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EFFECT OF INTERACTION BETWEEN BIOFERTILIZERS AND SALINE IRRIGATION WATER ON THE PRODUCTIVITY OF SAFFLOWER (*Carthamus tinctorius* L) IN NORTHERN SINAI - EGYPT

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ABSTRACT

Two field experiments were carried out during the two successive seasons (2007/2008 and 2008/2009) at the experimental station of El Sheikh Zowayed, Desert Research Center to study the effect of biofertilizer application (*Azotobacter chrococcum, Azospirillum lipoferum* and mixture of them) under three levels of saline irrigation water (2000, 3000 and 4000 μgl^{-1}) on growth and productivity of safflower (*Carthamus tinctorius* L).

The obtained results showed that, biofertilizer treatments significantly increased microbial activities in safflower rhizosphere (total bacterial counts, azotobacters and azospirilla densities, CO_2 evolution and dehydrogenase activity). Also, biofertilizers enhanced plant height, number of branches per plant, fresh and dry weight /plant. On the other hand, nitrogen content in soil and shoots plant at 35, 70 and 120 days from sowing increased by inoculation with biofertilizers during the two seasons. Number of heads /plant, head diameter, number of seeds /head, weight of 100 seed, stand, protein, phosphorus, oil content and oil yield at harvest significantly increased in biofertilizers treatments. Dual inoculation treatment gave the highest values of growth characters, yield and chemical composition of safflower plant as well as microbial activities in safflower rhizosphere.

Increasing salinity in irrigation water from 2000 to 4000 μgl^{-1} significantly decreased microbial activities in safflower rhizosphere, growth characters, yield, yield components and chemical contents of plant.

Interaction between biofertilizer treatments and salinity had a significant effect on microbial activities in safflower rhizosphere. Also, application of dual or individual biofertilizer with saline irrigation water improved plant growth and yield and yield components of safflower compared with uninoculated plants.

Keywords: Safflower variety (Giza 1), biofertilizers, salinity, microbial counts, growth, yield, chemical composition.

INTRODUCTION

Safflower provides three principle products: oil, meal and birdseed. Safflower oil is used by both food producers and industry. Safflower oil consists of two types with corresponding types of safflower varieties: those high in monounsaturated fatty acid (oleic) and those high in polyunsaturated fatty acid (linoleic).

Soil and water salinity are a wide spread problem in crop production. However, this problem is usually confined to arid and semi-arid regions. Saline conditions cause physical and chemical changes in soil and significantly decrease the soil productivity. The type as well as the

concentration of salts affect soil structure and interfere with the nutrition of plant. The anion of salt whether chloride or sulphate is also important. Several investigations concluded that increasing salinity decreased the yield components of safflower such as seed yield, biomass yield (dry weight), number of plant per hectare, 1000- seed weight, plant height, number of capitula per plant and capitula weight per plant, Mohammad *et al.* (2010).

Several investigations concluded that increasing salinity decreased the vegetatative growth characteristics and yield of safflower plants. Rumasz *et al.* (2002) and Muhammad *et al.* (2007) found that increasing salt concentration from 0 to 150 mm NaCl, significantly decreased dry root and shoot weight, fresh leaf weight and leaf area of *Beta vulgaris* L.

Biofertilizer is a natural organic fertilizer known that helps to provide all the nutrients required by the plants and helps to supply and increase the soil with natural and beneficial microorganism. Biofertilizers are the most advanced biotechnology necessary to support developing organic agriculture, sustaintable agriculture, green agriculture and safe agriculture. Mixed bacterial inoculation increased growth and yield of different plant species as compared with uninoculation. This was shown by (Rahim and Mirzaei, 2010.) who found that significant increase was observed on yield and yield component of safflower with applying biofertilizers. Also applying *Azotobacter* and *Azospirillum* increased seed yield and yield components by 35 and 21% respectively compared with control.

The aim of this study is to evaluate the role of biofertilizers under different levels of irrigation water salinity on the microbial activities. as well as the growth and productivity of safflower at North Sinai, Egypt.

MATERIALS AND METHODS

Determination of nitrogen fixing capacity by Azotobacter spp. And Azospirillum spp. in pure culture: Fixed Nitrogen in cultures media was determined after 7 days from incubation as mentioned by Bremner, (1965). Briefly, Azotobacter spp. and Azospirillum spp. isolates were grown in 10 ml Ashby medium or Dobereiner medium (without agar) in 20 ml test tube on a rotary shaker (125 rpm) under continuous airflow at 30^{o}C for 72 hr. Cell concentrations were determined as 10^{5} CFU ml^{-1} of each isolate by plate counts on agar Ashby or Dobereiner medium. The non-inoculated media served as control. Afterwards, the concentration of nitrogen in each liquid culture was measured by digestion and subsequent measurement by the Kjeldahl method (Bremner, 1965). The quantities of nitrogen reported represent the average of duplicate cultures after deducting the average of duplicate controls.

Two field experiments were carried out on safflower plant (*Carthamus tinctorius* L) at El- Sheikh Zowayed Research Station, Desert Research Center, North Sinai Governorate, Egypt, during two successive growing seasons (2007/2008 and 2008/2009).

Mechanical and chemical analysis of the experimental soil were carried out according to Richards (1954); Black (1965) and Jackson (1967) as shown in Table (1).

Each experiment included twelve treatments which were the combintation of three salinity levels of irrigation water (2000µgl^{-1}, 3000µgl^{-1},and 4000 µgl^{-1}) and three biofertilizer treatments (*Azotobacter chrococcum*, *Azospirillum lipoferum* and their mixture) in addition to controls (without bacterial inoculation).

Bacterial culture preparation.

