plant.

Key words: Mycorrhizal fungi, *Bacillus polymyxa*, *Saccharomyces cerevisiae*, phosphorus fertilization, *Allim cepa*

INTRODUCTION

Phosphorus (P) is an essential macroelement for plants, yet the total concentration of P in soils ranges from 0.02-0.5% and average approximately 0.05%, the variation being largely due to differences in the weathering intensity and parent material composition (Stevenson, 1986). Thus, to increase the availability of phosphorus for plants, large amount of fertilizers are used on a regular basis, yet after application, a large proportion of fertilizer phosphorus is quickly transferred to an insoluble form (Omar, 1997). In calcareous soils, phosphorus fertilizers are fixed by calcium carbonate through adsorption and precipitation, resulting in an efficiency of less than 20% (Tisdale *et al*., 1993). The solubilization of phosphate-bearing inorganic materials by microorganisms would seem to be an attractive solution that has been actively studied during the last decade. Several mechanisms, such as lowering the pH by acid production, ion chelation and exchange reaction in the growth environment, have been reported to play a role in P-solubilization by Phosphate-Solubilizing Microorganisms (PSMs) (Goldstein, 1986; Halder *et al*., 1991; Rajankar *et al*., 2007).

Arbuscular Mycorrhizae (AM) are widespread in nature and are fundamental component of the agro-ecosystem. They are stable mutually beneficial plant-fungus associations, in which the fungus is partly inside and partly outside the host and form a living link between root and soil (Bethlenfalvay *et al.*, 1997). One of the most dramatic effects of infection by mycorrhizal fungi on the host plant is the increase in phosphorus (P) uptake (Koide, 1991), mainly due to the capacity of the mycorrhizal fungi to absorb phosphate from soil and transfer it to the host roots (Asimi *et al.*, 1980). In addition, mycorrhizal infection results in an increase in the uptake of copper (Gildon and Tinker, 1983), zinc (Lambert *et al.*, 1979) and sulphate (Buwalda *et al.*, 1983). Resistance against biotic and abiotic stresses has been argued to be due to the effects of AM fungi on inducing plant hormones production (Sharma, 2003).

Phosphate solubilizing microorganisms such as bacteria are another sort of biofertilizers which have the ability to solubilize organic and inorganic phosphorus compounds by producing organic acid or phosphatase enzyme (Rashid *et al.*, 2004). Nonetheless, despite the known ability of yeasts to produce organic acids, there have been very few reports on their ability to solubilize inorganic phosphate (Kanti and Sudiana, 2002; Vassileva *et al.*, 2000; Hesham and Hashem, 2011). This study was conducted to compare the effects of inoculation with AM fungi (*Glomus mosseae*), bacteria strain (*Bacillus polymy*xa) and soil yeast strain (*Saccharomyces cerevisiae*) on growth, phosphorus content and yield of onion plants in calcareous soil under different levels of phosphorus fertilization.

MATERIALS AND METHODS

Preparation of microbial inoculum:

