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Phosphorus Efficiency in Wheat as Affected by Foliar Spray with Zinc, Humic Acid and Biofertilizer (*Bacillus megatherium* sp.) Addition Under Calcareous Soil Conditions

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ABSTRACT

To enhancing the efficiency use of phosphate fertilizer in calcareous soil by bio inoculation with (*Bacillus megatherium* sp.) as PGPR strain and foliar spray with Zn and humic acid (HA) on wheat (*Triticium aestavium* L., cv. Sakha 93) productivity and nutrients availability in soil after harvest, two field experiments were conducted at El-Nubaria Agricultural Research Station, Alexandria Governorate, Egypt during 2014 and 2015 seasons. The studied treatments involved three P application rates *i.e*, 0, 6.5 and 13 kg P fed.$^{-1}$, foliar spraying with Zn (1.5 kg $ZnSO_4$ /400 L water), humic acid (HA) 1.2 L/400 L water and Zn+HA without and with bio-inoculation with (*Bacillus megatherium* sp.). The results could be summarized as follow: Available N, P, K, Fe, Mn and Zn were significantly increased due to the above mentioned treatments. Foliar spraying with Zn and humic acid in combination with biofertilizer under different P fertilizer rates increased wheat yields and 1000-grain weight as well as N, P, K, Fe, Mn and Zn content and uptake by grains in combined data. Total chlorophyll content and carbohydrates were increased as well as protein content and protein yield. Highest yield efficiency (82.7%) and harvest index (44.7%) were obtained due to the treatment of 6.5 kg P fed.$^{-1}$ + Zn + bio and 6.5 kg P fed.$^{-1}$ + Zn, respectively. Apparent P-recovery (APR), P agronomic efficiency (PAE) and P use efficiency (PUE) were markedly decreased with increasing P-addition rates in presence or absence of bio fertilization. Hence, the plants treated with 6.5 kg P fed.$^{-1}$ + foliar spray with Zn, HA and Zn + HA when inoculated with *Bacillus megatherium* gave the highest mean values of APR, PUE and PAE. Generally, the treatment of P fertilization (13 kg P fed.$^{-1}$ combined with Zn + HA) when inoculated with *Bacillus megatherium* had superior effect in improving soil properties and increasing wheat production, protein content and nutrient uptake with compared to the other treatments under calcareous soil conditions but, in most of them without significant differences with lower rate of 6.5 kg P fed.$^{-1}$.

Keywords: Calcareous soil, P-fertilization, biofertilizer, Zn, humic acid and wheat.

INTRODUCTION

Wheat (*Triticum aestavium* L.) is one of the most important cereal crops in Egypt and has a high importance worldwide, measured either by cultivated area or production (Jagshoran *et al.,* 2004). Wheat has got an evolutionary history parallel to the history of human civilization; as it decides the feast or famine for millions of people even today. Wheat provides 37% of the total calories and 40% of the protein in the Egyptian people diet. Total production of wheat in Egypt reached 9.46 million tonns in 2012, produced from an area of 3.378 million feddan, (CAPMAS, 2014).

Thomas *et al.* (2009) stated that fertilizer management in calcareous soil pH value in the ranged of 7.6 – 8.3differs from that on non-calcareous soils because of the effect of soil pH on nutrient availability and chemical reactions that affect the loss or fixation of some nutrients. The presence of $CaCO_3$ directly or indirectly affects the chemistry and availability of N, P, K, Mg, Mn, Zn, Fe and Cu. The availability of these elements decreases when soil $CaCO_3$ increases to more than about 3% by weight.

Phosphorus (P) is one of the major plant growth limiting nutrients although it is abundant in soils in both inorganic and organic forms. Phosphate solubilizing micro-organisms (PSMs) are ubiquitous in soils and could play an important role in supplying P to plants in a more environmentally friendly and sustainable manner. Phosphorus is usually supplied to the plant in many different forms some of which are manufactured, *i.e.*, phosphoric acid and calcium super phosphate, while some others are common in nature such as rock phosphate, (Abou El-Yazeid and Abou-Aly 2011). Phosphorus is highly reactive with the calcium carbonates in alkaline and calcareous soils. A series of reactions between Ca and P reduces P solubility and its resulting availability to plants. In addition to Ca, other elements in alkaline soils including iron (Fe), aluminum (Al), and magnesium (Mg) can also react with P, reducing its solubility, (Leytem, 2008).

Zinc is an essential trace element for plants, animals and humans, but it is generally deficient in soil, which is the primary source of this nutrient. Wheat (*Triticum aestivum* L.) is the staple food for people living in Egypt, where potentially Zn-deficient calcareous soils (i.e. 0.5–1.0 mg DTPA-Zn kg^{-1}) are common, (Shivay *et al.* 2008). The low Zn concentration and high phytic acid content (i.e. inositol hexa phosphate) in wheat grain are the two main factors contributing to Zn deficiency in humans, (Cakmak, 2008) and Zhang *et al.* (2010). Zinc moves primarily by diffusion in calcareous soil. The diffusion rate from fertilizer granules to the rhizosphere can influence Zn absorption by plants. For these reasons, we hypothesized that the benefits of Zn application to potentially Zn-deficient soil are limited by slow diffusion rates. It is important to clarify the interactions among Zn diffusion rate, soil DTPA-Zn concentration and Zn absorption by wheat plants on potentially Zn-deficient soil, (Oliveira et al. 2010). Currently; $ZnSO_4$ is the most common Zn fertilizer, Hussain and Maqsood, (2010).

Plant growth promoting rhizobacteria (PGPR) have been known for long time to be involved in plant growth promotion via mechanisms such as phytohormone production, suppression of plant diseases and improved nutrient acquisition by nitrogen fixation, phosphate solubilization and metal accumulation (Martínez Viveros *et al*. 2010, Upadhyay and Srivastava 2014). Apart from this, PGPR are also known to be key players in bioremediation of contaminated soils due to their ability to accumulate metals (Zhuang *et al*. 2007). Also, this in metal- deficient soils, sufficing for the requirement of essential metals as shown for Zinc by Whiting *et al*. (2001).

Humic acid (HA) is one of the main organic fertilizers, which is an important component of humic substances. Humic acid is produced by the chemical and biological decomposition of organic material. Humic acid is a vital component of soil organic matter which improves the plant growth. It enhances soil fertility and improves its physical and chemical properties such as permeability, aggregation, water holding capacity, ion transport and availability through pH buffering Tan, (2003) and Turan *et al*. (2011).

The objective of this study is to compare the effects of (i) P fertilization (ii) foliar spray with $ZnSO_4$ and humic acid fertilizers, and (iii) with and without bio inoculation with phosphate-solubilising bacteria (*Bacillus megatherium var phosphaticium*) on yield components of wheat grown in calcareous soil.

MATERIALS AND METHODS

To find the effect of bio inoculation of wheat by *Bacillus megatherium and* foliar spray with Zn, HA and Zn + HA on enhancing the efficiency use of P fertilization as ordinary super phosphate (67.6 g P kg^{-1}) in calcareous soil and its effect on yield components of wheat, two experiments were conducted at El-Nubaria Agricultural Research Station, El-Bhara Governorate, Egypt during winter 2014/2015 and 2015/2016 seasons.