The fresh liquid cultures were prepared from pure local strains of *Azotobacter chrococcum* and *Azospirillum lipoferum* which previously isolated in Bunt and Rovira medium and semi solid malate medium from the rhizosphere of safflower plant grown in El Sheikh Zowayed area, respectively. They were purified and identified according to Bergey Manual (1984). Biofertilizers were added in the form of individual and mixed inoculations at the rate of ~10^{8} cfu/ml as soil treatment. Safflower seeds were treated before planting with individual or mixture of bacterial suspensions for three hours before transplanting (carboxy methyl cellulose 0.5% was used as an adhesive agent). Seed without microbial treatment was served as control.

The design of experiment was split plot with three replication, each split included 12 treatments which were the combination between three levels of saline irrigation water (2000, 3000 and 4000 µgl^{-1}) and four biofertilizer treatments. The main plots were devoted to saline irrigation water levels, while the sub-plots were occupied by the biofertilizer. The experimental plot area was 10.5 m^{2} (3/m x 3.5 m), consisting of 6 ridges, each of 50 cm width and 3.5 m length, 50 cm were between hills and four seeds were sown in each hill. Before sowing, sheep manure (15 m^{3}fed.) was mixed with the upper soil. 150 kg calcium super phosphate /fed. (15.5% P_2O_5) were added during seed-bed preparation before sowing and mixed with the surface layer. In addition, 150 kg ammonium sulphate / fed. (20.5% N) and 100 kg potassium sulphate / fed. (48% K_2O) were applied in two equal portions; after 15 and 21 days from sowing.

Safflower seeds (Giza1variety) were sown on 15th October in the two growing seasons; the plants were thinned to one plant per hill after fifteen days from sowing. The experiment was irrigated immediately after sowing by water pumped from a well (2000 µgl^{-1}). The analysis of irrigation water is given in Table (2). Chemical analysis of sheep manure is given in Table (3).

Table (1): Physical and Chemical analyses of soil experimental station

Depth (cm)	Physical properties					Chemical properties									
	Fine sand %	Coarse sand %	Silt %	Clay %	Texture	pH	E.C. dS.m^{-1}	Soluble anions(meq./L.)			Soluble cations (meq. /L.)				CaO3 %
								$HCO3^{-}$	Cl^{-}	$SO4^{=}$	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	
0 – 30	98.50	0.30	0.69	0.51	Sandy	7.81	0.31	0.87	1.05	1.20	1.04	0.35	1.56	0.17	1.45

Table (2): Chemical analysis of irrigation water of the Station

Irrigation water types	PH	E.C. $dS.m^{-1}$	Soluble cations, meq. /L				Soluble anions, meq. /L			
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	SO_4^{--}	Cl-
2000	7.7	3.13	2.2	2.8	26	0.34	0	2.3	3.34	25.7
3000	7.86	4.69	4.6	5.4	36	0.89	0	2.7	5.2	39
4000	7.91	6.25	8.5	9.7	43.4	0.9	0	3.4	9.9	49.2

Table (3): Chemical analysis of sheep manure.

Type of analysis		Type of analysis	
PH	7.91	**Soluble ions (meq\l)**	
Organic matter %	59.83	Phosphorus (P)	4.2
Organic carbon %	34.78	Potassium (K)	13.8
Total elements (%)		Calcium (Ca)	8.3
Nitrogen	2.31	Magnesium (Mg)	6.5
Phosphorus	0.51	Sodium (Na)	25.6
Potassium	1.01	**SD kg /m3**	466
Calcium	4.32		
Magnesium	0.26		
C /N ratio	15.06		
EC in dS $/m^{-1}$ (1:10)	5.79		

Determinations

Samples of rhizosphere and plants were taken after 35, 70 and 120 days from sowing to determine microbial activities, growth characters, chemical composition and yield and yield components.

A- Microbial determination:-

Total bacterial, *Azotobacter chrococcum* and *Azospirillum lipoferum* conts in the rhizosphere samples were counted on Bunt and Rovira medium (Bunt and Rovira, 1955), nitrogen deficient medium (Abd El Malek and Ishac, 1968) and semi solid malate medium (Dobereiner, 1978), respectively. Also, CO_2 evolution (µg/g dry soil/ hr.) and dehydrogenase activity (µg TPF g^{-1}. dry soil 24h.) in the rhizosphere were determined according to Pramer and Schmidt (1964) and Thalmann (1967), respectively.

B- Growth characters:

Three guarded plants were randomly taken from the three inner ridges of each experimental plot to measure plant height (cm), number of branches/plant, fresh weight / plant (g) and dray weight / plant (g).

C- Yield and its components:

Three inner ridges of each experimental plot were taken to measured number of heads/ plant, head diameter (cm), number of seeds / head, weight of 100 seeds (g), stand % and seed yield (Kg/fed.).

D- Chemical composition:

Chemical composition was determined in seeds after 120 days from sowing date as following.

1- Protein content: total nitrogen percentage was determined by using the modified microkjeldahl method as described by Peach and Tracey (1956). The protein content was calculated by multiplying the total nitrogen by 6.25 Tripath *et al*. (1971).

2- Phosphorus percentage was determined by ascorbic acid according to method reported by Frie *et al*. (1964).
3- Oil percentage was determined according to the method described in the official and tentative methods of American Oil Chemists (A.O.C.S. 1964).
4- Oil yield (kg / fed.) was calculated by multiplying seed yield Kg/fed by seed oil percentage.

Nitrogen content in soil samples and total nitrogen in shoots of plant were determined at 35, 70 and 120 days from sowing.

Statistical analysis:

All the obtained data were subjected to the proper statistical analysis of variance according to the procedure outlined by Snedecor and Cochran (1989). Mean values of treatments were differentiated by using L.S.D at 5% level as mentioned by Steel (1960).

