

## Influence of Organic and Biofertilizers on Some Soil Chemical Properties, Wheat Productivity and Infestation Levels of Some Piercing-Sucking Pests in Saline Soil

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### ABSTRACT

A field experiment was conducted at Port Said agricultural research station, Port Said Governorate, Egypt, during two successive winter seasons 2018/2019 and 2019/2020 to study the effect of humic acid, biofertilizer (*Azospirillum brasilense*) and their combination on some soil chemical properties, wheat (Masr 1) productivity and natural field infestation under saline soil conditions, compared to control (mineral fertilization only). The studied treatments were arranged within the experimental units in a complete randomized block design in three replicates. Results indicated that all treatments had improved soil chemical properties. There was a significant decrease in EC values in all treatments as compared to control. The best treatment was (humic acid + biofertilizer). Also, all treatments induced a significant increase in soil content of organic matter, while there was a slight decrease in pH values. Available macronutrients (nitrogen, phosphorous, and potassium) and micronutrients (iron, manganese, and zinc) were significantly increased in the soil by using all treatments. The highest increase was achieved with the combined treatment (humic acid + biofertilizer) followed by biofertilizer then humic acid. There was also a significant increase in the wheat content of macro and micronutrients as affected by all treatments. Also, the added treatments caused a significant increase in grains and straw yields as compared to control. The best addition was humic acid + biofertilizer, it increased the grains and straw yields to 2.06 and 2.20 ton/fed., respectively. The most abundant pest families collected were aphids, *Rhopalosiphum padi* Linnaeus (Aphididae), it was the common species followed by *Schizaphis graminum* Rondani; mites, *Oligonychus pratensis* Banks (Tetranychidae) and Thrips, *Thrips tabaci* Linderman (Thripidae).

**Keywords:** Saline soil, Humic acid, *Azospirillum brasilense*, Wheat, *Rhopalosiphum padi*, *Schizaphis graminum*, *Thrips tabaci*, and *Oligonychus pratensis*.

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### Introduction

Plant growth is influenced by a variety of stresses due to the soil environment, which is a major constraint for sustainable agricultural production. These stresses can be classified into two groups, Abiotic and biotic. Abiotic is stress due to the content of salinity, heavy metal in soils, drought, nutrient deficiency while biotic refers to the stresses due to plant pathogens and pest infestation such as insects, mites, viruses, fungi (Pravin-Vejan *et al.*, 2016).

Soil salinity is one of the most important threats to sustainable agriculture of arid and semi-arid regions of the world that salt stress is one of the most serious limiting factors for the crop. Salt affected soils occupy wide regions scattered all over the world, about 954 million of hectares, 0.9

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million ha of them in Egypt. The majority of saline soil is located in the northern-central part of the Nile Delta and on its eastern and western sides. Fifty-five percent of the cultivated lands of the northern Delta region, 20% of the southern Delta and middle Egypt region, and 25% of the Upper Egypt regions are salt-affected soils. Port-Said area parallels to the Suez Canal is one of the newly reclaimed salines that also faces salinity problems. Moreover, the northern regions are mainly saline or saline-sodic soils with heavy texture in Egypt (Shaban *et al.*, 2012).

The wheat plant (*Triticum aestivum* L.) is one of the most important cereal crops in Egypt. Wheat provides 37% of the total calories and 40% of the protein in the Egyptian people's diet. The total production of wheat in Egypt reached 8.6 million tons in 2018, produced from an area of 3.25 million feddan (FAO, 2018).

The use of humic substances in newly reclaimed soils is considered a vital subject, where these soils are characterized by poor fertility, low water holding capacity, high leaching, and alkaline pH (Waili Asal, 2010). Humic acid (HA) modifies the physical, chemical, and biological conditions in soil and affect the solubility of many nutrient elements by building complex forms or chelating with metal cations that improve the crop yield by forming aqueous complexes with micronutrients and enzymatically active complexes, which can be carrying on reactions that are usually assigned to the metabolic activity of living microorganisms. (Verlinden *et al.*, 2009). Humic acid (HA) suspensions based on potassium humate have been applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner that increased cell division, as well as optimizing uptake of nutrients and water and stimulating soil microorganisms for enhancing natural resistance against plant diseases and pest infestations, on the other hand, they increase the permeability of plant membranes and enhance the uptake of nutrients, that improve soil uptake of macro and microelements, making these nutrients more mobile and available to plant root systems. (Montaser *et al.*, 2011).

*Azospirillum braselence* is belonging to the group of plant growth-promoting rhizobacteria (PGPR). It is a Gram-negative nitrogen-fixing soil bacterium, lives in a close association with the roots of economically important crops. *A. braselence* enhances plant growth by producing plant growth-promoting substances, like auxins. This results in increasing the number of lateral roots and root hairs, enlarging the root surface and enabling a higher nutrient and water uptake, it also increased the plant growth and give protection against insect pests and pathogens. (Naeem *et al.*, 2018)

The using of humic acid combined with *A. braselence* is leading to an increase in wheat grain yield, straw yield, the weight of 1000 seeds and growth parameters (as plant dry weight, plant length) as compared to control, furthermore, a significant increase in seeds and shots content of nitrogen, phosphorous and potassium in the treated plots as compared to control (Massoud *et al.*, 2013).

