

## RESPONSE OF BARLEY GROWN IN SALT-AFFECTED SOIL TO BIO AND MINERAL FERTILIZERS

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Two field experiments were done to assess the effect of seed inoculation with single or dual plant growth promoting rhizobacterial (PGPR) strains (*Azotobacter chroococcum* and *Bacillus subtilis*) and three levels of NPK fertilization (50, 75 and 100%) on growth and productivity of barley (*Hordeum vulgare*) plants growing under saline condition at El-Monira village, El-Kharga Oasis, New Valley, during the two successive seasons 2016 and 2017. Spilt plot design in four replications was used, where NPK fertilization treatments occupied the main plots and biofertilization in the sub-plot. Analysis of variance revealed significant effect of biofertilizer application along with NPK fertilizer levels on all measured growth parameters (plant height, branching, spikes number, fresh and dry weight), soil biological activity (total microbial count, dehydrogenase activity and antioxidant capacity of soil), available nitrogen and phosphorus in soil, uptake of nitrogen and phosphorus, protein content of barley plants and yield and its related characters (spikes/m<sup>2</sup> numbers, 1000-grains weight and grain yield). Maximum traits were obtained by plants that were treated with 75% of NPK fertilizer and mixed cultures of *A. chroococcum* and *B. subtilis*. Thus, it could be concluded that the use of 75% chemical fertilizer along with co-inoculation with *A. chroococcum* and *B. subtilis* could promote the growth, productivity and disease resistance in barley under saline condition.

**Keywords:** Barley (*Hordeum vulgare*), salinity, microbial activity, biofertilizers, NPK fertilizers, soil antioxidant, New Valley

Barley (*Hordeum vulgare* L.) is an important and nutritious crop that could be used as fodder and cover crop to enhance soil fertility. It is rich in essential nutrients such as protein, carbohydrates, fat and fibers (Ghanbari et

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al., 2012). Barley is a moderate salt tolerance crop and has a potency to grow in a wide range of environmental stresses including drought, heating or saline conditions (Ayman, 2015).

Salt stress can directly or indirectly affect the physiological status of plant by altering its metabolism, growth, and development and hence lead to severe crop loss every year. In Egypt, plants are exposed to extreme climatic factors; such as drought and high temperatures. Under these conditions, dissolved salts may accumulate in soils because of the insufficient leaching of ions and unsuitable irrigation management (Eisa et al., 2012).

High salinity constitutes an environmental stress also for rhizospheric bacteria. Microbial activities and population were negatively affected by salinity as revealed by Yaseen and Yossif (2019). It inhibits various physiological processes, ranging from energy and nutrient uptake to inhibition of DNA replication and macromolecule biosynthesis (Bartels and Sunkar, 2005). In addition, causes an alteration of proteins involved in the initial attachment steps of bacteria to plant roots as well as inhibition of bacterial nodulation and nitrogen fixation activity, and also inhibit the bacterial mobility and chemotaxis toward plant roots (Jofre' et al., 1998).

The excessive use of chemical fertilizers adversely affects the soil quality as well as human health. Thus, there is a need to use ecofriendly alternative like plant growth promoting rhizobacteria (PGPR) to enhance the crop yield. The PGPR are a group of bacteria that colonize plant roots, causing an increase in plant growth and yield. They are being widely used as a substitute to chemical pesticides and mineral fertilizers due to their ecofriendly nature (Reddy et al., 2014). They promote plant growth through their ability to fix N<sub>2</sub>, dissolve mineral phosphates and other nutrients, support nutrition uptake, produce siderophore, hydrogen cyanide and growth promoting hormones (auxins, gibberellins and cytokinins), and antagonize phytopathogenic microorganisms or enhance plant resistance to pathogens (Bhattacharyya and Jha, 2012). Reducing the use of chemicals in agricultural areas has great interest in order to protect plant health and reduce production costs as well as environmental pollution. For this reason, many authors tried to use bio-fertilizers in a combination with less doses of chemical fertilizers (Kurokura et al., 2017 and Parlakova Karagöz and Dursun, 2019). Zafar-ul-Hye et al. (2015) found that the aminocyclopropane carboxylate deaminase containing bacteria alone and in combination with mineral fertilizers improved the root and shoot growth of maize seedlings in saline-sodic soil. Incorporation of PGPR with half dose of recommended chemical fertilizers enhanced the yield of sunflower plant by reducing the use and harmful effects of chemical fertilizer on soil health and environment (Tahir and Shehzadi, 2017).

The present study was done to investigate the effect of bio-fertilization treatments and different levels of mineral fertilization on growth and productivity of barley under saline condition.

## MATERIALS AND METHODS

### 1. Cultivar

Barley seeds cultivar Giza 123 (*Hordeum vulgare*) were obtained from Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

### 2. Microorganisms

The bacterial isolates used in this experiment were isolated from salt affected soils and identified as *Azotobacter chroococcum* and *Bacillus subtilis* according to Bergey's Manual of Systematic Bacteriology (Bergey et al., 1984).

