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The integration Effect between Mineral and bio-fertilization on Wheat Production in Soils High in Iron at the New Valley, Egypt

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HIGH Fe content in the New Valley soils hinders the absorption of both P and Zn by plants, which is negatively reflected on crop production. Field experiments were carried out in two successive seasons at two sites under the conditions of the New Valley soils in order to increase wheat yield by the integration between mineral (P and Zn) and bio-fertilization (Mycorrhiza and Azotobacter). The first experiment was carried out at El-Monera (sandy soil), El-Kharaga Oasis, while the second one was carried out at Al-Kaser (clayey soil), El-Dakhlla Oasis. Grains of wheat variety Sakha 93 was cultivated in both locations. Application of mineral P and Zn fertilizers along or with bio-fertilizers increased wheat yield parameters, nutrients content, total antioxidants, and phenols of wheat. Moreover, increasing the application rates of P and Zn decreased Fe and Mn contents in wheat and increased N, K, Zn and Cu contents in the straw and grains. The most effective treatment for yield, nutrients and biochemical contents was by applying P at a rate of 60 kg fed^{-1} (1 fed = 4200 m^2) in clay soils and 80 kg fed^{-1} in sandy soils with spraying Zn at a rate of 250 mg L^{-1} with the Mycorrhizae and Azotobacter applications. This treatment could achieve 5.45 and 2.21 Mg fed^{-1} for straw and grains respectively in the sandy soil while, 9.5 and 4.16 Mg fed^{-1} in the clay soil. This study revealed that combining bio- and mineral P and Zn fertilizers application could decrease the negative effect of high Fe content in soils and increased wheat production.

Keywords: New Valley soils, P, Zn, Mycorrhizae, Azotobacter, Iron content, Wheat

Several studies have reported that application of organic and/or bio-fertilizers along with mineral fertilizers increase yield and biochemical components of wheat. For example Tayebeh et al. (2010) found that the highest wheat grain yield was achieved when applied mineral N fertilizer and compost. Shah *et al.* (2010) reported that the application of N fertilizer as urea with 25% N from farm yard manure or poultry manure or city waste achieved optimum wheat yield and reduced fertilizer cost by 50%. Applications of humic acid with mineral fertilizers achieved highest yield of wheat as well (Tahir *et al.,* 2011). Fadl-Allah e*t al.* (2010) demonstrated that the use of bio-fertilizer with mineral N fertilizer optimized wheat production and nutrient contents. Singhal *et al.* (2012) reported that combining the application of mineral P along with mycorrhizae could achieve the highest yield of wheat. Castillo *et al.* (2012) demonstrated the beneficial effect of mycorrhizae on P uptake in wheat, barley and oats. Hasanpour *et al.* (2012) found that inoculation of mycorrhizae and/or Azotobacter significantly increased wheat grain yields. In the same respect, Mir *et al.* (2013) demonstrated that mycorrhiza and Azotobacter applications could improve wheat growth and seed production even under drought stress conditions. Moola *et al.* (2014) reported that the highest increase in wheat productivity, grain quality and nutrient uptake was recorded with the application of green manure + farmyard manure + bio-fertilizers. Barker and Tagu (2002) evidenced the beneficial effect of mycorrhizae by producing phyto-hormones like auxins and cytokines, which promotes plant growth and consequently the yield.

Soils with high content in Fe retard the uptake of other nutrients, like P and Zn. For instance, Wandruszka (2006) revealed that high Fe content in soil decreases P content in the leaf, stem and root of wheat. However, high P additions could reduce the accumulation of Fe into wheat leaves (Handreck, 2006). Furthermore, Li *et al.* (2007) reported that higher available soil P significantly decreased crop micronutrients, possibly because of their precipitation as metal phosphates. Hasina *et al.* (2011) stated that the growth performance of wheat was highly influenced by the application of two times spray of 0.5 % N, 0.5% K and 0.5% Zn solutions. Moghadam *et al.* (2012) reported that the wheat yield increased with foliar application of B and Zn. Niemi et al (2014) stated that application of Fe at high levels decreased the concentrations of Zn, Cu and Mn. The New Valley soils are high in Fe content causes severe problems regarding uptake of P and Zn by plants. Therefore, the present study was undertaken to study the possibility of improving yield and biochemical components of wheat under the New Valley conditions by integrating mineral P fertilizers, foliar spray of Zn and bio-fertilizers in order to overcome the problems caused by high Fe content.

Materials and Methods

Field experiments in two successive seasons (2012/2013-2013/2014) were carried out in split-split plot design with three replications in two sites at the New Valley, southern Egypt. The first site was at El-Kharaga Oasis (El-Monera villages) and the second was at El-Dakhlla Oasis (Al-Kaser). The first experiment (sandy soil) located at 27º 47.7'42" N and 30º 24.7' 56" E, while the second one (clay soil) located at 28º 20.6' 24" N and 31º 59.9' 58" E. The plot area was 90 m^2 at Al-Kaser location with flood irrigation system, while was 64 m^2 at the El-Monera with sprinkler irrigation system. The used cultivar in each site was Sakha 93, which is popular in these sites. The bio-fertilizer treatments constituted the main plots, Zn foliar application in sub plots and P treatments in sub-sub plots.

Table 1 shows the analytical data of soil and water at the experimental sites. A basal dose of N as ammonium nitrate and K as potassium sulfate was applied as 70 and 50 kg fed^{-1}, respectively in sandy soil, where 50 and 25 kg fed^{-1} was applied in clay soil. The fertilizers of N and K were divided to three equal split doses and applied at 20, 60, and 90 days after sowing following the general recommendation in the study area. Farm yard manure applied at rate 25 and 15 m^3 fed^{-1} for sandy and clay soils respectively. Four rates of P as calcium superphosphate and three treatments (without Bio_0 and with Bio_1,Bio_2) of bio-fertilizers (Microheyzae and Azotobacter) were applied as shown in Table 2. Phosphorus fertilizer was mixed with the soil during seedbed preparation. Foliar Zn was applied as zinc sulphate at two rates (0, 250 mg L^{-1}) at three events during the growing season.

