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Impact of Humic Acid Application, Foliar Micronutrients and Biofertilization on Growth, Productivity and Quality of Wheat (*Triticum aestivum,* L.)

Radwan, F. I., M. A. Gomaa, I. F. Rehab and Samera, I. A. Adam

Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt

ABSTRACT

This investigation aim to reduce the gap between production and human consumption in wheat by increase wheat productivity per feddan. In this respect, two field experiments were carried out at the Agriculture research station (Saba Basha) Alexandria, University, Egypt during 2012/2013 and 2013/2014 cropping winter seasons to study the impact of three humic acid rates (0, 2 and 4 kg/fed.), micronutrients (Zn, Mn and Zn +Mn), and biofertilization treatments (uninoculation, phosphorein and cerealine) as the third factor and their interactions on yield, its components and quality of wheat "cv. Misr1". The obtained results indicated that increasing humic acid application up to 4 kg/fed., significantly, increased most of the studied characters in both seasons. Also, foliar (Zn + Mn) mixture application gave the highest values of studied characters, however cerealine, generally, surpassed other biofertilizers treatment inoculation for all the studied characters, in both seasons of study. Interact Humic acid x micronutrients had significant effects on all studied traits in both seasons except spike length (cm) and grain phosphorus content in the first season and number of spikelets/spike in the two cropping seasons. Moreover, humic acid x biofertilization interactions had significant effects on number of spikes/m^2, grain and biological yields /fed., grain P and K contents in the two seasons, 1000- kernel weight (g), grain protein and N- content in the first season, straw yield and harvest index in the second season. On the other hand, interactions between micronutrients and biofertilizer had significant on grain yield tons/fed., number of spikes/m^2 and grain K content in the two seasons, 1000- kernel weight in the first season and biological yield/fed., besides harvest index in the second season. With the second order interaction had significant effects on number of spikes/m^2, grain yield/fed., and grain K content in both seasons, number of spikelets/spike in the first season and biological yield tons/fed, harvest index besides grain protein, protein and phosphorus contents in the second season.

Key words: Humic acid, micronutrients, biofertilization, wheat, yield, its components, quality.

Introduction

Wheat (*Triticum aestivum*, L.) is one of the most important crops used in human food and animal feed in Egypt. Increasing wheat production is an essential national target to fill the gap between production and consumption. Recently, great attentions of several investigators have been directed to increase the productivity of wheat to minimize the gap between the Egyptian production and consumption by increasing the cultivated area and wheat productivity per unit area.

Humic acid is water-soluble organic acid naturally present in soil organic matter. It can be recognized that humic substances (HS) have many beneficial effects on soil structure and soil microbial populations, as well as, increase modify mechanisms involved in plant growth stimulation, cell permeability and nutrient uptake causing increases (Mackowiak *et al.,* 2001, Atiyeh *et al*., 2002 and Rahmat *et al.,* 2010). On the other hand, Delfine *et al.,* (2005) investigated the effect of application of humic acid on growth and yield of durum wheat. Moreover, they specified that the application of humic acid caused a transitional production of plant dry mass with respect to unfertilized control. Application of the humic acid had statistically significant effect on Mg, Fe and Mn uptake. Humic acid raised the dry weight and N, P, K, Ca, Mg, Na, Fe, Cu, Zn and Mn uptake of plants at non limed pots and the amounts were found high at 0.1 % dose of humic acid. The second dose (0.2 %) was found much more effective on dry weight and nitrogen uptake at high lime conditions (Katkat *et al*., 2009). Better performance of FYM than humic acid with significant differences in many parameters including plant height, leaf area index, chlorophyll content, biological yield, grain yield, and N content of grains. However, more research is needed to evaluate the effectiveness of humic acid as an organic fertilizer and to compare higher humic acid rates with FYM due to increase crop production (Daur, 2013).

Micronutrients play a pivotal role in the yield improvement (Rehm and Sims, 2006). They are needed in trace amounts, but their adequate supply improve nutrients availability and positively affects on cell process physiological and that is reflected in yield as well (Taiwo *et al*., 2001). The use of Micronutrient is important

Corresponding Author: Samera Ibrahim Ali Adam, Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria, University.
E-mail: samiraibrahim98@yahoo.com

because of increasing economic and environmental concerns (Soleimani, 2006), Khan *et al*. (2006) reported that Cu, Fe, Mn and Zn contents of wheat leaf and grain increased with application of mineral fertilizers. grain yield, straw yield, 1000-grain weight and number of grains/spike, Fe, Mn and Zn concentration in flag leaves and grains as well as, protein content in grain were significantly increased by application of these elements (Mn +Zn) (Zeidan *et al*., 2010). Also, Zinc (Zn) is an essential mineral nutrient for plant and human growth, and dietary Zn deficiency is a worldwide nutritional problem. Foliar Zn application significantly improved the grain Zn concentration of maize by 27% and 37% and of wheat by 28% and 89% during the first and second growing seasons, respectively. The maize grain Fe concentrations during both growing seasons were also enhanced by foliar Zn application. The foliar application of Zn realized higher grain Zn recoveries of 35.2‰ and 42.9‰ in maize as well as 26.4‰ and 32.3‰ in wheat during the first and second growing seasons, respectively (Wang *et al*., 2012). Foliar application of zinc increased the number of fertile tillers and yield of wheat, however, have little effect on the agronomic characteristics of no-tilled crop with high nutrient content in soil (Zoz *et al*., 2012). Foliar Zn-enriched fertilizer applications greatly increased Zn concentration and bioavailability in both whole grain and grain fractions. Compared with foliar Zn alone, foliar Zn combined with N increased Zn concentration and bioavailability, whereas foliar Zn combined with P decreased Zn concentration and bioavailability. However, foliar Zn combined with P slightly increased the protein concentration compared to foliar Zn alone. Protein concentration significantly increased, whereas phytate concentration decreased, in whole grain and flour, both in soil N and foliar Zn-enriched N treatments. Therefore, foliar Zn plus N (with appropriate soil N management) may be a promising strategy for addressing dietary Zn micronutrient deficiencies, especially in countries where flour is a significant component of the daily diet (Li, 2015). Soil applied Zn at all levels significantly improved the maximum yield related traits of all wheat cultivars as compared with control. Though all the Zn levels excelled compared with control; however, the plots receiving Zn at 8 and12 kg ha-1 observed higher wheat output along with higher grain Zn contents (Nawaz *et al.,* 2015).

