J. Agric. Sci. Mansoura Univ., 31 (8): 5297 - 5311, 2006

IMPROVEMENT OF GROWTH YIELD AND ROOT COLONIZATION OF WHEAT CULTIVATED IN SALT AFFECTED SOIL INOCULATED BY *Azotobacter* AND *Azospirillum* WITH MINERAL NITROGENOUS FERTILIZER

Mansour, S. M.[1]; Nadia A. A. Ali[1], W.I.A. Saber[1] and Kh.M. Ghanem[2]

1- Microbiol. Dept., Soils, Water and Environ. Res. Institute., Agric. Res. Center, Giza, Egypt.

2- Environ. and Bio- Agric. Dept., Fac. Agric., Al-Azhar Univ., Cairo, Egypt.

ABSTRACT

The response and improvement of root colonization, yield, growth and N-uptake of wheat grown in salt affected soil (with pH value in the alkaline side) were studied. In a field experiment conducted and carried out during the season of 2005/2006, the biofertilizer inoculation (*Azotobacter chroococcum* and/or *Azospirillum brasilense*) in combined with different rates of N-fertilizer (ammonium nitrate at 0, 20, 40, 60 and 80 Kg. N fed^{-1}) were applied. The treatments were arranged in split plot design with three replicates. The results showed that there is an increment in *Azospirillum* count in wheat rhizosphere soil with single inoculation of *Azospirillum*, which gave highest number of *Azospirillum* after 60 days of planting, then reversible results were obtained at the end of cultivation period (120 days), where, the dual inoculation gave highest number of *Azospirillum* compared with the other treatments. Also, the inoculation of wheat grains with *Azotobacter*, led to gradual increases in the counts of *Azotobacter* in wheat rhizosphere soil up to 90 days, then, decreased at the end of cultivation period. All inoculated treatments gave higher counts of *Azotobacter* compared with the uninoculated treatment. Generally, the total N_2-fixers and total bacterial counts increased greatly in wheat rhizosphere soil in inoculation treatments compared with uninoculated treatments.

The results showed significant increases in plant dry weight, grain and straw yields as well as nitrogen uptake by wheat plants either by increasing the rate of mineral nitrogen or with inoculation by tested N_2-fixers. In addition, the dual inoculation with *Azotobacter chroococcum* and *Azospirillum brasilense* performed significantly greater followed by single inoculation with *Azotobacter* or *Azospirillum*. At any level of N-fertilizer, the inoculated treatments gave much higher straw and grain yields than the uninoculated one.

Finally, it could be concluded that in salt affected soil, the amount of mineral N fertilizer could be reduced by using biofertilizers, which in turn increases soil fertility as well as, minimizes the production cost and environmental pollution, which can occur by the excess use of chemical fertilizers.

Keywords: Biofertilizers, wheat growth and yield, ammonium nitrate, *Azospirillum brasilense*, *Azotobacter chroococcum*, root colonization, salt affected soil.

INTRODUCTION

Among cereal crops, wheat (*Triticum aestivum*, L.) is the major and most important crop in many countries, and it is the main winter cereal crop in Egypt. There are many attempts to increase wheat productivity in order to face the gap between consumption and production. Supplying crop plants with nitrogen fertilizer plays an essential role in improving its productivity, because nitrogen is considered as one of the limiting factors to achieve the

high yield of wheat crop. Application of mineral nitrogen may be results in environmental pollution in addition to its high cost. So, many efforts were done to decrease the utilization of chemical fertilizers by using biofertilizers, which might reduce financial costs. Fixation as an alternative or supplementary source of nitrogen for wheat plants has been the major approach in soil fertility management of nitrogen for wheat (Hamed, 1998; Kotb, 1998 and Saad El-Din & El-Metwally, 2003).

Hence, to obtain maximum yields of cereal crops, the maintenance of soil fertility at a high level is utmost important. The use of nitrogen fixing bacteria such as *Azotobacter, Azospirillum* and others is considered as an index to soil fertility and saving more than half recommended dose of mineral nitrogen fertilizer (Darmwal and Gaur, 1988 and Tantawey *et al.*, 2004). The beneficial effect of *Azotobacter* and *Azospirillum* are related not only to their N_2-fixing proficiency but also with their ability to produce anti-fungal compounds, growth regulators and siderophores (Pandey and Kumar, 1989). Single or dual inoculation of wheat grains with *Azotobacter chroococcum* and *Azospirillum brasilense* in sterilized soil have been extremely variable from significantly negative (Barber *et al.*,1976 and Albrecht *et al.*, 1977) to significantly positive stimulation of their population in wheat rhizosphere soil, and also, stimulated plant growth and significantly increased the concentration of indole acetic acid, P, Mg, N and total soluble sugars in wheat shoots (Bazzicalupo *et al.*, 1985; Charyulu *et al.*, 1985; Hegazi & Saleh, 1985, Elshanshoury, 1995 and Ali *et al.*, 2002).

Soil salinity has been found to reduce wheat yields usually when values of electrical conductivity are above 6 decisiments per meter (dS/m) throughout the root zone (Brady and Weil, 1966). Salinity affects grain germination, plant growth, nutrient uptake, and metabolism due to osmotic inhibition of water availability, toxic effects of salt ions and nutritional imbalance caused by such ions. In the life cycle of plant; germination, seedling and flowering stages are more critical for salt damage (Khan and Abdullah 2003).

Therefore, this study was undertaken to evaluate the impact of inoculation of wheat grains with *Azotobacter chroococcum* and/or *Azospirillum brasilense* on the bacterial colonization, growth, N-uptake and yield of wheat at different nitrogen levels, in salt affected soil especially when its pH in the alkaline side.

MATERIALS AND METHODS

Bacteria:

The non-symbiotic nitrogen fixing bacteria; *Azospirillum brasilense* and *Azotobacter chroococcum* were kindly obtained from Microbiol. Dept., Soils, Water and Environ. Res. Instit., Agric. Res. Center, Giza, Egypt. They were grown on liquid N-deficient medium (Döbereiner *et al.*, 1976) with shaking at 28-30°C for 48 h. Then the two strains were checked to nitrogenase activity before used. Thereafter, these strains were grown in modified Asby's medium (Abdel-Malek and Ishac, 1968) with shaking at 28-30°C for 24 h.

