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Improving the Productivity of Wheat *(Triticum aesitivum* L.) Cultivated under Saline Soil Using Some N_2-Fixers Halophillic Bacteria and Compost

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ABSTRACT

During the winter season of 2010 / 2011 field experiment was carried out to study the possibility of improving the productivity of saline tolerating wheat variety sakha 93 cultivated in saline soil by inoculation with certain N_2- Fixers halophillic bacteria i.e, *Azotobacter chrooccocum*, *Azospirillum lipoferum*, and *Bacillus polymyxa* isolated from El- Fayoum, saline soil. The tested saline soil was also treated with full recommended dose of NPK as mineral fertilizer, compost (plant source) at a rate of 10 m^3/ fed. and compost tea as organic fertilizers. Compost tea was added continuously with irrigation water till the end of the experiment. The obtained results revealed that, the three halophillic N_2- Fixers amended with compost tea had a positive effect on the growth and productivity of wheat as well as improved to uptake of macronutrients that reflected on the quality of wheat growth as compared to the control and other individual and dual treatments.

Key words: Wheat, Saline soil, N_2- Fixers, Halophillic bacteria, *Azospirillum*, *Azotobacter*, *Bacillus*, compost, compost Tea**.**

Introduction

Wheat is the world's most important and most widely grown cereal crop through many properties and uses of its grains and straw. Increasing grain yield of wheat is an important national goal to face the contentious increasing food needs of Egyptian population. Wheat production in Egypt increased from 2.08 in 1983 to 7.37 million ton in 2007. This increase was achieved by increasing wheat area from 1.83 to 2.71 million fed/ year and grain yield from 1.50 to 2.71 ton/ fed. in the same period. Plant density, sowing methods, fertilization, weed and diseases control are among the limited factors of wheat production (Kabesh *et al*., 2009).

Salinity is a natural feature of ecosystems in arid and semiarid regions and can also be induced by anthropogenic activities such as irrigation. Nearly 20 % of the world's cultivated lands or nearly half of all irrigated lands are affected by salinity (Siddikee *et al*., 2010). World estimates the area of saline soils ranged from 400 to 950 million hectares, 350 million of them are not strongly saline and have crop production possibilities. Salinity currently affects more than millions hectares of land. A direct effect of soil salinity on physicochemical and biological properties renders the salt – affected soils unsuitable for soil microbial processes and growth of the crop plants. Salinity stress decreases photosynthetic capacity due to the osmotic stress and partial closure of stomata. Plants also can suffer from membrane destabilization and general nutrient imbalance (Tanawy, 2009).

Composting is the biological decomposition of natural organic materials by soil organisms into stable organic matter. Compost improves the water-holding capacity and stability of soils and allows for easier root penetration by plants it may also reduce the need for commercial fertilizer. One of the most commonly seen effects where compost is used is improved soil structure and water infiltration. Better infiltration ensures water is able to move into the soil and percolate through it, rather than pooling on the surface, evaporating or running off slopes before it can do its job. Salts are then more easily flushed away with improved water infiltration. While not being able to reduce salinity, compost use improves plant growth in saline soils. (Huang, 1993).

Compost tea is a water extraction of compost. The microorganisms present in the compost are multiplied by adding selective nutritive substrata. As the amount of microbes in the extraction increases, the levels of dissolved oxygen decreases and thus air is constantly bubbled through the system to keep the extraction aerobic. After 48 hours this brewing process is complete and the compost tea then consists of nutritive materials (organic compounds, micro and macro elements) and a wide variety of beneficial bacteria, fungi, protozoa and nematodes. Compost tea’s efficiency depends on the quality of the compost, the system's extraction efficacy and the ability of the added food to increase the organisms without sacrificing the biodiversity (Ingham, 2005; Scheuerell, 2003; Scheuerell and Mahaffee, 2002).

Bacteria beneficially help the plant in a direct or indirect way, causing in the stimulation of its growth. Natural population of saline environments with better adaptive strategies, halophilic and or halotolerant bacteria

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are environmentally friendly option to apply as biocontrol mediators to ameliorate salt stress. Nitrogen fixing bacteria have been shown to modify the properties of saline soil by adding nitrogen to the soil (Shazia *et al*., 2010).

Free living diazotrophic bacteria such as *Azospirillum* and *Azotobacter* play an important role as biofertilizers. In order to have better biofertilizers for different crops a knowledge of diversity of these microorganisms is necessary. *Azospirillum* species are commonly found in soils and in association with roots of rice, maize, wheat and legumes. Rhizosphere colonization by *Azospirillum* species has been shown to stimulate the growth of a variety of plant species (Maurya *et al*., 2012).

Bacillus polymyxa is one of the oligonitrophilic bacteria which do not belong to *Azotobacter* and can fix atmospheric nitrogen at very low rates. *Bacillus polymyxa* reported by (Hino and Wilson, 1958) to fix about 0.85 – 2 mg nitrogen per gram sugar consumed, it was found to contain hydrogenase and its nitrogen fixation is sensitive to molecular oxygen (El- Hoseiny, 1972).

