Significance of Arbuscular Mycorrhizal Fungi and Phosphate Dissolving Bacteria to Enhance Phosphate Availability for Barley Plants Grown in Calcareous Soil

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ABSTRACT

Two field experiments were conducted to study the effect of Arbuscular Mycorrhizal fungi (VAM) and phosphate dissolving bacteria (PDB) application on soil phosphate availability to barley plant grown in calcareous soil. The experiments were carried out at experimental research station - Ras Sudr, Desert Research Center in winter seasons of 2015-2016 and 2016-2017. Biofertilizers treatments were: control, *Glomus macrocarpium* (VAM) and *Bacillus megatherium* (PDB) either single or mixed application. Phosphorus fertilizers were applied from two sources, mono super phosphate (MSP) and rock phosphate (RP) at rates of 50, 75 and 100% of the recommended dose. Application of MSP fertilizer significantly increase of grain, straw and biological yield during both growing seasons by 2.7, 2.1 and 2.3%, respectively as compared to rock phosphate fertilizer treatment. Dual inoculation with VAM and PDB increased significantly nitrogen and phosphorus concentrations in the grain and straw. The plant height, grain and straw dry weight per plant, 100 grain weight were increased by 7.5, 8.9, 14.8 and 15.8%, respectively and the grain, straw and biological yield increased significantly by 13.4, 20.6 and 18.1%, respectively compared with the un-inoculated treatments. The highest significant biological yield (5.248 t/ha) was obtained under MSP, 75, VAM+PDB treatments.

Key words: barley, salinity, biofertilization, Arbuscular Mycorrhizae Fungi

INTRODUCTION

here irrigation is feasible and other strategic crops such as wheat can be grown (Ahmed, 2005; Hussein *et al*., 2009 and Ahmed *et al*., 2013)

Phosphorus availability in soils can be one of the main factors limiting vegetation growth. Under conditions of limited P, microbes aid in mitigating P losses and increasing P availability. Vegetation clearly affects the microbial community and P cycling (Runyan and D'Odorico, 2013).

The phosphorus content in average soils is about 0.05 % (w/w) of which only 0.1 % is available to plants (Achal *et al*. 2007). Nearly 80 % of applied phosphorus may be unavailable to plants (Holford 1997). Global P fertilizer consumption for 2010 was approximately 37.6 Mt with an annual 3% increase in demand thereafter (Heffer and Prud'homme, 2010). Reserves of mineable rock phosphate (RP), which provide the base raw material for inorganic fertilizer production, are however relatively small and finite (Cordell and White, 2011).

The release of P adsorbed on the solid phase of the soil into soil solution is very slow, and consequently, phosphorus fertilizer efficiency remains low in calcareous soil (Delgado *et al*., 2002). The reaction of phosphate in soil has an important contribution to crop growth and fertilizer use efficiency (Sushanta *et al*., 2014). The availability of P to crops for uptake and utilization is declining in alkaline and calcareous soil due to the decreases of solubility of calcium phosphate minerals (Al Harbi *et al*., 2013 and Ghafoor, 2016).

Arbuscular mycorrhizal fungi (AMF) are found among the soil flora and interact with approximately 85% of the plants on the ground (Brachmann and Parniske, 2006). The association known as arbuscular mycorrhiza (AM) offers benefits such as improvements in the physicochemical conditions of soils, reduced erosion and is a component that must be given due consideration in integrated soil management in order to attain profitable levels of productivity without causing agroecosystem deterioration. it is imperative to further the knowledge that allows application of AMF as one of the fertilization technologies employed as part of the establishment and development of plantations in order to reduce the use of chemically synthesized fertilizers.

AM symbiosis can promote host plant growth by increasing the uptake of mineral nutrition such as P, Zn, and Cu (Javot *et al*., 2007). Assessments of spatial and temporal distribution of AM fungi in saline soil show that the abundance of AM fungi is inversely correlated with the level of soil salinity. The number of propagules or the infectivity of fungal isolates decreases with increasing salt (Azcon-Aguilar *et al*. 2003 and Owen *et al*., 2015)

Bio-resources is much interchangeable and confusing use of terms such as bio-inoculant, bio-fertilizer and bio-amendment in the literature. Bio-resources can be defined as any organic material applied to soil to improve soil quality, nutrient supply and plant growth. Mechanisms of plant growth promotion include hormone production, improved plant nutrition (mainly N and P). Bacteria promote plant growth through the

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production of a variety of stimulating compounds e.g. hormones, antibiotics and enzymes (Gray and Smith, 2005). P improvement mechanisms mediated by bacteria include the production of phosphatases (both alkali and acid), siderophores (Franco-Correa *et al*., 2010) and lowering of soil pH through acid secretion. Organisms that specifically mobilise native and legacy soil P and any insoluble source of P added (e.g. finely ground RP) are generally referred to as phosphate-solubilizing microorganisms (Jones and Oburger, 2011). Microbial solubilisation of P is widely thought of as the 'organic acid theory', in which the two mechanisms of P acquisition involve lowering of pH (directly dissolving mineral P, by proton extrusion) and/or by the release of organic acid anions that exchange for P on soil adsorption sites (Oburger *et al*., 2011 and Zhang *et al*., 2011).

