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Plant Growth Promoters Substances that Excreting from Bacteria and Cyanobacteria as Essential Factors for Alleviation Soil Salt Stress on Rice Plant

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Abstract Salt-tolerant of plant growth promoting rhizobacteria (PGPR) *Azotobacter chrocooccum*, *Azospirillum brasilense* and cyanobacteria *Anabaena oryzae* used to reduce the impact of salinity on plant growth and improved productivity. Laboratory experiments proved that all used bacteria excrete plant growth promoting substances such as exopolysaccharide (EPS), indole acetic acid (IAA), Catalase and Hydrogen peroxide (H_2O_2) with excellence for azospirillum in (EPS)and (IAA), while *Azotobacter chrocooccum* and cyanobacteria were superior in Catalase and Hydrogen peroxide (H_2O_2) with salinity that led to an increasing in Rice plant traits at harvest of Rice plant as a result of inoculation with three bacteria that increasing all macronutrients and micronutrients in both straw and grains of Rice harvest over the control beside decreasing of pH and EC in the soil after planting season. These confirm that inoculation by PGPR made as growth regulators considerably alleviate the soil salinity.

Keywords Exopolyscharide (EPS), Indole acetic acid (IAA), Catalase, Ammonia, *Azotobacter chrocooccum*, *Azospirillum brasilense*, *Anabaena oryzae*

1. Introduction

Azotobacter chrocooccum and *Azospirillum brasilense* are two association bacteria and the cyanobacteria (*Anabaena oryzae)* is one of the critical inhabitants of rice. These bacteria play a vital role in stimulating plant growth as they are actively colonize plant root and increase plant growth by production of plant growth promoting substances (PGPS) [1]. These PGPS such as plant growth hormones, P-solubilizing activity, N_2 fixation enhance plant growth and biological activity [2]. Worldwide, salinity is one of the most severe abiotic stresses that limit crop growth and productivity. Around 20% of worlds irrigated land is salt affected, with 2,500- 5,000 km^2 of production lost every year as a result of salinity [3]. The majority of salt-affected soils in Egypt are located in North of Nile Delta and on its Eastern and Western sides [4]. Soil salinity effects in and decreasing plant growth, photosynthesis, stomatal conductance, chlorophyll content and mineral uptake compared to soil without salinity [5]. Shukla *et al.* [6] reported that salinity adversely affects plant growth and development. Upadhyay *et al.* [7] mentioned that salt-tolerant PGPR can play an important role in alleviating soil salinity stress during plant growth as bacterial exopolysaccharide (EPS) can help to mitigate salinity stress by reducing the content of Na^+ available for plant uptake. The IAA producing bacteria made as growth regulators considerably alleviated salinity-induced dormancy of wheat seeds, so the root colonizing bacteria could produce phytohormone to alleviate salt stress of wheat grown under conditions of soil salinity [8].

Catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert approximately 5 million molecules of hydrogen peroxide that is harmful and must be removed as soon as it is produced as a by-product of respiration in the cell. Enzyme catalase decompose the hydrogen peroxide to water and oxygen [9]. Kalir and Poljakoff-Mayber [10] thought that the catalase may be formed in cells when growing under saline and extreme climatic conditions and this opinion was sureness when Saraf [11] reported that there was an enhancement of catalase activity in presence of the salt stress. Chelikani *et al.* [9] emphasized that hydrogen peroxide is harmful and must be removed as soon as it is produced as a by product of respiration in the cell. Enzyme catalases decompose the hydrogen peroxide to water and oxygen. Ammonium is an important source of nitrogen for plants. It is taken up by plant cells via ammonium transporters in the plasma membrane and distributed to intracellular compartments such as chloroplasts, mitochondria and vacuoles probably via different transporters in each case [12].

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Through these substances produced by our isolated bacteria it could be prove their helping roles to elevate the capable of the Rice plant to grow and production in saline soils.

