ENUMERATION OF RHIZOBACTERIA COUNT AND GROWTH CRITERIA OF SUGAR BEET PLANT AS AFFECTED BY BIOFERTILIZATION

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Abstract

Two field experiments were carried out at Sakha Agricultural Research Station, Kafer El-Sheikh governorate, Egypt during the two successive winter seasons of 2006/2007 and 2007/2008.Obtained results showed that increasing the applied level of nitrogen significantly increased root length, diameter, leaves and root fresh weight/plant. Using three N-fixing bacterial strains namely Azotobacter chroococcum, Bacillus polymyxa and Azospirillum brasilense recorded the highest values of root length, root diameter, leaves and root fresh weight/plant. Most of mineral nitrogen and N-fixing bacteria insignificantly affected juice impurities. The values of Log number of the tested bacteria at zero time were 3.65, 4.59, 3.7, 2.32 and 3.26, 3.32 for the 1st and 2nd seasons, for Azotobacter spp, Bacillus spp Azospirillum spp, and total bacteria counts, respectively. Results showed that in the first season inoculation with mixed bacteria was the superior treatment in increasing the log numbers of the tested bacteria which presented 6.23, 5.78, 5.45 and 5.63, 5.52, 5.75 after 120 and 180days for Azotobacter chroococcum, Bacillus polymyxa and Azospirillum brasilense, respectively.

INTRODUCTION

Sugar beet plant ranks the second sugar crops after sugar cane crop in the world as it provides about 40% of the world sugar production. The average cultivated area of sugar beet in Egypt has been increased from 17 thousand feddan in 1982 to 364.290 thousand feddan in 2009-2010. High mineral nitrogen levels are being added to sugar beet in order to maximize its productivity in clay soils (Abou-Zeid and Osman, 2005). One of the most important limiting factors in sugar beet cultivation is nitrogen. The use of N-fixing bacteria is economic importance to modern agriculture as they can partially replaced the cost of mineral N fertilizers. lowering production costs and reducing environmental pollution ensuring high yields. Bio-fertilizer has emerged as a promising component of integrating nutrient supply system in intensive agriculture. Therefore, attempts have been paid to the use of bio-fertilizer as being most cheap and safe for agricultural application.

They are extremely benefited in enriching soil fertility with those micro-organisms, which fix atmospheric N and make plant nutrients more available, (Aly *et. al.,* 2009). Khalil (2002) recorded that inoculation with *Azotobacter chroococcum* and *Bacillus megatherium* saved about 25 kg N/fed of mineral fertilizer, which reduced the cost of plant production and the environmental pollution, in addition to the increase of sugar yield and recoverable sugar/fed. Furthermore, inoculation with *Azospirlilum spp* increased sucrose content in sugar beet roots. Abou Zeid and Osman (2005), Soudi *et. al.,* (2008) and Aly *et. al.,* (2009) found that bacterial inoculation of sugar beet seeds caused insignificant increases in root quality and growth parameters but it was significantly increased root and sugar yields/fed. *Bacillus polymyxa* inoculation along with 40 kg N/fed gave root and sugar yields as those obtained by addition of 80 kg N/fed. Furthermore*, Bacillus polymyxa* inoculation along with the addition of the full N dose of 80 kg/fed gave a significant increase which amounted to 18 and 39% in root and sugar yields, respectively, compared to application of 80 kg/fed alone. Meanwhile, bacterial inoculation caused significant increases in root and sugar yields. The objectives of these experiments were to study the effect of inoculation with some nitrogen fixing bacteria, namely *Azotobacter chroococcum*, *Bacillus polymyxa* and *Azospirillum brasilense* under different levels of N-fertilizer, 25%, 50% and 100% of the recommended dose as well as their interaction on sugar beet plants growth.

MATERIALS AND METHODS

Two field experiments were carried out at Sakha Agricultural Research Station, Kafer El-Sheikh governorate, Egypt during the two successive winter seasons of 2006/2007 and 2007/2008.

I -Materials

Soil samples

The soil samples were taken from the experimental field at 30 cm depth air dried, mixed, grinned and sieved through. The preceding crop was Mize in the two seasons. Two mm mesh before analyses. Their mechanical and chemical analyses were determined according to the method of Jakson (1973). Table (1).