One rate of foliar NPK fertilizers divided to three doses and applied at the same times of Zn applications for all studied treatments except control as following; first $dose_1$ [1kg of (20/20/20)]/200L water, second $dose_2$ [0.75 kg of (20/20/20) + 0.25 kg of (0/80/0) + 0.25kg (0/0/50)]/200L water and third $dose_3$ [0.5kg of (20/20/20) + 0.35kg of (0/80/0) + 0.4 kg (0/0/50)]/200L water.

TABLE 1. Some physical and chemical properties of the soil and chemical analyses of the irrigation water at the experimental sites.

Depth cm	pH 1:1	EC dS m^{-1}	O.M	$CaCO_3$	Sand	Silt	Clay	CEC meq/100g	Texture
			%						
Sandy soil									
0-30	7.93	0.92	0.78	1.36	87.52	8.23	4.25	3.4	Sandy
30-60	7.68	0.65	0.39	1.16	85.37	9.45	5.18	4.3	Sandy
Clay soil									
0-30	8.48	2.43	1.23	1.94	21.46	25.31	53.23	29.6	Clayey
30-60	8.25	1.56	0.83	1.76	19.51	26.37	54.12	30.7	Clayey
Soluble cations and anions (meq L^{-1}), amount of total antioxidants and total phenols									
Sandy soil									
	Na	K	Ca	Mg	HCO_3	Cl	SO_4	T. phenol**	T.A.A*
0-30	3.42	1.22	1.98	2.58	1.85	3.99	3.36	323	85
30-60	2.51	0.95	1.32	1.74	1.20	3.20	2.12	186	54
Clay soil									
0-30	12.7	1.67	5.20	4.74	2.32	16.36	5.62	572	157
30-60	8.75	0.97	3.89	1.99	1.88	9.86	3.86	395	125
Available nutrients (mg kg^{-1})									
	N	P	K	Fe	Mn	Zn	Cu		
Sandy soil									
0-30	16.3	1.65	43	17.89	6.52	0.25	0.12		
30-60	14.2	1.16	55	19.93	8.54	0.28	0.13		
Clay soil									
0-30	39.6	2.93	246	31.9	12.9	0.58	0.32		
30-60	32.4	2.25	283	38.4	18.4	0.47	0.28		
Some chemical properties of irrigation water									
Soils	pH	EC	Na	K	Ca	Mg	HCO_3	Cl	SO_4
		dS/m	me/L						
Sandy	7.83	0.45	1.87	0.63	0.93	1.11	0.98	3.12	0.43
Clay	8.07	0.74	2.70	0.95	1.59	2.16	1.95	4.65	0.81

*: Total Antioxidants activity, µg of Ascorbic acid/ml extract, **:Total phenol, µmol of Gallic acid/ml extract

TABLE 2. Treatments of P (as P_2O_5), Zn and bio-fertilizers in wheat at the experimental sites.

Fertilizers	Sandy soil	Clay soil
	kg fed^{-1}	
Control	0	0
P_1	15	20
P_2	30	40
P_3	45	60
P_4	60	80
Zn	Zn (0, 250 mg L^{-1}) added as foliar spray	
Bio-fertilizer$_1$	Mycorrhizae (8 kg fed^{-1}) added to soil with P	
Bio-fertilizer$_2$	Mycorrhizae + (Azotobacater , 1% added as foliar spray with Zn)	

Azotobacter isolate (H-A) was used in the experiments. It has N_2-ase activity, 456µl$C_2H_4h^{-1}l^{-1}$, phosphate solubilization, 1.54 mg P l^{-1}, indole-3-acetic acid (IAA) was 0.16, gibberellic acid (GA3) was 3.2, Cytokinine was 26 µg ml^{-1}, enzyme production: Amylase was (+++), Protease was (+), Protease was (+) and Lipase was (+). Fresh liquid of *Azotobacter* culture of 48 hr old at a rate of 10^8CFU/ ml was added as foliar to plants at one rate (2L/200L) with Zn Foliar application.

For mycorrhiza, spores were isolated from soil pre-inoculated with mycorrhiza (*Glomusmacrocarpium*) by the wet-sieving and decantation method was used as described by Gerdeman and Nicolson (1963). The mycorrhiza was mixed with pure clay and used to inoculate the experiment plots with one rate (8 kg fed^{-1}) with seedbed preparation. Phosphate dissolving bacteria, (PDB) were Arbuscular Mycorrhizal Fungi (AMF), Bacillus sp and Pseudomonas fluorescens which achieved maximum yield when applied with the recommended dose of superphosphate (Neetu *et al.*, 2012). The densities of azotobacter in the phyllosphere were determined by cutting 1 cm^2 of leaf of each plant and transferred to bottles containing 100 ml sterilized water and shaken vigorously for 15 minutes (Mikhail, 2002) then was analyzed by using modified Ashby's medium. Microbiological analyses of rhizosphere of wheat plants were determined including total microbial counts by plating on Bunt and Rovira agar medium according to Abdel-Hafez (1966). The most probable number (MPN) of azotobacter was determined after incubating the tubes at $28\pm2C^o$ for 7 days on modified Ashby's medium ((Hill, 2000). The rates of CO_2 evolution were determined according to Page *et al*. (1984)

Initial Soil samples were collected from two layers (0-30 and 30-60 cm) for physical and chemical analysis. Plant samples were collected at physiological maturity. Yield components were determined as biological yield, grain weight, straw weight, weight of 1000 grain, weight of spikes/m^2 and number of spikes/m^2 in both seasons. Soil and plant samples were analyzed for macro and micro nutrients according to Cottenie *et al.* (1982). Soil analyses were accomplished according to Page *et al.* (1984) and Klute (1986). Total antioxidants measurements and total phenol in soil and wheat plants were determined according to Rimmer (2009). The analysis of variance (ANOVA) was used to determine the effect of treatments on yield parameters. Least significant differences (LSD) test was used to determine the differences between treatments means at 5% probability level according to Gomez and Gomez (1984).

