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Full Length Research Paper

The role of biofertilizers and/or some micronutrients on wheat plant (*Triticum aestivum* L.) growth in newly reclaimed soil

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The effect of biofertilizers (inoculation with different bacterial isolates), foliar spraying with some micronutrients and their interaction on growth, physiological parameters and nutrients content of wheat plants grown on new reclaimed soil were studied. The growth parameters, some physiological parameters and nutrient contents of wheat plants were significantly increased by inoculating wheat grains with different bacteria as compared with un-inoculated (control). The highest values of all the mentioned parameters were obtained by using *Azospirillum brasilense* followed by Azotabacter *chroococcum* and *Bacillus polymyxa* in decreasing order. Foliar spraying treatments significantly increased the growth parameters, physiological parameters as well as nutrients content of wheat plants as compared with control treatment. Highest values were obtained by using (Mn+Fe+Zn) treatment followed by Zn, Fe and Mn in decreasing order. Micronutrients in wheat plants differed as the foliar treatments were differed, so application of any micronutrient individually significantly increased its content and enhanced the content of other micronutrients in wheat. Used biofertilizers and foliar spraying with micronutrients significantly affected all the studied parameters of wheat plants, the highest were obtained by inoculating wheat grains with *A. brasilense* and spraying the plants with (Mn+Fe+Zn) treatment, while the lowest values were attained by un-inoculated grains (control) and spraying the wheat plants with tap water (control). It can be concluded that the data collected proves that the use of *Azospirillum, azotobacter* or *Bacillus*, in combination with foliar application with micronutrients (Mn+Fe+Zn) can lead to higher wheat yield.

Key words: Biofertilizers, wheat plants, nutrients content, physiological parameters, foliar spray, micronutrient, bacterial isolates.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the main diet for the Egyptian population, and is one of the crops which show a great response to foliar application with micronutrients (Serry et al., 1974). The increasing demand for production of crops and food for such vast population has led to an interest and necessity for the use of biofertilizers for the betterment of these crops and even for the soil health (Nishita and Joshi, 2010). Due to the excessive use of chemical fertilizers which generated several environmental problems, some of these problems can be tackled by the use biofertilizers, which are natural, beneficial and ecologically friendly (Mizakhani et al., 2009).

The use of biofertilizers in agricultural developing countries, still limited to minimize the high doses of chemical fertilizers as well as to lower the agricultural production costs.** Unfortunately, most of the newly reclaimed soils have poor nutritional and biological properties, which reflected on its productivity (El-Borollosy et al., 2000).

Today, emphasis is put on plant growth regulating bacteria, microorganisms that are capable of increasing the rate of plant growth, by direct or indirect mechanisms,

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secretion of vitamins and amino acids, auxin and fixing atmospheric nitrogen by *Bacillus*, *Azotobacter* and *Azosprillum* are among the direct mechanisms of increasing root development and plant growth (Akbari et al., 2007). While *Azotobacter* and *Azospirillum* inoculants are well adapted technologies to dry mountainous areas, these biofertilizers can significantly increase wheat and barley yield (Milani and Anthofer, 2008).

Moreover, biofertilizers are considered as the most important factor in reducing the application of chemical nitrogen fertilizers and minimizing the induced environmental pollution (Canbolat et al., 2006; El-Sirafy et al., 2006) and improve the soil fertility status to sustain crop yield (Mostafa et al., 2010).

The effect of plant growth promoting Rhizobacterial (PGPR) inoculation on growth and N2-fixation of tissue-cultured banana evaluated by Baset et al. (2010) who recorded that due to the PGPR inoculation, beside higher shoot growth and N yield, the inoculated banana plants showed higher growth attributes, leaf area, chlorophyll content and the total biomass was higher in compare to un-inoculated control. Accordingly, Zarabi et al. (2011) proved that different biofertilizers can positively affect on the growth increase of maize plant and phosphorus absorption,

Foliar application of micronutrients generally is more effective, less costly and accepted practice for many crops. In this respect, spraying of micronutrients to plants grown on some soil of Egypt, gave better growth and more yield (Abd-El Hamid and Sarhan, 2008; El-Desuki et al., 2010).

Micronutrients play an important role as essential trace elements in all living systems from bacteria to humans; they are essential component of number of hydrogenise, pretenses, peptidases and phosphohyrolases (Devlin and Withan, 1988; Massoud et al., 2005). Also, Muthaura et al. (2010) added evidence that application of biofertilizers can help to convert nutritionally important elements from unavailable to available form through biological processes.

However, micro elements as Fe, Zn, Mn and Cu are also added to foliar fertilizers used throughout the world as effective measure to compensate their deficiency. This has special importance in arid and semi arid regions where osmotic pressure promotes the absorption and activity of these elements influenced by the plant behavior and the foliar application timing (shehata et al., 2010).