A representative soil sample (0–30 cm) was taken before planting to determine physical and chemical properties. The soil was sandy Loam in texture (76.29% sand, 8.60% silt and 15.11% clay), having an EC of 3.73 dS $m^{–1}$ in its saturation extract and $CaCO_3$ and organic matter contents of 147 and 5.3 g $kg^{–1}$, respectively. Available nutrients were 32.8 mg N kg^{-1} (mineral N extracted with 2 M KCl), 3.21 mg P $kg^{–1}$ (extracted with 0.5 M Na-bicarbonate), 170 mg K $kg^{–1}$ (extracted with neutral 1.0 M NH_4OAC), 1.22 mg Fe $kg^{–1}$, 1.14 mg Mn $kg^{–1}$ and 0.51 mg Zn $kg^{–1}$ (extracted with DTPA) according to the some methods used for analysis the initial soil as Page *et al*. (1982).

The experiment was laid out in a 3-factor split-split plot in a randomized complete block design with three replications. The plot area was 50 m^2 (5 X 10 m). Main plots, were assigned to phosphorus fertilization rates, none, 6.5 and 13 kg P $fed.^{-1}$**.** Sub-plots were assigned to foliar treatments with Zn, humic acid (HA) and Zn+HA. Sub–sub plots were assigned to bio fertilizers: none and *Bacillus megatherium*. Each plot was sown with grains of wheat cultivar (*Triticum aestivum* cv. Sakha 93) on the 5^{td} and 10^{th} of November 2014 and 2015, and harvested on the 20^{th} and 24^{th} of April 2015 and 2016, respectively. Grains were inoculated with *Bacillus megatherium* strain (PGPR) biofertilizer supplement by Bio-fertilizer Production Unit, Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. Grains of wheat were coating with the gum media carrying the bacteria strain on the same day of sowing at rate of 700g per 60 kg grains. More of bacteria strain was added three times at 30, 45 and 55 days after sowing (DAS) as liquid spray on soil and plant at a rate of 20L per 400 L water $fed.^{-1}$.

Nitrogen and potassium were balanced in all the plots to insure that no differences between treatments existed. Nitrogen was added at 100 kg N $kg^{–1}$ soil as urea (460 g N kg^{-1}) in three equal splits at 21-day intervals after sowing. Potassium was added as potassium sulphate (400 g K kg^{-1}) at 200 kg K $fed.^{-1}$ in two equal splits, 30 and 45 DAS. Agricultural practices for growing wheat were carried out as recommended by the Ministry of Agriculture.

Foliar spraying with humic acid at rate of 1.2 L / 400 L water $fed.^{-1}$ within irrigation system was done twice at tillering stage, 30 days after sowing (DAS) and at booting stage (70 DAS). Zn fertilizer was sprayed at rate of 1.5 kg $ZnSO_4$ (21%) /400 L water $fed.^{-1}$ within irrigation system three times after 31, 45 and 60 DAS.

To collect the data from respected treatments, 1m^2 was randomly thrown at three different places and then averaged to measure straw and grain yields (Mg, Mega gram $fed.^{-1}$), (1 Mg = 10^6 g = 1000 kg). Whereas for plant height (cm), spike length (cm) and 1000 grain weight (g), 10 random plants were selected and averaged. The following parameters were calculated:

- Grain protein contents by multiplying grain N% by 6.25.
- Grain protein yield in kg $fed.^{-1}${protein content g kg^{-1} x grain yield Mg $fed.^{-1}$}.
- Harvest Index (HI): (grain yield / biological yield) x100
- Yield efficiency: (grain yield / straw yield) x 100.
- N, P and K content and uptake by grain.
- The chlorophyll content of leaf tissue was estimated following the method of Saric *et al.* (1967).
- Apparent P recovery (APR) by the equation described by Echeverria and Videla (1998), *i.e.*, ANR = [P uptake (fertilized plot) – P uptake (zero plot) / P fertilizer rate] X 100.
- Phosphorus agronomic efficiency (PAE) for P according to Craswell and Godwin (1984): grain yield (fertilized plot) - grain yield (zero plot)] / P fertilizer; yield and P fertilizer in $kgfed.^{-1.}$
- Phosphorus use efficiency (PUE) is the P applied to produce yield and is defined here as the amount of grain yield per unit of applied P (kg of grain yield kg^{-1} of P applied) as described by Angas *et al*. (2006).

Macro and micronutrients content of grain samples were determined in aliquots of digested solutions resulting from the digestion of grain samples by a mixture of H_2SO_4 and $HClO_4$ acids after drying in an oven at 70º C as described by Ryan *et al*. (1996).

Soil characteristics after wheat harvest.

After harvest, representative soil samples of the field were taken (0 – 30 cm layer) from each plot. Samples were analyzed for available N, P and K as well as Fe, Mn and Zn according to Page *et al.* (1982).

Statistical analysis

Statistical program MSTAT-C was used to analyze the data statistically analyzed using. Analysis of variance (ANOVA) was employed to test the overall significance of the data, while the least significance difference (LSD) test at $p \leq 0.05$ was used to compare the treatment means, Steel and Torrie (1997).

RESULTS AND DISCUSION

Available N, P and K in soil after harvest

Table 1 show that soil available N and K were significantly increased due to the treatments of P, Zn and HA fertilizations especially under inoculation with phosphorus dissolved bacteria (*Bacillus megatherium)*. Highest values were 43.8 and 222 mg kg^{-1} soil for N and K, respectively due to the treatment of P_{13} + (Zn+HA) under bio fertilization and 4.28 mg kg^{-1} soil for available-P when plants treated with P_{13} + HA under bio fertilization. The increases percentage reached 30.7, 25.4 and 30.1 %, for N, K and P, respectively compared with untreated.

The superiority of (13 kg P kg^{-1} + Zn +HA) when treated with bio fertilization could be due to the effect of microorganisms and their biological activity in build up the microflora and released active organic acids during microbial activity, thus enhancing solubilization of nutrient from the native and added sources Application of mineral-P might have improved the activities of microorganisms responsible for P transformation (Ewees and Abdel Hafeez, 2010). Humic substances act as a natural soil conditioner which improved soil properties and consequently soil productivity. These results are in accordance with those obtained by, Ali *et al.* (2011).

The used amendment sources could be arranged according to their effects in the following descending order: $P_{13} > P_{6.5} > P_0$ for phosphorus fertilization rate; Zn + HA> HA > Zn for treatments and Bio > without bio for bio inoculation effect. This trend was found true for available N, P and K content, which was significant for available N and K while insignificant for available P in the studied soil.