Field pests caused a great yield loss of wheat production; most of the arthropod pests were belongs to three major orders: Hemiptera, Thysanoptera, and Acari almost found to infest wheat in the field. Among these aphids are gaining importance since their population has increased over the last few years where, cereal aphid only, caused yield reduction estimated by up to 23% (Awadalla *et al.*, 2018). Wheat plants were infested by serious phytophagous mite species in different wheat-growing regions and cause huge damage (Ibraheem 2007). Thus, protection and increasing the productions of wheat will be highly appreciated; the cooperation between plant production and plant protection specialists is highly needed to reach this aim.

The objective of the present work aims to evaluate the effect of organic (humic acid) and biofertilizers (*Azospirillum braselence*) on some soil chemical properties, wheat productivity (*Triticum aectivum*, L.) (Masr1) under saline soil conditions, as well as the level of infestation of some piercing-sucking pests, which cause great damage to wheat plants.

## Materials and Methods

A field experiment was conducted at Port Said Agricultural Research Station, Port Said Governorate, Egypt, during two successive winter seasons 2018/2019 and 2019/2020 to study the effect of organic and biofertilizers on soil chemical properties and wheat productivity under saline soil conditions and pest infestation levels. The experiment was carried out in a complete randomized block design in three replicates with a 10.5 m<sup>2</sup> (3 X 3.5 m) plot area. The used organic fertilizer (humic acid) has the properties recorded in Table (1) which was analyzed according to the method described by Brunner and Wasmer(1978).

**Table 1:** Chemical properties of the used humic acid.

PH	EC (dSm <sup>-1</sup> )	O.M (%)	Macronutrients (%)			Micronutrients (mg kg <sup>-1</sup> )		
			N	P	K	Fe	Mn	Zn
7.69	2.86	71.28	2.06	0.39	3.53	386	253	31.58

The biofertilizer was nitrogen-fixing bacteria, *Azospirillum braselence*. The soil treatments understudy were as follows:

1. Control (mineral fertilization only).
2. Humic acid at rate 2 L/400 L water/fed.
3. Biofertilizer at rate 2 L/400 L water/fed.
4. Humic acid (1 L) + biofertilizer (1 L)/400 L water/fed.

The treatments were applied on soil and as a foliar application on the plant after 31, 45, and 65 days of sowing date. Wheat grains (Masr1) were sown on 5<sup>th</sup> December 2018 and 2019 in the two growing seasons. Wheat grains were obtained from the Crop Research Institute, Agriculture Research Center, Giza, Egypt.

Calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) was added at a rate of 200 kg calcium superphosphate/fed during soil preparation. Urea (46 % N) was used as N fertilizer at an application rate of 100 kg N/fed, where it's applied in 3 equal doses after 21, 45, and 60 days of planting. Potassium sulphate (48 % K<sub>2</sub>O) at 70 kg/fed was added on two equal doses after 21 and 45 days of planting. The wheat crop was harvested at mid of May of the two seasons.

### 1. Soil sampling:

Before planting as well as after harvesting of wheat plants, soil samples were taken at soil depths 0-30, 30-60, and 60-90 cm of the studied area, air-dried, ground, mixed, and sieved through a 2 mm sieve and analyzed for some chemical properties and available nutrients. The analysis data of soil samples before planting is shown in Table (2).

Soil pH and organic matter were estimated according to the methods described by Page *et al.* (1982). The total soluble salts (EC) were determined in soil paste extract as dSm<sup>-1</sup> according to Jackson (1973). Particle size distribution was carried out by the pipette method described by Klute (1986). The content of available macronutrients (N, P, and K) and micronutrients (Fe, Mn, and Zn) in the soil was determined according to the methods described by Cottenie *et al.* (1982).

**Table 2:** Mean of physical and chemical properties of the studied soil before planting.

Sand		Silt		Clay		Texture			
31.6		25.1		43.3		Clay			
Soil depth (cm)	EC (dS/m)	pH (1:2.5)	O.M (%)	Available macronutrients (mg.kg <sup>-1</sup> )			Available micronutrients (mg.kg <sup>-1</sup> )		
				N	P	K	Fe	Mn	Zn
0-30	10.12	8.17	0.55	36.0	3.83	168	2.71	1.62	0.55
30-60	10.20	8.20	0.53	35.8	3.79	166	2.25	1.42	0.40
60-90	10.24	8.22	0.52	34.9	3.67	162	2.10	1.19	0.31
<b>Mean</b>	<b>10.19</b>	<b>8.20</b>	<b>0.53</b>	<b>35.6</b>	<b>3.76</b>	<b>165</b>	<b>2.35</b>	<b>1.41</b>	<b>0.42</b>

### 2. Plant analyses:

Samples of wheat grains were taken from each replicate and ground. A 0.5 g powder of grains of each sample was digested by a concentrated digestion mixture of H<sub>2</sub>SO<sub>4</sub>/HClO<sub>4</sub> acids (Sommers and Nelson 1972). Nitrogen was determined by micro Keldahl, according to Cottenie *et al.* (1982). Phosphorus was determined by Spectrophotometric using ammonium molybdate/stannous chloride method (Chapman and Pratt 1978). Potassium was determined by a flame photometer, according to Page *et al.*, (1982). Fe, Mn, and Zn were determined by using Atomic Absorption (model GBC 932) (Cottenie *et al.*, 1982).