Biofertilizer contains live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic micro-organisms used for application to seed, soil or composting areas to accelerate microbial processes to augment the extent of availability of nutrients. Biofertilizer play a pivotal role for increasing the number of microorganisms and accelerate certain microbial process in the rhizospher of inoculated soil of plant, which can change the available forms of nutrients into plants (Zaki *et al*., 2007). Using of either organic or biofertilizer are considered a safe alternative for chemical fertilizers, which cause environmental pollution when they are used extensively (Basha, 2004 and Abdel-Aal *et al.,* 2007). Biofertilizers inoculation significantly increased most growth and yield parameters, yeast had superiority on Azotobacter. Moreover, mixed inoculums, generally, had more favorable effect on the majority of studied parameters than single inoculants (Amal *et al.,* 2011). Combined application of biofertilizers caused considerable increase in plant height over all the treatments. Tillering enhanced significantly due to application of bio-fertilizers either alone or in combination. Greater tillering was noticed when the crop received combined treatments than other treatments. Similar trend of results was also observed in case of yield components of wheat ie ears/m2, grains/ear and 1000 grain weight increased significantly when the crop received bio-fertilizers either alone or combined. Accordingly, the highest grain yield was recorded when the crop received combined bio-fertilizers (Singh and Prasad, 2011). Whereas, biofertilizer (*Azotobacter* sps.) treatment applied alone was very effective in promoting physiological parameters. When Biofertilizer was added to FYM the effect was better .Treatment of Jinong (J), the liquid organic fertilizer containing humic acid applied alone was very effective compared to Biofertilizer alone, FYM alone or Bf + F. Application of 0.2% J was generally better than 0.3% J. Moreover, Jinong + Biofertilizer was more effective than Biofertilizer + FYM except chlorophyll a, Addition of FYM to 0.2% J proved to be the best among the test applications. Combined treatment of Bf + FYM + Jinong showed very less or negative effect (Jan and Boswal*, 2015)*. The highest values of such traits were obtained in treatment (75% mineral N + biofertilizer with *Azotobacter* and *Azospirillum*). However, (50% mineral N + biofertilizer with *Azotobacter* and Azospirillum) resulted also higher values for the above mentioned traits comparing with (100% nitrogen and uninoculated) but the differences among the two treatments almost did not attain the statistical differences. They concluded that the biofertilizers (double-inoculation of *Azotobacter* and *Azospirillum*) of efficient strains could save 25 or 50 % of the recommended dose of mineral N (Abd El-Lattief, 2012). With respect to the response of multi-strain application, significant increments were recorded in number of seeds/main head, number of seeds/tiller and yield in the second season. Organic and biofertilizer led to an increase in number of reproductive tillers/plant, number of seeds/main head, number of seeds/tiller, number of seeds/plant, plant yield (g/plant), 1000 seed weight (gm) and yield (Mohammed *et al*., 2012).

The aim of this investigation was designed to study the effect of humic acid rates, micronutrients and biofertilization and their interaction on yield and its components, chemical compositions and technological characters in wheat crop.

Materials and Methods

Two field experiments were conducted at the Agriculture research station, the faculty of Agriculture (Saba Basha) Alexandria University, during 2012/2013 and 2013/2014 seasons to study influence of humic acid rates, some micronutrients and biofertilizer treatments and their interactions on growth, yield and its attributes and grain chemical contents of bread wheat “c.v. Misr1 “. Split- split plot design with three replicates was used in both seasons. Three humic acid rates (0, 2 and 4 kg/fed.(fed.=feddan=0.42 ha.), were randomly assigned in main plots, however, three foliar micronutrients treatments (Zn, Mn and Zn + Mn) by 2.5, 2.5 and 1.25+1.25 kg/fed., respectively, spraying 2 times after 45 and 60 days from sowing, were allocated the sub- plots and three biofertilizer experiments ((uninoculation, phosphorein and cerealine at 400 g/fed.) were randomly distributed in sub-sub plots. Wheat was sown on 15th and 18th November in the two growing winter seasons, respectively, after maize, seeding rate was 70 kg/fed., and plot size was (10.5 m^2) (1/400 feddan) with 3.5 m length and 3.0 m width. Humic acid treatment were carried out with irrigation at sowing, while, cerealine and Phosphorein applied and mixed will with wetted stick, wheat grains with Arabic gum. Other culture practices for wheat production were conducted as recommendations. Soil Physical and chemical analysis were carried out in the two cropping seasons and were shown in Table (1).