Results and Discussion

Effect of the integration between mineral and bio-fertilizers on wheat yield

Data presented in Table 3 showed that yield parameters of wheat were markedly affected by P and Zn treatments. Yield parameters of wheat were much higher in the clay soil than in the sandy soil. Increasing the rate of P and Zn increased yield parameters compared with other treatments. The best treatment appears to be P_4+Zn_1. Bio-fertilizers treatments increased yield parameters of wheat when combined with mineral fertilizers. The most effective treatment for wheat yield was (P_4+Zn_1 plus Mycorrhizae + Azotobacter) which achieved 5.45 and 2.21 Mg fed^{-1} for straw and grain, respectively in sandy soil, while 9.5 and 4.16 Mg fed^{-1} were achieved in clay soil.

Foliar Zn application increased yield about 51.9 and 57.8% above control treatment for straw and grains respectively in sandy soil, while 52.9 and 49.8% in clay soil. Yield components of wheat were increased with increasing P soil applications rates where the highest increases values at P_4(superior treatment) while the lowest increases values was at P_1 treatment in both studied soils. Straw and grain of wheat under superior treatment (P_4+Zn_1 plus Mycorrhizae +Azotobacter) were higher than control about 56.3 and 63% for straw and grains respectively in sandy soil while, 55.2 and 52.4% in the clay soil.

Mycorrhizae had higher increase in grains yield than Azotobacter, while Azotobacter had higher straw yield than Mycorrhizae. Combining both bio-fertilizers sources had higher significantly values of grains and straw yield than the individual sources.

These results could be due to that application of mineral and bio-fertilizers increasing available nutrients contents in the soil and consequently enhancing plant growth and yield. Mycorrhizae application increased available P in soil because it dissolves soil P, while Azotobacter application increased available N in soil since it is fixing N from the air. Moreover, bio-fertilizers produce hormones like auxins and cytokines which regulating plant growth and increase plant productivity. These facts about bio-fertilizers are barreled with Barker and Tagu (2002); Castillo *et al.* (2012); Amanullah *et al*. (2012); Kowsar *et al.*, (2014); and Garshasbi *et al.* (2014), where mineral fertilizers effect agreed with Hasina et al.,(2011); Moghadam et al.,(2012); Abbas *et al.* (2013); and Ehsan *et al.* (2014)

TABLE 3 . Effect of mineral and bio-fertilizers on wheat yield parameters in the studied soil.

Treatments			Straw	Grain	W. 1000 Grain	W. Spikes	Straw	Grain	W. 1000 Grain	W. Spikes
			Ton/fed		gm	kg/m^2	Ton/fed		Gm	kg/m^2
			Sandy soil				Clay soil			
Mineral fertilizer										
Bio_0	Zn_0	Control	2.21	0.76	23.9	0.39	3.80	1.80	33.8	0.93
		P_1	3.70	1.33	40.6	0.62	6.65	3.16	57.2	1.50
		P_2	4.18	1.58	48.2	0.71	6.98	3.26	61.5	1.57
		P_3	4.47	1.77	51.9	0.86	7.41	3.52	66.5	1.63
		P_4	4.80	1.95	56.5	0.94	7.75	3.59	70.9	1.73
	Zn_1	P_1	3.92	1.42	43.7	0.66	6.73	3.23	62.9	1.55
		P_2	4.30	1.67	49.8	0.76	7.34	3.29	63.4	1.63
		P_3	4.57	1.87	53.6	0.88	7.64	3.71	69.2	1.69
		P_4	4.81	1.99	60.5	1.04	8.22	3.77	73.0	1.77
Bio-fertilizers (Mycorrhizae)										
Bio_1	Zn_0	Control	2.24	0.78	25.1	0.41	3.83	1.86	35.4	0.99
		P_1	3.81	1.37	42.7	0.66	6.70	3.25	60.1	1.56
		P_2	4.35	1.64	51.1	0.77	7.08	3.39	65.2	1.61
		P_3	4.65	1.88	54.5	0.89	7.90	3.66	69.8	1.73
		P_4	5.04	2.06	60.5	0.98	7.92	3.72	72.6	1.75
	Zn_1	P_1	4.03	1.47	45.9	0.69	6.86	3.33	66.0	1.60
		P_2	4.47	1.74	52.7	0.81	7.98	3.41	67.2	1.68
		P_3	4.75	1.94	56.3	0.94	8.56	3.85	72.7	1.79
		P_4	5.05	2.11	64.7	1.09	8.94	3.96	75.3	1.81
Bio-fertilizers (Mycorrhizae+ Azotobacter)										
Bio_2	Zn_0	Control	2.28	0.80	26.4	0.43	4.15	1.91	40.0	1.03
		P_1	4.04	1.41	44.7	0.70	7.30	3.31	63.1	1.62
		P_2	4.64	1.70	55.1	0.82	7.92	3.52	69.1	1.68
		P_3	4.93	1.95	58.1	0.94	8.67	3.81	73.2	1.79
		P_4	5.39	2.15	64.7	1.04	8.77	3.86	77.6	1.81
	Zn_1	P_1	4.28	1.50	50.0	0.75	7.45	3.40	69.3	1.67
		P_2	4.79	1.87	57.7	0.86	8.68	3.55	71.2	1.78
		P_3	5.04	2.03	61.7	0.99	9.36	4.06	76.3	1.91
		P_4	5.45	2.21	69.2	1.13	9.50	4.16	78.6	1.93
$LSD_{0.05}$ Bio			0.082	0.026	1.23	0.017	0.20	0.048	1.35	0.03
$LSD_{0.05}$ Zn			0.007	0.005	0.19	0.003	0.03	0.008	0.18	0.01
$LSD_{0.05}$ P			0.059	0.027	0.76	0.013	0.10	0.042	0.79	0.02
$LSD_{0.05}$ Biox Zn			0.010	0.007	0.27	0.003	0.04	0.011	0.16	0.01
$LSD_{0.05}$ Bio x P			0.084	0.038	1.08	0.018	0.14	0.059	1.02	0.02
$LSD_{0.05}$ Zn x P			0.103	0.046	1.32	0.022	0.17	0.073	1.37	0.03
$LSD_{0.05}$ 3 factors			0.109	0.049	1.39	0.023	0.18	0.077	1.45	0.03