Table (1) Available macronutrients content (mg kg^{-1} soil) in soil after wheat harvest as affected by treatments

P application rate, kg P fed.$^{-1}$ (P)	Foliar treatment (F)	Bio addition (b)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
			Available-N			Available-P			Available-K	
0	Zn	34.8	33.5	34.1	3.64	3.29	3.46	181	177	179
	Humic acid, (HA)	36.4	35.1	35.7	3.83	3.51	3.67	191	185	188
	Zn +HA	38.5	37.1	37.8	4.05	3.63	3.84	196	192	194
	Mean	36.5	35.2	35.9 c	3.84	3.47	3.66	189	185	187 c
6.5	Zn	36.1	34.8	35.4	3.75	3.39	3.57	193	183	188
	HA	37.9	35.6	36.7	4.02	3.64	3.83	201	194	197
	Zn +HA	39.1	37.5	38.3	4.26	3.79	4.02	209	199	204
	Mean	37.7	36.0	36.8 b	4.01	3.60	3.81	201	192	197 b
13	Zn	40.3	36.9	38.6	3.94	3.76	3.85	207	191	199
	HA	43.0	38.2	40.6	4.28	3.97	4.12	217	199	208
	Zn +HA	43.8	38.6	41.2	3.92	4.07	3.99	222	210	216
	Mean	42.4	37.9	40.1 a	4.04	3.93	3.99	215	200	208 a
Grand Mean (bio)		38.9	36.4		3.96	3.67		202 a	192 b	
F -test		P:**	F:**	B:**	P: ns	F: ns	B: ns	P:**	F:**	B:**
		PxF: ns	Pxb:**	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns
			PxFxb: ns			PxFxb: ns			PxFxb: ns	
Mean of foliar treatment (F)										
Zn			36.0 c			3.63			189 c	
HA			37.7 b			3.87			198 b	
Zn +HA			39.1 a			3.95			205 a	

Available Fe, Mn and Zn in soil after Harvest

Data presented in Table 2 show that soil available Fe, Mn and Zn were significantly increased by application of different P fertilization rates and foliar spraying with Zn and HA especially under inoculation with phosphorus dissolved bacteria (*Bacillus megatherium)*. The highest values were 2.45, 1.53 and 0.72 mg kg^{-1} soil for Fe, Mn, and Zn, respectively owing to the treatment of P_{13} + (Zn+HA) under bio fertilization. The increases percentage reached 87.0, 29.7 and 33.3 %, for Fe, Mn, and Zn, respectively. These results agree with Zaki and Radwan (2006) who found that the applied P fertilizer and phosphate dissolving bacteria enhanced micronutrients availability in calcareous soil. biofertilizer treatments enhanced also the biological conditions in soil that caused nutrient uptake by plants to increase. These results may be attributed to one or more of the following reasons:

i. The presence of microbial media of bio-fertilizer, and in turn produces active organic and inorganic acids that led to decrease soil pH beside their ability to chelate metal ions (Fe, Mn and Zn). These chelated metal ions are held in forms available for plant and consequently they are found as strategic storehouse in organo-metalic compounds that are more suitable for uptake by plant roots (Mohammed, 2004).

ii. The effective role of microbial activity to reduce soil salinity stress, could be interpreted according to many opinions outlined by Ashmaye *et al.* (2008) who reported that many strains produce several phytohormones (i.e., indole acetic acid and cytokinins) and organic acids. Such products reduce the deleterious effect of Na-salts, and simultaneously improve soil structure, i.e., increase aggregate stability and drainable pores and hence accelerate leaching of soluble salts and soil profile with the drained water.

iii. The released soluble Ca^{2+} partially substitutes exchangeable Na and leads to reduce ESP value and formation of small clay domains. Such clay domains are coated with the released active organic acids, and then form coarse sizes of water stable aggregates which accelerate leaching of a pronounced content of soluble salts and accordingly reduce the ECe value (Ewees and Abdel Hafeez, 2010).

Table (2) Available micronutrient contents (mg kg^{-1} soil) in soil after wheat harvest as affected by treatments

P application rate, kg P $fed.^{-1}$ (P)	Foliar treatment (F)	Bio addition (b)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
		Available-Fe			Available-Mn			Available-Zn		
0	Zn	1.79	1.31	1.55	1.25	1.18	1.21	0.56	0.54	0.55
	HA	1.97	1.54	1.75	1.32	1.22	1.27	0.60	0.57	0.58
	Zn +HA	2.07	1.99	2.03	1.36	1.26	1.31	0.63	0.59	0.61
	Mean	1.94	1.61	1.78 b	1.31	1.22	1.26 b	0.59	0.57	0.58 b
6.5	Zn	1.87	1.35	1.61	1.28	1.20	1.24	0.60	0.55	0.57
	HA	2.20	1.60	1.90	1.39	1.28	1.33	0.65	0.61	0.63
	Zn +HA	2.40	2.02	2.21	1.44	1.31	1.37	0.68	0.64	0.66
	Mean	2.16	1.65	1.91 a	1.37	1.26	1.31 b	0.64	0.60	0.62 ab
13	Zn	1.95	1.37	1.66	1.35	1.25	1.30	0.61	0.60	0.60
	HA	2.25	1.73	1.99	1.46	1.35	1.41	0.67	0.63	0.65
	Zn +HA	2.45	2.05	2.25	1.53	1.39	1.46	0.72	0.68	0.70
	Mean	2.22	1.71	1.97 a	1.45	1.33	1.39 a	0.67	0.64	0.65 a
Grand Mean (bio)		2.11 a	1.66 b		1.38 a	1.27 b		0.64	0.60	
F -test		P: **	F: **	B: **	P: **	F: **	B: **	P: *	F: *	B: ns
		PxF: ns	Pxb: ns	Fxb: *	PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns
			PxFxb: ns			PxFxb: ns			PxFxb: ns	
Mean of foliar treatment (F)										
Zn			1.61 c			1.25 b			0.57 b	
HA			1.88 b			1.34 a			0.62 ab	
Zn +HA			2.16 a			1.38 a			0.66 a	

The availability of soil N, P and K as well as micronutrients in the studied calcareous soil were greatly affected by humic acid enriched with nutrients particularly P and Zn especially with bio inoculation with PDB.

The interaction between P, Zn and HA fertilization treatments showed that the values of available nutrients available were in ascending order of : Zn+HA > HA > Zn for available Fe and Zn as well as Zn+HA > HA > Zn for available Mn. As for P fertilization, the order was: $P_{13} \geq P_{6.5} > P_0$ for available Fe and Zn and $P_{13} > P_{6.5} \geq P_0$ for available Mn. In other words, the availability of micronutrients responded more to bio inoculation with (*Bacillus megatherium)*. These results are agreement with Van Hees *et al.* (2005).

Effect of treatments on growth parameters and yield of wheat:

Growth parameters

Some growth parameters of wheat plants are shown in Table 3. Application of P-fertilization and foliar spraying with Zn, HA and Zn+HA as well as bio fertilization significantly increased plant height, spike length and 1000-grain weight. The highest values (109 cm, 14.4 cm and 38.1 g) were recorded in the plants treated with 13 kg P $fed.^{-1}$ + (Zn + HA) + bio which caused increases of about 74.7%, 71.0% and 31.8%, respectively. The application of P-fertilization enriched with Zn and humic acids as well as inoculated with phosphorus dissolved bacteria was more effective on wheat yield than other treatments. The beneficial effects of HA on plant growth could be related to improved plant metabolism. Trevisan *et al.* (2010) attributed the growth stimulation effect of HA on plant growth to their interaction with physiological and metabolic processes; stimulating nutrient uptake and cell permeability, and the regulating mechanisms involved in plant growth stimulation. On the other hand, the increased occurred in growth parameters reflects the positive role of micronutrients in enhancing biochemical and physiological processes such as; photosynthesis, respiration, enzyme activity. These results are in agreement with those obtained by Yassen *et al.* (2010). The main effect of P-fertilization rate, concerning plant height, spike length and 1000-grain weight followed the order of: Zn + HA > HA > Zn for plant height and 1000-grain weight and followed the pattern: Zn + HA ≥ HA ≥ Zn for spike length.

As for the main effect of foliar treatments, results show progressive increase in plant height, spike length and 1000-grain weight. The pattern was: Zn + HA > HA > Zn for plant height and 1000-grain weight while, followed the pattern: Zn + HA ≥ HA > Zn for spike length Bio inoculation was superior for increasing available Fe, Mn and Zn in soil after harvest.

