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EFFECT OF BIO-FERTILIZER, PHOSPHORUS SOURCE AND HUMIC SUBSTANCES ON YIELD, YIELD COMPONENTS AND NUTRIENTS UPTAKE BY BARLEY PLANT

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ABSTRACT

Two field experiments were conducted at Faculty of Agriculture farm, Al-Azhar University, Nasr City, Cairo, Egypt, during 2016/2017 and 2017/2018 seasons, for evaluating the effects of phosphate bio-fertilization (mycorrhizal fungi,phosphorine and Without inoculation), two sources of phosphorus fertilizers (super phosphate, rock phosphate and without phosphate) and humic substances (humic acid, fulvic acid and without humic substances) as well as their interactions on yield, yield components and nutrients uptake of barley cv. Giza 123. These experiments contain 27 treatments; a split split plot design with three replicates.

The obtained results showed that the bio-fertilization were significantly increase of yield, yield components and nutrients uptake (nitrogen, phosphorus and potassium) compared without inoculation, it is worth mentioning inoculation Mycorrhizal fungi was superior to Phosphorine;inoculation Mycorrhizal fungi were significantly increase of yield components, phosphorus and potassium uptake by plant; phosphorus solubilizing bacteria play role in phosphorus nutrition by enhancing its availability to plants through slow release phosphorus from inorganic and organic soil by solubilization and mineralization. It is interesting to say that the applied phosphate source were significantly increase of yield,

yield components and nutrients uptake (nitrogen, phosphorus and potassium) compared without phosphorus, it is important to remember calcium super phosphate was superior to rock phosphate; the positive affect of super phosphate on yield behavior solubility nutrients also, absorbing various nutrients. The applications of humic substance were significantly increase of yield and nutrients uptake (nitrogen, phosphorus and potassium) compared without humic substance, additionally humic acid caused a significant increae grain, straw yields and nitrogen, phosphorus and potassium uptake by barley plant. Generally, from these results it can be concluded that the application of Mycorrhizal fungi, super phosphate and humic acid could be used as a complementary for mineral fertilizers to improve yield and nutrients uptake by barley plant.

Key words: Barley, humic acid and fulvic acid, mycorrhizal fungi, phosphate solubilizing bacteria (phosphorine), phosphorus sources.

INTRODUCTION

Barley (*Hordeum vulgare*, L.) a member of poaeceae family, is the fourth most important crop in the world and the main crop grown in the North Coastal Region of Egypt in a large scale as well as in the newly reclaimed soils. Barley considered one of the most adapted cereals to environmental conditions, which are not suitable for growing other cereal crops. Its grains are used as food and malting purposes, while straws provide an important source of roughage for feeding animals.

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

Plants absorb 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 is absorbed by Fe^{+2}, 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^{+2}, Ca^{+2} and Mg^{+2} in the acidic and calcareous, alkaline or normal soils (**Awasthi *et al.* 2011**). Therefore, the efficiency of P fertilization throughout the world is around 10-25%. Soil inoculation with phosphate solubilizing microorganisms such as phosphate solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) is usually effective on phosphate solubility. Phosphate solubilizing microorganisms enhanced plant growth and increase crop yields by one or more mechanisms such as phosphate solubilization and mineralization, production phytohormones, bioactive ingredients and organic growth promoting substances (**Khan *et al.* 2009**).Also, Phosphate solubilizing microorganisms enhancing phosphorus availability to plants by lowering the soil pH due to its organic acids production and can mineralized organic P by acid phosphatases. PSB and AMF are high potential as bio-fertilizers especially in P-deficient soils to enhance the growth and yield performance of crops. Ability of PSB and AMF with integrated to convert insoluble phosphorus into soluble one is an important trait in sustainable farming for increasing crops yield. (**Awasthi *et al.* 2011**).

Several studies investigated the effect of PSB and AMF alone or in combination with super phosphate or rock phosphate in barley crop. **Sahin *et al.*(2004)** indicated that inoculation of phosphate solubilizing bacteria (*Bacillus megatherium*) with 60 kg P_2O_5/ha significantly increased yield and yield component of barley relative to control plants. Also, **Mehrvarz and Chaichi, (2008)** indicated that seed inoculation by phosphate solubilizing microorganisms (mycroohizal plus 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 plants. In addition, **Thalooth *et al.* (2012)** indicated that application of biofertilizer (phosphorine and cerealine) improved yield and yield component of barley relative to 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, Ca, Mg, Fe, and Mn, consequently, increased yield and yield component of barley. **Wali *et al.* (2018)** indicated that seed inoculation by mycorrhizae combined with 50% P_2O_5 /fed increased grain yield of barley.