Wheat cultivar:

Wheat cultivar (Sakha 93) was kindly obtained from Wheat Dept., Field Crop Res. Institute, Agric. Res. Center, Giza, Egypt.

Inoculation procedure:

Prior to sowing, wheat grains were inoculated by soaking in liquid culture of *Azospirillum brasilense* ($1.3x10^7$ cells ml^{-1}, approximately) and/or *Azotobacter chroococcum* ($1.5x10^7$ cells ml^{-1}, approximately). Arabic gum was added to liquid culture as adhesive agent. Inoculated grains were air dried by spreading over a plastic sheet for short time before planting. The control treatment was done using uninoculated grains.

Experimental conditions:

A field experiment was carried out at Tag El-Ezz Agric. Res. Station, Dakahlia governorate, during the winter season of 2005/2006. The experiment aimed to study the effect of the inoculation with two strains of non-symbiotic N_2-fixing bacteria; *Azospirillum brasilense* and/or *Azotobacter chroococcum* on the growth, N-uptake, bacterial colonization and yield of wheat under salt affected soil and that tends to saline alkaline soil (pH value for this soil is 8.35). The experimental plots were planted with wheat grains (c.v. Sakha 93). Ammonium nitrate, (33.5% N.) was added at different levels *i.e.*, 0, 20, 40, 60 and 80 kg. N. $fed.^{-1}$. Each of studied N-level was divided into three doses at proportions of 1:2:2 then, applied at soil preparation, before the first irrigation and before the second irrigation. All other practices were done as usual.

Count of different Bacterial groups:

For enumeration the microbial communities, wheat rhizosphere soil samples at 30, 60, 90 and 120 days from sowing were collected, and (10 g.) root free soil were shaken for 1 hr. in 90 ml sterilized tap water and ten fold dilution were made.

The most probable number technique (M.P.N.) was used for enumeration of both *Azospirillum* and *Azotobacter*. Semi solid malate medium (Döbereiner *et al.*, 1976) was used for *Azospirillum* enumeration and modified Ashby's liquid medium (Abdel-Malek and Ishac, 1968) was used for *Azotobacter* enumeration. The pouring plate method technique was used for determination the total N_2-fixers and total bacterial count using the media of Watanabe & Barraquio (1979) and Collins & Lyne (1985), respectively. The counts of bacterial groups were expressed as log. c.f.u.$g.^{-1}$ oven dried soil at 105°C.

The studied characteristics:

Samples of wheat plants at 60, 90 and 120 days from sowing were taken from the inner area of each plot to determine dry weight (g. $plant^{-1}$) and N-uptake (mg. $plant^{-1}$). At the end of wheat life cycle, grains (ard. $fed.^{-1}$) and straw (ton $fed.^{-1}$) yields as well as yield components *i.e.*, grain weight $spike^{-1}$ (g), number of grains $spike^{-1}$, spike length (cm), number of spikelet $spike^{-1}$, and weight of 1000-grain (g), and N-uptake were determined (Jackson, 1973). All data were calculated on dry weight basis at 70°C.

Soil analysis:

The chemical analysis of soil was determined according to Richards, (1954) and Page, (1982). Particle size distribution of the soil sample was

carried out as described by Piper (1950), and the data were given in Table (1). This soil represents to salt affected soils. Regarding to the chemical analyses the soil is saline and the pH value is in the alkaline side. So it is tend to be saline alkaline soil.

Table (1): **Mechanical and some chemical properties of soil used for wheat cultivation (0-30 cm depth).**

Soil character			Value
Physical properties	Particle size distribution (%)	Sand	40
		Silt	20
		Clay	40
	Texture class		Clayey
Chemical analysis	E.C. dS m^{-1} soil paste		7.10
	pH 1:2.5 Soil: Water suspension		8.35
	E.S.P. (%)		11.00
	Soluble anions meq.l^{-1}	CO_3^{--}	0.00
		HCO_3^{-}	0.41
		Cl^{-}	1.85
		SO_4^{--}	2.65
	Soluble cations meq .l^{-1}	Ca^{++}	1.03
		Mg^{++}	0.62
		Na^{+}	3.20
		K^{+}	0.06
	Total Nitrogen (mg. kg^{-1})		620.00
	Organic matter (%)		1.04
	$CaCO_3$ (%)		3.13

Statistical analysis:

Data were analyzed with the statistical analysis software, CoStat (2005). All multiple comparisons were first subjected to analysis of variance (ANOVA). Comparisons among means were made using least significant differences (L.S.D.) at $P \leq 0.05$ according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

This experiment was conducted in salt affected soil with pH value (8.35) in the alkaline side of Tag El-Ezz Agric. Res. Station, Dakahlia governorate to study the effect of inoculation of wheat grains with *Azospirillum brasilense* and/or *Azotobacter chroococcum* under different levels of inorganic nitrogen fertilizer on root colonization, yield, growth and N-uptake by wheat plants.

1. **Impact of inoculation on the counts of some bacterial groups in wheat rhizosphere soil:**

The results presented in Tables (2,3,4 and 5) show the effect of *Azospirillum* and/or *Azotobacter* inocula in combined with different mineral N-levels, on total numbers of *Azospirillum*, *Azotobacter* and total N_2-fixers as well as total bacterial count in the rhizosphere soil of wheat, cultivated in salt affected soil, after 30, 60, 90 and 120 days from sowing.

