Middle East Journal of Applied Sciences, 4(4): 1075-1079, 2014
ISSN: 2077-4613

Effect of Mycorrhizal Fungi, Phosphate Solubilizing Bacteria and Phosphorus Rates on Yield and Yield Components of Barley (*Hordeum vulgare*, L.).

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ABSTRACT

Two field experiments were conducted at farm of Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt, during 2011/2012 and 2012/2013 seasons, to study the effect of phosphate biofertilizer treatments (Mycorrhizal fungi alone and Mycorrhizal fungi + phosphorine), different phosphorus rates (22.5, 16.9, 11.25 and 5.6 kg P_2O_5 /fad. from super phosphate and phosphate rock and control (without super phosphate or phosphate rock)) and their interaction on yield and yield components of barley (Giza 123 variety). Results indicated that the differences between phosphate biofertilizer treatments for leaf area index, plant height, spike length, 1000-grain weight, biological yield/fad., grain yield/fad. and harvest index were significant in the two seasons, while, spike weight and number of grains/ spike were significant in the first season only. Inoculation barley seeds with mycorrhizal fungi + phosphorine gave the highest values for all studied traits in the two seasons. In addition, results indicated the effect of phosphorus rates on all studied traits was significant in the two seasons. Phosphorus rates of 22.5, 16.9 and 11.25 kg P_2O_5 /fad. gave the maximum values for all studied traits in the two seasons, respectively. The differences between treatments of 22.5, 16.9 and 11.25 kg P_2O_5 /fad. as super phosphate or rock phosphate were insignificant for most studied traits in the two seasons.

The interaction between phosphate biofertilizer treatments and phosphorus rates were significant for all studied traits except spike length in the second season only and spike weight and number of grains/spike in the two seasons. In addition, results show that, application the plants by mycorrhizal fungi + phosphorine and 16.9 or 11.25 kg P_2O_5 increased the yield and yield components under the experiment soil condition.

Key words: Barley, mycorrhizal fungi, phosphate solubilizing bacteria (phosphorine), phosphorus rates (super phosphate, phosphate rock).

Introduction

Barley (*Hordeum vulgare*, L.) is considering one of the most adapted cereals to environmental conditions, which are not suitable growing other cereal crops. Barley is the main crop grown in a large scale in the North Coastal Region of Egypt and in the newly reclaimed lands. The total harvested area was 86800 fad. in 2013/2014 season (ASBWC,2014).

Phosphorus is one the most essential elements for barley growth and development after nitrogen (Tigre *et al.* 2014). However, the availability of this nutrient for plants is limited by different chemical reactions especially in arid and semi-arid soils. Phosphorus plays a significant role in several from vital functions such as photosynthesis, transformation of sugar to starch, protein information, nucleic acid production, nitrogen fixation and formation of oil. It is also, the part of all biochemical cycles in plants (Mehrvarz and Chaichi, 2008).

Plants acquire phosphorus from soil solution as phosphate anion. It is the least mobile element in the plant and soil contrary to other macronutrients. Pin precipitated form i.e. ortho phosphate ($H_2PO_4^{-1}$ or $H_2PO_4^{-2}$) is absorbed by Fe^{3+}, Al^{3+}, Ca^{2+} and Mg^{2+} in soil through legend exchange. A large amount of P applied as a fertilizer becomes immobile through precipitation reaction with highly reactive Fe^{3+}, Al^{3+}, Ca^{2+} and Mg^{2+} in the acidic and calcareous or alkaline or normal soils (Awasthi *et al.* 2011). These results led to that, efficiency of P fertilizer throughout the world is around 10-25% consequently, the use of phosphate solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) as inoculants in soil usually effective on phosphate solubility due to different mechanisms such as production and secretion of organic acids. Consequently, increases the phosphorus uptake by the plants and the crop yield (Khan *et al.* 2009). The ability of PSB and AMF with integrated to convert insoluble form phosphorus into soluble one is an important trait in sustainable farming for increasing crops yield. PSB and AMF play an important role in enhancing phosphorus availability to plants by lowering soil pH and by microbial production of organic acids and mineralization of organic P by acid phosphatases. These organisms besides providing P also facilitate the growth of plants by improving the uptake of nutrients and stimulating the production of some phytohormones. PSB and AMF are high potential as bio-fertilizers especially in P-deficient soils to enhance the growth and yield performance of crops (Awasthi *et al.* 2011). Several investigators studied the effect of PSB and AMF alone or in combination with super phosphate or rock phosphate in cereal crops. Sahin *et al.* (2004) indicated that inoculation of phosphate solubilizing bacteria