Therefore the present study was focused on Improving the productivity of wheat (*Triticum aesitivum* L.) cultivated under saline soil conditions using some N_2- fixers halophillic bacteria, compost and compost tea.

Materials and Methods

Soil:

The experiment was carried out in an experimental farm located at the Epshwai region, El- Fayoum governorate, Egypt. The soil texture of the experimental area was clay (Piper, 1950), soil pH reached 7.78 and EC 11.57 ds/m. (Page *et al*., 1982) in Table 1. Soil irrigated water (Jackson, 1973) was illustrated in Table 2. Physical and Chemical analysis of compost were illustrated in Table 3, moist content, Bulk density (Page *et al*., 1982), pH , EC (Richard, 1954), Organic matter and organic carbon (Black *et al*., 1965), Soluble nitrogen (ammonium and nitrate- nitrogen), Total phosphorus, Total potassium (Page *et al*., 1982), Total nitrogen (Jackson, 1973). compost tea (Ebtsam *et al*., 2009) were illustrated in Table 4.

Table 1. Mechanical and Chemical analysis of the tested saline soils.

Type of analysis	Value
Mechanical analysis (%)	
Coarse sand	1.5
Fine sand	19.8
Silt	30.1
Clay	47.3
Texture class	Clay
Phyxical- Chemical analyses (Anions and Cations) mq/ L	
pH (1: 2.5)	8.5
EC (1: 5)	11.57
Ca^{++}	13.02
Mg^{++}	7.98
Na^{+}	98.44
K^{+}	0.56
CO_3^{--}	--
HCO_3^{-}	1.24
Cl^{-}	72.96
SO_4^{--}	45.80
Available nutrients (mq/ Kg Soil)	
N	80.90
P	3.06
K	328.20
Fe	15.46
Mn	1.76
Zn	0.92
Cu	5.08

Table 2 . Physico- Chemical properties of the irrigated water at El- Fayoum governorate

Chemical properties	Value
pH (1 : 2.5)	7.78
EC (1 : 5) ds/m.	3.49
Anions and Cations (mq / L)	
Ca^{++}	6.19
Mg^{++}	3.72
Na^{+}	24.04
K^{+}	1.05
CO_3^{--}	--
HCO_3^{-}	3.99
Cl^{-}	19.20
SO_4^{--}	11.81

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Seeds:

Wheat (*Triticum aestivum* L.) variety Sakha 93, this variety tolerates the saline stress conditions. The seed were kindly obtained from Wheat Res. Dept., Field crop Res. Inst., Agric. Res. Center (ARC), Giza Governorate, Egypt.

Biofertilizers:

The halophilic nitrogen fixing bacteria namely *Azotobacter chrooccocum*, *Azospirillum lipoferum* and *Bacillus polymyxa* were isolated locally from a site near Lake Karoon saline cultivated soil then identified according to (Tarrand et al., 1978 and Bergy's Manual of Determinative Bacteriology. 1974). The strains were grown on N- deficient semi- solid malate medium (Dobereiner *et al*., 1976), modified Ashby's medium (Abd El-Malek and Ishac, 1968) and specific nitrogen deficient medium (Hino and Wilson, 1958), respectively. The cultures were supplemented with 4% NaCl (w/v) and mixed together in equal volumes for field inoculation. Then, the isolates were tested in the same specific medium free from NaCl. After the growth of all isolates on saline specific medium for each isolate type, the isolates were tested in the same specific medium free from NaCl to differentiate between the Halophilic and Halotolerant species, Where the isolates that didn't show sign of growth where the Halophilic ones. Whereas, the isolates that grow normally were Halotolerant N_2- fixers.

The N_2- fixer's cultures containing 10^6 cfu/ ml. were used. 100 ml. of each bacterial strain were used for single treatments, and 50 ml. of each bacterial strain were mixed just before inoculation for combined treatments. Bacterial cultures were coated onto wheat seeds using Arabic gum (40%) as an adhesive agent, than dried in shade for one hour before sowing.

Organic Fertilizers:

Compost:

The used compost was provided from Arabia Factory for compost subsequent to Arab organization for industrialization, at El- Katora province of El- sharkia Governorate. The physico- chemical propertion of the used compost are shown in Table (3). Moist content, Bulk density (Page *et al*., 1982), pH, EC (Richard, 1954), Organic matter and organic carbon (Black *et al*., 1965), Soluble nitrogen (ammonium and nitrate- nitrogen), Total phosphorus, Total potassium (Page *et al*., 1982), Total nitrogen (Jackson, 1973). Compost (Plant source) was mixed thoroughly with the outer soil profile (0- 15 cm.) at a rate of ten cubic meter/ fedden during soil preparation.