Straw and grains yields

As shown in Table 4, P application, Zn and HA as well as their combinations is significantly increased straw and grain yields and consequently the biological yield of wheat plants. On the other hand, bio inoculation significantly increased straw yield only. Ali *et al.* (2011) reported that P and Zn fertilization resulted in increasing straw, grain and total yield of wheat.

The highest yields of straw and grain (4.26 and 2.94 Mg $fed.^{-1}$), respectively were obtained with the application of 13 kg P $fed.^{-1}$ + (Zn +HA) + bio inoculation with *Bacillus megatherium* sp. treatment which resulted in relative increments of 38.3% and 21.0%, respectively.

When investigating the role of phosphorus in plants, El-Kabbany and Darwish (2002) concluded that phosphorus is part of the molecular structure of several vitally important compounds, notably nucleic acids. In addition, phosphorus plays an indispensable role in photosynthesis and respiration and is also essential for cell division and for the development of meristem tissues. The yield increase due to P application was perhaps related to the increased concentration and uptake of essential plant nutrients and the decreased concentration and uptake of toxic ions (Na^+ and Cl) and to the widening of the Ca/Na and K/Na ratios. These results are in harmony with those obtained by Helmy, (2008). These increases also, illustrate the pronounced effect of bio inoculation with phosphate dissolving microorganisms on improving soil characteristics, fertility and plant production, (Nour El-Dein and Salama, 2006).

Table (3) Yield components of wheat as affected by P and bio addition as well as foliar treatments

P application rate, kg P fed.$^{-1}$ (P)	Foliar treatment (F)	Bio addition (b)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
		Plant height (cm)			Spike length (cm)			1000-grain weight (g)		
0	Zn	69.2	62.4	65.8	9.78	8.42	9.10	32.1	28.9	30.5
	HA	75.1	68.4	71.7	12.0	10.6	11.3	33.7	30.1	31.9
	Zn +HA	79.8	72.6	76.2	12.7	11.4	12.0	35.0	31.1	33.1
	Mean	74.7	67.8	71.2 c	11.5	10.1	10.8 b	33.6	30.0	31.8 c
6.5	Zn	87.2	67.6	77.4	9.89	8.78	9.33	33.6	30.4	32.0
	HA	91.8	76.6	84.2	13.0	11.3	12.2	35.9	31.0	33.5
	Zn +HA	96.5	80.3	88.4	13.1	11.9	12.5	37.0	32.0	34.5
	Mean	91.8	74.8	83.3 b	12.0	10.7	11.3ab	35.5	31.1	33.3 b
13	Zn	95.7	77.9	86.8	10.2	8.94	9.56	36.1	31.9	34.0
	HA	103	86.8	94.9	13.9	11.8	12.9	36.8	32.1	34.5
	Zn +HA	109	91.3	100	14.4	12.0	13.2	38.1	33.8	35.9
	Mean	103	85.3	94.0 a	12.8	10.9	11.9 a	37.0	32.6	34.8 a
Grand Mean (bio)		89.8 a	76.0 b		12.1a	10.6 b		35.4 a	31.2 b	
F -test		P: **	F: **	B: **	P: **	F: **	B: **	P: **	F: **	B: **
		PxF: ns	Pxb: **	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns
			PxFxb: ns			PxFxb: ns			PxFxb: ns	
Mean of foliar treatment (F)										
Zn			76.7 c			9.33 b			32.1 c	
HA			83.6 b			12.1 a			33.3 b	
Zn +HA			88.2 a			12.6 a			34.5 a	

Table (4) Yield (Mg fed.$^{-1}$) of wheat as affected by P and bio addition as well as foliar treatments

P application rate, kg P fed.$^{-1}$ (P)	Foliar treatment (F)	Bio addition (b)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
		Straw yield			grain yield			Biological yield		
0	Zn	3.39	3.08	3.23	2.63	2.43	2.53	6.02	5.51	5.77
	HA	3.55	3.25	3.40	2.71	2.50	2.60	6.26	5.75	6.01
	Zn +HA	3.66	3.37	3.52	2.75	2.54	2.64	6.41	5.91	6.16
	Mean	3.53	3.23	3.38	2.69	2.49	2.59	6.22	5.72	5.98
6.5	Zn	3.42	3.23	3.33	2.83	2.61	2.72	6.25	5.84	6.05
	HA	3.70	3.35	3.52	2.89	2.69	2.79	6.59	6.04	6.32
	Zn +HA	3.79	3.41	3.60	2.92	2.71	2.81	6.71	6.12	6.42
	Mean	3.63	3.33	3.48	2.88	2.67	2.77	6.51	6.00	6.26
13	Zn	3.85	3.43	3.64	2.87	2.67	2.77	6.72	6.10	6.41
	HA	4.01	3.53	3.77	2.91	2.71	2.81	6.92	6.24	6.58
	Zn +HA	4.26	3.80	4.03	2.94	2.74	2.84	7.20	6.54	6.87
	Mean	4.04	3.58	3.81	2.91	2.71	2.81	6.95	6.29	6.62
Grand Mean (bio)		3.73 a	3.38 b		2.83	2.62		6.56	6.00	
F -test		P: ns	F: ns	B: **	P: ns	F: ns	B: ns	P: ns	F: ns	B: ns
		PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns
			PxFxb: ns			PxFxb: ns			PxFxb: ns	
Mean of foliar treatment (F)										
Zn			3.40			2.67			6.08	
HA			3.56			2.73			6.30	
Zn +HA			3.72			2.76			6.48	

Yield efficiency and harvest index

Values of yield efficiency as affected by P fertilization, Zn and HA whether applied solely or in combinations are shown in Table 5. Grain yield efficiency, which is the ratio of grain yield to straw yield at maturity varied between 69.0% - 82.7%. The plants treated with 6.5 kg P fed.$^{-1}$ + foliar spray with Zn + bio gave the highest yield efficiency giving increases of 19.9% as compared to the plants treated with 13 kg P fed.$^{-1}$ + foliar spray with HA + Zn under bio inoculation which gave lowest value. These results are in a harmony with those obtained by Helmy, (2008) who found that the highest yield efficiency value (94.2%) was obtained due to (phosphorus dissolving bacteria, PDB + 7.0 kg P fed.$^{-1}$ + 1.5 kg Zn fed.$^{-1}$).

Harvest index of wheat increased due to the addition treatments and varied between, 40.8% - 45.3%. Harvest index of plants treated with 6.5 kg P fed.$^{-1}$ + foliar spray with Zn + bio was the highest giving increase of 11.0%. The favourable effect of mineral P-fertilization is due to P being essential for plant growth. Therefore, the increase in P-fertilization rate would increase metabolic processes and physiological activities rate, and thus, increased yield with good quality of grains would occur (Russel, 1973).

Total chlorophyll and Carbohydrates

Data presented in Tables 5 and 6 shows that total chlorophyll and carbohydrate contents were significantly increased due to the addition of P fertilization, foliar spray with Zn and HA as well as biofertilization. The highest values of total chlorophyll and carbohydrate contents (38.8 mg/g F.W and 67.9 %) were obtained under application of 13 kg P fed.$^{-1}$ + (Zn + HA) + biofertilization causing 49.8% and 12.6% increases.