RESULTS AND DISCUSSION

Fixed nitrogen in cultures media

This laboratorial experiment was conducted on microbial strains (i.e., *Azotobacter, Azospirillum* and mixture of them) to evaluate the direct effect of irrigation water salinity, (i.e., 2000, 3000 and 4000ppm) on the ability of these microbial strains for N-fixation and compare their ability in soil which contain either beneficial or harmful microorganisms in addition to the presence of elements of fertilizers which may have a negative or positive effect on microbial activity.

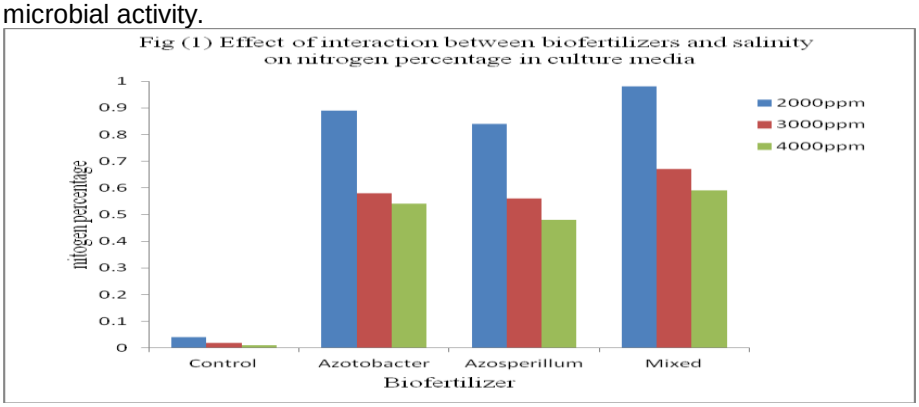

Figure (1) showed that the N-fixation under salinity level of 2000 ppm was high for all three treatments (i.e., *Azotobacter, Azospirillum* and mixture of them). While the N-fixation at 3000 ppm reduced by 35, 33 and 31%, respectively and the rate of reduction at 4000 ppm were 39, 43 and 34%, respectively. This shows that the activity of *Azotobacter* was higher in N-fixation at the three levels of salinity of irrigation water comparing to the activity of *Azospirillum* under the same levels that mentioned above. These results agreed with Faid, (2000) and EL-Tayeb (2000).

Interaction effect of biofertilizers and saline irrigation water on microbial activities in safflower rhizosphere:

1-Total microbial counts:

Data illustrated in Table (4) showed that increased salinity of irrigation water from 2000 mgl^{-1} to 4000 mgl^{-1} decreased total microbial counts of safflower rhizosphere at 35, 70 and 120 days from sowing in the two seasons. Co-inoculation with *Azotobacter* and *Azospirillum* gave the highest total microbial counts as compared with the others treatments in the two seasons. The positive response of growth as a result of Co-inoculation may be due to the fact that *Azotobacter* is free-living, nitrogen-fixing bacteria and is known to produce several plant growth promoting subustances. Abd El-Ghany (1996) and Abd El-Gawad (2008), confirmed these results that microbial inoculates improve fertility, increase the number and biological activities of desired microorganisms in root environment.

Data also show that increasing salinity from 2000µgl-1 to 4000μgl^{-1} caused a significant decrease in total microbial counts after 35, 70 and 120 days from sowing in the two seasons. The interaction between saline irrigation water and biofertilizer was significant at 35 days from sowing in the two seasons. The highest counts of all bacteria under study was after 70 days from sowing under all levels of saline irrigation water.

2- *Azotobacter chrococcum* densities:

Data in Table (4) also showed that the highest count of *Azotobacter* was at 70 days from sowing in the two successive seasons compared with the other periods. Increasing salinity of irrigation water from 2000μgl^{-1} to 4000 μgl^{-1} significantly decreased *Azotobacter* count at the three periods of plant growth i.e. 35, 70 and 120 days from sowing in the two seasons. The data are agreement with those recorded by (Hashem and Abd El-Ghany, 1992). The highest count of *Azotobacter* was obtained from the mixture of *Azotobacter* and *Azospirillum* in the two seasons.

3- *Azospirilla* densities:

Results in Table (5) reveal that biofertilizer treatments significantly affected azospirilla densities. The highest value of azospirilla densities was obtained by mixture inoculation with *Azotobacter* and *Azospirillum* at all sampling dates in two seasons. These data are in agreement with those recorded by Abd El-Gawad (2008).

Results in Table (5) indicate that increasing salinity in irrigation water up to 4000 μgl^{-1} significantly decreased azospirilla densities after 35, 70 and 120 days from sowing in the two seasons compared with 2000 μgl^{-1}.

Growing plants at high level of salinity (4000 μgl^{-1}) reduced *azospirilla* densities as compared to those irrigated by 2000 μgl^{-1} by 49.23, 63.21 and 42.98 % at 35, 70 and 120 days respectively, in the first season and by 45.78, 50.90 and 63.92 % at 35, 70 and 120 days respectively, in the second season.

4- CO_2 evolution:

The results shown in the Table (5) indicate that the rate of CO_2 evolution as a criterion for biological activity in the safflower rhizosphere gave the highest levels with mixed treatments of biofertilizer, followed by individual inoculation with *A. chroococcum* then *Azospirllum lipoferum*. The

development of high biological activity was observed when water salinity ranged between 2000 and 3000µgl^{-1} while activity decreased at 4000µgl^{-1}. Data of CO_2 evolution were almost in harmony with those of total microbial counts discussed before. These results agreed with that of El-Sayed (2006).

Table (4): Effect of interaction between biofertilization and saline irrigation on total bacterial counts and *Azotobacter* densities during 2007/2008 and 2008/2009 seasons.

