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RESEARCH ARTICLE

Evaluation of some biological and organic soil amendments in improving growth of wheat plant under irrigation with saline water

Zeinab M. Abdel Latif, A.A. Abobaker and H.M. Farrag *

Soil and Water Department, Faculty of Agriculture, South Valley University, 83523 Qena, Egypt.

Abstract

The present Preliminary experiments were conducted to examine the efficiency of different rates of some bio stimulate substances and local salinity adapted bacterial inoculants to facilitate wheat growth. Different doses of each biostimulate substances (s) were tested. Humic acid rates (0, 2, 3, 4 and 5 kg humic acid /fed), biofertilization treatment (most salinity adapted *Azotobacter* local isolates) and yeast extract soil treatment (0, 1, 2, 3 and 4 g / L) were examined for their effects on wheat plants growth. This experiment was conducted in the green house of agricultural experimental farm of department of soils and water, faculty of agricultural, south valley university- Qena governorate, Egypt. The growth parameters data showed that application of humic acid rates (4 and 5 kg humic acid/fed), biofertilization treatment (most salinity adapted *Azotobacter* local isolate A), yeast extract soil treatment (3 and 4 g / L) produced the highest significant parameters of the wheat plants; for the fresh and dry weight of shoot and root system. Increasing humic acid levels from 4 and 5 kg humic acid/fed did not recorded significant increases in all studied parameters. Also, increasing yeast extract levels as a soil treatment from (3 and 4 g / L) did not recorded significant increases in all studied parameters. The obtained results were clearly showed the promotion effects on growth parameters of wheat plants and the most efficient doses were selected for later use in the assessment experiment productivity of wheat plants.

Keywords: *Azotobacter;* Biofertilizer; biostimulate substances; Humic acid; Wheat plant; yeast extract.

1. Introduction

The properties of the soil must be modified by the addition of organic matter in order to increase plant productivity (Ask *et al.*, 2009). Application of organic fertilizer enhances the physical, chemical, and biological characteristics of soil and boosts soil fertility, increasing yield per unit area (Dolarslan and Gul, 2012). Humic compounds, which make up most of the soil's organic matter, influence plant growth both directly and indirectly (Sangeetha *et al.*, 2006). The uptake of humic substances into plant tissue, which results in a variety of biochemical outcomes, is what causes the direct effects. In contrast, the indirect effects involve the enhancement of soil properties like aggregation, aeration, permeability, water holding capacity, micronutrient transport, and availability. (Tan, 2003). It was reported that humic substances are effective in decreasing salinity levels of soils and reducing toxic effects of heavy metals (Gerzabek and Ulah, 1990). Regarding the effect of water shortage and salt level, use of humic substances for removing negative effects of elements in toxic quantities, and effects on plant growth were studied (Asık *et al.*, 2009), They reported that, humic acid application decreases adverse effects of salt. Moreover, microorganisms like nitrogen fixer Azotobacter spp. play an important role in stimulates vegetative growth through different mechanisms like, such as hormones, vitamins, nitrogen fixation, and

.*Corresponding author: **Hosny M. Farrag**
Email: hosnyfarraj@yahoo.com
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organic acid synthesis, as well as microbes like nitrogen fixer. (Muhammed *et al*., 2012). Moreover, PGPR (plant growth promoting rhizobacteria) can enhance a plant's competitiveness, root development pattern, growth, and reactions to environmental stressors. Moreover, PGPR has been demonstrated to promote systematic resistance (ISR) to bacterial, viral, and fungal diseases in a variety of crops, including tobacco, tomato, radish, and bean. (Glick,1995). Yeast as a nautral source of cytokinins-stimulates cell division and enlargement as well as the synthesis of protein, nucleic acid and chlorophyll (Kriag and Haber, 1980; Spencer *et al*., 1983; Casetlfranco and Beale, 1983; Fathy and Farid, 1996). This is because its content of many nutrient elements and being productive compounds of semi growth regulator compound like auxins, gibberellins and cytokinins (Glick, 1995). Therefore, the aim of this study aimed to evaluate the efficiency of different rates of some biostimulate substances and local salt tolerant bacterial inoculant to improving wheat growth under irrigation with saline water.

2. Materials and methods

2.1. Isolation and characterization of Azotobacter strains

The soil samples were collected from different saline soil cultivated areas in Qena governorate, including the following regions, mentioned below:

Region (I): Nag Hamady area.

Region (II): Abotesht area.