The aim of this study was to determine the effect of biofertilizers (inoculation of *Bacillus*, *Azotobacter* or *Azosprillum*) as well as foliar application with some micronutrients (Mn, Fe, Zn or Mn + Fe + Zn) on growth, chemical constituents and nutrients content of wheat plants grown on newly reclaimed soil.

MATERIALS AND METHODS

Pot experiment was conducted in the greenhouse of NRC, Dokki, Giza, Egypt, to investigate the effect of biofertilizer (inoculation with different bacteria), foliar spraying with some micronutrient (Mn, Zn, Fe) and their interaction on growth, physiological parameters and nutrients status of wheat plants grown on new reclaimed sandy soil (Table 1).

The experimental design was split plot with four replicates. Four biofertilizer treatments (un-inoculated, *Bacillus polymyxa*, *Azotobacter chroococcum* or *Azosprillium barasilense*) were used and randomly distributed in the main pots. The foliar treatments with micronutrients were randomly distributed in the sub plots as follows:

1. Without spraying (control).
2. Spraying with manganese at the rate of 200 mg Mn/L of spraying solution as manganese sulphate.
3. Spraying with iron at the rate of 300 mg Fe/L of spraying solution as ferrous sulphate.
4. Spraying with zinc at the rate of 200 mg Zn/L of spraying solution as zinc sulphate.
5. Spraying with solution containing 200, 300 and 200 mg/L of Mn, Fe and Zn together, respectively.

The biofertilizers were produced by the general organization for agriculture qualization, Ministry of Agriculture and Reclamation, Egypt (Abou-El-Naga, 1993).

Each bacterial isolate was grown on 100 ml of the convenient medium for 7 days on a gently laboratory shaker at 28℃ and then, the cultures were centrifuged at 5000 rpm for 20 min, the bacterial filtrate were separated for phytohormone-active substances extraction and determination as follow:

Phytohormone-active substances extraction

Phytohormone-active substances were extracted from bacterial culture filtrates as described by Cacciari et al. (1989). The bacterial culture filtrates were acidified with 1 M HCl to pH 2.8-3.0 and shaken with 1% (w/v) activated charcoal for 2 h to separate auxins and gibberellins, a part of the charcoal was extracted with 95% (v/v) aqueous acetone, the acetone was evaporated under vacuum at 30℃. The moist residue was extracted with ethyl acetate, which, was evaporated to dryness and dissolved in a known volume of methanol. Methanol fractions containing auxins and gibberellins were chromatographic by thin layer chromatography (TLC) and gas liquid chromatography (GLC).

For cytokines separation, another part of charcoal was extracted with 0.3 N NaOH, the filtrate was acidified with 1N H_2SO_4 and an excess of 0.1 N $AgNO_3$, was added. After 48 h the suspension was centrifuged at 5000 rpm, the $AgNO_3$ sediment was dissolved with 0.2 N HCl and centrifuged again. The supernatant was adjusted to pH 8.0 with NH_4OH and extracted with n-butanol. Cytokinins in the n-butanol fraction were analyzed by TLC and GLC.

Phytohormone-active substances analysis

Determination of Phytohormone-active substances was carried out by Gas liquid Chromatography according to Uapper et al. (1970). 0.1 ml of auxins and or cytokinins frctions was placed in 3 ml test tube and evaporated to dryness and then, the residue was derivative to their methyl ester-trimethylsily (MeTMSi) by adding 0.1 ml of bis (trimethylsilyl) acetamide (BSA). The test tube was then capped and allowed to stand at least 30 min before its contents were analyzed by GLC. 1 µL of the sample was injected into the split-splitles mode in a GLC consisting of a HP 5890 series II GC with operation and data analysis using chemostatin software. The GC column was HP-5 of 0.22 mm internal diameter and 25 m long, 0.1 µm film thickness eluted with nitrogen (5 ml/min0. the GC program was 220 to 250℃ at 6℃ min^{-1} then 2 min at 250℃, injector temperature was at 240℃ and detector temper ature was 270℃. The flow rates of hydrogen and air for the flame ionization

Table 1. Some physical and chemical analysis of the used soil were determined as described by Jackson (1982).

Mechanical analysis				Chemical analysis						
Sand %	Silt %	Clay %	Texture	$CaCO_3$ %	Soil pH	O.M %	E.C dsm^{-1}	Soluble N ppm	Available P ppm	Exchangeable K ppm
96.29	3.11	0.60	Sandy	2.22	7.59	0.50	0.11	36.62	4.30	21.52

Table 2. Production of Phytohormones (µg/L) and exo-polysaccharides (g/L) by isolated nitrogen fixers.

Isolated nitrogen fixers	Phytohormones			Exo-polysaccharides
	Auxins	Gibberellins	Cytokinins	
Azotobacter spp.	-	61.8	100.3	0.78
Azospirillum spp.	3.6	77.1	46.1	0.63
Bacillus spp.	46.3	97.2	196.8	1.19

detector were 30 and 300 ml min^{-1}, respectively.