		Total bacterial counts (Counts x 10^5 CFU/g dry soil)						*Azotobacter* densities (Counts x 10^4 CFU g dry soil)					
		Season 1			Season 2			Season 1			Season 2		
		35	70	120	35	70	120	35	70	120	35	70	120
2000 µgl^{-1}	Control	11.5	14.1	13.9	14.1	16.8	12.2	14.6	18.0	14.5	12.1	18.0	14.3
	Azotobacter	22.4	27.7	24.8	24.8	31.5	25.9	25.5	28.1	26.3	25.3	36.4	31.2
	Azospirllum	20.6	24.4	22.9	23.9	28.7	23.8	24.0	27.3	25.3	23.7	32.1	28.6
	Mixed	24.4	28.6	26.8	26.7	31.5	27.2	30.3	36.4	34.4	31.2	45.3	38.6
3000 µgl^{-1}	Control	11.8	12.2	12.2	8.7	12.6	13.1	11.5	16.3	13.1	12.6	17.6	12.3
	Azotobacter	20.1	25.9	23.8	20.4	27.7	24.6	22.4	27.3	25.8	23.7	32.2	28.6
	Azospirllum	18.1	22.9	21.2	19.6	25.6	23.3	21.3	26.2	23.5	23.0	29.4	24.5
	Mixed	22.9	26.4	25.9	22.4	28.6	26.7	26.6	35.4	32.6	28.1	43.0	37.0
4000 µgl^{-1}	Control	8.1	9.8	10.7	6.4	11.8	11.6	10.4	14.7	12.5	10.9	15.0	12.3
	Azotobacter	18.6	21	21.3	16.7	26.9	23.3	20.0	25.6	23.7	21.3	28.1	24.3
	Azospirllum	16.0	19.1	18.7	15.6	23.8	21.0	18.6	24.8	21.0	21.2	27.2	23.5
	Mixed	19.9	22.8	22.3	20	26.7	24.3	24.3	33.2	28.1	25.7	38.6	34.4
L.S.D. at 5% for													
	Salinity	0.06	1.83	3.38	0.04	0.19	0.80	0.19	1.08	2.36	1.48	3.73	0.19
	Biofertilizer	0.05	1.51	2.87	0.03	0.17	0.98	0.17	4.45	0.57	0.17	2.26	0.23
	Interaction	0.09	N.S	N.S	0.06	0.27	N.S	0.29	2.44	0.99	0.29	3.91	0.41

Initial microbial 55 x 10^2 cfu/gm dry soil
Initial *Azotobacter* densities 30 x 10^2 cfu/gm dry soil

Table (5): Effect of interaction between biofertilization and saline irrigation on azospirilla densities and CO_2 evoluted during 2007/2008 and 2008/2009 seasons.

		Azospirilla densities (Counts x 10^4 CFU g dry soil)						CO_2 evoluted (µg/g dry soil/ hr.)					
		Season 1			Season 2			Season 1			Season 2		
		35	70	120	35	70	120	35	70	120	35	70	120
2000 µgl^{-1}	Control	3.10	8.20	3.40	3.20	6.70	7.40	13.52	17.60	16.92	31.40	35.16	34.60
	Azotobacter	4.40	10.10	5.00	4.60	8.80	9.20	16.60	22.00	19.40	34.70	46.20	43.50
	Azospirllum	3.20	9.80	4.30	3.60	8.20	8.20	15.60	19.40	18.04	32.80	42.04	36.80
	Mixed	4.90	10.80	6.10	5.20	9.70	10.10	22.00	34.60	26.20	46.20	59.40	53.51
3000 µgl^{-1}	Control	2.20	4.90	2.60	2.50	4.30	5.10	12.60	16.40	15.60	30.6	34.7	32.80
	Azotobacter	3.60	6.90	4.30	3.60	6.60	7.10	15.60	21.20	18.30	36.80	43.50	42.04
	Azospirllum	2.50	5.70	3.40	2.70	5.90	5.90	14.52	18.30	17.60	32.80	40.30	37.20
	Mixed	4.10	7.40	5.40	4.50	7.10	7.60	20.10	32.80	24.90	42.04	46.20	43.50
4000 µgl^{-1}	Control	1.10	2.10	1.20	1.50	2.70	2.10	7.30	12.70	11.00	24.30	29.0	27.30
	Azotobacter	2.10	4.20	3.30	2.60	4.50	3.70	13.20	17.60	16.40	28.70	34.0	32.0
	Azospirllum	1.90	3.20	2.50	2.00	3.70	2.80	13.00	16.70	15.20	25.70	29.0	28.7
	Mixed	2.80	4.80	3.70	2.90	5.50	4.00	18.04	26.20	19.40	34.70	41.0	38.30
L.S.D. at 5% for													
	Salinity	0.57	1.13	0.87	0.87	0.02	0.49	1.71	1.13	1.37	1.71	2.11	1.73
	Biofertilizer	0.39	0.02	0.29	0.29	0.02	0.43	1.24	1.19	0.62	0.93	1.04	0.86
	Interaction	N.S	2.86	N.S	N.S	2.86	N.S	N.S	1.87	1.07	1.62	1.81	1.49

Initial *Azospirilla* densities 1.3 x 10^2 cfu/gm dry soil

5- Dehydrogenase activity:

Dehydrogenase activity was determined as a criterion of respiration rate and total microbial activity in the safflower plant under different investigated treatments. Data presented in Table (6) showed that inoculated soil with individual or mixed inoculants significantly gave higher values of dehydrogenase activity when compared with uninoculated soil. In addition, mixed inoculation with *A. chroococcum* and *Azospirillum* sp. gave a significant higher dehydrogenase activity than the soil inoculated with each one individually. In addition, dehydrogenase activity exhibited its dominant increase at 70 days after sowing during both seasons with different treatments. Data also revealed that the level of salinity of irrigation water used had a major impact in dehydrogenase activity reaching the highest activity at 2000 and 3000 μgl^{-1} when it went down at 4000 μgl^{-1}. In addition, dehydrogenase activity in various treatments were higher after 70 days. This may due to the difference in multiplication rate of different soil microorganisms which usually be maximum during flowering stage. Such differences could be attributed to the qualitative and quantitative changes in the nature of root exudates during different growth stages. These results are in harmony with Abd El-Gawad (1998) and Khalifa (2005).