- Arbuscular mycorrhizal fungus, *Glomus mosseae*, was obtained from a stock culture where an onion was the host plant. This specie reproduced by onion pot culture within 4 months in sterilized clay loam soil, by autoclaving twice at 121°C for 1 h. The inoculum contained 250 spores g^{-1} soils together with mycelium and mycorrhizal root fragment, was used at a rate 60 kg ha^{-1}. The number of spores in the soil sample was determined by wet sieving method (Gerdemann and Nicolson, 1963)
- *Bacillus polymyxa* strain was obtained from Desert Research Center, Egypt which used in large-scale production of biofertilizer called "Phosphorine". The strain was maintained on nutrient agar medium at 4°C in refrigerator. This strain was grown on Nutrient Broth (NB) medium for 5 days at 28°C. The counted number of viable cells in the culture at the time of use for inoculation was 1.7×10^8 CFU mL^{-1}
- Soil yeast strain (*Saccharomyces cerevisiae*) was previously isolated from composite sample of the clay soil (Hesham and Hashem, 2011). The strain was maintained on malt-yeast-glucose-peptone agar (YM) slants at 4°C in a refrigerator. Yeast strain was grown for 5 days at 25°C on Pikovskyaya's liquid medium (Rao and Sinha, 1963) having following composition: Glucose 10 g, $Ca_3(PO_4)_2$ 5 g, $(NH_4)_2SO_4$ 0.5 g, KCl 0.2 g, $MgSO_4.7H_2O$ 0.1 g, $MnSO_4$ trace, $FeSO_4$ trace (pH 7). The counted number of viable cells in the culture at the time of use for inoculation was 2.7×10^6 CFU mL^{-1}. The onion seedling inoculation with *Bacillus polymyxa* or *Saccharomyces cerevisiae* by dipping their roots in the culture for 1 h before planting. Carboxy Methyl Cellulose (CMC) was added at a rate of 0.5% to broth culture to increase its viscosity

Field experiment: Onion (*Allim cepa* L.) cultivar Giza-6 was planted at the El-gorahib Experimental Farm of Faculty of Agriculture, Assiut University during the season of 2012 to study the effect of inoculation with AM fungus (*Glomus mosseae*), phosphate solubilizing bacteria strain

Table 1: Some physical and chemical characteristics of a representative composite soil sample from the experimental site

Properties	Values
Clay	9.3
Silt	30.5
Sand	60.2
Texture grade	Sandy loam
Total $CaCO_3$ (%)	16.18
EC (dS/cm^{-1}) (1:1)	1.22
pH (1:1 suspension)	7.82
Total nitrogen (%)	0.04
Organic matter (%)	0.30
Available P ($t\ ha^{-1}$)	10

(*Bacillus polymyxa*) and soil yeast strain (*Saccharomyces cerevisiae*) on growth, yield and phosphorus content under different level of chemical phosphorus fertilizers. Some physical and chemical properties of soil are presented in Table 1. The experimental split plot design with four replicates was employed. The main plots were devoted to levels of P fertilizers (Super Phosphate 15.5% P_2O_5), consisting of P_1 = 50, P_2 = 100 and P_3 = 200 kg ha^{-1}, whereas the subplots were assigned for microbial inoculation: (1) Uninoculated, (2) Inoculated with AM fungus (*Glomus mosseae*), (3) Inoculated with phosphate solubilizing bacteria strain *Bacillus polymyxa* and (4) Inoculation with soil yeast strain (*Saccharomyces cerevisiae*). The plot area was 3×3.5 m and each plot contained 6 ridges each 3 m long. Fifteen seedlings were transplanted at 20 cm in between distance on each side of the ridge. Nitrogen in the form ammonium nitrate (33.5%) was added at rate 200 kg N ha^{-1} in two equal doses, the first after 25 days from transplanting and the second 25 days later.

Plant sampling, growth measurements and yield: Samples of 6 plants were taken from each plot after 60 days from transplanting for determination of growth parameters; plant height, shoots fresh and dry weights and roots fresh and dry weights. Dried shoots at 70°C were ground and submitted to the acid-digestion using a 2:1 H_2SO_4:$HClO_4$ acid mixture for the determination of P, K, N, Fe, Mn and Zn content. At harvest, total yield and average bulb diameter were determined for each plot.

Statistical analysis: The data reported in this study were the mean values based on the three replicates. Differences among treatments were tested by ANOVA and mean values among treatments were compared using Duncan's multiple range test at p = 0.05. Statistical analysis of the data was performed by using the statistical computer program (StatSoft, 1995).