1.1. *Azospirillum* counts as affected by tested N_2-fixers inoculation:

The results in Table (2) showed that inoculation of wheat grains with *Azospirillum brasilense* increased greatly the counts of *Azospirillum* in the rhizosphere soil of wheat, especially, at the biofertilization supplemented with high level of inorganic nitrogen (80 Kg N. fed.$^{-1}$) which reached 6.813 log. cycle g.$^{-1}$ dry soil after 60 days of sowing, thereafter, gradually decreased to reach up to 5.093 log. cycle after 120 days. *Azotobacter* inoculation also increased the number of azospirilla but these numbers are less than those of above, which reached 5.505 log. cycle at 80 Kg N. fed.$^{-1}$ after 60 days from sowing then, decreased slowly. In dual inoculation of *Azospirillum* and *Azotobacter* as well as in uninoculated one, the numbers of *Azospirillum* at the end of cultivation periods (120 days) tended to increase more than the single inocula. This means that dual inoculation of *Azospirillum* and *Azotobacter* enhanced and stimulated greatly the number of azospirilla with the prolongation of cultivation time up to 120 days. These results are in agreement with those reported by Ali *et al.*, (2002).

Table (2): Changes in counts of azospirilla in rhizosphere soil through different planting periods of wheat (log. c.f.u.g.$^{-1}$ oven dried soil).

Treatment		Time in days			
Inoculation	Ammonium nitrate (kg N fed.$^{-1}$)	30	60	90	120
Azospirillum	80	5.732	6.813	5.212	5.093
	60	5.633	5.954	5.328	5.107
	40	5.631	5.551	5.446	5.111
	20	5.491	5.505	5.265	5.114
	0	5.398	5.176	5.267	4.898
Azotobacter	80	5.407	5.505	5.398	4.718
	60	5.365	5.255	5.193	5.053
	40	5.309	5.342	5.362	5.104
	20	5.230	5.270	5.307	5.155
	0	5.146	5.162	5.146	5.230
Dual inoculation	80	5.041	5.176	5.362	5.462
	60	5.663	5.519	5.491	5.491
	40	5.631	5.519	5.480	5.467
	20	5.477	5.690	5.348	5.334
	0	5.147	5.146	5.146	5.083
Uninoculation (control)	80	4.699	5.146	4.732	5.362
	60	5.568	5.322	5.146	5.258
	40	5.599	5.398	5.380	5.380
	20	5.000	5.193	5.419	5.219
	0	4.447	5.056	5.342	5.119

Initial *Azospirillum* in soil was 3.898 log. cycle.

1.2. *Azotobacter* counts as affected by tested N_2-fixers inoculation:

Table (3) shows also that in the case of *Azotobacter* inoculation, the numbers of *Azotobacter* in rhizosphere soil of wheat plant increased gradually up to 90 days at 60 Kg N. fed.$^{-1}$ then decreased to 5.081 log. cycle at 120 days, whereas at 20 Kg N. fed.$^{-1}$ the numbers reached 5.243 log cycle

after 30 days of planting then decreased slowly to reach 5.080 log cycle at 120 days. Generally, the inoculation with *Azotobacter*, gave numbers of *Azotobacter* in rhizosphere soil of wheat more than that of *Azospirillum* inoculation. It was clear that dual inoculation caused slight increase in the numbers of *Azotobacter* than those of *Azospirillum* inoculation especially at low nitrogen levels after 120 days of planting. Also, it was noticed that the inoculation with *Azotobacter*, *Azospirillum* or dual inoculation recorded high number of *Azotobacter* than the uninoculated treatment during cultivation period at any level of nitrogen fertilizer.

Table (3): Changes in counts of *Azotobacter* in rhizosphere soil through different planting periods of wheat (log. c.f.u.g.$^{-1}$ oven dried soil).

Treatment		Time in days			
Inoculation	Ammonium nitrate (kg N fed.$^{-1}$)	30	60	90	120
Azospirillum	80	5.220	5.380	5.565	5.085
	60	5.176	5.462	5.574	5.090
	40	5.212	5.431	5.516	5.153
	20	5.455	5.380	5.438	5.093
	0	5.230	5.230	5.408	5.080
Azotobacter	80	5.398	5.204	5.556	5.086
	60	5.267	5.380	5.643	5.081
	40	5.757	5.467	5.618	5.080
	20	5.243	5.158	5.491	5.080
	0	5.342	5.322	5.556	5.125
Dual inoculation	80	5.389	5.241	5.418	5.093
	60	5.241	5.246	5.332	5.086
	40	5.238	5.104	5.418	5.181
	20	5.199	5.111	5.455	5.081
	0	5.155	5.100	5.580	5.085
Uninoculation (control)	80	4.924	5.021	5.057	4.852
	60	4.968	4.954	4.901	4.852
	40	5.041	4.944	4.903	4.847
	20	5.004	5.092	4.949	5.037
	0	5.021	5.004	5.004	4.847

Initial *Azotobacter* in soil was 2.699 log. cycle.

1.3. Count of total N_2-fixers as affected by tested N_2-fixers inoculation:

Results in Table (4) also showed that, in case of inoculation, there are pronounced increase in total count of nitrogen fixers with the prolongation of cultivation period than those without uninoculation. With *Azotobacter* inoculation, the numbers N_2-fixers reached up to 7 log cycle after 30 days under different levels of inorganic nitrogen and decreased slowly to the end of planting period (120 days). Also, with *Azospirillum*, the numbers of nitrogen fixers was found in the same trend as in *Azotobacter* inoculation, but, they were low compared to the inoculation of *Azotobacter*. While, the dual inoculation gave lower number than single inoculation treatments, after 30

days, then, give the same trend of single inocula up to the end of cultivation period. Our results are in agreement with those obtained by Ali *et al.*, (2002).

1.4. Total bacterial count as affected by tested N_2-fixers inoculation:

From the results tabulated in Table (5) it could be observed that inoculation with either *Azospirillum brasilense* or *Azotobacter chroococcum* increased and gave higher numbers of total bacterial counts. They reached 8.225 and 8.627 log cycle $g.^{-1}$ dry soil, respectively, compared with dual inoculation and uninoculated treatments which, gave 7.681 log cycle $g.^{-1}$ dry soil after 60 days and 7.872 log cycle $g.^{-1}$ dry soil after 30 days of cultivation at the same level of inorganic nitrogen fertilizer (80 Kg N. $fed.^{-1}$), respectively. Similar results were obtained by Ali *et al.*, (2002) and Hanna *et al.*, (2004).

