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PROFITABILITY OF USING HALOTOLERANT N_2-FIXERS AND ITS ROLE IN IMPROVING SOME SOIL PROPERTIES AND PRODUCTIVITY UNDER TOOR SINAI, SOUTH SINAI CONDITION

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ABSTRACT

Two winter wheat field experiments were designed in El-Gabeal at Toor Sinai, south Sinai during (2002-2003) and (2003-2004) seasons, to study the possible amelioration for some physical, biological and nutritional status of saline calcareous soils and its productivity using biotechnologies. Wheat (Sakha 8) was sown as a test crop for soil productivity The treatments comprised (1) halotolerant N_2-fixers biofertilizers as inoculation with (*Azospirillum* sp.+ *Klebsiella sp.*), N_2-fixing cyanobacteria (*Nostoc* sp.+ *Anabaena* sp.) and uninoculated. (2) applied N-levels, as 20, 40, 60, 80 and 100 kg N/fed. (3) methods of biofertilization as soaking grains, grain inoculation and (soaking+ grain inoculation).

Results showed improvements for several soil physical parameters. Bulk density, saturated hydraulic conductivity (SHC) and total porosity were ameliorated as a result of biofertilization referring to uninoculated and control (20 kg N/fed.) treatments. N_2-fixers cyanobacteria (*Nostoc* sp.+ *Anabaena* sp) by (soaking + grain inoculation) method was the best supporters in certain suitable soil structure, particularly in the presence of 60 kg N/fed treatment.

A positive effect of either grain or (soaking + grain inoculation) method was noticed by with both (*Azospirillum* sp. + *Klebsiella sp.*) and cyanobacteria (*Nostoc* sp + *Anabaena* sp.) inoculation on their densities and dehydrogenase activity in wheat rhizosphere plants, particularly at 75^{th} day of sowing in the presence of 100 kg N/fed *Klebsiella sp.* gave high densities compared with *Azospirillum* sp., While nitrogenase activity and available N in soil considerably increased in comparison to uninoculated and control (20 kg N/fed) treatments, particularly at 75^{th} day of sowing in the presence of 60 kg N/fed and inoculation with (*Azospirillum* sp.+ *Klebsiella sp.*) treatments.

Wheat grains and straw yields as well as N-fertilizer use efficiency were enhanced in response to different methods of biofertilization and N-levels, especially with soaking + grain inoculation method in the presence of 60 kg N/fed achieving relatively high increases compared to uninoculated treatments amended with 80 or 100 kg N/fed. Similar trends were also observed for the content of nitrogen, total amino acids and crude protein in grains and straw in response to inoculation with (*Azospirillum* sp.+ *Klebsiella sp.*) which in turn was more effective.

This means that (soaking+ grain inoculation) method with halotolerant N_2-fixing i.e., *Azospirillum* sp.+ *Klebsiella sp.*) and cyanobacteria (*Nostoc sp.* and *Anabaena* sp.) may save (20-40) kg N/fed.

Keywords: Nitrogen fertilizer, saline soil, calcareous soil, biofertilization, N_2-fixng bacteria, cyanobacteria, soaking, grains inoculation, wheat production and South Sinai.

INTRODUCTION

Toor Sinai soil suffers from two main problems, high content of $CaCO_3$ and high salinity of both soil and irrigation water. Sustainable development of such soils means creating desirable characteristics, *i.e.,* physical, biological and nutritional in one side and maintaining these characters as long as possible.

In an attempt to develop productive profitable and sustainable agriculture systems, several agriculturists turn to farming methods, which, are a base on biotechnologies, *i.e..*, *Azospirillum* sp., *Klepsiella* and cyanobacteria (*Nostoc* sp. and *Anabaena* sp.).

Regarding the effect of halotolerant N_2-fixing microbes on a possible amelioration for some physical properties, Rogers and Burns (1994) observed significant increase in the values of soil aggregates stability due to an increase in polysaccharide content of soils, when inoculated with N_2-fixing cyanobacteria. Also, due to the mucilaginous and fragile thalli of cyanobacteria, which, formed a compact grey substratum firmly holding the soil particles together which checked both wind- and water mediated soil erosion, particularly in light and sandy soils, resulting in an improvement in the water-holding capacity and aeration status of soil (Tiwari *et al.*, 1991). Such polysaccharide or extracellular mucilages of cyanobacteria can account for as much as 44% of their dry weight (Mandal *et al.,* 1999). Though, Omar and Hammouda (1998) added that the increases of both organic matter and exopolysaccharide, which, reached to 400 mg/days after planting are due to free nitrogen fixers inoculation (*Azospirillum* sp., *Klebsiella sp.* and *Azotobacter* sp.).

Boutros *et al.* (1987) and Reda *et al.* (2004) found that biofertilization technique with N_2-fixers (*Azotobacter chrococcum, Azospirillum* sp. and N_2-fixing Bacilli) either as single or mixture led to maximize the counts and activity of each of them in the new desert soil. El-Sheshtawy (2000) noticed that low doses of chemical nitrogen fertilizer, promote the association of microbes with plants. Although, El-Borollosy *et al.* (2000) and Mohamed (2004) added that heavy doses of inorganic N-fertilizers inhibited N_2-ase activity in rhizospheric soil of wheat plants. It reached their maximal levels at 75th day of cultivation then markedly dropped. Milesev *et al.* (1996) and Zaghloul *et al.* (1996) reported that wheat inoculation with *Azospirillum* sp. increased soil population of *Azospirillum* sp. and consequently increased dehydrogenase activity. Such enzyme is considered as an index for the biological activity in soils (Ghazal, 1980). While, Abd El-Rasoul *et al.* (2004) added that wheat inoculation with N_2-fixing cyanobacteria (*Nostoc* sp.) combined with 1/4 recommended N-dose led to increase soil dehydrogenase activity. They explained that inoculation with cyanobacteria increased the soil microbial biomass which in turn increased dehydrogenase activity due to the massive respiration process with increasing microorganisms population in soil.

