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ORIGINAL ARTICLE

Effect of nitrogen supply and *Azospirillum brasilense* Sp-248 on the response of wheat to seawater irrigation

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KEYWORDS

Azospirillum brasilense;
N-fertilization;
Seawater irrigation;
Wheat;
Friendly-microorganism

Abstract Response of wheat to *Azospirillum brasilense* Sp-248 inoculation with different N-fertilizer levels using seawater irrigation was investigated. All inoculated treatments increased plant height, shoot and root dry weight, and tiller number in compared with uninoculated treatments. Yield parameters measured were also increased due to the inoculation. In terms of the effect of saline irrigation, there were no significant differences in growth and yield parameters in plants treated with tap water and others irrigated with 8.0% seawater concentration. This would indicate a relatively high tolerance of *A. brasilense* to saline irrigation and its ability to reduce the deleterious effects of saline on growth by increasing the plant's adaptation. However, increasing the seawater concentration in the irrigation water to 16.0% significantly decreased all tested parameters. Inoculation treatments generally increased NPKCa contents and decreased sodium ratio of the grains in compared with the uninoculated treatments. Overall results clearly revealed that the *Azospirillum* inoculation saved about 20 units of N-fertilizer and that saving was made economically feasible by decreasing the chemical fertilizers needed, improving the nitrogen content and counteracting the effects of salinity.

1. Introduction

The extensive use of chemical fertilizers has disturbed the delicate ecological balance of the soil, contaminated groundwater, developed resistant races of pathogens and increased human health risks (Tawfik et al., 2006). Therefore, the development of eco-friendly-microorganisms, as alternatives to chemical fertilizers in agricultural applications, is urgently recommended. Different Plant-Growth Promoting Rhizobacteria (PGPR) such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Clostridium* and *Pseudomonas* have been used for their beneficial effects (Ozturk et al., 2003). Several studies have clearly showed positive effect of PGPR on growth of different crops in different climates and soils (Salantur et al., 2006).

Effects of *Azospirillum* on yield of several crop plants have been reviewed (Bhaskara and Charyulu, 2005). The inoculation of plants with *Azospirillum* can result in a significant change in various growth parameters in different cereals such as an increase in plant biomass, nutrient uptake, tissue N-content, plant height, leaf size, tiller numbers, root length and volume (Salantur et al., 2006). Mode of action of *Azospirillum* on plants are secretion of phytohormones, fixation of atmospheric

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nitrogen, reduction of nitrate and the enhancement of mineral uptake by plants (James, 2000).

Salinity is considered as one of the major limitations on crop productivity and quality in the world. It has been estimated that 10% of the world's cropland and as much as 27% of irrigated land may already be affected by salinity (Ali et al., 2002). It has been stated that one-third of the world's arable land resources are affected by salinity (Qadir et al., 2000). Biological activities are significantly reduced in such soil due to the effect of salinity stress. The negative effects of salinity stress on plant-growth include a reduction in growth rate and biomass, shorter stature, smaller leaves, osmotic effects, nutritional deficiency and mineral disorders (Parida and Das, 2005). Therefore, the use of PGPR to promote plant-growth in saline conditions is an important technology (Bacilio et al., 2004).

Saudi Arabia's agricultural development over the last three decades has been astonishing. Land under cultivation, less than 400,000 acres in 1976, reached millions of acres by the beginning of the 21st Century (Al-Amoudi and Moujahed, 2006). However, the lack of fresh water is one of the main constraints when it comes to developing high plant productivity. Consequently, the use of seawater irrigation is an ideal management practice for arid zones to meet the increasing demand for food. There have been many attempts to increase the wheat productivity in Saudi Arabia using microbial inoculation and N-fertilizers (Al-Amoudi and Moujahed, 2006). The current research aims to investigate the response of wheat to seawater irrigation under *Azospirillum* inoculation and different levels of N-fertilizer.

2. Materials and methods

2.1. Microorganism

Azospirillum brasilense Sp-248 was obtained from the Microbiology Department, Agriculture College, Mansoura University, Egypt.

2.2. Seed and soil

A commercial cultivar (Nagrany) of wheat (*Triticum sativum* L.) and agriculture soil used in this study were obtained from the Abha region, Saudi Arabia. Initial physiochemical characteristics of the soil were determined as shown in Table 1.

Table 1 Initial soil physiochemical characteristics.