The rhizosphere was removed from the soil soil samples and collected in sterilized clean plastic bags then transferred to the laboratory for *Azotobacter* spp. Isolation. Electrical conductivity (E.C dSm-1) of soil extract for the collected samples was measured according to (Richards, 1954). Ashby's modified medium for *Azotobacter* isolation (Abdel-Malek and Ishac, 1968) having the following composition, was used for isolation and subsequent cultivation of *Azotobacter* strains: sucrose, 10 g; K_2HPO_4, 0.2 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.2 g; K_2SO_4, 0.1 g; $CaCO_3$, 5 g; agar, 15.0 g and distilled water, 1000 ml. One gram of rhizosphere soil was added to 100 ml of the sterilized liquid medium in 250 ml capacity Erlenmeyer flask and, after 4 days incubation at 28 - 30 C^{o} a loop fool of the pellicle that was formed on medium surface was streaked on the nitrogen free agar medium in plates. After incubation, the developing *Azotobacter* colonies were repeatedly purified by single colony isolation, and then finally streaked on the nitrogen free medium containing 0.5 % sodium benzoate as sole carbon source. The purity of the isolated cultures was tested microscopically before studying their characteristics. The isolated cultures, 24 hours and 7 days old, grown on N-free broth and agar media, were examined microscopically for determination of cell morphology and Gram reaction. Two *Azotobacter* isolates were selected as a locally strains isolated from rhizosphere soils of Maize plants cultivated in saline soil of 3.37 ds/m for strain A and 3.8 ds/m for strain B), while, strain C were taken as a reference strain from Microbial Resource Centre, Faculty of Agriculture, Ain Shams University. The isolated cultures were characterized morphologically, examined for Gram stain to identify *Azotobacter* sp. According to Bergey's Manuals Systematic of Bacteriology, 1984.

2.2. Efficiency of the isolated diazotrophs to fix atmospheric nitrogen

The amount of nitrogen fixed by the selected isolated *Azotobacter* were determined by the microkjeldahl method according to Bremner and Mulvany (1982). The amounts of sucrose found in the media and cultures were determined calorimetrically according to the method described by Dubios *et al*. (1956).

2.3. Selection the most salt adaptive tolerance Azotobacter isolates

One ml of liquid culture of selected isolated *Azotobacter* were inoculated to Ashby's modified free nitrogen medium supplemented with different concentration of NaCl (0.02, 0.04, 0.08, 0.16, 0.32, 0.64 and 1.28 g/100ml) and incubated at 30°C with agitation at 100 rpm for 5 days then, at 24 hr intervals growth was measured in each culture by counting *Azotobacter* population. The most probable number (MPN) method of (Alexander, 1965) was used for estimation of *Azotobacter* population in strains cultures.

2.4. Evaluation of the biostimulant effects of different substances for wheat promoting growth

Three substances were tested for biostimulant activity on wheat plant. The description of the products can be found in Table (1).

Table 1. Identity of the tested substances.

NO.	Tested substances	Treatments concentration	Component
1	Biofertilizer (*Azoto*bacter) (Soil inoculation).	Azoto spp. (A)	*Azotobacter* (local strain) Isolated from soil with EC (3.47 dS/m)
		Azoto spp. (B)	*Azotobacter* (local strain) Isolated from soil with EC (3.8 dS/m)
		Azoto spp. (C)	Were taken from Microbial Resource Centre, Faculty of Agriculture, Ain Shams University.
2	Humic acids soil drench (Divided into 4 additions)	2kg/fed.	Water soluble fertilizer contain 10% K2O – in form of potassium humate. Humic acid (trade name MASHREK K-Humate) was supplied from a MASHREK company / Hebel monoband water soluble fertilizer Co.
		3kg/fed.	
		4kg/fed.	
		5kg/fed.	
3	yeast extract soil drench (four additions by 100 L / fed.)	1g/L	was supplied from TMMEDIA company: Solubility (2% 8oin.at 25 C)- soluble in distilled water, Clarity (2% 8oin.at 121 C)- Clear solution, pH (2% 8oin.at 25 C)- 5.5-6.5, Chloride (as NaCl)- 5% , Total nitrogen10%, Alpha Amino Nitrogen 3%, Total ash 15%
		2 g/L	
		3 g/L	
		4 g/L	

2.5. Pots experiment

Preliminary pots experiment were conducted in the screen house of agricultural experimental farm of department of soils and water, faculty of agricultural, south valley university, Qena governorate, Egypt to evaluate effect of different doses of each biostimulate substances and select the most efficient plant promoting dose (s) of each product on plant growth under normal conditions. Small pots (8 cm in diameter) were filled with 1 kg of the investigated soil. Treatments of the three biostimulate treatments were singly used on wheat plant plus the untreated control and arranged in the screen house in completely randomized block design with four replications**.** Some soil and irrigation water properties are in Table 2 and 3 respectively.

Table 2. Physical and chemical properties of the soil used in this experiment.