Sugar beet seeds

Seeds of sugar beet (variety multigerm Plemo) were planted on 17and 10 October in 2006 and 2007, respectively. These seeds were kindly supplied by the Sugar Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Mineral fertilizer used

Nitrogen fertilizer as urea (46.5%N) was added in two equal doses. Once at thinning and the 2nd one month later Phosphorus fertilization was applied as calcium super phosphate at 15 Kg P_2O_5 / fed during soil preparation.

In order to evaluate the fertility of the cultivated soil, the mechanical, chemical, cat ions and anions were measured. Obtained results are listed in Table 1.

Table 1. Physical and chemical properties of the experimental soil.

Parameters	Growing seasons	
	2006/2007	2007/2008
Mechanical analysis		
Sand%	26.07	28.82
Silt%	19.46	18.31
Clay%	54.47	52.87
T.class	clay	clay
Chemical analysis		
Available N (ppm)	46.72	47.20
Available P (ppm)	6.72	6.41
Available K (ppm)	290.18	284.00
PH1:2. 5soil suspension	8.50	8.30
Ec ds/m 1:5soil extraction	0.75	0.79
Cations and anions, me q / L		
Na^+	2.38	2.48
K^+	0.09	0.07
Ca^{++}	.0.82	0.93
Mg^{++}	0.55	0.56
HCO_3^-	0.93	1.03
CO_3^-	0.00	0.00
$C l^-$	1.95	2.26
SO_4^{--}	0.96	0.76

Bacterial strains used

Three nitrogen-fixing bacterial strains namely, Azotobacter chroococcum (A), Bacillus polymyxa (B) and Azospirillum brasilense(C) were kindly obtained from Agric Microbiology Res., Soils, Water and Environment Research Inst., ARC, Giza, Egypt.

Also , Bacterial count found in experimental soil (at time of cultivation) in the two growing seasons (Table 2).

Table 2. Bacterial count found in experimental soil (at time of cultivation) in the two growing seasons

Examined bacteria	2006-2007		2007-2008	
	Cfu/ml	log	Cfu/ml	log
Azotobacter spp.	4.5 $x10^3$	3.65	30.9 $x10^3$	4.59
Bacillus spp.	5.1 $x10^2$	3.70	2.1 $x10^2$	2.32
Azospirillum spp.	1.82 $x10^3$	3.26	2.1 $x10^3$	3.32
Total bacterial count	10.3 $x10^6$	7.11	7.7 $x10^6$	6.88

II. Methods

Preparation of bacterial inoculants

All the three bacterial strains were maintained on nutrient agar slopes (Difco Manual, 1984) and kept at 5ºC until use. Each of bacterial strain used was grown on its specific growth medium, *Azotobacter chroococcum* (A) was grown up to 7 days at 30°C on liquid Ashby's medium (Hegazi and Niemela, 1976). *Bacillus polymyxa* (B) was grown up to 3 days at 30°C on liquid Hino and Wilson medium ((Hino and Wilson, 1958) and *Azospirillum brasilense* (c) was grown up to 3days at 30°C on semi solid medium of (Döbereiner *et. al.,* 1976).

Seeds inoculation

The individual bacterial strain was grown up to maximum density to reach about 10^6-10^9 cells ml^{-1} in a specific growth medium mentioned above for appropriate period of time. Each inoculated seed of sugar beet received abound ant bacterial cells using 15% of Arabic gum as adhesive agent in the presence of peat moss as a carrier material. The uncoated seeds were treated only with 15% of Arabic gum solution and in the presence of peat moss to serve as control. The seeds were then allowed to dry in open air before sowing.

Experimental design

Soil used in these experiments in both cultivation seasons had received nitrogen fertilizer as urea (46.5%N) at ratios of 20, 40 and 80 kg N/fed which represent 25, 50 and 100 % of recommended does, respectively. Cultivation of sugar beet was at 17th October and 10th October in the first and second seasons, respectively. However, the harvesting dates were at 8th May and 13th May in the 1st and 2nd seasons, respectively. Each experiment included 15 treatments with three replicates using a split plot design.

N-Fertilizer occupied the main plots while the bacterial inoculation was randomly allocated in the sub-plots. The plot area was 14 m^2 (7x2 m) = 1/300 feddan.

