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Effect of Organic Acids and Plant Growth Promoting Rhizobacteria (PGPR) on Biochemical Content and Productivity of Wheat under Saline Soil Conditions

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ABSTRACT

An experiment was done to examine the survival ability of the plant growth promoting rhizobacteria PGPR under investigation (*Rhizobium leguminosarum* and *Paenibacillus polymyxa*) under salt stress to ensure their efficiency in saline soil when they used as PGPR inoculants. Data revealed the initial screening by spotting on media supplemented with different concentrations of salt (0.3, 0.6, 0.9, 1.2 and 1.5M NaCl) in which indicated that *R. leguminosarum* and *P. polymyxa* were able to grow at all concentrations of NaCl with different log numbers. However, the growth was reduced as the concentration of NaCl increased. Also, data revealed that both *R. leguminosarum* and *P. polymyxa* succeed to solubilize the phosphorus and produce IAA at the NaCl concentrations 0.3 and 1.5M NaCl. However, *P. polymyxa* show highest P-solubilization ability at different salt concentrations. The data also revealed the potentiality of testing PGPR in production of HCN and siderophores at different concentrations of NaCl, in which both PGPR have the ability to produce HCN and siderophores at 0.3 and 1.5M NaCl however *P. polymyxa* was superior in production of HCN and siderophore. A field experiment was conducted at the Sahl El-Hussinia, El-Sharkia governorate, Egypt during winter-growing season of 2018/2019 to study the effect of PGPR (*R. leguminosarum* and *P. polymyxa)* inoculants and foliar application of organic acids (humic and ascorbic acids) on wheat growth and productivity under salt stress. The experiment was laid out in randomized complete block design (RCBD).The results showed that, the best treatment was (AsA +HA+ mixture of PGPR) treatment has a high biological yield, which increased by 43.00%, grain yield by 43.11% and led to increases in 1000-grain weight by 35.19%. In addition, chemical constituents as (antioxidant activity, phenolic components and NPK uptake) of wheat crop gave the highest significant value by (AsA +HA+ mixture of PGPR) treatment. It is obvious from obtaining data in this study that all treatments play an important role in the alleviation of salt stress on wheat plants through their positive effect on biochemical contents of the plant.

***Keywords*:** PGPR, Chemical constituents, Humic acid, Ascorbic acid, Saline soil

Introduction

According to FAO (2014), wheat (*Triticum aestivum* L.) is the national staple food for over one-fifth of the human populace around the globe. The food and agriculture organization (FAO), during 2014-2015 confirmed that 9.4 million tons of wheat were produced and it is estimated that up to 11 million tons will be produced in 2015-2016 growing season in Egypt. Meanwhile, approximately 8.1 million ha of the Egyptian soil is cultivated with wheat (FAO, 2015). It is noticeable that the Egyptian population increases, thus the demand for wheat will be increased annually. So it has to expand the cultivated area with wheat; according to the economic affairs sector, Ministry of Agriculture and Land Reclamation in Egypt (FAO, 2015). Thus, the wheat productivity could be increased through resistance to a biotic stresses (Siahpoosh and Dehghanianb, 2011).

Agricultural productivity faces a great problem worldwide due to salinity, particularly in the arid and semiarid regions. In these regions, many factors such as low rainfall, high evaporation rate, poor irrigation water and its management and accumulation of salts in the top layer of the soil due to

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over-irrigation are responsible for the salinity of soils (Rady *et al*., 2013). While, the reduction in plant growth in saline soil could be according to the osmotic effect as a result of salt stress that caused an increase in growth inhibitors (i.e., abscisic acid) and decreased growth promoters (i.e., indole-3-acetic acid and gibberellins) (Rady *et al*., 2013). Also, Semida and Rady (2014) have referred the inhibition of growth to the distribution in the water balance of the stressed plants which lead to stomatal closure, ionic imbalance, reduction in photosynthesis and accumulation of toxic ions.

Since there is rapid growth of human population, the rising for food and usage of saline soils or water for crop production is demanded (El Sayed *et al*., 2016). Thus, efforts have been made to control salinity by various technological means, including the application of soil amendments (Semida *et al*., 2015).

Use of organic fertilizer such as humic acid, can meet the nutrient requirement of sustainable wheat production under saline soil conditions (Ahmed and Ismail, 2016). Ouni *et al*. (2013) defined humic acid, that it is mainly derived from the bio, chemical degradation of plant and animal residues and from microbial synthetic activity and they constitute a significant fraction of the soil organic matter (65-70%). Whereas, Asik *et al*. (2009) stated that, humic substances gave the highest values of available nutrients, yield and nutrient uptake by wheat plant in sandy soils.

Much attention has been paid to the soil application of humic acid to alleviate the inhibitory effects of soil salinity by improving the physical and chemical properties of soils, increasing soil water retention and providing the nutrients during plant growth and might show anti-stress effects under salinity conditions (Rady *et al*., 2016). In addition, (Osman and Rady, 2012) mentioned that, the one of mechanisms of humic acid to promote plant growth could be through the enhancement of nutrients uptake and reduction of the uptake of some toxic elements. They also added that, the application of humic acid as a soil amendment resulted in significant increases in crop yields in reclaimed saline soils, probably due to improvement of hydro-physical properties and nutrient availability of these soils.

Also humic acid has enormous impact on soils in which it can improve the chemical properties of soils through increment of soil microorganisms number, which enhance nutrient cycling, and reduce soil pH (Osman and Rady, 2012) thus leading to increase in the availability of mineral nutrients to plant roots.