RESULTS AND DISCUSSION

Plant growth and yield: Data in Table 2 show the main effects of inoculation with AM fungus *G. mosseae*, phosphate solubilizing bacteria strain *B. polymyxa* and soil yeast strain *S. cerevisiae* and different levels of P-fertilizer. The results showed that increasing the P-fertilization levels from 50-200 kg ha^{-1} had a significant influence on shoot and root dry weights and non-significant increases in plant height, average bulb diameter and total yield. The optimum P-level was 200 kg ha^{-1} which recorded the highest values for shoot and root dry weights when compared with the other two p-fertilization (50 and 100 kg ha^{-1}).

Table 2: Main effects of P-fertilization levels and inoculation with AM fungi (*Glomus mosseae*), bacteria strain (*Bacillus polymyxa*) and soil yeast strain (*Saccharomyces cerevisiae*) on growth of onion plants

Treatments	Plant height (cm)	Shoot weight (g $plant^{-1}$) Fresh	Shoot weight Dry	Root weight (g $plant^{-1}$) Fresh	Root weight Dry	Average bulb diameter (cm)	Total yield (t ha^{-1})
P-fertilizers							
50	49.45[a]	125.96[c]	13.62[c]	10.82[b]	0.997[b]	2.05[b]	19.17[b]
100	50.71[a]	128.95[b]	14.15[b]	11.33[b]	1.023[b]	2.40[b]	20.95[a]
200	50.98[a]	131.87[a]	14.48[a]	11.79[a]	1.081[a]	2.50[a]	21.30[a]
Inoculation treatment							
Uninoculated	47.33[c]	123.17[d]	13.43[b]	9.46[c]	0.866[c]	2.03[c]	18.30[b]
B. polymyxa	51.98[b]	131.31[b]	14.33[ab]	11.92[b]	1.103[b]	2.25[ab]	19.35[b]
S. cerevisiae	47.37[c]	126.43[c]	13.82[ab]	9.93[c]	0.909[c]	2.64[b]	18.52[b]
AM fungi (*G. mosseae*)	54.48[a]	134.81[a]	14.75[a]	13.94[a]	1.258[a]	2.86[a]	20.93[a]

Values in column followed by the same letter(s) are not significant by Duncan's multiple range test at 5% level significant

Table 3: Interaction effects o f P-fertilization levels and inoculation with AM fungi (*Glomus mosseae*), bacteria strain (*Bacillus polymyxa*) and soil yeast strain (*Saccharomyces cerevisiae*) on growth of onion plants

Treatment P-fertilizer and inoculation	Plant height (cm)	Shoot weight (g $plant^{-1}$) Fresh	Shoot weight Dry	Root weight (g $plant^{-1}$) Fresh	Root weight Dry	Average bulb diameter (cm)	Total yield (t ha^{-1})
50							
Uninoculated	42.66[e]	121.21[h]	13.14[d]	08.77[h]	0.807[c]	1.86[e]	18.84[e]
B. polymyxa	51.20[c]	127.03[e]	13.42[cd]	12.25[cd]	1.173[abc]	2.16[cde]	19.08[e]
S. cerevisiae	46.93[d]	123.85[g]	13.70[cd]	08.99[gh]	0.833[c]	2.70[abc]	18.88[e]
AM fungi (*G. mosseae*)	53.00[b]	131.75[c]	14.22[bc]	13.28[bc]	1.177[abc]	3.06[a]	19.90[d]
100							
Uninoculated	46.90[d]	123.60[g]	13.69[cd]	9.72[gh]	0.860[c]	1.96[de]	20.49[cd]
B. polymyxa	52.20[bc]	130.93[cd]	14.63[ab]	11.65[de]	1.047[abc]	2.30[bcd]	21.19[ab]
S. cerevisiae	47.60[d]	126.01[ef]	13.53[cd]	20.82[bc]	0.910[bc]	2.53[abc]	10.01[fg]
AM fungi (*G. mosseae*)	55.00[a]	135.28[b]	14.75[ab]	21.29[ab]	1.277[ab]	2.80[ab]	13.93[ab]
200							
Uninoculated	48.43[d]	124.69[fg]	13.45[cd]	9.89[fg]	0.930[abc]	1.98[de]	20.96[abc]
B. polymyxa	52.56[bc]	135.97[ab]	14.94[ab]	11.86[d]	1.090[ab]	2.32[bcd]	21.37[ab]
S. cerevisiae	47.60[d]	129.44[d]	14.24[bc]	10.80[ef]	0.983[ab]	2.70[abc]	21.30[ab]
AM fungi (*G. mosseae*)	55.46[a]	137.40[a]	15.28[a]	14.60[a]	1.320[a]	3.03[a]	21.60[a]