### 3. Plant Growth Promoting Properties of Bacterial Isolates

The abilities of bacterial isolates to fix nitrogen and dissolve phosphorus were determined by growing the isolates on Ashby's and Pikovskaya's media according to Subba Rao (1984) and Pikovskaya (1948), respectively. Production of indole-3-acetic acid (IAA) was assayed by colorimetric method (Patten and Glick, 2002). Production of exopolysaccharid (EPS) was tested using the method described by Emtiazi et al. (2004). Siderophore production was determined by the method of Rachid and Bensoltane (2005). Organic acid formation was determined using methyl red method described by Olutiola et al. (2000). Production of ammonia were determined according to Cappuccino and Sherman (1992).

### 4. Agricultural Experiment

Two field experiments were conducted in new reclaimed salt affected sandy soil at El-Monira village, El-Kharga Oasis (30.53 longitude, 25.45 latitude and elevation 78.8 m), New Valley Governorate, during the two cropping seasons of 2016 and 2017 to study the response of barley plant to mineral fertilizer levels (120, 180, and 240 kg/ha) and inoculation by *A. chroococcum*, *B. subtilis*, and their mixtures, and without inoculation as control. The fertilizer used was NPK 19-19-19, bacterial concentration was adjusted to  $10^8$  (CFU/ml) and was added at a rate of 20L/fed. Barley seeds were treated before planting with bacterial suspensions for three hours before planting (carboxy methyl cellulose 0.5% was used as an adhesive agent).

### 5. Physical and Chemical Analysis of Precultivated Soil and Irrigated Water

The physical and chemical analysis of experimental field soil was determined according to Page et al. (1982) and presented in table (1). The chemical analyses of irrigation water were pH 7.5 and EC 1.46 dS.m<sup>-1</sup>.

**Table (1).** Analysis of experimental soil and irrigation water.

Depth (cm)		Physical analysis %							Soil texture				
		Sand	Silt	Clay									
0-30		52.95	21.51	25.54			Sandy clay loam						
Chemical analysis													
pH	E.C dS.m <sup>-1</sup>	CaCO <sub>3</sub> %	O.M %	T.N %	Cation meq/l				Anion meq/l				
					Ca <sup>+2</sup>	Mg <sup>+2</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>-2</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-2</sup>	
8.64	8.21	4.34	0.58	0.09	25.2	7.9	43.4	5.3	0.9	15.9	50.9	6.2	
Chemical analysis of irrigation water													
pH	E.C dS.m <sup>-1</sup>	Cation meq./l				Anion meq./l							
		Ca <sup>+2</sup>	Mg <sup>+2</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>-2</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-2</sup>				
7.5	1.46	5.42	3.24	5.43	0.27	1.65	2.18	4.2	6.16				

## 6. Agronomic Data Recorded

### 6.1. Soil biological activity

Total microbial counts were estimated according to Allen (1959). Microbial activity was estimated by measuring the activity of dehydrogenase (DHA) of the rhizosphere soil (Casida et al., 1964), antioxidant activity of rhizosphere soil was evaluated following the extraction procedure of Rimmer and Abbott (2011). In brief, 2 g soil were extracted with 5 ml of 1 M NaOH for 16 h on a rotary shaker at room temperature, followed by centrifugation. The supernatants were heated at 90°C for 2 h then cooled, titrated with 4 M HCl to pH 2.0 and placed in disposable glass test tubes. The reducing power of soil extract was determined according to Bhalodia (2013).

### 6.2. Chemical composition

Seeds nitrogen content was determined by the modified microkjeldahl method as described by Peach and Tracy (1956). The protein content was calculated by multiplying the total nitrogen by 4.64 (Magomya et al., 2014). Phosphorus percentage was estimated by ascorbic acid according to method reported by Bender and Wood (2000).

### 6.3. Yield and yield components

At harvest, plant height (cm), number of branches, fresh weight (g), dry weight (g), number of spikes/m<sup>2</sup>, 1000-grain weight (g) and grain yield (kg/fad.) were measured.

## 7. Statistical Analysis

The data were analyzed using SPSS 21.1 software program (SPSS, 2014). The ANOVA test was used to determine the significant difference between treatments (LSD). After analysis the variance means were compared with each other according to Duncan's multiple range test at  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

### 1. Plant Growth Promoting Activities of Bacterial Isolates

Plant growth-promotion characteristics of two biofertilizer strains were evaluated and presented in table (2). Results revealed that the two strains had the capacity to solubilize phosphate and produce IAA, exopolysaccharide, organic acids and siderophores in iron-free medium. Microorganisms with PGP activities are useful in agricultural fields and can alleviate many biotic and abiotic stresses (Wang et al., 2016). Several studies reported that stress tolerance is improved in plants by PGP microbes through different mechanisms; such as producing growth regulators and antioxidant that can detoxify reactive oxygen species resulting in increased growth parameters and most importantly enhanced nutrient content, thus improving the health of plant under salt stress (Shahid et al., 2018 and Abbas et al., 2019).