Sand	Silt	Clay	Texture	pH (1:2.5)	EC (dS m^{-1})	Calcium Carbonate (%)	Organic Matter (%)	Total N (%)	Available P (mg/ kg)	Available K (mg /kg)
72	23	5	Sandy loam	8.21	0.53	9.92	0.73	0.014	4.42	115

Table 3. some chemical properties of irrigation water used in this experiment

pH	EC dS/m	RSC	SAR	Soluble cations (mmol L^{-1})				Soluble anions (mmol L^{-1})		
				Na^+	K^+	Ca^{2+}	Mg^{2+}	HCO_3^-	Cl^-	SO_4^{2-}
8.25	3.15	0.0	13.62	33.37	0.50	2.05	3.95	9.20	8.40	8.69

2.6. Recorded Data

Plant height (cm), plant fresh and dry weight (g) and roots fresh and dry weight were estimated at 35days after sowing by taking hole plants from each pot. Using MSTAT-C, one-way analysis of variance was used to examine all the data that were collected. The Least Significant Difference (L.S.D.) test with a probability of 5% was used to examine the differences in means between the various treatments.

3. Results and discussion

3.1. Isolation and Characterization of Azotobacter Isolates

Three *Azotobacter* strains were used in this investigation. The morphological, characteristic of the selected *Azotobacter* strains (A), (B) and (C), are presented in Table (4). On Ashby's medium, the isolates produced large cheesy mucoid, raised colonies. On sodium benzoate agar medium (0.5%) colonies of the isolates produced dark brown to black diffusible pigment. The vegetative cells of the three strains, were Gram negative rods with round ends, found single or in pairs. On Ashby's liquid medium, the two strains showed surface pellicle formation and capsulated non-spore forming, the cells result show that the selected strain were *Azotobacter chroococcum.*

3.2. Efficiency of the isolated strains to fix atmospheric nitrogen

The amount of nitrogen fixed by *Azotobacter chroococcum*, strains No. A, B and C determined by the microkjeldahl method according to Bremner and Mulvany (1982), were 9.9, 10.5 and 12.2 mg N/g sucrose respectively.

3.3. Selection the most salt adaptive tolerance Azotobacter isolates

Results as illustrated in Figure (1), showed that there was a negative strong relation between salt concentrations of media and growth, were with salt concentration increased, the growth of *Azotobacter* isolates decreased.

Table 4. Characterization of *Azotobacter* Isolates.

No.	Isolates	Sources	Gram test	Shape
1	*Azotobacter* spp. (A)	Maize rhizosphere soil With EC (3.5 ds/m)-Nage Hamady area.	G (-) ve	Rods, round ends
2	*Azotobacter* spp. (B)	Maize rhizosphere soil With EC (3.8 ds/m)-Abotesht area	G (-) ve	Rods, round ends
3	*Azotobacter* spp. (C)	Microbial Resource Centre, Faculty of Agriculture, Ain Shams University.	G (-) ve	Rods, round ends

Increasing of NaCl concentration in the media reduces the bacterial growth, where there was a gradual decreasing in the *Azotobacter*, accompanied with increasing of NaCl concentration until the maximum concentration (1.28 g/100ml) of salt where the growth decreased sharply, indicating that salt adaptive bacteria reached the maximum available tolerance against salt concentration of the media.

Figure (1) showed that the salt tolerance of strain A was higher than strains B and C, while strain A can still grow under salt concentration up to 1.28 g / L. and booth strains B and C cannot grow under salt concentration more than 0.32 g / L. These results are in conformity with the findings of Sivapriya *et al*. (2017). They reported that some strains of *Azotobacter* are able to grow at NaCl concentrations as high as 4 % and others cannot grow, when NaCl concentration is above 6%. Also, Nanis *et al*. (2018) reported that *Azotobacter* can grew until the maximum concentration (5g/100ml) of salt, after which the growth began to decrease sharply, indicating that salinity-adapted bacteria reached the maximum available tolerance against salt concentration of the media.