Bacterial inoculation treatments were without bacterial inoculation (control), seeds inoculated with either *Azotobacter chroococcum* (A), *Bacillus polymyxa* (B), *Azospirillum brasilense*(C) or seeds inoculated with mixture of all used bacteria (A+ B+C).

Measurements

Samples of three plants were collected after 120 and 180 days to estimate the growth parameters considering root length (cm), root diameter (cm), root fresh weight (Kg/plant), and leaves fresh weight (g/plant).Nutrient contents of roots such as nitrogen percent (%) was determined in roots by micro-kjeldahl method as reported by A.O.A.C. (1990). A flame photomemeter model E.E.L. was used to estimate potassium (K) and sodium (Na) as reported by Richards (1954).

Enumeration of N- fixing bacteria

Counting of N-fixing bacteria of the three of rhizosphere, i.e. *Azotobacter, Bacillus* and *Azospirillum* were performed in the of sugar beet plant after 120 and 180 days. These counting were done by plate method technique using modified Ashby's medium (Hegazi and Niemela, 1976), N-deficient medium (Hino and Wilson, 1958) and N-deficient medium (Döbereiner *et. al.,* 1976), for the three bacterial genera, respectively. The inoculated plates were incubated for 48 hrs at 30°C then the appeared bacterial colonies were counted.

Statistical analysis: The obtained results were subjected for Statistical Analysis according to the procedure outlined by Gomes and Gomes (1984).

RESULTS AND DISCUSSION

II. Plant Growth

Root length (Cm):

Data in Table 3 show that increasing the applied nitrogen dose significantly increased the root length of sugar beet plants. Results obtained in Table 3 also showed that even under the various N-levels using the combination of the three N-fixing bacteria recorded the highest values of this trait. This observation was fairly true in the two growth stages, i.e. 120 and 180 days after sowing as well as in the two growing seasons. However, it could be noted that the above mentioned interaction was significant in the two growing seasons except that when the sugar beet plants aged 120 days in the 1st cultivation season. The results obtained may be indicate to the close relationships between bacterial action and the available nitrogen as well as, it could be noted that application the three bacteria together was more effective than the individual one.

Table 3. Effect of nitrogen fertilizer levels and bio fertilization and their interaction on root length (cm) of sugar beet plant.

Nitrogen fertilizing dose	Biological treatment	Cultivation season (days after sowing)			
		2006/07		2007/08	
		120	180	120	180
	Control	14.967	16.833	15.400	15.300
	Azotobacter(A)	17.200	20.467	15.900	16.067
20 KgN/Fed	*Bacillus* (B)	16.733	19.600	14.467	14.833
	Azospirillum(C)	19.200	22.433	17.267	17.233
	A+B+C	20.433	23.300	18.700	19.00
	Control	15.500	17.767	13.700	13.900
	Azotobacter(A)	19.067	20.900	16.400	17.733
40 KgN/Fed	*Bacillus* (B)	18.200	20.433	15.433	16.400
	Azospirillum(C)	20.967	23.300	17.767	18.733
	A+B+C	21.100	23.733	20.067	20.100
	Control	17.200	18.200	14.433	14.433
	Azotobacter(A)	20.600	21.633	17.200	18.200
80 KgN/Fed	*Bacillus* (B)	19.267	21.100	15.467	16.367
	Azospirillum(C)	21.400	23.300	18.300	18.700
	A+B+C	22.867	24.200	21.000	21.633
	Control	15.889	17.600	14.511	14.544
	Azotobacter(A)	18.956	21.000	16.500	17.333
	Bacillus (B)	18.067	20.378	15.122	15.867
	Azospirillum(C)	20.522	23.011	17.778	18.222
	A+ B + C	21.467	23.744	19.922	20.244
LSD at 0.05 level of significance:					
Nitrogen fertilizer (N)		0.082	0.121	0.125	0.1682
Nitrogen fixation (F)		0.1756	0.1555	0.132	0.209
N x F		N.S	0.249	0.211	0.335

Root diameter (cm)