**Table (2).** Plant growth promoting activities of bacterial isolates.

Characteristics	Bacterial isolates	
	<i>A. chroococcum</i>	<i>B. subtilis</i>
Nitrogen fixation	+	-
Phosphate dissolving	+ (46 ug/ml)	+ (55 ug/ml)
IAA production	+ (7.2 ug/ml)	+ (4.7 ug/ml)
Siderophore production	+ (25 ug/ml)	+ (42 ug/ml)
Exopolysaccharide	+ (8.7 mg/ml)	+ (6.9 mg/ml)
Organic acid production	+	+
Ammonia production	-	+

### 2. Effect of Mineral Fertilizer Levels and Biofertilizers on Growth Parameters of Barley Plant During 2016 and 2017 Seasons Under Salt Conditions

The analysis of variance showed a significant difference for Interaction effect between bio-fertilizer and chemical fertilizer levels in terms of plant height, branches numbers, spikes numbers, fresh and dry weight (Table 3). Application of biofertilizers (mixed culture) in growing season 2016 increased about 29.7, 39.65 and 37.7% in plant height, 48.1, 73.9 and 63.3% in branches numbers, 42.7, 82.4 and 81.3% in spikes numbers, 29.3, 82.8 and 71.6% in fresh weight and 43.4, 64.7 and 45.2% in dry weight in comparison with control (without bio fertilizer) at 50, 75 and 100% of chemical fertilizers, respectively. While in growing season 2017 there was an increase in plant height by 54.1, 35 and 28.4%, in branches numbers by 57.1, 68.9 and 48.8%, in spikes numbers by 94.6, 73.6 and 54.1%, in fresh weight by 25.6, 75.2 and 62% and in dry weight by 49.7, 72.5 and 56.2% over the

control at 50, 75 and 100% of chemical fertilizers, respectively. Interaction effect between mixed culture and 75% of chemical fertilizers gave the highest growth parameters of barely plant growing under saline condition, when compared with the other two levels. Salinity is a major abiotic stress that influencing all crops in Egypt. Although barley is considered a salt tolerant crop species, productivity of barley is affected by osmotic and oxidative stresses. Babu et al. (2007) illustrated that salt stress led to decline in the rate of germination percentage and all growth parameters of barley genotypes. PGP bacteria enhanced plant growth under saline condition through producing growth promoting nutrients and growth regulators (Etesami and Maheshwari, 2018).

**Table (3).** Effect of mineral fertilizer levels and biofertilizers on growth parameters of barley plant during 2016 and 2017 seasons under salt conditions.