Humic substances, such as organic matter, humus, humate, humic acid, fulvic acid and humin, play a vital role in improving physical, chemical and biological properties of soils (**Varanini *et al.* 1995 and Mikkelsen, 2005**), nutrient uptake by plants, mineral availability **(Nardi *et al.* 2002 and Mauromicale *et al.* 2011)**, stimulate plant enzymes and hormones **(Mart, 2007)** as well as controlling soil-borne diseases **(Mauromicale *et al.* 2011).** Humic substances effect on the plant growth depending on the source and concentration, in addition to the molecular fraction weight. Low humic molecular size fraction easily reaches the plasma lemma of plant cells, leading to a positive effect on plant growth, due to the nutrient uptake, especially nitrate. Its effects on the intermediate metabolites are less understood, but it seems that humic substances may influence on both respiration and photosynthesis process **(Nardi *et al.* 2002)**.

Khalil *et al.* (2013) indicated that bio-fertilization combined with humic acid significantly increased yield, N, P and K contents of grain and straw of barley compared with control. Also, **Wali *et al.* (2018)** reported that the addition of 65 kg N fed^{-1} with 2 kg humic acid and mycorrhizal inoculation gave the best yield of barley.

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

MATERIALS AND METHODS

Two field experiments were conducted at the farm of Agriculture Faculty, Al-Azhar University, Nasr City, Cairo, Egypt, during 2016/2017 and 2017/2018 seasons, to study the effect of the integrated between phosphorus fertilizer sources, bio-fertilizes and soil application of humic or fulvic acids on yield and yield component of barley (*Hordeum vulgare*, L.) cultivar Giza 123. A split split plot design with three replicates was used.

A. The main plot was devoted to phosphate biofertilizer treatments of :

a_1. Inoculation with mycorrhizal fungi at rate of 500g/fed.

a_2. Inoculation with phosphorine (phosphate solubilizing bacteria) at rate of 500g/fed.

a_3. Without inoculation.

B. The sub-plot contained phosphorus fertilizer sources treatments of :

b_1. 22.5 kg P_2O_5 /fed. as calcium super phosphate (15.5% P_2O_5).

b_2. 22.5 kg P_2O_5 /fed. as phosphate rock (25% P_2O_5).

b_3. Without phosphate.

C. The sub sub-plot contained humic substances treatments of:

c_1. Soil application of humic acid at rate of 2 kg/fed.

c_2. Soil application of fulvic acid at rate of 2 L/fed.

c_3. Without humic substances.

Mycorrhizal fungi and phosphorine are biofertilizer products comprising *Glumus aggregatum* fungi and *Bacillus megatherium* var. phosphaticum bacteria, respectively. 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/fed. For seed inoculation, adhesive gum solution was added to the seeds and mixed carefully for about 5 minutes until all seeds were thoroughly coated. Seeds were sown directly after inoculation and irrigated. The phosphate was added during soil preparation, while. Nitrogen fertilizer rate (60 kg N/fed.) was 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 one 50 days after sowing. Potassium fertilizer was soil added at the rate of 24 kg K_2O /fed. as potassium sulphate (48% K_2O) in one dose with 1^{st} 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 (Giza 123) were sown at the rate of 40 kg/fed. in 1^{st} December for both seasons and the preceding crop was maize in both seasons. All other cultural practices were followed as recommended for barley fields. Soil analysis for the two seasons was carried out according to **Black (1965)** and **Jackson (1973)** and tabulated in **Table (1) and Table (2)** showed some chemical of the used rock phosphate rock phosphate, humic and fulvic acid. Plant samples were taken at harvesting time (120 days after planting), the sample of plant was dried at 70 ^{0}C. the determine of N, P and K in grain and straw of barley plant.

Table 1: some physical and chemical properties of investigated soil.

Soil characteristics	Values
Mechanical analysis	
Sand (%)	82.20
Silt (%)	7.60
Clay (%)	10.20
Texture class	Loamy sand
Chemical analysis	
pH (1:2.5)	7.4
EC (ds/m)	0.75
$CaCO_3$ (%)	1.35
Soluble ions in 1:5 soil water extract (meq/100g).	
Ca^{++}	1.13
Mg^{++}	1.03
Na^{+}	1.03
K^{+}	1.23
CO_3^{--}	0.00
HCO_3^{-}	1.76
SO_4^{--}	1.13
Cl^{-}	1.53

Table 2: Some chemical properties of rock phosphate, humic and fulvic acid used in the experiment.

Characteristics	Rock phosphate	Humic acid	Fulvic acid
pH	7.60	5.46	1.53
EC(dSm^{-1})	3.15	0.10	0.11
	Total macronutrients (%)	**Available macronutrients (ppm)**	
N	-	1.27	0.45
P	25.00	0.23	0.17
K	0.05	1.95	1.87

Studied attributes :

At harvesting time, ten individual plants were randomly chosen from each sub sub-plot to record the following attributes, while, grain yield/fed and straw yield/fed were taken from whole plot.

1- Plant height (cm).	2- Spike length (cm).
3- Spike weight (g).	4- Number of grains/spike.
5-1000-grain weight (g).	6- Grain yield (kg/fed).
7- Straw yield (kg/fed).	8- N, P and K uptake in grain and straw (kg/fed).