Grain protein content was obtained by multiplying grain N concentration by 5.95 according to the method given in AACC (2000). Total Chlorophyll and total proline were determined in the fresh weight of leaf samples taken after 85 days of planting. Chlorophyll was determined according to Saric *et al.* (1967), while proline content was determined according to Bates *et al.* (1973).

### 3. Evaluation of pest infestations of wheat:

The experimental area was divided into plots, and the visual count of all pests attacked ten tillers/ weekly beginning of the first half of February to evaluate the main insect and mite pests attacking wheat plants and their associated predators. Normal agriculture practices were carried out. No pesticides were used throughout the studied season. Mites, aphid species, thrips were counted weekly through the period from seedling appearance till dryness of the plants.

### 4. Seasonal density of pests in Wheat field:

Ten plants were picked up at random from each plot to investigate the number of the cereal pests. The collected specimens were kept in vials containing 75% ethyl alcohol with some drops of glycerin to keep their tissues soft and labeled for the date. Identification by Department of Classification and Crop and Cotton Acarology, Plant Protection Research Institute, Agriculture Research Center, Cairo, Egypt (Awadalla *et al.*, 2018). The mean density of total life stages (immature and adults) of these pests was counted.

### Statistical Analysis:

The data of this study were statistically analyzed through analysis of variance (ANOVA) and the least significant difference (LSD) at the 0.05 probability level to make comparisons among treatment means according to Gomez and Gomez (1984).

## Results and Discussion

### 1. Soil chemical properties:

#### 1.1. Soil electrical conductivity (EC):

It can be deduced from the data of EC in Table (3) show that the EC values (dS/m) were affected by soil treatments. There was a significant decrease in EC values in all treatments as compared to control. The combined treatment (humic + biofertilizer) was the best in decreasing EC values, followed by bio-treatment, while humic acid was the least in decreasing EC values. This may be due to the treatment of humic acid with *Azospirillum braselence* has positive effects in decreasing EC values due to that humic acid has many functional groups that give it a high ability to separate NaCl compound, that cause reduces ameliorate the deleterious effects of salt stress. Also, *A. braselence* uses humic acid as a carbon source, thus may stimulate *A. braselence* to produce phytohormones, which play an important role in decline soil salinity. Also, activation of bacteria in soil caused by humic acid and biofertilizers addition and the influence of biofertilizer or humic acid on total porosity, and improving soil aggregation and possible moving salt soil under irrigation water. In this respect, Sushila *et al.* (2017) suggested that the application of biofertilizers on saline soil decrease soil salinity because the biofertilizers activate microorganisms in soil and dehydrogenase enzyme production in the soil led to decrease the soil salinity compared with control. Mohamed (2012) reported that by application of humic acid, soil salinity was significantly decreased. Shaban and Attia (2009) showed that the values of EC were decreased with the increase in mineral fertilizer in combination with biofertilizer as compared with mineral fertilizers alone. The humic acid application led to decrease soil Na and EC likely due to high supplies of Ca, Mg and K. Ali *et al.* (2013) mentioned that humic acid application to soil led to decrease of soil salinity (EC), which may be due to improvement of the physical-chemical and biological properties of soil.

#### 1.2. Soil reaction (pH):

Data presented in Table (3) show that a slight decrease in pH values as affected by the studied soil treatments compared with control. The highest decrease in pH values was noticed with the combined treatments of humic acid and biofertilizer. The slight decrease in soil pH values may reflect the activity of microorganisms in decomposing organic matter and releasing organic acids. The positive impact of organic fertilizers on soil fertility improvement might be due to the following relationships. First, decomposition and mineralization of nutrients present in the organic material. These results reflected the release of some organic acids as a result of organic decomposition and increase microorganisms which reduces the soil pH while improving nutrient availability. Similar arguments are supported by Janpen *et al.* (2009). Ahmed *et al.* (2016) reported that the soil pH was decreased slightly due to the application of humic acid.