Table (4): Changes in counts of total N_2-fixers in rhizosphere soil through different planting periods of wheat (log. c.f.u.$g.^{-1}$ oven dried soil).

Treatment		Time in days			
Inoculation	Ammonium nitrate (kg N. $fed.^{-1}$)	30	60	90	120
Azospirillum	80	7.050	6.425	6.401	6.219
	60	7.046	6.338	6.515	6.200
	40	6.513	6.471	6.743	6.199
	20	6.969	6.599	6.408	6.203
	0	6.520	6.384	6.418	6.219
Azotobacter	80	7.394	6.384	6.479	6.250
	60	6.744	6.415	6.471	6.253
	40	6.813	6.502	6.458	6.243
	20	7.107	6.563	6.481	6.204
	0	7.033	6.473	6.515	6.315
Dual inoculation	80	6.486	6.606	6.221	6.248
	60	6.457	6.577	6.239	6.259
	40	6.429	6.415	6.229	6.255
	20	6.404	6.502	6.224	6.296
	0	6.178	6.398	6.243	6.182
Uninoculation (control)	80	6.555	6.307	6.208	6.004
	60	6.048	6.034	6.143	6.010
	40	6.014	6.047	6.093	6.012
	20	6.007	6.312	6.179	6.014
	0	6.006	6.116	6.114	6.010

Initial total N_2-fixers in soil was 4.505 log. cycle.

2. Growth, yield, and N-uptake of wheat as influenced by ammonium nitrate and inoculation with *Azospirillum brasilense* and/or *Azotobacter chroococcum*:

2.1. The effects on wheat growth during cultivation period:

During the cultivation period, wheat samples at 60, 90, 120 days from sowing were collected and analyzed to follow up the growth of wheat. It could be easily observed from Table (6) that the wheat dry weight and N-uptake at different stages of cultivation period increased greatly by increasing the rate of nitrogenous fertilizer, because it helps the plant to build up all metabolites

and subsequently improves growth parameters. Higher values of such criteria were observed when inorganic nitrogen was used with dual inoculation followed by *Azospirillum* and *Azotobacter* inoculation treatments. This is may be due to that these inoculants produced growth promptings and other substances as well as fixing much more amount of atmospheric nitrogen, thus these materials enhancing and stimulating the plant growth, yield and its containing from NPK. Similar results were obtained by El-Borollosy & Refaat (1982). They observed that inoculation with a mixture of *A. chroococcum* and *Azospirillum* sp. gave higher fresh and dry weights of maize plants, followed by inoculation with *Azotobacter* then *Azospirillum*.

Table (5): Changes in total bacterial counts in rhizosphere soil through different planting periods of wheat (log. c.f.u.g.$^{-1}$ oven dried soil).

Treatment		Time in days			
Inoculation	Ammonium nitrate (kg N fed.$^{-1}$)	30	60	90	120
Azospirillum	80	8.167	7.705	7.554	7.580
	60	8.113	7.740	7.611	7.512
	40	8.225	8.130	7.653	7.519
	20	8.104	7.724	7.598	7.520
	0	8.170	7.708	7.613	7.490
Azotobacter	80	8.627	7.556	7.585	7.499
	60	8.452	7.613	7.602	7.516
	40	8.051	7.607	7.504	7.496
	20	8.375	7.663	7.534	7.507
	0	8.334	7.693	7.569	7.507
Dual inoculation	80	7.496	7.681	7.513	7.498
	60	7.512	7.613	7.496	7.499
	40	7.503	7.645	7.498	7.496
	20	7.500	7.613	7.496	7.492
	0	7.499	7.556	7.499	7.479
Uninoculation (control)	80	7.872	7.550	7.217	7.185
	60	7.239	7.238	7.237	7.179
	40	7.204	7.255	7.265	7.181
	20	7.205	7.247	7.253	7.181
	0	7.203	7.238	7.209	7.180
Initial total colonies in soil was 5.255 log. cycle					

2.2. The effects on wheat yield and its components at the end of life cycle:

At the end of life cycle of wheat, samples of grains and straw were analyzed for their content of protein (%), then the N-uptake (kg. N. fed^{-1}) was determined. The results presented in Table (7) show increasing of N-uptake

at the end of wheat life cycle in all inoculated treatments over the uninoculated one, but the dual inoculation gave highest N-uptake especially with the use of high level of inorganic nitrogen followed by *Azospirillum* and *Azotobacter* inoculation. The increasing of N-uptake reflected on the protein content of grains and straw.

Table (6): Effect of ammonium nitrate and inoculation with *Azospirillum* and/or *Azotobacter* on wheat dry weight and N-uptake during cultivation period.

Treatments		Dry weight (g. plant^{-1})			N-uptake (mg. plant^{-1})		
Ammonium nitrate	Inoculation	Time in days					
		60	90	120	60	90	120
80 kg N fed.$^{-1}$	*Azospirillum*	2.96	6.76	8.69	62.17	155.37	243.05
	Azotobacter	2.85	6.57	8.45	55.50	137.29	230.79
	Dual inoculation	3.10	7.05	8.94	70.39	168.88	294.24
	Uninoculation	3.05	6.45	7.70	61.00	141.90	280.46
	Mean	2.99	6.71	8.45	62.26	150.86	262 13
60 kg N fed.$^{-1}$	*Azospirillum*	2.87	6.35	8.23	63.80	163.40	255.61
	Azotobacter	2.80	6.16	8.19	57 87	135.53	248 41
	Dual inoculation	3.00	6.57	8.28	71.08	171.00	305.93
	Uninoculation	2.70	6.25	7.17	51 30	132.50	157 74
	Mean	2.84	6.33	7.97	61.01	150.61	241.92
40 kg N fed^{-1}	*Azospirillum*	2.78	6.22	7.38	63.90	161.89	204.22
	Azotobacter	2.69	6.09	7.21	58.21	140.04	185.05
	Dual inoculation	2.91	6.36	7.71	71.86	170.00	231.03
	Uninoculation	2.42	5.70	6 68	41.69	114.00	133.60
	Mean	2.70	6.09	7.25	58.91	146.48	188.47
20 kg N fed.$^{-1}$	*Azospirillum*	2.53	5.89	6.66	48.94	129.52	170.97
	Azotobacter	2.39	5.65	6.34	44.58	113.94	149 96
	Dual inoculation	2.64	6.19	7.07	55.41	146.35	195.57
	Uninoculation	2.32	5.28	5.90	39.44	100.32	112.10
	Mean	2.47	5.75	6.49	47.09	122.53	157.15
'0 kg N fed^{-1}	*Azospirillum*	1.95	5.10	5.89	33.12	93.66	120.40
	Azotobacter	1.81	5.00	5.67	29.97	91.71	108.02
	Dual inoculation	2.07	5.19	5.99	37.35	95.25	131.61
	Uninoculation	1.78	4.58	5.39	27.60	73.28	91.58
	Mean	1.90	4.97	5.74	32.01	88.47	112.90
L.S.D. at $P \leq 0.05$	N x Inoculation	0.19	0.23	0.09	4.87	6.06	21.91
	N rate	0.44	0 15	0.31	1.79	2.55	12.13
	Inoculation	0.43	0.91	0.22	1.88	2.53	11.08