Ascorbic acid is an organic acid and one of the most powerful antioxidants, which increase the total organic components for both shoot and roots of the plant and mitigate the impact of salinity inhibitory to the plant metabolism (El Sayed *et. al*., 2015 a & b).

El Sayed *et al*. (2016) also reported that Ascorbic acid has a major role in the regulation of many critical biological processes such as photo-inhibition and cell elongation. Ascorbic acid also is involved in cell cycle and many other important enzymatic reactions (biosynthesis of ethylene, for example) (Smirnoff, 2000).

Furthermore, such positive effects of ascorbic acid in overcoming the adverse effects of salt stress were attributed to the stabilization and protection of photosynthetic pigments and the photosynthetic apparatus from oxidative damage (Khan *et al*., 2006).

Since, Plant growth and agriculture productivity are severely affected by soil salinity because salt levels detrimental to plant physiology have adverse effects on vast territories globally. Thus, Hamidi *et al*. (2009) have addressed the advantageous effects of bacterial inoculation on plant physiology and growth under salt stressed conditions, through diverse mechanisms such as the ability to produce normal plant growth promoting properties such as phytohormone (gibberellic acid, cytokines, indole acetic acid, symbiotic nitrogen fixation) which led to an increase in grain filling period.

Previous studies suggested that utilization of plant growth promoting bacteria PGPB has become a promising alternative to alleviate plant stress caused by salinity (Yao *et al*., 2010) and the role of microbes in the management of biotic and abiotic stresses is gaining importance. The subject of PGPR elicited tolerance to abiotic stresses has been reviewed recently (Yang *et al*., 2009).

Therefore, in the development and implementation of sustainable agriculture techniques, biofertilization has great importance in alleviating environmental pollution and deterioration of nature (Mehran *et al*., 2011 and Jalilian *et al*., 2012). Also Asal (2010) exhibited the role of biofertilizers in agriculture as a sustainable way of increasing crop yields and economize their production as well.

A good number of studies are underway to improve plant growth, and induce systemic tolerance to various abiotic stresses in plants such as salinity, drought and heavy metals through alteration of plant physiology using plant growth promoting rhizobacteria (PGPR) (Egamberdieva *et al*., 2016).

In this regard, Grover *et al*. (2011) reported that, bacteria belonging to different genera including *Rhizobium*, *Bacillus*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Burkholderia*, *Achromobacter*, *Azospirillum*, *Microbacterium*, *Methylobacterium*, *Variovorax*, *Enterobacter* etc. have been reported to provide tolerance to host plants under different abiotic stress.

Nishma *et al*. (2014) proved that many species of *Pseudomonas*, *Bacillus*, and *Azotobacter* can grow and survive in extreme environmental conditions, such as, tolerant to higher salt concentration, pH values, and even in dry soils with maximum temperature. Specially *A*. *Chroococcum* which found to be tolerated to a maximum NaCl concentration of 6% with a temperature of 45°C and also up to pH of 8.

The main purpose of this work was to evaluate the possible use of organic acids such as humic and ascorbic acids as foliar applications individually or in combination with PGPRs (*Rhizobium leguminosarum* and *Paenibacillus polymyxa*) to alleviate the harmful effects of salinity stress on wheat crop, explaining the role of these amendments and inoculations in improving growth and yield of wheat under salt stress conditions.

Material and Methods

Rhizobacteria:

Two locally isolates were used for inoculating wheat seeds, *Rhizobium leguminosarum* and *Paenibacillus polymyxa.* These microorganisms were kindly provided by the Biofertilizers Production Unit of Agric. Microbiol. Res. Dept., (SWERI), (ARC), Giza, Egypt.

Rhizobium leguminosarum was grown in yeast extract mannitol medium (Vincent, 1970), while the nutrient agar medium was used for cultivation and maintenance of *Paenibacillus polymyxa* (Dowson, 1957).

Assessment of growth and survival of the rhizobial strains under salt stress:

To determine the effect of salt on the growth of *Rhizobium leguminosarum* and *Paenibacillus polymyxa* cultures, yeast extract mannitol and nutrient broth media was supplemented with different concentrations of salt (0.3,0.6,0.9,1.2 and 1.5M NaCl) (Paul *et al.,*2014), while normal broth media without additional salt used as control. All flasks were inoculated with 0.2 ml of the starter culture previously prepared. Inoculated flasks were incubated in a rotary shaker at 28^{o}C for 3 days, their growth was visually assessed by the plate count technique (Vincent, 1970). The mean values were calculated from triple reading and expressed by log number.

Evaluation of PGP properties in vitro under salt stress:

Effect of salt on the plant growth promoting activities of the tested PGPR such as P-solubilization, indole acetic acid IAA production, hydrogen cyanide HCN production and siderophores production abilities was determined under different concentrations of salt (0.3 and 1.5 M NaCl). Three replicates per treatment and uninoculated controls were maintained.

- Phosphate solubilization:

The ability of *Rhizobium leguminosarum* and *Paenibacillus polymyxa* to solubilize phosphorus under salt was conducted quantitatively through supplementation of Pikovyskaya broth media (Pikovyskaya, 1948) with different concentrations of NaCl (0.3 and 1.5M). Then the amount of phosphate solubilized was measured according to Jackson (1973).

- IAA production:

L.B. Media supplemented with tryptophan and different concentrations of NaCl were used to determine the IAA production quantitatively by the tested PGPR (Hartmann *et al*., 1983).

-HCN production:

L.B. Media supplemented with glycine and different concentrations of NaCl were used to determine the HCN production qualitatively by PGPR under investigation (Hartmann *et al*., 1983). The intensity of color was noted.

