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The Potential Use of Microbial Inocula for Improving Wheat Productivity in Saline Soils

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WHEAT (*Triticumaestivum*-L.) is one of the main winter cereal crops in Egypt for grain production and straw. The combined use of mineral fertilizer and bio fertilizer is required so; wheat plants were inoculated with *Azospirillumlipoferum* strains and/or *Anabaena oryza* to evaluate plant growth parameters and productivity under salt affected soils. Salt-tolerant *A. lipoferum* isolates (A_{10} and A_{11}) have been isolated and identified by 16S rRNA sequencing. Wheat grains were inoculated with *A. lipoferum* strains and/or *A. oryza*. Inoculation with *A. lipoferum* and/or *A. oryza* increased root length compared with un-inoculated grains. Wheat plants inoculated with bacterial species grown in pots and soil experiments which had different salinity levels that arranged from normal (2.4 dSm^{-1}) to salty (6.9 and 11.4 dSm^{-1}) soils. The activity of enzymes urease and phosphatase in the wheat rhizosphere were determined. *A. lipoferum* species had the variable microbial count at different salinity levels. In addition, salinity had deleterious effects on the dry weight of plants, the number and dry weight of branches, spikes and grains, total chlorophyll, nitrogen and potassium concentrations. Furthermore, Na% was increased in shoot and grains of wheat plants. However, inoculation with nitrogen fixed *A. lipoferum* strains and/or *A. oryza* enhanced these parameters. Thus, inoculation with the salt-tolerant *A. lipoferum* strains (A_{10} and/or A_{11}) and/or *A. oryza* reduced the deleterious effect of salt stress on wheat plants and enhanced productivity as compared to un-inoculated plants which fertilized with full dose traditional mineral nitrogen.

Keywords: Salinity, Wheat, Inoculation, *Azospirillum lipoferum;* 16srRNA, Productivity.

Introduction

Cordovilla et al. (1994) estimated that 23% of agricultural soils are affected by high salinity. Most crops are impaired by growth of ever low levels of salinity. Soils infertility in arid and semi-arid partiality influenced by the presence of large quantities of salt, a problem that could be circumvented by introducing plants capable of surviving under these conditions (Kaya et al., 2009). To improve plant tolerance to salinity stress the use of biofertilizers and chemical treatments can enhance processes used naturally by plants to minimize the movement of Na^+ to the shoot (Hamdia and Shaddad, 2010). Wheat (*Triticumaestivum L.*) the main winter cereal crops in Egypt for grain production and straw and grows on an area of 3.39 million faddan (faddan = 4200m^2) with an annual production of about 9.28 million tones and with an average yield of 2.74 tons per faddan during 2014/2015 growing season (CLAC, 2015 and El-Temsah, 2017). The combined use of nitrogen, phosphorus,

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potassium fertilizers plays a great impact on wheat yield (Gill and Saleem, 1994). To maintain soil fertility, nitrogen can be returned to the soil through biological nitrogen fixation (BNF) or by applications of nitrogen fertilizer (Hamdia *et al*., 2005).

Wheat tolerance to salinity depends mainly on the capability of roots to (i) restrict or the control uptake of Na^+ and Cl^- and (ii) continued uptake of essential elements, particularly K^+ and Na^- (Gilroy and Jones, 2000). Production of phytohormones such as IAA, gibberellic acid (GA3), and kinetin are known to be involved in the regulation of plant responses to salinity stress (Cassan et al., 2001), they regulate the activation of a specific enzyme, which participates in RNA and protein synthesis (Hamdia and Brakat 1999), and stimulate physiological responses related to salt stress (Kaya et al., 2009). Biological nitrogen fixed increases the rhizosphere availability of Fe, Zn etc., through production of plant growth promoting substances (Kucey et al., 1989). In general, microorganisms play a significant role in soil fertility and plant productivity (Hamdia and El-Komy, 1998, Roomina et al., 2016 and Halmouch et al., 2016) by N_2-fixation, production of siderophores, ammonia excretion and production of phytohormones such as auxin (Baca et al., 1994). Microbes such as *A. lipoferum* synthesize and metabolize gibberellic acid (Piccoli et al., 1997), and other plant growth promoting substances (Steenhoudt and Vandeleyden, 2000), these phytohormones that improve root growth and thus increase adsorption of water and minerals that eventually enhance and/or improve wheat yields under salt stress (Hamdia et al., 2004). IAA produced by microbes can minimize the negative effects of abiotic stresses on plants ((Malhotra and Srivastava, 2009 and Hartmann and Bashan, 2009). *Azospirillum* salt-tolerant have significantly positive effects on coleoptiles, biomass production, water status, grain number weight per spike, the length of spike, nitrogen and protein contents of grains and straw as compared with non-inoculated seedlings (Zaied et al., 2009).

Cyanobacteria (blue-green algae) are capable both of carbon assimilation and N_2-fixation; they secrete a number of biologically active substances thereby enhancing productivity in a variety of environments (Makandar and Bhatnagar, 2010). These active substances include phytohormones, such as gibberellins, vitamins, amino acids (Rodriguez et al., 2006), cytokinins (Hussain and Hasnain, 2009) and auxin (Prasanna et al., 2010). They increase soil biomass after their decomposition, decrease soil salinity prevent weed growth and increase available soil phosphate by excretion of organic acids (Boghdady and Ali., 2013). A novel association between a N_2-fixing cyanobacterium and cereal could found Gantar et al. (1991). *Nostoc, Anabaena* and *Cylindrospermum* have the ability to form associations with the roots of wheat seedlings grown in liquid culture (Obreht et al., 1993). Nostoc and Anabaena also, play a role in maintaining soil fertility and productivity under salt stress conditions (Zhang et al., 2008) these effects are related to biomass production and secretion extracellular compounds that increase soil microbial activity and IAA production (Mazhar and Hasnain, 2011). However, increased soil total N and available P and play a crucial role by binding the hazardous Na^+ ion, thereby alleviating salinity and alkalinity stresses resulted in improveing seed germination and seedling growth of wheat (Zhang et al., 2008 and Roomina et al., 2016).

Materials and Methods

Grains used

Grains wheat (*Triticumaestivum L.* Sakha 93) was kindly supplied from the Department of Cereals, Field Crop Research Institute, Agricultural Research Center, Sakha Agriculture Research Station, Kafrelsheikh, Egypt.