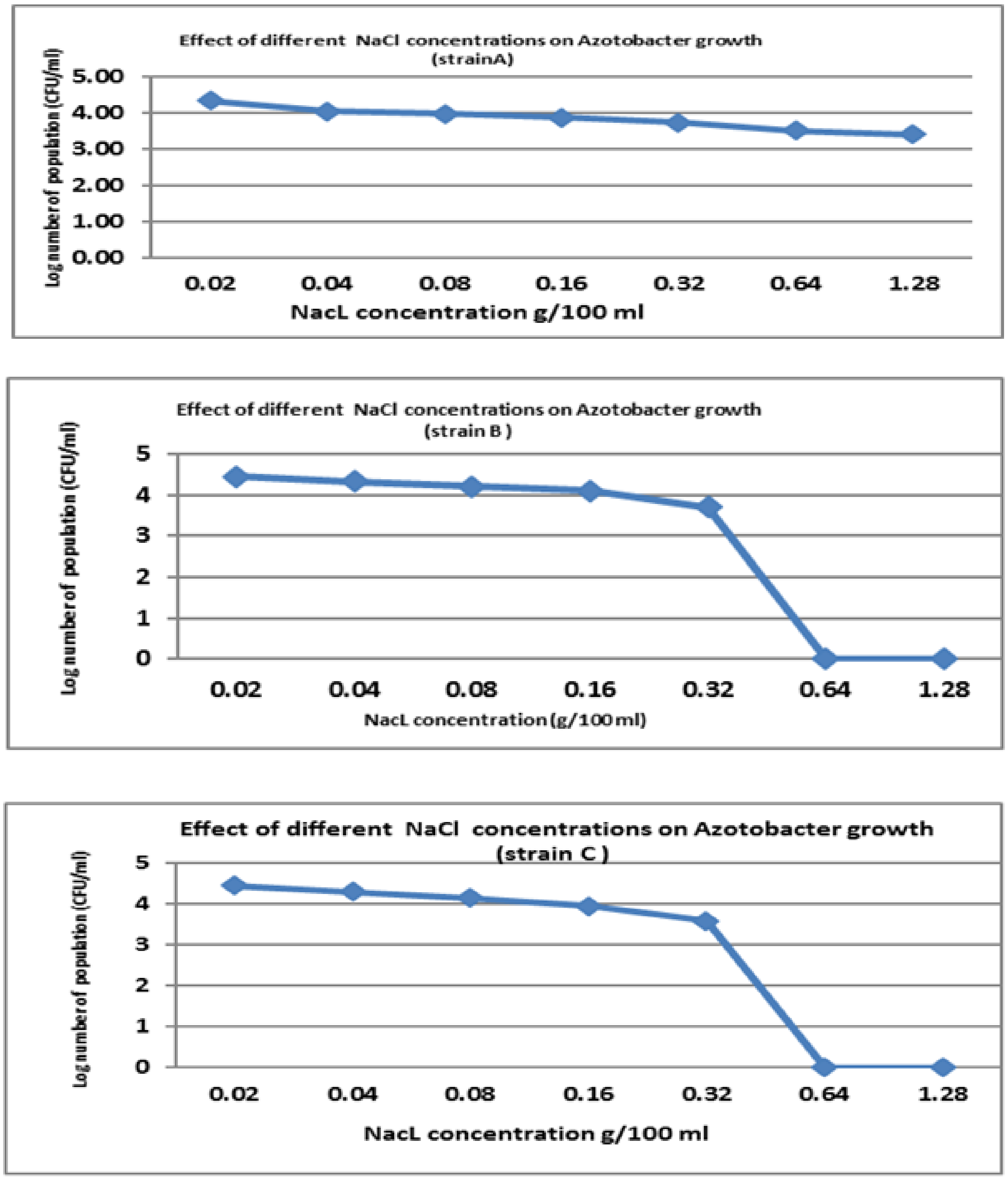


Figure 1. Effect of different NaCl concentrations on Azotobacter growth.

3.4. Evaluation of the biostimulant potential of different substances for Promoting Growth

3.4.1. Effect of soil treatment with different rates of humic acid on growth of wheat plant

Figure 2. clearly showed that, different humic acid rates positively affected plant height, plant fresh weight, plant dry weight, roots fresh weight and roots dry weight of wheat. The results showed that all parameters were increased gradually by increasing levels of added humic acid. The highest rate (5 kg humic acid /fed.) followed by a rate of (4 kg / fed.) recorded heighest values with no significant differences observed between the two rates.