Table 1: Some Physical and chemical properties of the experimental soil in2012/2013 and 2013/2014 seasons.

Soil properties																		
Season		A) Mechanical analysis						Soluble cations (1 : 2) (cmol/kg soil)			Soluble anions (1 : 2) (cmol/kg soil)							
	Clay %	Sand %	Silt %	Soil texture	PH (1 :1)	PH (1 : 1)	K+	Ca++	Mg++	Na++	CO3- - + HCO3-	Cl-	SO4-	CaCO3 (%)	Total N	Available P mg/kg	OM
2012/ 2013	38	32	30	Clay loam	8.20	8.31	1.52	9.4	18.3	13.50	2.90	20.4	12.5	6.50	1.00	3.70	1.4
2013/ 2014	37	33	30	Clay loam	3.80	3.70	1.54	8.7	18.5	13.8	2.80	19.8	12.6	7.00	0.91	3.55	1.40

At harvest, one square meter was randomly taken in each sub- sub plot to measure number of spikes/m^2. While, ten random spikes were chosen in each sub-sub plot to measure spike length (cm) and number of spikelets/spike. One thousand kernel weight (g) was determined as an average of three samples. Total biological, grain yields were determined by harvesting all plants in each sub-sub plot and converted to tons/fed.

Data obtained was exposed to the proper method of statistical analysis of variance difference among mean of different treatments as described by Gomez and Gomez (1984). The treatments means were compared using the least significant differences (L.S.D.) test at 5% level o probability by using the split- split model as obtained by CoStat 6.311, 1998-2005 as statistical program.

Results and Discussion

Yield and its components

Data in Tables (2) showed that humic acid application had significant effects on spike length, number of spike/m^2, number of spikelets/spike and 1000-kernel weight in the two seasons of study. Increasing humic acid rate from zero up to 4 kg/fed., led to gradually and significant increasing for the previous traits by 15.56% and 14.66% for spike length, 28.73% for number of spike/m^2, 23.52% and 29.03% for number of spikelets/spike and 16.90% and 23.90% for 1000- kernel weight in the first and the second seasons, respectively. These increase in the yield components referred to the favorable effect of humic acid for improving early plant growth and increasing dry matter accumulation in wheat grains. These results were generally agreed with these obtained Serenella *et al*. (2002), Zandanadi *et al*. (2007) and Rahmat *et al*. (2010).

Results in that table revealed that micronutrients application had significant effects on the studied traits in both seasons except 1000- kernel weight in the second season (Table, 2), where, zinc and manganese mixture had the tallest spikes (15.07 and 14.89 cm), highest number of spike/m^2 (318.74 and 326.96), highest number of spikelets/spike (40.19 and 40.69) and heaviest grains (50.19 and 49.07 g) in the two successive seasons. Conversely Zn application gave the lowest values, i.e. 13.52 and 13.59 cm; 67.04 and 266.85; 35.22 and 34.98 and 45.96 and 46.48 g for the previous characters in the first and the second seasons, respectively. These results indicate the importance of micronutrients especially pH of the soil of experiment was high (8.20 and 8.31) istle was successive seasons.

Results are shown in Table (2) also, indicated that wheat grain treated with phosphorein or cerealine biofertilizer were significantly superior than control treatment (uninoculation) for the studied traits in that table in both seasons except number of spikelets/spike in the second season. On the other hand, cerealine biofertilizer produced taller spikes (15.07 and 15.41 cm), higher number of spikelets/spike (41.41 and 38.98) and heavier grains (53.22 and 52.59 g) in the two successive seasons and higher number of spike/m^2 (335.0) only in the second season. These increases might be due to the stimulation effect of micro-organisms that produce plant phytohormonas as IAA, GAs and CKs, which promote plant growth and cell division, hence encouraging photosynthesis and assimilates accumulation (El-Khawas,1990) and (Hussein *et al* (2005). These results were in agreement with those obtained by Basha (2004), Ibrahim *et al*. (2004) and Zaki *et al*. (2007).

Table (2), also, showed the first and second order interaction, it was significant or not significant at 0.05 level of probability in these studied parameters.

Table 2: Spike length, number of spike/m^2, number of spikelets/spike and 1000- kernel weight (g) as affected by humic acid rates, micronutrient and bio-fertilization during 2012/2013 and 2013/2014 seasons.

Treatments	Spike length (cm)		No. of spikes/m^2		Number of sikelets/spike		1000-grain weight (g)	
	2012/2013	2013/2014	2012/2013	2013/2014	2012/2013	2013/2014	2012/2013	2013/2014
A) Humic acid rates								
Untreated	13.56b	13.44c	252.70c	258.22c	34.18c	33.72c	44.26c	45.26b
Humic acid 2kg/fed	14.11b	14.11b	301.15b	297.52b	37.56b	37.33b	47.59b	46.11b
Humic acid 4kg/fed	15.67a	15.41a	331.59a	332.41a	42.22a	43.51a	51.74a	52.36a
L.S.D at (0.05)	0.71	0.17	2.16	2.39	0.96	2.87	1.21	4.51
B)*Micronutrients*								
Zn	13.52b	13.59c	267.04c	266.85c	35.22c	34.98b	45.96c	46.48a
Mn	14.74a	14.48b	299.67b	294.33b	38.57b	38.88a	47.44b	48.44a
Zn + Mn	15.07a	14.89a	318.74a	326.96a	40.19a	40.69a	50.19a	49.07a
L.S.D at (0.05)	0.41	0.34	3.11	1.60	0.65	2.13	1.45	3.27
C)Biofertilization								
Uninoculation	13.52b	13.07c	281.41c	252.81c	34.15c	36.37a	42.56c	42.81c
Phosphorein	14.74a	14.48b	315.78a	300.33b	38.41b	39.20a	47.81b	48.59b
Cerealine	15.07a	15.41	288.26b	335.00a	41.41a	38.98a	53.22a	52.59a
L.S.D at (0.05)	0.65	0.35	1.53	1.89	0.48	2.87	2.37	2.87
Interactions								
AXB	N.S	*	**	**	N.S	N.S	*	N.S
AXC	N.S	N.S	**	**	N.S	N.S	**	N.S
BXC	N.S	N.S	**	**	N.S	N.S	**	N.S
AXBXC	N.S	N.S	**	**	**	N.S	N.S	N.S