Determination of total exo-polysaccharides

To determine the production of total exo-polysaccharides, the method described by Hebber et al. (1992) was performed; bacteria were grown in specific media overnight at 28℃ and cen trifuged at 15000 rpm for 15 min. The pellet obtained was suspended in 5.0 ml of 0.85%KCl solution and inoculate into a 250 ml serum bottle containing 100 ml culture medium. One hundred ml batch cultures were inoculated at 28℃ for 3 to 5 days with constant agitation in an orbital shaker (150 rpm).

The first step for producing total exo-polysaccharide by centrifugation of the culture solution at 18000 rpm, the pH of the supernatant was then adjusted to 7.25; polysaccharides were precipitated by the addition of 6.0 g of NaCl, followed by adding equal volume of 95% ethanol. The precipitate was recovered either by swirling a spatula or a glass rod or by centrifugation at 3000 rpm for 15 min. Polysaccharides were then dehydrated in an alchol series (60, 70, 80 and 95% ethanol) and then dried at 35℃.

Inoculation with different bacteria was performed by coating wheat grains using a sticking substance (Arabic gum, 5%) just before sawing. The grains were left for air drying about an hour in shaded place and sown, immediately in plastic pots 25 cm diameter and 17 cm depth, which were filled with 4 kg sandy soil/pot. Eight seedlings were planted in every pot then thinned to 5 after one week.

All pots were fertilized at the recommended rate of N, p and k fertilizers. Nitrogen was added as $NH_4 NO_3$ (33.5% N, at the rate of 250 gm/pot, phosphorus was added as P_2O_5 (15.5%), at the rate of 100 gm/pot, and potassium was added as K_2SO_4 (48% K_2O) at the rate of 200 gm/pot. 75 days after sowing, wheat samples were harvested and undergone the following parameters:

i. Growth parameters: Plant height (cm), leaf area (cm^2), dry weight of roots, shoots and whole plant (gm/pot), and number of tillers and leaves per plant were recorded.
ii. Physiological parameters: Soluble sugar %, protein %, polysaccharide %, Chl. a+b µg/cm leaf/plant, carotenoids in fresh weight µg/gm, IAA in fresh wt. mg/kg and Ps II µ*mole, were determined as described in standard methods carried out by Moran and Forath (1980), Osman and El-Shintinawy (1988), Biswal and Mohanty (1976) and A.O.A.C. (2000).
iii. Nutrients concentration: Nitrogen, P, K, Mg, Fe, Mn, Zn and Cu were determined in wheat plants as described by Cottenie et al. (1982).
iv. Statistical analysis: Were conducted according to Snedecor and Cochran (1990) methods.

RESULTS AND DISCUSSION

Determination of Phytohormone-active substances and exo-polysaccharides produced by bacterial isolates

The amounts of Phytohormone-active substances (auxins, gibbirellens and cytokines) were determined by gas liquid chromatography. The obtained data are recorded in Table 2.

Generally, the amounts of auxins produced from the cultures of any isolate were low and did not exceed 46.3 $\mu g.L^{-1}$. These low levels were in the range of these reported for *Azospirillum* spp.; by Cacciari et al. (1989), the highest amount remarked was that produced by *Bacillus* spp.

Gibberellins production of any culture isolates was higher than auxins production, the highest amount of gibberellins was that produced by *bacillus* spp. (97.2 $\mu g.L^{-1}$). Such, a discrepancy in the amounts of these phytohormones is apparent also in *Azotobacter* spp., values ranging from 10-100 $\mu g.L^{-1}$ (Azcon and Barea, 1975).

Amounts of cytokinins produced by the different bacterial isolates were highly increased than auxins and gibberellins production, their values ranged from 196.8 to 46.1 $\mu g.L^{-1}$, also the highest amounts were produced by *Bacillus* spp. 196.8 $\mu g.L^{-1}$.

The ability of selected isolates for exo-polysaccharides production was studied. Data presented in Table 2 revealed that all isolates produced variable quantities of

Table 3. Effect of different biofertilizers and foliar application with micronutrients on some growth parameters of wheat plants.