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(*Bacillus megatherium*) with 60 kg P_2O_5 /ha. significantly increased yield and yield components of barley compared with the control. Also, Mehrvarz and Chaichi, (2008) indicated that seed inoculation by phosphate solubilizing microorganisms (mycroohizal + bacteria) and different levels of phosphorus chemical fertilizer with 30 and 60 Kg P_2O_5/ha. increased physiological and growth traits of barley compared with sole or control. Suri and Choudhary, (2010) indicated that seed inoculation with mycorrhizal fungi and phosphate solubilizing bacteria in integration with 50 or 75% from recommended rate in alkaline soils increased yield and yield components of wheat by 15.7% compared with control. Lone *et al.* (2011) found that use of phosphate solubilizing bacteria (PSB) (*Bacillus* spp) with 60 kg/ha. rock phosphate significantly increased yield and yield components of aerobic rice compared with the control. Panhwar *et al.* (2011) found that application phosphate solubilizing bacteria and mycorrhizal fungi with 45 P_2O_5/ha. increased yield and yield components of wheat in semi and tropical soils. While, Zaefarian *et al.* (2011) found that application of four mycorrhizal strains included of (*Glomus mosseae, G. etanicatum, G. intraradices* and mixed strains (combination of *Glomus mosseae, G. igaspora hartiga* and *G. fasciculatum*) improved physiological traits and grain yield of barley. In addition, Thalooth *et al.* (2012) indicated that application of biofertilizer (phosphorine and cerealine) improved yield and yield components of barley compared with the control under water stress conditions. Sharma and Yadav, (2013) indicated that use of mycorrhizal fungi in semi-arid areas improved uptake of P, N, K, Zn, Cu, S, Fe, Mg, Ca and Mn, consequently, increased yield and yield components of barley.

Consequently, these the study aimed to investigate the effect of integrated between mycorrhizal fungi and phosphate solubilizing bacteria on yield and yield components of barley (*Hordeum vulgare*, L.) under different phosphorus rates, so as to reduce the need for P fertilizer application and maximize plant yield.

Materials and Methods

Two field experiments were conducted at farm of Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt, during 2011/2012 and 2012/2013 seasons, to study the effect of the integrated between mycorrhizal fungi and phosphate solubilizing bacteria on yield and yield components of barley (*Hordeum vulgare*, L.) Giza 123 variety under different phosphorus rates. A split plot design with four replicates was used.

A. The main plot was devoted to phosphate biofertilizer treatments of:
A1. Application mycorrhizal fungi alone.
A2. Application mycorrhizal fungi + phosphorine (phosphate solubilizing bacteria).
B. The sub-plots contained phosphorus rates (super phosphate or rock phosphate).
B1. 22.5 kg P_2O_5/fad. as calcium super phosphate (15.5% P_2O_5).
B2. 16.9 kg P_2O_5/fad. as calcium super phosphate.
B3. 11.25 kg P_2O_5/fad. as calcium super phosphate.
B4. 5.6 kg P_2O_5/fad. as calcium super phosphate.
B5. 22.5 kg P_2O_5/fad. as rock phosphate (18% P_2O_5).
B6. 16.9 kg P_2O_5/fad. as rock phosphate.
B7. 11.25 kg P_2O_5/fad. as rock phosphate.
B8. 5.6 kg P_2O_5/fad. as rock phosphate.
B9. Control (without application of phosphate).

Mycorrhizal fungi and phosphorine are biofertilizer products containing *Glumus aggregatum* fungi and *Bacillus megatherium* var. phosphaticum bacteria, respectively.

Phosphate biofertilizer treatments were by inoculated barley seeds by arbuscular mycorrhizal fungi (AMF) are mutualistic symbiosis living in root of 80% of land plant species and developing extensive belowground extra radical hyphae fundamental for the uptake of soil nutrients and their transfer to host plants, or in combination inoculated between mycorrhizal fungi and phosphate solubilizing bacteria, which, were mixture in ratio of 1:1 w/w.