The stimulatory effect of dizotophes on increasing crop yield attributed to not only to N_2-fixation activity or production of some compounds like polysaccharides, peptides, lipids during their growth in soils, but also to the production of growth promoters. Aly (2003) reported that *Azospirillum* sp. produces some biological activity substances, which, help in greater absorption of nutrients from soil. Also, Mandal *et al.* (1999) reported the effect of N_2-fixing cyanobacteria on growth and yield of crops in the presence of N-fertilizers has commonly been described to the production of growth-promoting substances, *i.e..*, gibberellins, cytokinin, auxin, abscisic acids, vitamins, antibiotics and amino acids.

The main target of this study is to evaluate the improvement parameters: physically, biologically and nutritionally for biotechnologies treated soils under different levels of inorganic N-fertilizer, as well as wheat production under Toor Sinai conditions.

MATERIALS AND METHODS

Two winter field experiments were designed in El-Gabeal at Toor Sinai, south Sinai during (2002-2003) and (2003-2004) seasons to study a possible amelioration for some properties of saline calcareous soils and its productivity using halotolerant N_2-fixers biofertilizers. The previous crop was wheat (Sakha 8). Soil analyses to 25 cm depth before planting at each season are recorded in Table (1) and irrigation water analysis was presented in Table (2).

Table (1): Some physical and chemical properties of El-Gabeal soil.

A-Physical properties

Particle size distribution (%)				Texture class	Bulk Density (g/cm^3)	Hydraulic conductivity (cm/h)	Total porosity (%)
Coarse sand	Fine sand	Silt	Clay				
18 1	62.1	12.3	7.5	Loamy sand	1.55	20.6	42.8

B. Chemical properties

pH	Ece	Soluble cations (meq/L)				Soluble anions (meq/L)				SAR	$CaCO_3$ (%)	O.M. (%)
(1:2.5)	dS/m	Ca^{2+}	Mg^{2+}	Na^+	K^+	CO_3^{2-}	HCO_3^-	$SO_4^=$	Cl^-			
8.1	10.9	44.1	6.21	51.3	1.62	--	4.63	29.9	68.7	11.8	51.3	0.21

Table (2): Chemical composition of the saline well water used for irrigation.

Ece	Soluble cations (meq/L)				Soluble anions (meq/L)				SAR
dS/m	Ca^{2+}	Mg^{2+}	Na^+	K^+	CO_3^{2-}	HCO_3^-	$SO_4^=$	Cl^-	
6.92	23 6	5.10	32.0	0.46	---	4.86	28.6	27.7	8.41

In a split-split plot design with four replicates, the plot consisted of 7 rows 3m long with 0.60 cm apart giving a plot area of 12.6 m^2. The experimental soil ploughed twice and fertilized with 150 kg/fed of superphosphate (15% P_2O_5) and 50 kg/fed potassium sulphate (48% K_2O). Five rates of mineral nitrogen fertilizer (20, 40, 60, 80 and 100 kg N/fed.) were applied with the form of ammonium nitrate 33.5%N, as the main plots. nitrogen applied at five equal split doses (at sowing and then every 20 days) before irrigation. Each main plot was divided into three subplots having; *i.e..* (*Azospirillum + Klepsiella*), uninoculated treatment (Unino.), inoculation with a

Table (3): Influence of N levels and biofertilization with dizotrophes by soaking and grain inoculation on soil bulk density, saturated hydraulic conductivity and total porosity after harvesting during two seasons (combined analysis).

N-fertilizer	Bulk density (g/cm3)									Saturated hydraulic conductivity (cm/h)									Total porosity (%)								
Levels	Unino.			Azo.+Kl.			Nos.+Ana.			Unino.			Azo.+Kl.			Nos.+Ana.			Unino.			Azo.+Kl.			Nos.+Ana.		
(kg/fed.)	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G
20*	1.53	1.52	1.53	1.51	1.49	1.50	1.52	1.50	1.50	20.1	20.3	20.4	20.4	20.3	20.1	20.3	20.0	20.1	43.2	43.6	43.4	44.1	46.1	47.1	44.6	46.8	47.3
40	1.52	1.52	1.52	1.51	1.48	1.47	1.50	1.48	1.47	19.8	20.0	20.2	18.8	17.5	17.5	19.3	18.0	17.6	43.5	43.5	43.6	44.7	47.0	48.0	45.1	47.4	48.7
60	1.54	1.50	1.51	1.49	1.46	1.46	1.49	1.45	1.44	19.7	19.9	20.1	19.0	18.4	18.0	18.6	17.9	17.2	43.7	43.6	43.7	45.6	47.9	48.9	46.6	48.8	50.7
80	1.50	1.51	1.52	1.51	1.50	1.50	1.50	1.49	1.49	19.7	20.1	20.0	19.3	18.9	18.5	19.3	18.2	17.6	43.7	43.7	43.8	45.6	46.7	47.8	46.2	48.5	49.8
100	1.50	1.51	1.52	1.51	1.50	1.50	1.51	1.50	1.50	19.8	20.1	20.0	19.5	19.1	18.7	19.3	18.9	18.0	43.8	43.9	43.9	45.5	46.8	47.9	46.3	48.6	49.6

* Control treatment = Uninoculated with bacteria under 20 kg/fed of N-fertilizer.