As for P fertilization, the main effect followed the order: 13 kg P fed.$^{-1}$ > 6.5 kg P fed.$^{-1}$ >0 kg P fed.$^{-1}$ for chlorophyll content and 13 kg P fed.$^{-1}$ ≥ 6.5 kg P fed.$^{-1}$ > 0 kg P fed.$^{-1}$ for carbohydrate content. On the other hand, the main effect of foliar treatments of Zn and HA shows descending order as follows: Zn + HA > HA > Zn for total chlorophyll and carbohydrate contents. The main effects of bio inoculation with *Bacillus megatherium* sp. showed a descending order of with bio > without bio. The increased amount of chlorophyll content in leaves indicates the photosynthetic efficiency, thus it can be used as one of the criteria for quantifying photosynthetic rate which occurred with biofertilization (Kowsar, *et al.*, 2014). Zinc is essential for the activity of various enzymes and delay the senescence of wheat plants through increasing the levels of indol acetic acid (IAA) and chlorophyll, and is associated with carbohydrate metabolism and protein synthesis (Marrschner, 1998).

Grain protein content and protein yield

It can be seen from results presented in Table 6 that the grain protein content and grain protein yield of wheat increased as affected by the treatments of P fertilization and foliar spray with Zn and HA as well as biofertilization and their combinations. Helmy, (2008) reported that protein content in wheat grain increased with high rates of mineral P fertilizer up to 14 kg P $fed.^{-1}$ + 1.5 kg Zn $fed.^{-1}$ + biofertilization. The favourable effect of mineral P-fertilization is attributed to its role as one of the most important constituents of all proteins and nucleic acids, and hence protoplasm and chlorophyll which led to an increase in metabolic processes and physiological activities necessary for more plant organs formation, more dry matter accumulation and enhancing the grain hilling rate, which finally increase the amount of protein in grain (Wortman *et al.*, 2011). Moreover, the effect of phosphate dissolving bacteria in dissolving insoluble P as well as secreting promoting growth substances, which would increase plant growth and grain yield. The phosphate dissolving bacteria utilize organic compounds as carbon and energy source and produce organic acids, which can solubilize insoluble inorganic phosphate. According to Metwally (2000), PDB could produce growth promoting substances, including auxins, gibberelines and cytokinins, which increase plant growth. These results are in a harmony with those obtained by Abedi *et al.* (2010) and Rana *et al.* (2012).

The highest values of protein content (17.4 and 506 kg $fed.^{-1}$) were obtained due to the treatment of 13 kg P $fed.^{-1}$ + (Zn + HA) + bio fertilization representing increase percentage of 31.8% and 57.6%, respectively. The treatments and its interaction had no significant effect on increasing protein content and protein yield

Table (5) harvest index, yield efficiency and total chlorophyll of wheat as affected by P and bio addition as well as foliar treatments

P application rate, kg P $fed.^{-1}$ (P)	Foliar treatment (F)	Bio addition (b) With	Without	Mean	With	Without	Mean	With	Without	Mean
		Yield efficiency			Harvest Index (HI)			Total chlorophyll (mg/g F.W.)		
0	Zn	77.6	78.9	78.3	43.7	44.1	43.8	27.5	25.9	26.7
	HA	76.3	76.9	76.5	43.3	43.5	43.3	30.8	27.0	28.9
	Zn +HA	75.1	75.4	75.0	42.9	43.0	42.9	35.1	27.9	31.5
	Mean	76.2	77.1	76.6	43.2	43.5	43.3	31.1	26.9	29.0 c
6.5	Zn	82.7	80.8	81.7	45.3	44.7	45.0	30.2	26.4	28.3
	HA	78.1	80.3	79.3	43.9	44.5	44.1	33.0	27.8	30.4
	Zn +HA	77.0	79.5	78.1	43.5	44.3	43.8	35.9	28.2	32.1
	Mean	79.3	80.2	79.7	44.2	44.5	44.3	33.1	27.5	30.3 b
13	Zn	74.5	77.8	76.1	42.7	43.8	43.2	34.9	27.2	31.0
	HA	72.6	76.8	74.5	42.1	43.4	42.7	35.9	27.9	31.9
	Zn +HA	69.0	72.1	70.5	40.8	41.9	41.3	38.8	29.3	34.1
	Mean	72.0	75.7	73.8	41.9	43.1	42.4	36.5	28.1	32.3 a
Grand Mean (bio)		75.8	77.7		43.1	43.7		33.6 a	27.5 b	
F -test		P: ns	F: ns	B: ns	P: ns	F: ns	B: ns	P: **	F: **	B: **
		PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: **	Fxb: **
			PxFxb: ns			PxFxb: ns			PxFxb: ns	
Mean of foliar treatment (F)										
Zn			44.0			78.7			28.7 c	
HA			43.4			76.8			30.4 b	
Zn +HA			42.7			74.5			32.5 a	

Yield efficiency: (grain yield / straw yield) x 100.

Harvest Index (HI): (grain yield / biological yield) x100

Table (6) Carbohydrate, protein (%) and protein yield (kg $fed.^{-1}$) of wheat grain as affected by P and bio addition as well as foliar treatments

P application rate, kg P $fed.^{-1}$ (P)	Foliar treatment (F)	Bio addition (b) With	Without	Mean	With	Without	Mean	With	Without	Mean
		Carbohydrate (%)			Protein (%)			Protein yield (kg fed^{-1})		
0	Zn	61.8	60.3	61.1	14.2	13.2	13.7	373	321	347
	HA	63.2	61.7	62.4	15.3	13.5	14.4	413	338	375
	Zn +HA	65.5	62.7	64.1	16.2	13.7	14.9	445	348	396
	Mean	63.5	61.6	62.5 b	15.2	13.5	14.3	411	335	373
6.5	Zn	62.7	61.2	61.9	15.1	14.2	14.6	426	370	398
	HA	64.9	62.9	63.9	16.3	14.6	15.4	471	392	432
	Zn +HA	67.8	63.8	65.8	17.0	14.9	15.9	496	403	450
	Mean	65.1	62.6	63.9 a	16.1	14.5	15.3	465	388	427
13	Zn	63.5	62.4	63.0	16.4	14.8	15.6	470	394	432
	HA	64.8	63.9	64.3	17.0	14.9	16.0	488	399	443
	Zn +HA	67.9	64.6	66.2	17.4	15.4	16.4	506	418	462
	Mean	65.4	63.6	64.5 a	16.9	15.0	16.0	488	404	446
Grand Mean (bio)		64.7 a	62.6 b		16.1 a	14.3 b		455	376	
F -test		P: **	F: **	B: **	P: ns	F: ns	B: *	P: ns	F: ns	B: ns
		PxF: ns	Pxb: ns	Fxb: **	PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns
			PxFxb: ns			PxFxb: ns			PxFxb: ns	
Mean of foliar treatment (F)										
Zn			62.0 c			14.6			392	
HA			63.5 b			15.3			417	
Zn +HA			65.4 a			15.8			436	

Macronutrients content and uptake

It can be seen from results presented in Tables 7 and 8 that N, P and K content and uptake by wheat grains increased owing to application of P fertilization and foliar spray with Zn and HA in presence or absence of biofertilization.