Table (6): Effect of interaction between biofertilization and saline irrigation on dehydrogenase activity during 2007/2008 and 2008/2009 seasons.

Salinity	Inoculation	Dehydrogenase activity (µg TPF g-1. dry soil 24h.)					
		First season			Second season		
		Days after sowing			Days after sowing		
		35	70	35	70	35	70
2000 µgl-1	Control	4.95	6.88	5.22	5.81	6.96	6.12
	Azotobacter	6.11	8.91	7.69	7.55	8.95	8.16
	Azospirllum	5.05	7.85	7.05	6.36	7.81	7.25
	Mixed	6.21	9.71	8.22	7.94	9.90	8.51
3000 µgl-1	Control	3.85	5.95	4.65	3.65	5.24	4.25
	Azotobacter	5.41	7.52	6.56	5.50	7.05	6.14
	Azospirllum	4.61	6.55	6.04	4.49	6.45	5.83
	Mixed	5.33	8.61	7.68	6.15	8.21	7.07
4000 µgl-1	Control	2.59	3.72	3.52	2.37	4.09	3.21
	Azotobacter	4.75	6.09	5.15	4.38	6.50	5.69
	Azospirllum	4.14	5.79	4.86	3.42	5.37	4.59
	Mixed	5.15	7.39	6.22	5.16	6.95	6.12
L.S.D. at 5% for							
	Salinity	0.85	0.57	0.57	0.54	0.83	0.68
	Biofertilizer	0.85	0.95	0.86	0.55	0.89	0.56
	Interaction	N.S	N.S	N.S	N.S	N.S	N.S

***- Initial DHA 0.70 µg TPF g-1. dry soil 24h.**

Nitrogen content in rhizosphere and shoots of safflower plant

Data presented in Table (7) indicated that biofertilizer treatments significantly increased the content of nitrogen in rhizosphere and in shoots of safflower compared with uninoculated plants. Increasing salinity up to 4000

µgl^{-1} had a significant decrease of nitrogen percentage in soil and in shoots of the plant either with biofertilizer application or without inoculation. Inoculation with mixture of *A. chroococcum* and *Azospirllum lipoferum* under 2000 µgl^{-1} gave the highest values of nitrogen content in soil and in shoots at all sampling date during the two seasons. This result is compatible with the finding of EL-Tayeb (2000) who found that inoculation with selected halo tolerant *Azospirllum* strains resulted in considerable increases of growth and yield of wheat plants grown under the saline conditions of Egyptian desert soil.

Interaction between biofertilizers and water irrigation salinity on growth characters of safflower

The results summarized in Tables (8 and 9) revealed that biofertilizer treatments significantly affected on plant height, number of branches per plant, fresh and dry weight / plant after 35, 70 and 120 days from sowing during the two seasons**.** The highest value of growth characters and survival plant (stand %) were obtained when plant were inoculated with mixture of *Azotobacter* and *Azospirillum***.** In addition to nitrogen fixation by these bacteria, they are also known to protect plants against pathogenic microorganisims either by discouraging their growth or by destroying them. These inoculants need more attention in view of their triple action of nitrogen fixation, bio-control, and production of plant growth regulators. The positive response of growth to inoculation with *Azospirillum* and *Azotobacter* was described by several investigators including Mahmoud *et al*. (2012) and Paritosh *et al.* (2013).

Table (7): Effect of interaction between biofertilization and saline irrigation on nitrogen percentage in (soil samples and shoots of plant).

Levels Of salinity (µgl^{-1})	Inoculation	Nitrogen (%)					
		Soil			Plant		
		Days after sowing					
		35	70	120	35	70	120
2000	Control	0.06	0.07	0.07	1.643	2.120	2.290
	Azotobacter	0.16	0.19	0.18	2.707	3.100	3.260
	Azospirllum	0.15	0.18	0.16	2.663	2.940	3.010
	Mixed	0.18	0.21	0.19	2.827	3.260	3.343
3000	Control	0.05	0.07	0.05	1.323	2.007	2.127
	Azotobacter	0.16	0.18	0.17	2.560	2.963	3.110
	Azospirllum	0.13	0.16	0.15	2.540	2.887	2.933
	Mixed	0.17	0.19	0.18	2.670	3.060	3.240
4000	Control	0.03	0.05	0.04	1.173	1.877	2.007
	Azotobacter	0.14	0.17	0.15	2.290	2.837	3.030
	Azospirllum	0.13	0.15	0.13	2.120	2.757	2.820
	Mixed	0.16	0.17	0.17	2.587	2.953	3.157
L.S.D. at 5% for							
	Salinity	0.01	0.02	0.01	0.02	0.02	0.66
	Biofertilizer	0.01	0.01	0.01	0.04	0.04	0.50
	Interaction	0.01	N.S	0.01	0.06	0.06	N.S

***- Initial total nitrogen in soil 0.02 (%)**

Data presented in Tables (8 and 9) clearly indicated that increasing salinity irrigation water up to 4000 µgl^{-1} significantly decreased plant height, number of branches per plant, fresh and dry weight / plant of safflower plant after 35, 70 and 120 days from sowing in the first and second seasons compared with 2000 µgl^{-1}. The gradual depression occurred in all the growth characters of safflower plant due to the irrigation with saline water. Thus, as salinity is a condition of excess salts in soil solution, it affects plant by increasing the osmotic pressure of the soil solution. These results are in agreement with those obtained by Ebrahim *et al.* (2010), Mostafavi (2011) and Aymen *et al.* (2012). They found that increasing salinity in irrigation water decreased growth characters of safflower plant.