NPK levels	Inoculation	2016					2017				
		Plant height	BN/p	SN/p	Fresh wt.	Dry wt.	Plant height	BN/p	SN/p	Fresh wt.	Dry wt.
50% (120 kg/ha)	Control	63.0 <sup>g</sup>	13.3 <sup>g</sup>	11.0 <sup>g</sup>	70.3 <sup>j</sup>	47 <sup>h</sup>	67.7 <sup>h</sup>	17.0 <sup>i</sup>	13.0 <sup>j</sup>	74.9 <sup>l</sup>	54.5 <sup>k</sup>
	PDB	73.7 <sup>f</sup>	15.0 <sup>fg</sup>	13.7 <sup>ef</sup>	82.8 <sup>h</sup>	51.7 <sup>gh</sup>	84.0 <sup>g</sup>	20.7 <sup>h</sup>	17.0 <sup>i</sup>	87.7 <sup>i</sup>	65.6 <sup>i</sup>
	Azot	79.3 <sup>ef</sup>	18.0 <sup>e</sup>	14.7 <sup>e</sup>	88.8 <sup>g</sup>	53.2 <sup>gh</sup>	98.3 <sup>c</sup>	23.3 <sup>f</sup>	21.7 <sup>fg</sup>	90.7 <sup>h</sup>	78 <sup>f</sup>
	Mixed	81.7 <sup>de</sup>	19.7 <sup>de</sup>	15.7 <sup>de</sup>	90.9 <sup>g</sup>	67.4 <sup>de</sup>	104.3 <sup>b</sup>	26.7 <sup>d</sup>	25.3 <sup>d</sup>	94.1 <sup>g</sup>	81.6 <sup>e</sup>
75% (180 kg/ha)	Control	74.7 <sup>f</sup>	15.7 <sup>f</sup>	13.7 <sup>ef</sup>	77.5 <sup>i</sup>	56.4 <sup>fg</sup>	85.7 <sup>g</sup>	20.3 <sup>h</sup>	19.0 <sup>h</sup>	83.6 <sup>k</sup>	62.2 <sup>j</sup>
	PDB	91.3 <sup>bc</sup>	20.7 <sup>d</sup>	17.0 <sup>cd</sup>	104.5 <sup>e</sup>	71.7 <sup>cd</sup>	97.3 <sup>cd</sup>	25.0 <sup>e</sup>	23.7 <sup>e</sup>	112.6 <sup>e</sup>	75.7 <sup>g</sup>
	Azot	96.7 <sup>b</sup>	23.3 <sup>bc</sup>	18.3 <sup>c</sup>	116.4 <sup>c</sup>	77.4 <sup>bc</sup>	105.0 <sup>b</sup>	29.7 <sup>c</sup>	28.7 <sup>c</sup>	121.4 <sup>c</sup>	90.9 <sup>c</sup>
	Mixed	104.3 <sup>a</sup>	27.3 <sup>a</sup>	25.0 <sup>a</sup>	141.7 <sup>a</sup>	92.9 <sup>a</sup>	115.7 <sup>a</sup>	34.3 <sup>a</sup>	33.0 <sup>a</sup>	146.5 <sup>a</sup>	107.3 <sup>a</sup>
100% (240 kg/ha)	Control	75.3 <sup>ef</sup>	15.3 <sup>fg</sup>	12.3 <sup>fg</sup>	79.2 <sup>hi</sup>	58.3 <sup>fg</sup>	89.0 <sup>f</sup>	21.7 <sup>g</sup>	20.3 <sup>gh</sup>	86.3 <sup>j</sup>	63.3 <sup>j</sup>
	PDB	87.7 <sup>cd</sup>	19.3 <sup>de</sup>	17.0 <sup>cd</sup>	96.0 <sup>f</sup>	63.5 <sup>ef</sup>	93.3 <sup>e</sup>	23.3 <sup>f</sup>	22.7 <sup>ef</sup>	106.9 <sup>f</sup>	72.7 <sup>h</sup>
	Azot	92.3 <sup>bc</sup>	21.3 <sup>cd</sup>	18.0 <sup>c</sup>	112.2 <sup>d</sup>	71.0 <sup>cde</sup>	95 <sup>de</sup>	26.7 <sup>d</sup>	26.0 <sup>d</sup>	115.9 <sup>d</sup>	87.7 <sup>d</sup>
	Mixed	103.7 <sup>a</sup>	25.0 <sup>b</sup>	22.3 <sup>b</sup>	135.9 <sup>b</sup>	84.7 <sup>b</sup>	114.3 <sup>a</sup>	32.3 <sup>b</sup>	31.3 <sup>b</sup>	139.8 <sup>b</sup>	98.9 <sup>b</sup>
<b>LSD at 0.05</b>		6.6	2.13	2.07	3.9	8.0	2.56	0.96	1.5	1.3	1.33

BN/p: Branches number / plant.

SN/p: Spikes number / plant

### 3. Effect of Mineral Fertilizer Levels and Biofertilizers on Soil Biological Activities in the Rhizosphere of Barley Plant during 2016 and 2017 seasons Under Salt Conditions

Microbial density as affected by application of different NPK levels and applied biofertilizers shown in table (4). Results indicated that total microbial counts were higher with application of 180 kg/ha than that obtained with 120 kg/ha or 240 kg/ha. The high dose of fertilizers used in cultivated systems may restrict the microbial growth and activities in soil (Nguyen et al., 2018).

Concerning the effect of biofertilizers, data showed an increase in microbial densities with biofertilizer application. It could be noticed that, dual inoculation significantly gave the highest results than single ones. Many studies showed that salinity reduces microbial activity, microbial biomass and changes microbial community structure (Singh, 2016; Wang et al., 2016 and Chen et al., 2017). Application of halotolerant bacteria with PGP properties enhanced the soil microbial biomass through production of osmolyte (Yasin et al., 2018).

**Table (4).** Effect of mineral fertilizer levels and biofertilizers on soil biological activities in the rhizosphere of barley plant during 2016 and 2017 seasons under salt conditions.