Nitrogen content and uptake

Results given in Table 7 reflect in significant increases in N content and uptake as affected by application of the treatments for wheat grains while, the effect of foliar spray with Zn and HA was significant in increasing grain N content only. The highest increase in N content and uptake (2.78% and 81.7 kg $fed.^{-1}$, respectively) was recorded in the plants treated with 13 kg P fed.$^{-1}$ + (Zn + HA) under bio inoculation with PDB. The positive effect of the PDB on nitrogen uptake can be attributed to

Table (7) N, P and K content (%) of wheat grain as affected by P and bio addition as well as foliar treatments

P application rate, kg P fed.$^{-1}$ (P)	Foliar treatment (F)	Bio addition (b)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
		N content			P content			K content		
0	Zn	2.27	2.11	2.19	0.32	0.28	0.30	2.47	1.38	1.92
	HA	2.44	2.16	2.30	0.44	0.31	0.38	2.70	1.53	2.11
	Zn +HA	2.59	2.19	2.39	0.48	0.34	0.41	2.78	1.60	2.19
	Mean	2.43	2.15	2.29	0.41	0.31	0.36 b	2.65	1.50	2.08 b
6.5	Zn	2.41	2.27	2.34	0.38	0.33	0.35	2.60	1.41	2.01
	HA	2.61	2.33	2.47	0.52	0.37	0.44	2.76	1.60	2.18
	Zn +HA	2.72	2.38	2.55	0.56	0.41	0.48	2.88	1.65	2.26
	Mean	2.58	2.32	2.45	0.48	0.37	0.43 a	2.75	1.55	2.15 b
13	Zn	2.62	2.36	2.49	0.41	0.36	0.38	2.64	1.94	2.29
	HA	2.72	2.39	2.56	0.61	0.41	0.51	2.81	2.11	2.46
	Zn +HA	2.78	2.47	2.63	0.69	0.44	0.57	2.94	2.18	2.56
	Mean	2.70	2.41	2.56	0.57	0.40	0.48 a	2.80	2.08	2.44 a
Grand Mean (bio)		2.57 a	2.30 b		0.49 a	0.36 b		2.73 a	1.71 b	
F -test		P: ns	F: *	B: ns	P: **	F: **	B: **	P: **	F: *	B: **
		PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: *	Fxb: ns
			PxFxb: ns			PxFxb: ns			PxFxb: ns	
Mean of foliar treatment (F)										
Zn			2.34			0.34 b			2.07 b	
HA			2.44			0.44 a			2.25 a	
Zn +HA			2.52			0.49 a			2.34 a	

Phosphorus content and uptake

Phosphorus content and uptake in wheat grains increased significantly as a result of P fertilization, foliar spray with Zn and HA as well as bio fertilization. The highest P content and uptake (0.69% and 20.3 kg fed.$^{-1}$, respectively) were given by13 kg P fed.$^{-1}$ + (Zn + HA) under bio inoculation with PDB with an increases of 146% and 199%. This reflects the important role of phosphorus dissolving bacteria in releasing P from in soluble forms beside their stimulating effect (Abd El-Rasoul *et al.,* 2003). These bacteria produce growth promoting substances which could influence the plant growth that roots become able to explore more soil and more zones, where phosphate ions were chemically liberated from rock phosphate fertilizer and making P more available to the crop, (Metwally, 2000). increasing plant growth due to increasing nutrients availability in the soil, and the promoting effect to the N supply.

These findings are in agreement with those reported by Ibrahim *et al.* (2008) and Helmy *et al.* (2013).

Effect of the treatments in increasing P-content followed the order of, 13 kg P fed.$^{-1}$ ≥ 6.5 kg P fed.$^{-1}$ > 0 kg P fed.$^{-1}$ for P fertilization, Zn + HA ≥ HA > Zn for foliar spray treatments and with bio > without bio inoculation representing an increases of 33.3% and 11.6% for P fertilization; 44.1% and 11.4% for foliar spray and 36.1% for bio fertilization, respectively. As for P-uptake, the pattern was: 13 kg P fed.$^{-1}$ ≥ 6.5 kg P fed.$^{-1}$ ≥ 0 kg P fed.$^{-1}$ for P fertilization and bio > without bio. Increases were 45.9% and 26.9% for P fertilization and 47.2% for bio fertilization, respectively.

Table (8) N, P and K uptake (Kg fed.$^{-1}$) of wheat as affected by P and bio addition as well as foliar treatments

P application rate, kg P fed.$^{-1}$ (P)	Foliar treatment (F)	Bio addition (b)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
		N-uptake			P-uptake			K-uptake		
0	Zn	59.7	51.3	55.5	8.42	6.80	7.61	65.0	33.5	49.3
	HA	66.1	54.0	60.1	11.9	7.75	9.83	73.2	38.3	55.8
	Zn +HA	71.2	55.6	63.4	13.2	8.64	10.9	76.5	40.6	58.6
	Mean	65.7	53.6	59.7	11.2	7.72	9.46 b	71.6	37.5	54.6
6.5	Zn	68.2	59.2	63.7	10.8	8.61	9.71	73.6	36.8	55.2
	HA	75.4	62.7	69.1	15.0	10.0	12.5	79.8	43.0	61.4
	Zn +HA	79.4	64.5	72.0	16.4	11.1	13.8	84.1	44.7	64.4
	Mean	74.3	62.1	68.3	14.1	9.90	12.0ab	79.2	41.5	60.3
13	Zn	75.2	63.0	69.1	11.8	9.61	10.7	75.8	51.8	63.8
	HA	79.2	64.8	72.0	17.8	11.1	14.5	81.8	57.2	69.5
	Zn +HA	81.7	67.7	74.7	20.3	12.1	16.2	86.4	59.7	73.1
	Mean	78.7	65.2	71.9	16.6	10.9	13.8 a	81.3	56.2	68.8
Grand Mean (bio)		72.9	60.3		14.0 a	9.51 b		77.4 a	45.1 b	
F -test		P: ns	F: ns	B: ns	P: **	F: ns	B: **	P: ns	F: ns	B: **
		PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns
			PxFxb: ns			PxFxb: ns			PxFxb: ns	
Mean of foliar treatment (F)										
Zn			62.8			9.34			56.1	
HA			67.1			12.3			62.2	
Zn +HA			70.0			13.6			65.4	

Phosphorus use efficiency (PUE)

Data of PUE parameter, which indicates yield produced by a unit weight of fertilizer phosphorus and also called nutrient to grain ratio, are shown in Table 9. The values of PUE markedly decreased as the phosphorus addition rates increased. P fertilization at low rate 6.5 kg P fed.$^{-1}$ was more efficient than 13 kg P fed.$^{-1}$ which indicates that no more P fertilizer is needed to raise the efficiency of P fertilization. This confirms that a reduction in P fertilization can be made. The highest PUE was obtained due to addition of 6.5 kg P fed.$^{-1}$ + (Zn + HA) especially, with bio inoculant of *Bacillus megatherium* which increased the efficiency use of phosphorus fertilization by 98.7% as compared with the high addition rate 13 kg P fed.$^{-1}$ + Bio +(Zn + HA).

Phosphorus agronomic efficiency (PAE)

Agronomic efficiency (kg grain/ kg P applied) gave similar trend like the aforementioned parameter (PUE). Fageria *et al.* (2011) stated that phosphorus agronomic efficiency decreased with increasing P rate. Greater agronomic efficiency at lower P rate indicates highest P utilization by wheat at a low P rate. The above two traits which behaved similarly, showed that plants absorb more P when it is of low level in the soil especially, under biofertilization with PDB. As the level of P increased the relative absorption of P went on decrease.