2. Materials and Methods

Laboratory tests

Salinity tolerant bacterial isolates namely, *Azospirillum brasilense, Azotobacter chrocooccum* and *Anabaena oryzae* were isolated from Egyptian saline soils as they are saline resistant. These isolates were grown individually on the media prepared as describe by Dobereiner *et al.* [13], Hegazy and Neimela [14] and Venkataraman [15] for the three isolates, respectively.

The bacteria were taken in pure culture to detect the plant growth promoting substances, the Indole acetic acid (IAA) production by bacteria and cyanobacteria estimated sited by Holt *et al.* [16] and Sergeeva *et al.* [17], respectively. Exopolysccharide (EPS) extraction and estimation in bacteria and cyanobacteria were done according to Sabra *et al.* [18] and Kaushik [19], respectively. Catalase reaction and the detection of Ammonia production according to Cappuccino and Sherman [20].

Field experiment

A field experiment was carried out to evaluate the effect of inoculation with *Azospirillum*, *Azotobacter* and *Anabaena* on Rice plant (*Oryza sativa* L.) as well as, nitrogen fertilizer was applied at recommended dose (100% N) as control , beside (100%) and (50%) of recommended dose + inoculation with bacteria.

The field experiment was conducted in salt affected clay soil at Sahl El-Hossinia region, El-Sharkia Governorate, Egypt. The soil properties of the experimental field were determined (in saturated soil paste) as described by Jackson [21] and summarized in table (1).

The deep sub-soiling plough was done in soil prepared stage, and establishment of field drains at a distance of 10 m between each of two drains and at 90 cm at drain beginning. The plot units are subjected to continuously and alternatively leaching processes before rice planting.

The three bacterial strains were grown in a broth culture contain 10^8 cell/ml^{-1} of either strain. Equal portion of each strain were mixed with peat and vermiculite neutralized with 5% $CaCO_3$ (2:1 w/v) and the moisture content of final product was adjusted at ca. 50%, these products were inoculated in Rice grains cultivar (Giza 178) that obtained from the Field Crop, Res. Inst., ARC. Giza, Egypt.

The grains were coated with (peat and vermiculite)-based inoculants of bacteria individually using Arabic gum as adhesive agent to form a bio-film of bacteria around seeds before planting, and dried in shadow before planting. A liquid bacterial culture was added in soil after 30 and 60 days of planting. After planting the recommended agriculture practices were carried out along the season. The NPK were added in the form of ammonium sulphate (46% N), Calcium super-phosphate (15% P_2O_5) and potassium sulphate (48% K_2O), respectively, at rates of 100 kg N/fed, 30 kg P_2O_5/fed and 100 kg K_2O/fed as a recommended dose, respectively. The nitrogen added at equal 3 doses after 21, 42 and 60 days of rice planting and the phosphate added during soil preparation, while potassium added in two equal split doses during soil tillage and after 42 days of planting. Plant samples were collected from each plot at 75th day to evaluate the enzymes dehydrogenase activity (DHA) according to Casida *et al.* [22] and nitrogenase activity (N-ase) according to Leth Bridage *et al.* [23].

At harvest, yields of both Rice grains and straw Rice were recorded after separating in ton fed^{-1}. Grain and straw were oven dried at 70°C up to constant dry weight, then weighed to obtain their dry matter per plant. The selected samples of plant were ground and digested using the methods described by Page *et al.* [24]. Macroelements contents (N, P and K) in seeds and straw were determined according to Cottenie *et al.* [25]. Microelements (Fe, Mn, Zn and Cu) were determined as described by Jackson [21], Page *et al.* [24] and Cottenie *et al.* [25].

Table (1). Chemical properties of soil before planting

Sample	Particulars size distribution (%)				Texture Classes	O.M (%)	$CaCO_3$ (%)
	C. sand	F. sand	Silt	Clay			
0 – 30	3.56	43.92	31.98	20.54	Clay	0.24	12

	pH (1:2.5)	EC (dS/m)	Cations (meq)				Anions (meq)			
			Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	CO^{-3}	HCO^{-3}	Cl^{-}	SO^{-4}
0 – 30	8.45	16.64	17.19	27.6	121	0.62	nil	12.83	105	48.58

	Macro elements (mg/kg)			Microelements (mg/kg)			
	N	P	K	Fe	Mn	Zn	Cu
0 – 30	27.18	3.64	183	1.19	6.91	0.81	0.66

The obtained data were subjected to statistical analysis a cording to Sendecore and Cochran [26] were mean values were compared using L.S.D. at 5% level.