Biological processes in the soil, such as microbial activity, tend to control the mineralization and immobilization of organic conversion of the insoluble forms of phosphorus to an accessible form by plants (orthophosphate), which is an important trait of phosphate-solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) (Fankem *et al*. 2006, Khan *et al*. 2007). In the last few years, the development of microbial inoculum containing phosphate-solubilizing microbes (PSM) gained attention of agriculturists (Fasim *et al*. 2002). Application of PSM, either individually or in combined form, remained successful for increasing yield of soybean, maize, wheat, mung bean and chickpea (Hameeda *et al*. 2008, Jha *et al*. 2011, Singh and Prakash 2012 and Minaxi *et al*. 2013). Sharma *et al*. (2013) observed increased germination, root and shoot length, fresh weight and proline content of chickpea seedlings by *Bacillus* sp. and *Pseudomonas* sp. under osmotic potential of up to 0.4 MPa over un-inoculated control. The strains were able to produce IAA and showed P solubilizing activity. Elkoca *et al*. (2010) demonstrated increased P, K and micronutrient in common bean as a result of Bacillus and Azospirillum inoculations. In another study, PGPR showed positive effects on plant growth of chickpea (Roopa *et al*. 2012) resulting in increased number of nodules, root, shoot growth and yield of plant under stress conditions (Egamberdieva *et al*. 2014).

The objective of this study was to investigate the role of Arbuscular Mycorrhizal Fungi and Phosphate Dissolving Bacteria in increasing availability of phosphate for barley grown in calcareous soil.

MATERIALS AND METHODS

Two field experiments were carried out at the Agricultural Experimental Station of Desert Research Center (D.R.C.) at Ras Sudr, South Sinai in two successive seasons 2015/2016 and 2016/2017. Seeds of barley (*Hordeum vulgare*) were sown in the last week of November 2015 and 2016 in plots (3×3.5m) in rows. The physical and chemical properties of the soil and irrigation water were determined according to the methods outlined by Page *et. al*. (1982) and presented in Tables 1 and 2.

Inorganic fertilization:

Nitrogen and potassium fertilizers were applied at rates of 167 kg N/ha. as NH_4NO_3 (33% N) and 107 kg K_2O/ha. (48% K_2O) into three equal doses. At seedling, tillering and heading growth stages. The dose of 10 m^3 organic manure was added by mixing with the upper 0-20 surface layer before seeds sowing.

Phosphate fertilizers were applied from two sources mono super phosphate (MSP) (15.5% P_2O_5) and Rock phosphate, at three (50, 75 and 100%) rates of the recommended dose (74 kg P_2O_5/ha) of the Ministry of Agriculture and Land Reclamation, and mixed with the soil during land preparation.

Table 1.Tthe main physical and chemical properties of the experimental soil (Average of both seasons)

Particle size distribution (%)				pH	E.C (dSm^{-1})	$CaCO_3$ (%)	Chemical analysis							
							Soluble cations (meq/l)				Soluble anions (meq/l)			
Sand	Silt	Clay	Texture				Na^+	Ca^{++}	Mg^{++}	K^+	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
69.28	17.2	13.52	Sandy loam	8.42	9.61	46.2	64	21.6	10.8	0.57	--	21.7	61.6	14.51

Available nutrients (mg/kg)							
N	P	K	Zn	Mn	Fe	Cu	Mo
32	3.7	108	0.61	4.28	2.5	0.44	0.028

Table 2.The chemical composition of irrigation water

pH	EC (dSm^{-1})	Soluble cations (meq/l)				Soluble anions (meq/l)				S.A.R
		Na^+	Ca^{++}	Mg^{++}	K^+	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	
7.62	8.01	48.32	25.93	5.35	0.48	---	2.41	58.5	20.6	10.4

Biofertilization:

Separation of Arbuscular Mycorrhizal (VAM): Different spores of mycorrhizae were isolated from soil pre-inoculated with mycorrhiza (*Glomus macrocarpium*) by wet-sieving and decantation method described by Gerdeman and Nicolson (1963). The VAM inocula was mixed with pure sand and kept in the refrigerator to be used in the inoculation.

Isolation of Phosphate dissolving bacteria (PDB): For isolation of phosphate dissolving bacteria different soil samples were collected from saline soil at different sites of South Sinai. The highest isolate for phosphate solubilization (DeFreitas *et al*., 1997) were selected for further study. Each isolate was grown on its specific medium containing different sodium chloride concentrations (2, 4, 6, 8 and 10%), also, at different incubation temperatures (25, 30, 40, 45 and 50°C) and different pH values (5-9) and the growth was measured at 600 nm. Selected PDB isolates were purified and identified according to Bergey´s manual of determinative bacteriology (1994). The selected isolates (*Bacillus megatherium*) were subjected to different biochemical tests for screening their hormonal (Rizzolo *et al.*, 1993) and enzymatic activity (Barrow and Veltham, 1993).