Media used

Medium 1: Nitrogen-free malate (NFM) used for isolating and growing *Azospirillum* spp**.** (Dobereiner and Day, 1976 and Bergey·s Manuals Systematic of bacteriology, 2005): Malic acid: 5.0 g, K_2HPO_4: 0.5 g, MgSO4.$7H_2O$: 0.2 g, NaCl: 0.1 g, $CaCl_2.2H_2O$: 0.002, Fe-EDTA (1.64% w/v aqueous): 4.0 ml, the trace element solution contained 2.0 ml (Bromothymol blue (0.5% alcoholic solution), 2.0 ml: $ZnSO_4.7H_2O$; 1.4 mg, $MnSO_4.4H_2O$: 1.6 mg, $FeSO_4.7H_2O$: 5.0 mg, $CaCl_2$. 6 H_2O, 20 mg) and vitamin solution 1.0 ml: (Biotin: 10.0 mg, Pyridoxin: 20.0 mg, KOH: 4.0 g), agar (Semi solid):1.75 gl^{-1}**.**

Medium 2: Watanabe medium for cyanobacteria growth this medium is modified (El-Nawawy et al., 1958). K_2HPO_4: 0.30 g, $MgSO_4.7H_2O$: 0.20 g, K_2SO_4: 0.20 g, $CaCO_3$: 0.10 g, glucose: 2.00 g, $FeCl_3$ 1% (freshly prepared): 0.20 ml. and microelements solution: 1.00 ml of distilled water, microelements solution, H_3BO_3: 2.80 g**,** $MnCl_2$: 1.80 g, $ZnSO_4.7H_2O$: 0.22 g, $CuSO_4.5H_2O$: 0.08 g, molybdic acid: 0.02 g, all

of these continents were dissolved in a liter of distilled water and pH adjusted to 7.50**.**

Microbial inoculants

Two *A. lipoferum* strains and the cyanobacterium *A. oryza* were kindly supplied from Microbiology Department of the Soils, Water and Environment Research Institute, Agricultural Research Center, Sakha Agriculture Research Station, Kafrelsheikh, Egypt.

DNA extraction, 16S ribosomal RNA (rRNA) amplification and sequencing

Genomic DNA of selected bacteria were extracted from a 10-ml bacterial culture grown overnight according to a method described by Dobereiner and Day, (1976). Bacterial pellets suspended in a mixture of TE buffer, SDS (10%) and proteinase K was inoculated for 1 hr at 37 C. NaCl (5 M) and CTAB/ NaCl solution (4.1 g NaCl and 10 g CTAB [N-cetyl-N,N,N,-trimethyl ammonium bromide] were dissolved in (pr-warmed 100 ml distilled water). The mixture was inoculated for 10 min at 65 C. The solution was extracted with 780 µl of chloroform- isoamyl alcohol (24:1), centrifuged for 5 min and the aqueous phase was further extracted with an equal volume of phenol-chloroform-isoamylalchohol (25:24:1). After centrifugation for 5 min, the DNA in the aqueous phase was precipitated with 0.6 vol. isopropanol and the precipitate was washed with 70% ethanol. The DNA pellet was dried and re-suspended in TE buffer (Abou-Shanab et al., 2006). 16S for ribosomal RNA (rRNA) amplification and sequencing by oligonucleotide primers with specificity for eubacterial 16S rRNA, namely primers 16Sa (GGCGAGGCTTAACA) and 16Sb (CCAGCCGCAGGTTCCCCT) Van Berkum and Fuhrmann (2000), were used to amplify the 16S rRNA gene fragments with template DNA originating form used A. lipoferum, PCR mixtures contained 300 mM Tris-HCL pH 9, 7.5 mM $MgCl_2$, 75 mM $(NH4)_2SO_4$, 10 mM each dDTPs, 10 pmol of each primer, 10-50 ng of the extracted DNA and 3U of Taq DNA polymerase were used in a reaction volume of 120 µl. (Van Berkum and Fuhrmann, 2000). PCR products were purified using QIAquick Spin columns. Aperkin Elmar 377 DNA sequences in combination with a Dye Deoxy Terminator cycle sequencing kit (Perkin Elmer, Fster City, CA) was used for sequencing of the purified PCR products. A database search of GenBank using BLAST (Altschul et al., 1997) was performed to identify bacterial species.

Preparation of Azospirillum cultural

The strains were grown in a 500 ml flask containing 250-ml liquid malate medium (Dobereiner and Day, 1976) at 30° C. After 3 days, the number of cells/ml of each culture was determined using a dropping plate method according to Somasegaran and Hoben (1985).

Effect of A. lipoferum strains and/or A. oryza *on wheat grains germination*

Ten grains of wheat were surface sterilized in $HgCl_2$ solution for 4 minutes then treated with ethyl alcohol for 3 minutes and washed several times with sterilized distilled water (Vincent, 1970). Grains were grown on plates containing 5 ml ($1x10^6$cfu) of each *Azospirillum* species and/or *A. oryza* with 3 replicates. The plates were incubated at 30° C for 3 days. Number and length of roots were determined. After 3 days, 3 grains (from each treatment) were taken and placed in 3 incubation tubes. The tubes contained solid media of nutrient solution free of nitrogen (Shrdleta et al., 1984) at 30 days, plants were harvested and analyses.

In vivo evaluation of microbial inoculation on wheat productivity (greenhouse and field experiments)

Greenhouse pots and field experiments were carried out at Sakha Agricultural Research Station, Kafrelsheikh to investigate the effect of inoculation with salt-tolerant efficient *A. lipoferum* strains (A_{10}+A_{11}) as well as *A. oryza* to reduce salt stress. The used pots were about 25 cm in diameter and 28 cm in high filled with 5 kg clay soil. Composite surface soil samples (0-20 cm depth) were taken just before conducting the experiment. The soil samples were air dried, crushed and sieved through 2 mm sieve, and subjected to chemical characterizations according to Richards (1954). Soil samples collected from the wheat field were determined and showed differences in their chemical compositions. The site showed normal (EC 2.4 dSm^{-1}) while the sites 2 and 3 had high salinity levels (EC 6.9 and 11.4 dSm^{-1}) respectively. Variations of EC value depended on soluble anions and cations of soils as shown in Table 1.

The pots and soil experiments design had a split with seven replicates; The experimental was performed at three salinity levels. Inoculation with different *A.lipoferum* strains and/or *A.oryza* was assigned as sup-factor. Results were recorded at 60 and 120 days after planting time.

TABLE 1. Chemical analysis of collected soils form field wheat sites.

Soil Exp.	EC dSm^{-1}	Soluble cations (meqL^{-1})				Soluble anions (meqL^{-1})				pH
		CO^{-3}	HCO^{-3}	Cl^{-1}	SO^{-4}	Mg^{++3}	K^{+}	Na^{+}	Ca^{++}	
Level (1)	2.4	0.0	8	20	4.44	6.65	0.22	18.1	5.49	7.5
Level (2)	6.9	0.0	9	22.5	26.6	8.90	0.43	21.2	23.8	7.8
Level (3)	11.4	0.5	7.25	85	5.25	27	0.65	33.1	30.9	6.8
Field	11.6	0.5	7.31	88	6.89	28	0.65	34.8	32.9	7.2

EC: Electric conductivity Exp. Experiment.

Genetic analysis of the *Azospirillum* strains A_{10} and A_{11} by 16S rDNA sequences data they are *A. lipoferum* strains

Fig. 1. Phylogenetic dendrogram showing the taxonomic positions of *Azospirillum* sp. type strains based on 16S rDNA partial sequences.