Values in column followed by the same letter(s) are not significant by Duncan's multiple range test at 5% level significant

Inoculation with *G. mosseae* or *B. polymyxa* significantly promoted root mass and vegetative plant growth and resulted in significant increases in plant height, shoot fresh and dry weights, root fresh and dry weights, average bulb diameter and total yield. Inoculation with *S. cerevisiae* caused non-significant increases in all growth parameters and total yield compared with uninoculated control. The highest total yield and average bulb diameter were obtained from the inoculation treatment of AM fungus *G. mosseae*, recording 14.4 and 40.8% increases, respectively over the uninoculated control.

The results presented in Table 3 show the interaction effect between inoculation with AM fungus *G. mosseae*, phosphate solubilizing bacteria strain *B. polymyxa* and soil yeast strain *S. cerevisiae* and the p-fertilization levels. The data showed that the promotion effect of inoculation with AM fungus *G. mosseae* and phosphate solubilizing bacteria strain *B. polymyxa* on the plant growth and total yield was more obvious at the low fertilization level 50 kg ha^{-1}, while increasing

Table 4: Main effects of P-fertilization levels and inoculation with AM fungi (*Glomus mosseae*), bacteria strain (*Bacillus polymyxa*) and soil yeast strain (*Saccharomyces cerevisiae*) on nutrient content of onion plants

Treatments	P	N	K	Fe	Mn	Zn
	(%)			(mg kg^{-1})		
p-fertilizers						
50	0.087^{b}	4.30^{b}	2.20^{b}	141.06^{a}	59.92^{a}	37.13^{a}
100	0.107^{b}	4.24^{ab}	2.43^{ab}	115.05^{b}	36.95^{b}	29.81^{b}
200	0.123^{a}	4.21^{ab}	2.80^{ab}	102.78^{c}	26.87^{c}	26.53^{b}
Inoculation treatment						
Uninoculated	0.110^{a}	4.31^{b}	2.10^{b}	137.62^{c}	57.63^{c}	37.64^{d}
B. polymyxa	0.130^{b}	4.80^{b}	2.33^{c}	143.42^{b}	63.61^{b}	43.13^{b}
S. cerevisiae	0.120^{c}	4.36^{b}	2.23^{bc}	140.03^{c}	60.42^{c}	40.58^{c}
AM fungi (*G. mosseae*)	0.128^{d}	4.88^{a}	2.90^{a}	149.04^{a}	68.27^{a}	49.31^{a}

Values in column followed by the same letter(s) are not significant by Duncan's multiple range test at 5% level significant