2.1.1. Densities of *Azospirillum* sp. and *Klebsiella sp.*:-

Data recorded in Table (4) showed that grain inoculated treatments of wheat plants remarkably increased the densities of *Azospirillum* sp. and *Klebsiella sp.* in the rhizosphere of the inoculated plants. Stimulation of microbial growth was more pronounced when (Azo.+Kl.) biofertilizaer was the source of nitrogen than in (Nos.+Ana.). These results are true in most of plant growth stages and in the presence of all tested N-doses. Such results may be because the nitrogenous materials released via nitrogen fixers microorganisms (cyanobacteria and *Azospirillum* spp.) into soil, which in turn enhanced the microbial growth. These explanations are in accordance with those stated by **Reda *et al.*, (2004)**.

In all cases, rhizospheric *Klebsiella sp.* were in high densities compared with *Azospirillum* sp. populations, since, their densities reached 80×10^4 cells/g dry rhizospheric soil at 75th day of sowing in mix_1 inoculated treatments amended with 100 kg N/fed. Microbial densities of two groups under different levels of N-fertilizer treatments gradually increased, reaching their maximal levels at 75th day, then decreased thereafter. Although, increasing N dose of chemical N-fertilizer resulted in an increase in microbial growth reaching its maximal in the presence of 100 kg N/fed. Also, it is not a surprising result that uninoculated plots gave the lowest count of all tested N_2-fixers in wheat rhizosphere.

2.1.2. Total count of cyanobacteria:-

Regarding soaking and grain inoculation with (*Azospirillum* sp.+ *Klebsiella sp.*) and/or cyanobacteria (*Nostoc* sp.+ *Anabaena* sp.) and their influence on total count of cyanobacteria, data in Table (4) indicated that densities of cyanobacteria in wheat rhizosphere plants increased by soaking, grain inoculation or (soaking+ grain inoculation) methods with a Mix_2 (*Nostoc* sp.+ *Anabaena* sp.) and Mix_1 (*Azospirillum* sp. + *Klebsiella sp.*). These results are true at the most of plant stages and in the presence of all tested N-doses compared with 20 kg N/fed treatment (control). Data also clarified that the inoculation with N_2-fixing cyanobacteria (*Nostoc* sp.+ *Anabaena* sp.) was more effective in increasing total count cyanobacteria than that of (*Azospirillum* sp.+ *Klebsiella sp.*) inoculation, particularly by (soaking + grain inoculation) method, in the presence of 100 kg N/fed treatment, when their densities reached (173×10^3 cfu/g dry rhizospheric soil.

It may be worthy to mention that total count cyanobacteria under different methods and N-fertilization levels gradually increased in wheat rhizosphere reaching their maximal levels at 75th day of cultivation and decreased thereafter.

2.2. N_2-ase activity in rhizospheric soil:-

In contrast to microbial densities in rhizosphere of wheat plants, when they increased by increasing N-dose, giving their maximal in 100 kg N/fed, N_2-ase activity showed an opposite trend up to 60 kg N/fed (Table 5).

Data also revealed that N_2-ase activity for inoculation with (Azo.+Kl.) treatment was appreciably higher than that detected in inoculation with (Nos. sp+Ana. sp) in rhizosphere of tested plants.

Table (4): Influence of N-levels and biofertilization with dizotrophes by soaking and grain inoculation on *Azospirillum* sp., *Klepsiella* sp. and total count of *Cyanobacteria* under different growth stages during two seasons (combined analysis).

