

Improving growth and yield of wheat plants culivated under saline conditions by application of some biological and organic amendments

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Abstract

Two experiments (pots and field) were performed, during two successive winter seasons (2022/2023 and 2023/2024), to evaluate the effect of some organic amendments (humic acid (9.53kg/ha) and yeast extract (3g/L) with application rate of (238.1L/ha.)) and *Azotobacter* bio-fertilizer (2.381L/ha.) on some soil chemical properties and growth and yield of wheat plants under saline conditions. The results indicated that application of treatments especially that contain humic acid led to noticeable increase in the soil organic matter and significantly increased in all plant growth parameters and increased the N, P and K content and uptake by wheat plants. On the other hand, application of these materials led to decrease in soil salinity, soil CaCO3 content and slightly lowering in soil pH. The highest effects on soil properties and plant growth parameters, yield parameters and nutrient content and uptake were obtained by *Azotobacter* combined with humic acid plus yeast extract. Generally, under saline soil conditions application rate of (238.1L/ha.) and *Azotobacter* bio-fertilizer (2.381L/ha.) which positively affected of soil properties which in turn alleviating the effect of salt stress on plant growth in saline soil and improve and increase growth and yield of wheat plant.

Keywords: amendments; Azotobacter; biological; saline; yeast extract.

1. Introduction

Salinity is one of the main abiotic stresses affecting crop plants and reducing production worldwide. Soil salinity affects over 1125 million hectares of land globally(Hossain, 2019). Therefore, Salinity is considered as the scourge of agricultural production. For this reason, there is a trend to improve the productivity and quality of main crops grown under salt stress by using sources that are safe for the environment, such as organic and

biological treatments. According to the Food and Agriculture Organization (FAO), salt stress, which affects 397 million hectares of wheat

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agriculture, poses a severe danger to food security(Organization, 2019). When saline water is used, there is a high risk of plant growth and a limiting factor for the productivity of the majority of key crops since the yearly production may be vulnerable to yield damage due to salt intake by the plant(Sahi et al., 2006). Furthermore, using low-quality water might result in the buildup of salts in the soil. By 2050, salinity is predicted to impact 50% of the world's arable land (Bartels and Sunkar, 2005). As a result, methods for saline irrigation water-based sustainable agriculture should focus on enhancing the chemical and physical properties of the soil (Mahmood et al., 2020) The properties of the soil must be modified by the addition of organic matter in order to increase plant productivity(Aşık et al., 2009). Humic acid is the active component of organic fertilizers,

and its application may provide an alternative to conventional soil fertilization and a rapid source of N, especially under semi-arid circumstances (Khan et al., 2010). Humic acid can be exploited as a low-cost organic fertilizer source to boost plant growth and yield, stress tolerance, as well as the physical qualities of the soil and complex metal ions (Zandonadi et al., 2007). Humic acid application decreased soil salinity (EC), which may be related to the enhanced physical, chemical, and biological characteristics of the soil (Ahmed and Ismail, 2016). soil microorganisms can fix nitrogen from the atmosphere, solubilize phosphate, create compounds that aid in growth, or accelerate the breakdown of plant waste. Furthermore, through a number of processes, including enhanced nutrient uptake, increased plant metabolic activity, and the secretion of growth-promoting chemicals like hormones, microorganisms-like the nitrogen fixer Azotobacter spp.—play a vital role in promoting vegetative growth (Yasin et al., 2012) In addition, Plant growth-promoting rhizobacteria (PGPR) are among the beneficial soil microbes that have been shown in earlier research to restore plant growth and yield in stressful environments. (Ji et al., 2020). In a previous study, it was found that, the application of PGPR has enhanced wheat cultivation in saline conditions (Desoky et al., 2020; Nawaz et al., 2020). Proteins, carbohydrates, nucleic acids, lipids, and variety elements are found in yeast extract, a natural stimulant that provides P, K, Na, Fe, Mg, S, Zn, Mn, Cu, and Si. Yeast contains hormones, biotin, B12, folic acid, thiamin, riboflavin, pyridoxine, and other growth-regulating substances (Manea, et al 2019). Yeast extract also offers the advantages of inexpensive manufacturing costs and a plentiful supply of raw materials(Tao et al., 2023). The aim of the study is evaluating the effect of some organic amendments (humic acid and yeast extract) and biofertilizers (*Azotobacter* spp.) on some soil chemical properties and growth and yield of wheat plants under saline conditions.

2. Materials and methods

The present study was carried out in laboratory, screen house and Agricultural Experimental Farm of Department of Soils and Water, Faculty of Agricultural, South Valley University, Qena, Egypt, which located at latitude 26°11' 25 N", and longitude 32° 44' 42" E, in hyper hot dry zoon; during the two successive winter seasons of (2022/2023 and 2023/2024). By using local salt-adaptive bacterial isolates and certain organic amendments, this work aimed to improve wheat yield and address the problem of low crop production and growth caused by soil salinity and irrigation water.

2.1. Soil and water sampling

Prior to cultivation, a representative soil sample was taken from the experimental location at a depth of 30 cm. After allowing the samples to air dry at room temperature, they were sieved through a 2 mm stainless steel sieve. After completely mixing the sieved soil, a subsample was taken for chemical analysis such as pH was measured. Particle size distribution, soluble cations and anions, calcium carbonate, organic matter. and EC. Also the chemical characteristics of the groundwater used in this study was analyses (Table 1 and 2).

