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COMPLEMENTED EFFECT OF HUMIC ACID AND BIOFERTILIZERS ON WHEAT (*TRITICUM AESTIVUM* L.) PRODUCTIVITY

BY

Abou-Aly, H.E. and Mady, M. A.
Botany Dept. Fac. of Agric., Moshtohor, Benha Univ., Egypt

ABSTRACT

The role of humic acid for enhancing biofertilization performance was studied on growth and yield of wheat in newly sand clay soil. Application of arbiscular mycorrhiza (AM) (*Glomus mosseae*) and plant growth promoting rhizobacteria (*Azotobacter chroococcum*) in combination with humic acid was evaluated. The results indicated that mycorrhizal root infection percentage significantly increased by application of humic acid with AM fungus. Inoculation with the biofertilizer agents increased phosphatase and dehydrogenase activity in wheat rhizosphere especially with AM inoculation. The highest values of enzymes activity were observed when the plants were treated by humic acid in the presence of biofertilizers especially with the dual inoculation. There were remarkable increases in available nutrients in rhizosphere of plants those inoculated with any of the two biofertilizers in combination with humic acid. Application of *A. chroococcum* and AM either alone or dual inoculation in the presence of humic acid gave considerable improvement in growth characteristics, photosynthetic pigments as well as nutrients uptake, total charbohydrates and crude protein of wheat plants when compared with either inoculated or uninoculated treatments without humic acid. Concerning endogenous phytohormones in wheat shoots, inoculation with *A. chroococcum* individually gave maximum value of auxins, while application of humic acid especially with dual inoculation of biofertilizers didn't have positive effect on auxins content. On the other hand, humic acid enhanced the effect of biofertilizers on increasing of cytokinins and gibberellins content in wheat shoots and reducing of abscisic acid. Moreover, application of humic acid gave the highest values of grain and straw yield when associated with dual inoculation or *A. chroococcum* individually. Also, maximum values of grain quality were obtained from plants those treated with dual inoculation and humic acid. Therefore, application of humic acid can be considered as a good approach in enhancement of biofertilizers performance in newly reclaimed soil.

Key words: Humic acid, biofertilizers, mycorrhizae, *A. chroococcum*, wheat, chlorophyll, endogenous hormones, grain yield

INTRODUCTION

Wheat enjoys a privileged position amongst food grain crops in the world in general and particularly in Egypt where it serves as a staple food for the majority of the population. Hence, under the prevailing circumstances, restoration and maintenance of soil fertility is a basic and critical problem, particularly in the newly reclaimed soil. This can be accomplished by adding organic material, biological active substances and plant growth-promoting microorganisms, in addition to other field practices (Akhtar *et al*., 2007). Soil organic contents are one of the most important parts that they directly affected the soil fertility and textures as well as increasing the microbial activities in the soil (Tejada *et al*., 2006).

In recent years, humic substances can be added to the soil for improvement the crop yield. From the point of view of producers, these chemical preparations have been perceived and accepted as a kind of hormone promoting the growth rather than improving the chemical and physical conditions of the soil (Cacco and Dell Agnolla, 1984). A benefit of humic acid is its ability to complex metal ions and can form aqueous complexes with micronutrients. It is the subject of studies in various areas of agriculture, such as soil chemistry, fertility, plant physiology as well as environmental sciences, because the multiple role by these materials can greatly improve plant growth and the plant nutrient uptake and was particularly important for the transport and availability of micronutrients (Bohme and Lua, 1997 and Turkmen *et al*., 2004). Also, humic acid may form an enzymatically active complex which can carry on reactions that are usually assigned to the metabolic activity of living microorganisms (Sellamuthu and Govindaswamy, 2003).

Microorganisms are important for agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers. Plant growth-promoting rhizobacteria (PGPR) can affect plant growth directly by the synthesis of phytohormones and vitamins, inhibiting plant ethylene synthesis, enhancing stress resistance, improving nutrient uptake, fixing atmospheric nitrogen, solubilizing inorganic phosphate and mineralizing organic phosphate (Lucy *et al*., 2004 and Cakmakci *et al*., 2007). One of the most often reported PGPR is *A. chroococcum*. The beneficial effect of these bacteria is attributed to IAA production and to some extent to non-symbiotic N_2-fixation. So, these bacteria can potentially be used to improve wheat nutrition of micronutrients (Rajaee *et al*., 2007). Arbuscular mycorrhizae (AM) are symbiotic associations formed between plants and soil fungi that benefit both partners. The role of AM in acquisition and sorption of nutrients from the soil has been recognized. Pronounced response had been obtained in the solubility of micronutrients in newly reclaimed soil when mycorrhiza was accompanied with organic substrates (Habashy *et al*., 2008).