N-fertilizer levels (kg/fed.)	Days after sowing	*Azospirillum* sp. $\times10^4$ cfu/g dry rhizospheric soil									*Klepsiella* sp. $\times10^4$ cfu/g dry rhizospheric soil									Total count of *Cyanobacteria* $\times10^3$ cfu/g dry rhizospheric soil								
		Unino.			Azo.+Kl.			Nos.+Ana.			Unino.			Azo.+Kl.			Nos.+Ana.			Unino.			Azo.+Kl.			Nos.+Ana.		
		S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G
	45	6.50	6.10	6.60	6.90	9.15	9.81	6.80	9.00	9.66	15.1	14.8	14.5	15.6	18.7	19.0	15.3	17.3	18.2	38.6	37.9	38.5	40.3	43.6	43.3	39.6	56.2	59.1
20*	75	8.15	8.21	8.10	8.79	22.4	26.1	8.24	20.3	25.0	23.5	22.9	23.1	24.1	28.0	28.6	24.1	26.6	27.7	47.1	42.4	42.0	45.1	51.3	52.0	45.8	68.7	70.3
(control)	120	2.66	2.41	26.0	2.70	5.31	5.63	2.66	8.01	8.22	6.67	6.51	6.73	6.91	7.57	7.90	6.81	7.06	7.14	6.75	6.80	6.70	6.67	6.91	7.13	7.20	8.20	8.90
	45	13.1	14.2	13.8	15.1	23.6	25.5	14.6	20.6	21.4	18.3	18.7	17.9	19.2	26.9	29.7	19.4	20.8	22.7	48.3	48.6	48.1	48.6	52.1	52.9	48.4	70.1	76.2
40	75	19.2	18.9	19.5	19.9	29.8	32.3	19.6	28.7	29.6	31.1	30.8	31.4	32.6	37.6	38.5	30.9	34.5	35.1	56.7	56.9	56.9	57.3	70.6	70.5	57.2	94.8	98.2
	120	6.21	6.34	6.07	7.06	13.7	15.1	6.65	12.6	13.0	9.16	9.07	8.99	9.29	15.4	16.9	8.96	16.3	16.9	8.13	8.20	8.17	8.21	8.91	8.88	8.17	13.6	14.1
	45	23.5	23.9	24.1	24.9	36.7	41.1	29.5	32.1	33.9	29.7	29.9	30.1	30.8	37.3	47.9	30.0	33.7	34.2	63.6	53.0	53.4	54.0	78.7	80.3	54.4	106	116
60	75	35.7	33.6	36.0	39.6	61.7	63.3	39.0	58.1	58.7	44.6	46.3	43.9	48.6	69.5	71.0	47.3	58.9	59.6	69.4	68.9	69.7	69.0	89.6	89.8	69.7	138	146
	120	8.91	9.10	9.16	10.1	14.6	14.7	9.98	13.9	4.10	15.3	16.0	15.7	15.4	17.7	18.3	15.0	16.8	17.1	8.96	9.00	88.50	9.17	9.19	9.31	8.99	18.7	20.3
	45	31.6	32.0	31.8	33.2	56.7	61.1	33.6	52.6	53.3	38.9	39.3	38.6	40.5	66.8	66.3	39.6	62.3	62.8	69.8	70.2	70.6	69.3	86.6	90.0	69.8	129	135
80	75	40.1	40.4	41.6	42.1	70.5	71.6	41.8	63.3	66.1	50.0	49.6	50.3	53.1	75.4	76.2	52.4	66.0	69.2	77.5	76.8	77.0	78.0	94.0	96.3	78.6	158	166
	120	10.3	10.7	9.96	10.9	15.1	15.9	10.6	14.9	15.3	16.9	16.6	17.0	17.3	17.9	18.9	17.4	17.3	17.8	9.09	8.86	9.08	8.96	9.96	9.87	9.11	18.5	19.2
	45	38.9	39.5	38.5	40.1	64.6	65.2	39.8	54.7	59.1	46.7	47.0	46.6	47.3	69.1	96.9	47.5	68.2	68.9	70.6	69.9	70.9	7.1	96.0	98.0	70.8	140	137
100	75	43.1	43.6	43.5	45.2	76.7	79.3	44.2	68.1	69.3	55.7	55.9	56.0	56.3	79.8	80.3	56.0	76.9	77.7	83.6	83.1	83.9	84.2	108	113	84.7	169	173
	120	11.1	11.0	10.9	12.3	16.3	16.8	11.9	15.9	16.3	17.3	18.1	17.6	17.5	18.3	18.9	17.1	18.0	18.5	9.85	10.0	9.80	10.2	10.6	10.5	9.93	19.8	20.3

* Control treatment = Uninoculated with bacteria under 20 kg/fed of N-fertilizer.

The highest N_2-ase activity value recorded in (soaking +grain inoculation) method was only 10.62 *u*mol C_2H_4/g dry soil/h in treatments inoculated with (Azo.+Kl.) after 75th day of cultivation in the presence of 60 kg N/fed, in comparison to 8.25 *u*mol C_2H_2/g dry soil/h for same treatment in presence of (Nos. sp+ Ana sp. inoculation.

It was generally observed that N_2-ase activities recorded in both inoculated and uninoculated plants gradually increased reaching their maximal levels at the 75th day of cultivation, then markedly dropped. These results are in line with those of Mohamed (2000) and Alkasas (2002).

2.3. Dehydrogenase activity in rhizospheric soil:-

Data in Table (5) revealed that similar to microbial densities, dehydrogenase activity in rhizosphere of wheat plants was more affected by (soaking+grain inoculation) method than that using in soaking or grain inoculation separately, particularly in the case of inoculation with a mixture of (*Azospirillum* sp.+ *Klebsiella sp.*) in the presence of 100 kg N/fed treatment. Inoculation with any of the tested N-fixers microorganisms to wheat plants increased the microbial population in wheat rhizosphere area which in turn increased the rate of respiration in this area and resulted in increasing the dehydrogenase activity. Dehydrogenase enzyme is one of the enzyme group that shares in the respiration process. This explanations in line with those of Zaghloul *el al.* (1996) and Abd El-Rasoul *et al.* (2004).

With respect to supplementation with graded levels of chemical N-fertilizer, data revealed that higher dose of N-fertilizer (100 kg N/fed) improved dehydrogenase activity in rhizosphere of wheat plants compared to lower ones in uninoculated treatment.

Again, and similar to the recorded N_2-ase activities, dehydrogenase activities in both inoculated and uninoculated plants gradually increased reaching their maximal levels at the 75th day of cultivation, then markedly dropped. These results are in line with those of Alkasas (2002).