**Table 3:** Chemical properties of the soil as affected by the studied treatments.

Treatments		Soil depth (cm)	EC (ds/m)	O.M (%)	pH (1:2.5)
Control		0 – 30	10.15	0.53	8.19
		30 – 60	10.23	0.52	8.21
		60 – 90	10.26	0.51	8.22
		Mean	10.21 <sup>c</sup>	0.52 <sup>d</sup>	8.21
Humic acid	After 1 <sup>st</sup> addition	0 – 30	8.56	0.58	8.06
		30 – 60	8.63	0.56	8.09
		60 – 90	8.68	0.55	8.11
		Mean	8.62	0.56	8.09
	After 2 <sup>nd</sup> addition	0 – 30	7.81	0.60	8.04
		30 – 60	7.83	0.57	8.05
		60 – 90	7.83	0.56	8.07
		Mean	7.82	0.58	8.05
	After harvest	0 – 30	7.72	0.61	8.04
		30 – 60	7.74	0.60	8.04
		60 – 90	7.79	0.59	8.05
		Mean	7.75	0.60	8.04
	Mean		8.06 <sup>b</sup>	0.58 <sup>c</sup>	8.06
Bio	After 1 <sup>st</sup> addition	0 – 30	7.52	0.61	8.11
		30 – 60	7.59	0.60	8.14
		60 – 90	7.60	0.60	8.15
		Mean	7.57	0.60	8.13
	After 2 <sup>nd</sup> addition	0 – 30	7.34	0.64	8.11
		30 – 60	7.40	0.62	8.12
		60 – 90	7.43	0.61	8.14
		Mean	7.39	0.63	8.12
	After harvest	0 – 30	7.18	0.67	8.08
		30 – 60	7.22	0.65	8.10
		60 – 90	7.23	0.64	8.11
		Mean	7.21	0.65	8.10
	Mean		7.39 <sup>b</sup>	0.63 <sup>b</sup>	8.12
Humic acid + Bio	After 1 <sup>st</sup> addition	0 – 30	6.61	0.71	7.96
		30 – 60	6.68	0.70	7.97
		60 – 90	6.73	0.67	8.00
		Mean	6.67	0.69	7.98
	After 2 <sup>nd</sup> addition	0 – 30	4.90	0.73	7.92
		30 – 60	4.97	0.73	7.94
		60 – 90	4.98	0.72	7.95
		Mean	4.95	0.73	7.94
	After harvest	0 – 30	4.82	0.76	7.90
		30 – 60	4.89	0.75	7.91
		60 – 90	4.91	0.74	7.94
		Mean	4.87	0.75	7.92
	Mean		5.72 <sup>a</sup>	0.72 <sup>a</sup>	7.95
L.S.D			0.69	0.02	-

### 1.3. Organic matter (O.M.):

Organic matter is regarded as the ultimate source of nutrients and microbial activity in the soil. It is clear from the data in Table (3) that all studied treatments had significantly increased the soil content of organic matter as compared with untreated. The best treatment in increasing soil organic matter was the combined treatment (humic acid + biofertilizer), it increased organic matter from 0.51 to 0.67 % (i.e. by about 26%). Humic acid stimulates the activity of microorganisms that led to an increase in organic matter. Also, the biofertilizer helps to a high extent in the decomposition of plant residues that increases soil organic matter content. Similar results were obtained by Khalil *et al.* (2013).

## 2. Macronutrients contents in the soil:

Regarding macronutrients availability, the results indicated that there were positive significant effects on nutrients availability (Table 4). Available nitrogen, phosphorous, and potassium in soil were significantly increased by using all treatments. The highest increase was achieved with the combined treatment (humic acid + bio) followed by biofertilizer then humic acid. Data indicated that the used biofertilizer gave suitable increases in available nitrogen; this may be due to that (*Azospirillum brasilense*) acts as nitrogen fixers.

**Table 4:** Available macronutrients of the soil as affected by the studied treatments.

Treatments	Soil depth (cm)	Available macronutrients (mg.kg <sup>-1</sup> )				
		N	P	K		
Control	0 – 30	37.5	3.83	168		
	30 – 60	36.3	3.80	171		
	60 – 90	35.1	3.78	170		
	Mean	36.3 <sup>d</sup>	3.80 <sup>d</sup>	170 <sup>d</sup>		
Humic acid	After 1 <sup>st</sup> addition	0 – 30	41.4	4.18	179	
		30 – 60	40.3	4.16	176	
		60 – 90	39.4	4.15	175	
		Mean	40.4	4.16	176	
	After 2 <sup>nd</sup> addition	0 – 30	43.6	4.24	183	
		30 – 60	42.8	4.22	180	
		60 – 90	40.5	4.19	179	
		Mean	42.3	4.21	181	
	After harvest	0 – 30	41.5	4.24	178	
		30 – 60	41.1	4.20	177	
		60 – 90	40.8	4.11	178	
		Mean	41.1	4.18	178	
	Mean	41.3 <sup>c</sup>	4.19 <sup>c</sup>	178 <sup>c</sup>		
	Bio	After 1 <sup>st</sup> addition	0 – 30	42.9	4.50	192
			30 – 60	42.8	4.44	189
			60 – 90	42.8	4.42	186
Mean			42.8	4.45	188	
After 2 <sup>nd</sup> addition		0 – 30	44.8	4.60	199	
		30 – 60	44.2	4.55	195	
		60 – 90	43.7	4.52	193	
		Mean	44.2	4.56	195	
After harvest		0 – 30	42.9	4.52	192	
		30 – 60	42.6	4.50	190	
		60 – 90	41.8	4.48	190	
		Mean	42.4	4.50	191	
Mean		43.2 <sup>b</sup>	4.50 <sup>b</sup>	192 <sup>b</sup>		
Humic acid + Bio		After 1 <sup>st</sup> addition	0 – 30	48.6	4.73	197
			30 – 60	48.5	4.71	196
			60 – 90	47.8	4.70	194
	Mean		48.3	4.71	196	
	After 2 <sup>nd</sup> addition	0 – 30	51.8	4.96	207	
		30 – 60	51.6	4.92	204	
		60 – 90	50.9	4.90	202	
		Mean	51.4	4.93	204	
	After harvest	0 – 30	51.4	4.83	201	
		30 – 60	50.2	4.80	198	
		60 – 90	49.1	4.78	197	
		Mean	50.2	4.80	199	
	Mean	50.0 <sup>a</sup>	4.81 <sup>a</sup>	200 <sup>a</sup>		
	L.S.D (0.05)		1.15	0.06	2.99	