- *, **: *significant difference at 0.05 level of probability.* - *N.S.: Not significant at 0.05 level of probability.*

Figures (1, 2, 3, and 4) indicated the interaction between humic acid rate and micronutrient effect on spike length, number of spikes/m^2 and 1000- kernel weight (g) in the first or second or both cropping seasons. Whereas, increasing humic acid at rate (4 kg/fed.) with Zn + Mn mixture produced the highest mean values of these traits, while the lowest ones were obtained from 0 kg/fed., of humic acid (untreated) with Zn. That might e due to the complementary effect of both factors on plant growth and dry matter accumulation. While, figures (5, 6, and 7) showed the interaction between humic acid rates and biofertilization treatments, where, the highest mean values were resulted from increasing humic acid rates up to 4 kg/fed., with cerealine biofertilizer while the lowest ones were obtained with 0 kg/fed., of humic acid (untreated) plus uninoculation treatments. These results proved that both humic acid and biofertilizer especially cerealine are important for stimulation plant growth and dry matter accumulation (El-Khawas, 1990) and (Hussein *et al* (2005). On the other hand, obtained results from figures (8, 9, and 10) reported that inoculation grain with cerealine biofertilizer produced the highest mean values of these characters with foliar spraying with Zn + Mn mixture.

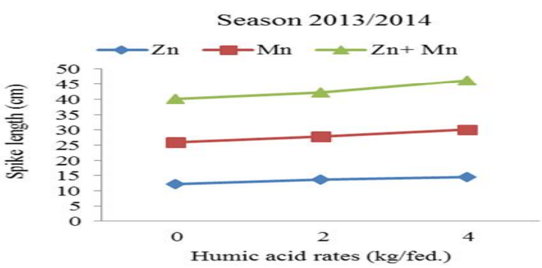

Fig. 1. Interaction between humic acid rates and micronutrient on spike length during season 2013/2014.

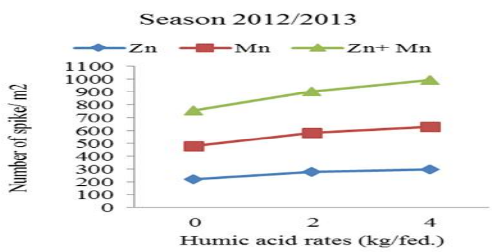

Fig. 2. Interaction between humic acid rates and micronutrient on number of spikes/m2 during season 2012/2013.

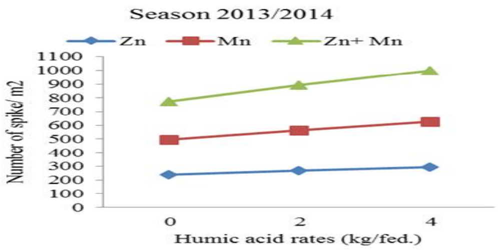

Fig. 3. Interaction between humic acid rates and micronutrient on number of spikes/m2 during season 2013/2014.

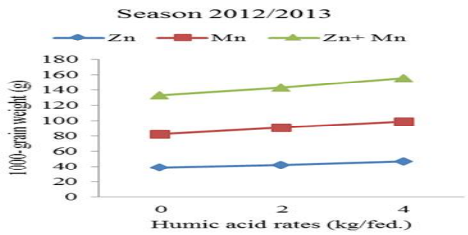

Fig. 4. Interaction between humic acid rates and micronutrient effect on 1000- kernel weight (g) during season 2013/2014.

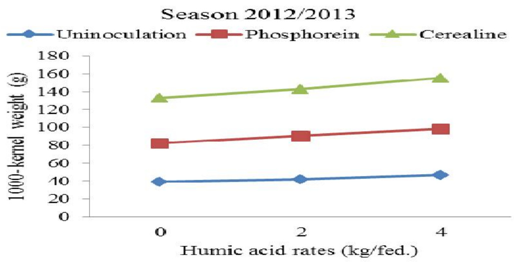

Fig. 5. Interaction between humic acid rates and biofertilizer on 1000- kernel weight (g) during season 2012/2013.

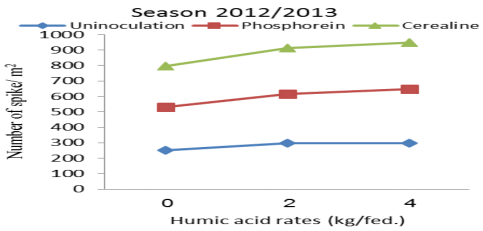

Fig. 6. Interaction between humic acid rates and biofertilizer on number of spikes/m2 during season 2012/2013.