The present work is designed to evaluate integration between humic acid as soil enhancer and biofertilizers with *A. chroococcum* and mycorrhiza for improving the growth and yield of wheat in newly reclaimed soil.

MATERIALS AND METHODS

The study was conducted on newly soil cultivated with wheat (*Triticum aestivum* L. c.v. Sakha 93) at El-Bostan region, El-Behera Govern., Egypt during winter seasons of 2006/2007 and 2007/2008. Interaction effects between humic acid and endomycorrhizal fungi (*Glomus mosseae*) combined with *A. chroococcum* on growth and yield of wheat were studied. Some physical and chemical properties of the experimental soil were estimated according to Jackson (1973) and Black *et al*. (1982), respectively (Table A).

Table (A): Physical and chemical analyses of the experimental soil.

Particle size distribution %				**Soil chemical properties**			
Sand	**Silt**	**Clay**	**Texture class**	**pH**	**$CaCo_3$ %**	**OM %**	**EC (ds/m^{-1})**
65.25	10.21	24.54	**Sandy clay loam**	8.16	14.27	0.97	1.43

Soluble cations and anions m mol/L							**Available nutrients (ppm)**						
Ca^{++}	**Mg^{2+}**	**Na^+**	**K^+**	**CO_3^{2-}**	**HCO_3^-**	**Cl^-**	**N**	**P**	**K**	**Fe**	**Mn**	**Zn**	**Cu**
7.92	4.25	9.19	0.63	0.00	2.96	11.81	25.8	3.1	13.7	2.1	0.62	0.39	0.36

Humic acid

Humic acid (85%) which contain 56% C, 4.5% H, 31% O and 4.5% N was obtained from Sphinx for International trade Company, Cairo, Egypt.

Mycorrhizal inoculation

Arbuscular mycorrhizal fungus (*Glomus mosseae*) was obtained from Agric. Microbiol. Dept., Soils, Water and Environment Res. Inst., Agric. Res. Center, Giza, Egypt. Micorrhizal inoculum consisted of root, hyphae, spores and growth media from a pot culture of onion plants which was previously infected with *Glomus mosseae* and grown for 4 months in pot culture. The standard inoculum (400 kg/fed.) contained about 270 spores/g. Spores of the fungus were measured by a wet-sieving and decanting technique (Gerdemann and Nicolson, 1963).

Azotobacter chroococcum

Growth regulators producing *Azotobacter chroococcum*-previously isolated and identified by El-Mehiy (2007) in Botany Dept., Fac. of Agric., Benha Univ., Egypt- was used as seed inoculants. The tested bacteria were grown on modified Ashby's medium (Abdel-Malek and Ishac, 1968) at 30˚C for 7 days just before seed inoculation to reach the final density of 25×10^8 cfu/ml. Grains of wheat were mixed with the suspension for 30 min. Arabic Gum (16%) was applied to the grains as an adhesive agent. The grains were left to air-drying in shade, and then the grains became ready for sowing.

Experimental design

Grains of wheat (Sakha 93) were successfully washed with water and air-dried. Then, grains were soaked in solution of humic acid (2g /L) for 2 hrs and/or cell suspension of *A. chroococcum.* The grains were sown on the 15^{th} and 17^{th} of November in the two growing seasons, respectively. The experiments were arranged in randomized complete block design with three replicates. The plot area was 10.5 m^2 (3x 3.5m). All plots except N_2-fixer treatments received nitrogen fertilizers at the rate of 200 kg/fed urea (46 % N) in two equal doses (before the first and second irrigation). While, *A. chroococcum* treatments were supplemented with a half dose of inorganic N-fertilizer. Calcium super phosphate (15.5% P_2O_5) and potassium sulphate (48 % K_2O) were added before cultivation in both seasons at the rates of 150 and 100 kg/ fed., respectively. Humic acid was added at the rate of 5 kg/fed. after 30 and 60 days from sowing in two equal doses, while mycorrhiza was added just before sowing. The other required culture practices for growing wheat were followed as recommended.