Treatments		Plant height (cm)	Leaf area (cm^2)	Dry weight (mg)			No. of tillers/plant	No. of leaves/plant
				Root	Shoot	plant		
Effect of biofertilizer								
Un-inoculated (control)		30.7	24.17	210.25	292.05	502.3	6.18	20.33
Bacillus polymyxa		34.37	28.85	220.75	315.5	536.25	6.44	21.48
Azotobacter chroococcum		36.42	30.72	275.95	327.05	553	6.55	21.7
Azospirillum brasilense		39.68	33.99	234.65	339.55	574.2	7.1	22.99
LSD. at 5%		0.27	0.42	5.1	11.19	12.06	0.09	0.12
Effect of foliar application								
Control		29.42	24.86	147.19	251.06	398.25	5.6	19.55
Mn		30.29	25.93	198.38	294.88	493.26	6.15	21.12
Fe		32.45	30.38	201.88	307.75	509.63	6.54	21.67
Zn		37.97	31.05	273.5	342.81	616.31	6.95	22.26
(Mn+Fe+Zn)		46.37	34.94	293.56	396.19	689.75	7.6	23.53
LSD at 5%		0.3	0.47	11.54	13.33	16.3	0.1	0.15
Interaction biof. x foliar application								
	Control	25.76	20.4	132.5	225.5	358	5.3	19
	Mn	26.55	21.2	183	271.5	454.5	5.8	20
Un-inoculated (control)	Fe	28.78	25.5	189	285.75	474.75	6.1	20.33
	Zn	32.68	25.68	262	297.25	559.25	6.7	20.6
	(Mn+Fe+Zn)	39.73	28.08	284.75	380.25	665	7	21.71
	Control	28.9	24.3	142.5	250	392.5	5.5	19.5
	Mn	29.65	25.05	197.5	286.25	483.75	6.2	21.4
Bacillus polymyxa	Fe	31.43	29.88	200.75	301.75	502.5	6.4	21.8
	Zn	35.95	30.48	272	348.75	620.75	6.8	21.8
	(Mn+Fe+Zn)	45.9	34.55	291	390.75	681.75	7.3	22.9
	Control	30	26	148.25	259.5	407.75	5.7	19.8
	Mn	31.45	26.78	203.75	303.25	507	6.2	21
Azotobacter chroococcum	Fe	33.23	31.7	204.75	315.75	520.5	6.45	21.7
	Zn	38.95	32.2	276.75	357	633.75	6.7	22.3
	(Mn+Fe+Zn)	48.48	36.9	296.25	399.75	696	7.7	23.9
	Control	33	28.73	165.5	269.25	434.75	5.9	20.1
	Mn	35.5	30.67	209.25	318.5	527.75	6.4	22.08
Azospirillum brasilense	Fe	36.38	34.45	213	327.75	540.75	7.2	22.86
	Zn	44.13	35.85	283.25	368.25	651.5	7.6	24.32
	(Mn+Fe+Zn)	51.4	40.23	302.25	414	716.25	8.4	25.6
LSD at 5%		0.6	0.93	3.04	2.63	4.55	0.2	0.23

exo-polysaccharides. It was also found that the highest amounts of the production of exo-polysaccharides were 1.19 g/L for *Bacillus* spp. The colonization of rice roots by an exo-polysaccharides over-producing *Azospirillum* spp. and *Klebsiella* spp. *were* investigated by El-khawas and Aduchi (1999).

Growth parameters of wheat plants

Table 3 indicated the influence of applied biofertilizers i.e. *B. polymyxa*, *A. chroococcum* and *Azospirillum brasilinse* on wheat growth parameters. Results show that all growth parameters (plant height; leaf area; roots, shoots

and whole plant dry weights; No. of tillers and leaves/ plant) were significantly increased with using biofertilizers as compared with the control treatment (un-inoculated). In this respect Zaki et al. (2009) and Radwan and Wafaa (2009) found highly significant increases in growth, grain yield and yield components by inoculation of barley and broccoli seeds with biofertilizers and multi strain inoculants of a symbiotic N-fixing bacteria. The highest values of our studied parameters in wheat growth were obtained by inoculate wheat grains with *A. brasilense* followed by those inoculated with *A. chroococcum* and *B. polymyxa* in decreasing order (Table 3). In this concern Yadava et al. (2011) pointed out the ability of *Azospirillum* inoculation in stimulating the relative elongation rate of shoots fresh and dry weight and improving crop yield of Maize.

The obtained results are in agreement with those obtained by El-Houssini, (2007) who stated that seed inoculation with *Azospirillum* spp. gave better and higher results than those of *Azotobacter*, Alizadeh et al. (2009) explained that inoculating wheat grains by *Azospirillum* spp. Improved the No. of fertile ear, No. of grain/ear, grain yield, grain protein and leaf area index.

The significant favorable effects of biofertilizers on growth and productivity of the treated plants may be explained on the bases of the beneficial effect of bacteria on nutrients availability, vital enzymes, and hormonal stimulating effect on plant growth or increasing of photosynthetic activity. Generally, there are several possible mechanisms affect on the growth of inoculated plants, one mechanism may be that bacteria can produce plant growth hormones such as gibberlic acid, indole-3-acetic acid, and cytokine, which promote plant growth (Barea and Brown, 1974); moreover, Brown (1974) suggested another mechanism, that diseases could be suppressed by bacterial inoculation, which in turn stimulate plant growth. The PGPR are known to participate in many important ecosystem processes, such as the biological control of plant pathogens, nutrient cycling, and/or seedling growth (Nivedhitha et al., 2008) so, growth of wheat plants might, therefore, be affected by one or more of these mechanisms.