- Siderophores production:

Siderophores production was detected by using Chrome azurol assay (CAS) developed by Schwyn and Neilands (1987). Bacterial cultures were spotted on CAS medium supplemented with different concentrations of NaCl and incubated at 28±20C for 7 days. Siderophores production was confirmed by the development of yellow to orange colored colony upon incubation.

Preparation of inoculants:

To prepare inoculants of rhizobacteria *(Paenibacillus polymyxa and Rhizobium leguminosarum*), vermiculite provided with 10% Irish peat was packed in polyethylene bags, then sealed and sterilized by gamma irradiation ($5.0x10^6$ rads). Bacterial culture ($1x10^9$ cfu/ml) from each kind was injected into the sterilized carrier to satisfy 60% of the maximal water holding capacity (10^7 cells/g carrier).

Field experiment:

A field experiment was conducted at the Sahl El-Hussinia, El-Sharkia governorate, Egypt, located at 31º00 14´N, 32º08. 14´E, during winter season of 2018/2019 to study the effect of PGPR (*R. leguminosarum and P. polymyxa*) inoculants and foliar application of organic acids (humic and ascorbic acids) on growth and productivity of wheat crop under salt stress. The main chemical and physical characteristics of used soil were carried out according to methods of Piper (1950) and Page *et al.* (1982) and the obtained data were recorded in Table (1).

Table 1: Some chemical and physical properties of the studied soil

a) Some chemical properties:

Depth (cm)	PH (1:2.5)	EC dS/m	Soluble cations and anions in saturated soil extract (meq/L)								Available nutrients (ppm)		
			Cations				Anions						
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Cl^{-}	HCO^{-}_3	$CO^{=}_3$	$SO^{=}_4$	N	Olsen-P	K
0-30	8.20	8.5	20.44	16.60	36.95	10.93	26.54	18.75	0.00	39.63	18.95	6.50	165.5

b) Some physical properties:

Depth (cm)	Particle size distribution (%)			Texture grade
	Sand	Silt	Clay	
0-30	18.6	25.6	55.8	Clay

The experiment was laid out in randomized complete block design (RCBD) with three replicates and each plot was 10.5 m^2. Wheat seeds were inoculated shortly using coating technique before sowing with *R. leguminosarum* and *P. polymyxa* individually or mixed together at a rate of 600g inoculum per 60 kg grains fed^{-1} using Arabic gum solution (16%) as a sticking agent. While, foliar application of humic solution as 600 mg L^{-1} (HA) (Fawy *et al*., 2017) and spraying ascorbic acid solution as 400 mg L^{-1} (As) in two sprays (Hashem and Hegab, 2018). The first spray was carried out after 30 days from germination and the other dose at the heading stage of plant growth.

The treatments were conducted as follows:

T1: *Paenibacillus polymyxa.*
T2: *Rhizobium leguminosarum.*
T3: *Paenibacillus polymyxa* + *Rhizobium leguminosarum.*
T4: Ascorbic acid (As.A) + *Paenibacillus polymyxa.*
T5: As.A + *Rhizobium leguminosarum.*

T6: As.A + *Paenibacillus polymyxa* + *Rhizobium leguminosarum.*
T7: Ascorbic acid.
T8: Humic acid (H.A.).
T9: H.A. + *Paenibacillus polymyxa.*
T10: H.A. + *Rhizobium leguminosarum.*
T11: H.A. + *Paenibacillus polymyxa* + *Rhizobium leguminosarum.*
T12: H.A. + As.A + mixture of *(Paenibacillus polymyxa* + *Rhizobium leguminosarum).*
T13: Control (untreated with organic acids or biofertilizers).

Grains of wheat (*Triticum aestivum, cv*. Egypt 1) were kindly supplied by the Wheat Research Department, Field Crops Research Institute, ARC, Giza, Egypt, were sown on November 17, 2018. The recommended dose of NPK fertilizer for wheat was as 90 kg N/feddan (as ammonium sulphate 20.5% N), 30 kg P_2O_5/feddan (as superphosphate 15% P_2O_5) and 50 kg K_2O/feddan (as potassium sulphate 48% K_2O) (Shehab El-Din and El-Shamy, 2003). Nitrogen was applied in four equal doses at10, 20, 30 and 40 days from sowing, while phosphorus was added during soil preparation and potassium was added after 15 and 30 days from sowing in equal two doses. Flood irrigation was practiced on the plants approximately every one month until harvest time was due. Wheat harvest took place on May 12, 2019.

Plant analysis:

-At vegetative stage:
Chlorophyll content was assayed according to Arnon (1949).
The root surface area was assayed depending on digital image analysis technique (Dutta Gupta *et al.,* 2013).
Leaf Proline content, in µg/g fresh weight, according to Bates *et al.* (1973).

Measurement of total antioxidant activity
The extract (0.1 ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. The mixture was cooled to room temperature, and then the absorbance of the solution was measured at 695 NM against the blank. The total antioxidant activity was expressed as ascorbic acid equivalents (AAE) in milligrams per gram of extract (Prieto *et al.,* 1999).

Measurement of total phenol compounds
Total phenolic constituents of plant extracts were performed employing the literature methods involving Folin-Ciocalteu reagent and gallic acid as standard (Slinkard and Singleton, 1977). Extract solution (0.1 ml) containing 1000 ug extract was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent was added and the flask was shaken thoroughly. After 3 min, 3 ml of solution 2% Na_2CO_3 was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated to all standard gallic acid solutions (0-1000 mg, 0.1 ml^{-1}) and standard curve was obtained. The obtained data were statistically analyzed according to Slinkard and Singleton, (1977).