NPK levels	Inoculation	2016			2017		
		TMC	AOA	DHNAS	TMC	AOA	DHNAS
50 % (120 kg/ha)	Control	35 <sup>f</sup>	1.0 <sup>i</sup>	1.0 <sup>h</sup>	38 <sup>l</sup>	1.3 <sup>i</sup>	1.1 <sup>j</sup>
	PDB	50 <sup>ef</sup>	1.7 <sup>g</sup>	1.5 <sup>f</sup>	66 <sup>i</sup>	2.2 <sup>g</sup>	1.5 <sup>h</sup>
	Azot	68 <sup>def</sup>	2.0 <sup>f</sup>	1.6 <sup>e</sup>	80 <sup>h</sup>	2.3 <sup>f</sup>	1.8 <sup>g</sup>
	Mixed	72 <sup>cde</sup>	2.0 <sup>lf</sup>	1.8 <sup>d</sup>	100 <sup>g</sup>	2.4 <sup>f</sup>	2.1 <sup>f</sup>
75 % (180 kg/ha)	Control	40 <sup>ef</sup>	1.3 <sup>h</sup>	1.2 <sup>g</sup>	46 <sup>j</sup>	1.7 <sup>h</sup>	1.4 <sup>h</sup>
	PDB	110 <sup>b</sup>	2.4 <sup>c</sup>	1.9 <sup>c</sup>	134 <sup>c</sup>	2.9 <sup>c</sup>	3.1 <sup>d</sup>
	Azot	170 <sup>a</sup>	3.9 <sup>b</sup>	2.1 <sup>b</sup>	194 <sup>b</sup>	4.3 <sup>b</sup>	3.5 <sup>b</sup>
	Mixed	174 <sup>a</sup>	4.1 <sup>a</sup>	2.4 <sup>a</sup>	210 <sup>a</sup>	4.5 <sup>a</sup>	3.7 <sup>a</sup>
100 % (240 kg/ha)	Control	40 <sup>ef</sup>	1.5 <sup>h</sup>	1.2 <sup>g</sup>	40 <sup>k</sup>	2.0 <sup>h</sup>	1.2 <sup>i</sup>
	PDB	106 <sup>bc</sup>	2.4 <sup>c</sup>	1.8 <sup>d</sup>	114 <sup>f</sup>	2.5 <sup>c</sup>	2.9 <sup>c</sup>
	Azot	122 <sup>b</sup>	3.1 <sup>d</sup>	1.9 <sup>c</sup>	176 <sup>d</sup>	4.2 <sup>d</sup>	3.1 <sup>d</sup>
	Mixed	140 <sup>bcd</sup>	3.4 <sup>c</sup>	2.1 <sup>b</sup>	192 <sup>c</sup>	4.5 <sup>c</sup>	3.3 <sup>c</sup>
<b>LSD at 0.05</b>		34	0.179	0.022	1.13	0.178	0.095

TMC: Total microbial count  $\times 10^5$  cfu /g dry soil

AOA: antioxidant activity of soil ug ascorbic acid /g dry soil

DHNAS: dehydrogenase umol TF /g dry soil

The impact of interaction between NPK levels and biofertilizers application on dehydrogenase activity was studied (Table 4), results showed that the highest dehydrogenase activity were obtained with application mixture of the two biofertilizers combined with 180 kg/ha. The trend of the above results was true in both investigated seasons. These results agreed with those obtained by Marwa et al. (2018).

The antioxidant system can be considered useful indicators of the soil biological status and soil quality (Cardelli et al., 2014). Data showed that the interaction between mixed culture of bio-fertilizer and 75% of chemical fertilizer level have the greatest effect on increasing the antioxidant activity of soil in both investigated seasons. The increase in antioxidant activity in soil led to plant protection against oxidative stress resulting from high salinity (Rimmer, 2006). Skwaryło-Bednarz and Krzepińko (2007) confirmed a close relationship between soil total antioxidant capacity in the soil and the microbial count in various soil types. Enzymatic and non-enzymatic antioxidant play an important role in plant growth and development, particularly in defense mechanisms. Prolonged exposure to environmental stress resulted in oxidative stress, causing pathological condition. Many authors suggested that peroxidases and phenolic compounds were involved in plant protection against oxidative stress (Kulbat, 2016).

#### **4. Effect of Mineral Fertilizer Levels and Biofertilizers on Available Minerals in the Rhizosphere of Barley Plant During 2016-2017 Seasons under Salt Conditions**

Salinity affects soil microbial communities and inhibits the mineralization of organic materials in soil, causing severe changes in the process of organic matter turnover (Chowdhury et al., 2011) and thus reduce the released of plant nutrients. Application of mixed biofertilizer cultures combined with mineral fertilizer levels significantly enhanced the availability of nitrogen and phosphorus in investigated soil (Table 5). Application of mixed biofertilizer cultures in growing season 2016/2017 increased about 96.4, 65.4 and 50.4% in available nitrogen and 59.6, 42.6 and 38.1% in available phosphorus over the control at 50, 75 and 100% of chemical fertilizers, respectively. While the application of mixed biofertilizer cultures in growing season 2017/2018 increased about 75.2, 87.2 and 75.6% in available nitrogen and 49.1, 74.3 and 58.1% in available phosphorus over the control at 50, 75 and 100% of chemical fertilizers, respectively. Similar results were obtained by Yaseen et al. (2018), who found that the most significant factor affected NPK availability in soil was biosurfactant producing bacteria. PGPR play an essential role in nutrient cycling in soils, they involved in processes such as oxidation, nitrification, ammonification, nitrogen fixation, and other processes which lead to decomposition of soil organic matter and releasing essential inorganic plant nutrients to the soil. It has been reported that production of organic acids by PGPR participate in the conversion of insoluble form of phosphorus to soluble forms (Kurokura et al., 2017).