Table1. Physical and chemical analysis of the soil used in this experiment

| Sand | Silt | Clay | Texture | %SP | O.M % | pH (1:2.5) | EC (dS m ⁻¹) | CaCO ₃ (%) |
|------|------|------|-----------------|------|----------|---------------|-----------------------------|--------------------------|
| 65 | 13.4 | 21.6 | Sandy clay loam | 32.0 | 1.26 | 7.80 | 5.5 | 6.56 |

| EC dS/m | SAR | RSC | Na^+ | Ca^{+2} | Mg^{+2} | \mathbf{K}^+ | CO3 ⁻² +HCO3 ⁻ | SO_4^{-2} | CL ⁻ |
|------------|-------|-------|--------|-----------|-----------|----------------|--------------------------------------|-------------|-----------------|
| | | | | (meq /L) | | | | | |
| 6.55 | 10.39 | 10.43 | 47.15 | 10.07 | 10.50 | 0.90 | 31.00 | 15.50 | 20.6 |

| Table 2. C | Groundwater | characteristics, | used for | ^r irrigation |
|------------|-------------|------------------|----------|-------------------------|
|------------|-------------|------------------|----------|-------------------------|

2.2. Pots experiment

Pots experiment was conducted during the winter season of (2022/ 2023) in the screen house. The objective of this experiment was aimed to evaluate the bio stimulant potential as shown in table 3 for promoting wheat growth, Yield, and soil physicochemical properties under saline conditions. Using plastic pots of 25 cm in diameter, 30 cm in height, with drainage hole in the bottom, the pot experiment was set up in a fully randomized treatment. Each pot held 7 kilograms of the dry soil under investigation. As shown in table (4), there were seven treatments in the experiment.

The soil moisture was adjusted to the field capacity using saline groundwater during the

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experiment time. After germination, the plants in each pot were reduced to ten plants. At planting, superphosphate fertilizer $(15.5\% P_2O_5)$ was applied at rate of 476.2 kg/ha (2.4 g/pot. Two weeks later, potassium sulphate (48% K₂O) at rate of 119.1 kg/ha (0.6 g/pot and 33.5% ammonium nitrate at rate of 866.68 kg/ha (4.3 g/pot) were added.

After 70 days from planting, samples of soil and wheat plants were taken for determination of plant height, shoots fresh and dry weights, roots fresh and dry weights, total plant fresh and dry weights, and N, P, K plant content. Also, soil samples from each treatment were taken for soil chemical analysis (EC, pH, organic matter (O.M) and calcium carbonate (CaCO₃ %).

| Table 5. The organic | Table 5. The organic and biomaterial used | | | | |
|----------------------|---|--|--|--|--|
| Treatment | Selected Treatment | | | | |
| Azotobacter | Azotobacter isolate (soil inoculation) 1 L /fed. | | | | |
| Humic acids | Humic acids (soil drench) 4kg/fed. | | | | |
| Yeast extract | Yeast extract (soil drench) 3g/L. (With application rate of 100 L/fed.) | | | | |
| | | | | | |

| Table 4. The experimental selected treatments (Pots experiment) | |
|--|--|

| Treatmen | nts NO. | Stimulate Treatments |
|----------|--------------|---|
| T1 | С | Control |
| T2 | Azoto | Azotobacter (Soil inoculation) |
| T3 | HA | Humic acids (soil drench) |
| T4 | Y | Yeast extract (soil drench) |
| T5 | Azoto+ HA | Azotobacter + Humic acids |
| T6 | Azoto + Y | Azotobacter + Yeast extract |
| T7 | Azoto+ HA +Y | Azotobacter + Humic acids + Yeast extract |

2.3. Field experiment In the winter of 2023-2024, a field experiment was carried out at the Department of Soils and Water's Experimental Research Farm, to evaluate the effect of positive treatments derived from pots experiment on the growth and yield of wheat, nutrients status, and some soil physical and chemical properties under saline conditions. Table (1) displays some of the physical and chemical properties of a representative soil sample (30 cm top-soil) from the field-testing site.

Wheat grains (*Triticum aestivum* L.) cv., Giza 168 were planted on the soil at the rate of 119 kg/ha. The distance between the lines is set to 15-16 cm and the planting depth is 3-5 cm from the soil surface. The plot area was 10 m² (3 m in length X 3.33 m in width). A composting filter mud cake, made from the organic waste of the Quos sugarcane factory, was applied to each plot at a rate of 9.52 tons per hectare as a general organic amendment.

At planting time, a base dose of 476.2 kg/fed super phosphate (15.5% P_2O_5) was administered. One month after planting, 866.68 kg of the nitrogen fertilizer (in the form of lime ammonium nitrate, 33.5% N) was added, and fifty days later, 119 kg of potassium sulphate (48% K₂O) was added (the Agriculture Ministry's recommended doses). Three replications and in completely randomized block design was used in the experimental setup.

The chemical characteristics of groundwater used in this study are shown in Table (2). In the second seasons selected treatments were reduced to 4 positive treatments instead of 7 treatments in the first season (Pots experiment), choosing those that proved most promotive in the first season. Tables (5) present the selected experimental treatments to field experiment. Plant and soil samples were collected from the experimental field at 70 day from planting, soil samples (0-15 cm) were taken from each plot air-dried and passed through 2-mm sieve and kept for some physical and chemical properties analyses. Moreover, Plant height (cm), root length (cm), shoots fresh and dry weights (g), roots fresh and dry weights (g), numbers of tillers per plant and N, P, K pant contents. Moreover, spike length (cm), weight of grains/spike (g), number of grains/spikes, 1000grains weight , total grain yield, straw yield and biological yield was recorded at harvest.