-At harvesting stage:
At maturity, 1 m2 at the center of each experimental plot was chosen to be harvested for the estimation of biological parameters (biological yields, dry weights of grain, 1000 grain weight and grain crude protein) and N, P and K of grains were determined in acid digested solution, which was prepared according to Cottenie *et al.* (1982).

Statistical analysis:
Data of the present work were statistically analyzed and the differences between the means of the treatments were considered significant when they were more than the least significant differences (L.S.D) at the 5% level by using a computer program of Statistix version 9 (Analytical software, 2008).

Results and Discussion

***In vitro* growth of PGPR under salt stress:**

Plant growth promoting rhizobacteria differ widely in their ability to survive under soil environmental stress, therefore, an experiment was done to examine the survival ability of the PGPR under investigation under salt stress to ensure their efficiency in saline soil when they used as PGPR inoculants. Data in Table (2) showed the initial screening by spotting on media supplemented with different concentrations of salt in which indicated that *Rhizobium leguminosarum* and *Paenibacillus polymyxa* were able to grow at all concentrations of NaCl with different log numbers. However, the growth was reduced as the concentration of NaCl increased, the survival of PGPR at high concentrations of salt may due to their physiologically adaptation to abiotic stress, thus they could survive in such harsh environment and help the plant to tolerate salt stress (Cho *et al*., 2015 & Egamberdieva *et al*., 2016). While, Ross *et al*. (2000) suggested that, bacterial inoculants surviving in stress condition have to be developed so that these formulations encapsulate the living cells, protect the microorganisms against many environmental stresses, release them to the soil, and ultimately enhance crop yield.

Table 2: Effect of salt stress on growth and survival of the tested PGPR

NaCl concentrations	*Rhizobium leguminosarum*	*Paenibacillus polymyxa*
Control	9.78	9.68
0.3M	9.58	9.49
0.5M	9.18	8.91
0.9M	8.60	8.08
1.2M	3.91	3.84
1.5M	3.05	2.89

Values are means of three replicates. Readings are taken in log. No

Effect of salt on plant growth promoting activities:

PGPR were observed to possess multiple plant growth promoting traits such as phosphate solubilization, IAA production, HCN production and siderophores production. The effect of salt on all these traits was determined .Effect of salt on the ability of PGPR under investigation to solubilize phosphate and produce IAA was shown in Table (3), data revealed that both *Rhizobium leguminosarum* and *Paenibacillus polymyxa* succeed to solubilize the phosphorus and produce IAA at the NaCl concentrations 0.3 and 1.5M NaCl. However, *Paenibacillus polymyxa* show highest P-solubilization ability at different salt concentrations. Comparing to *Rhizobium leguminosarum* where the amount of phosphorus, dissolved by *Paenibacillus polymyxa* was (260.4 and121.0 ppm) at 0.3 and 1.5M NaCl respectively, while the corresponding amounts dissolved by *Rhizobium leguminosarum* were (200.4 and 92.9 ppm) at 0.3 and 1.5M NaCl, respectively. Same trend was observed in the production of IAA, where, amount of IAA produced by the tested PGPRs was (70.89 and 46.6 µg ml^{-1}) for *P. polymyxa* and (61.9 and 40.3 µgml^{-1}) produced by *Rhizobium leguminosarum* at 0.3 and 1.5M NaCl, respectively.

Table 3: Some PGP properties under saline conditions

NaCl concentrations	P-solubilization (ppm)		Amount of IAA (µg ml^{-1})		HCN production		Siderophores production	
	P.p	**R.l**	**P.p**	**R.l**	**P.p**	**R.l**	**P.p**	**R.l**
Control	301.7	210.7	134.1	107.3	+++	++	+++	++
0.3M	260.4	200.4	70.89	61.9	++	+	++	+
1.5M	121.0	92.9	46.6	40.3	+	+	+	+
High +++	Medium ++	Low +	*R.l.=Rhizobium leguminosarum*		*P.p. = Paenibacillus polymyxa*			

The ability of the tested PGPR to dissolve the P and produce IAA may due to adaptation of PGPR to survive under salt stress and consequently carry out PGP activities. In this aspect, Paul *et al*. (2014) assumed that, although there was a decrease in all PGP activities in 0.3M NaCl compared to

control, these activities were not drastically reduced. Moreover, the salt concentration of saline soil used in agricultural activities is lower than 0.3 M NaCl.

Also Deshwal and Kumar (2013) presumed that there would not be a significant reduction in the PGP activities of these cultures under such saline conditions.

Gravel *et al* (2007) proved the positive role of PGPR as they synthesis and secret IAA which can enter plant cells and stimulate root growth; IAA can also stimulate Acc synthase to produce more ACC deaminase which has greater effect on plant to alleviate the stress.

Another PGP traits under salt stress was recorded. Data in Table (3) revealed the potentiality of testing PGPR in production of HCN and siderophores at different concentrations of NaCl, in which both PGPR have the ability to produce HCN and siderophores at 0.3 and 1.5 M NaCl, however *P. polymyxa* was superior in production of HCN and siderophores. The ability of PGPR to produce siderophores under salt stress was reported by Tank and Saraf (2010), who showed that PGPRs which able to produce phytohormones and siderophores in salt condition can promote growth of tomato plants under 2%NaCl stress. Ramadoss *et al*. (2013) also reported that *Bacillus sp.* with high salt tolerance was further characterized for the PGP activities including IAA production, P-solubilization, HCN and siderophores production.

High salinity is one of the most common environmental stress factors that adversely affect plant productivity by retarding the plant growth and development. To promote plant growth under saline condition, direct use of salt-tolerant bacteria has drawn considerable research interest both in industry and in academics (Ramadoss *et al*., 2013).