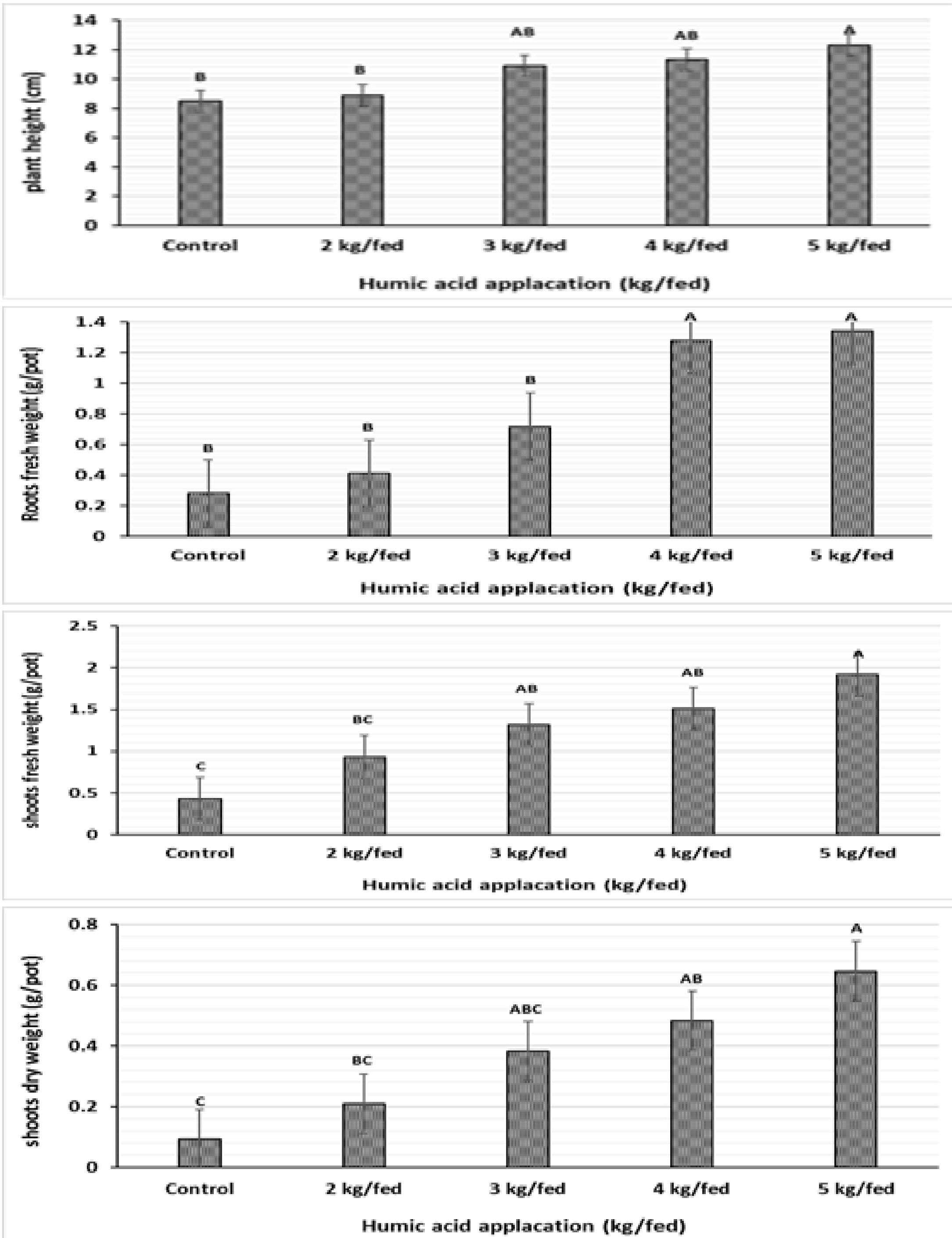
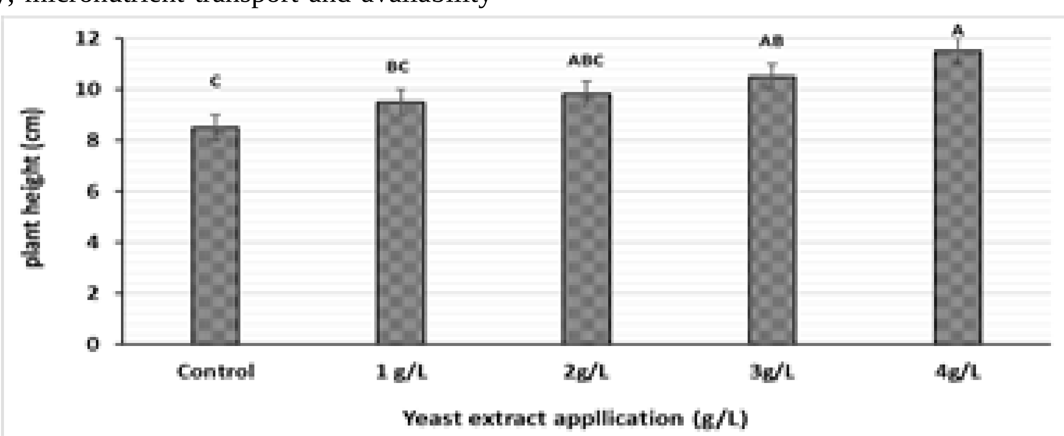


Figure 2. Effect of soil treatment with different rates of humic acid on growth of wheat Plant.