the p-fertilization to 200 kg ha^{-1} decreased the promotive effect of the inoculation. Furthermore, whereas inoculation with AM fungus *G. mosseae* at a P-fertilization levels of 200 kg ha^{-1} gave higher values for plant height, shoot fresh and dry weights, root fresh and dry weights, average bulb diameter and total yield, these were non-significant increases when compared with inoculation with AM fungus *G. mosseae* at a P-fertilization level of 100 kg ha^{-1}. This result indicates that onion inoculation with effective arbuscular mycorrhizae fungus *G. mosseae* could save approximately between 80-100 kg P-fertilizer ha^{-1}. Mycorrhizal fungi can play an important role in plant nutrient, particularly on soils with low P availability (Bagayoko *et al.*, 2000). Bolandnazar *et al.* (2007) obtained that AM fungi colonization effectively improved onion growth indices such as leave area index and hastened bulbing nearly 2 weeks leading to significantly greater bulb yield. Increasing plant size and yield (Charron *et al.*, 2001; Aguilera-Gomez *et al.*, 1999) and enhancing chlorophyll content (Morte *et al.*, 2000) by AMF colonization have been already reported. In contrast Yousefi *et al.* (2011) reported that Phosphate Solubilizing Bacteria (PSB) was more effective than Arbuscular Mycorrhizae (AM) on wheat growth and P-uptake. Sharif and Moawad (2006) suggested that VA- mycorrhizal fungal spores and root colonization varied in different crops from one site to another under different agro-ecological conditions. Arbuscular Mycorrhizae fungi inoculated to crop plants colonize the plant root system and increase the growth and yield of crop plants. The improved plant growth is due to increased nutrient uptake particularly P, Zn and other micronutrients, production of growth promoting substances, tolerance to drought and salinity and resistance to plant pathogens (Sharma, 2003).

Phosphorus content and other nutrients: The main effect of P application levels, inoculation with AM fungus *G. mosseae*, phosphate solubilizing bacteria strain *B. polymyxa* and soil yeast strain *S. cerevisiae* on nutrient content of onion plants was determined at 60 days after transplanting. Data in Table 4, show that, increasing P application levels had a significant increasing effect on P and K but a decreasing effect on N, Fe, Zn and Mn of onion plant at 60 day after transplanting. In case of P and K, increasing P fertilization levels from 50 to 200 kg ha^{-1} resulted in significant increases, namely: P content from 0.087-0.123% and K from 2.20-2.80%, respectively. The results of this study indicate that P application may interfere with the availability and uptake by onion of these nutrient elements. The results are supported by Mulder's chart of plant nutrient interactions which shows that higher P application reduce availability of Fe, Zn and Mn. Charron *et al.* (2001) reported that increasing in P-uptake by onion plant with increasing in P fertilizers application but Zn content tend to decrease.

In case of inoculation treatments, only inoculation with AM fungus (*G. mosseae*) had a significant increases in all mineral content in onion plant compared with uninoculated control or other biofertilizers inoculation treatment (*B. polymyxa* or soil yeast strain *S. cerevisiae*). But inoculation with phosphate solubilizing bacteria *B. polymyxa* only had significant increase in P content and non significant increases in N, K, Fe, Zn and Mn. While, inoculation with soil yeast *S. cerevisiae* had a non-significant increases in all minerals content of onion plant. These results are in agreement with those reported by Charron *et al*. (2001). Results of experiments suggest that AM fungi absorb N, P, K, Ca, S, Cu, Zn and other micro-elements from the soil and translocate them to associated plants (Turk *et al*., 2006). Microorganisms can increase the solubility of inorganic P by releasing protons, H^+ or CO_2 and organic acid anions such as citrate, malate and oxalate (Yousefi *et al*., 2011). Also, various mechanisms have been suggested for the increase in the uptake of phosphorous by mycorrhizal plant. These include; physical exploration of the soil; increased movement into mycorrhizal fungus hyphae; modification of the root environment; increased storage of absorbed P and efficient transfer of phosphorous to plant roots (Sharma, 2003).

CONCLUSION

The present study demonstrated the inoculation with Arbuscular Mycorrhizal Fungi (AMF) specie (*Glomus mosseae*) had the most effectively improved onion growth, phosphorus and other minerals content and total yield compared with other biofertilizers inoculation treatment phosphate solubilizing bacteria (*B. polymyxa* or soil yeast strain *S. cerevisiae*). The highest total yield and average bulb diameter were obtained from the inoculation treatment of AM fungus *G. mosseae*, respectively recording 14.4 and 40.8% increases over the uninoculated control.

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