Table 3 . Physical and Chemical properties of the used compost

Character	Value
Physical properties	
Color	Dark brown to gray
Bulk density (kg m^{-3})	714
Moisture content %	28.4
Chemical properties	
pH	7.6
EC (ds/ m)	2.45
Organic matter (%)	31.75
Organic carbon (%)	18.14
Ash (%)	72.0
Total nitrogen (%)	2.28
C: N ratio	1 : 16
Total phosphorus (%)	0.85
Total potassium (%)	3.7
Available N NH_4 (ppm)	100
Available N NO_3	250
Nematode (worm)	Nil
Total *E. coli* (cfu /g soil)	Nil
Weed seed	Nil

Where: E. coli: Eshericha coli, cfu: Colony forming unit

Compost Tea:

The compost was soaked in previously stored tap water (2: 10 w/v) to avoid the chlorine harmful effect on the compost microbial load. The compost extract was kept in shaded place for seven days and stirred from time to time. Then the extract was filtered and kept at room until used. Compost water extract (20 % concentration) was added continuously with the irrigation water at a rate of (5 liter/ feddan) till the end of the experiments (Ebtsam, *et al*., 2009). The physico- chemical and biological properties of compost extract were shown in Table (4).

Table 4. Chemical and microbiological characteristics of compost tea used in the experiment

Parameters	Measurements
- pH	7.2
- EC (ds/m)	4.6
- Total nitrogen (%)	0.09
- Total phosphorus (%)	0.07
- NH_4 – N (ppm)	15
- NO_3 – N (ppm)	5.6
- Dehydrogenase enzyme activity (TPF/ µg ml CWE)	67.7
- Total count of bacteria CFU/ ml CWE	0.03 X 10^7
- Total count of fungi CFU/ ml CWE	0.015 X 10^5
- Total count of actinomycetes (CFU/ ml CWE)	0.02 X 10^6

Mineral fertilizers:

Full recommended dose of NPK was added to the tested saline soil (Positive control) C_3. Where nitrogen was added as ammonium nitrate at a rate of 350 kg/ fed. In three equal doses often 30, 45 and 60 days respectively. Phosphours was applied as super phosphate 15.5 % P_2O_5 during soil preparation at a rate of 200 kg/ fed. Potassium was added as potassium sulphate at a rate of 50 kg/ fed. once before flowering stage (60 day from sowing).

Experimental design:

Field experiment was carried out in the growing winter season of 2010/ 2011.The experimental treatments were arranged in a complete randomized plot design (Each experimental plot size was 2 m length x 2 m width 4m^2) with three replicates as the following:

T_1- Plain soil (Negative control) C_1
T_2- Compost 10 cubic meter / feddan (Positive control) + compost tea. C_2.
T_3- 100 % NPK from recommended dose (Positive control) C_3.
T_4- *Azospirillum lipoferum*.
T_5- *Azospirillum lipoferum*. + Compost + compost tea.
T_6- *Azotobacter chroococcum*.
T_7- *Azotobacter chroococcum*. + Compost + compost tea.
T_8- *Bacillus polymyxa*.
T_9- *Bacillus polymyxa* + Compost + compost tea.
T_{10}- *Azospirillum lipoferum* + *Azotobacter chroococcum*..
T_{11}- *Azospirillum lipoferum* + *Azotobacter chroococcum*. + Compost + compost tea.
T_{12}- *Azospirillum lipoferum* + *Bacillus polymyxa*
T_{13}- *Azospirillum lipoferum* + *Bacillus polymyxa* + Compost + compost tea.
T_{14}-*Azotobacter chroococcum*. + *Bacillus polymyxa*
T_{15}- *Azotobacter chroococcum*. + *Bacillus polymyxa* + Compost + compost tea.
T_{16}- *Azospirillum lipoferum* + *Azotobacter chroococcum*.+ *Bacillus polymyxa*
T_{17}- *Azospirillum lipoferum* + *Azotobacter chroococcum* + *Bacillus polymyxa* + Compost + compost tea.

The studied characteristics:

The studied parameters are: plant length (cm), fresh and dry weights (g) of shoots and roots, total nitrogen, phosphorus, potassium, sodium and calcium percentage (N, P, K, Na and Ca %) in wheat plants were determined according to (Jackson, 1973). crude protein (%) was calculated based on total nitrogen % multiplying by the factor of 6.25 as described in (A.O.A.C, 1990), free proline content (%) was determined according to the method described by (Bates *et al*., 1973).Total Carbohydrates (%) of wheat were determined according to (Smith *et al*., 1964), measurements were carried out by using spectrophotometer (model spectronic 20 D). Weight of grain yield and 1000 grains weight, were determined at harvest.

Biological status of the tested saline soil:

Total count of soil microflora:

The average total count of bacteria (Difco, 1985), fungi (Allen, 1950) and actinomycetes (Jensen, 1930) was determined using plate count technique. *Azotobacter, Azospirillum* and *Bacillus*. in the rhizospheric zone of wheat were determined using the most probable number (cfu/ g dry soil) method described by (Cochran, 1950).

The N_2- fixers bacteria were undertaken to determine their Nitrogenase enzyme activity (µ mole C_2H_4 / g rhizosphere /h) was measured in wheat rhizospheric roots as acetylene reduction assay (ARA) by GC analysis using a 5880 HP chromatograph (Hewlett Packard Inc Palo Alto, CA, USA) with an ionization flame detector at 135°C according to (Somasegaran and Hoben, 1994), and the Dehydrogenase enzyme activity (µg TPF/ g dry soil/ day) in the rhizosphere of wheat plants was determined according to the method described by (Skujins, 1976).