Fertilizer application

In the pots experiment, nitrogen was added to the control with 100% traditional mineral fertilizer. Treatments inoculate with *A.oryza*, *A. lipoferum* (A_{10}), *A, lipoferum* (A_{11}), *A. lipoferum* (A_{10}+A_{11}), *A. lipoferum* (A_{10}) + *A. oryza*, *A. lipoferum* (A_{11}) +*A. oryza* and *A. lipoferum* (A_{10}+A_{11}) + *A. oryza* pots received 85% traditional mineral nitrogen (0.25g urea pot^{-1}) with a rate of 420 kg ha^{-1} urea. Nitrogen fertilizer was applied at sowing time was 120 kg ha^{-1}, and another two time during growth. The pots were fertilized with basal rate super phosphate at a rate of 240 kg ha^{-1} added to the soil before sowing and with potassium sulphate (at rate of 120 kg ha^{-1}). Fertilizers were added to inoculated and un-inoculated plans.

Plant growth analysis

60 and 120 days after sowing, plants were collected and subjected to the following analyses: Dry weight (g plant^{-1}) after drying in an oven at 70° C until a constant weight, total number and dry of branches, number and dry weight of spikes, number and dry weight of grains/plant and grain index (weight of 1000 grain) determined after 120 days after sowing.

Chemical analyses

For determination of N, K contents expressed as plant samples or grains were dried and 0.2 g were incubated in 5 ml H_2SO_4 and 1 ml perchloric acid in a conical flask for 24 h as described by Chapman and Parker (1963). The digested materials were completed to 50 ml H_2O and then distilled by a micro-Kjeldahl method and the nitrogen concentration of distillate was determined by titration against 0.02 normal H_2SO_4 according to Black et al. (1965) Phosphorus concentration of samples was determined calorimetrically according to the methods described by Snell and Snell (1967). Sodium and potassium contents were determined for the digested solution by using flame photometer (No, 712700 REG. DES No, 866150) as described by Jackson (1967). N, P, K and Na contents were calculated according to Black et al. (1965). Element content = element % x dry weight/100. Total chlorophyll was determined by a Minolta chlorophyll meter SPAD-502 for plant in the field at 60 days after sowing.

Enumeration of microbes

Microbial counts (colony forming unit, cfu) were determined by estimation of total number of microorganisms in the rhizosphere soil according to Allen (1959).

Estimation of phosphatase and urease activity in wheat rhizosphere soil after 70 days of sowing

1- Phosphatase activity of soil samples was determined by following the procedure of Tabatabai (1982). One gram of soil sample was placed in a 50 ml Erlenmeyer flask to which 0.2 ml toluene. 4 ml modified universal buffer MUB solution was added. One milliter of P-nitrophenyl phosphate solution was added (0.42 g of disodium P-nitrophenyl phosphate tetrahydrate dissolved in 40 ml of MUB) pH 11 and diluted to 50 ml with MUB stored in a refrigerator. This mixture was incubated at 37°C for 1 h. After incubation, 1 ml of 0.5 M $CaCl_2.H_2O$ and 4 ml of 0.5 M NaOH were added and the solution was mixed thoroughly. The mixture was filtered then through a filter paper (Whatman no. 42). The absorbance formation of a yellow color was measured at 420 nm against the reagent blank using a spectrophotometer (model 6705). Control was determined for each soil sample by following the same procedure described above except that the p-Nitrophenyl phosphate solution was added after the addition of 0.5 M $CaCl_2$ and 0.5 M NaOH (just before filtration). The phosphatase activity in the soil samples was expressed as µg p-Nitrophenyl formed per gram soil per hour with reference to the standard curve prepared by using standard concentrations of p-Nitrophenyl phosphate.

2-Determination of urease activity in soil samples were performed as reported by Pancholy and Rice (1973) except the ammonia liberated (hydrolysis of urea in the reaction mixture) was determined by nesslerization as described by Jackson (1973). Five grams of each freshly collected soil samples were placed in 100 ml Erlenmeyer flasks to which 0.5 ml toluene was added samples were incubated for 15 min at room temperature to permit complete penetration of toluene into the soil. 10 ml of phosphate buffer (17.85 g KH_2PO_4 per 500 ml added to 500 ml solution of K_2HPO_4 containing 20.66 g, pH 7.6) and 10 ml of 10% urea solution was added for each flask. For control flasks, the urea solution was replaced by an equal quantity of distilled water. The flasks were well shaken for five minutes and incubated at 30°C for 24 h. After incubation, the samples were filtered through a filter paper (Whatman No. 42). The remaining soil in the flask was supplemented with 15 ml of 1 N KCl solution shaken for five minutes and filtered. The volume of the total filtrate was adjusted to 100 ml in the volumetric flask using distilled water. One ml filtrate of each sample was transferred to a 50 ml volumetric flask, to which one ml of 10% sodium and potassium tartrate and one ml of 1% gum acacia solution and 5 ml of Nessler's reagent was added (Hg 3%, KI 3.5%, NaOH 12% and Water 81.5%). The volume was made to 50 ml with distilled water. The yellow color developed after 30 minutes was measured at 410 nm using a spectrophotometer against the reagent blank. The results obtained were expressed as mg of ammonia liberated per gram soil per day with using a standard curve for different concentrations of $(NH_4)_2SO_4$ differentiation by nesslerization.

Statistical analysis

The collected data were subjected to statistical analysis, using the analysis of variance (ANOVA). LSD range tests were used to compare differences between the means (Steel and Torrie, 1980).