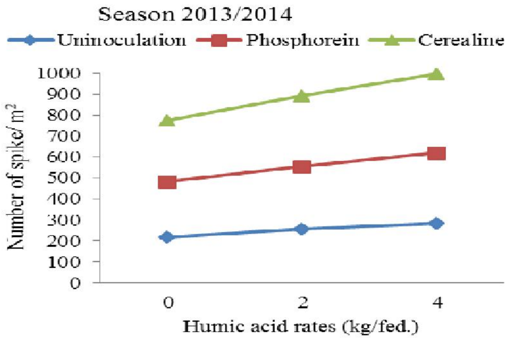

Fig. 7. Interaction between humic acid rates and biofertilizer on number of spikes/m2 during season 2013/2014.

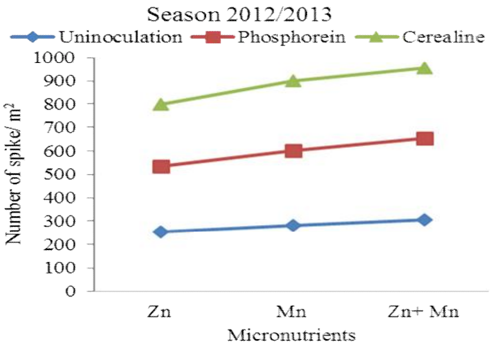

Fig. 8. Interaction between micronutrient and biofertilizer on number of spikes/m2 during season 2012/2013.

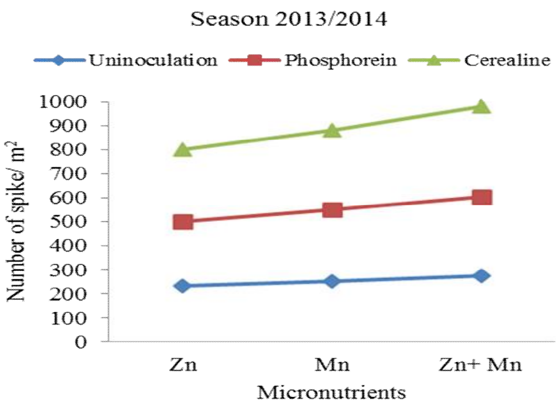

Fig. 9. Interaction between micronutrient and biofertilizer on number of spikes/m2 during season 2013/2014.

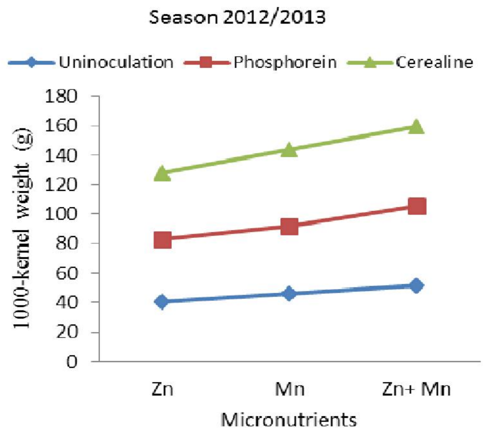

Fig. 10. Interaction between micronutrient and biofertilizer on 1000- kernel weight (g) during season 2012/2013.

Data are shown in Table (3) indicated that both grain and biological yields and harvest index were significantly affected with humic acid rates, micronutrients application and biofertilizer treatments in the two seasons. The highest humic acid application rate (4kg/fed) produced the highest grain yield (2.77 and 2.80 ton /fed), biological yield (6.42 ton /fed) in both seasons, however, differences between 3 and4 kg humic per feddan did not reach the significance level for harvest index in the two seasons. Superiorly of grain yield with 4 kg of humic acid per feddan might due to its favourable effect on number of spike/m^2, number of spikelets/spike, 1000-grain weight, biological yields and harvest index in the two seasons. Mixture of zinc + manganese application produced the highest grain yield (2.65 and 2.65 ton/fed) and biological yield (6.55 and 6.45 ton /fed) and lowest harvest index (40.36 % and 41.09%) in the two successive seasons. These results were in harmony with these obtained by (Soleimani, 2006) and Gueins *et al*. (2003).

On the other side, cerealine as biofertilizer significantly increased grain yield (2.90 and 2.86 tons/fed), biological yield (6.64 and 6.72 tons/fed.) and harvest index (43.67 % and 42.56 %) in the first and the second seasons, respectively, compared to phosphorein.

Data presented in Tables (3) showed that increasing either humic acid levels or zinc, manganese mixture application led to increase grain yield/fed in the two seasons. However, the highest grain yields (2.78 and 2.78 tons/fed.) and (2.80 and 2.85 ton /fed), resulted from manganese separate or mixed with zinc at 4 kg humic acid rate in the two successive seasons, respectively. However, manganese separate or mixed with zinc without humic acid application produced (6.62 and 6.67 ton /fed) besides zinc or manganese application at4 kg humic acid /fed gave (6.43 and 6.67 tons/fed.) of biological yield/fed in the first season, while the highest biological yield (6.73 ton /fed) in the second season resulted from spraying zinc + manganese mixture with 4kg/fed of humic acid.

Promotion in plant growth and the nutrients uptake with the addition of humic acid had been reported by various researchers (Chen and Aviad, 1990; David, *et al*., 1994; Fagbenro and Agboda, 1993).