**Table (5).** Effect of mineral fertilizer levels and biofertilizers on available minerals in the rhizosphere of barley plant during 2016-2017 seasons under salt conditions.

NPK levels	Inoculation	2016		2017	
		Available nitrogen (mg/kg soil)	Available phosphorus (mg/kg soil)	Available nitrogen (mg/kg soil)	Available phosphorus (mg/kg soil)
50 % (120 kg/ha)	Control	8.4 <sup>i</sup>	4.7 <sup>f</sup>	11.7 <sup>j</sup>	5.7 <sup>h</sup>
	PDB	15.5 <sup>f</sup>	6.8 <sup>de</sup>	16.8 <sup>i</sup>	7.3 <sup>g</sup>
	Azot	15.8 <sup>f</sup>	6.2 <sup>e</sup>	18.3 <sup>h</sup>	6.8 <sup>g</sup>
	Mixed	16.5 <sup>e</sup>	7.5 <sup>cd</sup>	20.5 <sup>g</sup>	8.5 <sup>f</sup>
75 % (180 kg/ha)	Control	13.0 <sup>h</sup>	6.1 <sup>e</sup>	17.9 <sup>h</sup>	7.0 <sup>g</sup>
	PDB	17.4 <sup>d</sup>	8.0 <sup>b</sup> c	24.7 <sup>e</sup>	11.4 <sup>bc</sup>
	Azot	19.4 <sup>c</sup>	7.5 <sup>cd</sup>	29.3 <sup>c</sup>	11.1 <sup>cd</sup>
	Mixed	21.5 <sup>a</sup>	8.7 <sup>ab</sup>	33.5 <sup>a</sup>	12.2 <sup>a</sup>
100 % (240 kg/ha)	Control	13.9 <sup>g</sup>	6.3 <sup>e</sup>	18.0 <sup>h</sup>	7.4 <sup>g</sup>
	PDB	17.3 <sup>d</sup>	7.7 <sup>c</sup>	23.2 <sup>f</sup>	10.2 <sup>e</sup>
	Azot	19.4 <sup>c</sup>	7.4 <sup>cd</sup>	28.0 <sup>d</sup>	10.7 <sup>de</sup>
	Mixed	20.9 <sup>b</sup>	8.7 <sup>ab</sup>	31.6 <sup>b</sup>	11.7 <sup>ab</sup>
LSD at 0.05		0.52	0.72	0.44	0.66

### 5. Effect of Mineral Fertilizer Levels and Biofertilizers on Chemical Contents of Barley Plant During 2016 and 2017 Seasons Under Salt Conditions

Application of N<sub>2</sub>-fixing and P-solubilizing PGPR strains significantly promoted nitrogen and phosphorus contents in barely plants as well as protein contents (Table 6). The highest N, P and protein contents were obtained from mixed inoculation combined with 75% of NPK level, which increased N contents of plant by 134 and 156%, P content by 60.1 and 94.6% and protein content by 135.2 and 156.1% compared with the control treatment in the two seasons, respectively. The previous studies have been reported similar findings on cowpea (Marwa et al., 2018), rice (Mohaseb et al., 2019), wheat (Attia and El Salam, 2016). El-Mekser et al. (2016) recorded a significant increase in nitrogen, phosphorus and potassium level in biofertilized treatments corn plants relative to control, they also observed a significant increase in water- and salt-soluble proteins (albumins and globulins) fractions percentage by biofertilization application.

**Table (6).** Effect of mineral fertilizer levels and biofertilizers on chemical contents of barley plant during 2016 and 2017 seasons under salt conditions.