W: Weight

Effect of the integration between mineral and bio-fertilizers on nutrients uptake by wheat

Regarding the effect of mineral P and Zn on nutrients uptake, Tables 4 and 5 showed that application of P and Zn increased N, P, K, Zn and Cu uptake at harvest. On the other hand, Fe and Mn uptake by wheat have decreased. These results could be due to the antagonistic effect between the absorbed Fe and P.

TABLE 4 . Effect of mineral and bio-fertilizers on wheat nutrients uptake at the first site (sandy soil).

Treatments			Nutrients uptake of straw							Nutrients uptake of grain						
			N	**P**	**K**	**Fe**	**Mn**	**Zn**	**Cu**	**N**	**P**	**K**	**Fe**	**Mn**	**Zn**	**Cu**
			Kg/fed			**g/fed**				**Kg/fed**			**g/fed**			
Mineral fertilizers																
Bio_0	Zn_0	Control	5.3	1.8	4.6	690	365	5	3.5	2.2	1.1	1.3	271	140	3	1.4
		P_1	13.7	5.6	15.5	1062	570	13	6.1	5.9	2.5	3.7	464	229	8	2.5
		P_2	17.1	10.0	18.0	1137	619	16	7.1	7.6	4.4	4.9	529	254	10	3.1
		P_3	19.2	13.9	21.0	1140	621	21	7.8	9.0	6.4	6.0	582	271	13	3.5
		P_4	22.6	17.3	24.0	1133	610	25	8.5	11.1	8.0	7.0	608	277	15	4.0
	Zn_1	P_1	16.1	6.3	16.9	1031	517	251	7.6	6.7	3.1	4.4	447	223	118	3.0
		P_2	19.8	10.8	20.6	1079	520	297	9.4	9.0	5.3	6.0	506	247	141	3.7
		P_3	22.9	15.1	24.2	1074	512	340	10.1	10.8	7.3	7.3	552	247	163	4.4
		P_4	25.0	17.8	26.5	1029	495	370	11.0	11.9	8.6	8.4	571	251	177	4.9
Bio-fertilizers (Mycorrhizae)																
Bio_1	Zn_0	Control	6.0	3.1	4.9	629	340	7	3.6	2.5	2.0	1.4	266	137	3	1.5
		P_1	14.9	10.7	16.4	964	518	14	6.4	6.3	4.7	4.0	455	230	9	2.8
		P_2	18.7	15.7	19.1	1070	561	17	7.6	8.2	6.4	5.7	533	254	11	3.5
		P_3	20.9	20.9	22.8	1102	563	23	8.3	10.0	9.2	7.3	594	278	15	4.1
		P_4	24.7	26.7	26.2	1094	570	29	9.1	12.2	11.3	8.9	622	286	18	4.6
	Zn_1	P_1	17.7	12.5	17.7	923	463	284	8.3	7.5	5.4	4.9	467	219	125	3.4
		P_2	21.0	17.4	21.9	961	487	355	10.1	9.4	7.5	7.0	529	251	151	4.2
		P_3	23.3	22.8	25.2	988	466	399	10.9	11.3	10.1	8.5	568	246	174	4.8
		P_4	27.3	27.8	31.3	990	460	447	11.9	13.3	12.0	10.1	584	260	192	5.3
Bio-fertilizers (Mycorrhizae+ Azotobacter)																
Bio_2	Zn_0	Control	6.6	3.6	5.7	613	340	7	3.8	2.8	2.3	1.8	266	137	4	1.7
		P_1	21.4	11.7	18.2	1002	533	15	6.9	8.9	5.2	4.4	458	219	10	3.0
		P_2	26.0	17.6	22.7	1086	561	20	8.3	11.2	7.3	6.6	530	241	12	3.7
		P_3	30.6	23.7	27.1	1090	562	25	9.1	14.0	9.9	9.0	595	257	16	4.3
		P_4	36.1	29.6	32.3	1137	577	32	10.1	16.6	12.0	10.5	630	258	20	4.9
	Zn_1	P_1	24.0	14.6	21.0	1010	479	319	9.0	9.8	5.7	5.3	453	222	131	3.5
		P_2	29.2	20.6	24.9	1073	508	378	11.0	13.1	8.4	8.0	540	252	166	4.5
		P_3	33.3	24.7	29.7	1068	474	428	11.9	15.2	11.2	9.9	552	250	187	5.2
		P_4	38.7	31.1	35.4	1046	480	488	13.2	18.3	13.0	11.7	586	256	207	5.7
$LSD_{0.05}$ Bio			1.70	1.55	0.89	13	8.3	7.1	0.24	0.71	0.58	0.35	1.7	1.3	1.9	0.12
$LSD_{0.05}$ Zn			0.14	0.08	0.14	4	4.5	18.3	0.13	0.07	0.05	0.06	1.1	0.7	7.8	0.04
$LSD_{0.05}$ P			0.43	0.42	0.44	9	4.1	5.7	0.13	0.21	0.17	0.15	6.4	2.5	2.5	0.06
$LSD_{0.05}$ Bio x Zn			0.12	0.12	0.20	5	6.3	25.8	0.18	0.10	0.06	0.05	1.6	1.1	6.8	0.03
$LSD_{0.05}$ Bio x P			0.61	0.59	0.63	13	5.7	8.1	0.19	0.30	0.24	0.21	8.2	3.5	3.6	0.09
$LSD_{0.05}$ Zn x P			0.75	0.73	0.77	16	7.0	9.9	0.23	0.37	0.30	0.26	11.0	4.3	4.4	0.11
$LSD_{0.05}$ 3 factors			0.79	0.77	1.09	22	9.9	14.0	0.24	0.39	0.32	0.28	15.6	6.1	6.2	0.12

TABLE 5 . Effect of mineral and bio-fertilizers on wheat nutrients uptake at the second site (clay soil).