Results in Table 3 reveal that all the foliar spraying treatments significantly increased the growth parameters under study comparing with control plant. The combined treatment (Mn+Fe+Zn) had the superior effect on all the growth parameters followed by Zn, Fe or Mn treatments in decreasing order. The increasing in growth parameters may be due to increasing in the rate of photochemical reactions and activities of the carboxylation enzymes as well as carbonic anhydrate. Similar results were obtained by El-Fiki et al. (2008) and Knany et al. (2009) who stated that growth and yield parameters were positively affected by the application of Zn, Mn and Fe either individually or in mixtures, and the maximum response were observed with the triple treatment (Mn+ Fe+Zn), followed by double and single treatments in decreasing order, they reviled that the highest values to the suitable balance among them, and this enables the plants to grow well, so growth and yield components increased.

Foliar treatment with Zinc significantly increased all the studied growth parameters may be attributed to the effect of Zn on producing the growth substances, auxin, which in turn encourages the meristematic activity of plants led to cell division and enlargement (Devlin and Withan, 1988).

The stimulative effect of zinc on growth can be also attributed to the effect of Zn on protein content and its effect as a material component of some enzymes or regulatory for others. Zinc has essential role in tryptophane synthase and metabolism (Romheld and Marschner, 1991).

Data presented in Table 3 indicated that the interaction between biofertilizers and foliar spraying with micron-trients significantly affected plant height, leaf area, dry weigh of roots, shoots and the whole plant, No. of tillers and leaves/ plant. Generally, the highest values of all mentioned parameters were obtained by inoculating wheat grains with *A. brasilense* and spraying the plants with the combined (Mn+Fe+Zn) treatment. Whereas, the lowest values were attained by using the un-inoculated treatment and sprayed the wheat plants, with tap water (control treatment). These results may show the positive effect of both biofertilizers and micronutrients application, which could increase decomposition of organic matter and thereby release more nutrients in available form for growing wheat plants, results are going with those obtained by Muthaura et al. (2010) and Rokhzadi et al. (2008) who proved that when living microorganisms applied to seed, plant surface or soil, they colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of nutrients to the host plant.

Physiological parameters

It is of interest to mention that inoculation of wheat grains with biofertilizers (*B. polymyxa*, *A. chroococcum* or *Azosprillum brasilense*) significantly increased the studied physiological parameters (soluble sugar, protein content, polysaccharide, Ps II, chl. a+b, carotenoids and indole acetic acid) as compared with un-inoculated treatment (Table 4). The highest values of the chemical constituents were obtained when wheat grains were inoculated with *A.* brasilense followed by *Azotobacter chroococcum* and *B. polymyxa* in decreasing order. The stimulatory effect of biofertilizers may be attributed not only to N. fixation activity, but also to the production of growth promoting substances such as IAA, gibberellins and cytokinin like substances with positive effect on plant growth (Fayez et al., 1985). In this concern, El-Borollosy et al. (2000) observed that *A. chroococcum* produces some B-vitamins (thiamine, nicotinic acid, pentothenic acid, biotin and pyridoxine),

Table 4. Effect of different biofertilizers and foliar application with micronutrients on some physiological parameters of wheat plants.

Treatments		Soluble sugar %	Protein %	Polysaccharide %	Ch. a+b µg/cm leaf/plant	Carotenoids µg/gm	IAA mg/kg	Ps II µ mole DCPIP reduced/mg chl./h.
Effect of biofertilizer								
Un-inoculated (control)		9.91	9.27	25.83	25.83	1.29	13.03	75.49
Bacillus polymyxa		11.69	10.3	31.29	31.29	1.27	14.3	80.24
Azotobacter chroococcum		12.24	11.71	33.21	33.21	1.38	14.95	83.88
Azospirillum brasilense		14.68	12.64	36.21	36.21	1.56	15.95	88.19
LSD. at 5%		0.06	0.23	0.1	0.69	0.11	0.13	0.18
Effect of foliar application								
Control		8.94	6.29	18.24	22.52	1.12	10.11	65.6
Mn		10.99	8.74	18.81	26.66	1.17	13.5	77.36
Fe		11.58	11.06	20.56	28.17	1.32	15.11	78.55
Zn		13.47	11.55	23.33	37.22	1.39	16.06	86.51
(Mn+Fe+Zn)		16.53	15.71	28.04	43.61	1.61	18.02	101.73
LSD at 5%		0.07	0.25	0.11	0.77	0.12	0.14	0.21
Interaction biof. x foliar application								
	Control	6.15	6.56	13.18	17.9	0.98	8.15	60.4
	Mn	9.08	6.81	13.28	18.63	0.99	11.85	70.25
Un-inoculated (control)	Fe	9.6	9.31	16.18	21.88	1.12	13.95	73.25
	Zn	11.23	10.41	20.3	32.13	1.12	14.45	80.25
	(Mn+Fe+Zn)	13.5	13.26	23.13	38.6	1.25	16.73	93.28
	Control	8.78	7.06	18.2	21.93	1.11	9.85	64.28
	Mn	10.2	7.78	19.43	27.35	1.12	13.6	76.28
Bacillus polymyxa	Fe	11.15	10.52	20.23	28.08	1.29	14.88	77.45
	Zn	12.9	10.91	22.25	36.98	1.33	15.8	84.5
	(Mn+Fe+Zn)	15.43	15.25	27.4	42.13	1.51	17.38	98.7
	Control	10.05	9.17	19.73	24	1.2	10.8	67.3
	Mn	11.55	9.92	19.9	29.5	1.23	13.83	79.7
Azotobacter chroococcum	Fe	11.75	11.29	21.53	30.1	1.37	15.5	79.88
	Zn	14.03	11.75	24.4	38.28	1.4	16.68	88.55
	(Mn+Fe+Zn)	17.25	16.43	29.33	44.15	1.69	17.95	103.95