This experiment included the following treatments:

1- Control.
2- Humic acid.
3- Arbiscular mycorrhiza (AM)
4- *A. chroococcum.*
5- Humic acid + AM
6- *A. chroococcum* + Humic acid
7- *A. chroococcum* + AM.
8- *A. chroococcum* **+** AM + Humic acid

Microbial activities

Microbial activities of the plants rhizosphere after 45 days from sowing were conducted. Mycorrhizal infection was microscopically estimated on a sample of fresh root as described by Giovannetti and Mosse (1980) after clearing and staining (Vierheilig *et al*., 1998). The samples were analyzed for dehydrogenase activity according to the method described by Casida *et al*. (1964) while phosphatase activity was determined by the method given by Drobnikova (1961). Rhizosphere samples were analyzed for available nitrogen according to Page *et al*. (1982), available phosphorus was determined according to (A.P.H.A, 1992), available potassium according to Chapman and Pratt (1961) and available Fe and Zn were determined according to Page *et al*. (1982).

Sampling and collecting data

Nine plants of wheat from each treatment were randomly taken at 70 and 100 days after sowing to measure different morphological characteristics (plant height (cm), number of tillers/ plant, leaves dry weight (g/plant) and total leaf area (cm^2 /plant) using the disk methods according to Derieux *et al.* (1973).

Photosynthetic pigments

Chlorophyll a, b and carotenoids were colorimetrically determined in fresh leaves of wheat plants at 70 and 100 days after sowing during the two seasons according to the methods described by Wettstein (1957) and calculated as mg/g fresh weight.

Chemical composition

Samples from wheat leaves at 70 and 100 days after sowing and grains at harvest were taken to determine total nitrogen (Horneck and Miller, 1998), phosphorus (Sandell, 1950), potassium (Horneck and Hanson, 1998). Also NPK uptake was calculated after determination of NPK according to (Chapman and Pratt, 1961). Total carbohydrate was determined according to (Dubois *et al*., 1956). Crude protein was calculated according to the following equation: Crude protein= Total nitrogen x 5.75 (A.O.A.C., 1990).

Endogenous phytohormones

Endogenous phytohormones were quantitatively determined in wheat shoots at 80 days after sowing in the second season using High- Performance Liquid Chromatography (HPLC) according to Koshioka *et al*. (1983) for auxin (IAA), gibberellic acid (GA_3) and abscisic acid (ABA) while, cytokinins were determined according to Nicander *et al*. (1993).

Yield characteristics

At harvest, three plants were randomly taken /plot from each treatment for estimation of number of spikes/plant, grain yield (g)/plant, straw yield (g)/plant and weight of 1000 grains (g).

Statistical analysis

Data obtained in this study were statistically analyzed by using the least significant differences test (L.S.D) according to Senedecor and Cochran (1980).

RESULTS AND DISCUSSION

Mycorrhizal colonization and soil enzymes

Results of mycorrhizal colonization percent shown in Table (1) exhibited a gradual increase with inoculation by AM fungi, while it showed no significant increase with individual application of humic acid or *A. chroococcum* comparing to the control treatment. Mycorrhizal root infection was significantly increased by application of humic acid in combination with AM fungi. The results were in agreement with those obtained by Habashy *et al*. (2008) who reported that organic compounds significantly increased colonization of mycorrhiza. It was also noticed from Table (1) that individual application of humic acid or biofertilization with AM or *A. chroococcum* significantly increased phosphatase and dehydrogenase activity in wheat rhizosphere as compared to the control treatment. The combined inoculation with *A. chroococcum* and AM increased enzymes activity more than the individual inoculation. Also, the highest values of enzymes activity were recorded in rhizosphere of the plants that treated with humic acid in the presence of biofertilizer especially the dual inoculation. This may be due to the mechanisms of *Azotobacter* and AM on soil properties, also *Azotobacter* require large amounts of available carbon for their survival in soil. Addition of humic acid may be of special importance in restoring optimal levels or organic matter for plant growth and for microbial activity which associated with enzymes activity (Karaca *et al.* (2006). These results showed a good agreement with Sellamuthu and Govindaswamy (2003) who reported an increase in enzymes activity with application of humic acid. They also reported that the microbial population and soil enzymes in the rhizosphere could be built up for the efficient utilization of nutrients.