2.4. Methods of analysis:

2.4.1. Methods of soil and water analysis

Soil texture: were determined using the micropipette method (Shirazi and Boersma, 1984).Total calcium carbonate (CaCO₃): was determined using Scheibler calcimeter according to Jackson, (1973). Soil pH: was measured in 1:2.5 (soil : water) suspentions using a glass electrode Jenway 3510 pH Meter according toJackson (1973). Electrical conductivity (EC dSm⁻¹) of the 1:5 ratio of soil to water extract was estimated using an electrical conductivity meter after shaking for 30 minutes and measured by using 4510 conductivity meters (JENWAY, UK) according to (Richards, 1954). The oxidizable organic carbon contents were measured. It was

determined according to the modified Walkely and Black method. (USDA, 1996).

Table 5. The selected experimental treatments (field experiment)

| NO. | Treatments |
|-----------|--|
| T1 | Control |
| <i>T2</i> | Azotobacter +Humic acid (AZOTO + HA) |
| Т3 | Azotobacter +Yeast extract (AZOTO + Y) |
| T4 | Azotobacter +Humic acid + Yeast extract (AZOTO+ HA +Y) |

2.4.2. Methods of plant analysis

Dried plant samples (at 70 C°) were digested with the acid mixture of sulphuric acid and hydrogen peroxide to determine nitrogen, phosphorus and potassium content according to (Jackson, 1973). Total Nitrogen was determined following the micro-Kjeldahl method as described by (Jackson, 1973). Total phosphorus was determined spectrophotometrically using the chlorostannus-phosphomolybdic acid method in a sulfuric acid system (Jackson, 1973).Total potassium was determined using the flame photometer method described by (Page, 1982).

2.5. Statistical analyses

MSTAT-C (Russell, 1994) was used to analyze all the data, and the LSD test was used to compare the treatment averages at a 0.05 percent probability level.

3. Results and discussion

3.1. Pots experiment

Specific objectives of this experiment were to test whether the single or combined application of humic acids, yeast extract and microbial bio effectors *Azotobacter* can promote synergy and more efficacy on improving some soil properties, wheat plant growth and nutrients uptake.

3.1.1. Effect of application biological and organic treatments on some soil chemical properties.

3.1.1.1. Effect on the Electric conductivity

Regarding the effect of different treatments on some chemical properties of the soil; the results in table (6) and figure (1) indicate that the electric conductivity (EC) of the soil treated in different treatments were lower compared to control. Results showed that, The EC value of soil treated with HA + Azoto + Y recorded the highest lowering compared with all treatments. The relative decreases due to application of different type of substances were; 18.20, 25.50, 14.50, 27.30, 18.20 and, 34.50% for treatments of (Azoto), (HA), (Y), (Azoto + HA), (Azoto + Y) and, (HA + Azoto + Y), respectively compared with the control treatment. Humic acid's ability to enhance soil aggregation and enhance of water flow from soil, which leaches the excess soluble salts, may be the cause of this. These findings concurred with those published by (Mohamed, 2012 and Wang et al., 2019) who reported that, different types of humic substances showed decrease in soil conductivity (EC). In addition, Kang et al (2015) reported that yeast may effectively mitigate the negative effects of salt stress on cultivated plants. Plants cultivated under salt stress benefited from yeast extract, which also mitigated the negative effects of salinity stress on vegetative growth (Nassar et al., 2016). Also, the combined treatment (humic acid + biofertilizer) was the best in lowering EC values It could be due to activation of bacteria in soil and the influence of biofertilizer or humic acid on total porosity and improving soil aggregation, and possible moving salt from soil under the effect of irrigation water (Alakhdar et al., 2020).

3.1.1.2. Effect on soil pH

With respect to the impact on soil pH, humic acid application produced a minor decrease in soil pH (table 6). These slight pH decreases are due to soil buffering capacity, which is caused by the immediate protonation of minerals and organic material that occurs in the soil or is intentionally added to the soil. Motojima et al. (2012) reported that, The soil pH decrease was improved when humic acid extracted from solubilized excess sludge was applied to salinealkaline soil. They stated that the quicker pH drop brought about by humic acid treatment would enhance crop germination Humic acid improved the saline-alkaline soil is thought to be that the carboxyl groups in the acid generated H^+ , which neutralized the OH^- in the soil solution and lowered the pH of the soil. Additionally, Mohamed (2012) found that, applying humic acid considerably lowered the pH of the soil.

3.1.1.3. Effect on the soil organic matter

Data presented in Table (6) and Figure (1) reveal that, there was an increase in soil organic matter content of soil with addition of all substances, in comparison with that of the control treatment. As illustrated in figure (1) the organic matter content values significantly increased in all treatments except of sole application of yeast extract that was recorded non-significant increase. The strongest effect on soil organic matter content was obtained with the combined application of humic acid (HA) and yeast extract (y) plus biofertilizer (Azoto). The effectiveness of different treatments on the organic matter soil content can be arranged in the following order: (HA + Azoto + Y) > (Azoto + HA) > (HA) > (Azoto + Y) > (Azoto) > (Y) and the percentage of increase in soil organic matter content in by using of different treatments were, 61.11, 32.5, 26.9, 23.0 ,21.4, and 5.6%. Humic acid encourages the activity of microbes, which increases the amount of organic matter (Alakhdar et al., 2020; Heba et al., 2013). Also, microorganisms in bio fertilizer help to rebuild the soil's organic matter and restore the natural nutrition cycle (Gaur et al., 2010).