3. Results and Discussion

The *Azospirillum brasilense* (AS), *Azotobacter chrocooccum* (AT) and *Anabaena oryzae* (Cy) were evaluate for their ability to form plant growth promoters substances detected in laboratory tests.

Laboratory trials

The data in table (2) summarized the results of Exopolysccharide (EPS), indole acetic acid (IAA), Catalase and Ammonia production of the three bacterial used in presence of Nacl in their cultural media compared to those produced by the media avoid Nacl, results showed that the *Azospirillum* growth with Nacl was superior in the production of both EPS and IAA compared to those recorded by both *A. chrocooccum*, and *A. oryzae*. The values of both EPS and IAA recorded by all tested bacteria in presence of Nacl were higher than those given without Nacl. On the other respect, *Azotobacter* and *cyanobacteria was* superior in catalase production with adding of Nacl, while the three bacteria were equal in ammonia production in both normal culture and plus Nacl and the *Azospirillum* was superior in culture plus Nacl in case of IAA. These results are reflected in the observation of Noumavo *et al.* [27] when mentioned that both *Azotobacter* and *Azospirillum* as the plant growth promoting bacteria that could produce exopolysaccharide, indole acetic acid, catalase and ammonia.

Regarding to cyanobacteria, Srivastava *et al.* [28] and Arora *et al.* [29] proposed that the cyanobacteria, including exopolysaccharide production, afford resistance to higher salinity and added that the biological process of cyanobacteria responsible for reduction of molecular nitrogen into ammonia in the nitrogen fixation. Sergeeva *et al.* [17] found an evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria and that evidence emphasized by Ahmed *et al.* [30]. Tel-Or *et al.* [31] found a high activities of catalase in vegetative cells of two species of *Nostoc muscorum* and *Synechococcus* and in the heterocysts of *N. muscorum*. Catalase is an enzyme present in the cell of an aerobic bacteria and that's why the action of Catalase test pronounced a high reaction in case of *Azotobacter* and cyanobacteria because they are a highly anaerobic in comparison with a microaerophilic *Azospirillum* [32].

Field trial

An inoculation experiment was carried out in field to evaluate the effect of the three bacterial selected (*Azospirillum*, *Azotobacter* and cyanobacteria) on the Rice plant with application of mineral nitrogen (N), Full of Recommended dose (100% N) which is a farmer application and considered as a control in addition to the half dose of Recommended (50% N) added to the bacterial inoculation.

Soil enzymes activity

Data in table (3) pointed out the measure of dehydrogenase activity (DHA) and nitrogenase activity (N-ase) in the rhizosphere area of rice plant after 75th day of planting. Results showed the Correlation relationship between both enzymes compared to those recorded by (100% N) treatment. However, the highest dehydrogenase and nitrogenase activity was recorded by the treatment of *Azospirillum* with (50% N). The corresponding values were 1.95 ml H^2 100g soil^{-1} h^{-1} (DHA) and 2.26 mmole C_2H_2 g dwt^{-1} h^{-1}. These values were significantly different than those recorded by the control treatment (100% N) and other inoculated tested treatments. The inoculation with half dose of mineral nitrogen (50% N) led to high values of both enzymes comparing with bacterial inoculation and full dose of mineral nitrogen (100% N) in all bacterial types. *Azotobacter* announced high efficiency in (DHA) with no significant comparing with the control.

These results are confirmed by those observed by Nain *et al.* [33] when said that *Azotobacter*, *Azospirillum* and cyanobacteria raise the dehydrogenase and nitrogenase in soil significantly.