Recorded results in Table 4 clearly show that the effect of bio-fertilization on root diameter of sugar beet plants at the different growth stages. Data in Table 4 obviously showed the root diameter statistically responded to the supplied nitrogen. This response was continuously up to 80 Kg N/ fed at 120 days in the 1st season and at 120 and 180 days in the 2nd season. Meanwhile when the plant aged 180 days in the 1st season, application of 40 kg N/fed was enough to produce the highest value of the trait. However, based upon the general view it could be concluded that application of 80 kg N/fed was still the effective dose on this characteristic in the two growing seasons. This finding is in a good line with that reported by Azzazy (2004)

The collected results pointed out that the highest values of root diameters of sugar beet plants were absolutely recorded when sugar beet seeds were treated with the combination of the three tested bacterial strains to be *Azotobacter chroococcum* (A), Bacillus *polymyxa* (B) and *Azospirillum brasilense*(C). These values were followed by *Azospirillum* then *Azotobacter* that came later. Also, it was clearly shown that the lowest effect on this trait was that of control treatment. The influence of bio-fertilizing bacteria on sugar beet growth has been reported by Hilal (2005).

As to the effect of the interaction between the studied factors, the results given in Table 4 demonstrated that root diameter of sugar beet plants recorded a significant difference due to influence the interaction between the studied factors. The highest values of these traits were found when sugar beet seeds treated with the combination between N-level of 40 and 80 kg N/fed and the mixture of the three N-fixing bacteria (A + B + C) of the2nd stage at the 1st and 2nd seasons, respectively.

Table 4. Effect of nitrogen fertilizer levels and bio fertilization and their interaction on root diameter (cm) of sugar beet plant.

Nitrogen fertilizing dose	Biological treatment	Cultivation season (days after sowing)			
		2006/07		2007/08	
		120	180	120	180
	Control	6.733	9.500	8.500	8.500
	Azotobacter(A)	8.400	9.733	8.200	10.133
20 KgN/Fed	*Bacillus* (B)	8.100	11.500	8.267	9.600
	Azospirillum(C)	8.867	11.633	9.300	9.300
	A+B+C	9.633	13.400	9.967	10.600
	Control	7.433	9.600	7.200	8.133
	Azotobacter(A)	8.500	11.967	9.267	10.900
40 KgN/Fed	*Bacillus* (B)	8.300	13.067	8.633	10.500
	Azospirillum(C)	10.633	10.867	9.667	12.667
	A+B+C	11.833	13.500	10.933	13.467
	Control	8.400	10.167	7.500	8.253
	Azotobacter(A)	9.967	11.200	9.400	12.700
80 KgN/Fed	*Bacillus* (B)	9.267	10.167	9.133	12.233
	Azospirillum(C)	10.400	11.067	9.900	12.300
	A+B+C	11.833	10.167	11.500	13.500
	Control	7.522	9.756	7.733	8.296
	Azotobacter(A)	8.956	10.967	8.956	11.244
	Bacillus (B)	8.556	11.578	8.678	10.778
	Azospirillum(C)	9.967	11.189	9622	11.422
	A+ B + C	11.100	13.356	10.800	12.522
LSD at 0.05 level of significance:					
Nitrogen fertilizer (N)		0.175	0.1357	0.0909	0.183
Nitrogen fixation (F)		0.145	0.1838	0.133	0.67
NxF		0.232	0.294	0.213	0.588

Leaves fresh weight (g. /plant)

Results given in Table 5 show the values of leaves fresh weight (g./plant). These results pointed out that the highest values of root dimensions i.e. root length and root diameter were attained with the treatment in which the three N-fixating bacteria (A+B+C) with the highest N-level of 80 kg N/fed.

Obtained figures pointed out that leaves fresh weight attained an ascending increment in the various growth stages in the two growing seasons. However, this influence was statistically significant when the plant aged 120 and 180 days in the 1st cultivation season and 180 days in the 2nd cultivation season. In general, it was obviously clear that application the highest N-dose i.e. 80 kg.N/fed over passed the other nitrogen levels with respect to its effect on leaves fresh weight/plant. This finding is in accordance with that reported by Azzazy (2004).

Data illustrated in Table 5 proved that N-fixing bacteria play a distinct role in the plant growth may be through their effect on N-element availability in rhizosphere in which the plant grown. The mixture of the three examined bacterial strains

(A+B+C) produced a relative advantage in the values of leaves fresh weights / plant compared with that of other N-fixing bacteria used in individual form as well as with control treatment. It was also clear that *Azospirillum brasilense (c)* treatment attained the 2nd highest effect on this trait followed by *Azotobacter chroococcum (A)* treatment. This observation is in a good line with that of Hilal (2005).