Total nitrogen was determined using Kjeldahl method, phosphorus was determined colorimetrically, potassium by flame photometer **(Black, 1965).**

Statistical analysis:

Data were subjected to the proper statistically analysis as the technique of analysis of variance (ANOVA) of split split plot design as mentioned by **Gomez and Gomez (1984)**. The treatment means were compared by using Least Significant Difference (LSD) test at 5% level of significance as outlined by **Waller and Duncan (1969)**. The error mean squares of split split plot design were homogenous (Bartlett's test), the combined analysis was calculated for all the studied characters in both seasons. The data collected were analyzed using MSTAT-C (**Nissen, 1989)** statistical package.

RESULTS AND DISCUSSION

Yield and its components:

Plant height, yield and its components (spike length, weight, number of grains/spike and 1000-grain weight) of barley as affected by bio-fertilization, phosphate source and humic substances are presented in **Tables (3, 4 and 5).** The inoculation by mycorrhizal fungi or phosphorine significantly enhanced plant height, yield and its components than without bio-fertilizer treatment. While mycorrhizal fungi inoculation recorded highest results than phosphorine in this respect.Positive effect of mycorrhizal fungi or phosphorine on growth, yield and yield component owing to reducing soil pH by organic acids realization and mineralized organic Phosphorus by phosphate fertilizer. besides, the ability of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria 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).**

Concerning to phosphate source, plants subjected to super phosphate application revealed dominating results than plants that subjected to rock phasosphate. Calcium super phosphate provided P to the plant at the initial stage of growth, until P from the rock phosphate became available **Bekele and Hofner (1993)**. Also, super phosphate has positive effect on yield behavior solubility nutrients such as phosphorus, sulphur and some micronutrients also absorbing various nutrients.

Regarding to humic substances (humic and fulvic acids), humic substances significantly increased the yield and its components relative to without humic substances treatment. Also, humic acid was superior to fulvic acid or without humic substances. These enhancements may be due to the humic substances that are the major components (65-70%) of soil organic matter, increase plant growth enormously due to increasing cell membrane permeability, respiration, photosynthesis, oxygen and phosphorus uptake, and supplying root cell growth. A distinct effect of humic acid was observed among plants. Many researchers noted the enhancing effect of humic acid on growth, yield, and nutrient uptake by many crops **(David, 1991; Neri *et al*., 2002 and El-Desuki, 2004).** Indirect effects are mainly exerted through properties such as: enrichment in soil nutrients, increase of microbial population, higher cation exchange capacity (CEC), and improvement of soil structure; whereas direct effects are various biochemical actions exerted at the cell wall, membrane or cytoplasm and mainly of hormonal nature **(Varanini and Pinton, 2001 and Chen *et al*., 2004).** The hormone like activities of humic acid is well documented in various papers, in particular auxin, cytokinin and gibberellins.

The interaction between phosphate biofertilizer treatments and phosphorus source was significant on most studied traits, except spike length in the combined analysis, as shown as in **Tables 3, 4 and 5**. The maximum values for these traits were obtained from inoculation with mycorrhizal fungi and calcium super phosphate application. Interaction mycorrhizal fungi × super phosphate increased grain and straw yield the values were 35.26 and 34.85 %, respectively, compared with without inoculation × without phosphorus source treatment.

The interaction between phosphate biofertilizer treatments and humic substances was significant on most studied traits, except plant height and 1000-grain weight in the combined analysis. The maximum values for these traits were obtained from inoculation with mycorrhizal fungi and humic acid application. Interaction mycorrhizal fungi × humic acid increased grain and straw yield the values were 9.99 and 9.96 %, respectively, compared with without inoculation × without humic substances.

The interaction between phosphorus source treatments and humic substances was significant on most studied traits, except spike length only in the combined analysis. The maximum values for these traits were obtained from application of calcium super phosphate with humic acid. Interaction calcium super phosphate × humic acid increased grain and straw yield the values were 49.61 and 49.42 %, respectively, compared with without phosphorus source and without humic substances.

Interaction of mycorrhizal fungi × calcium super phosphate × humic acid gave the highest values for all studied traits in the combined analysis, except spike length and number of grains/spike, as shown as in Tables 3, 4 and 5. This treatment significantly increased the grain and straw yield by about 81.28 and 80.91 %, respectively, than control plants. These results are in harmony with those obtained by **Sahin *et al*. (2004)**, **Lone *et al.* (2011), Mesbah and El-Sheshtawy (2014) and Panhwar *et al.* (2011)** They indicated that use mycorrhiza fungi or phosphate solubilizing bacteria with levels of phosphorus as super phosphate or rock phosphate increased yield and yield components. The promotion effect of bio-fertilizers may be owing to the effect of non-symbiotic phosphate, solubilizing microorganisms in exerting a positive influence on plant growth by the synthesis of phytohormones and enzymes (as ACC deaminase) that modulate the plant hormones level additionally, inorganic phosphate solubilization and organic phosphate mineralization, which change phosphorus to available for plants **(Rodriguez and Fraga,1999).** On the other hand, humic acid enhanced plant growth, yield and nutrient uptake in barley plants **(Roozbahani, 2015).** Application of mycorrhiza in the presence of humic acid gave considerable improvement in growth characteristics, photosynthetic pigments as well as nutrients uptake, total charbohydrates and crude protein of plants when compared with either inoculated or uninoculated treatments without humic acid **(Abou-Aly and Mady, 2009 and Wali *et al.* 2018).**

Table 3: Effect of bio-fertilizer, phosphate source ,humic substances and their interaction on plant height, spike length and spike weight of barley in combined analysis.