Treatments			Nutrients uptake of straw							Nutrients uptake of grain						
			N	P	K	Fe	Mn	Zn	Cu	N	P	K	Fe	Mn	Zn	Cu
			Kg/fed			g/fed				Kg/fed			g/fed			
Mineral fertilizers																
Bio_0	Zn_0	Control	16.0	4.6	13.3	1577	745	29	11.7	9.4	3.1	5.8	922	392	19	6.8
		P_1	50.5	14.6	39.2	2687	1210	83	21.0	28.8	9.8	16.7	1558	648	55	12.5
		P_2	54.4	21.6	42.6	2701	1208	96	23.4	30.0	13.7	17.9	1549	626	59	14.1
		P_3	60.0	31.9	50.4	2705	1200	108	26.4	33.4	18.7	21.8	1602	644	69	16.2
		P_4	65.1	39.5	56.6	2658	1163	119	28.9	34.5	23.3	23.7	1562	628	74	17.0
	Zn_1	P_1	53.2	16.2	42.4	2598	1184	595	30.1	31.3	11.3	18.4	1563	610	358	17.6
		P_2	60.2	26.4	47.7	2708	1204	687	33.7	34.5	16.1	19.4	1530	569	374	18.5
		P_3	64.9	34.4	55.8	2705	1169	746	35.8	40.4	21.5	24.5	1651	612	444	21.3
		P_4	71.5	44.4	61.7	2770	1159	832	38.9	43.0	26.0	25.6	1576	573	466	22.5
Bio-fertilizers (Mycorrhizae)																
Bio_1	Zn_0	Control	17.6	8.0	13.8	808	372	31	12.1	10.6	4.5	6.3	487	214	21	7.3
		P_1	52.3	22.8	41.5	2573	1159	88	21.7	30.6	12.4	18.2	1521	634	59	13.8
		P_2	55.9	30.4	47.4	2577	1154	104	24.0	32.5	16.6	20.7	1515	610	65	15.2
		P_3	65.6	42.7	60.0	2678	1193	125	28.3	36.2	21.2	25.3	1548	622	73	17.3
		P_4	67.3	48.3	64.2	2503	1093	133	30.0	38.3	26.0	27.2	1488	599	80	18.5
	Zn_1	P_1	56.3	24.7	45.3	2518	1146	656	30.7	33.0	14.0	20.0	1532	599	384	18.6
		P_2	67.0	37.5	57.5	2769	1229	776	36.8	36.8	18.4	22.2	1490	556	404	19.5
		P_3	74.5	48.8	69.3	2816	1216	870	40.9	43.9	23.9	28.1	1594	589	467	22.9
		P_4	79.6	57.2	75.1	2771	1162	948	43.2	46.7	28.9	30.1	1525	554	497	24.3
Bio-fertilizers (Mycorrhizae+ Azotobacter)																
Bio_2	Zn_0	Control	22.4	10.4	16.2	834	382	41	13.3	16.4	5.0	6.9	476	208	23	8.0
		P_1	71.5	27.0	47.5	2665	1197	108	24.0	47.7	13.6	19.2	1473	612	64	14.5
		P_2	93.5	34.8	58.6	2709	1212	124	27.2	52.1	18.3	23.6	1478	598	72	15.9
		P_3	107.5	51.2	72.8	2740	1214	139	31.6	58.3	24.4	29.0	1501	602	82	18.7
		P_4	112.3	55.3	78.9	2543	1114	153	33.7	60.6	28.2	31.3	1420	571	86	19.7
	Zn_1	P_1	82.7	29.1	51.4	2593	1185	736	33.9	50.3	15.3	21.1	1486	581	394	19.6
		P_2	105.0	39.9	68.6	2830	1259	878	40.6	54.7	19.9	25.6	1459	543	439	20.8
		P_3	118.9	57.1	84.2	2864	1236	996	45.4	64.6	26.4	32.9	1563	581	515	24.8
		P_4	125.4	62.7	88.4	2708	1131	1053	46.8	67.8	31.2	34.9	1473	562	549	25.9
$LSD_{0.05}$ Bio			7.06	2.62	2.97	36	17	17	3.91	0.75	0.95	0.90	31	12	6	0.42
$LSD_{0.05}$ Zn			0.48	0.26	0.40	5	1	37	0.28	0.12	0.13	0.61	1	2	20	0.28
$LSD_{0.05}$ P			1.37	0.84	1.08	35	15	12	0.71	0.43	0.43	0.48	20	7	7	0.27
$LSD_{0.05}$ Bio x Zn			0.68	0.37	0.56	7	2	53	0.40	0.10	0.18	0.86	1	3	17	0.24
$LSD_{0.05}$ Bio x P			1.93	1.18	1.53	50	21	18	1.01	0.61	0.61	0.68	28	10	9	0.38
$LSD_{0.05}$ Zn x P			2.37	1.45	1.87	61	26	22	1.24	0.74	0.74	0.83	34	12	11	0.47
$LSD_{0.05}$ 3 factors			2.50	1.53	1.98	65	28	30	1.31	0.78	0.78	1.18	36	13	16	0.49

The soil application of Mycorrhizae increased P uptake by straw and grain, while foliar application of Azotobacter increased N uptake by straw and grain. The application of bio-fertilizers (from both sources) with mineral P and Zn deceased Fe and Mn uptake.