Statistical analysis:

The data were computed using analyses of variance ANOVA according to (Steel and Torrie, 1984). Least significant differences (LSD) were calculated from ANOVA Tables.

Results

Enumeration of soil microflora in El- fayoum saline soil:

As shown in Table (5), the dynamics of microorganisms varied according to soil type where the number in saline soil differs from other soil types in addition soil texture as well. The populations of microbial groups from the selected saline soil, El- Fyoum region indicated that the numbers of bacteria represented more domination than actinomycetes and fungi. The populations of bacteria recorded 0.35 X 10^6 CFU, whereas actinomycetes recorded 2.176 X 10^5 CFU and fungi recorded 0.26 X 10^4 CFU respectively.

The microbial community in the saline soil involved a lot of microbial groups, some of them are known as halotolerance and halophillic N_2– fixers. The populations of diazotrophic genous in specific media differs from one genus to another where the N_2– fixing bacilli that grown and enriched on specific nitrogen deficient medium (Hino and Wilson, 1958) recorded the highest populations 16.0 X 10^5 followed by *Azospirillum* 2.8 X 10^5 whereas *Azotobacter* record the least populations respectively.

The isolates of N_2– fixers, *Azospirillum*, *Azotobacter* and *Bacillus* were tested for their ability to fix atmospheric nitrogen and the three isolates proved that they had the ability to fix atmospheric nitrogen as pure isolates grown on specific nitrogen deficient media. Although the populations of *Azotobacter* were fewer than both *Azospirillum* and *Bacillus*. These isolates recorded the highest activity of N- ase enzyme where its value were 15.23 µ mole, while *Azospirillum* and *Bacillus* recorded 13.651, 13.6 respectively.

Table 5. The average plate count of total Bacteria, Fungi and Actinomycetes as well as the average count of certain N_2- fixer's bacteria and N- ase activity in the saline soil at El- fayoum governorate

The microbial count	The average plate count cfu/ g dry weight x 10^6	
Bacteria	0.3500	
Fungai	0.0026	
Actinomycetes	0.2176	
N_2- fixers bacteria	The average most probable number (MPN) x 10^5	N – ase activity (Mmole C_2 H_4/ g rhizosphere/ h)
Azospirillum	2.800	13.651
Azotobacter	2.100	15.23
Bacillus	16.000	13.60

Effect of inoculation with N_2- fixer's bacteria, compost and compost tea on the plant height (shoot and root) cm. and number of tillers of wheat (Triticum aestivum L.) grown in saline soil at two growth stages:

Data illustrated in Table (6) revealed that the plant height as a vegetative parameter was significantly affected with the treatment T_{17} that included the mixture of N_2- fixers + compost, where the combined inoculation with mixture of N_2- fixers surpassed the single inoculation either in presence or absence of compost as an organic soil amendment specially at 70 and 90 days after sowing. The highest shoot and root length recorded with T_{17} where this treatment gave 50.80 and 91.60 cm shoot length, 12.90 and 18.00 cm root length respectively.

In concern, the number of tillers, there was markedly increase T_{17} (*Azospirillum lipoferum* + *Azotobacter chroococcum* + *Bacillus bolymyxa* + Compost + Compost tea) still the one that obtained more tillers than others treatments including controls. It recorded the mean value 9.67 and 10.33 at 70 and 90 day respectively.

Effect of inoculation with N_2- fixers bacteria, compost and compost tea on the plant fresh and dry weights (shoot and root) gm. of wheat (Triticum aestivum L.) grown in saline soil at two growth stages

Treatment T_{17} that included (*Azospirillum lipoferum* + *Azotobacter chrooococcum* + *Bacillus polymyxa* + Compost + compost tea) is considered the superior one comparing to the controls and the other treatments. It obtained the highest significant increase in fresh and dry weights of shoots and roots respectively. It recorded 29.70 and 132.70 g shoot fresh weight, 7.20 and 54.70 g shoot dry weight after 70 and 90 days respectively. T_{17} also recorded those highest root fresh and dry weights, where it gave 2.20 and 14.20 g root fresh weight, 1.03 and 7.10 g root dry weight at both periods respectively. The significant increase in fresh and dry weights of both shoots and roots of wheat plants is considered an indication of the positive role of bio and organic fertilizers on plants growth during different time intervals.