Bio-fertilizer	Phosphate source	Plant height (cm)				Spike length (cm)				Spike weight (g)			
		Humic substance				Humic substances				Humic substances			
		Humic acid	Fulvic acid	Without humic substances	Mean	Humic acid	Fulvic acid	Without humic substances	Mean	Humic acid	Fulvic acid	Without humic substances	Mean
Mycorrhizal fungi	Super phosphate	111.00	110.25	109.00	110.08	10.75	10.25	9.25	10.08	4.42	3.49	3.14	3.68
	Rock phosphate	108.75	108.00	107.25	108.00	10.50	10.00	9.00	9.83	4.11	3.41	3.05	3.52
	Without phosphate	108.50	105.75	98.25	104.17	10.00	9.75	8.88	9.54	3.19	3.12	3.01	3.11
Mean		109.42	108.00	104.83	107.42	10.42	10.00	9.04	9.82	3.91	3.34	3.07	3.44
Phosphorine	Super phosphate	108.50	107.75	103.00	106.42	10.75	10.25	9.00	10.00	3.85	3.63	3.03	3.50
	Rock phosphate	108.25	105.00	102.50	105.25	10.75	10.00	8.75	9.83	3.09	3.05	2.78	2.97
	Without phosphate	105.00	104.00	99.50	102.83	10.50	9.50	8.25	9.42	2.96	2.61	2.34	2.64
Mean		107.25	105.58	101.67	104.83	10.67	9.92	8.67	9.75	3.30	3.10	2.72	3.04
Without inoculation	Super phosphate	105.50	104.50	98.50	102.83	9.75	8.83	8.29	8.96	3.39	3.04	2.87	3.10
	Rock phosphate	105.00	104.00	98.00	102.33	9.25	8.50	8.25	8.67	3.02	2.55	2.27	2.61
	Without phosphate	97.00	95.25	91.50	94.58	9.00	8.50	7.50	8.33	3.04	2.40	2.24	2.56
Mean		102.50	101.25	96.00	99.92	9.33	8.61	8.01	8.65	3.15	2.66	2.46	2.76
Mean	Super phosphate	108.33	107.50	103.50	106.44	10.42	9.78	8.85	9.68	3.89	3.39	3.01	3.43
	Rock phosphate	107.33	105.67	102.58	105.19	10.17	9.50	8.67	9.44	3.41	3.00	2.70	3.04
	Without phosphate	103.50	101.67	96.42	100.53	9.83	9.25	8.21	9.10	3.06	2.71	2.53	2.77
	Mean	106.39	104.94	100.83	104.06	10.14	9.51	8.57	9.41	3.45	3.03	2.75	3.08
LSD at 5% level													
Bio-fertilizer (A)					0.732				0.190				0.460
Phosphate source (B)					0.655				0.170				0.412
Humic substances (C)					0.632				0.164				0.397
A × B					1.135				N.S				0.713
A × C					N.S				0.285				0.081
B × C					1.094				N.S				0.688
A × B × C					1.895				N.S				1.191

Table 4: Effect of bio-fertilizer, phosphate source , humic substances and their interaction on number of grains/spike and 1000-grain weight of barley in combined analysis.

Bio-fertilizer	Phosphate source	Number of grains/spike				1000-grain weight (g)			
		Humic substances				Humic substances			
		Humic acid	Fulvic acid	Without humic substances	Mean	Humic acid	Fulvic acid	Without humic substances	Mean
Mycorrhizal fungi	Super phosphate	57.75	56.25	53.50	55.83	53.75	51.83	48.50	51.36
	Rock phosphate	57.00	54.50	49.50	53.67	51.95	50.13	48.08	50.05
	Without phosphate	53.00	50.50	47.50	50.33	50.43	48.38	47.35	48.72
Mean		55.92	53.75	50.17	53.28	52.04	50.11	47.98	50.04
Phosphorine	Super phosphate	56.00	54.00	48.50	52.83	53.35	51.13	48.20	50.89
	Rock phosphate	56.00	53.25	47.75	52.33	51.25	50.05	47.66	49.65
	Without phosphate	47.00	43.50	38.50	43.00	48.48	48.13	46.28	47.63
Mean		53.00	50.25	44.92	49.39	51.03	49.77	47.38	49.39
Without inoculation	Super phosphate	51.75	49.25	44.75	48.58	52.93	49.10	48.08	50.04
	Rock phosphate	49.25	43.50	38.00	43.58	49.33	48.30	47.03	48.22
	Without phosphate	45.00	39.50	35.50	40.00	47.60	44.70	42.63	44.98
Mean		48.67	44.08	39.42	44.05	49.95	47.37	45.91	47.75
Mean	Super phosphate	55.17	53.17	48.92	52.41	53.34	50.69	48.26	50.76
	Rock phosphate	54.08	50.42	45.08	49.86	50.84	49.49	47.59	49.31
	Without phosphate	48.33	44.50	40.50	44.44	48.84	47.07	45.42	47.11
	Mean	52.53	49.36	44.83	48.91	51.01	49.08	47.09	49.06
LSD at 5% level									
Bio-fertilizer (A)					0.229				0.460
Phosphate source (B)					0.229				0.412
Humic substances (C)					0.229				0.397
A × B					0.396				0.713
A × C					0.396				N.S
B × C					0.396				0.688
A × B × C					N.S				1.191