3.1.1.4. Effect on soil Calcium Carbonate

The data in table (6) and figure (1) show that, decreasing in the $CaCO_3$ % content was from 6.56 % in the soil before wheat planting to 6.0, 5.46,6.36, 5.29, 5.8 and, 5.09 % by adding (*Azotobacter*), (humic acids), (yeast extract), (Azotobacter + humic acid), (*Azotobacter* + yeast extract) and, (*Azotobacter* + humic acid + yeast extract), respectively.

Table 6. Effect of application biological and organic treatments on some soil chemical properties.

| Stimulate Treatments | | pН | $EC (dSm^{-1})$ | Organic Matter | CaCO ₃ (%) |
|----------------------|---------------------|------|-----------------|----------------|-----------------------|
| | | | | (%) | |
| T1 | Control | 7.80 | 5.50a | 1.26f | 6.56a |
| T2 | Azotobacter (Azoto) | 7.77 | 4.50c | 1.53d | 6.00c |
| T3 | Humic acids (HA) | 7.76 | 4.10d | 1.60c | 5.46e |
| T4 | Yeast extract (Y) | 7.78 | 4.70b | 1.33e | 6.36b |
| T5 | Azoto + HA | 7.74 | 4.00f | 1.67b | 5.29f |
| T6 | Azoto + Y | 7.75 | 4.50d | 1.55d | 5.80f |
| T7 | Azoto + HA + Y | 7.72 | 3.60e | 2.03a | 5.09g |



Figure 1. Effect of application biological and organic treatments on some soil chemical properties

Moreover, the obtained results show that examined treatments which contain humic acid resulted in higher decreases in the CaCO₃% content compared to the control and other treatments. The beneficial impact of organic enhancing the materials on chemical characteristics of soil may result from the release of CO₂ during the breakdown process, which lowers the precipitation of Ca^{2+} and CO_3^{-2} ions in the form of CaCO₃ (Sekhon and Bajwa, 1993). Humic acid treatment may have a positive impact on soil chemical characteristics because it releases H+ from HA, which lowers pH, dissolves CaCO₃, and causes it to seep down from the soil profile (Mindari et al., 2014; Raychev et al., 2001). Also, (Gadd, 1999) said that acidity serves the dual purpose of increasing ion solubility by acidifying the substrate.

3.1.2. Effect of application biological and organic treatments on the wheat growth characteristics under saline conditions

3.1.2.1. Plant height

Plant height showed highly significant increases with addind the bio and organic compound as compared with the control (table 7 and figure 2). The highest plant height was obtained by using the combination treatment of (*Azotobacter*+ Humic acid+ Yeast extract) which recorded increase reached to 65.9% compared to the control followed by combination of *Azotobacter* + Humic acid which recorded 54.5% and combination of *Azotobacter* + Yeast extract which recorded 52.3% and 52.3%, while the lowest values were recorded on the plant with single treatments alone (T2, T3 and T4) and the control treatment. The increments in plant growth may be due to the enhancement effect of biostimulation substances that improved the chemical properties of soil. These results are similar to those of (Allah and Mohmeed, 2003; Gowda et al., 2010; Ibrahim et al., 2008). Also, these results agree with (Türkmen, 2005) who reported that humic acid application positively affected the plant growth parameters.

3.1.2.2. Shoots fresh and dry weight

Data in table (7) and figure (2) showed that soil sole application with single treatments of humic acids, yeast extract and Azotobacter as well as combined addition of these treatments resulted in significant increases in the fresh weight of wheat plant representing 67.0, 91.12 and 60.8% for Azotobacter, humic acids, and yeast extract application over the control, respectively, while the results showed that the combined addition of (Azotobacter + Humic acids) or (Azotobacter + Yeast extract) and (Azotobacter + Humic acids + Yeast extract) gave higher fresh weight than these treatments solely recorded 98.3%, 98.3% and 133.6% comparing to control, respectively. The highest mean of fresh were obtained with treatments of (Azotobacter + Humic acids + Yeast extract). Additionally, the dry weight data showed a trend that was nearly identical to the one previously observed in the fresh weight of the shoots, indicating that the addition of the various treatments in the presence of humic acid had an impact on the dry weight of wheat plants.

| Stimulate Treatments | | Plant height | Shoots fresh | Shoots dry | Roots fresh | Roots dry |
|----------------------|----------------|--------------|--------------|------------|-------------|-----------|
| | | (cm) | weight | weight | weight | weight |
| | | | (g/plant) | (g/ plant) | (g/plant) | (g/plant) |
| T1 | Control | 22.00c | 5.18d | 1.69d | 0.31d | 0.21d |
| T2 | AZOTO | 29.50b | 8.65c | 2.79c | 0.60c | 0.34c |
| Т3 | НА | 31.00b | 9.19bc | 3.19b | 0.73b | 0.39abc |
| T4 | Y | 30.00b | 8.33c | 3.18b | 0.63c | 0.35bc |
| T5 | AZOTO + HA | 34.00ab | 10.27ab | 3.42b | 0.88a | 0.40ab |
| T6 | AZOTO + Y | 33.50ab | 10.27ab | 3.38b | 0.80a | 0.39abc |
| T7 | AZOTO + HA + Y | 36.50a | 12.10a | 4.03a | 0.90a | 0.43a |

Table 7. Effect of biological and organic application on wheat growth characteristics.