Available nutrients in wheat rhizosphere

Data in Table (2) show that significant increases in available macronutrients (N, P and K) and some micronutrients (Fe and Zn) were observed when wheat plants received humic acid or individually inoculated with *A. chroococcum* or AM as compared to control plants. Application of humic acid with either AM or *A. chroococcum* exhibited values of available nutrients greater than the treatments of biofertilizers without humic acid. Also, application of humic acid with the dual inoculation gave the maximum values of available nutrients. This may be due to the ability of humic acid to complex metal ions in agricultural systems, also humic acid can form aqueous complexes with soil nutrients, though not to the same extent as many synthetic chelating agents. Since humic acid binds to soil colloidal surfaces, it is not easily leached (Mackowiak *et al*., 2001). On the other hand, the function of all mycorrhizal systems depends on the ability of the fungal symbiont to absorb inorganic and/or organic nutrients available in soil (Turkmen *et al*., 2005). Also, Habashy *et al*. (2008) found that a pronounced response had been obtained in the solubility of nutrients when mycorrhiza was accompanied with organic substances compared to AM inoculation or organic substances added alone. This may be due to that the addition of organic substances which improved the physical properties of the soil, and increased the supplying power of available nutrients to plants.

Table (1): Mycorrhizal colonization and activity of some soil enzymes in the rhizosphere of wheat as affected by humic acid and biofertilizers after 45 days from sowing during the two growing seasons (S_1 and S_2).

Characters / Treatments	Mycorrhizal colonization %		Phosphatase (µg inorganic-P/g soil/day)		Dehydrogenase (mg of TPF/g soil/ 24 h)	
	S_1	S_2	S_1	S_2	S_1	S_2
Control	9.3	15.8	26.7	32.1	23.4	31.1
Humic acid (HA)	11.6	23.2	39.7	46.8	38.4	36.2
Mycorrhiza (AM)	43.6	61.7	45.8	49.2	42.6	51.3
A. chroococcum	15.2	28.6	35.4	38.6	41.2	48.2
AM + HA	56.3	71.2	46.9	50.4	69.1	55.7
***A. chroococcum* + HA**	12.8	25.3	37.9	49.7	51.4	57.8
***A. chroococcum* +AM**	68.9	61.7	57.6	51.6	63.8	52.5
***A. chroococcum* +AM +HA**	53.1	67.5	61.5	53.5	82.3	69.4
LSD at 5%	6.1	5.7	3.8	3.5	4.6	3.2

Table (2): Available nutrients of wheat rhizosphere as affected by application of humic acid and biofertilizers after 45 days from sowing during the two seasons (S_1 and S_2).

Characters / Treatments	Available-N (ppm)		Available-P (ppm)		Available-K (ppm)		Available-Fe (ppm)		Available-Zn (ppm)	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Control	41.4	56.8	3.88	5.12	17.4	19.2	2.53	2.74	0.54	0.56
Humic acid (HA)	66.2	72.4	6.31	7.83	18.9	20.6	3.65	2.95	0.72	0.62
Mycorrhiza (AM)	71.4	65.9	6.21	9.15	21.5	23.7	3.19	3.62	0.53	0.61
A. chroococcum	69.3	71.6	5.70	6.83	20.6	19.4	2.46	3.37	0.58	0.51
AM + HA	89.2	96.5	8.64	9.98	21.7	23.4	4.26	4.83	0.78	0.84
***A. chroococcum* + HA**	91.6	87.0	6.61	7.04	23.7	25.8	4.05	4.94	0.60	0.75
***A. chroococcum* + AM**	87.3	80.7	8.23	9.70	25.0	23.0	4.52	4.02	0.72	0.67
***A. chroococcum* + AM + HA**	98.4	106.3	9.34	9.61	25.8	23.7	4.71	4.28	0.89	0.77
LSD at 5%	10.8	11.3	1.12	0.8	1.06	0.83	0.66	0.37	0.07	0.05