Chlorophyll content:

Impact of foliar spray with ascorbic acid (ASA) and humic acid (HA) and PGPR inoculants individually or mixed together on chlorophyll content of wheat plants under saline soil conditions is shown in Table (4). Data revealed significant increment in chlorophyll contents by all treatments compared to control. However, treatment of ASA combined with HA and mixture of PGPRs gave highest chlorophyll content (3.8033 mg/g fresh leaf), followed by ASA combined with mixture of PGPRs, ASA treatment and HA combined with mixture of PGPR inoculants where the amounts of chlorophyll were (3.5000, 3.1900 and 3.1200 mg/g fresh leaf), respectively. Humic acid led to higher concentrations of nutrients, including K, leading to a corresponding increase in chlorophyll (Rady, 2011). While Beltagi (2008) reported that application of ASA increased Chl-a content in cowpea plants under high salinity conditions. The data also revealed reduction in chl. content in untreated plant and could be due to the adverse effect of salinity on photosynthesis. Arora *et al*. (2011) reported that plant tolerance to salinity could be enhanced by the use of biological approaches such as the use of salt-tolerant PGPRs.

Root surface area:

Data in Table (4) revealed the root surface area affected by foliar application of ASA and HA and PGPR inoculants individually or mixed together. Data statically analyzed show significant increase in root surface area with all treatments comparing to control (untreated), whereas, the treatment (ASA +HA+ mixture of PGPR) has highest root surface area (104.86 cm^2). Previous studies have shown that humic acid (HA) promotes root growth and the formation of lateral roots, and enlarges the root's effective absorption area. HA also improves biomass, overall activity and absorption ability of the root system (Jindo *et al*., 2012), also Chen *et al.* (2017) reported that HA treatment significantly increased root diameter and root surface area. These results indicate that the application of HA enlarged roots, and promoted the differentiation from adventitious root to storage root, that reflect root development. Ascorbic has a beneficial effect on the root surface area as well, in which, ascorbic acid can accelerate shoot and root growth under salt stress (Beltagi, 2008). Concerning to effect of PGPRs, Ashraf *et al*. (2004) reported that root inoculation of wheat plants with exopolysaccharide-producing bacterial isolates (*Aeromonas hydrophila*, *Bacillus insolitus*, and *Bacillus* sp.) native to the salt-affected soils, through increasing extent of soil aggregation around roots, provided a "blanket salt-tolerant cover" to the roots, regulating activities of the stress phytohormones promoted and controlled growth of roots of the inoculated wheat plants grown under salt stress conditions.

Table 4: Effect of PGPR and organic amendments on chlorophyll content and root surface area

Treatments	Chlorophyll content mg/g fresh leaf	Root surface area Cm^2
Paenibacillus polymyxa	2.1200 G	75.60 H
Rhizobium leguminosarum	1.7500 H	71.17 J
Mixture (Mix.)	3.0400 CD	83.47 F
AsA + *P. polymyxa*	3.0000 CD	99.95 B
AsA + *R. leguminosarum*	2.9733 D	85.82 E
AsA+ Mix.	3.5000 B	95.22 D
AsA	3.1900 C	84.63 EF
HA	2.5467 E	77.19 G
HA+ *P. polymyxa*	2.3400 F	74.02 I
HA+ *R. leguminosarum*	2.1467 G	75.55 H
HA + mix	3.1200 CD	96.73 C
HA+ AsA + Mix.	3.8033 A	104.86 A
Control	1.6600 H	63.93 K
L.S.D. 0.05	0.1912	1.2136

ASA *= Ascorbic acid* ***HA=*** *Humic acid* ***Mix=*** *(Paenibacillus polymyxa + Rhizobium leguminosarum*)

Effect of PGPRs and organic amendments on wheat yield characters:

After the final harvest the biological yield, grain yield, 1000-grain weight and grain crude protein were determined for all plots. Declining in yield and production due to salinity could be caused by high concentrations of NaCl, which can reduce growth by the accumulation of high concentrations of both Na^+ and Cl^- simultaneously, however, high Cl^- concentration reduces the photosynthetic capacity and quantum yield due to chlorophyll degradation which may result from a structural impact of high Cl^-concentration (Davenport *et al*., 2007and Møller *et al*., 2009). While, high Na^+ interferes with K^+ and Ca^{2+} nutrition and disturbs efficient stomata regulation which results in a depression of photosynthesis and growth (Tavakkoli *et al*., 2010). Thus exogenously applied of ascorbic acid (AsA) and humic acid as a foliar spray and/or inoculation with PGPR promoted the growth of wheat cultivars under saline conditions as shown in Table (5).

Table 5: Effect of PGPR and organic acids amendment on some yield components of wheat plants grown in saline soil

Treatments	Biological yield (ton/fed)	Grain yield (ton/fed)	1000 grain (g)	Grain crude Protein%
Paenibacillus polymyxa	6.01 ABC	2.04 ABC	124.24 D	10.69BCDE
Rhizobium leguminosarum	5.89 ABC	2.00 ABC	133.26 C	9.73DEF
Mixture (Mix.)	6.51 AB	2.37 A	126.70 D	12.83AB
AsA + *P. polymyxa*	5.91 ABC	2.21 AB	138.48 BC	14.25A
AsA + *R. leguminosarum*	6.49 AB	2.21 AB	138.91 BC	10.26CDEF
AsA + Mix.	6.96 A	2.31 A	136.08 C	9.98DEF
AsA	5.47 BC	1.86 BC	125.78 D	11.54BCDE
HA	6.97 A	2.01 ABC	138.35 BC	11.40BCDE
HA+ *P. polymyxa*	6.55 AB	2.23 AB	121.59 D	11.69BCD
HA+ *R. leguminosarum*	6.31 AB	2.14 AB	121.41 D	9.26EF
HA +mix	6.80 A	2.37 A	143.86 AB	12.35ABC
HA+ AsA +Mix.	7.05 A	2.39 A	147.72 A	14.49A
Control	4.93 C	1.67 C	109.27 E	8.77F
L.S.D. 0.05	1.2153	0.4135	6.2440	2.3425