Fresh liquid culture of *B. megatherium* was used for single inoculation at the rate of 10^8 colony forming unit (cfu)/ml or in combinations with *Glomus macrocarpium.*

Rhizosphere soil sample were collected at different stages of plant growth and analyzed for: total microbial counts on Bunt and Rovira medium Nautiyal (1999). PDB counts using Pikovskaya's agar medium (PVK) Goenadi *et. al.*, (2000). For the determination of phosphatase activity; disodium phenylphosphate served as enzyme substrate (Őhlinger, 1996).

Assessment of VAM infection: The staining method of Phillips and Hayman (1970) was used for preparing root samples for microscopic observation. The gridlines intersect method of Giovannetti and Mossa (1980) was used to estimate the VAM infection percentage, as follows:

$$\text{Root colonization \%} = \frac{\text{No. of Positive intersect points}}{\text{Total number of observed intersect points}} \times 100$$

Plant samples and Analysis: Plant samples were taken at harvesting from each treatment, washed by tap water then by distilled water (Chapman and Pratt, 1961), dried at 70 °C, and ground using stainless steel equipment's for the determination of N, P and K as follows: total nitrogen using the micro kjeldahl method (A.O.A.C,1980), phosphorus, using dry ashing technique according to Cottenie *et al*. (1982).

Growth parameters: At heading and harvesting stages, the plants were taken from each plot for estimating plant height, fresh and dry weights.

Yield and yield components: At harvest, one square meter from each plot was taken to determine grains, straw and biological yields.

Statistical analysis: The obtained data were exposed to proper statistical analysis of variance according to Gomez and Gomez (1984). LSD at 0.05 level of significance was used for the comparison between means.

RESULTS AND DISCUSSION

Soil microbial activity:

PDB counts:

Table 3 clearly showed that there are high variations of PDB counts between all treatments in barley rhizosphere in both the two successive seasons. The highest PDB counts are recorded with mixed treatment and 100% mineral phosphate fertilizer (being 92×10^{-2} cfu / g dry soil). In the case of rock phosphate, the highest PDB counts are recorded with mixed treatment and 100% mineral phosphate (being 82×10^{-2} cfu / g dry soil). These results agree with those found by Copetta *et al*. (2006). Another study showed that the increase of soil phosphorus availability was due to PDB action (Yousufinia *et al*., 2013).

Phosphatase enzyme:

Table (3) clearly showed that phosphatase activity recorded significant increase due to mixed biofertilization treatments. Mixed biofertilization treatment with 100% MSP recorded the highest phosphatase activity (being 0.47 and 0.48 mg phenol/g soil/24h) at the first and second growing season, respectively. In the case of rock phosphate, the highest phosphatase activity was recorded with mixed biofertilization and 100% rock phosphate (being 0.41 and 0.44 mg phenol/g soil/24h) at the first and second growing season respectively. George *et al*. (2002) stated that Phosphatase enzyme is able to mineralize organic phosphate into inorganic phosphates that provides high phosphate availability for plant.

Mycorrhizal infection and number of spores:

The root colonization of barley plants and number of spores / g soil in the rhizosphere soil were affected by microbial inoculation. The percent of root colonization was higher in the barley inoculated with mixed treatments and 75% rock phosphate (46.6 and 44.7 in the two seasons for mycorrhizal infection, 14.7 and 14.6

for number of spores /g soil) compared to non-inoculated plants (6.1 and 6.8 in the two seasons for mycorrhizal infection, 9.6 and 9.9 for number of spores /g soil).

Mycorrhizal infection and number of spores were increased under rock phosphate treatments by 33.7 and 27.7% and 5.5 and 3.0% compared to MSP treatments. Bahadori *et al*. (2013) found that mixed inoculation have positive effect on increasing root colonization and numbers of VAM spores. These results also agree with

Table 3. Effect of biofertilizers application, mineral and rock phosphate on microbial activity in rhizosphere of Barley plant grown in the two seasons