Soil characteristics	
Texture	45.7 sand, 16 silt and 26.2 caly (%)
pH (d.d. H_2O)	7.85
Organic matter	8.58%
Nitrates	8.80 ppm
Phosphates	3.00 ppm
Magnesium	0.07% ppm
Bicarbonate	0.05% ppm
Calcium	0.05% ppm
Chlorides	0.44% ppm
EC.	133 ds m^{-1}

2.3. Growth conditions

The *A. brasilense* culture was grown on a liquid N-deficient medium (Dobereiner and Day, 1976). After incubation at 30 °C for 48 h, the cells were harvested by centrifugation at 6000 rpm for 20 min. Supernatant was discarded and the pellet was washed twice with saline solution and resuspended at a concentration of 10^6 CFU ml^{-1}. Ten milliliter of the inoculum was used for inoculation at the rate of 2.8 ml kg^{-1}.

2.4. Experimental design

A pot (3.5 kg) experiment was carried out from December 2007 until March 2008 in a greenhouse of the Science College, King Khalid University. The experiment involved three factors: seawater irrigation (tap water (140 ppm saline, as a control), 8.0% of the seawater concentration (4650 ppm saline) and 16.0% of the seawater concentration (9300 ppm saline)), bacterial inoculation and N-fertilization. It consisted of 23 treatments with three replications. The pots were arranged in randomized a complete block design and distributed every two weeks throughout the experiment to compensate for any local temperature fluctuations within the greenhouse. Five seeds were sown in each pot and thinned to three plants per pot after the full emergence of the first leaf. Ammonium nitrate, (33.5% N) was added at different levels (0, 40, 60 and 80 Kg N fed^{-1}) in two equal doses.

2.5. Bacteriological enumeration

For the enumeration of the microbial communities, samples of wheat rhizosphere soil were collected at 30, 60 and 90 days from sowing, and then 10 g root free soil was shaken for 1 h in 90 ml sterilized tap water and serial dilutions were made (Mansour et al., 2006). The most probable number technique was used for the enumeration of *Azospirillum* using the liquid N-deficient medium. Colony count plate method was used for the determination of the total bacterial count using the medium of Collins and Lyne (1985).

2.6. Plant-growth analysis

At the end of the experiment, the wheat plants were harvested to evaluate the effect of the applied treatments on plant-growth. The harvested plants were subjected to the following determinations: plant height (cm), dry weight of roots (g $plant^{-1}$), dry weight of shoots (g $plant^{-1}$), number of tillers, number of spikelets/main spike, spike length (cm), dry weight of spikes/plant, grain yield/plant and grain index (weight of 1000 grain). The grains were then dried to a constant weight at 70 °C, and then thoroughly ground to fine powder. Total nitrogen was measured using the semi-micro Kjeldahl method (Jackson et al., 1973). Phosphorus was also determined according to Chapman and Pratt (1978). The content of sodium and potassium were determined in the digested material using the flame photometer method (Brown and Lilland, 1946). Calcium was determined by the Versinte method according to Jackson (1967).

2.7. Statistical analysis

Analysis of variance (ANOVA) was used for data analysis. If the data and residuals were not normally distributed or did not have equal variance, even after transformation, then the Kruskal–Wallis test was used. All analyses were performed at $P \leqslant 0.05$ using MINITAB, version 13.1.

3. Results and discussion

3.1. Effect of treatments on wheat growth

Growth parameters of wheat at the time of harvesting were greatly increased by the use of N-fertilizer and bacterial inoculation (Table 2). These parameters increased with increases in the concentrations of N-fertilizer, because nitrogen helps the plant to build up all metabolites and subsequently improves the growth (Mansour et al., 2006). The addition of N-fertilizer at a concentration of 80 Kg N Fed^{-1} increased the plant height, the number of tillers, and the dry weight of roots and shoots in compared with untreated plants (control). All the growth parameters measured for plants treated with N-fertilizer and bacterial inoculation were significantly greater in compared with the plants treated with N-fertilizer alone. These results were in agreement with those reported in some previous studies (e.g. Akhter et al., 2004; Rothballer et al., 2005). They reported that the stimulatory effect of bacterial inoculation is probably due to the bacterial production of growth-promoting substrates such as nitrogen, phosphorus, nitrite and indole-3-acetic acid in the plant rhizosphere. Furthermore, it was found that microbial inoculation not only increased the nutritional assimilation of the plants, but also improved soil properties, such as organic matter and total N-content (Zahiroddini et al., 2004).