Growth parameters

As shown in Table (3), the growth parameters of wheat plants as plant height, number of tillers/plant, dry weight of leaf/ plant and total leaf area/plant were significantly increased by individual application of humic acid and biofertilizers. Inoculation with *A. chroococcum* in the presence of humic acid or AM significantly increased number of tillers and total leaf area/plant at 70 and 100 days after sowing during the two seasons. In this regard, El-Mehiy (2007) reported that *A. chroococcum* possess a great variety of properties that are interest in the development of biofertilizers including production of growth promoting plant hormones (especially auxins, gibberellins and cytokinins) as well as N_2-fixation. Maximum stimulatory effect of the biofertilizers was obtained when they associated with humic acid application after 70 and 100 days from sowing in the two seasons. These results are in agreement with Turkmen *et al*. (2005) who reported that humic acid application positively affected the plant growth parameters. The mechanism of humic acid that is active in promoting plant growth is not completely known. However, increasing cell membrane permeability, oxygen uptake and root cell elongation are of plant growth factors which were reported. (Russo and Berlyn, 1990).

Photosynthetic pigments

Data in Table (4) indicated that different photosynthetic pigments i.e., chlorophyll a, b and carotenoids in wheat leaves were positively responded to application of humic acid, biofertilizers and their combinations at 70 and 100 days after sowing during the two seasons. Moreover, the interaction between humic acid and dual inoculation with *A. chroococcum* and AM gave the highest values of total pigments during the two seasons as compared with individual treatments and control plants. Generally, these results are to be considered as a good explanation to the obtained data regarding the favorable role of biofertilizers and humic acid on growth parameters (Table 3) that enhanced photosynthetic efficiency and increased dry matter accumulation. Ebrahim and Ali (2004) found that application of *Azotobacter* improved chlorophyll a, b and charotenoids content of wheat leaves.

Table (3): Growth characters of wheat as affected by humic acid and biofertilizers after 70 and 100 days from sowing during the two seasons (S_1 and S_2).

Characters / Treatments	70 days after sowing							
	Plant height cm/ plant		No. of tillers /plant		Dry weight of leaf g/ plant		Total leaf area cm^2/ plant	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Control	36.4	41.7	3.9	4.1	4.05	4.12	1250.3	1330.1
Humic acid (HA)	46.4	47.3	5.1	5.4	4.45	4.70	1380.1	1410.2
Mycorrhiza (AM)	43.5	45.7	5.6	5.5	4.90	5.10	1451.2	1490.4
A. chroococcum	50.4	46.9	5.0	5.4	5.22	5.38	1514.7	1540.3
AM + HA	49.7	52.4	5.9	6.0	5.88	5.72	1640.2	1690.4
***A. chroococcum* + HA**	47.6	50.7	5.7	5.9	5.40	5.92	1701.3	1780.1
***A. chroococcum* + AM**	44.7	49.2	5.2	5.7	5.42	5.60	1550.2	1617.1
***A. chroococcum* + AM + HA**	40.6	43.4	6.1	6.3	6.59	6.44	1843.2	1820.2
LSD at 5%	3.12	3.23	0.5	0.62	0.42	0.60	81.2	97.43
	100 days after sowing							
Control	86.2	89.1	5.2	5.7	5.95	6.25	1401.0	1480.7
Humic acid (HA)	98.5	97.2	7.7	7.4	9.20	9.62	1680.4	1885.9
Mycorrhiza (AM)	94.7	95.2	6.9	6.4	10.60	10.35	1835.2	1820.3
A. chroococcum	96.5	97.5	7.2	7.7	9.40	9.65	1620.1	1790.7
AM + HA	100.4	101.6	8.2	8.4	10.70	10.60	1875.2	1910.5
***A. chroococcum* + HA**	98.7	99.4	7.9	7.3	10.25	10.75	1890.3	1905.1
***A. chroococcum* + AM**	103.8	99.8	7.3	7.8	10.20	9.90	1870.2	1850.8
***A. chroococcum* + AM + HA**	92.5	93.5	8.8	8.6	11.20	11.10	1980.0	1960.5
LSD at 5%	5.12	5.9	0.57	0.62	1.25	1.32	102.2	107.1

Table (4): Photosynthetic pigments, total carbohydrates and crude protein as affected by humic acid and biofertilizers after 70 and 100 days from sowing in the two seasons (S_1 and S_2).