P	Dose*	Bio	Mycorrhizal Infection (%)		Mycorrhiza No. of spore/g soil		PDB counts ($\times 10^2$cfu/g dry soil)		Phosphatase enzyme (mg phenol/g soil/24h)	
			1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
MSP	50	Control	6.1	6.8	9.6	9.9	24	29	0.11	0.12
		VAM	31.9	33.2	13.7	14.2	53	64	0.29	0.35
		PDB	8.6	10.1	9.8	10.1	69	76	0.34	0.36
		VAM+PDB	33.1	33.4	13.9	14.4	77	79	0.37	0.40
		mean	19.9	20.9	11.8	12.2	56	62	0.28	0.31
	75	Control	8.2	7.3	9.4	9.7	25	32	0.15	0.16
		VAM	25.4	27.9	13.5	14.0	57	66	0.33	0.36
		PDB	7.9	8.8	9.7	10.1	71	74	0.35	0.38
		VAM+PDB	26.7	28.1	13.8	14.3	78	81	0.42	0.44
		mean	17.1	18.0	11.6	12.0	58	63	0.31	0.34
	100	Control	7.2	7.9	8.9	9.2	28	33	0.16	0.19
		VAM	17.3	19.8	13.4	13.6	52	61	0.35	0.39
		PDB	7.6	8.1	9.8	10.1	65	73	0.39	0.41
		VAM+PDB	18.9	21.2	13.4	14.0	80	92	0.47	0.48
		mean	12.8	14.3	11.4	11.7	56	65	0.34	0.37
	mean		16.6	17.7	11.6	12.0	57	63	0.31	0.34
RP	50	Control	8.2	8.9	10.1	10.3	21	23	0.13	0.15
		VAM	43.7	43.1	14.5	14.4	42	49	0.22	0.25
		PDB	11.2	12.1	10.4	10.5	56	63	0.28	0.32
		VAM+PDB	46.6	44.7	14.7	14.6	73	78	0.31	0.34
		mean	27.4	27.2	12.43	12.45	48	53	0.24	0.27
	75	Control	8.2	9.1	9.9	10.0	24	29	0.14	0.17
		VAM	35.4	33.6	14.3	14.5	52	63	0.27	0.3
		PDB	10.3	11.1	10.2	10.3	64	75	0.35	0.36
		VAM+PDB	35.4	36.1	14.5	14.6	70	76	0.39	0.43
		mean	22.3	22.5	12.2	12.4	53	61	0.29	0.32
	100	Control	7.9	8.1	9.4	9.5	27	29	0.17	0.19
		VAM	24.5	27.2	14.1	14.3	55	58	0.28	0.31
		PDB	8.3	9.1	10.3	10.5	63	70	0.37	0.42
		VAM+PDB	26.4	28.1	14.2	14.4	74	82	0.41	0.44
		mean	16.8	18.1	12.0	12.2	55	60	0.31	0.34
	mean		22.2	22.6	12.2	12.3	52	58	0.28	0.31
$LSD_{0.05}$		Bio	0.05	0.05	0.03	0.03	0.12	0.13	0.0006	0.0007
		Dose	0.04	0.04	0.02	0.02	0.10	0.11	0.0005	0.0006
		P	0.03	0.03	0.02	0.02	0.08	0.09	0.0005	0.0005

(MSP) mono superphosphate, (RP) rock phosphate, (*) Percentage of recommended dose

Table 4. Effect of biofertilizers application, mineral and rock phosphate on macronutrients content of grain and straw of barley

P	Dose*	Bio	Grain N (%) 1st season	Grain N (%) 2nd season	Grain P (%) 1st season	Grain P (%) 2nd season	Grain K (%) 1st season	Grain K (%) 2nd season	Straw N (%) 1st season	Straw N (%) 2nd season	Straw P (%) 1st season	Straw P (%) 2nd season	Straw K (%) 1st season	Straw K (%) 2nd season
MSP	50	Cont.	1.54	1.55	0.32	0.33	0.403	0.390	0.56	0.57	0.17	0.17	1.93	2.01
		VAM	1.65	1.65	0.42	0.43	0.416	0.416	0.58	0.58	0.18	0.19	2.01	2.10
		PDB	1.56	1.55	0.40	0.41	0.416	0.403	0.57	0.59	0.18	0.18	2.02	2.11
		VAM+PDB	1.66	1.67	0.48	0.50	0.416	0.403	0.60	0.58	0.19	0.19	2.10	2.19
		mean	1.60	1.61	0.41	0.42	0.413	0.403	0.58	0.58	0.18	0.18	2.02	2.10
	75	Cont.	1.52	1.53	0.35	0.35	0.416	0.403	0.57	0.56	0.18	0.18	2.00	2.04
		VAM	1.62	1.64	0.46	0.48	0.416	0.403	0.61	0.59	0.21	0.20	2.10	2.18
		PDB	1.57	1.54	0.45	0.48	0.403	0.416	0.59	0.60	0.22	0.20	2.14	2.15
		VAM+PDB	1.66	1.65	0.49	0.51	0.403	0.403	0.61	0.60	0.23	0.22	2.16	2.20
		mean	1.59	1.59	0.44	0.46	0.410	0.406	0.60	0.59	0.21	0.20	2.10	2.14
	100	Cont.	1.55	1.54	0.36	0.37	0.416	0.416	0.57	0.57	0.19	0.18	2.17	2.20
		VAM	1.63	1.64	0.48	0.49	0.416	0.429	0.58	0.59	0.20	0.20	2.20	2.22
		PDB	1.57	1.55	0.49	0.50	0.403	0.429	0.60	0.59	0.20	0.20	2.18	2.26
		VAM+PDB	1.68	1.66	0.51	0.53	0.403	0.429	0.60	0.61	0.21	0.22	2.17	2.24
		mean	1.61	1.60	0.46	0.47	0.410	0.426	0.59	0.59	0.20	0.20	2.18	2.23
	mean		1.60	1.60	0.43	0.45	0.411	0.412	0.59	0.59	0.20	0.19	2.10	2.16
RP	50	Cont.	1.55	1.54	0.31	0.32	0.416	0.416	0.56	0.57	0.16	0.17	2.00	2.08
		VAM	1.66	1.64	0.40	0.41	0.416	0.416	0.58	0.58	0.18	0.19	2.10	2.18
		PDB	1.56	1.55	0.39	0.41	0.429	0.403	0.58	0.59	0.18	0.19	2.11	2.15
		VAM+PDB	1.68	1.66	0.45	0.47	0.429	0.429	0.59	0.60	0.19	0.20	2.21	2.21
		mean	1.61	1.60	0.39	0.40	0.423	0.416	0.58	0.59	0.18	0.19	2.11	2.16
	75	Cont.	1.54	1.53	0.33	0.34	0.416	0.403	0.57	0.57	0.17	0.16	2.10	2.11
		VAM	1.64	1.64	0.43	0.45	0.429	0.416	0.59	0.61	0.20	0.19	2.04	2.18
		PDB	1.55	1.56	0.43	0.44	0.442	0.403	0.59	0.60	0.21	0.19	2.08	2.20
		VAM+PDB	1.66	1.67	0.47	0.48	0.442	0.403	0.58	0.61	0.23	0.21	2.10	2.19
		mean	1.60	1.60	0.40	0.43	0.432	0.406	0.58	0.60	0.20	0.19	2.08	2.17
	100	Cont.	1.55	1.54	0.34	0.35	0.429	0.416	0.57	0.57	0.17	0.17	2.14	2.02
		VAM	1.64	1.65	0.46	0.47	0.429	0.416	0.58	0.59	0.20	0.19	2.08	2.16
		PDB	1.57	1.56	0.47	0.49	0.442	0.403	0.60	0.60	0.19	0.19	2.12	2.20
		VAM+PDB	1.67	1.66	0.49	0.51	0.416	0.403	0.61	0.60	0.21	0.21	2.14	2.18
		mean	1.61	1.60	0.44	0.46	0.429	0.410	0.59	0.59	0.19	0.19	2.12	2.14
	mean		1.61	1.60	0.41	0.43	0.428	0.411	0.58	0.59	0.19	0.19	2.10	2.16
$LSD_{0.05}$		Bio	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.0004	0.0004	0.004	0.004
		Dose	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.0004	0.0003	0.005	0.004
		P	0.002	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.0003	0.0003	0.003	0.003