ASA = Ascorbic acid **HA=** Humic acid **Mix=** (*Paenibacillus polymyxa+ Rhizobium leguminosarum*)

In view of the results obtained from this study, it is obvious that all treatments show significant increase in all yield parameters (biological yield, grain yield, 1000-grain weight and grain crude protein) as compared to control (untreated). Whereas, (AsA +HA+ mixture of PGPRs) treatment has a high biological yield, which increased by 43.00%, grain yield by 43.11% and led to increases in 1000-

grain weight by 35.19% over the control treatment (untreated with organic amendments or PGPRs). Same trend was obtained in grain crude protein results, where, (AsA + HA + mix. of PGPR) treatment show highest grain crude protein percentage (14.49 %) followed by (AsA+ *P. polymyxa*) treatment (14.25 %). The positive effect of ascorbic acid may be attributed to its crucial role in improvement of leaf area and leaf chlorophyll content which in turn enhances vegetative and reproductive growth, carbohydrates accumulation and seed set (Smirnoff, 2000; Athar *et al*., 2009). The results of yield components showed that the highest grain yield was crucial due to the higher number of grains/ one spike and number of spikes/m^2 and secondly, to the higher mean 1000-grain weight which are the vital yield. Fawy *et al.* (2017) reported the combination of 48 mg organic manure ha^{-1} + 240 kg N ha^{-1} with spraying with humic acid solution of 600 mg L^{-1} + ascorbic acid solution of 1000 mg L^{-1} gave the highest positive response of plant height (cm) of 118, 26.4, 3.97 and 16.8 for plant height, number of branches per plant, 1000-seed weight, seed weight per quinoa plant and seed yield, respectively. El-Galad *et al*. (2013) reported the positive impact of humic acid application on plant growth through increment of seed yield, pods yield and 1000 grain weight under saline soil conditions.

Also, PGPR may improve plant growth and yield through direct mechanisms which may act on the plant itself and affect growth by means of plant growth regulators, solubilization of minerals, and fixation of atmospheric nitrogen. This in agree with Whipps (2001) who reported the beneficial role of many bacterial genera, such as *Azospirillum*, *Bacillus*, *Pseudomonas*, *Rhizobium*, *Serratia*, and *Streptomyces*, which are plant growth promoters under saline conditions, and their intimate relationship with plants to mitigate salt stress, and hence promote the plant growth. Shrivastava and Kumar (2015) also reported that, the minimum yield (1.74 g per plant) was observed at the highest salinity application level with no biofertilizer application.

Effect of PGPR and organic amendments on NPK uptake of wheat grains:

Data in Table (6) recorded that the NPK uptake in a grain of wheat under saline soil conditions was significantly affected by the treatments. The combination of ASA and HA with mixture of PGPRs (*Rhizobium leguminosarum* and *Paenibacillus polymyxa*) gave higher uptake than each individually or than control. The highest significant values of N, P and K uptake of grains (59.25, 6.06 and 10.45kg/fed) were obtained by (AsA+ HA+ mixture of PGPRs) treatment, respectively. Results of this study agree with the findings by Abdel- Rahaman *et al*. (2015) who found that the foliar sprays with ASA and HA on sorghum gave highest NPK uptake, this is may due to the presence of humic acid which increase cation exchange capacity and enhance soil fertility, converting the mineral elements into forms available to plants (Yilmaz, 2007). In this concern, Talaat (2003) also reported an increment in NPK uptake by sweet pepper foliar sprayed with ascorbic acid. Shawky (2003) referred the superior role of ascorbic acid in an increment of potassium concentration in sweet pepper plants growing under saline conditions, and he assumed that the protection of plant against salt stress by the exogenous supply of AsA is believed to be caused indirectly as a result of its effect on K uptake which plays an essential role in many metabolic processes. Whereas, in the present of AsA under various of NaCl salinity caused an increased significantly in P contents of tomato plant compared to control. El Sayed *et al.* (2016) concerned to PGPR and their role in enhancement of NPK uptake by wheat grains may due to PGP traits by which they make mineral elements into available forms through different mechanisms such as P-solubilization, siderophore, HCN and IAA production. These in agreement with Wahyudi *et al*. (2011) who reported the ability of PGPR isolates to solubilize tri-calcium phosphate in vitro shows the possible application of the isolates in crop fields. Improvement of phosphorus nutrition is one of the factors involved in plant growth promotion by PGPR that may improve plant phosphorus acquisition by solubilizing organic and inorganic phosphate sources through phosphatase synthesis or by lowering the pH of the soil (Saharan and Nehra, 2011).