Table 6. The plant height (shoot and root) cm. and number of tillers of wheat (*Triticum aestivum* L.) grown in saline soil at two growth stages under different treatments of fertilizers

Treatments	Shoot		Root		Number of tillers	
	70 day	90 day	70 day	90 day	70 day	90 day
T_1 ($Control_1$)	31.00	60.50	8.30	10.50	1.33	1.33
T_2 ($Control_2$)	36.60	70.30	9.00	10.80	2.33	3.33
T_3 ($Control_3$)	46.10	80.50	11.60	12.30	2.67	3.00
T_4	39..07	77.50	10.40	12.00	3.33	3.33
T_5	46.70	75.70	9.70	13.00	3.67	4.67
T_6	47.00	78.67	9.20	12.60	4.33	4.33
T_7	49.10	81.20	9.90	12.00	4.33	6.33
T_8	44.77	77.80	10.90	14.60	5.33	6.67
T_9	45.53	83.20	10.80	14.50	5.67	7.33
T_{10}	43.80	78.50	9.00	10.57	6.67	8.00
T_{11}	44.30	82.40	9.50	12.80	9.67	9.67
T_{12}	39.70	80.20	10.20	11.20	6.67	9.33
T_{13}	41.00	83.00	10.40	11.50	8.67	9.67
T_{14}	41.00	79.10	11.20	12.80	7.67	9.67
T_{15}	42.30	86.00	11.50	12.50	8.67	9.67
T_{16}	46.00	87.60	12.30	13.30	9.33	10.00
T_{17}	50.80	91.60	12.90	18.00	9.67	10.33
$L.S.D._{0.05}$	0.893	0.872	0.758	0.843	0.873	0.921

Table 7. The plant Fresh and dry weights (Shoot and Root) gm. of wheat (*Triticum aestivum*) grown in saline soil at two growth stages under different treatments of fertilizers

Treatment	Fresh weight (g)				Dry weight (g)			
	Shoot		Root		Shoot		Root	
	70 day	90 day	70 day	90 day	70 day	90 day	70 day	90 day
T_1 ($Control_1$)	4.20	25.10	0.57	1.40	0.80	12.40	0.14	0.70
T_2 ($Control_2$)	5.10	30.20	0.67	3.20	1.60	16.00	0.17	1.80
T_3 ($Control_3$)	9.39	61.60	1.00	8.90	2.50	25.20	0.43	1.60
T_4	9.04	79.20	0.97	10.83	1.70	31.40	0.20	3.50
T_5	9.41	116.80	1.90	12.20	1.80	52.50	0.30	5.20
T_6	11.43	78.10	1.30	11.00	2.40	33.00	0.33	5.50
T_7	12.20	117.90	1.40	11.90	2.60	54.70	0.73	5.70
T_8	12.80	82.40	1.50	10.80	3.60	36.30	0.33	4.70
T_9	15.50	103.70	1.70	12.90	3.70	45.70	0.43	4.90
T_{10}	13.80	79.10	1.50	7.10	3.60	35.20	0.43	2.10
T_{11}	20.30	89.20	2.10	10.20	7.00	38.60	0.53	3.60
T_{12}	14.90	85.17	1.20	7.60	4.40	36.10	0.37	3.00
T_{13}	18.20	89.50	1.60	8.20	6.10	38.10	0.43	3.40
T_{14}	18.63	90.90	1.30	6.90	5.00	40.10	0.37	1.60
T_{15}	25.50	94.70	1.70	7.40	6.40	41.00	0.37	3.00
T_{16}	26.80	115.70	2.10	12.80	5.10	49.80	0.93	3.10
T_{17}	29.70	132.70	2.20	14.20	7.20	54.70	1.03	7.10
$L.S.D._{0.05}$	0.288	0.419	0.250	0.023	0.288	0.00	0.250	0.699

Effect of inoculation with N_2- fixers bacteria, compost and compost tea on the total chlorophyll (a,b) (mg/ g dr. wt.), Nitrogenase activity (µ mole C_2H_4 / g rhizosphere /h) and Dehydrogenase activity (µg TPF/ g dry soil/ 24 hrs) in wheat (Triticum aestivum L.) grown in saline soil at two growth stages:

Data in Table (8) show the effect of inoculation with N_2- fixers bacteria and compost on the total chlorophyll (mg/ g dr. wt), Nitrogenase activity (µ mole C_2H_4/g rhizosphere /h.) and Dehydrogenase enzyme activity (µg TPF/g dry soil/ 24 hrs) in wheat (*Triticum aestivum* L.) rhizosphere at different growth intervals. For chlorophyll, the effect of application of organic and biofertilizers positively helped in the healthy state of plant during growth intervals and consequently the optimum photosynthetic processes. T_{17} that included (*Azospirillum lipoferum* + *Azotobacter chroococcum* + *Bacillus polymyxa* + Compost + Compost tea) is considered the superior one where it recorded the highest Chlorophyll content at both 70 and 90 days respectively, Followed by T_7 that included (*Azotobacter chroococum* + Compost + Compost tea) where their values 69.87 and 69,73 at 70 days, whereas they recorded 71.73 and 73.82 at 90 days respectively. All these treatments in addition to the controls obtained less total chlorophyll content.