NPK levels	Inoculation	2016			2017		
		Total N content	Total P content	Total protien	Total N content	Total P content	Total protien
50 % (120 kg/ha)	Control	0.89 <sup>g</sup>	0.27 <sup>i</sup>	5.47 <sup>g</sup>	1.15 <sup>f</sup>	0.30 <sup>f</sup>	7.19 <sup>f</sup>
	PDB	1.33 <sup>e</sup>	0.34 <sup>g</sup>	8.33 <sup>e</sup>	1.61 <sup>e</sup>	0.40 <sup>de</sup>	10.06 <sup>e</sup>
	Azot	1.91 <sup>cd</sup>	0.36 <sup>f</sup>	11.94 <sup>cd</sup>	2.33 <sup>c</sup>	0.42 <sup>d</sup>	14.58 <sup>c</sup>
	Mixed	2.03 <sup>bc</sup>	0.42 <sup>e</sup>	12.68 <sup>bc</sup>	2.56 <sup>b</sup>	0.46 <sup>c</sup>	16.00 <sup>b</sup>
75 % (180 kg/ha)	Control	1.07 <sup>f</sup>	0.33 <sup>h</sup>	6.67 <sup>f</sup>	1.23 <sup>f</sup>	0.37 <sup>e</sup>	7.69 <sup>f</sup>
	PDB	1.79 <sup>d</sup>	0.49 <sup>c</sup>	11.21 <sup>d</sup>	1.96 <sup>d</sup>	0.58 <sup>b</sup>	12.25 <sup>d</sup>
	Azot	2.11 <sup>b</sup>	0.48 <sup>cd</sup>	13.19 <sup>b</sup>	2.67 <sup>b</sup>	0.57 <sup>b</sup>	16.69 <sup>b</sup>
	Mixed	2.51 <sup>a</sup>	0.53 <sup>a</sup>	15.69 <sup>a</sup>	3.15 <sup>a</sup>	0.72 <sup>a</sup>	19.69 <sup>a</sup>
100 % (240 kg/ha)	Control	1.15 <sup>f</sup>	0.35 <sup>fg</sup>	7.21 <sup>f</sup>	1.28 <sup>f</sup>	0.38 <sup>e</sup>	7.98 <sup>f</sup>
	PDB	1.90 <sup>cd</sup>	0.48 <sup>cd</sup>	11.89 <sup>cd</sup>	2.10 <sup>d</sup>	0.56 <sup>b</sup>	13.13 <sup>d</sup>
	Azot	2.04 <sup>bc</sup>	0.47 <sup>d</sup>	12.73 <sup>bc</sup>	2.63 <sup>b</sup>	0.55 <sup>b</sup>	16.46 <sup>b</sup>
	Mixed	2.19 <sup>b</sup>	0.51 <sup>b</sup>	13.69 <sup>b</sup>	3.00 <sup>a</sup>	0.71 <sup>a</sup>	18.77 <sup>a</sup>
<b>LSD at 0.05</b>		0.08	0.01	1.03	0.19	0.03	1.19

### 6. Effect of Mineral Fertilizer Levels and Biofertilizers on Yield and its Components of Barley Plant During 2016 and 2017 Seasons Under Salt Conditions

The results in table (7) reveal that the combination between PGPR inoculations and mineral fertilizer levels significantly enhanced yield and yield components of barley plants growing under saline conditions. Results showed that spikes/m<sup>2</sup> numbers (SN), 1000-grains weight (GW) and grain yield (GY) of barley plant in each of the two seasons significantly increased by biofertilizers application compared with the control. The lowest SN, GW and GY were recorded in the control treatment whereas the highest SN (392 and 441), GW (41.33 and 46.67 g) and GY (2.41 and 2.92 ton fed<sup>-1</sup>), were recorded from mixed PGPR inoculations combined with 75% of NPK fertilizer level in two years, respectively.

The increase in yield and its component in treatments received biofertilizer mainly attributed to the beneficial effect of biofertilizer application to the soil led to improved soil physical, biological and chemical properties and in turn resulted in more release of available nutrients to plant root (Boostani et al., 2014). Similar results were reported on rice, soybean, canola and barley plants where the inoculation with PGPR along with reduced level of chemical fertilizers mostly increased growth and yield of each crop

(Hafeez, 2019; Egamberdieva et al., 2017 and Dadashzadeh et al., 2018). The application of biofertilizer with beneficial PGP properties improved leaf chlorophyll, plant nutrient uptake and grain protein content. Naher et al. (2016) revealed that the use of chemical fertilizer can be minimized by 50% and improve rice yield with the supplement of 5 ton ha<sup>-1</sup> of bio-organic fertilizer.

**Table (7).** Effect of mineral fertilizer levels and biofertilizers on yield and its components of barley plant during 2016 and 2017 seasons under salt conditions.