Results and Discussion

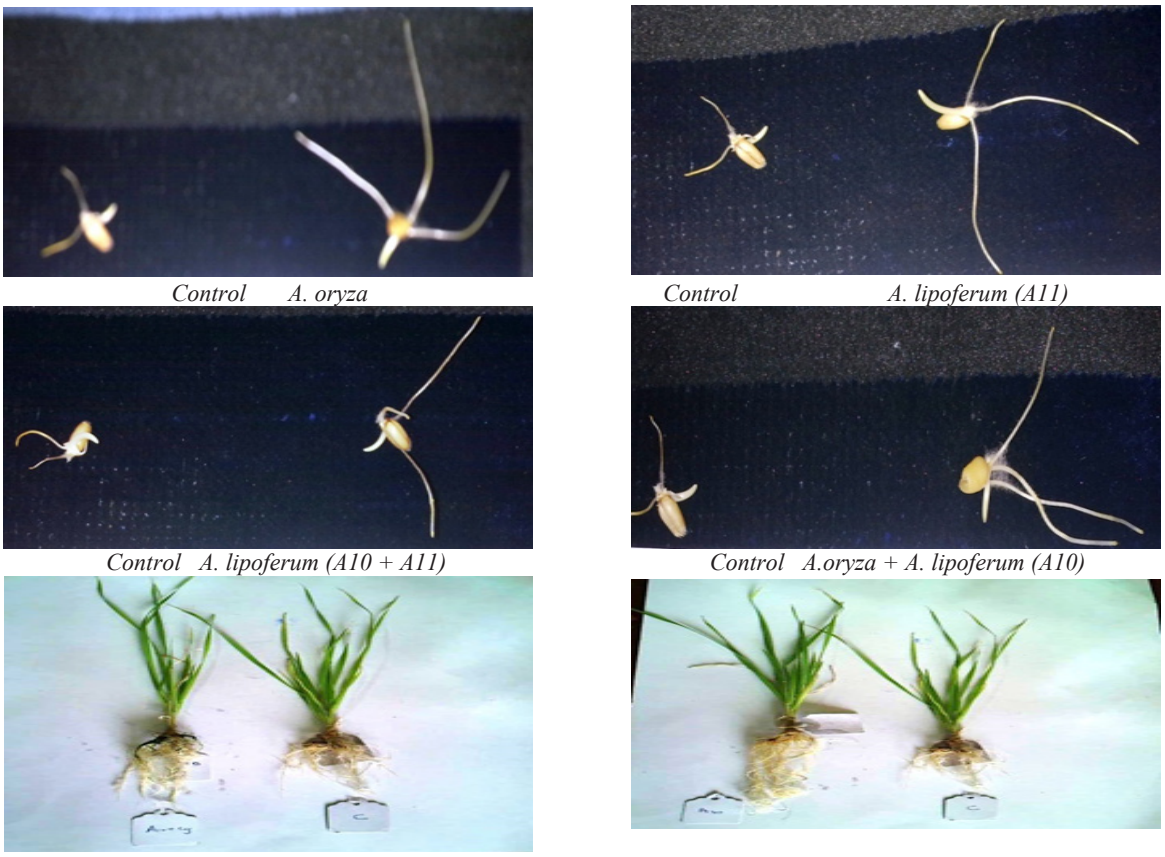
Effect of *A.lipoferum* strains and/or *A. oryza*inocula on germination of wheat seedling grains, (number and length of wheat roots) was carried out in the laboratory at 3 days after sowing. Results in Table 2 revealed significantly increase germination rate grains and highly increase of numbers and length of radicals (cm/root) for all inoculated treatments as compared with controls (un-inoculated) plants. The *A. lipoferum* (A_{10}) + *A. oryza* treatment gave a highly significant rising number of germinated grains and radicals with 48% and 50% increase compared to un-inoculated control plants. In addition, the results showed that inoculation with different treatments was leading a highly significant acceleration of shoot and root development results an increased dry weight of plants (g/pant) compared with control un-inoculated plants. The treatments with *A. lipoferum* (A_{10}+A_{11}) caused highest dry weight, which reached up to 0.905 g/plant as shows in Table 3 and Fig. 1. This observation is similar to Dobbelaere et al. (2003) who reported that inoculations of wheat grains with *Azospirillum* stimulate number of grains and radicals. A possible explanation for some of the growth promoting effects of *Azospirillum* on plants is the production of several phytohormones that alter metabolism and morphology of plants, leading to a better mineral and water absorption and consequently larger and healthier plants. In unicellular algae, phytohormones may lead to larger cell population. Our results also, agree with those of Bashan et al., (2004) and Bottini et al., (2004) who reported that *Azospirillum* species are well known for their ability to produce plant hormones in *vitro*, among which are indoles, mainly IAA and gibberellins. Moreover, cyanobacteria can significantly improve germination and seedling growth of

wheat particularly in salty soils which suggesting a crucial role in binding hazardous Na^+, thereby alleviating salinity and alkalinity stresses. Also, soaking of grains wheat by cyanobacterail culture increased germination rate and coleoptile lengths of seeds (Karthikeyan et al., 2008).

TABLE 2. Effect of inoculation with *A. lipoferum* stains and/or *A. oryza* on germination after 3 days and growth parameters after 30 days.

Treatments	Seedling Experiment 3 days			Tube experiment 30/days		
	GR	No. of Radicals plant^{-1}	Root length cm plant^{-1}	Shoot length cm plant^{-1}	Root length cm plant^{-1}	Dry weight g plant^{-1}
Control	4.333	1.667	2.1	12.900	11.02	0.753
Cyano.	7.667	3.000	3.267	15.300	14.10	0.820
A_{10}	7.667	2.667	2.933	16.025	13.67	0.870
A_{11}	8.000	3.333	3.033	16.300	13.85	0.878
A_{10}+A_{11}	7.667	3.000	3.000	15.800	13.40	0.905
A_{10}+Cyano.	8.333	3.333	3.133	16.150	13.85	0.865
A_{11}+Cyano	8.000	3.000	3.200	16.425	14.00	0.873
Mix	7.000	3.333	3.267	15.875	13.35	0.868
Means	7.33	2.917	2.992	15.575	13.41	0.854
LSD 5 %	1.280	0.775	0.166	1.76	0.83	0.0208
Significant	**	**	**	**	**	**

Germination rate of grains from 10 grain plat^{-1} A: *Azospirillum* strains. Cyano.: *Anabaena oryza*. Mix: *Azospirillum* strains+ *Anabaena oryza* I: Inoculation S: Salinity SI: Interaction between Salinity and Inoculation *: significant N.S: Not significant. **: Highly significant. Control: 100% N-fertilizer (un-inoculated)



Control *A. oryza* *Control* *A. lipoferum (A11)*

Control *A. lipoferum (A10 + A11)* *Control* *A.oryza + A. lipoferum (A10)*

Control *A. lipoferum (A10 + A11)* *Control* *A.oryza + A. lipoferum (A10)*

Fig. 2. Effect of A. lipoferum strains and/or A. oryza on wheat grains 3 days after sowing.

As shown in results in Fig. 2 increased shoot lengths (cm/shoot), root depth (cm/root) and dry weight of plants (g/pant) was found for inoculated plans as compared to non-inoculated controls. These results are similar to those of Dobbelaere et al. (1999) who found that auxin produced by *Azospirillum* appear to play a major role in plant growth promoting. Likewise, Verma et al. (2001) reported that root morphology and enhance the root cell proliferation, results increased formation of lateral roots and root hairs. Yegorenkova et al. (2001) reported that *Azospirillum* plays an important role in the formation of associations with other rhizobacteria.