Table 6: Effect of PGPR and organic acids amendment on some nutrients uptake of inoculated wheat grown in saline soil

Treatments	N-uptake kg/fed	P-uptake kg/fed	K-uptake kg/fed
Paenibacillus polymyxa	38.35CDE	1.197EFG	6.09EF
Rhizobium leguminosarum	34.37DE	1.073FG	2.72G
Mix.	51.54ABC	1.48DEF	9.24AB
As.A + *P. polymyxa*	56.64AB	4.49B	6.76CDEF
As.A+ *R. leguminosarum*	40.03CD	3.26C	5.51F
As.A+ Mix.	41.79CD	5.55A	6.51DEF
As.A	38.53CDE	1.93D	3.18G
HA	47.64ABCD	1.39DEF	7.50CDE
HA + *P. polymyxa*	46.19ABCD	1.48DEF	8.45BC
HA + *R. leguminosarum*	35.58DE	1.43DEF	7.21CDEF
HA + Mix.	43.93BCD	1.87DE	8.02BCD
HA + AsA + Mix.	59.25A	6.06A	10.45A
Control	25.77E	0.67G	2.03G
L.S.D.0.05	13.368	0.6750	1.7104

ASA = Ascorbic acid **HA=** Humic acid **Mix = (***Paenibacillus polymyxa+ Rhizobium leguminosarum***)**

Effect of PGPR and organic amendments on some biochemical contents of wheat Plants:

In order to understand the physiological responses of wheat plants to the inoculation under salinity stress, proline, phenols and total antioxidants were examined. Plants can use several strategies to avoid salt injury at all levels of the organization. Among them, osmotic adjustment, enhancement of antioxidant defense systems and increase the photosynthetic ability are the most important ones (Zhu, 2000; Xiong and Zhu, 2002).

Enzymes play an important role in lowering the reactive oxygen species levels and helping the plant defense against salt stress, from the results mentioned in Table (7) and Figure (1 &2), it is clear that total antioxidant activity and total phenolic contents of wheat plant were significantly affected by different treatments.

Table 7: Effect of PGPR and organic amendments on some biochemical contents of wheat plants grown in saline soil

Treatments	Proline µg/g fr.wt	Phenols (µg GAE/mg ext.)	Antioxidants (µg AAE /mg ext)
Paenibacillus polymyxa	2.74 E	0.92 F	206.41 E
Rhizobium leguminosarum	3.81 D	0.69 F	191.17 G
Mixture	3.67 D	4.18 E	232.12 B
AsA + *P. polymyxa*	3.91 D	4.47 DE	265.46 A
AsA + *R. leguminosarum*	6.82 B	0.66 F	182.59 H
AsA + Mix.	3.87 D	0.98 F	189.26 G
AsA	5.38 C	6.04 B	232.12 B
HA	0.67 G	4.04 E	208.31 E
HA+ *P. polymyxa*	0.43 G	5.18 C	214.03 D
HA+ *R. leguminosarum*	0.55 G	6.90 A	201.64 F
HA + Mix.	1.97 F	4.83 CD	220.69 C
HA + AsA + Mix.	1.74F	6.60 A	201.64 F
Control	8.62 A	0.27 F	171.83 I
L.S.D.0.05	0.2894	0.4349	2.0757

ASA = Ascorbic acid **HA=** Humic acid **Mix = (***Paenibacillus polymyxa+ Rhizobium leguminosarum)*

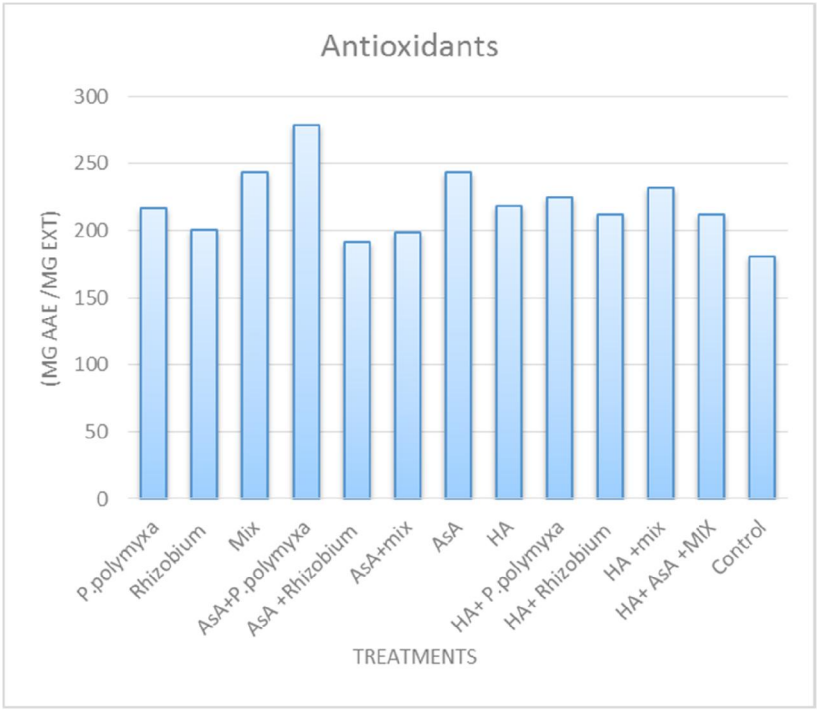

Fig. 1: Effect of PGPR and organic amendments on antioxidants content of wheat plants grown in saline soil.