3. Available N in soil:

The availability of soil nitrogen was affected by different methods of biofertilization, inoculation and inorganic N-fertilizer treatments (Table 5). Available N was increased with application of N-doses, inoculation with (*Azospirillum* sp.+ *Klebsiella sp.*) or (*Nostoc* sp.+ *Anabaena* sp.) as well as grain or (soaking+grain inoculation) methods compared with control (20 kg N/fed) and uninoculated treatments. (Soaking+ grain inoculation) method was more pronounced particularly with inoculation treatments than soaking or grain inoculation individually.

The effect of inoculants on the availability of N in soil was more obvious with the mixture of *Azospirillum* sp. and *Klebsiella sp.* than the mixture of Cyanobacteria (*Nostoc* sp.+ *Anabaena* sp.) inoculants. Also, data showed that application of 60 kg N/fed increased N available in soil. However, application of inorganic-N beyond this level was slightly increased N available, particularly inoculation with (*Azospirillum* sp.+ *Klebsiella sp.*) at the 75th day from sowing.

Table (5): Influence of N- levels and biofertilization with dizotrophes by soaking and grain inoculation on nitrogenase activity, dehydrogenase activity and available nitrogen in soils under different growth stages during two seasos (combined analysis).

N-fertilizer levels (kg/fed.)	Days after sowing	N_2-ase activity (umole C_2H_4/g soil/h)								
		Unino.			Azo.+Kl.			Nos.+Ana.		
		S	G	S+G	S	G	S+G	S	G	S+G
20* (control)	45	1.00	1.09	1.10	1.06	1.36	1.45	1.00	1.22	1.26
	75	1.16	1.13	1.16	1.19	1.46	1.58	1.13	1.30	1.31
	120	0.81	0.73	0.79	0.96	1.30	1.39	0.83	1.11	1.20
40	45	1.75	1.80	1.79	1.78	2.86	2.99	1.70	2.55	2.60
	75	3.95	3.90	3.86	3.99	6.04	6.30	3.93	4.88	4.93
	120	1.10	1.14	1.12	1.16	2.66	2.71	1.16	2.01	2.19
60	45	2.96	3.07	2.99	3.11	4.81	5.13	2.98	4.36	4.93
	75	5.75	5.69	5.71	5.76	9.74	19.62	5.73	7.96	8.25
	120	2.13	2.19	2.12	2.16	9.21	4.37	2.13	2.98	3.56
80	45	2.64	2.70	2.66	2.70	3.06	3.33	2.68	2.96	3.01
	75	2.99	4.06	4.00	4.11	4.53	4.81	4.10	4.39	4.56
	120	0.76	0.79	0.80	0.83	1.18	1.32	0.84	0.96	1.17
100	45	1.89	1.92	1.90	1.89	1.93	1.93	1.88	1.89	1.90
	75	3.25	3.27	3.30	3.30	3.31	3.30	3.28	3.30	3.29
	120	1.88	0.86	0.89	0.90	0.71	0.69	0.58	0.70	0.70

N-fertilizer levels (kg/fed.)	Days after sowing	Dehydrogenase activity (ug TPF/g soil/day								
		Unino.			Azo.+Kl.			Nos.+Ana.		
		S	G	S+G	S	G	S+G	S	G	S+G
20* (control)	45	8.04	8.16	8.11	8.15	8.18	8.21	8.13	8.16	8.18
	75	11.4	12.0	12.1	11.9	14.6	17.1	12.1	13.9	14.2
	120	4.05	4.71	4.71	4.33	6.20	7.03	4.91	5.56	6.23
40	45	20.3	19.8	20.0	21.2	27.6	32.5	20.6	25.1	29.9
	75	36.3	35.6	37.1	36.9	42.1	48.0	36.1	39.3	40.2
	120	17.6	17.0	17.3	17.9	26.8	26.9	17.0	21.6	22.9
60	45	32.1	32.7	32.0	32.9	40.1	47.6	32.4	38.3	41.7
	75	49.0	48.2	48.6	49.3	56.5	62.4	49.0	52.3	59.6
	120	20.7	19.9	20.6	20.9	25.8	30.1	20.2	23.4	27.0
80	45	41.6	41.9	42.1	42.5	48.9	55.9	42.2	44.6	48.3
	75	60.9	60.3	60.0	62.4	69.5	73.9	61.8	66.5	73.1
	120	38.4	34.6	35.0	35.9	39.9	45.3	35.3	38.7	42.8
100	45	54.3	54.9	54.0	54.9	60.6	68.3	54.6	59.2	64.4
	75	72.1	71.9	71.5	72.4	78.4	85.8	72.0	76.4	81.9
	120	44.6	42.2	43.3	44.0	46.3	49.5	42.6	42.9	48.0

N-fertilizer levels (kg/fed.)	Days after sowing	Available Nitrogen (ppm)								
		Unino.			Azo.+Kl.			Nos.+Ana.		
		S	G	S+G	S	G	S+G	S	G	S+G
20* (control)	45	98.7	96.3	97.9	91.0	126	130	90.1	115	125
	75	120	123	119	112	137	139	115	129	134
	120	83.0	81.0	80.9	77.6	101	109	78.9	91.6	99.0
40	45	113	111	112	106	136	147	110	125	131
	75	135	135	133	129	159	166	132	145	152
	120	89.7	89.1	98.9	89.0	113	119	89.3	106	110
60	45	148	146	149	140	153	166	146	150	161
	75	169	164	165	170	190	199	166	184	189
	120	101	98.3	106	98.1	119	125	98.7	112	119
80	45	156	150	153	150	156	170	152	154	169
	75	174	168	176	176	197	203	170	193	199
	120	109	99.2	100	99.3	122	126	101	118	123
100	45	158	149	156	156	159	176	155	159	171
	75	179	179	177	180	199	207	176	199	201
	120	110	104	111	108	124	125	113	121	126

* Control treatment = Uninoculated with bacteria under 20 kg/fed of N-fertilizer.