T1 T2 T3 **T4 T5 T6** T7 **Stimulate Treatments**

Figure 2. Effect of biological and organic application on wheat growth characteristics

Also, the findings indicated that treatments that resulted in the greatest increase in the plants' dry weight were (Azotobacter + Humic acids + Yeast extract) It showed a 138.5% rise in comparison to the control therapy. Activating the synthesis of biological materials, promoting rhizosphere microbes. generating phytopathogenic controllers, and enhancing nitrogen fixation and element absorption are some of the ways that Azotobacter chroococcum may promote wheat growth development and element absorption. It may also promote yield. (Lenart, 2012). Applyed dry yeast as a foliar spray promotes plant development, which results in notable increases in growth and yield (Fawzy, 2007).

0.2

0

3.1.2.3. Roots fresh and dry weight

Data in table (7) and figure (2) revealed that, roots fresh and dry weight significantly increased as a result of soil additions in both single treatments of Azotobacter, humic acid, yeast extract and their combined treatments comparing to control. Obtained data revealed an increase in roots fresh weight reached to 93.5, 135.5, 103.2, 183.9, 158.1 and, 190.32% over control with application of Azotobacter, humic acid, yeast extract, (Azotobacter +Humic acid), (Yeast extract + Azotobacter) and (Azotobacter + Humic acid + Yeast extract), respectively. Also, the results of roots dry weight recorded significant increases reached to 61.9, 85.7, 66.7, 90.5, 85.7, and 104.7% for the previous treatments over the control, respectively.

These results confirm the stimulating influence of combined treatments of *Azotobacter* biofertilizer treatment mixed with humic substances and yeast extract on roots fresh and dry weight, were recorded increase in roots fresh and dry weight more than sole application of humic acids, *Azotobacter* or yeast extract. These results indicated that a synergetic effect between humic acid and both *Azotobacter* N-fixer and yeast extract.

Abd El-Razek et al. (2020) reported that, due to the beneficial effects of humic acid and biohumic in promoting root development and the generation of thin lateral roots, applying organic manure, humic, and bio-humic improves nutritional status by activating nutrient uptake. Moreover, Duca et al. (2014) found that, Auxin, indole acetic acid, gibberellic acid, and cytokinins are among the hormones that the plant growth promoting rhizobacteria (PGPR) can create. Dey et al. (2004) found that, Azotobacter may promote the synthesis of auxins and gibberellins, which are plant growth hormones. This could improve the growth of plant roots, which in turn could improve productivity, agricultural nodulation, and nitrogen fixation.

3.2. The second experiment (Field experiment)

This field experiment carried out to evaluation of the biostimulant potential of selected treatments derived from pots experiments on some soil chemical properties, nutrients status and wheat growth and yield under saline conditions.

3.2.1. Effect of application of selected biological and organic treatments on some soil chemical properties

3.2.1.1. Effect on the Electric conductivity (EC) of the soil

Data of EC in Table (8) and Figure (3) show that soil EC values (dSm⁻¹) were affected by application biological and organic treatments. Data indicate that all treatments significantly decreased the salinity level in soil after 70 days. Application of *Azotobacter* combined with humic acid plus yeast extract (T4), caused noticeable decrease in salinity level from 5.5 in control to 3.8 (dSm⁻¹) followed with *Azotobacter* combined with humic acid treatment (T2) which decreased up to 4.5 dSm-1, and the lowest decreasing value recorded with (T4), in comparison to the control; were the percentage of decreases in EC reached to 30.9, 18.2 and 14.5% for T4, T2 and T3 comparing to control.

El-Kamar, (2020) found that Applying both liquid and dried yeast waste significantly reduced soil EC, which may be explained by soluble salts leaching with irrigation water. The organic acid produced by microorganisms' activity speeds up the loss of soluble salt, and the addition of humic acid and their complementary effects in combination treatments resulted in the greatest drops in soil EC values in saline conditions. Additionally, the treatment of humic acid with Azotobacter and yeast extract may be the cause of this. This

| Т | reatments | рН | EC (dSm ⁻¹) | O.M(%) | $CaCO_3(\%)$ |
|----|----------------|------|----------------------------|--------|--------------|
| T1 | Control | 7.80 | 5.50a | 1.30c | 6.60a |
| T2 | Azoto + HA | 7.75 | 4.50b | 2.43b | 5.47b |
| Т3 | Azoto + Y | 7.78 | 4.70c | 1.83c | 5.90c |
| T4 | Azoto + HA + Y | 7.70 | 3.80d | 3.34a | 4.66d |

Table 8. Effect of application of selected treatments on some soil chemical properties



Figure 3. Effect of application of treatments on some soil chemical properties

treatment has the advantage of lowering EC values because humic acid has a large number of functional groups that enable it to effectively separate NaCl compounds, thereby mitigating the negative effects of salt stress. (Ahmed and Ismail, 2016). In arid land circumstances, lower soil EC is a good sign of soil quality that can promote plant growth. (Bello et al., 2023).

3.2.1.2. Effect on soil pH

Applying biological and organic treatments (T2, T3 and T4) to the soil slightly decreased in soil pH compared with the control treatment (table 8 and figure 3). The dropped in soil pH from 7.80 for the control to 7.70 with all treatments. Slight decrease in pH values in the soil may be due to microorganisms breaking down organic materials and producing organic acids that reduce soil pH. Furthermore, the beneficial impact of yeast extract on reduce soil pH may be due to yeast's direct or indirect ability to alter the pH of the soil around the roots (Al-Rawi and Aldouri, 1991). Also, according to the study by (El-Kamar, 2020), The chemical soil parameters were significantly impacted by the application of both liquid and dried yeast waste, as the pH of the soil dropped from 8.3 to 8.1.