The results obtained showed that a negatively effect on shoot and root length, dry weight, total chlorophyll content, N, P, K content of shoots and grains for the same incubation experiment this reminded by Zaied et al. (2009), Somayeh et al. (2012) Boghdady and Ali (2013) and Roomina et al. (2016) who showed that salinity caused reduction in growth parameters compared with control plants un-inoculated. The observed decrease in root and shoot length with increasing salinity concentrations also, in agreement with Munns (2002) who reported that salinity reduces the ability of plants to take up water and results in reduced growth rate. As, salt-tolerant *Azospirillum* bacteria enhance root growth Boghdady and Ali (2013), inoculation with salt-tolerant *Azospirillum* increases vegetative growth of wheatunder salt stress Somayeh et al. (2012) and Verma et al. (2001). Khosravinejad et al. (2008) and Roomina et al. (2016) indicated that high salinitycaused degradative effect of chlorophyll and reduces net photosynthetic rates, transpiration rates and stomatal conductance in various plant species, theses effect are high concentrations of ions (Na^+, Cl^-, SO^2) that accumulate in cells, these by inactivating enzymes and inhibiting photosynthesis (Coradovill et al., 1999). Our experiment data conducted that inoculation with *A. lipoferum*strains (A_{10} and/or A_{11}) and/or *A. oryza*exhibited significant increase of shoot and root length in shoot and root length, dry weight, total chlorophyll content, N, P, K content of wheat shoots and grains of wheat as reported in Tables 3, 4 and 5. These agree with Somayeh et al. (2012), Boghdady and Ali (2013) and Roomina et al. (2016) showed that in the presence of salt stress, plant height was significantly greater in inoculated plants than in un-inoculated**,** inoculation with *A. lipoferum* strains (A_{10} and/or A_{11}) and/or *A. oryza* as compared to N-fertilized un-inoculated plants gave significantly increased in total chlorophyll contents. Karthikeyan et al. (2008) reported that soaking of wheat seeds in a cyanobacteria culture enhanced chlorophyll contents of wheat plants, caused also, a significantly increased N, P, K contents of wheat plants as compared to un-inoculated plants. Likewise, Somayeh et al. (2012) and Boghdady and Ali (2013) who investigated inoculation with salt-tolerant *Azospirillum* and other diazotrophs increased mineral uptake by plants. This was likely due to specific enhancement of normal ion uptake mechanisms or promotion of root development. In addition, Begum and Islam (2011) proposed that inoculation with cyanobacteria enhances microbial proliferation and increases organic matter and available N concentrations in soil surfaces. Plant inoculation with *Azospirillumbrasilense* promoted greater uptake of NO^{3-}, K^+ and H_2PO_4 in wheat experiments with (Saubidet et al. 2000). Stimulating effects on N, P and K uptake were found in inoculated wheat crops with *A. brasilense* (Diaz-Zorita and Grove, 2006). Also, Brahmaprakash and Sahu (2012) suggested that an increase of phosphate uptake observed in roots inoculated with *Azospirillum* could be the result from an increase in acid phosphatase activity**.** In this context, an increase of Na contents in wheat plants by exposed to high salinity concentrations as reported by Saqib et al. (2006). In other study, inoculation with *A. lipoferum* strains (A_{10} and/or A_{11}) and/or *A. oryza* as compared to N-fertilizer un-inoculated control plants results a significant decrease in Na contents of wheat, this results are similar to those of Saqib et al. (2006) and Roomina et al. (2016).

Actually, our obtained results reveal a correlation between a decrease in enzyme activity (urease and phosphatase) of wheat rhizosphere soil and an increase in the salinity concentration (Table 6). On the other hand, inoculation with *A. lipoferum* strains (A_{10} and/or A_{11}) and/or *A. oryza* compared to N-fertilized un-inoculated control caused a significant increase in enzyme activity of urease and phosphatase. The measurements were carried out to study the effect of the microorganisms on the availability of minerals plants. Notably Boghdady and Ali (2013) and Roomina et al. (2016) reported that co-inoculation increase the activity of urease and phosphatase in soil when compared to un-inoculated control or individual inoculation.

Table 6 Indeed, showed that inoculation with

A. lipoferum strains (**A_{10}** and/or **A_{11}**) and/or *A. oryza* inoculation showed a positive effect on total microbial counts of soil wheat plants above the un-inoculated plants under normal and salinity soils. In drought or osmotic stress conditions, accumulated betaines and/or proline are probably these substances are available for microorganisms in the rhizosphere. In *Azospirillum*, osmotolerance is a species-specific character that declines in the order *A. halopraeferens, A.brasilense, A. lipoferum* and *A. amazonense* (Reinhold et al., 1987).

TABLE 3. Influence of inoculation with *A. lipoferum* strains and/or *A. oryza* on root and shoot length of wheat plants after 60 days in pot experiments with different salinity levels.

Treatments	Shoot length (cm/plant)			Root length (cm/plant)		
	Salinity levels (dSm^{-1})					
	2.4	**6.92**	**11.4**	**2.4**	**6.92**	**11.4**
Control	47.5	43.05	38.00	17.0	12.25	10.5
Cyano.	50.75	44.75	41.00	22.75	20.50	15.00
A_{10}	51.15	47.25	41.75	30.00	25.75	20.00
A_{11}	54.02	48.50	42.50	29.25	23.25	18.25
A_{10}+A_{11}	54.75	49.75	42.50	25.25	22.00	14.00
A_{10}+Cyano.	55.00	49.75	46.75	26.00	20.75	14.50
A_{11}+Cyano	52.97	49.00	47.25	27.75	22.00	15.25
Mix	52.8	48.25	46.00	28.50	23.00	14.75
Means	52.36	47.53	43.21	25.81	21.18	15.28
LSD 5 %	**S****		**1.490**	**S****		**0.597**
	I**		**0.865**	**I****		**0.771**
	SI**		**1.49**	**SI****		**1.337**

A :Azospirillum strains. Cyano.: Anabaena oryza. Mix: Azospirillum strains+ Anabaena oryza I: Inoculation S: Salinity SI: Interaction between Salinity and Inoculation *: significant N.S: Not significant. **: Highly significant. Control: 100% N-fertilizer (un-inoculated).

TABLE 4. In pot experiment; Influence of inoculation with *A.lipoferum* strains and/or *A. oryza* on dry weight and chlorophyll content of wheat plants after 60 days.

Treatments	Total chlorophyll			Dry weight (g/plant)		
	Salinity levels (dSm^{-1})					
	2.4	**6.92**	**11.4**	**2.4**	**6.92**	**11.4**
Control	47.72	47.27	46.17	2.30	1.97	1.74
Cyano.	51.92	49.22	47.55	2.87	2.72	2.13
A_{10}	50.70	48.57	47.35	3.85	3.02	2.20
A_{11}	50.50	47.92	46.97	3.62	3.19	2.27
A_{10}+A_{11}	50.55	47.75	46.70	3.55	3.10	2.35
A_{10}+Cyano.	49.80	46.50	46.00	3.22	2.70	2.30
A_{11}+Cyano	48.95	47.22	46.20	3.20	2.75	2.23
Mix	49.42	47.75	46.50	3.59	3.06	2.28
Means	49.99	47.77	46.68	3.27	2.81	2.19
LSD 5 %	**S****		0.658	**S****		0.982
	I**		0.692	**I****		0.0682
	SI^{NS}		1.99	**SI****		0.118

A : *Azospirillum* strains. Cyano.: *Anabaena oryza*. Mix: *Azospirillum* strains+ Anabaena oryza I: Inoculation S: Salinity SI: Interaction between Salinity and Inoculation *: significant N.S: Not significant. **: Highly significant. Control: 100% N-fertilizer (un-inoculated)