Phosphorine, *Bacillus megatherium* var. phosphaticum was used a phosphate solubilizing bacteria and mycorrhizal fungi were used as solubilizing and transfer for phosphorus. Phosphorine biofertilizer was produced from General Organization for Agric. Equalization Fund (G.O.A.E.F.), while mycorrhizal was produced from Biofertilizer unit, Soils and Water Research Institute, Agriculture Research Center, Giza, Egypt. The biofertilizer was used at the rate of 500g/fad., adhesive solution of gum was added to the seeds and mixed carefully for about 5 minutes until all seeds were thoroughly coated. Seeds were sown directly in the same day then irrigated. The phosphate was added during soil preparation, while. Nitrogen fertilizer rate (60 kg N/fad.) added as ammonium nitrate (33.5% N) in three equal portions, the first portion was added at 21 days after sowing, the second at 35 days after sowing and the third 50 days after sowing. Potassium fertilizer was soil added at the rate of 24 kg K_2O/fad. as potassium sulphate (48% K_2O) in one dose with 1st does of nitrogen fertilizer. The experimental unit area in both seasons was 10.5m^2 (3×3.5m). there were 15 rows in each plot spaced 20 cm apart. Grains of barley var. Giza 123 were sown at the rate of 40 kg/fad. in 1st December in first and second seasons and the preceding crop was cowpea in both seasons. All other cultural practices were followed as recommended in barley fields.Soil analysis for two seasons was carried out according to Jackson, (1973) as shown in Table (1).

Table 1. Mechanical and chemical analysis of experimental soil in 2011/2012 and 2012/2013 seasons .

Mechanical analysis	2011/2012 season	2012/2013 season
Sand %	57.58	56.45
Silt %	15.30	15.89
Clay %	27.12	27.66
Texture class	Sandy clay loam	Sandy clay loam
Chemical analysis		
pH	8.00	7.95
EC (mmohs/cm)	0.93	0.91
Available P (ppm)	8.11	7.98

Studied attributes:

1- Leaf area index (LAI) was determined at 75 days from sowing and calculated by the following formula:

$$\text{Leaf area index (LAI)} = \frac{\text{Leaf area per plant}}{\text{Ground area per plant}}$$

Leaf area per plant (cm^2) = length × maximum width × 0.79 according to Voldeng and Simpson (1967).

At harvesting time, ten individual plants were chosen at random from each sup plot to record the following attributes, while, biological yield/fad. and grain yield/fad. were taken from whole plot.

2- Plant height, in cm.
3- Spike length, in cm.
4- Spike weight, in g.
5- Number of grains/spike.
6- 1000-Grain weight, in g.
7- Biological yield/fad., in kg.
8- Grain yield/fad., in ardab (one ardab =120 kg).
9- Harvest index (HI) computed as:

$$\text{Harvest index (HI)} = \frac{\text{Grain yield (kg/fad).}}{\text{Biological yield (kg/fad).}}$$

The obtained data of plant parameters were statistically analyzed according to the methods suggested by Gomez and Gomez (1984). Means were compared by using the L.S.D. values at 5% level of significance.

Results and Discussion

The effect of phosphate biofertilizers on growth, yield and yield components of barley in 2011/2012 and 2012/2013 seasons are presented in Tables (2 and 3).

Results indicate that the differences between phosphate biofertilizer treatments for leaf area index, plant height, spike length, 1000-grain weight, biological yield/fad., grain yield/fad. and harvest index were significant in the two seasons, while, spike weight and number of grains/spike were significant in the first season only. Inoculation barley seeds with mycorrhizal fungi + phosphorine gave the highest values for all studied traits compared with mycorrhizal fungi alone in the two seasons. Positive effect of use mycorrhizal fungi with phosphorine on growth, yield and yield components may be due to effective on phosphate solubility due to different mechanisms of microorganism such as production or secretion of organic acids, reduce soil pH with organic acids and carbonic acid, consequently, mineralization of organic Phosphorus, improving the phosphorus uptake and nutrients such as N, K, Zn, Fe, Mg, Mn, S and Cu, beside, the ability of phosphate solubilizing bacteria and arbuscular mycorrhizal fungi with integrated on production or secretion of some phytohormones, that led to enhance the growth, yield and yield components. These results are in agreement with Mehrvarz and Chaichi (2008), Suri and Choudhary (2010), Awasthi *et al*. (2011) and Lone *et al*. (2011).