The interaction between biofertilizer and irrigation water salinity had a significant effect on growth characters of safflower plants at all sampling dates in the second season (Tables, 8 and 9). The highest value of growth characters of safflower plants were recorded when irrigated by 2000 µgl^{-1} and inoculated with mixed *Azotobacter* and *Azospirillum* at all sampling in both seasons, Kaci *et al*., (2005) reported that, *Azospirillum* and *Azotobacter* are known to deliver a number of benefits including plant nutrition, disease resistance, and tolerance to adverse soil and climatic conditions. Their function ranges from stress alleviation to soil bioremediation or as a biological tool for sustainable agriculture.

Interaction between biofertilizers and water irrigation salinity on yield and its components of safflower

The results summarized in Tables (10) show that biofertilizer treatments had positive significant effects on number of heads /plant, head diameter, number of seeds / head, weight of 100 seed, seed yield (Kg/fed) and stand % during the two seasons compared with uninoculated plants.

Mixed inoculations with *Azotobacter* and *Azospirillum* gave the highest values of yield and its components and survival plant of safflower as compared with the control treatment. The positive response of yield as a result of inoculation with *Azotobacter* and *Azospirillum* may be due to the high ability of these microbes in N_2-fixation and the secretion of several compounds that increase soil fertility and decomposition of organic materials that increase the plant's ability to grow and increase productivity. Seed yield and yield components of safflower have been significantly affected by the inoculation with *Azotobacter* and *Azospirillum*, because these biofertilizers can fix atmospheric nitrogen, increase phosphorus availability in soil and enhanced absorb elements by safflower plant (Mirzakhani *et al*., 2009, Mohammad *et al.* 2010, Mahmoud *et al*., 2012, Omid and Jalilian, 2012, Raouf, 2012, Mina *et al*., 2013 and Paritosh *et al.*, 2013). The depression effect of salinity on plants may not show water deficit symptoms and metabolize normally under the applied salinity levels, the additional energy requirements for maintaining normal metabolism demand substantial photosynthetic diversions for growth. This leads to a reduction in yield, light interception and light utilization efficiency which attributed to partial stomata closure and simultaneously decrease in Co_2 fixation that ultimately reduce growth and yield (Aymen *et al*. 2012, Neeta 2012 and Mostafavi, 2011).

8-9

Data presented in Table (10) show that the interaction between salinity and biofertilizer had a significant effect on head diameter, number of seeds / head, weight of 100 seed. The highest value of yield characters of safflower plant were recorded with irrigated by 2000 μgl^{-1} and inoculated with co-inoculation.

In general it can be said that the use of biofertilizers with safflower plant at all levels of salinity of irrigation water gave positive results as compared to the control. This is due to the high ability of these microbes in N_2-fixating atmosphere and the secretion of several compounds that increase soil fertility and decomposition of organic materials that increase the plant's ability to grow and increase productivity under those levels of salinity of irrigation water. In addition, **Abou-Aly *et al*. (2012)** reported that application of biofertilizers as bio stimulate for pepper grown in saline soil can improve plant defense against saline stress conditions, increase productivity and enhanced plant defense to stress through the decreasing of proline accumulation and increasing of some compounds as an indicator to plant resistance for saline stress.

Interaction effect of biofertilizer and water irrigation salinity on chemical composition of safflower

Results in Table (10) revealed that biofertilizer treatments had a significant effect on protein, phosphorus, oil percentage and oil yield at harvest. The highest value of chemical composition was obtained by using dual inoculation with *Azotobacter* and *Azospirillum*. These results confirmed by the work of Omid and Jalilian. (2012), Mina *et al.* (2013) and **Paritosh** *et al.* (2013).

Results also show that increasing salinity in irrigation water significantly decreased chemical composition of safflower plants. Increasing salinity from 2000 to 4000 μgl^{-1} decreased protein, phosphorus, oil percentage and oil yield. Such reduction in protein content may be due to failure of plants to make full utilization of nitrogen compounds, the accumulation of nitrogen compounds is more rapid than their utilization in building more cells and organs. These results are in agreement with those obtained by Siddiqee (2010).

The interaction between salinity and biofertilizer had a significant effect on protein, oil percentage and oil yield of safflower. The highest value of protein, phosphorus, oil percentage and oil yield was obtained by plants irrigated 2000 μgl^{-1} and inoculated with both biofertilizers.

10

Conclusion

From the obtained results it can be said that, application of dual inoculation with *A. chroococcum* and *Azospirllum lipoferum*. or individually improve plant growth and increase productivity due to the ability of these microbes to do many of the tasks next to its ability to fix high amount of nitrogen, secretion of several hormones and thawed several of soil elements needed by the plant during the period of growth and can tolerate high levels of salinity of irrigation water used. Also, reduced the economically production and the hazard of the doses of mineral fertilizers.