Concerning, the effect of organic and biofertilizers on the activity of nitrogenase enzyme activity in the rhizospheric area of wheat plants. Treatment T_{17} that included (*Azospirillum lipoferum* + *Azotobacter chroococcum* + *Bacillus polymyxa* + Compost + Compost tea) obtained the highest activity of nitrogenase enzyme activity. It was noticed that the activity increased with increasing the time intervals, where at 70 days T_{17} recorded 0.73 µ moles C_2H_4/g rhizosphere/ h., where at 90 days it gave 2.36 µ moles C_2H_4/g rhizosphere/ h. The control treatments exhibited the least activity. The inoculation of wheat with single N_2- fixers either alone or with compost gave less activity than the compound ones.

Table 8. Total Chlorophyll (a,b) (mg/g dr.wt.), Nitrogenase activity (µ moles C_2H_4/g rhizosphere/h) and Dehydrogenase activity (µg TPF/g dry soil/24 hrs) in wheat (*Triticum aestivum*) grown in saline soil at two growth stages under different treatments of fertilizers .

Treatments	Chlorophyll mg/g dr.wt		Nitrogenase activity µ moles C_2H_4/g rhizosphere/h			Dehydrogenase activity µg TPF/g dry soil/24 hrs		
	70 day	90 day	70 day	90 day	At harvest	70 day	90 day	At harvest
T_1 (Control$_1$)	29.99	33.81	0.06	0.40	0.08	1.176	1.752	1.488
T_2 (Control$_2$)	32.90	37.62	0.09	0.72	0.11	2.208	3.984	2.520
T_3 (Control$_3$)	36.00	36.09	0.03	0.20	0.04	0.744	2.064	1.056
T_4	38.50	44.26	0.17	1.00	0.12	2.544	5.400	2.808
T_5	59.69	67.48	0.28	1.16	0.20	2.592	6.072	2.880
T_6	57.99	60.84	0.26	2.00	0.19	2.568	5.688	2.780
T_7	69.73	73.82	0.29	2.24	0.21	2.976	9.432	2.808
T_8	54.10	53.49	0.13	1.12	0.13	2.832	5.304	3.096
T_9	56.16	57.98	0.19	1.32	0.19	3.000	7.824	4.488
T_{10}	48.97	55.62	0.17	0.96	0.17	3.744	5.040	3.672
T_{11}	49.60	58.04	0.35	1.40	0.17	4.272	7.128	3.792
T_{12}	48.16	59.91	0.17	1.56	0.17	2.832	5.712	2.712
T_{13}	50.41	60.74	0.25	1.60	0.18	3.312	6.864	3.984
T_{14}	47.26	59.84	0.17	1.16	0.17	2.568	5.184	2.304
T_{15}	47.50	61.86	0.18	1.44	0.17	2.664	5.520	3.192
T_{16}	60.66	68.84	0.34	1.36	0.21	5.016	6.408	4.824
T_{17}	63.87	71.73	0.73	2.36	0.26	5.064	9.672	4.848
L.S.D.$_{0.05}$	0.821	0.650	0.403	0.009	0.008	0.014	0.009	0.011

Regarding, dehydrogenase enzyme activity, this enzyme is considered as an indicator of the abundance of microbial groups in rhizospheric area. Treatment T_{17} that involved mixture of N_2- fixers + compost (*Azospirillum lipoferum* + *Azotobacter chroococcum* + *Bacillus polymyxa* + Compost + Compost tea) obtained the highest activity of this enzyme that reflected the increase of microbial activity in soil and the symbiotic relations among the microorganisms. The activity increased with the periods it recorded 5.064 and 9.672 µg TPF/ g dry soil/ 24 hrs during 70 and 90 days respectively. The controls and other treatments gave less activity. At 130 days the activities of both nitrogenase and dehydrogenase enzymes recorded values less than those obtained at 70 and 90 days. This due to the decrease of populations in the soil and plant roots as plants reached the maturity stage and hence the migration of microorganisms.

Effect of inoculation with N_2- fixers bacteria, compost and compost tea on the Proline content (mg/g dry weight) and minerals (%) in wheat grains (Triticum aestivum L.) grown in saline soil at two growth stages under different treatments of fertilizers:

Data in Table (9) show the effect of inoculation with N_2- fixer's bacteria and compost on proline content (mg/g dry weight) and minerals (%) in wheat grains (*Triticum aestivum* L.) cultivated in saline soil. Many plants accumulate proline when they exposed to environmental stresses. As shown in Table (9), the significant increase of amino acid proline with Treatment T_{17} (*Azospirillum lipoferum* + *Azotobacter chroococcum* + *Bacillus polymyxa* + Compost + Compost tea) and T_{15} (*Azotobacter chroococcum* + *Bacillus polymyxa* + Compost + Compost tea) during growth intervals where they recorded 15.96, 15.50 and 16.73 with T_{17} and 17.29, 17.02 and 18.05 (mg/g dry weight) with T_{15} respectively. The controls and other treatments attained less proline content increase of proline accumulation in T_{17} and T_{15} relay on the role of microorganisms either represented in the N_2- fixers or the groups found in compost that help plant to tolerate salty stress during growth.

The results in Table 9 showed the increase of Nitrogen, Phosphorus and Potassium as main macro elements in grain of wheat with the inoculated treatments particularly T_{17} it obtained the significant increase of these elements. Whereas, there was markedly decrease in both sodium and calcium where the two elements attained the lowest values at all.