In addition, results in Tables 2 and 3 illustrate that the effect of phosphorus rates on all studied traits were significant in the two seasons. Phosphorus at the rates of 22.5, 16.9 and 11.25 P_2O_5 kg/fad. gave the maximum for all studied traits in the two seasons, respectively. The differences between treatments 22.5, 16.9 and 11.25 P_2O_5 kg/fad. for calcium super phosphate, 22.5 and 16.9 P_2O_5 kg/fad. for rock phosphate were insignificant for most studied attributes in the two seasons. Positive effect of the high rate of phosphorus on these traits may be due to the phosphorus essential role as element limited for growth plant after nitrogen in many physiological processes of barley, especially in arid and semi- arid soils. Phosphorus plays a significant role in several from vital function, such as, photosynthesis, transformation of sugars to starch, protein information, nucleic acid production, nitrogen fixation and oil formation.It is also, the part of all biochemical cycles in plants (Mehrvarz and Chaichi 2008).

Table 2. Effect of phosphate biofertilizers, phosphorus rates and their interaction on leaf area index, plant height (cm), spike length (cm), spike weight (g) and n. of grains/ Spike of barley in 2011/2012 and 2012/2013 seasons.

Bio-fertilizers (A)	Phosphate rate (kg P_2O_5 /fad.) (B)	Leaf area index		Plant height (cm)		Spike length (cm)		Spike weight (g)		N. of grains/ Spike	
		2011/2012 season	2012/2013 season	2011/2012 season	2012/2013 season	2011/2012 season	2012/2013 season	2011/2012 season	2012/2013 season	2011/2012 season	2012/2013 season
Mycorrhizal fungi	22.5 as super phosphate	5.61	5.36	107.33	106.66	7.33	7.06	1.73	1.56	53.33	50.66
	16.9 as super phosphate	5.57	5.42	104.12	105.33	7.36	7.13	1.70	1.53	52.66	50.00
	11.25 as super phosphate	5.51	5.35	103.17	107.00	7.40	7.20	1.72	1.60	52.33	49.50
	5.60 as super phosphate	4.69	4.32	93.26	91.00	5.93	5.70	1.23	1.06	44.00	44.66
	22.5 as rock phosphate	5.58	5.26	105.00	105.33	7.23	7.00	1.66	1.56	52.00	47.33
	16.9 as rock phosphate	5.55	5.23	103.02	104.00	7.20	6.86	1.64	1.46	51.33	48.66
	11.25 rock phosphate	5.50	5.22	101.11	103.00	7.18	6.90	1.63	1.53	51.00	50.00
	5.60 as rock phosphate	4.66	4.16	92.00	91. 33	5.96	5.40	1.16	0.90	42.66	42.66
	control	4.01	3.90	90.31	90.66	5.53	5.20	1.06	0.76	36.66	38.00
Means		5.18	4.91	99.92	100.47	6.79	6.49	1.50	1.33	48.44	46.83
Mycorrhizal fungi + Phosphorine	22.5 as super phosphate	5.84	5.61	111.62	109.33	8.13	7.50	1.83	1.66	56.00	51.66
	16.9 as super phosphate	5.77	5.58	108.13	107.66	8.10	7.40	1.76	1.63	54.66	50.33
	11.25 as super phosphate	5.80	5.57	107.16	107.33	8.11	7.50	1.80	1.62	55.33	51.00
	5.60 as super phosphate	4.77	4.65	95.00	91.66	6.20	5.80	1.33	1.16	45.33	43.80
	22.5 as rock phosphate	5.82	5.55	109.66	108.66	8.06	7.33	1.76	1.63	54.00	49.00
	16.9 as rock phosphate	5.76	5.53	107.33	107.33	8.03	7.33	1.73	1.56	53.66	50.66
	11.25 rock phosphate	5.75	5.52	105.66	106.66	8.00	7.40	1.72	1.58	52.66	50.00
	5.60 as rock phosphate	4.74	4.32	93.50	91.00	6.16	5.50	1.31	1.76	42.66	41.66
	control	4.16	4.02	91.33	89.33	5.63	5.33	1.17	1.66	35.00	37.00
Means		5.37	5.15	103.27	102.10	7.38	6.78	1.60	1.35	49.84	47.23
Over all mean for phosphate rate (B)	22.5 as super phosphate	5.72	5.84	109.47	107.99	7.73	7.28	1.78	1.61	54.66	51.16
	16.9 as super phosphate	5.67	5.50	106.12	106.49	7.73	7.26	1.73	1.58	53.66	50.15
	11.25 as super phosphate	5.65	5.46	105.16	107.16	7.75	7.35	1.76	1.61	53.83	50.25
	5.60 as super phosphate	4.73	4.48	94.13	91.33	6.06	5.75	1.28	1.11	44.50	44.23
	22.5 as rock phosphate	5.70	5.40	107.33	106.99	7.64	7.16	1.71	1.59	53.00	48.16
	16.9 as rock phosphate	5.65	5.38	105.17	105.66	7.61	7.10	1.68	1.51	52.49	49.66
	11.25 rock phosphate	5.56	5.37	103.38	104.83	7.59	7.15	1.67	1.55	51.38	50.00
	5.60 as rock phosphate	4.68	4.24	92.75	91.16	6.06	5.45	1.23	0.83	42.66	42.16
	control	4.05	3.96	90.82	89.99	5.58	5.26	1.11	0.71	35.83	37.61
L.S.D. at 5%											
Bio-fertilizers (A)		0.12	0.26	1.55	1.40	0.07	0.08	0.04	N.S.	0.73	N.S.
Phosphate rate (B)		0.26	0.56	2.93	2.86	0.14	1.61	0.10	0.09	1.54	3.26
A×B		0.37	0.79	3.25	3.06	0.21	N.S.	N.S.	N.S.	N.S.	N.S.