These results could be due to the role of Mycorrhizae in dissolving P in soil and Azotobacter in fixing N from the air. The superior treatment was application of P_4+Zn_1 along with Mycorrhizae + Azotobacter compared with the other treatments at both sites. These results demonstrate that high Fe content and its negative effect on plant growth and yield could be decreased by mineral P and Zn. This is in agreement with the results obtained by Ewa & Jolanta (2005); Kefyalew *et al.* (2006); Wandruszka (2006); Li *et al.* (2007); Yassen *et al.* (2010); and Niemi *et al.* (2014).

The sandy soil had higher response to mineral and bio-fertilizers application than the clay soil. That is logically because of the lower nutrient contents in sandy soils than clay soil, these are facts agreed with obtained by Abbas *et al.*, (2012), Abbas *et al.* (2011) and Nadim *et al.* (2011)

Effect of the integration between mineral and bio-fertilizers on biochemical components in wheat

Total phenols and antioxidants contents in straw and grain increased with increasing mineral P and Zn application in the two studied soils. Combining application of mineral and bio-fertilizers further increased total phenols and antioxidants contents in straw and grains of wheat (Table 6). These components contents in straw were higher than that in grains. These results agreeable with that obtained with Li *et al.* (2013).

The bio-fertilizer (Mycorrhizae + Azotobacter) increased total phenols and total antioxidants contents in straw and grains. The superior treatment was P_4 + Zn_1 combined with application of Mycorrhizae + Azotobacter compared with the other treatments in both soil types. These result agreed with that obtained by Yoshie *et al.* (2008) and Lachman *et al.* (2011).

Regarding the effect of soil type, sandy soil had higher response to mineral (P + Zn) and bio-fertilizers than clayey soil in respect of biochemical. However, the clay soil achieved the highest contents. This is agreed with Ríos *et al.* (2009); Vaher *et al.* (2010); and Li *et al.* (2013).

Effect of the integration between mineral and bio-fertilizers on microbial densities and CO_2 evolution

Microbial densities

Generally, the microbial densities in rhizosphere at vegetation stage in all treatments were significantly higher than control as shown in Table 7. The highest counts in rhizosphere were obtained from plants treated with P_4+Zn_1 and Mycorrhizae + Azotobacter recording mean values of 92 and 965×10^2 cfu g^{-1} dry soil in sandy and clay soils, respectively. The counts declined toward harvesting stage which could be attributed to the unfavorable moisture content of soil due to soil dryness.

The maximum phosphate dissolving bacterial (PDB) counts of wheat rhizosphere was 12.9 and 59 $X10^2$CFU/g D.S) for sandy and clay soils respectively under superior treatment conditions. This result agreed with the obtained by Singhal *et al.* (2012), Castillo *et al.* (2012) and Hasanpour *et al.* (2012) who indicated that the combing the application of mineral P along with mycorrhiza could achieve the highest yield and P uptake by wheat.

TABLE 6. Effect of mineral and bio-fertilizers treatments on biochemical components of wheat in studied soils.

Treatments			Sandy soil				Clay soil			
			[1]T. antioxidants µg *Asc/ml		T. phenols µmol **Gal/ml		T. antioxidants µg Asc/ml		T. phenols µmol Gal/ml	
			Grains	Straw	Grains	Straw	Grains	Straw	Grains	Straw
Mineral fertilizers										
Bio_0	Zn_0	Control	38	52	119	176	72	93	200	294
		P_1	53	73	158	245	107	137	298	439
		P_2	56	77	165	259	113	145	313	461
		P_3	59	81	182	273	120	154	333	491
		P_4	64	88	195	296	130	166	348	513
	Zn_1	P_1	57	78	169	264	115	147	313	461
		P_2	61	83	183	283	120	154	328	483
		P_3	66	90	198	306	128	165	347	511
		P_4	72	99	212	333	134	171	359	529
Bio-fertilizers (Mycorrhizae)										
Bio_1	Zn_0	Control	43	59	125	188	80	104	212	319
		P_1	60	82	166	262	120	157	321	483
		P_2	66	91	178	289	127	166	339	509
		P_3	69	95	193	302	134	174	362	544
		P_4	75	103	198	328	143	187	373	560
	Zn_1	P_1	65	89	181	284	128	167	329	495
		P_2	71	97	195	310	134	174	358	538
		P_3	77	106	207	337	142	185	377	566
		P_4	83	114	215	363	151	197	385	578
Bio-fertilizers (Mycorrhizae+ Azotobacter)										
Bio_2	Zn_0	Control	49	65	132	196	88	113	227	336
		P_1	67	89	175	268	128	166	332	492
		P_2	75	99	184	300	134	173	359	532
		P_3	79	105	197	316	144	187	375	556
		P_4	86	114	208	344	153	197	390	578
	Zn_1	P_1	73	97	188	292	138	178	344	510
		P_2	78	103	195	312	146	188	377	558
		P_3	84	111	206	336	154	199	394	584
		P_4	93	123	219	372	161	208	404	598
$LSD_{0.05}$ Bio			3.1	3.7	2.5	6.2	4.1	5.6	7.2	11.5
$LSD_{0.05}$ Zn			0.3	0.4	0.8	1.4	0.4	0.6	0.8	1.1
$LSD_{0.05}$ P			0.7	0.9	1.5	2.8	1.2	1.6	3.1	4.7
$LSD_{0.05}$ Biox Zn			0.4	0.6	1.1	2.0	0.6	0.8	1.1	1.6
$LSD_{0.05}$ Bio x P			0.9	1.2	2.2	3.9	1.7	2.2	4.4	6.6
$LSD_{0.05}$ Zn x P			1.1	1.5	2.7	4.8	2.1	2.7	5.4	8.1
$LSD_{0.05}$ 3 factors			1.2	1.6	2.8	5.1	2.2	2.9	5.7	8.5

[1] T: Total, Asc*: µg Ascorbic acid/ml extract, Gal**: µmol of Gallic acid/ml extract

TABLE 7 . Effect of mineral and bio-fertilizers applied on total microbial counts, PDB counts and CO_2 evolution in rhizosphere and azotobacter densities in phyllosphere of wheat in studied soils.