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3.2.1.3. Effect on soil organic matter

Results in Table (8) and Figure (3) Showed that, the relative increases in soil O.M% reached to 86.9, 40.77 and 156.9 % for T2, T3, and T4 treatments, respectively compared to the control (T1). When compared to the control, applying the investigated treatments to the soil generally resulted in increases in the O.M. content, reaching its maximum value in the soil amended with combined treatments T4 (Azotobacter combined with humic acid plus yeast extract), followed by T2 (Azotobacter combined with humic acid), while applying Azotobacter combined with yeast treatment (T3) produced a lower O.M. increasing value. Humic acid increases the amount of organic matter by stimulating the activity microbes. of Additionally, biofertilizer plays a significant role in the decomposition of plant residues, which raises the content of organic matter in the soil. This is to be expected, as high humic acid concentrations significantly increase soil fertility. This outcome is comparable to the findings of (Melero et al., 2007) Who found that the amount of organic carbon rose when organic amendments were applied. When soil organic matter rose from 0.93 to 1.15, the application of both liquid and dried yeast waste significantly changed the chemical characteristics of the soil. (El-Kamar, (2020).

3.2.1.4. Effect on soil Calcium Carbonate (CaCO₃ %)

Data presented in Table (8) and Figure (3) show that, soil CaCO₃ values were affected by biological and organic treatments. Generally, CaCO₃ values were lower in soil treated with treatments of (Azotobacter combined with humic acid plus yeast extract (T4); caused higher decrease in $CaCO_3$ %. followed with Azotobacter combined with humic acid treatment (T2) and the lowest decreasing value recorded was with Azotobacter combined with yeast treatment (T3); in comparison to the control. The relative decreases in CaCO₃ content due to different treatments were 29.4, 17.1 and 10.6 % for T4, T2 and T3 comparing to control, respectively. Humic acid treatment may have a positive impact on soil chemical characteristics because it releases H⁺ from HA, which lowers pH, dissolves CaCO₃. (Mindari et al., 2014; Raychev et al., 2001). Also, (Gadd, 1999) reported that the double function of acidity is to acidify the substrate thus enhancing ion solubility.

3.2.2. Effect of application of selected biological and organic treatments on wheat growth and yield characteristics

3.2.2.1. Growth parameters

The results in Table (9) show that, The highest values of plant height, root length, number of tillers, shoot fresh weight, shoot dry weight, roots fresh weight and roots dry weight was detected on the plants which treated by Azotobacter combined with humic acid plus yeast extract (T4) followed by Azotobacter combined with humic acid (T2) were the higher relative increases over control reached to 46.8 and 41.5% for plant height; 61.3 and 55.4% for root length; 177.4 and 155.5% for number of tillers; 252.7 and 224.5% for shoot fresh weight; 180 and 147.7% for shoot dry weight; 474.4 and 389.0% for roots fresh weight addition to 562.5 and 298.8% for roots dry weight for T4 and T2 over control, respectively.

| Table 9. Effect of application of Azotobacter combined with humic | acid or yeast on wheat growth Parameters |
|--|--|
|--|--|

| Treatments | | Plant | Root | Number | Shoot | Shoot | Root fresh | Root |
|------------|--------------|---------|--------|---------|-----------|-----------|------------|-----------|
| | | height | length | of | fresh | dry | weight(g) | dry |
| | | (cm) | (cm) | tillers | weight(g) | weight(g) | | weight(g) |
| T1 | Control | 50.20c | 5.60c | 1.37c | 26.30c | 9.00c | 3.90b | 2.40b |
| T2 | Azoto + HA | 71.03ab | 8.70ab | 3.50ab | 85.33ab | 22.30b | 19.07a | 9.57a |
| T3 | Azoto + Y | 69.07b | 7.30b | 2.90bc | 66.67b | 19.97b | 10.90b | 5.67b |
| T4 | Azoto + HA+Y | 73.67a | 9.03a | 3.80a | 92.77a | 25.20a | 22.40a | 15.90a |

3.2.2.2. Component of Yield parameters

Data in Table (10) showed that, all treatments had positive impact on yield parameters compared with control. Results concerning the effect of application selected bio stimulant substances showed significant increase in spike length, weight of grains/spike, number of grains/spike and 1000-grain weight. Also, application of combined treatment T4 produced the longest spikes height (11.20 cm) followed by T2 (9.20cm). The least spike length produced from treatment T3 were 8.3 cm. T4 Spike length were increased by 36.10 % significantly compared with the control. An increase in vegetative growth characteristics could be the cause of the observed rise in yield components. Humic acid, a naturally occurring polymeric composition that can be utilized to improve soil fertility and plant crop productivity under saline soil conditions, may be the cause of this outcome. (Alakhdar et al., 2020).

In addition, Data presented in Table (10) reveal that all treatments under the study on weight of grains/spike(g) was significantly increased compared to those of untreated control. The highest recorded values of weight of grains/spike (g) were found with that of application T4 followed with T2 and T3 recording increasing reached to 90.75, 32.90 and, 21.40% compared to control treatment, respectively. Increased moisture content may contribute to a high wheat yield in plants treated with a combination of biofertilizers. incressed of moisture content, helps to increase the nutrient availability to plants and, consequently, the overall yield.