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تـأثير التفاعل بـين الأسـمدة الحيويـة ومياـة الـرى المالحـة علـى أنتاجيـة القـرطم فـى شمال سيناء- مصر

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قسم خصوبة وميكروبولوجيا الأراضي مركز بحوث الصحراء

أقيمت تجربتان حقليتان خلال موسمي ٢٠٠٧- ٢٠٠٨ / ٢٠٠٨-٢٠٠٩ بمحطة تجارب مركز بحوث الصحراء بمنطقة الشيخ زويد بشمال سيناء، لدراسة تأثير ثلاثة معاملات من التسميد الحيوى (الأزوتوباكتر – الأزوسبيريلليم - وخليط من الأزوتوباكتر و الازوسبيريلليم). و ثلاثة مستويات من ملوحة ماء الرى (٢٠٠٠ , ٣٠٠٠ و ٤٠٠٠ جزء فى المليون) والتفاعل بينهما على صفات النمو والمحصول وكذلك التركيب الكيماوى لنبات القرطم والأعداد الكلية للميكروبات والنشاط الحيوى فى منطقة الريزوسفير. وكانت أهم النتائج المتحصل عليها مايلى:

وجد أن إضافة الأسمدة الحيوية لها تأثير مرتفع على نمو وإنتاجية نبات القرطم حيث ظهر لها تأثير على نسبة البقاء وطول النبات وعدد الأفرع وعدد الرؤوس على النبات والوزن الطازج والوزن الجاف كما أن لها تأثير إيجابى على كمية المحصول من البذور والزيت وعلى محتوى البذور من البروتين والفوسفور. كما أن لها أثر واضح على الأعداد الكلية للميكروبات والنشاط الحيوى لها خلال فترة نمو النبات.

وقد أدى زيادة تركيز ملوحة ماء الرى من ٢٠٠٠ إلى ٤٠٠٠ جزء فى المليون إلى نقص معنوى فى نسبة البقاء وطول النبات وعدد الأفرع وعدد الرؤوس على النبات والوزن الطازج والوزن الجاف و كمية المحصول من البذور والزيت وعلى محتوى البذور من البروتين والفوسفور وكذلك الأعداد الكلية للميكروبات والنشاط الحيوى لها خلال فترة نمو النبات.

وقد أظهر التلقيح المشترك لبذور القرطم بخليط من الازوتوباكتر و الازوسبيريلليم مع الرى بماء ملوحتة ٢٠٠٠ جزء فى المليون الى الحصول على أعلى القيم من النشاط الميكروبى فى منطقة الريزوسفير وكذلك صفات النمو والمحصول ومكوناتة والتركيب الكيماوى فى البذور خلال فترة نمو النبات.

قام بتحكيم البحث

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Table (8) Effect of interaction between biofertilization and saline irrigation on plant height and number of branches/plant of Safflower during 2007/2008 and 2008/2009 seasons.

Irrigation	Inoculation	Plant height (cm)						Number of branches/plant					
		First season			Second season			First season			Second season		
		Days after sowing			Days after sowing			Days after sowing			Days after sowing		
		35	70	120	35	70	120	35	70	120	35	70	120
2000 µgl^{-1}	Control	33.3	81.9	114.3	30.3	115.8	132.2	5.2	7.1	9.2	4.8	8.1	10.2
	Azotobacter	36.8	171.6	174.7	42.7	176.2	176.9	6.8	12.3	15.4	8.3	12.7	16.8
	Azospirllum	35.7	162.3	170.4	42.2	165.8	173.4	6.4	11.4	13.1	6.1	12.1	14.7
	Mixed	41.2	177.4	182.4	44.4	187.3	184.3	9.1	18.7	20.9	10.9	20.8	23.3
3000 µgl^{-1}	Control	30.4	77.2	110.4	27.3	115.3	125.1	4.4	5.9	6.8	4.4	7.2	9.2
	Azotobacter	35.2	154.7	160.7	38.9	146.7	160.4	6.2	11.2	14.2	7.3	12.1	15.9
	Azospirllum	33.7	142.4	149.3	36.7	145.4	151.3	5.3	8.7	11.7	5.2	10.3	14.3
	Mixed	38.4	162.3	172.8	41.4	170.1	174.4	7.1	15.3	18.3	8.1	16.8	20.8
4000 µgl^{-1}	Control	27.2	72.3	100.4	23.1	100.2	120.3	2.3	4.3	5.3	3.4	6.4	7.3
	Azotobacter	31.6	146.8	155.9	38.3	150.4	160.2	4.4	9.1	11.2	5.2	9.3	12.7
	Azospirllum	30.3	135.4	142.2	33.8	137.9	153.1	3.2	7.4	8.9	4.3	8.2	12.2
	Mixed	36.4	153.9	168.7	39.2	156.6	170.9	5.1	11.8	14.3	6.4	13.4	17.8
L.S.D. at 5% for													
	Salinity	0.33	19.64	9.16	0.19	8.66	9.82	1.10	0.29	0.32	0.57	2.01	0.29
	Biofertilizer	0.29	8.61	5.22	0.17	4.04	2.86	0.42	0.48	0.40	0.51	0.45	0.38
	Interaction	0.50	N.S	N.S	0.29	3.83	4.95	N.S	0.83	0.69	0.89	1.11	0.65

Table (9) Effect of interaction between biofertilization and saline irrigation on fresh and dry weight/plant of Safflower during 2007/2008 and 2008/2009 seasons.