Table 4. Contd.

	Control	10.78	8.67	21.83	26.23	1.2	11.63	70.4
	Mn	13.13	10.43	22.63	31.15	1.33	14.73	83.23
Azospirillum brasilense	Fe	13.8	13.1	24.28	32.63	1.5	16.1	83.63
	Zn	15.75	13.11	26.38	41.48	1.7	19.3	92.73
	(Mn+Fe+Zn)	19.95	17.89	32.3	49.55	1.98	20	110.98
LSD at 5%		0.14	0.5	0.22	1.53	0.24	0.28	0.41

gibberllins, cytokinine, amino acids and other auxin compounds which considerably stimulate plant growth and consequently chemical constituents. Mahfouz and Sharaf El-din (2007) found that application of biofertilizer which was a mixture of *Azotobacter, Azospirilum* and *Bacillus* increased total carbohydrates in the dry fennel plant. Moreover, Mahmoud et al. (2006) and Santa et al. (2004) observed that the contents of nitrogen, total amino acids and crude protein in shoots being inoculated with *Azospirillum* sp. were more pronounced in wheat plants.

The obtained results in this study are in agreement with those obtained by Aly et al. (2009), Zaki et al. (2009) and Bashan et al.(2006), they found a significant increases in photosynthetic pigments (chl. a, b and carotenoids), crude protein, soluble sugar, polysaccharide, total soluble solids (T.S.S.), and sucrose in sugar beet, broccoli and wheat plants respectively inoculated with biofertilizers presowing, such as *B. polymyxa*, *A. chroococcum* and *Azospirillum* as compared with un-inoculated treatment. Data in Table 4 revealed that all the mentioned physiological parameters were significantly increased by spraying the plants with micronutrients as compared with the control plants. The highest values obtained by spraying plants with (Mn+Fe+Zn) treatment followed by Zinc, Fe and Mn treatment in decreasing order.

The favorable effect of foliar application with the used micronutrients may be due to the beneficial effect of these micronutrients on plants and their involvement in the other process, carbohydrate and nitrogen metabolism as well as plant resistance to diseases and the adverse environmental conditions. Micronutrients are essential for organization and rapid alternation of nutritional compounds with plant owing to their great importance in contribution to direct the enzymes way in metabolism (Massoud et al., 2005). The superior effect of foliar application with Zn comparing with those of Fe and Mn may be due to the essential role of Zinc in plant physiology, where it activates some enzymes such as dehydrogenises, pretenses, and peptidases. Moore and Patrick (1989) and Romheld and Marchener (1991), reported that the basic functions of Zn in plants are related to the metabolism of carbohydrates, proteins, auxins, RNA and ribosome functions. Zinc stimulates plant resistance to dry and hot weather and to bacterial and fungal diseases (Weinberg, 1997). Moreover, El-Kabbany (1992) and Farahat et al. (2007) stated that foliar application of Zn, promoted the content of chl. a, chl. b, carotenoids and total soluble sugars, such increments might be attributed to the significant increase in photosynthetic pigments content, which reflected on photosynthesis process and led to an increase in carbohydrates content of wheat. The obtained results in this study are in accordance with those obtained by Farahat et al. (2007), which added that soluble sugar content was significantly affected as a result of foliar application with different micronutrients. Concerning the effect of the interaction between different biofertilizers and foliar application with micronutrients as shown (Table 4) significantly affected all the physiological parameters of wheat plants. The highest values were obtained when the wheat grains were inoculated with *A. brasilense* and the plants sprayed with solution contained (Mn+Fe+Zn) treatment. While the lowest values were obtained when the grains were not inoculated with any bacteria (control) and plants sprayed by tap water. Our results reflect the positive combined effect of both nutrients and biofertilizer in enhancing plant growth and metabolism. The data obtained by Shehata et al. (2010) ensured that plants treated with the combination of mineral nitrogen fertilization and soil inoculation with the biofertilizer (*A. chroococcum* and/or *Azospirillium lipoferum*) recorded the highest and significant values of growth parameters, i.e. plant height, leaf number as well as fresh and dry weight of leaves,

Table 5. Effect of different biofertilizers and foliar application with micronutrients on nutrients content of wheat plants.