Also, data presented in Table (10) showed that the maximum number of grains/spike being pronounced with that of the plots treated with *Azotobacter* combined with humic acid plus yeast extract (T4) which recorded the highest significant increase in the number of grain /spike

(36.70%) compared with control, while no significant increases were recorded as a result of application other treatments of Azotobacter combined with humic acid (T2) or Azotobacter combined with yeast extract (T3) revealing the pronounced positive significant increasing of Azotobacter combined treatment with humic acid plus yeast extract (T4) comparing with other treatments. This is due to the part that organic fertilizer and yeast extract play in boosting photosynthetic efficiency and providing appropriate opportunities to reduce the circumstances of dropping flowers by reducing their degree of competition for the food supply (Alfatlawi and Alrubaiee, 2020).

Table 10. Effect of application of Azotobacr combined with humic acid or yeast on component of yield and yield parameters

| Treatments | | Spike length (cm) | Weight of grains/spike (g) | Number of grains/spikes | 1000-grain weight (g) | Grain yield (ton/fed.) | Straw yield (ton/fed.) | Biological yield (ton/fed.) |
|------------|-----------------|----------------------|----------------------------------|-------------------------|-----------------------------|------------------------------|------------------------------|-----------------------------------|
| T1 | Control | 8.23b | 1.73c | 39.00b | 41.20c | 0.70c | 0.86b | 1.50d |
| T2 | Azoto + HA | 9.23b | 2.30b | 42.70b | 56.30b | 1.50b | 1.46a | 2.90 b |
| Т3 | Azoto + Y | 8.30b | 2.10b | 41.00b | 47.00bc | 1.20bc | 1.30a | 2.50c |
| T4 | Azoto + HA+Y | 11.20a | 3.30a | 53.30a | 79.30a | 1.90a | 1.60a | 3.50a |

Moreover, the effect of the different treatments on weight of 1000-seed (g) are illustrated in Table (10)Weight of 1000 grains were increased under all the treatments as compared with the control. These increases in seed weight/1000 seed and are pronounced under of Azotobacter combined treatment with humic acid plus yeast extract (T4) more than the other treatments which recorded higher increases reached to 92.50% followed with T2 (36.70%) and T3 (14.10%) comparing to control. Abdel Latif et al. (2023), reported that inoculation of wheat with combined treatment (humic acid and yeasts extract) plus tested Azotobacter enhanced seedling growth as indicated from the significant increases in plant height, shoot and root fresh and dry weights which recorded the highest value compared to control.

Also, results illustrated in Table (10) show that, different treatments significantly increased the grain and straw yields compared to the control treatment. The best increases grain yield was obtained with treatment T4 followed with treatments T2 and T3. It is clear from the data that the combinations of *Azotobacter* with humic acid plus yeast extract (T4) gave the highest values in this respect compared with the control. Grain yield increased from 0.77 to 1.90, 1.50 and to 1.20 ton/fed. recording increase percent 146, 94.80, and 55.80 % for T4, T2 and T3 over the control treatment respectively. Similarly, straw yield increased from 0.86 to 1.67, 1.46,

and 1.30 ton/fed. recording 94.10, 69.80and 51.20% for previous treatment over the control respectively. Also, results show that all combined selected treatments significantly stimulated and enhanced the formation of biological yield of wheat recording increases over the control treatment. The best biological yield increases were obtained with treatment T4 followed by treatments T2 and T3. The highest values were found with T4 treatment was the average values of biological yield were 3.57, 2.96 and 2.5 ton/fed. and the relative increases % were 119, 81.50 and 53.40 % compared with control for T4, T2 and T3, respectively.

The observed increase in yield components could be the result of a rise in vegetative growth characters, which would then lead to an increase in the synthesis of metabolites. This may be explained by the fact that this addition resulted in the biggest decrease in soil EC and pH values, which increased the amount of nutrients that were available and promoted healthy plant growth and increased the yield of wheat. (Shahin et al., 2015). Furthermore, because of its high concentration of hormones like auxin and cytokinins and its ability to improve the accumulation of carbohydrates, yeast extract has been proposed to have a beneficial role in vegetative and fruiting growth by enhancing flower production and their set-in certain plants. (Barnett et al., 1990). Additionally, the reason for this is because organic fertilizer increases the number of spikes and grains per spike, which positively reflects the growth in total grain yield by providing the plant with more possibilities to benefit from the nutrients (Muhammad at al., 2014).

3.2.3. Effect of application of selected biological and organic treatments on N, P and K content and their uptake by wheat plants: 3.2.3.1. Nitrogen content and uptake

The data presented in Table (11) and Figure (4) reveal that, there was an increase in nitrogen content and N-uptake by wheat plants as a result

of addition of different substances, in comparison with that of the control treatment. These results indicating that treated soil with *Azotobacter* combined with humic acid plus yeast extract (T4) had a clear influence on N-content and N-uptake by wheat plants that the N-content of wheat plant increased from 1.45 in control treatment to 2.43, 2.22 and 2.66 % and N-uptake from 130.02 in control (T1) to 538.65, 425.50 and 780.13 mg/plant due to application of T2, T3 and T4, respectively.

These results are consistent with those of Kabesh et al. (2009), who discovered that the combination of organic fertilizer and biofertilizers significantly increased the nitrogen content and uptake of wheat plants. These could be the result of raising the amount of nitrogen in the soil and speeding up specific microbial activities in the plant rhizosphere. According to a different study by Hassan et al. (2017), when K humate was sprayed in conjunction with nitrogen fertilizer, mean values demonstrated an increase in N availability as compared to when N fertilizer was treated alone. This outcome might be the consequence of K humate's effective chelating qualities, which lessen nutrient loss via leaching and runoff.