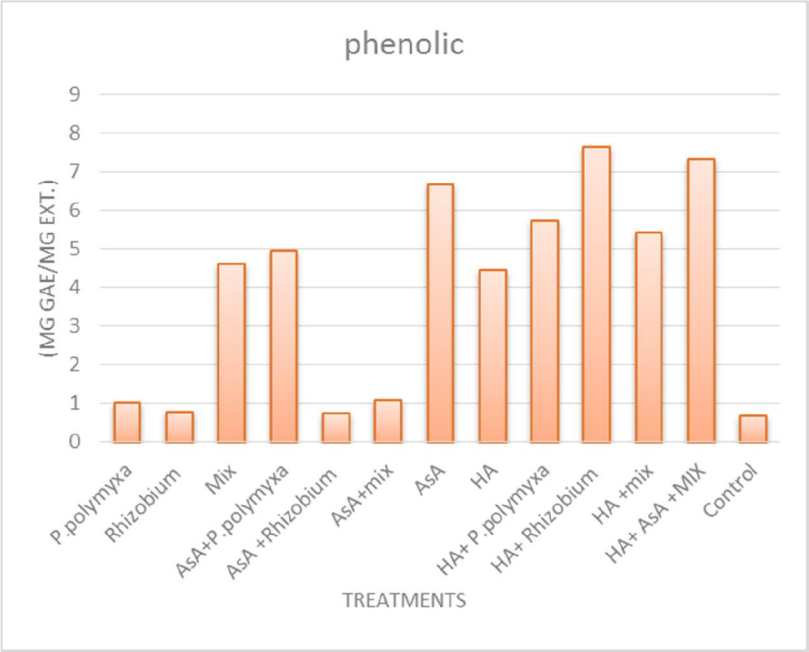

Fig. 2: Effect of PGPR and organic amendments on phenolic content of wheat plants grown in saline soil.

The combination of ASA with mixture of PGPRs (*Rhizobium leguminosarum* and *Paenibacillus polymyxa*) gave higher content than each individually or than control. The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture free radicals. Some of the flavonoid glycosides prevent oxidant injury and cell death by several mechanisms (Ślusarczyk *et al*., 2009; Fu *et al*., 2011). The total antioxidant

activity of grain increased by using (AsA + *P. polymyxa*) treatment which gave values of 265.46 µg of Ascorbic acid/mg ext followed by (AsA). Athar *et al*. (2008) reported an increase in antioxidant enzyme activities in wheat plants after AsA application.

The phenolic play vital roles in plants such as protection against herbivores and pathogens, cementing material joining phenolic polymers to cell wall polysaccharides (Wallace and Fry 1994), regulation of cell growth and cell division (Binns *et al*., 1987). The highest values in phenolic content were obtained from HA + *R. leguminosarum* and HA+ AsA + mixture PGPRs treatments followed by AsA which, were (6.90, 6.61 and 6.04 expressed as gallic acid equivalent µg GAE/mg ext.). The trend of these results agreed with those reported by (Wierdak and Zawiślak, 2016; Fawy *et al*., 2017 and Hashem and Hegab, (2018) on quinoa.

It is obvious from obtaining data in Table (7) and Figure (3) that all treatments play an important role in the alleviation of salt stress on wheat plants through their positive effect on biochemical contents of plant, which is evident by significant reduction of the proline content of treated plants comparing to untreated one.

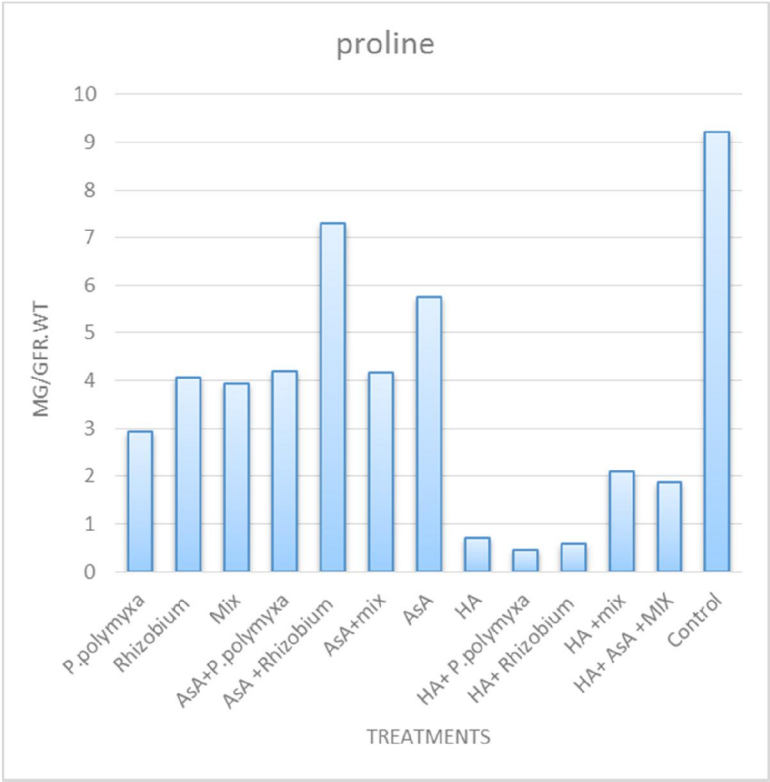

Fig. 3: Effect of PGPR and organic amendments on proline content of wheat plants grown in saline soil.

Humic acid application individually or in combination with PGPR treatment significantly decreased proline compared to control and this obtained result may be attributed to the crucial role of HA in mitigating the deleterious effects of soil salinity (Rady *et al*., 2016), Vimal *et al*. (2018) also assayed for PGPR and their plant growth promoting potential to mitigate the saline stress of wheat plants.

Conclusion

Previous results showed that the application of ascorbic acid (AsA) and humic acid (HA) as a foliar spray and inoculation with PGPR treatment gave the highest significant yield, and chemical constituents as (antioxidant activity, phenolic components, chlorophyll content and NPK uptake) of wheat crop. In addition, application of organic acids (humic acid and ascorbic acid) and PGPRs inoculants improve plant stress-defense responses resulting better plant performance under stress in

direct and indirect manners. Also, we can recommend that the application of these amendments and PGPRs may provide a useful way to reduce the adverse effects of salinity stress on wheat plants grown in saline soil.

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