Treatments		Macronutrients (mg/g)				Micronutrients (mg/kg)			
		N	P	K	Mg	Fe	Mn	Zn	Cu
Effect of biofertilizer									
Un-inoculated (control)		14.6	10.8	25.15	6.57	782.75	293.25	44.96	6.75
Bacillus polymyxa		16.59	11.78	26.34	7.66	818.5	315.2	47.88	8
Azotobacter chroococcum		17.91	12.67	27.1	8.24	837.65	324.45	49.5	8.65
Azospirillum brasilensel		19.84	13.96	28.28	8.71	861.19	337.7	52.45	10.2
LSD. at 5%		0.14	0.11	0.2	0.1	20.03	7.75	0.2	0.7
Effect of foliar application									
Control		11.9	9.78	22.47	5.77	780.69	215.56	24.42	6.44
Mn		13.91	10.85	25.38	7.33	808.44	334.94	40.55	7.25
Fe		17.28	11.5	26.42	7.59	849	325.44	49.95	8.44
Zn		18.27	13.06	28.52	8.61	825.75	344.81	55.48	9.25
(Mn+Fe+Zn)		24.82	16.32	30.8	9.61	862.06	367.5	73.1	10.63
LSD at 5%		0.16	0.12	0.22	0.12	22.42	6.84	0.22	0.8
Interaction biof. x foliar application									
	Control	10.3	7.65	21.03	4.25	746	202.25	22.08	4.5
	Mn	10.78	9.58	23.7	5.73	751	312.25	38.08	5.75
Un-inoculated (control)	Fe	14.78	10.15	24.78	6.5	801.5	307.5	44.85	7.25
	Zn	16.4	12.38	26.45	7.75	784	312.5	51.05	7.5
	(Mn+Fe+Zn)	20.73	14.23	29.8	8.6	831.25	331.75	68.75	8.75
	Control	11.13	9.4	22.2	5.68	771.75	211.5	24.1	6
	Mn	13.38	10.65	24.43	7.3	806.25	333.25	40.35	7.25
Bacillus Polymyxa.	Fe	16.83	10.75	25.88	7.33	841.5	321	49	8.5
	Zn	17.38	12.73	28.78	8.38	831.25	347.25	54.33	8.5
	(Mn+Fe+Zn)	24.23	15.38	30.4	9.6	841.75	363	71.63	9.75
	Control	12.6	10.78	22.83	6.35	792.75	216.5	25.18	7
	Mn	14.73	11	25.8	7.88	825.5	341.5	41.15	7.5
Azotobacter Chroococcum.	Fe	17.8	11.8	28.88	8.08	861.5	331.75	51.23	8.75
	Zn	18.4	13.03	28.9	8.95	836	353	56.95	9.5
	(Mn+Fe+Zn)	26.03	16.73	31.08	9.83	872.5	379.5	73	10.5
	Control	13.55	11.3	23.8	6.8	812.25	232	26.33	8.25
Azospirillum brasilense.	Mn	16.75	12.15	27.58	8.4	851	352.75	42.6	8.5
	Fe	19.7	13.3	28.15	8.43	891.5	341.5	54.7	9.25
	Zn	20.88	14.1	29.93	9.35	851.75	366.5	59.6	11.5
	(Mn+Fe+Zn)	28.3	18.93	31.93	10.58	902.75	395.75	79	13.5
LSD at 5%		0.31	0.25	0.44	0.23	44.27	1.65	0.44	0.69

chlorophyll carotenoids, N, P and K contents in tissues of celeriac leaves.

Nutrients content

Regarding the effect of inoculation with the applied biofertilizers, on the concentration of macro (N, P, K and Mg) and micronutrients (Fe, Mn, Zn and Cu) in wheat straw (Table 5), results show that various treatments significantly increased all the mentioned nutrients as compared with those of un-inoculated plants (control). The highest values of the nutrients concentration were recorded when wheat grains were inoculated with *Azospirillum brasilense*, followed by *Azotobacter chroococum* and *B. polymyxa* in decreasing order.

The favorable effect of inoculating wheat grains before planting on nutrients concentration may be due to one or more of the following reasons:

1. Increasing water and mineral uptake from the soil (Sarig et al., 1984).
2. Increasing of root surface area, root hairs and root elongation as affected by *Azotobacter* (Sunjaravelu and Muthukrishinan, 1993).
3. Increasing the ability to convert N_2 to NH_4 and thus make it available to plant (Hanafy et al., 1997).
4. Enhancing the production of biological active fungicidal substance, which may change the microflora in the rhizosphere and affect the balance between harmful and beneficial organisms (Apte and Shendi, 1981).