(MSP) mono super phosphate, (RP) rock phosphate, (*) Percentage of recommended dose

the earlier findings of Garbaye (1994) who reported that bacteria, such as those of genus bacillus, produce phytohormones and cohabit in the rhizosphere with VAM fungi which could stimulate the plant-fungus interaction.

Nutrient concentration:

Table (4) showed that N, P and K percentages in barley grains and straw were markedly influenced by biofertilization treatments, mineral and rock phosphate application. The highest N, P and K concentrations were recorded in mixed biofertilization and MSP at 100% of recommended rate (being 1.66, 0.53 and 0.43% for total N, P and K in grain, respectively at the second season). These results are in agreement with those found by Teakle and Tyerman (2010). Inoculation with AMF+PDB significantly increased the nitrogen concentration in grain and straw in both seasons compared to other biological treatments. At the same time, N concentration in grain and straw were increased by 8.4, 8.5% and 5.64, 5.68% in the two seasons, respectively compared to the control treatment. While N concentration was not significantly affected by phosphorus types or dose treatments, the highest significant P concentrations in grain and straw were recorded under MSP, 100% of recommended dose, and VAM+PDB treatments in two seasons. On contrast, K concentration was not significantly affected by application P or biological treatments. Farzaneh *et al*. (2011) and Wang *et. al.* (2015) stated that AMF colonize roots of host plants and promote plant growth due to improved uptake of nutrients.

Growth Parameters:

Data illustrated in Table (5) showed a significant difference due to biofertilizers (VAM and PDB), MSP and rock phosphate treatments on the growth parameters of barley plant. Application of MSP fertilizer significantly increase plant height, grain dry weight and straw dry weight per plant and 100 grain weight during both growing seasons by 1.2, 5.2, 1.7 and 5.0% compared to rock phosphate fertilizer treatment, respectively. Simultaneous application of 100% of the recommended dose of P fertilizer produced the highest growth parameters. Dual inoculation with (VAM and) PDB had the highest enhancement effect on the growth parameters and increased significantly the plant height, grain and straw dry weight per plant, 100 grain weight by 7.5, 8.9, 14.8 and 15.8% when compared with un-inoculated treatments, respectively. At the same time, single inoculation with (VAM) or (PDB) increased significantly the all plant growth parameters by 5.4 and 5.1%, 6.9 and 6.4%, 13.0 and 11.5%, and 11.2 and 9.1% due to VAM and PDB, respectively as compared to un-inoculated treatments. Shaalan (2005) reported that inoculated seeds with bio-fertilizer such as *Azospirillum, Azotobacter and Pseudomonas* gave better plant growth due to the increased nutrients uptake by plant. Microbial inoculation also led to improving soil attributes such as organic matter content and increased P content. Zahir *et al*. (1998), Shaukat *et al*. (2006b), Nourinia *et al*. (2007) and Xu *et al*. (2010) reported an increase of plant height of corn and barley by applying VAM, *Azotobacter and Pseudomonas*.