Table 5: Effect of bio-fertilizer, phosphate source, humic substances and their interaction on grain and straw yields of barley in combined analysis.

Bio-fertilizer	Phosphate source	Grain weight (kg/fed)				Straw weight (kg/fed)			
		Humic substance				Humic substances			
		Humic acid	Fulvic acid	Without humic substances	Mean	Humic acid	Fulvic acid	Without humic substances	Mean
Mycorrhizal fungi	Super phosphate	2092.21	1847.23	1700.07	1879.84	2790.67	2464.16	2268.64	2507.82
	Rock phosphate	1861.26	1812.12	1406.20	1693.19	2482.89	2417.53	1877.72	2259.38
	Without phosphate	1560.47	1539.00	1399.36	1499.61	2083.12	2054.7	1868.49	2002.10
Mean		1837.98	1732.78	1501.88	1690.88	2452.23	2312.13	2004.95	2256.44
Phosphorine	Super phosphate	1921.05	1727.85	1596.84	1748.58	2562.51	2305.52	2131.41	2333.15
	Rock phosphate	1784.41	1623.37	1503.90	1637.23	2380.61	2166.55	2007.59	2184.92
	Without phosphate	1601.88	1546.09	1398.98	1515.65	2137.76	2063.64	1867.84	2023.08
Mean		1769.11	1632.44	1499.91	1633.82	2360.29	2178.57	2002.28	2180.38
Without inoculation	Super phosphate	1900.05	1726.12	1553.1	1726.42	2534.41	2303.17	2073.17	2303.58
	Rock phosphate	1574.12	1546.13	1371.06	1497.10	2101.13	2063.90	1831.41	1998.81
	Without phosphate	1539.14	1476.07	1154.13	1389.78	2054.74	1982.03	1542.55	1859.77
Mean		1671.10	1582.77	1359.43	1537.77	2230.09	2116.37	1815.71	2054.06
Mean	Super phosphate	1971.10	1767.07	1616.67	1784.95	2629.20	2357.62	2157.74	2381.52
	Rock phosphate	1739.93	1660.54	1427.05	1609.17	2321.54	2215.99	1905.57	2147.70
	Without phosphate	1567.16	1520.39	1317.49	1468.35	2091.87	2033.46	1759.63	1961.65
	Mean	1759.40	1649.33	1453.74	1620.82	2347.54	2202.36	1940.98	2163.62
LSD at 5% level									
Bio-fertilizer (A)					16.42				21.73
Phosphate source (B)					14.70				19.45
Humic substances (C)					14.17				18.75
A × B					25.46				33.68
A × C					24.55				32.47
B × C					24.55				32.47
A × B × C					42.51				56.24

Nutrients uptake in grain and straw yields:

Results presented in **Tables 7 and 8** indicated significant effects due to bio-fertilizer for uptake of phosphorus and potassium in grain and straw yields by barley plant in combined analysis. Inoculation barley seeds with mycorrhizal fungi or phosphorine gave the highest values for all studied traits compared with uninoculated seeds. While, mycorrhizal fungi inoculation recorded highest results than phosphorine on uptake of phosphorus and potassium in grains and straw yields. Positive effect of mycorrhizal fungi or phosphorine on the uptake of phosphorus and potassium in grain and straw yields owing to produce organic acids and they could increase uptake of nutrients by reducing the pH of the soil. These results are in accordance with those reported by **Nogueira *et al*. (2007**) assessed the impact of AMF and some PGPRs on nutrient uptake in soybean and reported the role of AMF in N uptake as result of indirect symbiosis of AMF and plant. They believed that AMF increased the uptake of elements such as P, Fe and Mn and it increased the uptake of nutrients by increasing the level of hyphae at the root surface, and this increase was independent from N uptake. These results are in agreement with **Abou- Aly and Mady (2009), Khan, *et al*., (2009), Thalooth, *et al.* (2012), Heydari and Maleki (2014) and Wali *et al.* (2018).**

In addition, Results in **Tables 6, 7 and 8** illustrate that the effect of phosphorus source on all studied traits was significant in combined analysis. Calcium super phosphate gave the maximum values of nutrients uptake compared with rock phosphate or control. Super phosphate application significantly improved NPK uptake than rock phosphate. These results may be owing to the additional nutrients such as Ca, S and other micronutrients in super phosphate. Also, Calcium super phosphate provided P to the plant at the initial stage of growth, until P from the rock phosphate became available **Bekele and Hofner** (**1993)**. Similar results were obtained by **Csatho *et al*. (2009)** and **Foereid (2017)** they found that rock phosphate had low P availability at the same level of P.