In terms of evaluating the effect of saline irrigation, there were no significant differences in growth parameters in plants treated with tap water and others irrigated with an 8.0% seawater concentration. Similar results were obtained by Hamdia et al. (2005) who proved that bacterial inoculation reduced the deleterious effects of NaCl on growth by improving the tolerance feature of plants and increasing plant adaptation to saline irrigation. The common mechanism of osmotic-stress adaptation is considered to be due to the intracellular accumulation of organic solutes such as glycine, betaine, proline and glutamate (Rai, 1991; Alvarez et al., 1996). The increase in roots dry weight in all bacterial inoculation treatments matched with the results obtained by Akbari et al. (2007). They stated that the auxin and nitrite produced by *Azospirillum* can promote wheat growth by stimulating root elongation, root dry weight and the development of lateral roots. However, increasing the

Table 2 Effect of treatments on wheat growth parameters (LSD at $P \leqslant 0.05$; 0.01). Each value represents mean ± S.E. of triplications.

Treatments		Plant height (cm/plant)	No. of tillers/plant	Dry weight (g/plant)	
Seawater concentration (%)	N-fertilizer and/or *Azospirillum*			Roots	Shoots
Tap water	Control	39.46	1.66	0.27	1.61
	40 N	45.83	2	0.36	1.75
	60 N	51.66	2.33	0.49	2.10
	80 N	60.36	3	0.60	2.66
	Azo.	41.80	2	0.41	2.10
	40 N + Azo.	48.1	3	0.58	2.40
	60 N + Azo.	62.30	3.33	0.77	2.86
	80 N + Azo.	63.06	3.66	0.80	3.16
8.0%	Control	31.46	1.33	0.20	1.37
	40 N	38.36	1.66	0.26	1.60
	60 N	46.50	2	0.34	1.81
	80 N	51.50	2.3	0.43	2.24
	Azo.	40.36	2	0.38	1.88
	40 N + Azo.	44.26	2.66	0.50	2.10
	60 N + Azo.	52.66	3	0.64	2.53
	80 N + Azo.	52.06	3	0.60	2.61
16.0%	Control	21.16	1	0.14	1.07
	40 N	24.63	1	0.20	1.26
	60 N	34.86	1	0.26	1.38
	80 N	30.76	1	0.24	1.74
	Azo.	23.56	1	0.23	1.30
	40 N + Azo.	29.83	1.33	0.30	1.51
	60 N + Azo.	38.5	1.33	0.37	1.83
	80 N + Azo.	33.86	1	0.31	1.41
Seawater irrigation effects		**	**	**	**
N levels effect		**	NS	**	**
Inoculation effect		**	**	**	**
Saline × N-fertilization		NS	NS	*	NS
Saline × inoculation effects		NS	NS	**	*
Saline × N × inoculation effects		NS	NS	**	**

NS = Non significant.
* = Significant.
** = Highly significant.

seawater concentration in the irrigation water up to 16.0% significantly decreased all investigated growth parameters compared with the those plants treated with tap water. The reduction in growth and yield under high salinity levels could be due to a reduction in photosynthesis, a disturbance in mineral uptake, protein synthesis or carbohydrate metabolism as reported by Ashour et al. (2004).

3.2. Effect of treatments on wheat yield

Wheat yield was increased with increases in N-fertilizer (Table 3). However, the highest wheat yield increase was obtained from the plants treated with bacterial inoculation and N-fertilizer, particularly at 60 Kg N Fed^{-1}. All inoculated treatments showed a high yield in compared with uninoculated treatments. The highest values of spikelets number, spike length, weight of main spike, grains yield/plant and grain index were obtained from the plants treated with 60 Kg N Fed^{-1} and bacterial inoculum. The increase in grain yield was mainly derived from increasing the number of fertile tillers as reported by Ozturk et al. (2003) and Salantur et al. (2006). However, the wheat yield treated only with 80 Kg N Fed^{-1} showed no significant in compared with that treated with 60 Kg N Fed^{-1} and inoculation. Thus, the inoculation with bacteria saved about 20 units of N-fertilizer and that saving is economically feasible.

With regard to saline irrigation, there was no significant difference in yield parameters in plants treated with tap water and others irrigated with 8.0% seawater. Similar results were reported by Dubey and Rani (1989) and Zahiroddini et al. (2004), in which they stated that the bacterial inoculation positively affected all the growth and yield criteria as well as the salinity tolerance of the plants. They also reported that the stress conditions enhanced the growth-promoting effects of *Azospirillum* on plants. Moreover, inoculation markedly altered the selectivity ions in that it restricted sodium uptake and enhanced the uptake of potassium and calcium (Hamdia et al., 2005). Data obtained also emphasized that the addition of high levels of N-fertilizer with bacterial inoculation caused a reduction in the rate of increase of the yield parameters. This might be due to promoting the vegetative growth over the reproductive one when the N-fertilizer level was increased more than the optimum conditions (60 Kg N Fed^{-1} with inoculation).