Table 9. The proline content (mg/g dry weight) and minerals (%) in wheat grains (*Triticum aestivum* L.) grown in saline soil under different treatments of fertilizers.

Treatments	Proline content (mg/g dry weight)			Minerals %				
	70 day	90 day	At harvest	N	P	K	Na	Ca
T_1 (Control$_1$)	21.12	22.62	23.04	1.53	0.96	6.39	0.96	0.050
T_2 (Control$_2$)	20.04	21.11	22.01	1.63	1.38	4.80	0.78	0.037
T_3 (Control$_3$)	21.25	22.97	22.85	2.00	1.48	7.81	0.36	0.045
T_4	19.73	20.77	20.98	1.65	1.14	5.00	0.53	0.026
T_5	18.84	19.81	19.61	1.72	1.28	5.27	0.52	0.026
T_6	19.55	20.76	20.52	1.73	1.10	4.90	1.02	0.033
T_7	18.25	19.45	19.66	1.81	1.14	5.08	0.42	0.048
T_8	19.11	30.58	20.17	1.55	1.21	4.25	0.94	0.048
T_9	18.05	19.15	19.58	1.58	1.30	5.05	0.94	0.055
T_{10}	18.91	19.00	19.91	1.75	1.23	4.78	0.38	0.050
T_{11}	18.41	18.61	19.21	1.83	1.30	4.86	0.99	0.049
T_{12}	18.12	18.47	18.90	1.91	1.21	4.09	0.97	0.050
T_{13}	17.51	17.76	18.04	1.95	1.28	4.94	0.58	0.049
T_{14}	17.92	17.61	18.22	1.76	1.33	4.35	1.02	0053
T_{15}	17.29	17.02	18.05	1.80	1.36	5.03	1.19	0.045
T_{16}	16.62	16.88	17.75	2.04	1.44	5.26	0.55	0.061
T_{17}	15.96	15.50	16.73	2.21	1.52	7.97	0.40	0.067
L.S.D.$_{0.05}$	0.831	0.819	0.843	0.103	0.063	0.505	0.099	0.007

Effect of inoculation with N_2- fixers bacteria, compost and compost tea on the crude Protein (%), total carbohydrate, biological yield (ton/ fed.) and 1000 grain weight (g) of wheat (Triticum aestivum L.) in saline soil under different treatments of fertilizers:

As shown in Table (10) The inoculated plants recorded the highest yield parameters than un-inoculated ones where T_{17} (*Azospirillum lipoferum* + *Azotobacter chroococcum* + *Bacillus polymyxa* + Compost + Compost tea) that acts the mixture of N_2- fixers + compost when this treatment recorded the highest crude protein (%),total carbohydrates and all yield parameters included 1000 grain weight (g), and biological yield (ton/fed.) (Grains and Straw yield).

Table 10. Crude Protein (%), Total Carbohydrate (%), Biological yield (ton/ fed.) and 1000 grain weight (g) of wheat (*Triticum aestivum* L.) in saline soil under different treatments of fertilizers.

Treatments	Crude Protein %	Total Carbohydrates %	1000 grain weight (g)	Grain yield (ton/fed)	Straw yield (ton/fed)	Biological yield* (ton/ fed)
T_1 (Control$_1$)	9.11	13.900	26.73	0.89	2.14	3.25
T_2 (Control$_2$)	9.68	14.830	35.02	1.87	4.13	6.36
T_3 (Control$_3$)	11.92	14.830	39.77	1.73	4.82	6.86
T_4	9.20	14.710	41.20	2.41	5.72	8.59
T_5	9.40	14.770	41.90	2.87	6.05	9.47
T_6	9.82	13.930	41.90	2.58	6.08	9.15
T_7	10.25	14.560	47.81	2.64	6.86	10.01
T_8	10.29	14.120	44.48	2.38	5.76	8.61
T_9	10.42	15.030	46.56	2.74	5.77	8.75
T_{10}	10.50	14.250	41.94	2.54	5.99	9.03
T_{11}	10.89	14.430	42.82	2.64	6.27	9.38
T_{12}	11.33	14.010	44.99	2.79	6.43	9.76
T_{13}	11.59	14.210	45.03	3.18	7.08	10.68
T_{14}	10.49	14.210	47.13	2.82	6.52	9.70
T_{15}	10.72	15.243	49.22	3.11	7.12	10.68
T_{16}	12.16	14.490	48.47	2.83	7.00	10.37
T_{17}	13.13	15.330	49.51	3.55	7.13	10.87
L.S.D.$_{0.05}$	0.626	0.852	0.906	0.36	0.551	0.672

**Biological yield = grain yield + straw yield*

Discussion

It was of interest and applied values to found that the tested saline soil used in this investigation have high counts of the main groups namely Bacteria, Fungi and Actinomycetes as well as high counts of Non-symbiotic nitrogen fixers (Table 5). This is considered a key factor for their ability to survive at the saline soil used in this study. Some microbial groups are move domination than others. This in agreement with (Jolly *et al*., 2010) who stated that soil microorganisms are the biological components of soil that constitute less than 0.5 % (w/w) of the soil mass, they play an important role in soil processes. Under salt stress, some microorganism like members of *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Bacillus polymyxa* are not only fix atmospheric nitrogen but also produce certain plant growth promoting hormones. So the existence of these microbes in different counts have improved growth and yield of cereal crops like wheat (Zahra *et al*., 2013).