These results are harmony with those reported by Mohamed (2004) using a mixture of (*Azospirillum* sp.+*Klebsiella sp.*) and Abd El-Rasoul *et al.* (2004), using cyanobacteria.

4. Grains, straw yield and N-fertilizer use efficiency by wheat plants:

Stress conditions of directly reflected on grain, straw yield and N-fertilizer use efficiency by wheat plants (Table 6). In general, very low yield components and N-fertilizer use efficiency were attributed to 20 kg N/fed particularly with uninoculated treatments whereas (1.88, 1.78 and 1.81 ard./fed), (0.91, 0.95 and 0.93 ton/fed) and (13.6, 12.9 and 13.1 kg grains/N fertilizer unit) were recorded for grain yield, straw yield and N-fertilizer use efficiency in soaking, grain inoculation and (soaking+ grain inoculation) methods, respectively, while leveling the N-dose to 100 kg N/fed increased yield components and N-fertilizer use efficiency. Different methods of biofertilization with halotolerant diazotrophs namly (*Azospirillum* sp. + *Klebsiella sp.*) and cyanobacteria (*Nostoc* sp.+ *Anabaena* sp.) had a significant influence on grain, straw yields and N-fertilizer use efficiency, particularly (soaking+ grain inoculation) method with (*Azospirillum* sp.+ *Klebsiella sp.*) treatment in the presence of 60 kg N/fed compared with uninoculated treatments amended with 80 or 100 kg N/fed whereas (13.0, 13.3 and 13.9), 2.59, 2.62 and 2.73) and 31.4, 32.1 and 33.6). This means that (soaking+ grain inoculation) methods with diazotrophs can be save (20-40) kg N/fed.

The stimulatory effect of N_2-fixers on increasing crop yield clearly appeared in this work can be attributed not only to N_2-fixation activity of both inoculated diazotrophs, but also to the production of promoters and number of components like polysaccharides, peptides, lipids during their growth in soils. Aly (2003) revealed that *Azospirillum* spp. has the ability to produce some biological active substances which help in greater absorption of nutrients from soil, which positively affect the plant growth. Also, Mandal *et al.* (1999) reported the effect of N_2-fixing cyanobacteria on growth and yield of crops in the presence of N-fertilizers has commonly been described to the production of growth-promoting substances, *i.e.*., gibberellins, cytokinin, auxin, abscisic acids, vitamins, antibiotics and amino acids. Studies carried out by Pachpande (1990) and Abd-Alla *et al.* (1994) reported that soaking grains of wheat with extract of N_2-fixing cyanobacterium promoted germination and their subsequent growth and development. Also, Abd-Alla *et al.* (1994) added that inoculation of wheat with N_2-fixing cyanobacteria either a live or killed lead to a significant increase in dry matter accumulation over controls. Mandal *et al.* (1999) reported that the effect of N-fixing cyanobacteria on growth and yield of crops in the presence of N-fertilizers has commonly been described to the production of growth-promoting substances *i.e.*. gibberellins, cytokinin, auxin, abscisic acids, vitamins, antibiotics and amino acids.

Table (6): Influence of N-levels and biofertilization with dizotrophes by soaking and grain inoculation on grains and straw yield of wheat plants as well as the fertilizer use efficiency during two seasons (combined analysis).

N-fertilizer levels (kg/fed.)	Grain yield (ard./fed.)								
	Unino.			Azo.+Kl.			Nos.+Ana.		
	S	G	S+G	S	G	S+G	S	G	S+G
20*	1.88	1.78	1.81	1.99	2.04	2.21	1.90	1.97	2.11
40	3.85	3.88	3.83	5.30	5.52	5.66	4.80	4.87	4.91
60	6.42	6.46	6.40	13.0	13.3	13.9	11.2	11.9	12.8
80	10.7	10.6	10.7	13.3	13.6	14.1	12.4	12.9	13.7
100	14.2	14.2	14.3	13.9	14.0	14.6	13.8	13.8	14.3

N-fertilizer levels (kg/fed.)	Straw yield (ton/fed.)								
	Unino.			Azo.+Kl.			Nos.+Ana.		
	S	G	S+G	S	G	S+G	S	G	S+G
20*	0.91	0.95	0.93	1.16	1.18	1.28	1.00	1.04	1.19
40	1.16	1.20	1.19	1.27	1.34	1.47	1.21	1.28	1.36
60	1.54	1.52	1.56	2.59	2.62	2.73	2.33	2.40	2.55
80	1.98	2.00	2.09	2.61	2.68	2.76	2.46	2.52	2.66
100	2.70	2.63	2.65	2.73	2.69	2.78	2.68	2.69	2.74

N-fertilizer levels (kg/fed.)	Fertilizer use efficiency**								
	Unino.			Azo.+Kl.			Nos.+Ana.		
	S	G	S+G	S	G	S+G	S	G	S+G
20*	13.6	12.9	13.1	14.4	14.8	16.0	13.8	14.3	15.3
40	14.0	14.1	14.0	19.2	20.0	20.6	17.4	17.7	17.8
60	15.5	15.6	15.5	31.4	32.1	33.6	27.1	28.8	30.9
80	19.4	19.2	19.9	24.1	24.7	25.6	22.5	23.4	24.8
100	20.6	20.6	20.7	20.2	20.3	21.2	20.0	20.0	20.7

* Control treatment = Uninoculated with bacteria under 20 kg/fed of N-fertilizer.