Apparent phosphorus recovery (APR)

The apparent phosphorus recovery (APR) parameter indicates the proportion of fertilizer P recovered by the plants. As shown in Table 9, mean of APR was greatest when 6.5 kg P $fed.^{-1}$ was added in combination with bio inoculation by *Bacillus megaterium* var phosphaticum compared to the other rate of 13.kg P fed^{-1} and gave 44.5% recovery. This means that application of a low rate of P caused an enhancing effect on plant growth through causing the roots to explore a greater soil volume and absorb more P from the soil. These results are in agreement with those obtained by Sweeney *et al.* (2000) and Fageria *et al.* (2011). As for the individual effect of treatments highest APR value (54.6 %) was obtained owing to treatment of 13 kg P fed^{-1} + (Zn +HA) under bio inoculation.

Potassium content and uptake.

K content and uptake.

The results presented in Tables 7 and 8 show that application of different P fertilization rates, foliar spray with Zn and HA as well as bio inoculation with PDB significantly increased K content. El-Kabbany and Darwish, (2002) reported that application of P increased K content in wheat grains. Sobh, *et al*. (2000) stressed the importance of PDB in increasing uptake of nutrients by plants. The marked effect of Zn in increasing K-uptake is a reflection of the essential role of Zn for the activity of various enzymes caused delaying the senescence of wheat plants through increasing the levels of indole acetic acid (IAA) and chlorophyll, (Marrschner, 1998).

The highest K content and uptake of grains (2.94% and 86.4 kg fed^{-1}) were obtained due to the addition of 13 kg P fed^{-1} + (Zn + HA) with PDB causing 113% and 158% increases, respectively.

Regarding the effect of bio fertilization treatments, data show that inoculation with PDB gave grater K content and uptake as compared with without inoculation with increases of 59.6% and 71.6%, respectively.

Micronutrients content and uptake

Tables 9 and 10 clarify the content and uptake of Fe, Mn and Zn (mg/kg)in wheat grains as affected by the tested treatment. Fe, Mn and Zn contents were increased significantly and the treatment of 13 kg P fed^{-1} + (Zn + HA) with PDB was superior to the other treatments. This promoting effect could be related to the P supplementary effect of PDB to plants due to the role of these bacteria in improving the availability of soil elements (Table 2) which were likely attributed to several reasons: 1) Enhancing the chelation of metal ions by humic acid, organic legands and / or other organic function groups which may promote the mobility of metal from solid to liquid phase in the soil environment and 2) Lowering the redox statues of iron and manganese, leading to reduction of higher Fe^{3+} & Mn^{4+} to Fe^{2+} and Mn^{2+} and / or transformation of insoluble chelated forms into more soluble ions, Paramasivam et al (2005).

Table 9. PUE, PAE (kg kg^{-1} P) and APR (%) of wheat grains as influenced by P fertilization, foliar spray with Zn and HA as well as bio fertilization

P application rate, kg P $fed.^{-1}$ (P)	Foliar treatment (F)	Bio addition (b)					
		With	Without	With	Without	With	Without
		Phosphorus use efficiency (PUE)		Phosphorus agronomic efficiency (PAE)		Apparent phosphorus recovery (APR)	
0	Zn	--	--	--	--	--	--
	HA	--	--	--	--	--	--
	Zn +HA	--	--	--	--	--	--
6.5	Zn	435	402	3.08	2.77	36.6	27.8
	HA	445	414	2.77	2.92	47.7	34.6
	Zn +HA	449	417	2.62	2.62	49.2	37.8
	Mean	443	411	2.82	2.77	44.5	33.4
13	Zn	221	205	1.85	1.85	26.0	21.6
	HA	224	208	1.54	1.62	45.4	25.8
	Zn +HA	226	211	1.46	1.54	54.6	26.6
	Mean	224	208	1.62	1.67	42.0	24.6

Table (10) Fe, Mn and Zn content (mg/kg) of wheat grain as affected by P and bio addition as well as foliar treatments

P application rate, kg P $fed.^{-1}$ (P)	Foliar treatment (F)	Bio addition (b)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
		Fe content			Mn content			Zn content		
0	Zn	71.4	69.4	70.4	41.6	39.9	40.7	25.0	20.4	22.7
	HA	81.8	72.0	76.9	42.7	40.6	41.7	26.4	21.7	24.0
	Zn +HA	83.1	73.6	78.3	43.0	41.0	42.0	27.2	22.0	24.6
	Mean	78.8	71.6	75.2 b	42.4	40.5	41.5 c	26.2	21.4	23.8 b
6.5	Zn	72.9	70.6	71.8	44.8	40.7	42.7	25.5	21.0	23.2
	HA	83.2	72.8	78.0	45.7	41.5	43.6	27.2	22.4	24.8
	Zn +HA	84.4	74.4	79.4	46.1	42.5	44.3	28.4	22.8	25.6
	Mean	80.2	72.6	76.4 a	45.5	41.6	43.5 b	27.1	22.0	24.6 a
13	Zn	73.0	72.6	72.8	47.6	42.4	45.0	25.9	21.9	23.9
	HA	83.7	73.6	78.7	48.1	43.8	46.0	27.6	22.5	25.0
	Zn +HA	84.5	75.1	79.8	48.2	44.0	46.1	28.8	23.3	26.0
	Mean	80.4	73.8	77.1 a	48.0	43.4	45.7 a	27.4	22.6	25.0 a
Grand Mean (bio)		79.8 a	72.7 b		45.3 a	41.8 b		26.9 a	22.0 b	
F -test		P: **	F: **	B: **	P: **	F: **	B: **	P: **	F: **	B: **
		PxF: ns	Pxb: ns	Fxb: **	PxF: ns	Pxb: **	Fxb: ns	PxF: ns	Pxb: ns	Fxb: ns
			PxFxb: ns			PxFxb: ns			PxFxb: ns	
Mean of foliar treatment (F)										
Zn			71.7 c			42.8 b			23.3 c	
HA			77.8 b			43.7 a			24.6 b	
Zn +HA			79.2 a			44.1 a			25.4 a	

The main effect of P fertilization followed the sequence: 13 kg P fed^{-1} > 6.5 kg P fed^{-1} > 0 kg P fed^{-1} for Fe and Zn content as well as 13 kg P fed^{-1} > 6.5 kg P fed^{-1} > 0 kg P fed^{-1} for Mn content. The pattern was: Zn + HA > HA > Zn for Fe and Zn content as well as Zn + HA ≥ HA > Zn for Mn content as affected by foliar spray treatment. The main effect regarding bio fertilization shows with bio > without bio; with respective increases of 9.77%, 8.37% and 22.3% for Fe, Mn and Zn content, respectively.

As for Fe, Mn and Zn uptake, the increases were in significant due to P fertilization and foliar spray with Zn and HA while, the bio inoculation effect was significant. Highest Fe, Mn and Zn uptake of (248, 142 and 84.6 kg fed.$^{-1}$, respectively) due to the treatment of 13 kg P fed^{-1} + (Zn + HA) with PDB with an increases of 46.7%, 46.5% and 70.2%, respectively.