As for harvest index, the highest values (46.30 % and 4.24 %) in the first season, resulted from zinc application without humic acid using and manganese with humic acid at 4kg/fed. conversely, manganese applied with 3 or 4 kg/fed., of humic acid produced the highest harvest index (43.37 % and 43.48%) and that was along with zinc application under at 4kg humic acid per feddan which produced (44.59%). Concerning with humic acid x biofertilization effects, data in Table (9) stated that grains treated with cerealine and 4kg/fed of humic acid produced the highest grain yield (3.17 and 3.12 tons/fed.) in the two successive seasons, biological yield (7.09 tons/fed.) in the first season, harvest index (45.32%) in the second season. In the contrast, the highest biological yield in the second season (6.91 ton/fed) resulted from phosphorein under 4 kg/fed., of humic acid.The previous results indicated that humic acid application enhanced the micronutrients uptake (Hosam El-Din, 2007) and spraying with micronutrients led to activation of physiological process and uptake of plant nutrients (Arif *et al*., 2006 and Nadim *et al*., 2012). Also, nitrogen fixation bacteria are important for increasing the endogenous phytohormones which increase mineral absorption (Hosam El-Din, 2007 and Zaki *et al*. (2007). Also, Table (3) pointed out the first and second order interaction, it was significant or not significant at 0.05 level of probability in these studied parameters.

Table 3: Grain yield (tons/fed.) and biological yield (tons/fed.) and harvest index (%) as affected by humic acid, micronutrient and biofertilization during 2012/2013 and 2013/2014 seasons.

Treatments	Grain yield (ton/fed)		Biological yield (ton/fed)		Harvest index (%)	
	2012/2013	2013/2014	2012/2013	2013/2014	2012/2013	2013/2014
A) Humic acid rates						
Untreated	2.37c	2.33c	6.08b	6.05b	38.98b	38.51b
Humic acid 2kg/fed	2.59b	2.61b	6.04b	6.09b	42.88a	42.86b
Humic acid 4kg/fed	2.77a	2.80a	6.42a	6.42a	43.05a	43.61a
L.S.D (0.05)	0.05	0.03	0.16	0.09	2.54	0.87
B) Micronutrients						
Zn	2.50c	2.49c	5.62c	5.88c	44.48a	42.35a
Mn	2.58b	2.60b	6.37b	6.23b	40.50b	41.73ab
Zn + Mn	2.65a	2.65a	6.55a	6.45a	40.36b	41.09b
L.S.D (0.05)	0.04	0.04	0.16	0.08	1.97	0.80
C) Biofertilization						
Uninoculation	2.28c	2.34c	5.68c	5.53c	40.14c	42.31a
Phosphorein	2.55b	2.55b	6.22b	6.33b	41.00b	40.35b
Cerealine	2.90a	2.86a	6.64a	6.72a	43.67a	42.56a
L.S.D (0.05)	0.03	0.05	0.22	0.14	2.42	1.49
Interactions						
AXB	*	*	**	**	**	**
AXC	**	**	**	**	N.S	**
BXC	*	*	N.S	*	N.S	*
AXBXC	*	*	*	**	N.S	*

*- *, **: significant difference at 0.05 level of probability.* *- N.S.: Not significant at 0.05 level of probability.*

Figures (11, 12, 13, 14 and 15) indicated the interaction between humic acid rate and micronutrient effect on grain yield tons/fed., biological yield tons/fed and harvest index (%)in the first or second or both cropping seasons, 2012/2013 and 2013/2014. Whereas, increasing humic acid at rate (4 kg/fed.) with Zn + Mn mixture produced the highest mean values of these traits, while the lowest ones were obtained from 0 kg/fed., of humic acid (untreated) with Zn. That might e due to the complementary effect of both factors on plant growth and dry matter accumulation.

While, figures (16, 17, 18, 19 and 20) showed the interaction between humic acid rates and biofertilization treatments, where, the highest mean values were resulted from increasing humic acid rates up to 4 kg/fed., with cerealine biofertilizer while the lowest ones were obtained with 0 kg/fed., of humic acid (untreated) plus uninoculation treatments. These results proved that both humic acid and biofertilizer especially cerealine are important for stimulation plant growth and dry matter accumulation (El-Khawas, 1990) and (Hussein *et al* (2005).

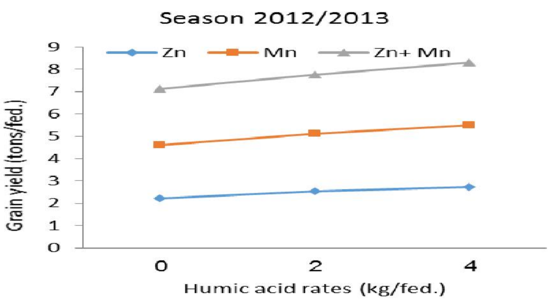

Fig. 11. Interaction between humic acid rates and micronutrient on grain yield (tons/fed.) during season 2012/2013.

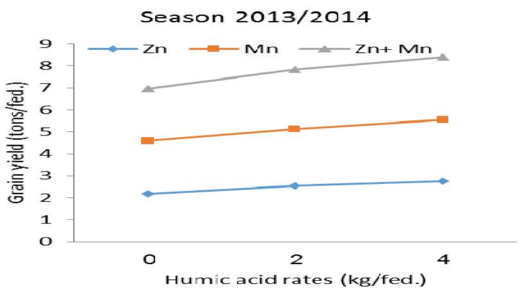

Fig. 12. Interaction between humic acid rates and micronutrient on grain yield (tons/fed.) during season 2013/2014.