** Fertilizers use efficiency = $\frac{\text{Grain yield (ardeb/fed.)} \times 145}{\text{N-fertilizer level}}$

5. Chemical composition of grains and straw of wheat plants:

5.1. Total nitrogen content:

Data in Table (7) indicated that total nitrogen content in grains and straw of wheat plants, followed the same trend of microbial densities and activities, in the presence of different doses of N-fertilizer together with the three methods of grain treatments. Thus their N contents increased with increasing N-dose in soaking, grain inoculation and (soaking + grain inoculation) treatments, till they reached their maximum (1.94, 1.94 and 2.00%) in grains and (0.66, 0.68 and 0.70%) in straw at soaking, grain inoculation and (soaking + grain inoculation) methods treatments with (*Azospirillum* sp.+ *Klebsiella sp.*) respectively, amended with the 100 kg N/fed. These results are in agreement with Amara and Dahdoh (1997) who stated that indol-3-acetic acid (IAA) and cytokinin are excreated by *Azospirillum* spp. Such hormones induced the proliferation of lateral roots and root hairs which increase nutrient absorbing surfaces. Also, Mussa *et al.* (2003) added that the nitrogen fixing cyanobacteria inoculation to wheat is recently established to substitute partially or entirely the mineral nitrogen utilization.

Concerning the response to N-biofertilization, data indicated that N-content in shoots of wheat plants considerably increased in the case of (soaking+ grain inoculation) method with (*Azospirillum* sp. + *Klebsiella sp.*) rather than N-fixing cyanobacteria (*Nostoc* sp. + *Anabaena* sp.). Diazotrophs application with 60 kg N/fed in all methods gave slightly less total N figures in comparison to treatments amended with the 100 kg N/fed without inoculation. Also, El-Sawah (2000) reported a significant increase in N content of maize plants when grains were soaked with *Azospirillum brasilense* and *Bacillus megatherium*.

5.2. Total amino acids and crude protein:

Data in Table (7) revealed that the content of amino acids and crude protein in wheat grains and straw increased by different methods of biofertilizers compared with uninoculated treatment. Responses were more pronounced in the presence of N-fertilizer, reducing its maximal, particularly in the presence of 100 kg N/fed.. Again, and similar to total N content, amino acids and crude protein contents in grains and straw were more affected by (soaking + grain inoculation) method than other methods, particularly inoculation with (*Azospirillum* sp.+ *Klebsiella sp.*) compared with N_2-fixing cyanobacteria. Diazotrophs together with 60 kg N/fed in all methods, mostly gave nearly similar development obtained by the application of 80 or 100 kg N/fed together without inoculation treatment.

Table (7): Influence of N- levels and biofertilization with dizotrophes by soaking and grain inoculation on total nitrogen, total amino acids and crud protein in grain and straw of wheat plants at harvesting during two seasons (combined analysis).

N-fertilizer levels	Total N (%)									Total amino acids (mg/100 g)									Crud protein (%)								
	Unino.			Azo.+Kl.			Nos.+Ana.			Unino.			Azo.+Kl.			Nos.+Ana.			Unino.			Azo.+Kl.			Nos.+Ana.		
(kg/fed.)	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G	S	G	S+G
Grains																											
20*	1.34	1.40	1.41	1.42	1.44	1.46	1.40	1.41	1.44	5.58	5.83	5.87	5.92	6.00	6.08	5.83	5.87	6.00	7.97	8.33	8.39	8.45	8.57	8.69	8.33	8.39	8.57
40	1.53	1.56	1.55	1.66	1.70	1.76	1.56	1.62	1.69	6.55	6.68	6.64	7.11	7.27	7.49	6.77	6.94	7.27	9.10	9.28	9.22	9.88	10.1	10.4	9.40	9.84	10.1
60	1.68	1.70	1.70	1.80	1.87	1.90	1.73	1.75	1.80	7.50	7.56	7.58	6.03	8.33	8.48	7.73	7.80	8.03	10.0	10.1	10.1	10.7	11.1	11.3	10.3	10.4	10.7
80	1.77	1.75	1.76	1.86	1.90	1.96	1.79	1.82	1.88	7.67	7.59	7.67	8.10	8.25	8.54	7.81	7.88	8.18	10.5	10.4	10.5	11.1	11.3	11.7	10.7	10.8	11.2
100	1.64	1.83	1.86	1.94	1.94	2.00	1.91	1.95	1.99	8.03	7.88	8.10	8.40	8.40	8.76	8.32	8.47	8.61	11.0	10.8	11.1	11.5	11.5	12.0	11.4	11.6	11.8
Straw																											
20*	0.23	0.24	0.22	0.26	0.29	0.33	0.26	0.29	0.31	0.96	1.00	0.92	1.09	1.21	1.37	1.09	1.21	1.29	1.37	1.43	1.31	1.55	1.73	1.96	1.55	1.73	1.84
40	0.27	0.30	0.26	0.39	0.48	0.53	0.36	0.42	0.46	1.16	1.29	1.13	1.67	2.06	2.27	1.54	1.80	1.97	1.61	1.79	1.55	2.32	2.86	3.15	2.14	2.50	2.74
60	0.36	0.35	0.38	0.50	0.58	0.62	0.43	0.50	0.57	1.61	1.56	1.70	2.24	2.59	2.77	1.92	2.24	2.54	2.14	2.08	2.26	2.98	3.45	3.69	2.56	2.98	3.39
80	0.48	0.50	0.46	0.60	0.63	0.68	0.55	0.59	0.64	2.09	2.18	2.00	2.61	2.74	2.96	2.39	2.56	2.78	2.86	2.98	2.74	3.57	3.75	4.05	3.27	3.51	3.81
100	0.64	0.66	0.65	0.66	0.68	0.70	0.84	0.65	0.68	2.78	2.87	2.83	2.87	2.93	3.04	2.32	2.83	2.96	3.81	3.93	3.87	3.93	4.01	4.17	3.81	3.87	4.05