Yield Parameters:

The impact of various treatments on barley yield parameters (grain, straw and biological yield) was shown in Table (6). Data showed a significant difference for biofertilizers (VAM and *PDB*), MSP and rock phosphate treatments on the yield parameters of barley plant. Application of MSP fertilizer significantly increase grain, straw and biological yield during both growing seasons by 2.7, 2.1 and 2.3%, respectively compared to rock phosphate fertilizer treatment. At the same time, application of 100% of recommended dose of P fertilizer produce the highest yield parameters. Dual inoculation with VAM and PDB had the highest enhancement effect on yield parameters and increased significantly the grain, straw and biological yield by 13.4, 20.6 and 18.1% when compared with un-inoculated treatments. On the other hand, single inoculation with VAM or PDB increased significantly the all yield parameters by 8.9 and 5.5%, 13.4 and 8.2%, and 11.8 and 7.3% for VAM and PDB, respectively as compared to un-inoculated treatments. the microbial inoculation increased plant growth and yield parameters especially in cereals, by producing growth promoting nutrients and improving soil attributes such as organic matter content and increased nutrients content. The obtained results are in agreement with those found by Mousavi and Seghatoleslami (2011) and Rahim *et al*. (2013).

Generally, comparing means of biological yield of barley are shown in Fig. (1). The highest significant biological yield (5.248 t/ha) was obtained with MSP, 75, VAM+PDB treatments while the lowest biological yield (4.153 t/ha) was recorded under RP,50, control. No significant differences of biological yield was observed between each of RP,75, VAM+PDB and MSP, 50, VAM+PDB; MSP, 100, PDB and MSP, 75, VAM; RP, 100, PDB and RP, 100, VAM; MSP, 50, PDB and RP, 50, PDB. Inoculated seeds of barley with VAM or PDB increased the solubility of phosphorus in soil and produced plant promoting materials which lead to increase plant growth and yield parameters.

CONCLUSION

This study was conducted to investigate the role of microbial inoculation with VAM and PDB on the concentration of N, P and K in barley plants, growth parameters and yield of barley grown in calcareous soil conditions. It can be concluded that application of MSP fertilizer significantly increase grain, straw and biological yield compared to rock phosphate fertilizer. Dual inoculation with VAM and PDB increased significantly nitrogen and phosphorus concentration in grain and straw. Also the plant height, grain and straw dry weight per plant and 100 grain weight and increased significantly the grain, straw and biological yield when compared with un-inoculated treatments. The highest significant biological yield was obtained under MSP, 75, VAM+PDB treatments. Using microbial inoculation would reduce inorganic chemical fertilizers and reduce the environmental pollution, simultaneously, to give a sustainable and productive agricultural system in the long term.

Table 5. Effect of biofertilizers, mineral and rock phosphate application on the growth parameters of Barley grown in the two seasons

P	Dose*	Bio	Plant height (cm)		Grain Dry weight (g/plant)		Straw Dry weight (g/plant)		100 grain weight (g)	
			1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
MSP	50	Control	69.8	69.1	17.5	17.7	27.3	27.1	35.7	36.4
		VAM	72.7	72.2	18.8	19.1	30.9	30.1	41.5	42.3
		PDB	73.2	72.4	18.9	19.2	29.7	29.7	39.9	40.6
		VAM+PDB	75.4	73.8	19.1	19.5	31.2	30.6	43.1	43.9
		mean	72.8	71.9	18.6	18.9	29.8	29.4	40.1	40.8
	75	Control	70.8	69.3	17.7	18.0	27.8	27.4	37.9	38.7
		VAM	73.9	73.1	19.4	19.8	32.0	31.1	43.1	44.0
		PDB	74.1	73.3	19.2	19.5	31.4	29.8	42.7	43.1
		VAM+PDB	75.9	75.0	19.7	20.0	32.1	31.5	44.2	45.2
		mean	73.7	72.7	19.0	19.3	30.8	30.0	42.0	42.8
	100	Control	71.2	70.1	18.8	19.1	29.2	29.1	43.4	44.2
		VAM	75.0	74.3	19.4	19.8	32.2	31.3	46.8	47.6
		PDB	75.2	74.5	19.3	19.6	32.0	29.9	45.4	46.2
		VAM+PDB	76.4	75.6	19.8	20.4	33.6	31.6	48.2	49.1
		mean	74.5	73.6	19.3	19.7	31.8	30.5	46.0	46.8
		Mean	73.6	72.7	19.0	19.3	30.8	29.9	42.7	43.4
RP	50	Control	69.2	69.4	16.7	17.0	26.4	25.9	34.0	34.8
		VAM	72.0	72.3	17.8	18.2	31.1	31.0	38.4	39.2
		PDB	72.4	72.4	17.9	18.3	31.0	30.1	37.9	38.6
		VAM+PDB	73.6	73.8	18.1	18.5	31.2	30.5	41.1	41.9
		mean	71.8	72.0	17.6	18.0	29.9	29.4	37.9	38.6
	75	Control	69.7	69.8	16.8	17.1	27.6	27.4	36.1	36.8
		VAM	73.1	73.4	18.4	18.8	30.3	30.1	41.3	42.1
		PDB	73.3	73.7	18.3	18.6	30.8	30.7	40.5	41.3
		VAM+PDB	74.9	74.9	18.7	19.1	31.4	31.1	42.2	43.0
		mean	72.8	73.0	18.1	18.4	30.0	29.8	40.0	40.8
	100	Control	70.1	69.9	17.8	18.1	27.8	27.6	42.1	41.7
		VAM	74.2	74.5	18.5	18.9	31.7	31.6	45.3	46.2
		PDB	74.5	74.7	18.3	18.6	31.3	31.0	43.5	44.2
		VAM+PDB	75.7	75.8	18.9	19.3	32.7	32.1	45.8	46.7
		mean	73.6	73.7	18.4	18.7	30.9	30.6	44.2	44.7
		Mean	72.7	72.9	18.0	18.4	30.3	29.9	40.7	41.4
$LSD_{0.05}$		Bio	0.15	0.15	0.04	0.04	0.06	0.06	0.09	0.09
		Dose	0.13	0.13	0.03	0.03	0.05	0.05	0.07	0.08
		P	0.11	0.11	0.03	0.03	0.04	0.04	0.06	0.06