In case of free living bacteria, Abou-El-Eyoun *et al.* [34] found that inoculation with *Azotobacter* in maize increased significantly both of N_2-ase and dehydrogenase over the control. In addition, Abou-Zeid and Bakry [35] investigated that *Azotobacter* and *Azospirillum* with half dose of ammonium sulphate scored the highest value of N_2-ase enzyme activity in rhizosphere of potato plants compared with full dose of ammonium sulphate as well as increased microbial populations and activity of microbial enzymes i.e. dehydrogenase.

Table (2). Exopolysccharide (EPS), indole acetic acid (IAA), Catalase and Ammonia production in the three tested bacterial strains

Microorganisms	EPS(g m^{-1})		IAA(µgm^{-1})		Catalase		Ammonia	
	Normal medium	medium + NaCl	Normal medium	medium + NaCl	Normal medium	medium + NaCl	Normal medium	medium + NaCl
Azospirillum brasilense	0.144	0.224	8.2	9.2	+	++	++	+
Azotobacter chrocooccum	0.135	0.191	7.82	8.60	++	+++	++	+
Anabaena oryzae	0.147	0.154	7.22	8.31	++	+++	++	+

The sign of (+) depending on the intensity of the color mode in ammonia or strength of reaction in Catalase

Concerning cyanobacteria, Swarnalakshmi *et al.* [36] reported that soil inoculation with nitrogen-fixing blue-green algal strains led to a significant influence on redox potential and significant increase in dehydrogenase and nitrogenase soil activity. Prasanna *et al.* [37] showed that the activities of all the enzymes had increased when combinations of bacteria and cyanobacteria inoculated in Rice plant.

Recently, and in two separated reports of Ghazal *et al.* [38, 39] who discussed the effect of nitrogen and cyanobacteria on rhizosphere soil biological activity and showed that cyanobacteria can increase the soil enzymatic activity in the first report and added that the soil biological activity for maize plants rhizosphere was assessed in terms of total count bacteria, carbon dioxide evolution, dehydrogenase (DHA) and nitrogenase activities in the second.

Table (3). Dehydrogenase activity (DHA) and nitrogenase activity (N-ase) as affected by bacterial inoculation in Rice root zone after 75 day from planting

Treatments	DHA(ml H_2 100g soil^{-1} h^{-1})	N-ase (mmole C_2H_2 g dwt^{-1} h^{-1})
100 % N	1.06	0.92
AS + 50% N	1.95	2.26
AS + 100% N	1.11	1.62
AT + 50% N	1.70	1.84
AT + 100% N	1.21	1.35
Cy + 50% N	1.55	1.96
Cy + 100% N	1.11	1.42
LSD at 0.05	0.41	0.54

Biological harvest plant

The results of Rice plant harvest concluded in tables (4, 5 and 6) where the table (4) revealed that inoculated rice plants with the bacterial tested increased the grain yield and the weight (1000) grains over the control (100% N) with superior by the plants treated with (AS + 50%) followed by (Cy + 50%) while (AT + 50%) cam in the third level followed by (AS + 100%), (AT + 100%) and (Cy + 100%), respectively. The data emphasized on the effect of biofertilizer of bacteria and cyanobacteria on all measured traits including straw grains and weight (1000) grains (g) and showed that the adding 100% mineral fertilizers recommended dose combined with biofertilizers led to decreasing in grains and weight (1000) grains compared to biofertilizers with 50%, while 100% mineral fertilizers alone scored highest value of straw.

The results of micronutrients in Rice plants summarized in table (5) and illustrated that the highest value of the nitrogen in the plant straw recorded 1.39% with inoculation of both AS + (50% N) and Cy + (50% N) with followed by AT + (50% N) that recorded 1.37% with no significant comparing to the control, the highest value of the nitrogen in the plant grains recorded with inoculation of AS + (50% N) followed by AT + (50% N) and Cy + (50% N) came in third rank. Concerning of phosphorus in the rice straw, the results showed that the inoculation with the Cy + (50%N) was superior and AS + (50% N) came in second rank, while AT + (50% N) and Cy + (100% N) came together in the third rank. Phosphate in the rice grains increasing with the inoculation by AS + (50% N) and Cy + (50%) with the same value 0.32% and came in the first rank followed by AT + (50% N) in the second rank. The results of potassium in the straw showed that the cyanobacteria with (50% N) came in the first rank with the highest value followed by *Azotobacter* with (50% N) and Cy + (100% N) with the same values, while *Azospirillum* with (50% N) and 100% N came at the last. Inoculation of AS + (50%N) and AS + (100% N) increasing potassium in the rice grains highly and occupied first and second rank followed by Cy with two of nitrogen doses application insignificantly comparing with control. The untreated treatment that taken the 100% N (control) was recorded the lowest value of potassium in straw and grains.