Also, the increase in the content (mg.kg<sup>-1</sup>) of available nitrogen, phosphorous and potassium was may be due to the decomposition of organic materials released acids that reduced soil pH which caused nutrients to be more soluble hence more available for plant uptake. Similar results were obtained by

El-Kouny (2007) who found that the application of organic materials caused a substantial increase in total N, available P and K availability were significantly increased in cases of used organic (humic acid) as well as Bio (N-fixing bacteria) Fertilizers. Also, Khalil *et al.* (2013) found an increase in available soil content of N, P, and K after the application of *Azospirillum brasilense*.

### 3. Micronutrients contents in the soil:

Data presented in Table (5) show that there was an increase in soil content (mg.kg<sup>-1</sup>) of available micronutrients (Fe, Mn, and Zn) which are considered as a result of the used treatments. The best treatment in increasing available Fe, Mn, and Zn was humic acid in combination with biofertilizer.

**Table 5:** Available micronutrients in the soil as affected by the studied treatments.

Treatments	Soil depth (cm)	Available macronutrients (mg.kg <sup>-1</sup> )				
		Fe	Mn	Zn		
Control	0 – 30	2.86	1.71	0.59		
	30 – 60	2.40	1.50	0.43		
	60 – 90	2.22	1.20	0.32		
	Mean	2.49 <sup>c</sup>	1.47 <sup>c</sup>	0.45 <sup>c</sup>		
Humic acid	After 1 <sup>st</sup> addition	0 – 30	2.88	1.77	0.60	
		30 – 60	2.46	1.61	0.51	
		60 – 90	2.40	1.43	0.37	
		Mean	2.58	1.60	0.49	
	After 2 <sup>nd</sup> addition	0 – 30	2.93	1.85	0.63	
		30 – 60	2.55	1.69	0.59	
		60 – 90	2.34	1.50	0.45	
		Mean	2.61	1.68	0.56	
	After harvest	0 – 30	2.91	1.81	0.62	
		30 – 60	2.50	1.64	0.55	
		60 – 90	2.36	1.44	0.43	
		Mean	2.59	1.63	0.53	
	Mean	2.55 <sup>c</sup>	1.64 <sup>b</sup>	0.53 <sup>b</sup>		
	Bio	After 1 <sup>st</sup> addition	0 – 30	2.95	1.80	0.61
			30 – 60	2.65	1.71	0.55
			60 – 90	2.41	1.37	0.50
Mean			2.67	1.62	0.55	
After 2 <sup>nd</sup> addition		0 – 30	2.98	1.89	0.66	
		30 – 60	2.76	1.76	0.63	
		60 – 90	2.49	1.43	0.59	
		Mean	2.74	1.69	0.63	
After harvest		0 – 30	2.97	1.82	0.63	
		30 – 60	2.71	1.73	0.60	
		60 – 90	2.48	1.39	0.54	
		Mean	2.72	1.64	0.59	
Mean		2.71 <sup>b</sup>	1.66 <sup>b</sup>	0.59 <sup>a</sup>		
Humic acid +Bio		After 1 <sup>st</sup> addition	0 – 30	3.03	1.98	0.65
			30 – 60	2.70	1.92	0.56
			60 – 90	2.50	1.76	0.52
	Mean		2.74	1.87	0.58	
	After 2 <sup>nd</sup> addition	0 – 30	3.12	2.06	0.69	
		30 – 60	2.84	2.00	0.63	
		60 – 90	2.69	1.87	0.60	
		Mean	2.88	1.98	0.64	
	After harvest	0 – 30	3.09	2.02	0.66	
		30 – 60	2.79	1.94	0.60	
		60 – 90	2.58	1.80	0.55	
		Mean	2.82	1.92	0.60	
	Mean	2.82 <sup>a</sup>	1.93 <sup>a</sup>	0.61 <sup>a</sup>		
	L.S.D (0.05)		0.08	0.06	0.04	

This may be due to the increase of soil organic matter as a result of adding organic and biofertilizers leading to a decrease in soil pH values that causes solubility and availability in micronutrients. Also, Wu *et al.* (2006) found that the activity of *Azotobacter chroococcum*, *Bacillus megatherium*, and *Bacillus mucilaginosus*, led to an increase of water dissolved organic carbon concentration and a decreased in pH value, which enhanced metal mobility and bio-availability. Also, Hussein and Hassan (2011) reported that potassium humate importance due to their ability to chelate micronutrients, thus increasing their bio-availability. El-Galad *et al.* (2013) indicated that the application of potassium humate to saline soil gave the highest soil available Fe, Mn, and Zn values after harvesting.