3.3. Effect of treatments on some chemical compositions of wheat

The content of nitrogen, potassium, calcium and phosphorus in the plants treated with bacterial inoculum was significantly greater than in the uninoculated plants (Table 4). Further-

Table 3 Effect of treatments on wheat yield parameters (LSD at $P \leqslant 0.05; 0.01$). Each value represents mean ± S.E. of triplications.

Treatments		No. of spike lets/main spike	Spike length (cm)	Dry weight of spikes (g/plant)	Grain yield (g/plant)	Grain index (weight of 1000 grains)
Seawater concentration %	N-fertilizer and/or *Azospirillm*					
Tap water	Control	13.33	9.23	1.54	1.16	39.33
	40 N	14.00	9.60	1.74	1.44	41.67
	60 N	15.66	10.33	2.47	2.12	44.4
	80 N	18.00	10.90	2.91	2.47	48.10
	Azo.	14.00	9.43	1.65	1.27	40.37
	40 N + Azo.	16.00	10.27	2.30	1.83	43.73
	60 N + Azo.	18.00	11.16	3.00	2.55	49.83
	80 N + Azo.	18.33	11.23	3.11	2.65	50.97
8.0%	Control	11	8.16	1.10	0.93	32.63
	40 N	11.66	8.53	1.45	1.23	36.40
	60 N	12.66	9.10	1.81	1.71	40.13
	80 N	14.33	9.50	2.50	2.13	43.63
	Azo.	11.33	8.80	1.34	1.01	35.83
	40 N + Azo.	13.00	9.13	1.86	1.49	39.53
	60 N + Azo.	15.00	9.97	2.23	2.10	44.37
	80 N + Azo.	12.33	9.73	2.13	1.84	43.70
16.0%	Control	8.66	6.43	0.48	0.32	16.93
	40 N	9.33	6.63	0.75	0.47	17.77
	60 N	11	7.00	0.91	0.66	19.57
	80 N	12.66	7.33	1.26	0.90	21.63
	Azo.	9.00	6.53	0.60	0.41	17.86
	40 N + Azo.	10.66	7.13	1.01	0.72	20.73
	60 N + Azo.	12.66	7.66	1.24	0.93	23.70
	80 N + Azo.	10.66	7.40	1.04	0.72	18.20
Seawater irrigation effects		**	**	**	**	**
N levels effect		**	**	**	**	**
Inoculation effect		**	**	**	**	**
Saline × N-fertilization		NS	NS	NS	**	NS
Saline × inoculation effects		NS	NS	NS	**	NS
Saline × N × inoculation effects		NS	NS	NS	**	NS

NS = Non significant.
** = Highly significant.

Table 4 Effect of treatments on chemical analysis (%) of wheat grains (LSD at $P \leqslant 0.05$; 0.01). Each value represents mean ± S.E. of triplications.

Treatments		N	P	K	Na	Ca	Protein
Seawater concentration (%)	N-fertilizer and/or *Azospirillum*						
Tap water	Control	3.21	0.301	0.100	0.110	0.076	20.06
	40 N	3.76	0.440	0.124	0.150	0.101	23.52
	60 N	3.94	0.510	0.142	0.182	0.118	24.63
	80 N	4.03	0.611	0.176	0.206	0.127	25.19
	Azo.	3.58	0.360	0.134	0.118	0.089	22.38
	40 N + Azo.	3.81	0.530	0.156	0.134	0.177	23.81
	60 N + Azo.	4.26	0.650	0.210	0.162	0.135	26.63
	80 N + Azo.	4.25	0.667	0.190	0.184	0.154	26.57
8.0%	Control	3.16	0.281	0.096	0.141	0.086	19.75
	40 N	3.69	0.411	0.106	0.188	0.145	23.06
	60 N	3.83	0.474	0.131	0.268	0.161	24.00
	80 N	3.91	0.531	0.163	0.310	0.182	24.44
	Azo.	3.45	0.311	0.126	0.154	0.112	21.56
	40 N + Azo.	3.77	0.481	0.144	0.162	0.125	23.53
	60 N + Azo.	4.10	0.615	0.196	0.224	0.148	25.63
	80 N + Azo.	4.06	0.653	0.178	0.260	0.166	25.39
16.0%	Control	3.01	0.221	0.060	0.207	0.118	18.81
	40 N	3.28	0.361	0.076	0.266	0.191	20.50
	60 N	3.40	0.382	0.110	0.301	0.291	21.25
	80 N	3.54	0.450	0.131	0.384	0.366	22.13
	Azo.	3.32	0.245	0.084	0.191	0.163	20.75
	40 N + Azo.	3.34	0.401	0.126	0.251	0.209	20.88
	60 N + Azo.	3.57	0.521	0.157	0.280	0.311	22.33
	80 N + Azo.	3.63	0.410	0.140	0.332	0.371	22.69
Seawater irrigation effects		*	*	*	*	*	**
N levels effect		*	*	**	*	*	**
Inoculation effect		*	**	*	*	*	**
Saline × N-fertilization		**	**	NS	*	*	**
Saline × inoculation effects		**	**	*	*	*	**
Saline × N × inoculation effects		**	**	NS	*	*	**