Treatments			Total microbial counts (counts X10^{2}CFUg^{-1}D.S)				PDB counts in rhizosphere (counts X10^{2}CFUg^{-1} D.S)				Azotobacter count in phyllosphere (counts X10^{2}CFUg^{-1} F.L)				CO_2 evolution (mg 100g^{-1} dry soil 24 hr^{-1})			
			Sandy soil		Clay soil		Sandy soil		Clay soil		Sandy soil		Clay soil		Sandy soil		Clay soil	
			St_1	St_2	St_1	St_2	St_1	St_2	St_1	St_2	St_1	St_2	St_1	St_2	St_1	St_2	St_1	St_2
Mineral fertilizers																		
Bio_0	Zn_0	Control	24	21	297	240	4.4	3.8	21	13	2.8	2.3	3.7	3.1	26	22	32	27
		P_1	37	33	450	364	6.6	4.7	29	16	3.2	2.9	3.8	3.4	32	29	35	32
		P_2	41	37	529	428	7.8	5.5	34	19	3.5	3.2	4.3	3.9	37	33	41	37
		P_3	43	38	599	484	8.9	6.7	39	23	4.0	3.6	4.9	4.4	41	38	47	42
		P_4	47	42	663	536	9.9	7.9	45	27	4.4	4.0	5.4	4.9	45	41	52	47
	Zn_1	P_1	41	37	495	400	6.8	5.1	32	17	3.4	3.1	4.2	3.8	34	31	39	35
		P_2	46	41	604	488	7.9	5.8	38	20	3.9	3.5	4.7	4.3	41	37	47	43
		P_3	50	45	673	544	8.1	6.9	40	24	4.3	3.9	5.3	4.9	46	42	52	48
		P_4	52	46	717	580	10.2	8.1	49	28	4.9	4.5	5.8	5.3	49	45	56	51
Bio-fertilizers (Mycorrhizae)																		
Bio_1	Zn_0	Control	32	26	376	304	4.8	4	23	14	3.1	2.6	3.9	3.2	31	26	41	34
		P_1	47	38	539	436	8.1	6.2	35	21	3.8	3.5	4.2	3.8	35	32	42	38
		P_2	51	42	613	496	9.6	7.3	41	25	4.4	4.0	4.9	4.5	39	36	48	43
		P_3	54	44	698	564	10.9	8.8	47	30	4.9	4.5	5.5	5.0	44	40	54	49
		P_4	59	48	777	628	12.2	10.4	53	36	5.4	4.9	6.1	5.6	49	45	60	55
	Zn_1	P_1	53	43	599	484	8.3	6.7	39	23	4.1	3.7	4.6	4.2	38	35	47	42
		P_2	56	46	678	548	9.7	7.7	46	26	4.8	4.4	5.6	5.1	43	39	53	48
		P_3	59	48	762	616	9.9	9.1	47	31	5.5	5.0	6.2	5.7	48	44	59	54
		P_4	64	53	831	672	11.5	10.7	55	37	5.8	5.3	6.6	6.0	55	50	65	59
Bio-fertilizers (Mycorrhizae+ Azotobacter)																		
Bio_2	Zn_0	Control	38	31	430	348	5.4	4.7	26	16	41	34	56	47	34	29	47	39
		P_1	69	56	648	524	8.6	6.8	39	23	48	44	60	55	41	37	50	46
		P_2	72	60	732	592	10.7	8.6	48	29	54	49	68	62	46	41	57	52
		P_3	80	66	836	676	11.4	9.7	54	33	63	57	77	70	53	48	65	59
		P_4	87	71	900	728	12.4	11.7	58	40	69	63	83	76	58	53	70	64
	Zn_1	P_1	72	60	683	552	9.2	7.9	43	27	53	48	63	58	44	40	53	48
		P_2	79	65	782	632	10.9	9.1	52	31	59	54	72	66	50	45	61	55
		P_3	85	70	886	716	11.2	10.2	55	35	66	60	82	75	56	50	69	63
		P_4	92	75	965	781	12.9	11.1	59	38	73	67	89	81	62	56	75	68
$LSD_{0.05}$ Bio			5.8	4.3	36	29	0.43	0.49	2.1	1.7	11	10	14	12	1.9	1.7	3.0	2.7
$LSD_{0.05}$ Zn			0.3	0.2	3	2	0.18	0.18	0.9	0.6	0.6	0.1	0.6	0.1	0.2	0.2	0.2	0.2
$LSD_{0.05}$ P			0.7	0.6	8	7	0.10	0.08	0.4	0.3	0.2	0.3	0.3	0.4	0.4	0.3	0.5	0.4
$LSD_{0.05}$Biox Zn			0.2	0.2	4	3	0.25	0.25	1.3	0.9	0.8	0.2	0.8	0.2	0.2	0.2	0.3	0.2
$LSD_{0.05}$ Bio x P			1.0	0.8	12	9	0.14	0.12	0.6	0.4	0.3	0.4	0.4	0.4	0.6	0.5	0.6	0.5
$LSD_{0.05}$ Zn x P			1.2	1.0	14	12	0.17	0.14	0.7	0.5	0.4	0.5	0.5	0.6	0.7	0.6	0.8	0.7
$LSD_{0.05}$3factors			1.3	1.1	15	12	0.24	0.20	1.0	0.7	0.6	0.7	0.7	0.8	1.0	1.0	0.8	0.8

St_1= vegetating stage, St_2= harvesting stage, F.L= fresh leaf, D.S=dry soil, PDB= Phosphate dissolved bacteria

Data also showed that the Azotobacter densities from the phyllosphere of wheat plants were 2.8 and 3.7 X10^2 cfu cm^{-2} leaf in control treatment in sandy and clay soils, respectively. While these values increased to reach 73 and 89 ×10^2 cfu/ cfu /cm^2 leaf with treating the plants with P_4+Zn_1 along with Mycorrhizae + Azotobacter in sandy and clay soils, respectively. These results agreeable with that obtained by Khin (2011) who reported that mixture of biofertilizer and zinc sulphate gave better results than individual treatments.