Table 3. Effect of phosphate biofertilizers, phosphorus rates and their interaction on 1000-Grain weight (g), biological yield (kg/fad.), grain yield (ardab/fad.) and harvest index of barley in 2011/2012 and 2012/2013 seasons.

Bio-fertilizers (A)	Phosphate rate (kg P_2O_5 /fad.) (B)	1000-Grain weight (g)		Biologicl yield (kg/fad.)		Grain yield (Ardab/fad.)		Harvest index	
		2011/2012 season	2012/2013 season	2011/2012 season	2012/2013 season	2011/2012 season	2012/2013 season	2011/2012 season	2012/2013 season
Mycorrhizal fungi	22.5 as super phosphate	46.33	44.33	4750.66	4599.66	17.05	16.26	43.06	42.39
	16.9 as super phosphate	46.00	43.00	4733.66	4593.33	17.04	16.19	43.17	42.29
	11.25 as super phosphate	46.33	43.33	4738.33	4592.66	17.03	16.20	43.16	42.33
	5.60 as super phosphate	35.66	33.33	4000.33	3824.66	14.07	13.36	42.21	41.92
	22.5 as rock phosphate	44.00	43.66	4610.33	4571.66	16.31	16.15	42.45	42.39
	16.9 as rock phosphate	43.00	43.66	4603.00	4565.66	16.26	16.15	42.38	42.46
	11.25 rock phosphate	43.00	43.00	4604.00	4562.33	16.23	16.14	42.30	42.44
	5.60 as rock phosphate	33.00	32.00	3888.33	3744.66	13.56	13.10	41.86	42.00
	control	30.66	30.33	3351.00	3268.00	11.62	11.04	41.61	40.55
Means		40.88	39.04	4364.40	4258.07	15.46	14.95	42.46	42.08
Mycorrhizal fungi + phosphorine	22.5 as super phosphate	52.66	51.00	4798.66	4620.66	17.34	16.61	43.37	43.14
	16.9 as super Phosphate	51.33	50.66	4797.33	4613.00	17.32	16.57	43.34	43.11
	11.25 as super phosphate	51.66	50.33	4794.33	4612.66	17.32	16.57	43.35	43.12
	5.60 as super phosphate	37.00	34.66	3997.00	3858.00	14.04	13.56	42.23	42.45
	22.5 as rock phosphate	51.66	49.33	4769.33	4605.66	17.19	16.57	43.24	43.17
	16.9 as rock phosphate	51.00	48.66	4756.66	4594.66	17.13	16.52	43.22	43.16
	11.25 rock phosphate	51.33	48.33	4760.66	4584.00	17.15	16.52	43.22	43.26
	5.60 as rock phosphate	32.66	31.33	3849.33	3766.33	13.71	13.42	42.75	42.75
	control	31.33	31.00	3371.00	3282.00	11.79	11.24	41.97	41.20
Means		45.63	43.92	4432.70	4281.88	15.89	15.30	42.97	42.82
Over all mean for phosphate rate (B)	22.5 as super phosphate	49.50	47.66	4774.66	4610.16	17.19	16.44	43.21	42.76
	16.9 as super phosphate	48.66	46.83	4765.50	4603.16	17.17	16.38	43.25	42.70
	11.25 as super phosphate	49.00	46.83	4766.33	4602.66	17.18	16.39	43.25	42.72
	5.60 as super phosphate	36.33	34.00	3998.66	3841.33	14.05	13.50	42.22	42.18
	22.5 as rock phosphate	47.83	46.50	4689.83	4588.66	16.75	16.36	42.84	42.78
	16.9 as rock phosphate	47.00	46.16	4679.83	4580.16	16.69	16.34	42.80	42.81