TABLE 5. In pot experiment; Influence of inoculation with *A. lipoferum* strains and/or *A. oryza* on N, P, K-content of wheat plants after 60 days

Treatments	N-content (mg/plant)			P-content (mg/plant)			K-content (mg/plant)			Na-content (mg/plant)		
	Salinity levels (dSm^{-1})											
	2.4	6.92	11.4	2.4	6.92	11.4	2.4	6.92	11.4	2.4	6.92	11.4
Control	42.8	36.9	27.5	1.66	1.37	1.15	56.7	42.8	30.9	10.2	11.0	9.5
Cyano.	55.2	53.3	40.4	2.16	1.91	1.45	95.5	72.9	48.4	10.5	11.9	9.9
A_{10}	85.0	58.9	40.9	2.95	2.14	1.52	125.0	75.0	45.6	14.5	13.5	10.2
A_{11}	86.1	62.0	43.4	2.76	2.20	1.57	117.0	87.3	48.3	9.8	12.1	10.1
A_{10}+A_{11}	78.2	60.2	42.9	2.77	2.06	1.60	105.6	75.5	58.5	9.8	12.8	10.7
A_{10}+Cyano.	78.0	52.1	44.9	2.55	1.94	1.65	99.6	70.0	49.6	11.9	10.8	10.7
A_{11}+Cyano	73.6	53.3	42.8	2.66	1.99	1.59	90.22	67.3	45.2	10.0	11.4	9.5
Mix	84.6	58.4	43.7	2.92	2.30	1.28	110.6	64.0	46.2	10.8	11.1	9.2
Means	75.7	54.4	40.9	2.55	1.99	1.47	100.3	69.3	46.6	10.9	11.8	10.02
LSD 5 %	**S****		**5. 40**	**S****		**0.335**	**S****		**3.18**	**S****		**0.547**
	I**		**4.85**	**I****		**0.516**	**I****		**5.18**	**I****		**0.631**
			8.17	**SI****		**0.893**	**SI****		**2.79**	**SI****		**1.095**

A : *Azospirillum* strains. Cyano.: *Anabaena oryza*. Mix: *Azospirillum* strains+ *Anabaena oryza* I: Inoculation S: Salinity SI: Interaction between Salinity and Inoculation *: significant N.S: Not significant. **: Highly significant. Control: 100% N-fertilizer (un-inoculated)

TABLE 6. In pot experiment; Influence of inoculation with the best salt-tolerant *A.lipoferum* strains and/or *A. oryza* on the urease, phosphataes activity and the total microbial count (bacteria and fungi) in soil of wheat after 60 days with in soils rhizosphere.

Treatments	Urease activity (mg NH_4-Ng^{-1} soil d^{-1}			Phosphatase activity (µg $PNPg^{-1}$ soil h^{-1}			Total microbial count (log number/g soil)		
	Salinity levels (dSm^{-1})								
	2.4	6.92	11.4	2.4	6.92	11.4	2.4	6.92	11.4
Control	2.91	2.70	2.51	0.44	0.39	0.32	5.40	3.66	3.20
Cyano.	3.03	2.85	2.63	1.14	0.43	0.39	7.70	5.70	4.30
A_{10}	3.03	2.69	2.69	0.76	0.43	0.38	7.16	7.23	4.90
A_{11}	3.26	3.04	2.78	0.96	0.78	0.42	7.43	5.70	4.26
A_{10}+A_{11}	3.66	3.20	2.71	1.03	0.85	0.48	6.56	6.06	4.03
A_{10}+Cyano.	3.33	3.36	2.75	0.64	0.56	0.52	6.96	5.46	4.36
A_{11}+Cyano	4.00	3.25	2.99	1.20	0.84	0.66	7.13	5.46	4.36
Mix	3.03	2.94	2.88	1.12	0.79	0.51	6.80	3.20	4.20
Means	3.28	3.00	2.74	0.914	0.637	0.464	6.89	5.61	4.20
LSD 5 %	**S****		**0.1045**	**S****		**0.05069**	**S****		**0.06208**
	I**		**0.153**	**I****		**0.067**	**I****		**0.482**
	SI**		**0.2657**	**SI****		**0.1165**	**SI^{NS}**		

A :*Azospirillum* strains. Cyano.:*Anabaenaoryza*. Mix: *Azospirillum* strains+ *Anabaenaoryza*I: Inoculation S: Salinity SI: Interaction between Salinity and Inoculation *: significant N.S: Not significant. **: Highly significant. Control: 100% N-fertilizer (un-inoculated)

In pots and field experiments (Tables 7, 8 and 9), results showed reduction in number of branches and spikes, dry weight of shoots, spikes, grains and dry weight of 1000 grain, shoots and grains at high salinity levels. This results agreed with Saqib et al. (2004), (2005), Saqib et al. (2006), Somayeh et al. (2012) and Boghdady and Ali (2013) showed that N, P and K contents, plant parameters and

grain yield of wheat decreased significantly with increasing salinity. In conclusion, inoculating with *A.lipoferum* strains (A_{10} and/or A_{11}) and/or *A. oryza* resulted significant increase in number of branches and spikes, dry weight of shoots, spikes, grains and 1000 grain dry weight of where compared to un-inoculated plants under normal and high salinity conditions. These results reminisced to those of with Zaied et al. (2009), Somayeh et al. (2012) and Boghdady and Ali (2013) who found that inoculation of wheat with salt-tolerant *Azospirillum* sp. increased in their growth and yield of wheat. Also, Somayeh et al. (2012) and Boghdady and Ali (2013) observed a significant increase in shoot dry weight and root dry weight of salt-tolerant *Azospirillum sp*. were inoculated on wheat. An observation by Verma et al. (2001) and Saqib et al. (2006) indicated that inoculation had a positive effect on shoot and root dry matter accumulation during wheat vegetative growth, grain number, dry weight of 1000 grain and kernel weight at the time of harvest. Efficiency of *Azospirillum* strains for their ability to enhance and/or improve yields under salt stress had also, reported by found that co-inoculation could result in higher grain yield with better quality in comparison to single inoculation (Lakshminarayana 1993, Askary et al., 2009, Somayeh et al. 2012 and Boghdady and Ali 2013). In addition, Kirlwood (2008) found that application of exopolysaccharides (EPS) produced by cyanobacteria significantly improves grain germination and seedling growth of wheat particularly in salt-affected soils. EPS seems to play a crucial role by binding hazardous Na^+ ions, thereby alleviating salinity and alkalinity stresses for plants crops. In recent years, *Azospirillum* sp. co-inoculation with other symbiotic microorganisms creates a successful system of biological nitrogen fixation in wheat that can lead to many profits for plants. Co-inoculation of wheat grains with *A. brasilense* and *R. meliloti* had positive effects on the grain yield of wheat as compared to either single inoculation or un-inoculated plants.