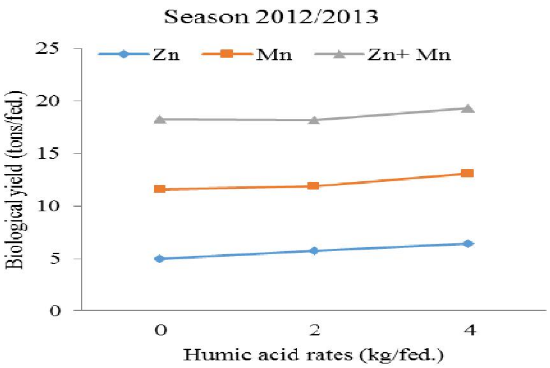

Fig. 13. Interaction between humic acid rates and micronutrient on biological yield (tons/fed.) during season 2012/2013.

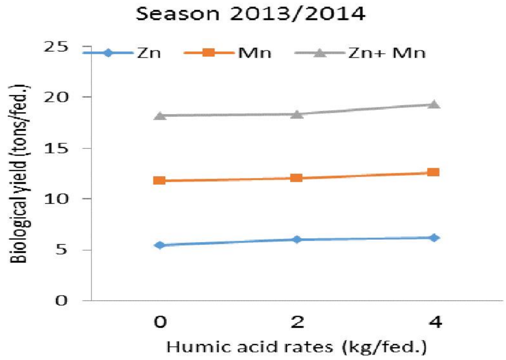

Fig. 14. Interaction between humic acid rates and micronutrient on biological yield (tons/fed.) during season 2013/2014.

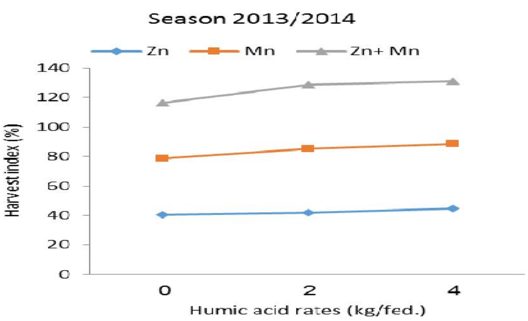

Fig. 15. Interaction between humic acid rates and micronutrient on harvest index (HI %) during season 2013/2014.

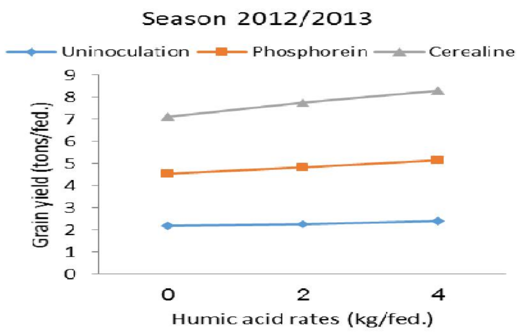

Fig. 16. Interaction between humic acid rates and micronutrient on grain yield (tons/fed.) during season 2012/2013.

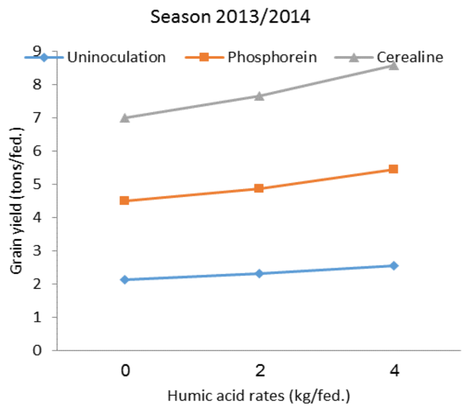

Fig. 17. Interaction between humic acid rates and biofertilizer on grain yield (tons/fed.) during season 2013/2014.

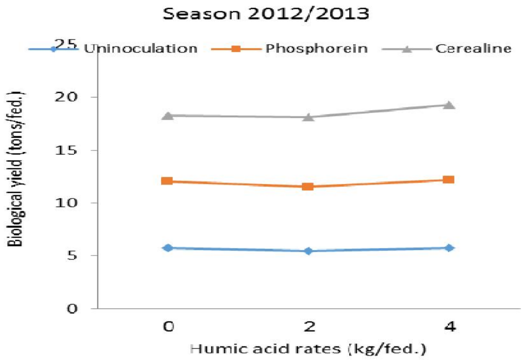

Fig. 18. Interaction between humic acid rates and biofertilizer on biological yield (tons/fed.) during season 2012/2013.

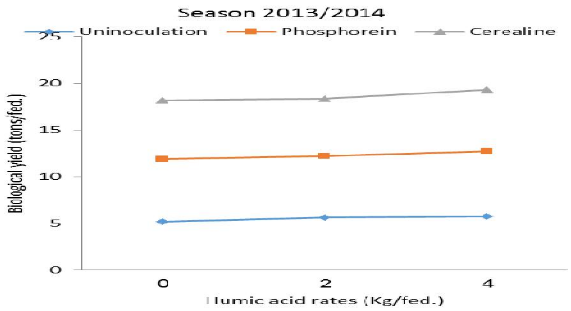

Fig. 19. Interaction between humic acid rates and biofertilizer on biological yield (tons/fed.) during season 2012/2013.