Characters / Treatments	Chlorophyll a mg/g F.W		Chlorophyll b mg/g F.W		Carotenoids mg/g F.W		Total pigments mg/g F.W	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
70 days after sowing								
Control	0.57	0.60	0.38	0.36	0.41	0.43	1.36	1.39
Humic acid (HA)	0.79	0.80	0.42	0.46	0.55	0.57	1.76	1.84
Mycorrhiza (AM)	0.67	0.71	0.44	0.48	0.51	0.52	1.62	1.71
A. chroococcum	0.78	0.81	0.51	0.50	0.54	0.56	1.83	1.87
AM + HA	0.87	0.84	0.50	0.52	0.73	0.77	2.10	2.13
***A. chroococcum* + HA**	0.79	0.83	0.52	0.54	0.71	0.74	2.02	2.11
***A. chroococcum* + AM**	0.75	0.77	0.49	0.51	0.62	0.69	1.86	1.97
***A. chroococcum* + AM + HA**	0.99	0.94	0.57	0.60	0.75	0.72	2.31	2.26
LSD at 5%	0.21	0.23	0.19	0.17	0.15	0.12	0.36	0.39
100 days after sowing								
Control	0.59	0.61	0.37	0.34	0.45	0.44	1.41	1.39
Humic acid (HA)	0.81	0.79	0.41	0.43	0.54	0.58	1.76	1.80
Mycorrhiza (AM)	0.71	0.74	0.51	0.54	0.57	0.59	1.79	1.87
A. chroococcum	0.71	0.73	0.58	0.59	0.61	0.62	1.91	1.94
AM + HA	0.87	0.89	0.63	0.62	0.72	0.74	2.21	2.25
***A. chroococcum* + HA**	0.83	0.87	0.62	0.64	0.70	0.73	2.15	2.25
***A. chroococcum* + AM**	0.72	0.78	0.61	0.59	0.64	0.63	1.97	2.00
***A. chroococcum* + AM + HA**	0.92	0.90	0.63	0.62	0.77	0.76	2.32	2.28
LSD at 5%	0.22	0.19	0.12	0.15	0.21	0.18	0.32	0.34

Nutrients uptake and some bioconstituents in leaves

Table (5) clearly indicates that application of both humic acid and biofertilizers significantly increased NPK uptake, total carbohydrates and crude protein content in wheat leaves at 70 and 100 days after sowing during the two seasons as compared with control treatment. Moreover, combination between humic acid and dual inoculation with *A. chroococcum* and AM increased NPK uptake nearly more than two times at 70 days and nearly more than three times at 100 days compared with control treatment. Furthermore, the addition of humic acid associated with both biofertilizers increased nutrients uptake with an pronounced effect, and parallel trend for their increases in the soil (Table 2). This may be attributed to the enhancing effect of humic acid and mycorrhiza on soil physical properties to release nutrients in the rhizosphere which supply a power of available nutrients to plants. The obtained data were in agreement with Turkmen *et al.* (2005) and Habashy *et al.* (2008). Also, Rajaee *et al.* (2007) reported that inoculation of wheat with *A. chroococcum* had a positive effect on nutrients uptake.

Regarding total carbohydrate and crude protein, the same positive trend was observed with application of humic acid and biofertilizers. All treatments showed a significant increase and the maximum one obtained by the interaction between *A. chroococcum* and AM in the presence of humic acid. In this respect, high content of total carbohydrates is a direct result for high rates of photosynthesis with great efficiency that was preceded with large photosynthetic area (Table 3) and high content of photosynthetic pigments (Table 4). The present results are in agreement with those of Ebrahim and Ali (2004).

Table (5): Nitrogen, phosphorus and potassium uptake in wheat leaves as affected by humic acid and biofertilizers after 70 and 100 days from sowing in the two seasons (S_1 and S_2).