CO_2 evolution

The CO_2 evolved from rhizosphere (mg CO_2 $100g^{-1}$ dry soil 24 hr^{-1}) was periodically determined to detect the microbial activity as influenced by mineral (P + Zn) and bio-fertilizers (Mycorrhizae + Azotobacter) treatments.

Data in Table 7 show that the CO_2 evolution increased in rhizosphere reaching maximum levels at 40 days after sowing (vegetation stage) then decreased during harvesting stage in both soils. In both soils, rhizosphere of control treatment (without inoculation with bio-fertilizers) gave the lowest CO_2 evolution values. Results also showed that CO_2 evolved in rhizosphere of wheat were markedly affected by application of P and Zn treatments. Increasing P and Zn rates led to increase yield parameters to reach maximum value in the treatment of the P4 + Zn1. CO_2 evolution values in rhizosphere were much higher in clayey soils compared with sandy soils. Bio-fertilizers **(**Mycorrhizae) and (Mycorrhizae + Azotobacter) treatments increased CO_2 evolution in the presence of mineral fertilizers. The most effective treatment was NP_4K+Zn_1 plus Mycorrhizae + *Azotobacter* which produced 61.57 and 55.97 (mg CO_2 $100g^{-1}$ dry soil 24 hr^{-1}) in vegetation and harvesting stages, respectively, in sandy soil. While produced 75.08 and 68.25(mg CO_2 $100g^{-1}$ dry soil 24 hr^{-1}), respectively, in clayey soil. These result agreeable with that obtained by Amanullah *et al.* (2012); Kowsar *et al.* (2014); and Garshasbi *et al.* (2014).

Conclusions

Application of mineral (P + Zn) and bio-fertilizers (Mycorrhizae+ Azotobacter) increased yield parameters, nutrients contents, nutrients uptake and biochemical components of wheat grown in the New Valley soils with high Fe content . These parameters were much higher in the clay soils than in the sandy soils. Bio-fertilizers **(**Mycorrhizae) and (Mycorrhizae+Azotobacter) treatments increased yield parameters of wheat in the presence of mineral fertilizers by increasing the available N and P in soils. The most effective treatment appeared to be applying P at a rate of 60 kg fed^{-1} in clay soil and 80 kg fed^{-1} in sandy soil with spraying Zn at a concentration of 250 mg L^{-1} combined with the application of Mycorrhizae and Azotobacter which achieved 5.45 and 2.21 Mg fed^{-1} for straw and grains respectively in the sandy soil, while 9.5 and 4.16 Mg fed^{-1} in the clay soil. This study revealed that the combination of bio- and mineral P and Zn fertilizers could decrease the negative effect of high Fe content in soils and increased wheat production.

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تاثير التكامل بين التسميد المعدنى والحيوى على انتاجية القمح تحت ظروف ارتفاع الحديد فى اراضى الوادى الجديد، مصر

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ارتفاع محتوى الحديد فى اراضى الوادى الجديد يعيق امتصاص كلا من الفوسفور والزنك بواسطة النباتات ، والذى

انعكس سلبيا على انتاجية المحصول. التجارب الحقلية نفذت خلال موسمين متتاليين فى موقعين تحت ظروف اراضى الوادى الجديد بهدف تحسين محصول القمح بواسطة التكامل بين الاسمدة المعدنية (الفوسفور والزنك) والحيوية (Mycorrhizae + Azotobacter). التجربة الاولى تمت فى المنيرة (اراضى رملية) فى الواحات الخارجة ، بينما الثانية تمت فى القصر(اراضى طينية)، الواحات الداخلة. حبوب القمح صنف سخا 93 زرعت فى كلا من موقعين الدراسة.

اضافة الاسمدة المعدنية للفوسفور والزنك بصورة فردية او مع الاسمدة الحيوية قد زاد من قياسات المحصول ومحتوى المغذيات ومضادات الاكسدة الكلية والفينولات الكلية والتسميد الحيوى للفوسفور زادت من قياسات المحصول ، محتوى المغذيات والممتص منها ومحتوى مضادات الاكسدة الكلية والفينولات الكلية فى القمح. وعلاوة على ذلك، زيادة معدلات اضافة الفوسفور والزنك خفضت من تركيز الحديد والمنجنيز فى القمح وزادت من محتوى النتروجين والفوسفور والبوتاسيوم والزنك والنحاس فى القش والحبوب. المعاملة الاكثر تاثيرا للمحصول ومحتوى العناصر الغذائية والممتصة ومحتوى المواد البيوكميائية (مضادات الاكسدة الكلية والفينولات الكلية) فى القش والحبوب هى تكون باضافة الفوسفور عند المعدل 80 كجم/ فدان فى الاراضى الطينية و 60 كجم/فدان فى الاراضى الرملية مع رش الزنك بالمعدل250 ملجرام/لتر مع اضافة الاسمدة الحيوية (Mycorrhizae + Azotobacter) . هذة المعاملة حققت 5.45 و2.21 طن/ فدان لكل من القش والحبوب على التوالى لنبات القمح النامى فى التربة الرملية بينما تكون 9.5 و 4.16 طن/ فدان فى التربة الطينية. هذة الدراسة توضح ان الاتحاد بين الفوسفور والزنك المضاف خفض من التاثير السلبى لارتفاع محتوى الحديد فى الاراضى وزادت من انتاجية القمح