Table (4). Effect of bacterial inoculation treatments on Rice plant traits at harvest

Treatments	Weight yield (ton/fed)		Weight (1000 grains) (g)
	Straw	Grains	
100% N	0.893	0.321	11
AS + 50% N	0.890	0.385	17
AS + 100% N	0.869	0.369	15
AT + 50% N	0.843	0.372	14
AT + 100% N	0.835	0.351	12
Cy + 50% N	0.839	0.374	18
Cy + 100% N	0.815	0.343	21
L.S.D at 0.05	0.085	0.03	2.22

Results in table (6) recorded the results of micronutrients concentration in rice crops as affected by biofertilizers, these results observed that the trained of iron in straw and grain were in parallel as the treatment that inoculated with cyanobacteria with application 50% N recorded a heist values while treatments were inoculated with *Azospirillum* and *Azotobacter* came in the second and third rank, respectively. In case of manganese the adding of the full dose of nitrogen were superior in both straw and grains. In straw the high values recorded when plants inoculated with cyanobacteria, *Azotobacter* and *Azospirillum* consecutively, while the contents of manganese grains recorded the high values (82.19, 81.96 and 81.59 mgkg^{-1}) with *Azotobacter*, *Azospirillum* and cyanobacteria, respectively. The contains of straw and grains of zinc mineral were similar as treated with 50% N combined with cyanobacteria was superior in zinc content flowed by *Azospirillum* and *Azotobacter*, respectively. Copper contains in straw show that inoculation with *Azospirillum* + 50% was superior flowed by *Azotobacter* and cyanobacteria + 50% with the same value, while in grains cyanobacteria was in the top, while *Azotobacter* and *Azospirillum* came in the second and third rank.

Table (5). Macronutrients concentration in rice crops as affected by biofertilizers

Treatments	N %		P %		K %	
	Straw	Grains	Straw	Grains	Straw	Grains
100% N	1.38	2.24	0.17	0.26	1.92	1.75
AS + 50% N	1.39	2.58	0.24	0.32	2.11	2.05
AS + 100% N	1.33	2.45	0.19	0.25	2.01	1.98
AT + 50% N	1.37	2.56	0.21	0.29	2.96	1.84
AT + 100% N	1.29	2.49	0.17	0.24	2.94	1.82
Cy + 50% N	1.39	2.51	0.25	0.32	3.05	1.91
Cy + 100% N	1.36	2.48	0.21	0.26	2.96	1.95
L.S.D at 0.05	0.37	0.415	0.059	0.071	0.49	0.17

Table (6). Micronutrients concentration in rice crops as affected by different biofertilizers

Treatments	Fe (mg/kg)		Mn (mg/kg)		Zn (mg/kg)		Cu (mg/kg)	
	Straw	Grains	Straw	Grains	Straw	Grains	Straw	Grains
100% N	128	139	71.32	78.58	45.78	55.12	6.58	4.93
AS + 50% N	146	153	74.66	79.78	57.42	59.29	6.98	6.21
AS + 100% N	142	149	75.24	81.96	54.60	57.36	6.82	5.97
AT + 50% N	137	144	74.89	78.34	53.44	55.69	6.88	6.53
AT + 100% N	132	139	78.63	82.19	51.86	53.59	6.74	6.32
Cy + 50% N	149	159	77.59	80.97	58.35	60.23	6.88	6.57
Cy + 100% N	145	152	78.96	81.59	56.84	58.96	6.71	6.42
L.S.D at 0.05	10.89	9.27	4.55	5.32	6.73	4.47	1.24	0.81