The inoculation with N2- fixers and the amendment with compost and compost tea as alternatives to mineral fertilization is considered to be the ideal way to cultivate wheat under saline soil conditions. Increase of nitrogenase activity in N2 – fixers strain to fix atmospheric nitrogen and nitrogen fixing capacity by *Azotobacter chroocpccum* in pure culture is resemble (Ridvan, 2009). The findings in Table (6) indicated that the reduction of plant (shoot height and root length) and number of tillers in controls due to salt stress where NaCl had both osmotic and ionic effects which decreased the amount of water and increased the concentration of salt in plant cells. However, the bacterial inoculation with the aid of compost (plant source) and its water extracts could relief the effect of salt stress and improved growth parameters in cereals including plant height, root length and number of tillers, this is clearly obtained with treatment T_{17} (*Azospirillum lipoferum* + *Azotobacter chroococcum* + *Bacillus polymyxa* + Compost + Compost tea) and that matched with the findings of (Burd *et al.*, 2000 and Zahra *et al.*, 2013). The physiological behavior of wheat to increase its surface roots to make a net of roots in the profile of 0 - 10 cm under soil surface besides the increase of tillers as a kind of facing salt stress.

The increasing values of fresh and dry weights of both shoots and roots during the growth intervals in Table (7), in treatments that inoculated with N_2 – fixers + compost + compost tea than the controls and individuals ones was due to the ability of the N_2 – fixers beside fixing atmospheric nitrogen, they could stimulate plant growth including the supply of mineral nutrients to the plant and direct plant growth promotion by delivering plant. Growth hormones like Indole acetic acid, Gibbrellic acid and cytokinenes are known to be the plants growth at different stages i.e. germination, heading and harvesting (Abd El- Ghany *et al.*, 2010).

Our finding in Table (8) indicate that, application compost led to an enhancement of soil aggregation process, subsequently soil penetrability resistance decrease whereas compost extracts reduce the effect of salt stress and promotes the plant growth that reflected the healthy state of plants where low level of salinity increased cell wall synthesis, cell enlargement and photosynthetic activities increase the amount of total chlorophyll in plants, as mentioned by (Abd El- Ghany *et al.*, 2010 and Zahra *et al.*, 2013).

In Table (8), The increasing of N_2 – ase activity in the rhizosphere of wheat plants resulted from abundances of nitrogen fixers (*Azospirillum lipoferum*, *Azotobacter chroococcum* and *Bacillus polymyxa*) to colonize the plant roots where the greater release of plant exudates and availability of carbon substrates beside organic matter will have higher activity fixers of this enzyme owing to the increasing of nitrogen fixers populations (Difuza and Zulfiya, 2008).

The activity of dehydrogenase reflects the oxidative capacity of the microbial biomass and it has been suggested as a good indicator of soil quality. Salinity affects negatively on dehydrogenase enzyme. The reduction of enzyme activity in saline soils could be due to the osmotic dehydration of the microbial cells that liberate intracellular enzymes.The inoculation with beneficial microorganisms and organic matter represented in compost and its extracts helped in increasing the respiration and consequently increase in dehydrogenase enzyme (Abd elbasset *et al.*, 2010).

Proline may play a protective role against the osmotic potential generated by salt. The increasing of concentration of proline especially with T_{17} (*Azospirillum lipoferum* + *Azotobacter chroococcum* + *Bacillus polymyxa* + Compost + compost tea) was due to the existence of halotolerent bacteria that can accumulate or synthesize organic compatible solutes, such as glutamine, proline and glycine betaine, that showed a positive effect on plant growth (Kobra *et al.*, 2013).

The effect of salt stress increased the absorption of Na^+. Whereas the absorption of K^+ decreased in the roots. Inoculation with N_2- fixers + compost extracts increased the accumulation of K^+ and decreased Na^+ and consequently increased the uptake of N, P and K and other nutrients and finally the microorganisms can produce substances that promote growth like exopolysaccharied that could bind some cations including Na^+ (Silini *et al.*, 2012).

Grain and Straw yield besides crude protein and total carbohydrates content were increased in T_{17} (*Azospirillum lipoferum* + *Azotobacter chroococcum* + *Bacillus polymyxa* + Compost + compost tea) than control and other treatments could be attributed to the role of N_2- fixers and organic matter amendments in improving plant growth through higher uptake of water and nutrients from soil which decreased the negative effects of salt and nutrient thereby enhancing plant yield (Ashraf *et al.*, 2006).

Conclusion

This study can support the usefulness of inoculation with halophillic nitrogen fixers and soil amendment with compost and compost tea for the production of wheat plants grown under saline soil conditions.

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