(MSP) mono super phosphate, (RP) rock phosphate, (*) Percentage of recommended dose

Table 6. Effect of biofertilizers application, mineral and rock phosphate on yield parameters of Barley grown in the two seasons

P	Dose*	Bio	Grain yield (t/ha)			Straw yield (t/ha)			Biological Yield (t/ha)		
			1st season	2nd season	mean	1st season	2nd season	mean	1st season	2nd season	mean
MSP	50	Control	1.476	1.499	1.488	2.737	2.808	2.773	4.213	4.308	4.260
		VAM	1.595	1.642	1.618	3.070	3.094	3.082	4.665	4.736	4.701
		PDB	1.523	1.547	1.535	2.832	2.904	2.868	4.355	4.451	4.403
		VAM+PDB	1.666	1.714	1.690	3.284	3.356	3.320	4.950	5.069	5.010
		mean	1.565	1.601	1.583	2.981	3.040	3.011	4.546	4.641	4.593
	75	Control	1.476	1.523	1.499	2.666	2.808	2.737	4.141	4.332	4.236
		VAM	1.642	1.690	1.666	3.165	3.237	3.201	4.808	4.927	4.867
		PDB	1.547	1.571	1.559	2.975	2.999	2.987	4.522	4.570	4.546
		VAM+PDB	1.737	1.761	1.749	3.451	3.546	3.499	5.188	5.307	5.248
		mean	1.601	1.636	1.618	3.064	3.148	3.106	4.665	4.784	4.724
	100	Control	1.499	1.523	1.511	2.785	2.832	2.808	4.284	4.355	4.320
		VAM	1.666	1.714	1.690	3.237	3.308	3.273	4.903	5.022	4.962
		PDB	1.642	1.642	1.642	3.189	3.284	3.237	4.831	4.927	4.879
		VAM+PDB	1.737	1.737	1.737	3.380	3.451	3.415	5.117	5.188	5.153
		mean	1.636	1.654	1.645	3.148	3.219	3.183	4.784	4.873	4.828
		Mean	1.601	1.630	1.615	3.064	3.136	3.100	4.665	4.766	4.715
RP	50	Control	1.428	1.452	1.440	2.666	2.761	2.713	4.094	4.213	4.153
		VAM	1.571	1.571	1.571	3.023	3.094	3.058	4.593	4.665	4.629
		PDB	1.523	1.547	1.535	2.808	2.904	2.856	4.332	4.451	4.391
		VAM+PDB	1.618	1.642	1.630	3.118	3.142	3.130	4.736	4.784	4.760
		mean	1.535	1.553	1.544	2.904	2.975	2.939	4.439	4.528	4.483
	75	Control	1.499	1.523	1.511	2.737	2.832	2.785	4.236	4.355	4.296
		VAM	1.571	1.571	1.571	3.070	3.118	3.094	4.641	4.689	4.665
		PDB	1.523	1.547	1.535	2.904	2.927	2.916	4.427	4.474	4.451
		VAM+PDB	1.666	1.690	1.678	3.308	3.356	3.332	4.974	5.046	5.010
		mean	1.565	1.583	1.574	3.005	3.058	3.032	4.570	4.641	4.605
	100	Control	1.499	1.499	1.499	2.808	2.856	2.832	4.308	4.355	4.332
		VAM	1.618	1.642	1.630	3.142	3.189	3.165	4.760	4.831	4.796
		PDB	1.618	1.642	1.630	3.142	3.165	3.154	4.760	4.808	4.784
		VAM+PDB	1.642	1.666	1.654	3.356	3.427	3.392	4.998	5.093	5.046
		mean	1.595	1.612	1.604	3.112	3.159	3.136	4.706	4.772	4.739
		Mean	1.565	1.583	1.574	3.007	3.064	3.035	4.572	4.647	4.609
$LSD_{0.05}$		Bio	0.003	0.003	0.003	0.006	0.006	0.006	0.01	0.01	0.001
		Dose	0.003	0.003	0.003	0.005	0.006	0.006	0.008	0.008	0.008
		P	0.002	0.002	0.002	0.004	0.005	0.006	0.007	0.007	0.007