#### 4. Macro and micronutrients in the wheat plant:

It is clear from the data in the table (6) that all treatments significantly increased the concentrations of macro (nitrogen, phosphorous, and potassium “%”) and micronutrients (iron, zinc, and manganese mg.kg<sup>-1</sup>) in wheat plants in the treated plots as compared to the untreated ones. The highest increase was found in the plots treated by humic acid + biofertilizer, followed by biotreatment then humic acid treatment.

These increases may be attributed to the role of microorganisms in improving micronutrients availability, which was likely attributed to several reasons: 1) releasing of these nutrients through the microbial decomposition of organic materials in the soil; 2) lowering the pH of soil making the nutrients more available; and 3) lowering the redox statues of iron and manganese leading to reduction of higher Fe<sup>3+</sup> and Mn<sup>4+</sup> to Fe<sup>2+</sup> and Mn<sup>2+</sup> and/or transformation of insoluble chelated forms of micronutrients into more soluble ions (Helmy *et al.*, 2013). These results are in agreement with the findings of Khafagy *et al.* (2019), who deduced a significant increase in pea plant content of macro and micronutrients as a result of the application of humic acid in addition to biofertilizers. Moreover, El-Ghamry *et al.*, (2009) reported that the application of potassium humate has significant increases of N, P, and K content in seed and straw of faba bean plants. Ahmad *et al.*, (2013) revealed the increase in the nutrients N, P, and K uptake in the shoot and seeds of pea as affected with potassium humate application under calcareous soil conditions. The positive effect of potassium humate on the uptake of nutrients might be due to its effect on the constancy of membrane permeability and correlated by the surface activity of potassium humate containing both hydrophilic and hydrophobic sites. El-Beheidi *et al.* (2005) found that the applied of biofertilizers alone or combined with mineral N and P fertilizers significantly increased for N, P, and K concentration in pea seeds compared with control.

**Table 6:** Macro-micronutrients concentration in wheat plants after 85 days.

Treatments	N	P	K	Fe	Mn	Zn	Protein	Proline	Chlorophyll
	(%)			(mg kg <sup>-1</sup> )			(%)	(µg/mg)f.w.	(mg/g. f.w)
<b>Control</b>	1.99 <sup>d</sup>	0.31 <sup>d</sup>	2.20 <sup>c</sup>	85.00 <sup>d</sup>	41.19 <sup>d</sup>	14.80 <sup>d</sup>	11.84 <sup>d</sup>	30.12 <sup>a</sup>	29.78 <sup>d</sup>
<b>Humic acid</b>	2.30 <sup>c</sup>	0.46 <sup>c</sup>	2.64 <sup>b</sup>	95.11 <sup>c</sup>	45.10 <sup>c</sup>	18.55 <sup>c</sup>	13.69 <sup>c</sup>	20.24 <sup>c</sup>	35.77 <sup>c</sup>
<b>Bio</b>	2.40 <sup>b</sup>	0.49 <sup>b</sup>	2.66 <sup>b</sup>	99.80 <sup>b</sup>	49.60 <sup>b</sup>	19.17 <sup>b</sup>	14.28 <sup>b</sup>	22.44 <sup>b</sup>	36.89 <sup>b</sup>
<b>Humic acid + Bio</b>	2.62 <sup>a</sup>	0.53 <sup>a</sup>	3.03 <sup>a</sup>	103.10 <sup>a</sup>	56.20 <sup>a</sup>	22.40 <sup>a</sup>	15.59 <sup>a</sup>	15.22 <sup>d</sup>	38.19 <sup>a</sup>
<b>L.S.D (0.05)</b>	0.067	0.026	0.037	2.42	0.877	0.201	0.122	0.209	0.058

Also, there was a significant increase in the wheat content of protein, proline, and chlorophyll as a result of the applied treatments. These results are in agreement with those found by Zaghloul *et al.* (2015), who indicated that the highest values of total protein in pea seeds were observed in plants inoculated with biofertilizers. Meganid *et al.* (2015) reported that the potassium humate application has positive effects on chlorophyll content under salinity stress. Bakry *et al.* (2015) found that the potassium humate at a rate of 20 mg/l caused significant increases in total chlorophyll compared with control. This positive effect of potassium humate on photosynthetic pigments could be attributed to an increase in CO<sub>2</sub> assimilation and photosynthetic rate.