Characters / Treatments	N-uptake		P-uptake		K-uptake		Total carbohydrates mg/g D.W		Crude protein mg/g D.W	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
70 days after sowing										
Control	86.6	84.4	11.5	11.9	98.4	85.2	487.3	492.7	123.1	117.9
Humic acid (HA)	163.5	163.5	18.6	20.4	152.4	156.9	560.7	571.4	211.3	200.1
Mycorrhiza (AM)	172.7	184.3	16.0	17.5	155.3	151.9	542.7	560.5	202.7	207.9
A. chroococcum	168.0	181.5	19.8	20.1	169.6	163.8	548.4	555.7	185.2	194.0
AM + HA	219.6	208.2	25.2	24.3	194.6	188.4	588.3	593.8	214.8	209.3
***A. chroococcum* + HA**	199.2	208.6	22.4	25.3	168.2	193.2	590.6	611.7	212.2	202.7
***A. chroococcum* + AM**	191.8	200.4	21.3	21.2	174.5	174.1	568.2	570.1	203.6	205.9
***A. chroococcum* + AM + HA**	255.0	242.4	31.3	28.9	223.0	222.8	614.8	624.4	222.5	216.2
LSD at 5%	27.3	31.4	7.5	9.3	21.8	26.4	37.9	41.4	11.3	15.7
100 days after sowing										
Control	117.8	134.0	17.0	18.0	132.3	146.8	508.1	511.7	113.9	123.3
Humic acid (HA)	329.3	339.1	38.1	42.1	307.2	313.1	615.3	622.8	205.6	202.7
Mycorrhiza (AM)	366.7	365.8	36.0	37.7	343.9	323.7	591.4	598.4	199.0	203.3
A. chroococcum	308.7	325.2	32.3	33.7	311.6	311.2	602.1	610.7	188.9	193.8
AM + HA	410.8	394.3	44.0	43.4	333.8	340.2	630.8	637.4	220.8	213.9
***A. chroococcum* + HA**	387.4	390.7	41.7	45.6	329.8	352.0	658.1	664.2	217.4	209.0
***A. chroococcum* + AM**	366.1	360.8	37.3	39.1	342.7	334.6	622.4	625.3	206.4	209.6
***A. chroococcum* + AM + HA**	443.2	426.2	52.0	49.4	377.4	375.7	673.2	680.4	222.7	220.8
LSD at 5%	55.3	43.1	8.5	9.4	12.3	17.6	38.6	34.1	13.5	12.4

Endogenous phytohormones

According to the data in Table (6), *A. chroococcum* gave maximum values of auxins in wheat shoots compared with all treatments, but inoculation of these bacteria with humic acid or humic acid with AM led to a decrease in auxins content compared to control. Gibberellins and cytokinins were improved by inoculation with *A. chroococcum* or AM and reached the highest values when the biofertilizers were supported by humic acid. Many investigators reported the role of plant growth promoting rhizobacteria such as *A. chroococcum* in the production of hormones such as gibberellins, auxins and cytokinins (El-Mehiy, 2007 and Rajaee *et al*., 2007). On the other hand, abscisic acid, as growth inhibitor, was decreased with using AM or humic acid application while dual inoculation with AM and *A. chroococcum* in the presence of humic acid recorded maximum reduction of abscisic acid content in wheat shoots.

Yield and its components

Data in Tables (7 & 8) showed that, number of spikes, grain yield, weight of thousand grains and straw yield of wheat as well as chemical composition of wheat grains significantly increased in response to any of the tested biofertilizer compared to control. Also, humic acid had positive effect on the same parameters. Moreover, humic acid application triggered and increased the positive effects of *A. chroococcum* and AM inoculation when wheat plants were inoculated with both biofertilizers in the presence of humic acid.

Table (6): Endogenous phytohormones in wheat shoots as affected by humic acid and biofertlizers applications at 80 days after sowing during second season.