Ali *et al.*, (2002) showed that the increasing in nitrogen uptake and protein content (%) can be attributed to the ability of *Azospirillum brasilense* and *Azotobacter chroococcum* to fix atmospheric nitrogen together with high production of growth promoting substances that enhance root development and function and stimulate seed germination, shoot and root length, and subsequently increased nutrients uptake by wheat plants.

Table (7): Effect of ammonium nitrate and inoculation with *Azospirillum*, and/or *Azotobacter* on N-uptake and protein content of wheat at harvesting.

Treatments		Grain			Straw			N-uptake (kg. fed^{-1})
NH_4NO_3	Inoculation	N (%)	N-uptake	Protein (%)	N (%)	N-uptake	Protein (%)	
80 kg N fed.$^{-1}$	*Azospirillum*	1.953	54.889	12.206	0.473	17.864	2.956	72.75
	Azotobacter	1.913	54.081	11.956	0.431	16.766	2.694	70.85
	Dual inoculation	2.117	60.514	13.231	0.511	20.900	3.194	81.41
	Uninoculation	1.852	51.365	11.575	0.241	9.439	1.506	60.80
	Mean	1.959	55.212	12.242	0.414	16.242	2.588	71.45
60 kg N fed.$^{-1}$	*Azospirillum*	1.833	50.710	11.456	0.427	15.785	2.669	66.49
	Azotobacter	1.843	51.807	11.519	0.401	15.505	2.506	67.31
	Dual inoculation	1.992	55.776	12.450	0.469	17.588	2.931	73.36
	Uninoculation	1.760	31.064	11.000	0.389	10.892	2.431	41.96
	Mean	1.857	47.339	11.606	0.422	14.942	2.634	62.28
40 kg N fed.$^{-1}$	*Azospirillum*	2.099	47.889	13.119	0.497	17.478	3.106	65.37
	Azotobacter	2.209	53.646	13.806	0.411	14.933	2.569	68.58
	Dual inoculation	2.317	54.137	14.481	0.477	15.010	2.981	69.15
	Uninoculation	1.711	25.579	10.694	0.330	9.845	2.063	35.42
	Mean	2.084	45.313	13.025	0.429	14.316	2.680	59.63
20 kg N fed.$^{-1}$	*Azospirillum*	1.831	30.028	11.444	0.352	10.361	2.200	40.39
	Azotobacter	1.756	28.711	10.975	0.348	10.208	2.175	38.92
	Dual inoculation	1.937	33.675	12.106	0.401	13.393	2.506	47.07
	Uninoculation	1.329	17.277	8.306	0.320	8.501	2.000	25.78
	Mean	1.713	27.423	10.708	0.355	10.616	2.220	38.04
0 kg N fed.$^{-1}$	*Azospirillum*	1.798	23.716	11.238	0.333	6.993	2.081	30.71
	Azotobacter	1.691	21.535	10.569	0.311	6.500	1.944	28.03
	Dual inoculation	1.830	25.345	11.438	0.344	7.602	2.150	32.95
	Uninoculation	1.101	12.056	6.881	0.220	3.351	1.375	15.41
	Mean	1.605	20.663	10.031	0.302	6.112	1.888	26.77
L.S.D. at 0.05	N X Inoculation		4.21			2.33		
	N rate		1.92			1.01		
	Inoculation		1.63			0.98		

They also show that the N-fertilization of wheat plants increased the protein content and that subsequently improves the grain quality. This is due to the influence of N availability at critical stages of spike initiation and the development on plant metabolism in way leading to increase synthesis of amino acids and their incorporation into grain protein. Darwiche (1994) indicated that any increase in N-fertilization was followed by an increase in protein percentage in wheat grain.

Results presented in Table (8) clearly showed that wheat yield and its attributes were highest and increased greatly with the increasing of nitrogen dose and significantly increased with the inoculation by N_2-fixing bacteria.

All inoculated treatments showed significant increases in both grains and straw yields compared to uninoculated treatments irrespective of inorganic nitrogen fertilizer levels (Table, 8). However, highest values of

these parameters were observed with the dual inoculated treatment followed by *Azotobacter* and *Azospirillum* inoculation. For uninoculation treatments the application of 0, 20, 40, 60 and 80 Kg N. fed.$^{-1}$ gave 7.30, 8.67, 9.97, 11.77 and 16.49 ardab fed.$^{-1}$ for grain and 1.52, 2.66, 2.98, 2.80 and 3.92 ton fed.$^{-1}$ for straw yields, respectively.

Table (8): Effect of NH_4NO_3 and inoculation with *Azospirillum* and/or *Azotobacter* on wheat yield and its components.