* Control treatment = Uninoculated with bacteria under 20 kg/fed of N-fertilizer

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الأستفادة من مثبتات الأزوت المقاومة للملوحة وعلاقتها بتحسين بعض خـواص التربة وإنتاجيتها تحت ظروف طور سيناء – جنوب سيناء

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صممت تجربتين حقليتين على محصول القمح صنف سخا ٨ فى منطقة الجبيل بطـور سـيناء محافظة جنوب سيناء خلال الموسمين الشتويين (٢٠٠٢-٢٠٠٣)، (٢٠٠٣-٢٠٠٤) لدراسة مدى الإستفادة من مثبتات الأزوت المقاومة للملوحة فى تحسين بعض خواص التربة وإنتاجيتها تحت ظروف منطقة الجبيل بطور سيناء.

وتضمنت المعاملات الأتى:-

١- معاملات التسميد النيتروجينى المعدنى بمعدلات ٢٠، ٤٠، ٦٠، ٨٠، ١٠٠كجم/فدان

٢- معاملات التسميد الحيوى بمثبتات الأزوت المقاومة الملوحة:-

- معاملة التلقيح بخليط من سلالتى الأزوسبيرلاً والكليبسيلا
- معاملة التلقيح بخليط من سلالتى النوستوك والأنابينا من الطحالب الخضراء المزرقة والمثبتة للنتروجين
- معاملة بدون تلقيح

٣- معاملات طرق التسميد الحيوى:-

طريقة نقع الحبوب فقط، طريقة تلقيح الحبوب فقط وطريقة النقع ثم تلقيح الحبوب معا.

وقد تبين من النتائج تحسنا ملحوظا فى الخواص الطبيعية تحت الدراسة لكل من الكثافة الظاهرية والتوصيل الهيدروليكى والمسامية الكلية عند استخدام معاملات التسميد الحيوى حيث كانت طريقـة النقـع والتلقيح معا هى أفضل الطرق المستخدمة خاصة عند إستخدام الطحالب الخضراء المزرقة فى وجود تسميد نيتروجينى معدنى بمعدل ٦٠ كجم/فدان وذلك مقارنة بمعاملة المقارنة (٢٠ كجم/فدان) والمعاملة بدون تلقيح.

وأوضحت النتائج أيضا تأثيرا إيجابيا لكل من طريقة تلقيح الحبوب فقط وطريقة النقع ثم تلقـيح الحبوب بكل من خليط (الأزوسبيرلا و الكلبسيلا) وكذلك الطحالب الخضراء المزرقة على أعدادها وعلـى نشاط أنزيم الديهيدروجينيز فى منطقة الريزوسفير خاصة عند عمر ٧٥ يوم من الزراعة وتحت معدل تسميد نيتروجين معدنى ١٠٠ كجم/فدان. وكانت أعداد الكلبسيلا أكثر من أعداد الأزوسبيرلا. بينما سجلت النتـائج أعلى نشاط لإنزيم النيتروجينيز وأعلى محتوى من النيتروجين الميسر بالتربـة مقارنـة بـالكنترول (٢٠ كجم/فدان) والمعاملة بدون تلقيح، وخاصة عند استخدام طريقة النقع مع تلقيح الحبوب بخليط مـن سـلالتى الأزوسبيرلا والكلبسيلا فى وجود تسميد نيتروجينى معدنى بمعدل ٦٠ كجم/فدان عند عمر ٧٥ يـوم مـن الزراعة.

كما أنعكس تأثير النتائج والمعاملات على النمو حيث سجلت نتائج مشجعة فقد زاد الوزن الجاف لكل من محصول الحبوب والقش وكذا محتواهما من النيتروجين والأحماض الأمينية والبـروتين بـإجراء التسميد الحيوى والمعدنى. وأيضا أعطت مؤشرات ناجحة لكفاءة الاستفادة من التسميد النيتروجينـى حيـث سجلت أعلى كفاءة عند استخدام طريقة (النقع والتلقيح معا) للتسميد الحيوى بخليط من سلالتى الأزوسبيرلا والكلبسيلا فى وجود تسميد نيتروجينى معدنى بمعدل ٦٠ كجم/فدان. وهذا يعنى أنه يمكن توفير حوالى مـن (٢٠-٤٠) كجم نيتروجين للفدان.