The rate of 4 kg/fed. recorded 11.33 cm, 1.52 g, 0.483, 1.28 g and 0.36 g/plant for plant height, shoots fresh and dry weight and roots fresh and dry weight of wheat, respectively, while at the 5 k humic acid /fed. the values for all measurements being 12.33 cm, 1.72 gm and 0.65 g, 1.34 g, and 0.44 gm respectively, comparing with. control treatments which recorded 8.5 cm, 0.43 g, 0.093 g, 0.28 g and 0.11 g for previous parameters, respectively (Fig. 2). As shown in Figure (2), humic acid soil treatment by 5 and 4 kg humic acid /fed. Resulted in significant highest increases in wheat plant growth parameters compared with untreated treatment (control), the results indicated that no significant differences between 5 and 4 kg / fed. Which meaning that treatment of 4 kg / fed. had similar effect that obtained by 5 kg / fed. Several studies have evaluated the effect of the organic matter content on the fertility of soils (Loveland & Webb, 2003 and Pan *et al.*, 2009). Gümüs and seker (2015) reported that, humic acid applications can improve chemical and physical properties of soil such as EC, soil organic carbon, total nitrogen, modulus of rupture and aggregate stability. Sangeetha *et al.* (2006), reported that, the humic Substances, the major component of soil organic matter has both direct and indirect effects on plant growth. The direct effects are those that require the uptake of humic substances into the plant tissue resulting in various biochemical outcomes, whereas the indirect effects involve the improvement of soil properties, such as aggregation, aeration, permeability, water holding capacity, micronutrient transport and availability (Tan, 2003). Abd El-Razek *et al*. (2020) reported that, Adding organic manure, humic and bio-humic, which was composed of humic acid plus liquid cultures of Azotobacter chroococcum, Bacillus megaterium, and Bacillus circulans, not only plays an important role in increasing the water holding capacity but also improves the nutritional status in order to activate nutrient uptake and beneficial microorganisms because humic acid and bio-humic have a positive effect on improving the root development, use of organic fertiliser boosts soil fertility and enhances the physical, chemical, and biological characteristics of the soil, increasing yield per unit area.(Dolarslan and Gul, 2012). Mona and Wael (2019), reported that, humic acid application has a beneficial effect not only to plant growth and soil but to biofertilizers applied as well. They added that, biofertilization and humic acid application have the potential to improve Olive seedling growth under salinity stress.

3.4.2. Effect of soil treatment with different rates of yeast extract on growth of wheat plant

Figure (3) shows that, progressive significant growth of wheat plant in all growth parameters was achieved with the application of different rates of yeast extract. When 4 g / L. of yeast extract was applied to the soil, the plant height of wheat reached 11.4 cm, and recorded 1.38 g, 0.55 g,0.72 g and 0.37 g for shoot fresh weight, shoot dry weight, roots fresh weight and roots dry weight, respectively, which was significantly higher than that under the control.