Treatments		Grain weight/spike (g)	No. of grains/spike	Spike length (cm)	No. of spikelet/ spike	1000-grain weight (g)	Grain yield (ard. fed^{-1})	Straw yield (ton fed^{-1})
NH_4NO_3	Inoculation							
80 kg N fed.$^{-1}$	*Azospirillum*	2.50	48.21	11.25	20.09	50.97	18.74	3.78
	Azotobacter	2.17	44.08	11.18	18.46	47.64	18.85	3.89
	Dual inoculation	2.17	43.44	11.19	19.37	47.72	19.06	4.09
	Uninoculation	2.09	46.00	11.37	17.25	44.87	16.49	3.92
60 kg N fed.$^{-1}$	*Azospirillum*	2.10	42.48	11.58	18.06	49.81	18.44	3.70
	Azotobacter	1.84	37.77	11.13	16.45	49.12	18.67	3.75
	Dual inoculation	1.85	38.19	10.88	16.27	47.83	18.74	3.87
	Uninoculation	1.67	31.80	10.88	15.90	47.59	11.77	2.80
40 kg N fed^{-1}	*Azospirillum*	1.99	40.55	10.43	19.42	48.23	15.21	3.52
	Azotobacter	2.19	40.55	10.49	20.86	53.50	16.19	3.63
	Dual inoculation	2.37	43.73	11.17	21.29	50.50	15.58	3.15
	Uninoculation	2.16	45.43	11.42	22.13	48.09	9.97	2.98
20 kg N fed.$^{-1}$	*Azospirillum*	1.85	39.48	10.13	19.24	46.18	10.93	2.94
	Azotobacter	2.19	45.13	10.76	17.48	47.75	10.90	2.93
	Dual inoculation	2.27	47.10	10.78	19.56	47.20	11.59	3.34
	Uninoculation	2.12	54.38	10.78	20.23	45.14	8.67	2.66
0 kg N fed^{-1}	*Azospirillum*	1.45	32.21	8.80	15.81	44.35	8.79	2.10
	Azotobacter	1.88	40.11	10.21	18.60	46.69	8.49	2.09
	Dual inoculation	2.11	41.98	10.40	19.46	47.98	9.23	2.21
	Uninoculation	1.89	43.37	11.17	19.27	43.99	7.30	1.52
Effect of Ammonium nitrate (kg N fed.$^{-1}$)								
80		2.23	45.43	11.25	18.94	48.25	18.78	3.92
60		1.86	37.56	11.12	16.82	48.85	16.90	3.53
40		2.18	42.56	10.88	21.06	50.21	14.24	3.32
20		2.11	46.52	10.61	19.69	46.78	10.52	2.97
0		1.70	39.42	10.15	18.57	45.93	8.63	1.98
Effect of inoculation								
Azospirillum		1.98	40.58	10.44	18.75	48.05	14.42	3.21
Azotobacter		2.06	41.53	10.75	18.86	49.42	14.63	3.28
Dual inoculation		2.16	42.89	10.89	19.29	48.54	14.82	3.31
Uninoculation		1.87	44.20	11.12	19.16	46.01	11.38	2.78
L.S.D. at 0.05	N x Inoculation	0.09	3.20	1.11	2.38	2.07	1.06	0.94
	N rate	0.05	1.13	1.06	2.25	1.24	1.78	0.91
	Inoculation	0.04	1.09	1.05	1.71	1.12	0.92	0.46

These results may be attributed to the high efficiency of bacteria presented in inoculated grains to fix atmospheric nitrogen and to produce some biologically active substances, *e.g.*, IAA, ALA, gibberellins and cytochinine-like substances. These results are in line with those reported by Kotb (1998) and Ali *et al.*, (2002). They showed higher grain and straw yields when they use inoculated grains of wheat than uninoculated ones in both silty clay loam and sandy soils.

It is worth to mention that the dual inoculation by *Azotobacter* and *Azospirillum* recorded the highest values of grain and straw yields (19.06 & 18.74 ard. fed.$^{-1}$ and 4.09 & 3.87 ton. fed.$^{-1}$, respectively) at 80 and 60 Kg N. fed.$^{-1}$. In addition, the yield at 80 Kg N. fed.$^{-1}$ without inoculation recorded lower result than inoculation treatments at 60 Kg N. fed.$^{-1}$. Also, the same result was obtained with 60 Kg N. fed.$^{-1}$ without inoculation and with inoculation treatments at 40 Kg N. fed.$^{-1}$. Thus, the inoculation save about 20 units of N-fertilizer and that saving was economically feasible. Therefore, it seams from the data that the recommended dose of chemical N-fertilizer could be reduced by using biofertilizer, which in turn minimizes the production costs and environmental pollution, which can occur with the excess use of chemical fertilizers.

With respect to wheat yield components (Table, 8), inoculation of wheat grains by *Azospirillum* in combined with high levels of inorganic nitrogen (80 and 60 Kg N. fed.$^{-1}$) recorded the highest values of grain weight/spike, number of grains/spike, spike length, number of spikelet/spike and 1000-grain weight, followed by either *Azotobacter* or dual inoculation. On the other hand, at low levels of N (0, 20 and 40 Kg N. fed.$^{-1}$) the mixed inoculation and single with *Azotobacter* gave the highest results followed by *Azospirillum* inoculation. In all cases, the inoculated treatments gave better results than the uninoculated and control ones.

It is worth to mention that seed inoculation increased all values of wheat yield and its components at all levels of N-fertilizer (ammonium nitrate). Shams El-Din & Abdrabou (1995) and Kotb (1998) stated significant increases in number and weight of grain/spike by inoculation of wheat grains by N_2-fixing bacteria.

In summary, the effect of soil salinity on wheat growth could be neutralized by the inoculation of wheat grains with *Azospirillum* and/or *Azotobacter*, which improved the yield, and growth as well as protein content of wheat in salt affected soil. However, these inocula alleviated the adverse effect (s) of salinity, particularly, when plants were inoculated with both bacteria. This alleviation was enough for the plant to be able to overcome the harmful effects of salinity. Therefore, we recommend inoculating wheat grains with such bacteria when wheat is cultivated in salt affected soil especially when its pH in the alkaline side. Moreover, the addition of *Azotobacter chroococcum* and *Azospirillum brasilense* to wheat grown soil, is very useful because, these non-symbiotic nitrogen fixing bacteria saved more than ¼ recommended dose of mineral nitrogen fertilizer and increased soil fertility as well as increased greatly wheat yield and its quality under such adverse conditions.