Characters / Treatments	Auxins		Gibberellins		Cytokinins		Total promoters		Cytokinins /Auxins		Abscisic acid (ABA)	
	µg/g F.W	±% Relative to control	µg/g F.W	±% Relative to control	µg/g F.W	±% Relative to control	µg/g F.W	±% Relative to control	µg/g F.W	±% Relative to control	µg/g F.W	±% Relative to control
Control	86.7	0.0	60.2	0.0	132.2	0.0	279.1	0.0	1.52	0.0	1.56	0.0
Humic acid (HA)	89.4	+3.1	87.9	+46.0	166.6	+26.0	343.9	+23.2	1.86	+22.4	1.25	-19.9
Mycorrhiza (AM)	110.9	+27.9	70.8	+17.6	170.5	+29.0	352.2	+26.2	1.54	+1.3	1.32	-15.4
A. chroococcum	132.7	+53.1	80.4	+33.6	168.4	+27.4	381.5	+36.7	1.27	-16.4	1.50	-3.8
AM + HA	90.3	+4.2	95.7	+59.0	172.4	+30.4	358.4	+28.4	1.91	+25.7	1.48	-5.1
A. chroococcum + HA	85.8	-1.1	90.3	+50.0	186.3	+40.9	362.4	+29.8	2.17	+42.8	1.20	-23.1
A. chroococcum + AM	114.7	+32.3	79.9	+32.7	168.5	+27.5	363.1	+30.1	1.45	-4.6	1.38	-11.5
A. chroococcum + AM+ HA	82.4	-5.0	102.4	+70.1	193.7	+46.5	378.6	+35.6	2.35	+54.6	0.96	-38.5

Table (7): Yield components of wheat as affected by humic acid and biofertilizers applications during the two growing seasons (S_1 and S_2).

Characters / Treatments	No. of spikes/ plant		Grain yield g/plant		Weight of 1000 grains		Straw yield g/plant	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Control	5.76	6.11	6.35	6.80	42.60	43.50	8.95	8.70
Humic acid (HA)	7.25	7.30	8.40	8.65	50.47	52.60	10.70	10.95
Mycorrhiza (AM)	6.70	6.40	7.75	7.90	48.80	49.20	10.50	10.25
A. chroococcum	6.59	6.96	8.25	8.45	51.20	50.80	10.40	10.35
AM + HA	7.15	7.22	9.45	9.15	52.80	52.85	11.60	11.23
A. chroococcum + HA	7.42	7.60	10.90	10.35	53.35	53.60	11.80	11.75
A. chroococcum +AM	6.90	6.93	9.30	9.70	50.20	51.30	11.20	10.90
A. chroococcum + AM + HA	8.59	8.70	11.72	11.20	55.70	54.42	12.45	12.80
LSD at 5%	0.46	0.31	0.45	0.52	2.05	1.76	0.59	0.64

Table (8): Chemical composition of wheat grains as affected by humic acid and biofertilizers applications during the two growing seasons (S_1 and S_2).

Characters / Treatments	N mg/g D.W		P mg/g D.W		K mg/g D.W		Total carbohydrates mg/g D.W		Crude protein mg/g D.W	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Control	16.7	19.4	2.26	2.56	6.12	6.75	728.2	715.4	96.0	111.6
Humic acid (HA)	21.8	19.8	3.94	3.85	9.80	9.15	734.7	738.8	125.4	113.9
Mycorrhiza (AM)	20.7	20.9	2.35	2.41	8.75	8.20	730.5	744.2	119.0	120.2
A. chroococcum	21.4	19.9	2.82	2.74	7.25	7.40	752.1	794/5	123.1	114.4
AM + HA	21.8	20.6	2.48	2.33	7.90	7.88	760.4	755.2	125.4	118.5
A. chroococcum + HA	21.3	20.5	2.55	2.68	8.25	8.42	750.3	749.8	122.5	117.9
A. chroococcum + AM	20.3	21.2	2.71	3.66	9.95	10.15	769.6	763.5	122.5	121.9
A. chroococcum + AM + HA	22.4	21.7	4.12	3.95	10.70	10.80	787.1	770.4	128.8	124.8
LSD at 5%	1.05	0.95	0.12	1.07	1.25	2.11	12.88	18.50	7.41	5.20

The stimulatory effect of humic acid with dual inoculation on wheat yield would be expected since these applications promoted microbial activities (Tables 1 & 2), growth parameters (Table 3), increased photosynthetic pigments (Table 4), increased nutrients uptake and total carbohydrates (Table 5) as well as endogenous phytohormones (Table 6) as previously resulted and discussed in this work. These findings are supported by Turkmen *et al*. (2005) and Akhtar *et al.* (2007). They reported that the combined application of *Azotobacter* or mycorrhiza with humic substances increased plant yield.

This study clearly indicated that humic acid could have positive effect on plant growth and yield by acting as soil enhancer and as well as by improving its physical properties. Also, the combined application of humic acid with the potent biofertilizers is a good tool for growth and yield promotion as well as improving soil health, particularly in newly soil.

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