NPK levels	Inoculation	2016			2017		
		No. of spikes m <sup>-2</sup>	Weight of 1000-grains (g)	Grain yield (ton fed <sup>-1</sup> )	No. of spikes m <sup>-2</sup>	Weight of 1000-grains (g)	Grain yield (ton fed <sup>-1</sup> )
50% (120 kg/ha)	Control	306.3 <sup>k</sup>	14.67 <sup>h</sup>	1.37 <sup>k</sup>	213.0 <sup>i</sup>	17.33 <sup>i</sup>	1.66 <sup>j</sup>
	PDB	309.0 <sup>j</sup>	22.00 <sup>g</sup>	2.06 <sup>h</sup>	265.3 <sup>g</sup>	20.33 <sup>h</sup>	2.34 <sup>g</sup>
	Azot	322.3 <sup>h</sup>	26.33 <sup>f</sup>	2.10 <sup>g</sup>	302.7 <sup>f</sup>	21.33 <sup>g</sup>	2.43 <sup>f</sup>
	Mixed	329.0 <sup>g</sup>	28.67 <sup>e</sup>	2.20 <sup>d</sup>	312.7 <sup>f</sup>	25.67 <sup>f</sup>	2.49 <sup>e</sup>
75% (180 kg/ha)	Control	328.0 <sup>g</sup>	21.33 <sup>g</sup>	1.61 <sup>i</sup>	247.3 <sup>h</sup>	25.67 <sup>f</sup>	1.87 <sup>h</sup>
	PDB	352.3 <sup>e</sup>	32.67 <sup>d</sup>	2.17 <sup>e</sup>	384.7 <sup>de</sup>	36.00 <sup>d</sup>	2.50 <sup>e</sup>
	Azot	371.0 <sup>c</sup>	36.33 <sup>bc</sup>	2.26 <sup>c</sup>	401.7 <sup>bc</sup>	42.67 <sup>b</sup>	2.79 <sup>b</sup>
	Mixed	392.0 <sup>a</sup>	41.33 <sup>a</sup>	2.41 <sup>a</sup>	441.0 <sup>a</sup>	46.67 <sup>a</sup>	2.92 <sup>a</sup>
100% (240 kg/ha)	Control	317.7 <sup>i</sup>	22.67 <sup>g</sup>	1.58 <sup>j</sup>	224.0 <sup>i</sup>	26.33 <sup>f</sup>	1.82 <sup>i</sup>
	PDB	343.0 <sup>f</sup>	31.33 <sup>d</sup>	2.14 <sup>f</sup>	373.3 <sup>e</sup>	34.67 <sup>e</sup>	2.43 <sup>f</sup>
	Azot	362.3 <sup>d</sup>	35.33 <sup>c</sup>	2.21 <sup>d</sup>	390.3 <sup>cd</sup>	38.67 <sup>c</sup>	2.58 <sup>d</sup>
	Mixed	378.0 <sup>b</sup>	37.33 <sup>b</sup>	2.34 <sup>b</sup>	410.0 <sup>b</sup>	43.00 <sup>b</sup>	2.74 <sup>c</sup>
<b>LSD at 0.05</b>		2.28	1.93	0.19	11.42	0.76	0.02

## CONCLUSIONS

Microbial inoculation of barley seeds with dual culture of *A. chroococcum* and *B. subtilis*, could reduce costly mineral fertilizers in barley production even in saline soils and provide plant nutrition requirement resulted in an increase in growth and productivity of barley plant. It could increase mineral concentration in the grain, thus reduced hidden hunger. It also improves soil biological structure and availability of nutrient. It can be concluded that bacteria used in this study may well be appropriate alone or in combination to achieve sustainable and ecological agricultural production in this region.

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## استجابة الشعير المزروع في التربة المتأثرة بالملوحة للأسمدة الحيوية والمعدنية

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أجريت تجربتان حقليتان لتقييم تأثير التلقيح الحيوي لنبات الشعير بالبكتريا المعززة لنمو النبات (أزوتوباكتر كروكوم وباسلس سبتلس) منفردة او مزدوجة مع استخدام ثلاثة مستويات من التسميد المعدني NPK (٥٠، ٧٥ و ١٠٠٪) على إنتاجية نبات الشعير (*Hordeum vulgare*) تحت ظروف الملوحة في الوادي الجديد، خلال الموسمين ٢٠١٦ و ٢٠١٧. كان تصميم التجربة قطع منشقة مرة واحدة مع استخدام أربع مكررات، حيث احتل التسميد المعدني القطع الرئيسية والتسميد الحيوي القطع الشقية. أظهر تحليل التباين أن تطبيق الأسمدة الحيوية إلى جانب مستويات سماد NPK له تأثير معنوي على جميع معايير النمو التي تم قياسها (طول النبات - عدد الأفرع/نبات - عدد السنابل/نبات - الوزن الطازج والجاف للنبات)، النشاط البيولوجي للتربة (إجمالي عدد الميكروبات، نشاط انزيم الهيدروجينيز ومحتوى التربة من المركبات المضادة للأكسدة)، النيتروجين والفوسفور المتاح بالتربة، امتصاص النبات للنيتروجين والفوسفور، محتوى نباتات الشعير من البروتين وكذلك المحصول وجودته (عدد السنابل/م<sup>2</sup> - متوسط وزن الألف حبة - محصول الحبوب). تم الحصول على الصفات القصوى من النباتات التي تم معاملتها بنسبة ٧٥٪ من الأسمدة NPK وإضافة مخلوط من البكتريا. وبالتالي، يمكن أن نخلص إلى أن استخدام ٧٥٪ من الأسمدة الكيماوية جنباً إلى جنب مع التلقيح المشترك للبكتريا يمكن أن يعزز نمو وإنتاجية الشعير تحت ظروف الأراضي الملحية.