REFERENCES

Abdel-Malek, Y. and Y.Z. Ishac (1968). Evaluation of methods used in counting Azotobacters. J. Appl. Bacteriol., 31: 267 – 275.

Albrecht, S. L.; Y. Okon and R. H. Burries (1977). Effect of light and temperature on the association between *Zea mays* and *Spirillum lipoferum*. Plants Physiol., 60 : 528.

Ali, Nadia A. A., S.D. Darwish and S.M. Mansour (2002). Effect of *Azotobacter chroococcum* and *Azospirilla brasilense* inoculation and anhydrous ammonia on root colonization, plant growth and yield of wheat plant under saline alkaline condition. J. Agric. Sci. Mansoura Univ., Egypt. 27(8): 5575-5591

Barber, L. E.; J. D. Tiekema; S. A. Russel and H. J. Evans (1976). Acetylene reduction (nitrogen – fixation) associated with corn inoculated with *Spirillum*. Appl. Environ. Microbiol., 32 : 108.

Bazzicalupo, M.; E. Cresta and F. Favilli (1985). An "*in Vitro*" assay for evaluating the *Azospirillum* wheat association. In: *Azospirillum* III. Genetics. Physiology. Ecology. Kligmuller W. (Ed.). Springer-Verlag. Berin-Heidlberg-New York – Tokyo. P. 139.

Brady N.C. and R.R. Weil (1966). The Nature and Properties of Soils. Eleventh Edition. Prentice Hall, Upper Saddle River, New Jersey. 740 pp.

Charyulu, P. B. B. N.; A. K. Fourcassie; A. K. Bardouche; L. Rondro Horiosa; A. M. N. Omar; P. Weinhard; R. Marie and J. Balandreau (1985). Field inoculation of rice using in vitro selected bacterial and plant genotypes. In: *Azospirillum* III. Genetics. Physiology. Ecology. Kligmuller W. (Ed.). Springer-Verlag. Berin-Heidlberg-New York – Toko. P . 163.

Collins, C.H. and Lyne (1985). Microbiological methods. 5th ed. Butter Worths, London, 167-181.

CoStat (2005). CoHort Software, version 6.311. 798 Lighthouse Ave. PMB 320 Monterey, CA, 93940, USA.

Darmwal, N. S. and A. C. Gaur (1988). Association effect of celluloytic fungi and *Azospirillum lipoferum* on yield and nitrogen uptake by wheat. Plant and Soil, 107: 211 -218.

Darwiche, A. A. (1994). Agricultural studies on wheat. Ph. D. thesis, Fac. of Agric. Zagazig Univ., Egypt.

Döbereiner, J.; L. E. Marriel and M. Nery (1976). Ecological distribution of *Spirillum lipoferum* Beijernick. Can. J. Microbiol. 22: 1464 – 1473.

El-Borollosy, M. A. and A. A. Refaat (1982). Phyllosphere inoculation with asymbiotic nitrogen fixing bacteria. Research Bulletin No. 1176, Fac. Agric., Ain Shams Univ., Egypt.

Elshanshoury, A. R. (1995). Interaction of *Azotobacter chroococcum*, *Azospirillum* brasilense and *Streptomyces mutabilis*, in relation to their effect on wheat development. J. Agron. Crop. Sci. 175: 119 – 127.

Gomez K. N. and A. A. Gomez (1984). Statistical procedures for agricultural research. John Wiley and Sons, New York, 2nd ed.

Hamed M.F. (1998). Wheat response to inoculation, source and rate of nitrogen fertilization. J. Agric. Sci. Mansoura Univ., Egypt. 23(3): 1021-1027.

Hanna Mona M., Elham M. Aref. and F. M. Ghazal (2004). Effect of cyanobacteria-wheat association on wheat production and soil fertility J. Agric. Sci. Mansoura Univ., Egypt. 29(5): 2941-2948.

Hegazi, N. A and H. Saleh (1985). Possible contribution of *Azospirillum* spp. to the nutritional status of wheat plant grown in sandy soils of Gasim-Saudi Arabia In : *Azospirillum* III Genetics. Physiology. Ecology. Klinmuller (Ed). Springer-Verlag. Berlin-Heidelberg – New York – Tokyo.

Jackson, M. L. (1973). Soil Chemical Analysis. Printic–Hall of Indian, Private Limited, New Delhi.

Khan M. A. and Z. Abdullah (2003). Reproductive physiology of two wheat cultivars differing in salinity tolerance under dense saline-sodic soil. Food, Agric & Environ.1 (3&4) : 185-189.

Kotb, M. Th. A (1998). Response of wheat to biofertilizer and inorganic N and P levels. The regional symposium on Agro-technologies based on Biological Nitrogen Fixation for Desert Agriculture. April 14 – 16, 1998, El-Arish, North Sinai Governate P. (291- 301).

Pandey, A. and S. Kumar (1989). Potential of *Azotobacter* and *Azospirillum* as biofertilizer for upland agriculture: a review. J. of Scientific and Industrial – Research, 48(3): 134-144.

Page A. L. (1982). Methods of soil analysis, part 2, Chemical and microbiological properties, 2nd Edition. Agronomy series 9, ASA, SSSA, Madison, Wis, USA.

Piper C. S. (1950). Soil and plant analysis. Inter science Publisher Inc. New York.

Richards L. A. Ed. (1954). Diagnosis and improvement of saline and alkali soils. USA, Hand book No. 60.

Saad El-Din Samia A. and I.M. El-Metwally (2003). Response of wheat and faba bean plants and their associated weeds to some weed control methods. J. Agric. Sci. Mansoura Univ., Egypt. 28(8): 5931-5944.

Shams El-Din, G. M. and R. Th. Abdrabou (1995). A study on the effect of biological fertilization, nitrogen rates and weed control on yield and its components of wheat. Annals Agric. Sci., Moshtohor, 33(3): 973-886.