## 5. Wheat grains and straw yields:

The effect of humic acid and biofertilizer treatments on wheat yield is shown in table (7). It can be deduced that there was a significant increase in weight of 1000 seeds, grains, and straw yields in all additions as compared to control. The best addition was humic acid in combination with biofertilizer, it increased the grains and straw yields to 2.06 and 2.20 ton/fed., respectively. This may be attributed to that this addition caused the highest decrease in soil EC and pH values that led to an increase in the availability of nutrients and provides a healthy environment for plant growth and increase wheat yield. The obtained increase in yield components may be due to an increase of vegetative growth characters and an increase, in turn, the number of metabolites synthesized. This result may be due to the humic acid is a natural polymeric composition can be used to increase soil fertility and pea yield productivity under saline soil conditions. The humic acid can improve plant growth and increase yield components. These results are in harmony with those obtained by Mazhar *et al.* (2011). Also, Khalil *et al.* (2013) found an increase in wheat yield after the application of different addition levels of *Azospirillum brasilense*.

**Table 7:** Effect of different treatments on wheat productivity

Treatments	Weight of 1000 seeds	Yield (ton/fed)	
	(g)	Grains	Straw
Control	52 <sup>d</sup>	1.02 <sup>d</sup>	1.24 <sup>d</sup>
Humic acid	57 <sup>c</sup>	1.31 <sup>c</sup>	1.53 <sup>c</sup>
Bio	59 <sup>b</sup>	1.64 <sup>b</sup>	1.89 <sup>b</sup>
Humic acid + Bio	63 <sup>a</sup>	2.06 <sup>a</sup>	2.20 <sup>a</sup>
L.S.D (0.05)	0.93	0.017	0.020

## 6. Seasonal density of pests infesting wheat plants:

The field experiments showed that the pests infested wheat plants sown in treated saline soil was significantly affected by biofertilizer (*A. braselence*) and humic acid treatment. Pest populations were in response to biofertilizer, humic acid, and, their mixed treatments compared with control were presented in Table (8). Highly significant decreases were observed after treatment by biofertilizer combined application with humic acid. The most pests found to be as the following orders:

### Order: Hemiptera

In this study, two aphid species were recorded attacking the wheat plants, Bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus), and Green cereal- bug aphid, *Schizaphis germanium* (Rondani) (Family: Aphididae). *Rhopalosiphum padi* was appeared in a few numbers beginning of the agriculture season but quickly increased in number during April, while *S. germanium* appeared in a few numbers, with *R. padi* at the beginning of March. The minimum population (1284 aphids) was recorded on plants that were treated with a mixture of biofertilizer and humic acid. Aphid insects have attacked wheat plants during vegetative growth until the yellow mature stage causing harmful damage to the yield (Table 8).

### Order: Acari

The mite species, *Oligonychus pratensis* (Banks) (Family: Tetranychidae) was appeared in a few numbers beginning of March, feeding on high leaves. The total number/ treatment was 156, 218, 338, and 424 mite individuals by the use of a mix of biofertilizer + humic acid, biofertilizer, humic acid, and control, respectively.

### Order: Thysanoptera

*Thrips tabaci* Linderman, (Family: Thripidae) was attacked wheat plants during the two seasons feeds on hidden parts of the plants. The maximum thrips population (532 individuals) was recorded with fertilization by humic acid followed by bacterial strains treatment (261 individuals), while treatment with both of biofertilizer and humic acid treatment, the Thrips, *Thrips tabaci* was in a low number.

**Table 8:** Mean of seasonal abundance of aphid, thrips, and mites infesting wheat plants cultivated in a newly reclaimed saline area under treatments by organic and biofertilizer during the successive growing seasons of 2018-2019/ 2019- 2020.