	11.25 rock phosphate	47.16	46.00	4682.33	4573.33	16.69	16.33	42.76	42.85
	5.60 as rock phosphate	32.83	31.66	3868.83	3755.33	13.64	13.21	42.30	42.37
	control	31.00	30.66	3361.00	3275.00	11.70	11.14	41.79	40.88
L.S.D. at 5%									
Bio-fertilizers (A)		0.57	0.41	10.48	4.37	0.02	0.02	0.11	0.08
Phosphate rate (B)		1.22	0.90	22.24	9.27	0.04	0.05	0.23	0.18
A×B		1.72	1.28	31.46	14.12	0.06	0.07	0.33	0.28

While, positive effects of 11.25 and 16.19 kg P_2O_5 /fad. may be due to increased phosphorus fertilizer use efficiency and rock phosphate due to inoculation with mycorrhizal fungi and phosphate solubilizing bacteria, which, led to convert insoluble from phosphorus into soluble, that from through production or secretion of organic acids, decrease soil PH with organic acids or carbonic acids, consequently, mineralization of organic phosphorus, improving phosphorus uptake nutrients such as N, K, S, Fe, Mn, Zn and Cu beside, the ability of phosphate solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) on secretion some phytohormons, with addition to, the main function of AMF is phosphorus transportation. Extra- radical mycelium of AMF easily access P from soil and deliver to root cortical cells as polyphosphate, which, at least translocate to host plant after solubilization and it is sketchy that external hyphae deliver up to 80% of P requirement of the plant. Suri and Choudhary (2010). All these the functions led to improving the growth, yield and yield components of barley. These results are in agreement with Sahin *et al.* (2004), Mehrvarz and Chaichi (2008), Lone *et al.* (2011), Panhwar *et al.* (2011) and Sharma and Yadav (2013). They indicated that use mycorrhiza fungi and phosphate solubilizing bacteria with 11.25 and 16.19 kg P_2O_5 /fad. as super phosphate or rock phosphate increased yield and yield components.

The interaction between phosphate biofertilizer treatments and phosphorus rates was significant on most studied traits in the two seasons, except spike length in second season, spike weight and number of grains/spike in both seasons. The maximum values for these traits were obtained from inoculation with mycorrhizal fungi + phosphorine and 22.5 kg P_2O_5 /fad. as rock phosphate.

It could be concluded that the best grain yield was obtained from inoculation barley seeds with combination from mycorrhizal fungi + phosphorine and use 11.25 kg P_2O_5 /fad. as calcium super phosphate, 16.9 or 22.5 kg P_2O_5 /fad. as rock phosphate under experiment soil condition.

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