Table (11) Fe, Mn and Zn uptake (g fed.$^{-1}$) of wheat as affected by P and bio addition as well as foliar treatments

P application rate, kg P fed.$^{-1}$ (P)	Foliar treatment (F)	Bio addition (b)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
		Fe-uptake			Mn-uptake			Zn-uptake		
0	Zn	188	169	179	109	96.9	103	65.8	49.7	57.8
	HA	222	180	201	116	101	109	71.4	54.2	62.8
	Zn +HA	228	187	208	118	104	111	74.8	55.8	65.3
	Mean	213	179	196	114	101	108	70.7	53.2	62.0
6.5	Zn	206	184	195	127	106	117	72.2	54.7	63.5
	HA	240	196	218	132	112	122	78.7	60.3	69.5
	Zn +HA	246	202	224	135	115	125	83.0	61.7	72.4
	Mean	231	194	212	131	111	121	78.0	58.9	68.4
13	Zn	210	194	202	137	113	125	74.4	58.5	66.5
	HA	244	199	222	140	119	130	80.4	60.9	70.7
	Zn +HA	248	206	227	142	121	132	84.6	63.9	74.3
	Mean	234	200	217	140	118	129	79.8	61.1	70.5
Grand Mean (bio)		226 a	191 b		128	110		76.1 a	57.7 b	
F -test		P: ns PxF: ns	F: ns Pxb: ns PxFxb: ns	B: ** Fxb: ns	P: ns PxF: ns	F: ns Pxb: ns PxFxb: ns	B: * Fxb: ns	P: ns PxF: ns	F: ns Pxb: ns PxFxb: ns	B: ** Fxb: ns
Mean of foliar treatment (F)										
Zn			192			115			62.6	
HA			214			120			67.7	
Zn +HA			220			123			70.6	

SAMMARY

In calcareous soils there are many factors that affect P and Zn availability, such as high pH, high $CaCO_3$ and low organic matter (Alloway, 2008). The lower bioavailability of P and Zn in soil directly affects grain P concentration. (Cakmak, 2002).

The addition of humic acid with P produced significant and economical wheat yield with maximum nutrient accumulation and increased crop productivity by increased nutrient uptake. Phosphorus is of high mobility in plants and when deficient it may be translocate from old plant tissue to young active growing areas.

On the other hand, zinc absorption by plants involves a number of steps (Lasat *et al.*, 1998). First, adequate Zn bioavailability was necessary in the rhizoshpere. There are two pathways for Zn to move from the soil solution to the rhizosphere, mass flow and diffusion. In calcareous soils, diffusion is the dominant pathway for Zn to reach root zones. However, diffusion of Zn in calcareous soils is low due to low soil moisture, low organic matter and high pH (Alloway, 2008). Consequently, there is not sufficient available Zn reaching the rhizosphere. However, the combined of humic acids with P or Zn were more effective on translocation, agronomic efficiency (AE) and apparent nutrient recovery (ANR) for wheat plants which might be due to the availability of soil microorganisms to convert the unavailable forms of nutrients elements to available forms (Ali *et al.*, 2011).

CONCLUSION

From the above mentioned results and discussion it can be concluded that, use of phosphorus materials such as super phosphate enriched with Zn and humic acids and inoculated with bacteria-dissolving phosphorus, maximizes the benefit from it and increases the released of phosphorus and other nutrients in calcareous soil. Enrichment of humic substances with the macro or micronutrients was more economic and secures not only a waste of money, but also keep the environment clean. Inoculation of wheat grains with bio-fertilizer (*Bacillus megaterium* var. phosphaticum) and foliar spraying with Zn and humic acid enhanced the efficiency use of P fertilization in calcareous soil alleviate the adverse effects of $CaCO_3$ stress on soil and plant and hence increased the wheat productivity and grains quality. Utilization of phosphorus fertilizer decreased to 49 % by integrating biological and low mineral phosphorus fertilizers rate without yield loss.

Increasing the productivity of wheat crop with good grain quality under newly reclaimed calcareous soil conditions of Egypt was achieved not only by using high rates of P-mineral fertilizers, but also by better management of its application to the soil through a moderate level of 6.5 kg P fed.$^{-1}$ and inoculation of grains with PDB. On the other hand, such management will decrease the enormous consumption of chemical P-fertilizers and meanwhile will minimize health and environmental risks which are prospectively fulfilled. Also, it could be consider as a strategy to achieve sustainable agricultural in calcareous soil.

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كفاءة التسميد الفوسفاتي للقمح تحت ظروف الأراضي الجيرية تحت تأثير الرش بالزنك ، حامض الهيوميك والتلقيح الحيوي بـ (***Bacillus megatherium* sp.**)

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تم دراسة تأثير التسميد الحيوي ببكتريا (*Bacillus megatherium* sp.) و الرش بالزنك وحامض الهيوميك علي رفع كفاءة التسميد الفوسفاتي (سوبر فوسفات) بمعدلات مختلفة في الأرض الجيرية بمحطة النوبارية للبحوث الزراعية - محافظة الأسكندرية - جمهورية مصر العربية خلال موسم الشتاء لعامين متتالين هما 2014/2015 و 2015/2016 على نبات القمح صنف سخا 93 و تأثير ذلك علي إنتاجية القمح وجودة الحبوب و أمتصاصه لبعض العناصر الكبري و الصغري وتحسين بعض صفات التربة وقد تم إضافة السوبر فوسفات بمعدلات 0 ، 6.5 و 13 كجم فو للفدان وتم الرش بالزنك وحامض الهيوميك مع التلقيح أو عدم التلقيح بالبكتريا المذيبة للفوسفات. وقد أظهرت النتائج أن هناك زيادة معنوية في محتوى التربة من العناصر الكبري و الصغري الميسرة وذلك بعد الحصاد نتيجة للمعاملات تحت الدراسة. أدي الرش بالزنك و حامض الهيوميك مع التلقيح الحيوي و المعدلات المختلفة من التسميد الفوسفاتي إلي زيادة المحصول ومساهماتة ، وزن 1000 حبة ، المحتوي و الممتص بواسطة الحبوب من عناصر ن ، فو ، بو ، حديد ، منجنيز و زنك (متوسط الموسمين). أدت أيضاً المعاملات تحت الدراسة إلي زيادة الكلوروفيل الكلي و الكربوهيدرات و كذلك البروتين الكلي ومحصولة بواسطة الحبوب. أعلي كفاءة محصولية (82.7 %) ،و دليل الحصاد (44.7%) تم التحصل عليها نتيجة معاملة الإضافة (6.5 كجم فو فدان$^{-1}$ + الرش بالزنك) مع التسميد الحيوي و (6.5 كجم فو فدان$^{-1}$ + الرش بالزنك)، علي التوالي. أنخفضت قيم الفسفور الكلي المستعاد APR, ، و كفاءة الفسفور المستخدم PUE والكفاءة المحصولية للفسفور PAE مع زيادة معدل السوبر فوسفات المستخدم خاصة عند أضافتة مع التسميد الحيوي وقد أعطت النباتات المعاملة بمعدل (6.5 كجم فو فدان$^{-1}$) مع الرش بالزنك أو حامض الهيوميك أو كلاهما معاً في وجود التسميد الحيوي أعلي متوسطات للكفاءات السابقة للفسفور وهو ما يوضح دور التسميد الحيوي في خفض الكمية المستخدمة من التسميد الفوسفاتي المعدني دون خفض الإنتاجية من الشعير معنويا. كانت المعاملة (سوبر فوسفات بمعدل 13 كيلوجرام P فدان$^{-1}$ + الرش بالزنك و حامض الهيوميك معاً) مع التلقيح بالبكتريا المذيبة للفوسفات هي أحسن معاملة مقارنة بباقي المعاملات تحت الدراسة لمعظم القياسات و التقديرات تحت الدراسة للقمح النامي تحت ظروف الأراضي الجيرية و أن كان في أغلبها بدون فرق معنوي مع المعدل المنخفض 13 كيلوجرام P فدان$^{-1}$.