The results of harvest were arrangement with the result found by Zayed *et al.* [40] when they investigated the impact of eco-friendly organic fertilizers and biofertilizers on the reduction of chemical fertilizer use in rice production under saline soil conditions, In the first year the application of *Azospirillum brasilense* culture + half dose of chemical nitrogen fertilizer had the second best ranking after the full dose of nitrogen (control) with no significant. In the second year of the study, the treatment with *Azospirillum brasilense* culture combined with rice straw compost significantly increased rice grain yield and yield components over the control. The same bacteria showed an positive effect when Saad and Mostafa [41] used *Azospirillum brasilense* as inoculation in wheat plant with different N-fertilizer levels using seawater irrigation and that led to increasing in all the yield parameters and growth measured with no significant differences compared with plants treated with tap water and others irrigated with 8.0% seawater concentration. The *Azospirillum* inoculation saved about 20 units of N-fertilizer and that saving was made economically feasible by decreasing the chemical fertilizers needed, improving the nitrogen content and counteracting the effects of salinity. Sartaj *et al.* [42] reported that *Azotobacter chroococcum* has manifested its significance in plant nutrition and its contribution to soil fertility. It form a good association when inoculated in an appropriate plant as it synthesizes auxins, cytokinins, and GA–like substances, and these growth materials are the primary substances controlling the enhanced growth.

Chaudhary *et al.* [43] reported that Inoculation with salinity tolerant *Azotobacter* strains caused significant increase in total nitrogen, biomass and grain yield of wheat. The survival of *Azotobacter* in the soil was also highest in all the treatments at 30, 60 and 90 days after sowing at all the fertilization treatments, they also added that some of the major PGPR activities such as Hydrogen cyanide (HCN), Catalase production, Exopolyscharides production, Ammonia production and phosphorous solubilization were very important in plant growth stimulation. Srivastava *et al.* [28] when they reported that Low salinity favored the presence of heterocystous cyanobacteria. Changes cyanobacteria in rice fields were correlated to salinity resilient physiologies of certain cyanobacteria, including exopolysaccharide production, afford resistance to higher salinity. Also, Prasanna *et al.* [37] reported that when combinations of bacteria and cyanobacteria showed significantly increased in all microbial biomass inoculated and plant biomass were highly correlated with soil microbial carbon besides N (nitrogen) savings about half dose of recommended. They illustrated the positive effects of co-inoculation of bacterial and cyanobacterial integrated nutrient management of rice crop. Cyanobacterial production of extracellular polymers, mainly EPS is well documented [44] and these EPS possible application for binding sodium ions from saline medium, thereby alleviating salt stress for germinating seeds and increase the plant growth and harvest [29].

The state of soil after harvest

1- Soil nutrients after harvest

Macronutrients and micronutrients available that remained in soil after rice harvest were summarized in table (7) which the results of macronutrients showed that the nitrogen contents in soil after treated with *Azospirillum* plus full dose of nitrogen (AS + 100% N) recorded the highest value followed by the treatments of both (As + 50% N) and (Cy + 100% N) in the second order significantly compared to the control (100% N). *Azotobacter* and full dose of nitrogen (AT + 100% N) sorting in the third position insignificantly compared to the control. Concerning Phosphorus saving in soil the results show that cyanobacteria (Cy + 100% N), (AS +100% N) and both of (AT + 100% N) and (AS + 50% N) are in sequence order with no significant. The cyanobacteria with 100% N and 50% N caused a highest potassium content in soil followed by AS +100% N, whereas AT + 100% N came after that and the values were significant.

The results of micronutrients measured in soil after planting the cyanobacteria were obvious superior in case of ferrous with two of nitrogen levels, but in manganese and zink *Azospirillum* and 100% N were exceeded, while in case of cupper *Azotobacter* with 50% N had the best value.

Results of the residual elements in soils were the importance of microorganism's parameters as soil microorganisms are active transformation agents of both the mineral and the organic components of the soil, they play an essential role in plant nutrient cycles, i.e., soil fertility. These results have been confirmed by Rifat *et al.* [45] they reported that PGPR as a biofertilizer helps in fixing N_2, solubilizing mineral phosphates and other nutrients as well as enhancing tolerance to stress.