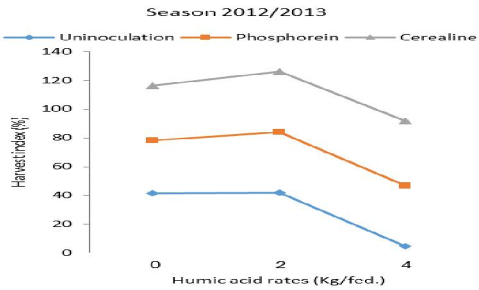

Fig. 20. Interaction between humic acid rates and biofertilizer on harvest index (HI %) during season 2012/2013.

Chemical composition of grains

Data in Table (4) illustrated the three studied factors and their interactions on crude protein, nitrogen, phosphorus and potassium content in the two seasons. Generally, increasing humic acid rate up to 4kg/fed produced, significantly, high protein content (14.06 % and 13.31%), N- percent (2.25 and 2.13), phosphorus percent (0.70 and 0.71) and potassium (2.87 and 2.60) in the first season and the second seasons, respectively. Also, Zn and Mn mixture gave the highest values (13.36, 13.01, 2.14, 2.08, 0.66, 0.65, 2.44 and 2.34) for the previous traits, respectively. Data, also, revealed that inoculation with cerealine produced the highest protein content and nitrogen % in the second season, phosphorus % in the first season and potassium % in both seasons.

With respect to humic acid rates x micronutrients interaction effect on the studied traits, data in Table (4) revealed that zinc, manganese mixture spraying at 4kg humic acid gave the highest values of chemical composition of wheat grains, i.e. (14.75% and 14.13%) for protein content, (2.36 and 2.26) for nitrogen percent, (3.37% and 3.17%) for potassium percent in the two successive seasons and 0.78% for phosphorus percent in the second season.

Concerning to humic acid rate x biofertilization treatments, results in Table (4) showed that 4 kg/fed., of humic acid with Phosphorein inoculation produced the highest grain content of crude protein (14.99%) and nitrogen percent (2.39%) in the first season, phosphorus in the second season (0.78%), however cerealine with the same humic acid rate gave the highest potassium percentage (3.30% and 2.89%) in the two successive seasons and both Phosphorein and cerealine under the same humic acid rate gave the highest values for phosphorus percent (0.72% and 0.75%) in the first season. These results were well agreement with those obtained by Shahryari *et al.* (2009) and Mirzamasoumzadeh *et al.* (2012).

On the other side, inoculation with cerealine and spraying with 4 kg/fed., of humic acid per feddan produced the highest potassium percentage (2.74 % and 2.59 %) in the first season and the second seasons, respectively.

Concerning the second order interaction effects, Table (4) indicated that zinc + manganese mixture application combined with 2 or 4kg humic acid per feddan for inoculated wheat grains produced the highest crude protein content (15.18% and 15.52%) and nitrogen percent (2.43 % and 2.48 %) in the first season.

However, 4kg of humic acid with zinc + manganese mixture foliar spraying and inoculated with cerealine gave the highest potassium percentage (3.91% and 3.66%) in the two successive seasons, while grains inoculated with phosphorein with the same humic acid and mixture of zinc + manganese gave the highest phosphorus percent (0.84%) in wheat grains in the second season. Similar results were obtained by El-Sayed and Hammad (2007) and El-Mantawy (2008).

Table 4: Crude protein (%) and NPK (%) as influenced by humic acid rates, micronutrient and bio-fertilization during 2012/2013 and 2013/2014 seasons.

Treatments	Crude protein (%)		Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	2012/2013	2013/2014	2012/2013	2013/2014	2012/2013	2013/2014	2012/2013	2013/2014
B)Humic acid rates								
Untreated	11.14c	10.82c	1.78c	1.73.c	0.54c	0.54c	1.78c	1.71c
Humic acid 2kg/fed	12.23b	11.83b	1.96b	1.89b	0.60b	0.58b	1.95b	1.86b
Humic acid 4kg/fed	14.06a	13.31a	2.25a	2.13a	0.70a	0.71a	2.87a	2.60a
L.S.D (0.05)	0.34	0.08	0.06	0.01	0.05	0.02	0.04	0.04
B)Micronutrients								
Zn	11.70c	10.95c	1.87c	1.75c	0.55b	0.55c	1.95c	1.82c
Mn	12.37b	12.01b	1.98b	1.92b	0.63a	0.63b	2.22b	2.03b
Zn + Mn	13.36a	13.01a	2.14a	2.08a	0.66a	0.65a	2.44a	2.34a
L.S.D (0.05)	0.35	0.19	0.06	0.03	0.03	0.02	0.02	0.07
C)Biofertilization								
Uninoculation	11.68c	10.78c	1.87c	1.78c	0.55c	0.58b	1.95c	1.83c
Phosphorein	13.18a	11.84b	2.10a	1.89b	0.62b	0.66a	2.18b	2.11b
Cerealine	12.57b	13.36a	2.01b	2.14a	0.66a	0.60b	2.47a	2.24a
L.S.D (0.05)	0.54	0.21	0.09	0.03	0.04	0.02	0.02	0.08
Interactions								
AXB	*	**	*	**	N.S	**	**	**
AXC	*	N.S	*	N.S	*	**	**	**
BXC	N.S	N.S	N.S	N.S	N.S	N.S	**	*
AXBXC	N.S	**	N.S	**	N.S	**	**	**

**, **: significant difference at 0.05 level of probability.* - *N.S.: Not significant at 0.05 level of probability*

Conclusion

In Conclusion using humic application of wheat plants with mixture of micronutrients i.e., Zn + Mn plus grain inoculation with cerealine produced highest mean values from yield, it components and quality of most of studied characters under Alexandria conditions.

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