Regarding to humic substances (humic and fulvic acids), it significantly increased uptake of nitrogen, phosphorus and potassium in grain and straw yields relative to without humic substances treatment in combined analysis. Also, humic acid was superior to fulvic acid. These enhancements may be due to the humic substances increase plant growth enormously due to increasing cell membrane permeability, respiration, photosynthesis, oxygen and phosphorus uptake, and supplying root cell growth. Many researchers noted the enhancing effect of humic acid on growth, yield, and nutrient uptake by many crops **(David, 1991; Neri *et***

***al*., 2002 and El-Desuki, 2004).** Indirect effects are mainly exerted through properties such as: enrichment in soil nutrients, increase of microbial population, higher cation exchange capacity (CEC), and improvement of soil structure; whereas direct effects are various biochemical actions exerted at the cell wall, membrane or cytoplasm and mainly of hormonal nature **(Varanini and Pinton, 2001 and Chen *et al*., 2004).** Similar results were obtained by **(Bohme and Lua, 1997 and Turkmen *et al*., 2004).** They found that humic substances improved plant growth and nutrient uptake.

The interaction between phosphate biofertilizer treatments and phosphorus source was significant on nutrients uptake in grain and straw yield in the combined analysis, as shown as in **Tables 6, 7 and 8**.The maximum values for these traits were obtained from inoculation with mycorrhizal fungi and calcium super phosphate application. Similar results were obtained by **Fernández Bidondo *et al.*, (2012) and Heydari and Maleki (2014).** They found that application of mycorrhizal is playing significant roles in the optimization of P solubilization, increase of nutrient levels and mineralization of organic phosphate.

The interaction between phosphate biofertilizer treatments and humic substances was significant on nutrients uptake in grain and straw yield in the combined analysis, as shown as in **Tables 6, 7 and 8**.The maximum values for these traits were obtained from inoculation with mycorrhizal fungi and humic acid application. These results are in harmony with those obtained by **Abou-Aly and Mady (2009).** They found that application of humic acid with mycorrhizal exhibited values of available nutrients greater than the treatments of biofertilizers without humic acid. This may be due to that the addition of organic substances which improved the physical properties of the soil, and increased the supplying power of available nutrients to plants.

The interaction between phosphorus source treatments and humic substances was significant on nutrients uptake in grain and straw yield in the combined analysis. The maximum values for these traits were obtained from application of calcium super phosphate with humic acid.

Maximum NPK uptake in grain and straw was obtained by the treatment of mycorrhizal plus super phosphate and humic acid as gave 59.09, 7.68 and 24.31 kg/fed for grain, respectively and 46.10, 7.26 and 16.67 kg/fed for straw of barley plants, respectively.

Table 6: Effect of bio-fertilizer, phosphate source, humic substances and their interaction on nitrogen uptake by grain and straw yields of barley in combined analysis.

Bio-fertilizer	Phosphate source	N- uptake by grain (kg/fed)				N- uptake by straw (kg/fed)			
		Humic substance				Humic substances			
		Humic acid	Fulvic acid	Without humic substances	Mean	Humic acid	Fulvic acid	Without humic substances	Mean
Mycorrhizal fungi	Super phosphate	59.09	49.88	45.11	51.36	46.10	39.69	23.44	36.41
	Rock phosphate	44.15	42.44	32.18	39.59	35.36	32.75	14.94	27.68
	Without phosphate	35.87	34.31	30.49	33.56	24.04	20.45	12.86	19.12
Mean		46.37	42.21	35.93	41.50	35.17	30.96	17.08	27.74
Phosphorine	Super phosphate	54.07	47.54	40.05	47.22	36.68	29.34	26.32	30.78
	Rock phosphate	50.65	42.19	33.46	42.10	32.95	28.63	19.62	27.07
	Without phosphate	44.08	36.46	31.27	37.27	28.88	27.54	22.69	26.37
Mean		49.60	42.06	34.93	42.20	32.84	28.50	22.88	28.07
Without inoculation	Super phosphate	51.77	45.14	37.3	44.74	33.73	28.65	24.21	28.86
	Rock phosphate	39.9	37.54	30.48	35.97	28.38	26.17	22.12	25.56
	Without phosphate	29.38	23.36	12.99	21.91	23.32	15.99	10.45	16.59
Mean		40.35	35.35	26.92	34.21	28.48	23.60	18.93	23.67
Mean	Super phosphate	54.98	47.52	40.82	47.77	38.84	32.56	24.66	32.02
	Rock phosphate	44.90	40.72	32.04	39.22	32.23	29.18	18.89	26.77
	Without phosphate	36.44	31.38	24.92	30.91	25.41	21.33	15.33	20.69
	Mean	45.44	39.87	32.59	39.30	32.16	27.69	19.63	26.49
LSD at 5% level									
Bio-fertilizer (A)					0.62				0.85
Phosphate source (B)					0.56				0.76
Humic substances (C)					0.54				0.73
A × B					0.96				1.31
A × C					0.93				1.26
B × C					0.93				1.26
A × B × C					1.61				2.19

Table 7: Effect of bio-fertilizer, phosphate source, humic substances and their interaction on phosphorus uptake by grain and straw yields of barley in combined analysis.