3.2.3.2. Phosphorus content and uptake

Data presented in Table (11) and Figure (4) show that combined humic acid, yeast extract and Azotobacter biofertilizer (T4) gave the highest P-content and P-uptake (2.06 % and 600.6 mg/plant) followed by T2 (Azotobacter combined with humic acid) recorded 1.78 % and 394.4 mg/plant, while T3 (humic acid+ yeast extract) gave the lowest P-content and P-uptake values (1.24 % and 238.7 mg/plant). The increments in P-content and P-uptake may be due to the beneficial effect of humic acid which improved the physical - chemical and biological properties of soil. So, it may increase soil exchange capacity, increasing available of some

| Treatments | | N% | N Uptake (mg/plant) P% | | P Uptake (mg/plant) | K% | K Uptake (mg/plant) |
|------------|----------------|-------|---------------------------|-------|------------------------|-------|------------------------|
| T1 | Control | 1.45d | 130.02c | 0.90b | 80.30d | 0.23d | 20.62c |
| T2 | Azoto. + HA | 2.43b | 538.65b | 1.78a | 394.40b | 0.81b | 179.55b |
| T3 | Azoto. + Y | 2.22c | 425.50b | 1.24b | 238.70c | 0.62c | 118.83b |
| T4 | Azoto. + HA+ Y | 2.66a | 780.13a | 2.06a | 600.60a | 1.42a | 416.53a |

 Table 11. Effect of application of selected biological and organic treatments on some nutrient concentrations by wheat plants







nutrient in soil and this reflect in stimulating both P-content and P-uptake. Through its chelation capability, humic acid can influence the solubility of insoluble phosphorus compounds in soil, and plants can swap chelated metals for other metals (Tan, 2003). The most remarkable feature of humic acids in soil and other environments, according to Filip and Bielek (2002), is their capacity to interact with metal ions and soil minerals to create complexes with a variety of properties, particularly phosphorus, and to increase chemical stability.

3.2.3.3. Potassium content and uptake

Data in Table (11) and figure (4) demonstrate the effect of organic substances in combination with *Azotobacter* bio-fertilizer on K-content and K-uptake by wheat plants. The results pointed out that there were significant increases in Kcontent and K-uptake by wheat plants due to the combined application of organic materials and biofertilizer compared to the control. Obtained data revealed that the maximum values of K content and K-uptake (1.42 % and 416.53 mg/plant and 0.81 % and 179.55 mg/plant) were recorded in plants received *Azotobacter* combined with humic acid plus yeast extract (T4) followed *Azotobacter* combined with humic acid (T2) treatments, respectively.

However, the lowest values of the K-content and K-uptake (0.62 % and 118.83 mg/plant) respectively, were found under T3 treatment (*Azotobacter* combined with yeast). This may be explained by the effect of adding organic materials, which increased plant metabolic activity and encouraged metabolite migration from roots and stems to leaves, perhaps increasing the proportion of nutrients in leaves and stems. (Sikander, 2001).

It has also been demonstrated that, applying humic acid to plants in saline conditions has enhanced nitrogen uptake. (Khaled and Fawy, 2011; Mohamed, 2012 and Rady et al., 2016). Also, (Selim et al., 2012). Applying humic acid enhanced plant growth by facilitating the uptake of nutrients such as K, Zn, Mn, P, and N. In order to counteract the effects of salinity, the stimulating impact seems to be greatest when humic acid administration and bio-fertilization are coupled. as shown by (Abdelhamid et al., 2011) They found that humic acid and bio fertilization might be used in conjunction with mineral fertilizers to increase plant yield and quality. This could be because humic acid and Azotobacter treatment increased plant fresh and dry weight compared to other treatments.

4. Conclusion

The results in Pots experiment revealed that, application of humic acid alone or mixed with Azotobacter and Yeast extract led to increase of organic matter content, plant dry weight and plant fresh and dry weight. On other hand application of humic acid alone or mixed with Azotobacter and Yeast extract led to decreased of soil electric conductivity, soil pH and CaCO₃ content. The effectiveness of different treatments on the soil chemical properties and wheat growth characteristics can be arranged in the following order: (humic acid + Azotobacter+ Yeast) > (Azotobacter + humic acid) > (humicacid) > (Azotobacter + Yeast) > (Azotobacter) > (Yeast).

In addition, the results in field experiments revealed that, this experiment carried out to evaluation of the biostimulants potential of treatments derived selected from pots experiments (Humic acid + Azotobacter), (Yeast extract + Azotobacter) and (Azotobacter + Humic acid + Yeast extract). on wheat growth and yield, nutrients status and some soil chemical properties under saline conditions, the results indicate that, application of selected treatments especially that contain humic acid led to caused noticeable increase in the soil organic matter, Also, application of selected treatments led to significantly increased in plant height, root length, number of tillers, shoot fresh weight, shoot dry weight, roots fresh weight and roots dry weight. Also, application of selected treatments led to increase in spike length, weight of grains/spike, number of grains/spike and 1000-grain weight, in addition, increased in N, P and K content and uptake by wheat plants. On the other hand, application of selected treatments led to decrease in soil salinity, soil CaCO3 content and slightly decreasing in soil pH. The highest effect on soil properties and plant growth parameters, yield parameters and nutrient content and uptake with treated by Azotobacter combined with humic acid plus yeast extract.

Generally, under saline soil conditions application of combined treatment of humic acid component as organic source (4kg/fed.), yeast extract (3g/L) with application rate of four time and (100L/fed.) Azotobacter biofertilizer (1L/fed.) which positively affected of important soil properties which in turn reduced the deleterious effect of salt stress on plant growth in saline soil and improve and increase growth and yield of wheat plant.

Authors' Contributions

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