Pots and field experiments (Tables 8, 10 and 11) show that N, P, K contents of shoots and grains of wheat decreased by increasing the salinity concentrations. Somayeh et al. (2012) and Boghdady and Ali (2013) reported that as increased salinity levels cause decrease in N, P, K

TABLE 7. In pot experiment; Influence of inoculation with *A. lipoferum* strains and/or *A. oryza* on number of branches, spike and dry weight of shoot and spikes shoot plants after 120 days.

Treatments	No. of branches			Dry weight of shoot (g/plant)			No. of spikes			Dry weight spikes (g/plant)		
	Salinity levels (dSm^{-1})											
	2.4	**6.92**	**11.4**	**2.4**	**6.92**	**11.4**	**2.4**	**6.92**	**11.4**	**2.4**	**6.92**	**11.4**
Control	14.66	13.00	10.00	7.56	6.96	6.40	12.00	10.33	8.66	6.46	5.16	3.40
Cyano.	17.66	14.66	12.66	11.26	10.40	7.06	14.66	12.66	10.66	7.06	5.86	5.20
A_{10}	18.66	17.00	12.66	11.83	10.50	8.60	15.00	13.33	10.00	7.46	6.03	5.20
A_{11}	18.00	17.33	13.33	13.03	11.73	8.52	15.33	13.66	10.66	7.40	5.20	5.00
A_{10}+A_{11}	17.00	17.33	11.33	11.23	10.60	8.93	16.00	13.66	10.00	7.33	5.40	4.03
A_{10}+Cyan.	18.33	17.00	11.66	14.26	11.83	7.79	14.33	13.00	10.33	7.86	6.33	4.00
A_{11}+Cyano	16.00	16.33	12.00	12.66	11.96	7.73	14.00	12.33	11.00	7.60	5.53	4.02
Mix	17.00	16.00	11.66	12.53	10.60	7.53	14.33	12.66	11.66	7.16	5.80	3.13
Means	17.16	16.08	11.91	11.80	10.57	7.82	14.45	12.70	10.25	7.29	5.66	4.37
	S**		**2.412**	**S****		**0.581**	**S****		**0.9086**	**S****		**0.285**
LSD 5 %	**I****		**1.505**	**I****		**0.762**	**I****		**1.269**	**I****		**0.521**
	SI**		**2.606**	**SI****		**1.320**	**SI****		**2.198**	**SI***		**0.904**

A :*Azospirillum* strains. Cyano.:*Anabaenaoryza*. Mix: *Azospirillum* strains+ *Anabaenaoryza*I: Inoculation S: Salinity SI: Interaction between Salinity and Inoculation *: significant N.S: Not significant. **: Highly significant. Control: 100% N-fertilizer (un-inoculated).

TABLE 8. In pot experiment; Influence of inoculation with *A. lipoferum* strains and/or *A. oryza* on dry weight of grains and 1000 grain N, P, and K contents after 120 days.

Treatments	Dry weight of grains (g/plant)			Dry weight of 1000 grain (g /plant)			N-content (mg/plant)			P-content (mg/plant)			K-content (mg/plant)		
	Salinity levels (dSm-1)														
	2.4	**6.92**	**11.4**	**2.4**	**6.92**	**11.4**	**2.4**	**6.92**	**11.4**	**2.4**	**6.92**	**11.4**	**2.4**	**6.92**	**11.4**
Control	2.83	2.33	2.11	25.80	23.40	21.10	166.2	146.0	129.5	7.4	6.8	6.5	138.7	122.1	115.3
Cyano.	3.27	2.69	2.20	27.00	25.20	22.23	255.6	211.3	149.7	11.97	10.6	6.8	270.0	218.0	134.9
A_{10}	3.10	2.72	2.27	27.56	25.26	24.23	306.2	242.5	208.3	12.1	10.2	8.5	241.1	205.8	160.0
A_{11}	3.11	2.75	2.26	27.40	25.36	22.53	340.0	241.6	182.7	14.3	12.3	9.7	279.7	246.6	163.0
A_{10}+A_{11}	3.12	2.70	2.16	27.03	25.26	22.50	288.9	250.6	199.8	12.2	10.8	8.6	251.4	226.4	157.6
A_{10}+Cyan.	3.09	2.75	2.23	26.63	25.13	23.13	371.0	260.1	156.9	14.6	11.7	7.6	293.4	235.5	140.3
A_{11}+Cyano	3.03	2.74	2.22	26.30	25.10	23.26	316.4	254.8	164.0	13.1	11.7	7.5	274.2	244.3	144.3
Mix	3.09	2.75	2.14	26.13	25.46	23.33	300.8	239.6	151.4	13.4	10.2	7.3	246.8	214.6	143.3
Means	3.08	2.68	2.20	26.73	25.02	22.79	293.1	230.8	167.8	12.4	10.5	7.87	249.4	214.1	144.8
LSD 5 %	**S****		**0.1542**	**S****		**0.578**	**S****		**5.43**	**S****		**0.981**	**S****		**1.415**
	I**		**0.134**	**I****		**0.624**	**I****		**25.15**	**I****		**1.085**	**I****		**1.533**
	SI**		**0.233**	**SI^{NS}**			**SI****		**43.57**	**SI***		**1.87**	**SI****		**2.65**

A :*Azospirillum* strains. Cyano.:*Anabaenaoryza*. Mix: *Azospirillum* strains+ *Anabaenaoryza*I: Inoculation S: Salinity SI: Interaction between Salinity and Inoculation *: significant N.S: Not significant. **: Highly significant. Control: 100% N-fertilizer (un-inoculated)

TABLE 9. In field experiment; Influence of inoculation with *A. lipoferum* strains and/or *A. oryza* on plant growth parameters of wheat plants after 120 days.

Treatments	No. Branches	D.W g plant^{-1}	No. of Spike	D.W of Spike (gplant^{-1})	D.W of Grains Tonsfeddan^{-1}	D.W of 1000 Grains (g)
Control	15.00	1.89	14.5	2.873	**0.328**	19.27
Cyano.	17.00	2.25	15.6	3.973	**0.476**	24.2
A_{10}	18.16	2.18	17.1	3.963	**0.520**	22.08
A_{11}	15.83	2.06	15.5	3.755	**0.599**	22.33
A_{10}+A_{11}	16.33	2.23	15.0	3.922	**0.522**	22.37
A_{10}+Cyano	16.16	2.18	15.5	3.81	**0.585**	21.1
A_{11}+Cyano	16.33	2.25	15.5	4.16	**0.5418**	24.5
Mix	15.83	2.46	14.1	4.360	**0.5376**	21.68
Means	16.33	2.19	15.3	3.853	**0.328**	22.19
LSD 5%	**0.9722**	**0.13**	**1.07**	**0.6774**	**0.3688**	**0.7254**
Significant	**	**	**	**	**	**

A :*Azospirillum* strains. Cyano.:*Anabaenaoryza*. Mix: *Azospirillum* strains+ *Anabaenaoryza*I: Inoculation S: Salinity SI: Interaction between Salinity and Inoculation *: significant N.S: Not significant. **: Highly significant. Control: 100% N-fertilizer (un-inoculated)

TABLE 10. in field experiment; Influence of inoculation with *A. lipoferum* strains and/or *A. oryza* on wheat shoots 120 days of inoculation with salinity levels.