Bio-fertilizer	Phosphate source	P- uptake by grain (kg/fed)				P- uptake by straw (kg/fed)			
		Humic substance				Humic substances			
		Humic acid	Fulvic acid	Without humic substances	Mean	Humic acid	Fulvic acid	Without humic substances	Mean
Mycorrhizal fungi	Super phosphate	7.68	6.34	5.67	6.56	7.26	6.16	5.53	6.32
	Rock phosphate	6.27	5.74	4.22	5.41	6.29	5.73	4.19	5.40
	Without phosphate	4.57	4.11	3.55	3.99	5.00	4.79	3.99	4.59
Mean		6.17	5.40	4.48	5.35	6.18	5.56	4.57	5.44
Phosphorine	Super phosphate	6.98	5.64	4.56	5.73	6.41	5.69	4.91	5.67
	Rock phosphate	5.71	4.76	4.16	4.88	5.80	5.06	4.29	5.05
	Without phosphate	5.02	4.69	3.97	4.56	4.78	4.48	3.99	4.42
Mean		5.90	5.03	4.23	5.05	5.66	5.08	4.40	5.05
Without inoculation	Super phosphate	6.53	5.47	4.26	5.42	6.17	5.22	4.49	5.29
	Rock phosphate	5.30	4.80	3.75	4.62	5.04	4.62	3.97	4.54
	Without phosphate	4.32	3.94	1.81	3.36	4.59	4.10	1.95	3.55
Mean		5.38	4.74	3.27	4.46	5.27	4.65	3.47	4.46
Mean	Super phosphate	7.06	5.82	4.83	5.90	6.61	5.69	4.98	5.76
	Rock phosphate	5.76	5.10	4.04	4.97	5.71	5.14	4.15	5.00
	Without phosphate	4.64	4.25	3.11	4.00	4.79	4.46	3.31	4.19
	Mean	5.82	5.05	3.99	4.96	5.70	5.09	4.15	4.98
LSD at 5% level									
Bio-fertilizer (A)					0.083				0.091
Phosphate source (B)					0.075				0.081
Humic substances (C)					0.072				0.078
A × B					0.129				0.140
A × C					0.125				0.135
B × C					0.125				0.135
A × B × C					0.216				0.234

Table 8:Effect of bio-fertilizer, phosphate source ,humic substances and their interaction on potassium uptake by grain and straw yields of barley in combined analysis.

Bio-fertilizer	Phosphate source	K- uptake by grain (kg/fed)				K- uptake by straw (kg/fed)			
		Humic substance				Humic substances			
		Humic acid	Fulvic acid	Without humic substances	Mean	Humic acid	Fulvic acid	Without humic substances	Mean
Mycorrhizal fungi	Super phosphate	24.31	17.71	15.56	19.19	16.67	14.73	12.73	14.71
	Rock phosphate	18.31	16.46	11.8	15.52	15.20	14.31	11.13	13.55
	Without phosphate	15.03	13.58	11.17	13.26	12.11	11.17	9.43	10.90
Mean		19.22	15.92	12.84	15.99	14.66	13.40	11.10	13.05
Phosphorine	Super phosphate	19.33	14.27	11.85	15.15	15.99	13.58	11.80	13.79
	Rock phosphate	18.11	14.6	11.69	14.80	15.20	12.98	10.22	12.80
	Without phosphate	14.34	13.07	10.51	12.64	11.18	10.29	8.73	10.07
Mean		17.26	13.98	11.35	14.20	14.12	12.28	10.25	12.22
Without inoculation	Super phosphate	18.18	13.36	11.03	14.19	15.97	13.06	10.67	13.23
	Rock phosphate	12.97	12.56	10.29	11.94	11.44	10.26	7.86	9.85
	Without phosphate	10.96	9.48	5.80	8.75	8.39	8.04	4.88	7.10
Mean		14.04	11.80	9.04	11.63	11.93	10.45	7.80	10.06
Mean	Super phosphate	20.61	15.11	12.81	16.18	16.21	13.79	11.73	13.91
	Rock phosphate	16.46	14.54	11.26	14.09	13.95	12.52	9.74	12.07
	Without phosphate	13.44	12.04	9.16	11.55	10.56	9.83	7.68	9.36
	Mean	16.84	13.90	11.08	13.94	13.57	12.05	9.72	11.78
LSD at 5% level									
Bio-fertilizer (A)					0.288				0.097
Phosphate source (B)					0.258				0.087
Humic substances (C)					0.249				0.083
A × B					0.447				0.150
A × C					0.431				0.145
B × C					0.431				0.145
A × B × C					0.746				0.250

Conclusion:

It can be concluded that, the application of Mycorrhizal fungi, super phosphate and humic acid indicated that the combined effect of humic acid with the potent biofertilizers is a good tool for promotion barley growth and yield, nutrients uptake by barley plant, particularly in newly reclaimed soils.