As well as Mirzakhani et al.(2009) evaluate the effect of *A. chrooccocum* inoculation on seed yield and yield components of safflower, which significantly affected, because the biofertilizers can fix atmospheric nitrogen and increase phosphorus availability in soil and enhanced elements absorbance by safflower. El-Desuki et al. (2010) stated that all tested vegetative growth parameters as well as pea pods content of N, P, K, Mn, Fe, total protein and total carbohydrate were significantly increased and improved with using the highest level of biofertilizers. While the study of Rajeawari (2011) revealed that *Azotobacter,* even at low densities, can activity fix nitrogen, the pot culture experiment of his study showed a significant increases in shoot, root length of the wheat plants, he recommend that *Azotobacter* can survive in soil and fix atmospheric nitrogen, and can be used as a suitable biofertilizer in order to reduce the usage of chemical fertilizer which is potent harmful substances mainly protochemicals. Moreover, Montemurro et al. (2008) confirmed the important role of biofertilizers in reducing soil pH value, by secreting acid such as propionic, fumaric and succinic which brought about the dissolution of nutrients bound to organic materials and render them available for growing plants. The obtained results in this study are in harmony with those obtained by Aly et al. (2009) and Zaki et al. (2009), who stated that inoculating the grains before plantation with different biofertilizers significantly increased the concentration of different nutrients (N, P, K, Fe, Mn, Zn and Cu) in different plant organs.

It is appear from data in Table (5), that spraying wheat plants with different micronutrients led to significant increases in the nutrients concentration of wheat plants as these values compared with those obtained by the control treatment. These results are in good agreement with those obtained by Gamal El-Din and Reda (2005) on wheat plants. Results also show that the highest values of macronutrients were obtained by using foliar solution contains (Fe+Mn+Zn), followed by solution contains Zn, Fe and Mn respectively in decreasing order. Results indicated that micronutrients concentration in wheat plants differed as the foliar treatments were differed. This means that the application of any element individually significantly increased its content in wheat plants, and also enhanced the content of the other micronutrients. In this concern Nassar (1997) found that addition of micronutrients (Fe+Mn+Zn) to wheat plants, simultaneously give an additional enhancing effect of N, P and K content as compared with the individual application. Moreover, the superior effect of the triple treatment may be due to the suitable balance between the aforementioned micronutrients (Fe, Zn, and Mn) which enable plants to grow well and absorb more quantities of N, P and K. Results in this study are in good agreement with those obtained by Hanafy et al. (2008) and Mahmoud et al. (2008) who stated that spraying micronutrients on plants significantly increased plant content of N, P, K, Fe, Mn, Zn and Cu as well as chlorophyll content a, b, a+b and carotenoids.

The interactions between the main investigated factors (un-inoculated treatment, inoculated with *B. polymyxa*, *A. chroococcum* and *Azospirillum brasilense*) and sub main treatments (foliar application with Mn, Fe, Zn and (Mn+Fe+Zn) and control treatments) had a significant effect on all the studied nutrients (macro and micronutrients). The highest values of nutrients concentration were appeared when the wheat grains were inoculated with *A. brasilense* bacteria and plants sprayed with solution contained (Mn+Fe+Zn) treatment, while the lowest were found when wheat grains did not inoculated and plants sprayed with tap water (control).

Interaction improved the nutritional status of wheat plants, over that of the individual treatment of each. These results may be attributed to the efficiency of biofertilizers in the soil, was higher to fix atmospheric nitrogen as well as production of physiologically active compounds to stimulate root growth and the solubilization availability of elements from soil native sources which subsequently improved the nutritional status of whole plant tissues. It can be concluded from the obtained results that foliar application with (Mn+Fe+Zn) could be recommended for using with inoculation by biofertilizers especially *A. brasilense* bacteria in order to enhance the nutritional status of wheat plants grown on the new reclaimed sandy soil.

CONCLUSION AND RECOMMENDATION

To prevent the environmental pollution from extensive application of fertilizers, the effective microorganisms could be recommended to farmers to insure the public health and a sustainable agriculture. The data collected proves that the use of *Azospirillum, azotobacter* or *Bacillus*, in combination with foliar application with micronutrients (Mn+Fe+Zn) can lead to higher wheat yield.

The technology is cheap, easy to handle. Therefore, it can serve as a strategic component for farmers to cope with the harsh environments and to increase livelihood resilience in newly reclaimed soils in Egypt and similar areas. Further researches must apply to quantify the

numerous effects of biofertilizers on growth and yield of crop plants. The local community should be sensitize on the use of biofertilizon to improve farming and thus help alleviate poverty.

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