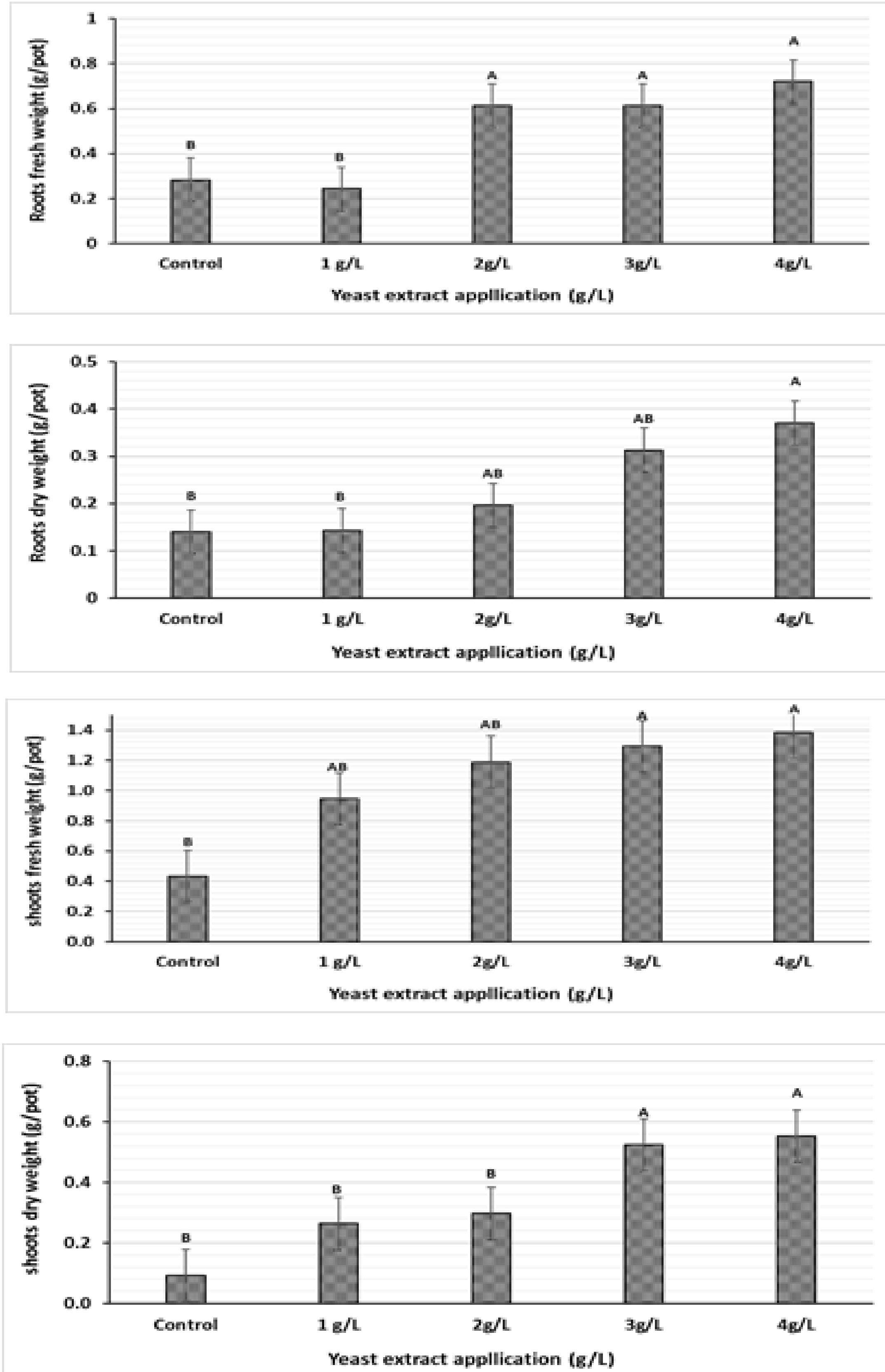


Figure 3. Effect of soil treatment with different rates of yeast extract on growth of wheat plant.

The results also showed that no significant differences with application of 3 g / L. of yeast extract, this meaning that soil treatment with 3 g/L of yeast extract gave the same promotion effects. Xi *et al.* (2019) reported that, using yeast extract to improve soil qualities and encourage seedling growth was a good idea. By presumably boosting the concentration of auxin and cytokinin and facilitating an osmotic adjustment, the yeast extract application to rhizosphere soil had a substantial impact on root characteristics. He continued by saying that because of the nutrients in the yeast extract, the soil's fertility might be raised due to the higher levels of organic matter, total nitrogen, and total phosphorus. Moreover, the pH and calcium carbonate level both slightly decreased, and the mineralization of amino acids followed. Fathy and Farid, 1996 reported that, as a naturally occurring supply of cytokinins, yeast promotes cell growth and division as well as the production of protein, nucleic acid, and chlorophyll. The benefits of yeast extract are related to its high nutrient content and ability to produce semi-growth-regulating chemicals including auxins, gibberellins, and cytokinins. (Glick, 1995). Also, Amer, 2004 reported that, yeast extract is rich in effective constituents, such as low-molecular- weight organic matter, amino acids, nucleotides, peptides, nitrogen, phosphorus, and trace elements. The application of yeast significantly increased the vegetative growth, yield, and quality of vegetables such as cucumber (*Cucumis sativus* L.), Shehata et al. (2012), head lettuce (*Lactuca sativa* L.) Fawzy (2007), potato (*Solanum tuberosum* L.), Ahmed *et al*. (2011), eggplant (*Solanum melongena* L.) El-Tohamy *et al*. (2008) , turnip (*Raphanus sativus* L.) (Shafeek *et al*. 2015) and soybean (*Glycine max* L.) Merrill) (Dawood *et al*., 2013) and also led to an increase in elemental content, such as N, P, K, Fe, and Zn, in vegetables.

3.4.3. Effect of soil inoculation with the most salinity adapted Azotobacter isolate on growth of wheat plant

As shown in Figure (4), inoculation of wheat with all tested *Azotobacter* isolates enhanced seedling growth. However, the increases in growth parameters were more pronounced in case of strain (A) as indicated from the significant increases in plant height, shoot and root fresh and dry weights which recorded the highest value compared to uninoculated control.

Hindersah, *et al*. (2020) reported that, as a biofertilizer, biostimulants, and bioprotectant, Azotobacter works to promote plant growth. Azotobacter uses nitrogen fixation as a method to make accessible nitrogen for root absorption. Indole acetic acid, cytokinins, and gibberellins are found in the liquid culture of Azotobacter, which stimulates plant development through the manufacture of phytohormones. Exopolysaccharide synthesis and plant protection are two indirect effects of Azotobacter. the secretion of substances that promote plant growth, such as hormones, vitamins, nitrogen fixation, and organic acid production, as well as the improvement of plant metabolic activity and nutrient uptake, are all important ways that microorganisms, such as the nitrogen fixer Azotobacter spp., in stimulates vegetative growth (Muhammed *et al*., 2012). In addition, Plant growth-promoting rhizobacteria (PGPR) can enhance a plant's growth, nutrition, pattern of root growth, competitiveness, and reactions to outside stressors. Moreover, PGPR has been demonstrated to promote systematic resistance (ISR) to bacterial, viral, and fungal diseases in a variety of crops, including tobacco, tomato, radish, and bean. (Glick,1995).