NS = Non significant.
* = Significant.
** = Highly significant.

more, the same treatment decreased the content of sodium in the wheat plants. These results coincide with those obtained by Wu et al. (2005) and Tawfik et al. (2006). The highest N-content was obtained from the wheat plants treated with 60 Kg N Fed^{-1} and bacterial inoculation. The possible mechanisms leading to higher nitrogen content were explained by Ali et al. (2002). They reported that the transfer of atmospheric nitrogen to the plant through bacterial nitrogen fixation and the high growth-promoting substances produced by rhizobacteria, enhance root development and subsequently increased the nutrient uptake by the wheat plants.

Regarding to saline irrigation, the results showed that an increase in saline irrigation levels significantly increased the content of sodium and calcium while decreasing the potassium and phosphorus content as well as the nitrogen content. However, moderate saline irrigation (8.0% seawater) had a slight effect on the chemical content in all wheat plants treated with bacterial inoculum. The plant adaptation to moderate saline irrigation under bacterial inoculation was associated with lower Na/K ratio and a greater capacity for osmotic adjustment (Lacerda et al., 2005). The addition of bacterial inoculum showed a significant decrease in fertilizer requirement, an improvement in the crude protein content and an ability to counteract the effects of salinity (Ashour et al., 2004; Zahirod-dini et al., 2004). An increase in the concentration of seawater irrigation water to 16.0% significantly decreased all investigated parameters compared with the tap water treatment. Reduction in the nitrogen content under high salinity levels may be due to a disturbance in nitrogen metabolism, the inhibition of nitrate absorption or the decrease in the availability of amino acids and the denaturation of the enzymes involved in amino acid and protein synthesis (Sher-Mohamed and Mohamed, 1994). Furthermore, Kader and Lindberg (2005) reported that the greatest accumulation of sodium by plants at high salt concentrations may be attributed to damage of the protoplasm of the plant cells. As a result, selective salt absorption is replaced by passive absorption which causes an abnormal accumulation of salts in the plant organs. The depressing effect of salinity on potassium and phosphorus content could be attributed to the difficulty of its uptake due to competition with the high concentration of the sodium in the root medium.

3.4. Changes in bacterial counts during the cultivation period

There was a negative correlation between microbial numbers of *Azospirillum* and the content of N-fertilizer during the cultivation period (Table 5). Furthermore, there was an increase

Table 5 Changes in bacterial counts during cultivation periods (LSD at $P \leqslant 0.05; 0.01$). Each value represents mean ± S.E. of triplications.