	Control			Humic acid			biofertilizer			Humic acid + biofertilizer		
	Aphids	Thrips	Mites	Aphids	Thrips	Mites	Aphids	Thrips	Mites	Aphids	Thrips	Mites
7/2	4 <sup>c</sup>	-	-	7 <sup>b</sup>	-	-	8 <sup>b</sup>	-	-	12 <sup>a</sup>	-	-
14/2	13 <sup>a</sup>	-	-	9 <sup>b</sup>	-	-	13 <sup>a</sup>	-	-	8 <sup>b</sup>	-	-
21/2	18 <sup>a</sup>	-	-	11 <sup>b</sup>	-	-	19 <sup>a</sup>	-	-	12 <sup>b</sup>	-	-
28/2	21 <sup>a</sup>	2	3	18 <sup>b</sup>	1	2	21 <sup>a</sup>	-	-	12 <sup>c</sup>	1	-
7/3	17 <sup>c</sup>	81 <sup>a</sup>	11	21 <sup>b</sup>	88 <sup>a</sup>	6	27 <sup>a</sup>	5 <sup>c</sup>	3	16 <sup>c</sup>	12 <sup>b</sup>	1
14/3	87 <sup>a</sup>	103 <sup>a</sup>	62 <sup>a</sup>	57 <sup>c</sup>	96 <sup>a</sup>	17 <sup>c</sup>	65 <sup>b</sup>	15 <sup>c</sup>	12 <sup>c</sup>	32 <sup>d</sup>	25 <sup>b</sup>	29 <sup>b</sup>
21/3	135 <sup>d</sup>	114 <sup>a</sup>	94 <sup>a</sup>	270 <sup>a</sup>	107 <sup>b</sup>	33 <sup>b</sup>	209 <sup>c</sup>	23 <sup>c</sup>	31 <sup>b</sup>	224 <sup>b</sup>	18 <sup>d</sup>	25 <sup>b</sup>
28/3	296 <sup>b</sup>	122 <sup>a</sup>	105 <sup>a</sup>	272 <sup>c</sup>	93 <sup>b</sup>	43 <sup>c</sup>	361 <sup>a</sup>	45 <sup>c</sup>	62 <sup>b</sup>	156 <sup>d</sup>	20 <sup>d</sup>	45 <sup>c</sup>
4/4	483 <sup>a</sup>	92 <sup>a</sup>	75 <sup>a</sup>	442 <sup>b</sup>	51 <sup>c</sup>	78 <sup>a</sup>	302 <sup>c</sup>	73 <sup>b</sup>	44 <sup>c</sup>	276 <sup>d</sup>	34 <sup>d</sup>	12 <sup>d</sup>
11/4	607 <sup>a</sup>	94 <sup>a</sup>	43 <sup>c</sup>	540 <sup>b</sup>	47 <sup>c</sup>	102 <sup>a</sup>	405 <sup>c</sup>	81 <sup>b</sup>	47 <sup>b</sup>	396 <sup>d</sup>	42 <sup>c</sup>	33 <sup>c</sup>
18/4	215 <sup>b</sup>	59 <sup>a</sup>	31 <sup>b</sup>	221 <sup>a</sup>	41 <sup>b</sup>	57 <sup>a</sup>	140 <sup>c</sup>	19 <sup>c</sup>	19 <sup>b</sup>	140 <sup>c</sup>	12 <sup>c</sup>	11 <sup>c</sup>
25/4	14 <sup>b</sup>	9	-	9 <sup>c</sup>	8	-	59 <sup>a</sup>	-	-	-	-	-
Total	1910 <sup>a</sup>	676 <sup>a</sup>	424 <sup>a</sup>	1877 <sup>b</sup>	532 <sup>b</sup>	338 <sup>b</sup>	1629 <sup>c</sup>	261 <sup>c</sup>	218 <sup>c</sup>	1284 <sup>d</sup>	164 <sup>d</sup>	156 <sup>d</sup>

Means followed by the same letter (s) are not significantly differed by the least significant Difference (Duncan, 1955).

Our results indicated that the use of humic acid and biofertilizer (*A. braselence*) individually or mixed were significantly effective on the resistance of wheat plants against some piercing-sucking pests as *Rhopalosiphum padi*, *Schizaphis germanium*, *Thrips tabaci*, and *Oligonychus pratensis*. Furthermore, wheat plants treated with a mixture of biofertilizer + humic acid were aggregated with the lowest population of pests. These results could be attributed to the indirect effects of biofertilizer + humic acid in the induction of systematic resistance thus enhancing the resistance against pests by the synthesis of physical and chemical barriers in the host plant. Moreover, these substances help to improve the phosphorus and nitrogen uptake /assimilation and growth-regulating phytohormone activities which helps the wheat plants to uptake and translocate the micro and macronutrients (zinc, iron, nitrogen, and manganese) as well as improve the plant health in a better way. Moreover, the most consistent increases in the percentages of protein, proline, and chlorophyll contents were reported when grown wheat plants which treated with mixed treatment, tables (6 and 8). Other studies showed that the addition of biofertilizer + humic acid could increase the accumulation of phenolic compounds, phytoalexins, and activities of defense enzymes/genes and inhibit the crop pests through the release of different volatile and diffusible metabolites (e.g. pyoluteorin and pyrrolnitrin) (Meena *et al.*, 2000 and Rajendran *et al.*, 2007). The present study showed that the wheat plants treated with humic acid alone (without adding biofertilizer) attacked with high populations of aphids, thrips, and mites. This could be referred to the level of infestation, source, and type of humic substances, these results were in agreement with Arancon *et al.*, 2006. There are several studies on the significant effects of biofertilizer + humic acid on the biological characteristics of other crops, Zebelo *et al.*, (2016) revealed a systemic resistance to *Spodoptera exigua* by fertilizing of biofertilizer (PGPR) due to increased plant hormones. The relative growth rate and the relative consumption rate of *Helicoverpa armigera* larvae were reduced in cotton plants treated with *Pseudomonas gladioli* because of an increase in the content of polyphenol and terpenoids in cotton (Qingwen *et al.*, 1998). Recently, rhizobacteria could increase plant health and resistance to herbivore insects by triggering systemic defense responses (Rashid and Chung 2017). Furthermore, the application of humic substances and biofertilizer (PGPR) might lead to induced resistance of plants against some pests as *Aphis gossypii* Glover reared on cucumber (Fahimi *et al.*, 2014).

## Conclusion

Treatment of soil and wheat plants by biofertilizer (*Azospirillum braselence*) combined with humic acid led to improvement of saline soil properties, and increase wheat plant productivity than other treatments. Therefore, this mix could induce resistance in wheat against the *Rhopalosiphum padi*, *Schizaphis graminum*, *Thrips tabaci*, and *Oligonychus pratensis* under saline soil conditions.

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