Prasanna *et al.* [37] reported that when combinations of bacteria and cyanobacteria showed significantly increased in soil microbial carbon besides N_2 (nitrogen) savings with half dose of recommended.

Abou-Zeid and Bakry [35] and Perotti *et al.* [46] indicated that the inoculation with N_2-fixing bacteria (*Azotobacter chroococcum* and *Azospirillum lipoferum*) in combination with inoculated potato tubers with phosphate dissolving bacteria improved soil fertility status and availability of macro and micronutrients in the rhizosphere of potato plants. Shaban *et al.* [47] reported that the available nitrogen, phosphorus, potassium , iron, manganese and zinc in soil, after sesame crop harvest, significantly increased by inoculation by inoculation of salt tolerant *Azospirillum brasilense* under different rates of mineral N_2 fertilizer. Prasanna *et al.* [48] reported that using associative microorganisms with cyanobacteria in rice crops improve the nutrient mobilization and improve the nutrient status of soils and crops.

2- Soil pH and EC after harvest

Table (8) show that the inoculation all the biofertelizers used led to decreasing in Ec and pH of soil after harvest and the results clarifying that decreasing in soil pH were insignificant comparing to the control (100% N) and the least decrease happened with AS + 50% N inoculation that scored 8.21 and the highest decrease recorded 8.09 in case of cyanobacteria with 100% N. The value of EC was dramatically and significantly decreased compared to the control (before planting) the application of 100% N only led to Ec decreasing from 16.64 (d.S/m) before planting to 12.67 after harvests. Inoculation by AT + 100% N scored the highest decreases in Ec scored 7.1 (d.S/m) followed by the value 7.32 (d.S/m) that scored with inoculation by Cy + 100% N then AS + 100% N in the third level of decreasing with value 7.98 (d.S/m).

These findings were in a good harmony with those obtained by Tantawy and Shaban [49] determined that the decreasing in pH and EC may be due changing in cations of Na^+ and Ca^{++} and anions of HCO^{-3} and SO^{-4} besides the production of organic acids by bacteria and endophytic bacterial inoculants and these results means that the bio-fertilizer alleviate soil salinity. Brady and Weil [50] investigated that the decomposition of applied organic materials and biofertilizer resulted in reduction in soil pH through forming various acids, acid forming compounds and/or active microorganisms, released from these additions. Shaban *et al.* [47] found that the soil pH and EC tended to decrease after planting season by increasing the mineral N_2 fertilizer rate with all bio-treated combinations.

Table (7). Macro-micronutrients available contents in soil after rice harvest

Treatments	Macroelements (mg/kg)			Microelements (mg/kg)			
	N	P	K	Fe	Mn	Zn	Cu
Before planting	27.18	3.64	183	1.19	6.91	0.81	0.66
100% N	40	3.82	194	1.35	7.02	0.89	0.69
AS + 50% N	51	4.09	208	1.64	7.88	0.98	0.79
AS + 100% N	53	4.12	212	1.69	7.92	0.99	0.85
AT + 50% N	43	4.06	211	1.74	7.72	0.89	0.91
AT + 100% N	47	4.09	214	1.76	7.76	0.91	0.81
Cy + 50% N	42	4.08	216	1.77	7.83	0.93	0.83
Cy + 100% N	51	4.13	218	1.79	7.88	0.95	0.85
L.S.D at 0.05	7.25	0.32	15.41	0.32	0.75	0.09	0.198

Table (8). pH and EC in soil after rice harvest

Treatments	pH (1:2.5)	EC (dSm^{-1})
Before planting	8.45	16.64
100% N	8.40	12.67
AS + 50% N	8.21	8.20
AS + 100% N	8.20	7.98
AT + 50% N	8.20	8.80
AT + 100% N	8.17	7.10
Cy + 50% N	8.13	8.23
Cy + 100% N	8.09	7.32
LSD at 0.05	1.19	1.05

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