Treatments	Shoot			Grains		
	N-Content (mg/plant)	P-Content (mg/plant)	K-Content (mg/plant)	N-Content (mg/plant)	P-Content (mg/plant)	K-Content (mg/plant)
Control	35.9	1.76	33.9	31.4	6.27	32.1
Cyano.	43.8	2.32	49.3	41.3	8.00	41.4
A_{10}	48.3	2.21	48.5	50.1	9.32	47.4
A_{11}	44.0	2.30	45.2	53.0	9.48	52.0
A_{10}+A_{11}	47.5	2.20	47.4	47.6	8.65	51.4
A_{10}+Cyano	46.6	2.25	48.6	45.4	8.37	43.3
A_{11}+Cyano	48.7	2.25	49.9	44.7	9.38	45.6
Mix	51.2	2.20	56.3	53.1	9.05	48.6
Means	45.8	2.40	48.1	45.8	8.56	45.27
LSD 5%	**4.417**		**4.818**	**9.244**	**1.336**	**7.514**
Significant	**	**NS**	**	**	**	**

A :*Azospirillum* strains. Cyano.:*Anabaenaoryza*. Mix: *Azospirillum* strains+ *A. oryza*I: Inoculation S: Salinity SI: Interaction between Salinity and Inoculation *: significant N.S: Not significant. **: Highly significant. Control: 100% N-fertilizer (un-inoculated)

TABLE 11. in field experiment; Influence of inoculation with *A. lipoferum* strains and/or *A. oryza* on Na-content of shoot, Na- content and protein% of grains wheat after 120 days.

Treatments	Shoot	Grains	
	Na-Content (mgplant^{-1})	Na-Content (mgplant^{-1})	Protein %
Control	29.53	8.22	9.910
Cyano.	31.82	8.30	10.46
A_{10}	30.23	8.77	11.35
A_{11}	27.78	9.37	11.01
A_{10}+A_{11}	28.27	7.32	10.93
A_{10}+Cyano	28.05	7.82	10.76
A_{11}+Cyano	30.97	7.72	10.56
Mix	33.22	8.17	10.82
Means	**29.9**	8.21	10.279
LSD 5%	**3.079**	**--**	**0.3957**
Significant	**	**NS**	**

A :*Azospirillum* strains. Cyano.:*A.oryza*. Mix: *Azospirillum* strains+ *A.oryza*I: inoculation S: Salinity SI: Interaction between Salinity and Inoculation *: significant N.S: Not significant. **: Highly significant. Control: 100% N-fertilizer (un-inoculated).

contents of shoots and grains of wheat.

As show, inoculation with *A.lipoferum* strains (A_{10} and/or A_{11}) and/or *Anabaena oryza* exhibited significant increased N, P, K contents of shoots and seeds wheat as comparison with un-inoculated wheat plants under normal and salinity conditions. N_2-fixation contributes to the N balance of plants within inoculated roots (Okon et al., 1994). Karthikeyan et al. (2008) reported that soaking of wheat grains by cyanobacterial culture increased N % and N contents of wheat plants.

Also, Diaz-Zorita and Grove (2006) reported that inoculation of wheat with *Azospirillumbrasilense* resulted in greater N, P, and K content in winter wheat flag leaves and grain compared with control. Kirlwood (2008) indicated that inoculation with cyanobacteria enhanced microbial proliferation and increased organic matter content and available N, P, K nutrients in soil surface. Salt-tolerant

Azospirillum bacteria are important in agriculture as they are known to improve soil health and to increase crop productivity caused by factors like N_2 fixation, siderophores, ammonia excretion, phytohormones and antifungal properties etc. In addition, Madhaiyan et al. (2009) and Roomina et al. (2016) showed that, *Azospirillum*sp. co-inoculation with other symbiotic microorganisms increased phosphatase enzyme in soil when compared to un-inoculated control or individual inoculation.

Cyanobacteria play a major role in improvement of soil physical and chemical properties (Kimand Kim, 2008). Furthermore, results showed an increase of Na-content of wheat shoots and seeds by rising salinity concentrations. In this context, Saqib et al. (2006) found that, significant increase in Na-content due to increased salinity. Otherwise, inoculation with *A. lipoferum* strains (A_{10} and/or A_{11}) and/or *Anabaena oryza* compared with N-fertilizer un-inoculated control gave a significant disprove affect Na% of wheat plants above the un-inoculated plants. This agreed with Saqib et al. (2006) who documented that, wheat seedling inoculated with *Azospirillum* decreased Na-content in shoots and grains compared with un-inoculated. Munns and James (2003) illustrated that Na accumulation in leaves was the most important character that correlated with the salt resistance of wheat.

Generally, it was obviously noticed protein% of wheat grains was disproved affect by increasing salinity concentrations (Table 11). This agreed with Saqib et al. (2006) and Somayeh et al. (2012) reported that with increased salinity levels caused decreased protein % of grains. Otherwise, Data showed that inoculated with *Azospirillumlipoferum* strains (A_{10} and/or A_{11}) and/or *Anabaena oryza* exhibited significant increased protein wheat grains above the un-inoculated plants under normal and salinity soils. In this context, Zaied et al. (2009) indicated thatsalt-tolerant *Azospirillum* strain inoculants exhibited significant increase grains protein content above un-inoculated wheat plants. However, saline soils may impose specific ionic effects on plants because high concentrations of ions (Na^+, Cl^-, SO^{2-}) accumulate in cells, inactivating enzymes and inhibiting protein synthesis. Wheat salt tolerance depends mainly on the capability of roots for (a) restricted or controlled uptake of Na^+ and Cl^- and (b) continued (b) uptake of essential elements, particularly K^+ and NO^- (Gilroy and Jones, 2000). However, *A.lipoferum* strains (A_{10} and/or A_{11}) and/or *A. oryza* treatments appeared significant increase in grains protein% in the two experiments above un-inoculated plants. These results agreed with, Somayeh et al. (2012), Boghdady and Ali (2013) and Roomina et al. (2016) concluded that increased of grain protein found with inoculation of wheat with salt-tolerant *Azospirillum* was in response to increased inorganic N uptake by roots.

Conclusion

Finally, it be concluded that inoculation withsalt-tolerant associative *A.lipoferum* strains (A_{10} and /or A_{11}) and /or *Anabaena oryza* of wheat plants enhanced the productivity and could help in the soil reclamation by improves soil characteristic.

Abbreviations

A: *Azospirillum* strains. Cyano.: *Anabaena oryza*. Mix: *Azospirillum* strains+*Anabaena oryza*I : Inoculation S : Salinity SI : Interaction between Salinity and Inoculation *: significant N.S: Not significant. **: Highly significant. Control: 100% N-fertilizer (un-inoculated) *Azospirillumlipoferum* strains and/or *Anabaena oryza*.

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