Tantawey Eman, A.; Nadia, M. Ghalab and A. O. Abd EL-Naby (2004). Microbial survival of toshky virigin soil and the influence of bacterial inoculation on yield productivity of wheat and clover. J. Agric. Sci. Mansoura Univ., Egypt. 29(11): 6559-6568.

Watanabe, I. and W.L. Barraquio (1979). Low levels of fixed nitrogen required for isolation of free living N_2-fixing organisms from rice roots. Nature (London), 277:565-566.

J. Agric. Sci. Mansoura Univ., 31 (8), August, 2006

تحسين النمو والمحصول والمجاميع الميكروبية حول جذر القمح المنزرع في أرض ملحية ملقحة بالأزوتوباكتر و الآزوسبيريللم مع سماد نيتروجيني معدني.

سعيد محمد منصور[1]، نادية عبد الهادي عوض علي[1]، وسام الدين إسماعيل علي صابر[1] و خالد محمد غانم[2]

١- قسم الميكروبيولوجيا- معهد بحوث الأراضي والمياه والبيئة - مركز البحوث الزراعية - الجيزة - مصر.

٢- قسم البيئة والزراعة الحيوية - كلية الزراعة - جامعة الأزهر - القاهرة - مصر.

نظرا لما للتسميد الحيوي من أهمية قصوى في زيادة خصوبة التربة الزراعية وتقليل معدلات التلوث فضلا عن زيادة إنتاج المحاصيل كما وجودة، ونظرا لما لملوحة التربة من تأثيرات عكسية على نمو وإنتاجية المحاصيل، فقد تم تنفيذ هذا البحث في تجربة حقلية خلال الموسم الشتوي ٢٠٠٥/٢٠٠٦ بمحطة البحوث الزراعية بتاج العز بمحافظة الدقهلية بهدف دراسة تأثير التلقيح بالأزوتوباكتر أو الآزوسبيريللم أو خليط منهما في وجود مستويات مختلفة من نترات النشادر (صفر و٢٠ و٤٠ و٦٠ و٨٠ كجم نيتروجين/فدان) علي نمو ومحصول نبات القمح، والنيتروجين الممتص، والمحتوي البروتيني للحبوب والقش وكذلك المجاميع البكتيرية حول جذور نبات القمح تحت ظروف الأراضي الملحية التي تميل درجة الـ pH فيها إلي القلوية.

وقد أوضحت الدراسة النتائج التالية:

١- وجد أن التلقيح المنفرد بالآزوسبيريللم أدى إلي زيادة أعداد الآزوسبيريللم في تربة ريزوسفير نبات القمح حيث وصل العدد إلى أقصاه عند ٦٠ يوم من الزراعة مقارنة مع التلقيح بالأزوتوباكتر ثم انخفضت هذه الأعداد حتى ١٢٠ يوم لتعطي معاملة التلقيح المختلط من الآزوسبيريللم والأزوتوباكتر أعلي قيم لأعداد الآزوسبيريللم عن بقية المعاملات. كما وجد أن التلقيح المنفرد بالأزوتوباكتر أدى إلي زيادة أعداد الأزوتوباكتر حول ريزوسفير نبات القمح حتى ٩٠ يوم من الزراعة ثم حدث انخفاض في أعدادها عند ١٢٠ من الزراعة، إلا أن كل المعاملات الملقحة أعطت قيم أعلي لأعداد الأزوتوباكتر مقارنة بالمعاملات غير الملقحة.

٢- زادت أعداد الميكروبات الكلية المثبتة للنيتروجين الجوي في جميع المعاملات الملقحة عن غير الملقحة، كذلك ازداد العدد الكلي للبكتريا في التربة زيادة ملحوظة عند التلقيح بالآزوسبيريللم والأزوتوباكترعنه في حالة التلقيح الخليط والمعاملات غير الملقحة.

٣- كانت هناك زيادة معنوية في الوزن الجاف والمحصول بزيادة معدل الأزوت المعدني وخاصة مع التلقيح الخليط عن التلقيح المنفرد والأخير أكثر من غير الملقح. كما أن جميع المعاملات الملقحة أدت إلي زيادة معنوية في محصول الحبوب والقش وأيضا النيتروجين الممتص وبالتالي المحتوي البروتيني عن المعاملات الغير ملقحة.

٤- أدي التسميد الحيوي إلي توفير ٢٠ وحدة من السماد النيتروجيني المستخدم مما أدي إلي تقليل معدلات التسميد وبالتالي التلوث، فضلا عن زيادة خصوبة التربة والمحصول حيث تفوقت المعاملة بـ٦٠ كجم أزوت/فدان مع التلقيح علي المعاملة ٨٠ كجم أزوت/فدان بدون تلقيح في محصولي الحبوب والقش، مما يشير إلي أهمية دور التلقيح الحيوي.

٥- أدت معاملة التلقيح الخليط مع جميع مستويات التسميد الأزوتى إلي الحصول علي أعلي قيم لمحصول الحبوب والقش ووزن حبوب السنبلة ووزن الألف حبة وكذلك محتوي الحبوب والقش من النيتروجين الممتص وتلاها في التأثير التلقيح المنفرد بأي من الأزوتوباكتر أو الآزوسبيريللم.

والنتائج المتحصل عليها من هذه الدراسة تشير بوجه عام إلي أهمية التلقيح بمثبتات النيتروجين الجوي وخصوصا في الأراضي الملحية المتجهة إلي القلوية مما يؤدي إلي تقليل استخدام السماد المعدني بمعدل ٢٥% تقريبا، وذلك يقلل من تكاليف الإنتاج ويحد من التلوث البيئي الناتج عن الإفراط في استخدام هذه الأسمدة المعدنية، بالإضافة إلي ما تلعبه الأسمدة الحيوية من دور هام في إنتاج بعض منشطات النمو في منطقة الريزوسفير، وزيادة قدرة النبات علي تحمل الملوحة، مما يؤثر تأثيرا إيجابيا علي نمو المجموع الجذري في هذه النوعية من الأراضي وما يتبع ذلك من زيادة في المجموع الخضري وامتصاص العناصر الغذائية من التربة. علاوة على دورها الفعال في زيادة خصوبة التربة.