Treatments		Cultivation periods (days)					
		Total bacterial count (CFU × 10^6/g dry soil)			Viable count of *Azospirillum* (log MPN/g dry soil)		
Seawater concentration %	N-fertilizer and/or *Azospirillum*	30	60	90	30	60	90
Tap water	Control	16.90	33.75	19.86	3.741	4.359	3.871
	40 N	26.71	85.30	63.72	4.410	4.928	4.652
	60 N	25.17	74.07	47.20	4.295	4.827	4.607
	80 N	20.36	42.53	35.73	4.006	4.262	4.078
	Azo.	47.91	123.8	104.1	4.463	4.922	4.633
	40 N + Azo.	79.31	196.3	131.4	5.100	5.574	5.306
	60 N + Azo.	66.50	169.3	116.2	5.033	5.519	5.078
	80 N + Azo.	52.43	122.8	74.34	4.941	5.233	4.868
8.0%	Control	13.80	28.38	17.01	3.592	4.114	3.830
	40 N	22.99	79.15	52.26	4.356	4.798	4.550
	60 N	18.77	65.40	39.28	4.226	4.736	4.470
	80 N	14.45	30.55	18.51	3.906	4.226	3.825
	Azo.	37.96	115.7	77.02	4.339	4.818	4.462
	40 N + Azo.	70.77	180.2	122.4	5.057	5.479	5.172
	60 N + Azo.	58.00	158.5	85.38	4.981	5.408	4.981
	80 N + Azo.	39.52	97.60	53.84	4.853	5.113	4.654
16.0%	Control	06.74	15.18	8.770	2.185	3.633	3.285
	40 N	13.91	33.71	16.18	4.019	4.549	4.152
	60 N	10.48	25.33	13.97	3.956	4.468	4.025
	80 N	08.10	15.23	09.90	3.637	3.969	3.608
	Azo.	20.72	75.28	25.48	4.036	4.503	4.123
	40 N + Azo.	50.45	114.2	83.68	4.868	4.988	4.799
	60 N + Azo.	39.44	90.91	61.57	4.757	4.853	4.461
	80 N + Azo.	24.21	57.25	30.02	4.235	4.533	3.838
Seawater irrigation effects		**	**	**	*	*	*
N levels effect		**	**	**	**	*	**
Inoculation effect		**	**	**	*	*	*
Saline × N-fertilization		NS	**	**	NS	**	*
Saline × inoculation effects		NS	NS	NS	*	*	**
Saline × N × inoculation effects		NS	**	**	NS	*	**

NS = Non significant.
* = Significant.
** = Highly significant.

in the *Azospirillum* count during the first 60 days of cultivation for all the treatments. However, this increase was followed by a significant reduction in the *Azospirillum* count between days 60 and 90. These results were in agreement with the findings of Ali et al. (2002), who reported that the survival of free-living N_2-fixing bacteria in the rhizosphere region was associated with the presence of chemicals exuded by the plant roots and the extra presence of combined nitrogen. These probably explained the suppression of *Azospirillium* in the rhizosphere soil of wheat treated with high levels of nitrogenous fertilizer. Inoculation with *Azospirillum* resulted in a considerable increase in the density of *Azospirillum* colonized in the rhizosphere region.

The *Azospirillum* count remained almost unchanged at moderate levels of saline irrigation (8.0% seawater). However, at high saline irrigation levels (16.0% seawater) the *Azospirillum* count decreased significantly. The reduction in the *Azospirillum* count was about 1.7% at moderate seawater irrigation levels, while it was about 10.5% at high seawater irrigation levels after 60 days of cultivation. The results also revealed that the effective colonization of *Azospirillum* in wheat rhizosphere soil was obtained from the treatments receiving 40 Kg N Fed^{-1} at day 60 of cultivation from the soil amended with 40 Kg N Fed^{-1} and irrigated with tap water. Similar results were also seen for the treatments irrigated with 8.0% seawater concentration. This would indicate a relatively high tolerance of *A. brasilense* Sp-245 to saline irrigation (Bashan et al., 2004; Barassi et al., 2006).

Total bacterial count (TBC) density in the rhizosphere soil demonstrated a similar trend to that of the *Azospirillum* population (Table 5). It was clearly observed that TBC density significantly increased up to 60 days from sowing for the inoculated treatments compared to the uninoculated treatments. Thereafter, there was a marked drop in the bacterial counts during the remaining period of cultivation. Similar results were reported by Mansour et al. (2006). Among the different N-fertilizer levels tested, the TBC survived better at 40 Kg N Fed^{-1} showing the highest microbial numbers in the second month of sampling. On the other hand, a similar trend was observed using sea water irrigation. The TBC was decreased slightly at moderate levels of saline irrigation. However, the highest reduction of TBC was observed at 16.0% seawater irrigation, confirming the negative effect of high salt concentration.

4. Conclusion

Bacterial inoculation saved about 20 units of N-fertilizer and that saving was economically feasible. Therefore, the recommended dose of chemical N-fertilizer could be reduced by using bacterial inoculation which, in turn, minimizes production costs, environmental pollution, increases soil fertility and wheat yield. The growth of wheat plants irrigated with 8.0% seawater was stimulated by the presence of *Asospirillum*, which improved the growth and yield parameters by alleviated the adverse effects of salinity.

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