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تأثير التسميد الحيوى ومصادر الفوسفور والمواد الهيوميه على المحصول ومكوناته وامتصاص العناصر الغذائيه لنبات الشعير

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أجريت تجربتان حقليتان بمزرعة كلية الزراعة - جامعة الأزهر خلال موسمى الزراعة 2016/ 2017 و 2018/2017م بهدف دراسة تأثير التسميد الحيوى ومصادر الفوسفور والمواد الهيوميه على المحصول ومكوناته وإمتصاص العناصر المغذية لنبات الشعير صنف جيزة 123. نفذت التجارب بإستخدام تصميم القطع المنشقة مرتين فى ثلاث مكرارت. اشتملت الدراسة على 27 معاملة تمثل التوافقات بين مستويات الثلاث عوامل تحت الدراسة وهم التسميد الحيوى (فطر الميكورهيزا ، الفوسفورين و بدون تسميد حيوى) وزعت عشوائي على القطع الرئيسة و مصادر الفوسفور (سوبر فوسفات الكالسيوم ، صخر الفوسفات و بدون إضافة الفوسفور) وزعت عشوائي على القطع الشقية والمواد الهيوميه (حامض هيوميك ، حامض فولفيك و بدون إضافة مواد هيوميه) وزعت عشوائي على القطع تحت الشقية، ويمكن تلخيص نتائج هذه الدراسة كما يلى:

ادى التسميد الحيوى الى زياده معنويه فى المحصول ومكوناته وامتصاص العناصر الغذائيه (النيتروجين والفوسفور والبوتاسيوم) مقارنة بدون التلقيح ، كما لوحظ ان تلقيح حبوب الشعير بفطر الميكروهيزا تفوق على المخصب الحيوى الفوسفورين، حيث ادى التلقيح بفطر الميكروهيزا

الى زيادة معنويه فى المحصول ومكوناته، كما تجدر الاشاره ايضاً الى زياده امتصاص الفوسفور والبوتاسيوم فى نبات الشعير. بالاضافه الى ماسبق وجد ان فطر الميكروهيزا والبكتيريا المذيبه للفوسفات تلعب دوراً هاماً فى اذابة وتيسر عنصر الفوسفور للنبات من خلال الانطلاق البطئ للفوسفور من المركبات الغير عضويه والعضويه هو مايعرف بعمليتى الاذابه والمعدنه للفوسفور. كما ادى التسميد الفوسفاتى الى زياده معنويه فى المحصول ومكوناته وامتصاص العناصر الغذائيه (النيتروجين والفوسفور والبوتاسيوم) مقارنة بدون اضافة فوسفات، ومن الجدير بالذكر ان السوبر فوسفات الكالسيوم اعطى نتائج ايجابيه افضل من صخر الفوسفات فى المحصول ومكوناته ، وزياده ذوبان وتيسر وامتصاص النيتروجين والفوسفور والبوتاسيوم فى نبات الشعير. ادى اضافة المواد الهيوميه الى زيادة معنويه فى المحصول ومكوناته وامتصاص العناصر الغذائيه (النيتروجين والفوسفور والبوتاسيوم) مقارنة بدون اضافة مواد هيوميه، وبخصوص اضافة حامض الهيوميك ادى الى زيادة زياده معنويه فى المحصول ومكوناته افضل من حامض الفولفيك، كما تجدر الاشاره ايضاً الى زياده امتصاص النيتروجين والفوسفور والبوتاسيوم فى نبات الشعير. ومن الملاحظ ان اعلى القيم التى تم الحصول عليها كانت عند خلط حامض الهيوميك مع سوبر فوسفات الكالسيوم والتلقيح بفطر الميكروهيزا ويرجع السبب فى ذلك الى التاثير الايجابى بين سوبر فوسفات الكالسيوم وحامض الهيوميك فى وجود فطر الميكروهيزا والذى يعمل على اذابة الفوسفور و زيادة المحتوى الخصوبى للتربه وامتصاص وتيسر العناصر الغذائيه (النيتروجين والفوسفور والبوتاسيوم) لنبات الشعير بالمقارنه بالكنترول. بوجه عام ادى اضافة حامض الهيوميك والسوبر فوسفات العادى والتلقيح ب بفطر الميكروهيزا منفرده او مختلطه مع بعضها للتربه الى زيادة معنويه فى المحصول ومكوناته والمحتوى الخصوبى للتربه وامتصاص العناصر الغذائيه لنبات الشعير.