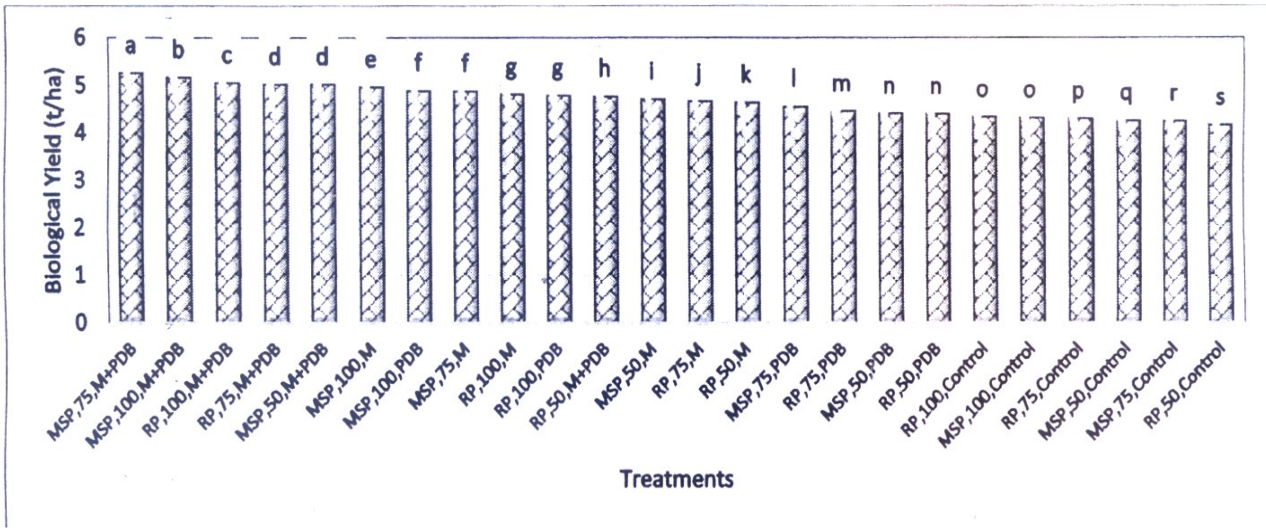

Fig. 1. Effect of applied P, dose and biological treatments on average Biological yield of Barley.

(M) = VAM

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الملخص العربي

أهمية فطريات الميكوريزا والبكتريا المذيبة للفسفور على زيادة الفوسفات المتاح للشعير فى أرض جيرية

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أجريت تجربتين حقليتين لدراسة تأثير فطريات الميكوريزا الجذرية والبكتيريا المذيبة للفوسفور على تيسر عنصر الفوسفور لنباتات الشعير المنزرعة في ارض جيرية. تم اجراء التجربتين في المحطة البحثية برأس سدر – مركز بحوث الصحراء في الموسم الشتوى للعامين ٢٠١٥-٢٠١٦ و ٢٠١٦-٢٠١٧. وقد تضمنت معاملات التسميد الحيوى التلقيح بفطريات الميكوريزا (VAM) والبكتريا المذيبة للفوسفور(PDB) كل منهما منفرداً أو مجتمعين بالإضافة الى معاملة الكنترول. تم إضافة السماد الفوسفورى في صورة سوبر فوسفات وصخر الفوسفات بثلاث مستويات وهى ٥٠ و ٧٥ و ١٠٠% من الكميات الموصى بها. أوضحت النتائج المتحصل عليها أن أضافة سماد السوبر فوسفات الأحادى أنتج زيادة معنوية في محصول الحبوب والقش والمحصول البيولوجى بمعدل ٢,٧ و ٢,١ و ٢,٣% بالترتيب بالمقارنة بمعاملات صخر الفوسفات. وقد أدى التلقيح المزدوج من فطريات الميكوريزا والبكتريا المذيبة للفوسفور (VAM+PDB) الى زيادة معنوية في تركيز النيتروجين والفوسفور في الحبوب والقش. وأيضاً زيادة معنوية لكل من طول النبات والوزن الجاف للحبوب والقش لكل نبات وكذا وزن ١٠٠ حبة بنسب ٧,٥ و ٨,٩ و ١٤,٨ و ١٥,٨% على التوالى. كما أدت لزيادة معنوية لمحصول الحبوب والقش والمحصول البيولوجى بنسب ١٣,٤ و ٢٠,٦ و ١٨,٨% على التوالى بالمقارنة بمعاملة عدم التلقيح. هذا وقد خلصت النتائج ان أعلى محصول بيولوجى من الشعير (٥,٢٤٨ طن/هكتار) كانت ناتجة عن معاملة التلقيح المزدوج بكل من فطريات الميكوريزا والبكتريا المذيبة للفوسفات (VAM+PDB) بالإضافة الى ٧٥% من المعدلات الموصى بها من سماد سوبر فوسفات الأحادى.