Irrigation	Inoculation	Fresh weight						Dray weight					
		First season			Second season			First season			Second season		
		Days after sowing						Days after sowing					
		35	70	120	35	70	120	35	70	120	35	70	120
2000 µgl^{-1}	Control	107.2	249.7	300.2	92.3	270.2	311.9	9.7	19	14.9	8.9	21.5	15.8
	Azotobacter	260.4	550.3	590.4	280.1	546.8	615.2	17.7	57.4	56.0	19.2	58.1	59.2
	Azospirllum	230.3	370.1	4102	254.6	432.4	511.7	15.7	54.3	47.1	17.6	56.2	59.9
	Mixed	284.6	574.9	601.7	337.8	600.3	670.4	21.2	59.1	63.3	27.1	65.4	71.5
3000 µgl^{-1}	Control	184.7	205.8	284.6	220.7	250.2	304.7	6.7	13.2	13.0	6.4	19.1	24.6
	Azotobacter	205.2	245.2	384.9	237.6	422.4	500.3	12.3	36.8	33.6	14.8	47.9	45.6
	Azospirllum	204.6	284.6	375.3	236.3	364.1	410.2	11.5	34.3	32.7	13.2	43.6	42.8
	Mixed	250.4	490.3	520.1	287.4	504.8	561.6	15.7	41.4	44.1	28.2	51.1	57.7
4000 µgl^{-1}	Control	90.3	150.2	203.7	185.9	214.6	284.8	2.9	12.8	13.0	5.9	17.4	20.2
	Azotobacter	110.1	270.4	304.9	205.6	400.3	440.2	12.9	28.5	23.6	14.2	34.3	40.1
	Azospirllum	114.8	217.7	300.3	166.7	310.1	370.1	9.6	23.4	22.8	12.9	33.3	38.1
	Mixed	190.3	264.9	351.6	230.8	415.7	460.4	13.3	37.4	31.9	16.2	43.1	51.7
L.S.D. at 5% for													
	Salinity	10.47	4.79	5.97	3.11	18.89	4.39	0.38	0.44	0.42	1.07	1.89	5.00
	Biofertilizer	5.59	4.36	4.37	4.05	16.51	7.57	0.33	0.80	0.99	0.58	1.65	3.89
	Interaction	9.69	7.54	7.56	7.01	28.58	13.10	0.57	1.58	2.41	1.00	2.86	6.74

Table (10) Effect of interaction between biofertilization and saline irrigation on yield components and chemical contents of Safflower at harvest Yield and its components

Irrigation	Inoculation	First season									
		Yield and its components						Chemical contents			
		No. of heads /plant	Diameter of head (cm)	No. of seeds /head	Weight of 100 seed(g)	Seed yield Kg fed^{-1}	Stand %	Protein (%)	Phosphorus (%)	Oil (%)	Oil yield kg/fed
2000 µgl^{-1}	Control	6.9	2.50	32.48	3.02	405.05	19.4	5.25	0.27	27.15	117.43
	Azotobacter	14.0	3.04	48.28	3.72	715.69	20.4	8.29	0.34	32.18	248.43
	Azospirllum	11.8	2.99	47.08	3.55	680.00	19.4	7.21	0.33	30.69	225.53
	Mixed	19.7	3.25	51.18	4.08	726.52	21.6	10.03	0.35	38.41	297.99
3000 µgl^{-1}	Control	5.6	2.43	28.48	2.48	383.76	15.9	4.68	0.23	21.44	104.23
	Azotobacter	12.5	2.53	32.53	3.26	520.35	18.2	6.97	0.31	25.87	166.93
	Azospirllum	10.7	2.43	32.43	3.21	507.95	17.3	6.92	0.30	23.86	152.43
	Mixed	18.0	2.78	45.75	3.44	587.85	20.0	7.10	0.32	30.11	214.53
4000 µgl^{-1}	Control	3.8	2.37	27.13	1.84	372.90	8.4	3.92	0.19	17.71	90.73
	Azotobacter	9.4	2.43	29.72	3.08	467.35	15.5	6.73	0.27	23.74	143.97
	Azospirllum	8.5	2.39	27.80	3.05	428.76	12.6	6.08	0.25	20.86	119.03
	Mixed	14.9	2.48	31.37	3.19	488.45	16.4	6.97	0.30	25.01	157.03
L.S.D. at 5% for											
	Salinity	0.851	0.159	0.717	0.159	0.319	1.328	1.461	0.027	0.053	2.018
	Biofertilizer	1.009	0.186	1.169	0.186	0.398	1.514	2.470	0.053	0.053	2.311
	Interaction	0.271	0.009	0.197	0.009	0.042	0.664	0.807	N.S	7.967	1.548
	Second season										
2000 µgl^{-1}	Control	10.5	3.10	39.08	7.22	419.65	28.2	9.4	0.45	37.79	133.87
	Azotobacter	17.6	3.64	54.88	7.92	730.29	35.0	12.4	0.52	42.82	264.87
	Azospirllum	15.4	3.59	53.68	7.75	694.60	34.0	11.4	0.51	41.33	241.97
	Mixed	23.3	3.85	57.78	8.28	741.12	36.2	14.2	0.53	49.05	314.43
3000 µgl^{-1}	Control	9.2	3.03	35.08	6.68	398.36	30.5	8.8	0.41	32.08	120.67
	Azotobacter	16.1	3.13	39.13	7.46	534.95	32.8	11.1	0.49	36.51	183.37
	Azospirllum	14.3	3.03	39.03	7.41	522.55	31.9	11.1	0.48	34.50	168.87
	Mixed	21.6	3.38	52.35	7.64	602.45	34.6	11.2	0.50	40.75	230.97
4000 µgl^{-1}	Control	7.4	2.97	33.73	6.04	387.5	23.0	8.1	0.37	28.35	107.17
	Azotobacter	13.0	3.03	36.32	7.28	481.95	30.1	10.9	0.45	34.38	160.41
	Azospirllum	12.1	2.99	34.40	7.25	443.36	27.2	10.2	0.43	31.50	135.47
	Mixed	18.5	3.08	37.97	7.39	503.05	31.0	11.1	0.48	35.65	173.47
L.S.D. at 5% for											
	Salinity	1.062	0.159	0.876	0.053	0.133	1.514	0.345	0.053	0.451	15.19
	Biofertilizer	1.169	0.186	1.169	0.053	0.159	1.726	0.478	0.053	0.505	17.37
	Interaction	0.451	0.009	0.295	6.108	0.007	0.886	0.045	N.S	0.074	88.53