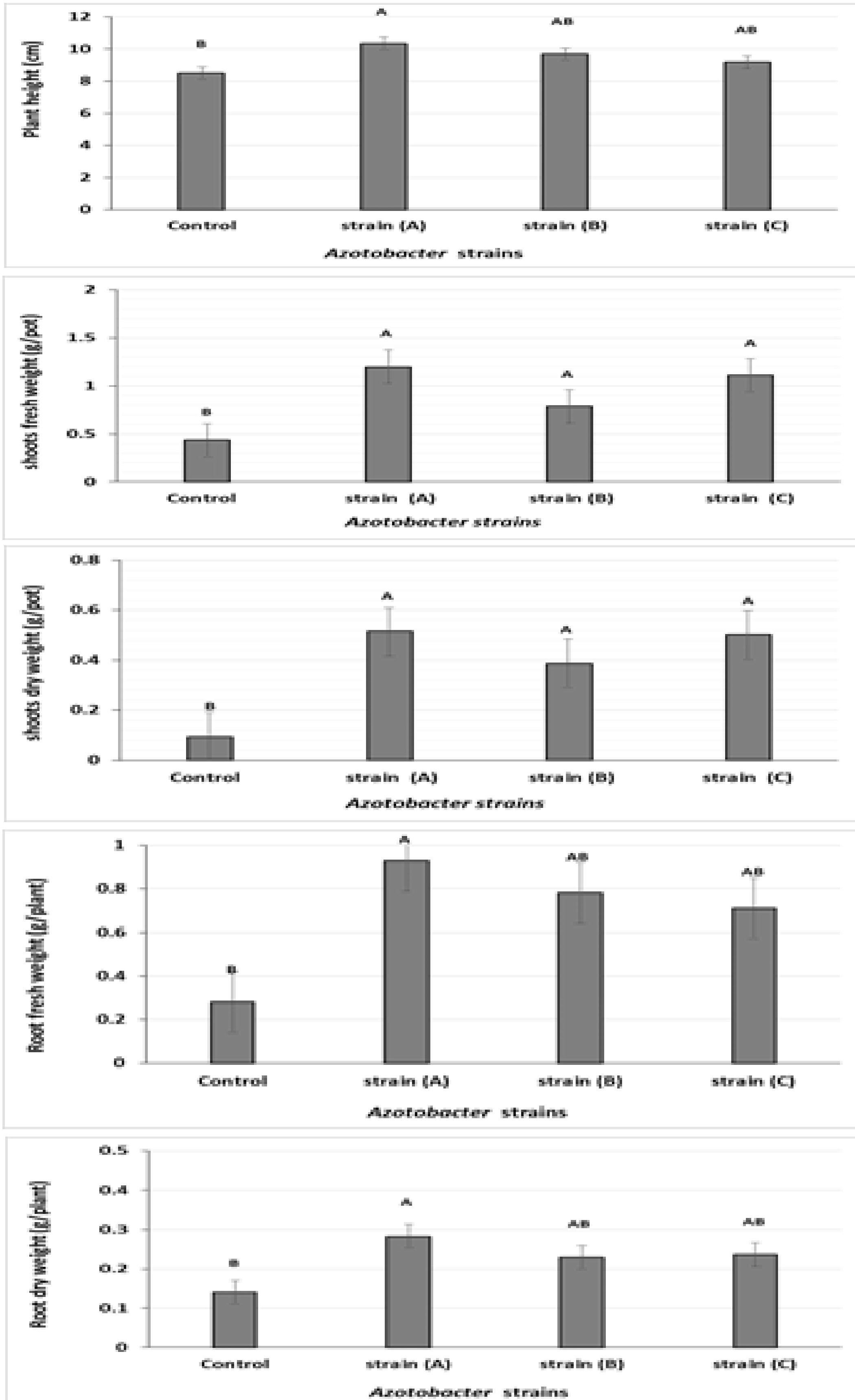


Figure 4. Response of wheat to soil inoculation with the most salinity adapted *Azotobacter*.

4. Conclusions

Application of humic acid led to improvement physical, chemical and microbiological properties of soils and enhances of plant growth with the treatment of 4 kg humic acid /fed compared to other humic acid treatments. In addition, the application of the yeast extract to rhizosphere soil had a significant effect on plant growth.

Overall, the application of yeast extract can be considered as a method for both a plant growth promoter and soil conditioner. It was the best treatment yeast extract soil drench by 100 L / fed., of the concentration 3 g/ L. On the other hand, growth of wheat was improved by using Azotobacter spp. Also, It was verified in this study that the application of humic acid, yeast extract and selected Azotobacter spp. strain had a beneficial effect on promoting wheat seedling growth and the amount applied to the soil was the key factor in improving the growth.

These treatments played a crucial role in enhancing the growth of wheat seedlings. So, this study is an exploration the important of like biostimulate substances application. The effects of greater levels of these biostimulate substances on other species need to be studied. As a superior plant growth promotion and soil conditioner under irrigation with saline water, these substances could probably be applied in combination with them or with other materials or even applied in other regions and soils.

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All authors are contributed in this research.

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Institutional Review Board Statement

All Institutional Review Board Statements are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable.

Conflicts of Interest

The authors disclosed no conflict of interest starting from the conduct of the study, data analysis, and writing until the publication of this research work.

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