Data in Table 5 also revealed that the studied factors showed that applying 80 kg N/fed with the mixture of the three N-fixing bacterial strains recorded the highest values of leaves fresh weight/plant, i.e. 1058.667and1166.0g/plant in the 1st cultivation season which corresponding to 506.667 and 913.667 g/plant in the 2nd cultivation season for120 and180 days, respectively. However, this superiority over the other treatments was significant only in the 2nd cultivation season.

Table 5. Effect of nitrogen fertilizer levels and bio-fertilization and their interaction on leaves fresh weight (g./plant) of sugar beet plant.

Nitrogen fertilizing dose	Biological treatment	Cultivation season (days after sowing)			
		2006/07		2007/08	
		120	180	120	180
	Control	265.002	481.667	376.00	454.667
	Azotobacter(A)	270.667	625.333	336.333	477.667
20 KgN/Fed	Bacillus (B)	298.667	594.333	280.333	574.667
	Azospirllum(C)	380.000	717.667	311.667	582.000
	A+B+C	412.333	746.333	366.333	617.667
	Mean	325.334	633.067	334.133	541.333
	Control	510.667	584.667	239.333	419.333
	Azotobacter(A)	648.667	709.667	344.400	659.667
40 KgN/Fed	Bacillus (B)	521.333	658.667	306.333	602.000
	Azospirllum(C)	742.000	778.667	376.333	721.333
	A+B+C	794.667	968.000	415.667	754.333
	Mean	643.467	739.933	336.333	631.333
	Control	637.667	670.667	290.333	554.667
	Azotobacter(A)	792.000	893.333	329.667	665.333
80 KgN/Fed	Bacillus (B)	706.000	718.000	360.333	725.000
	Azospirllum(C)	896.333	992.333	337.00	711.333
	A+B+C	1058.667	1166	506.667	913.667
	Mean	818.133	888.200	364.800	714.000
	Control	471.112	579.000	301.889	530.556
	Azotobacter(A)	570.444	742.778	336.667	600.889
	Bacillus (B)	508.667	657.000	315.667	593.889
	Azospirllum(C)	672.778	829.333	341.667	669.111
	A+ B + C	755.222	960.333	429.556	750.000
LSD at 0.05 level of significance:					
Nitrogen fertilizer (N)		20.74	30.638	N.S	16.142
Nitrogen fixation (F)		28.05	28.552	11.160	18.862
N x F		N.S	N.S	17.891	30.24

Root fresh weight (g. /plant)

Result shown in Table 6 illustrate the influence of N-level and bio-fertilization and their interaction on root fresh weight g. / plant for sugar beet plants at the various growth stages of the two growing seasons.

The present data showed that root fresh weight/plant statistically responded to the examined N-levels. This result was fairly true in the different growth stages of the two growing seasons. Increasing the addition of N-dose from 20 to 40 and 80 kg N/fed positively increased the values of root fresh weight. This finding may be explained by the possibility of more N-addition in plant rhizosphere. The effective role of nitrogen fertilizer on root fresh weight was also found by Azzazy (2004)

With respect to the influence of N-fixing bacterial strains on the values of root fresh weight/plant, results given in Table 6 revealed that the effect of the studied N-fixing bacterial strains on root fresh weight was as similar as its effect on leaves fresh weight. The results obtained appeared that the mixture of the three N- fixing bacteria (A+B+C) recorded the highest values of root fresh weight followed by the treatment in which *Azospirillum brasilense (c)* was used then *Azotobacter chroococcum(A)* treatment. This result coincides with that claimed by Hilal (2005). Once more the effect of the interaction between the mixtures of the three N- fixing bacteria and N-level on the values of root fresh weight/plant was shown in Table 6. Fertilizing of sugar beet plants with 80 kg N/fed together with the combination between N-fixing bacteria produced the highest root fresh weight values in the two growing seasons. Whereas this effect was significant only in the 2nd cultivation season as can be seen in the same Table. This result may be indicate to that application of 80 kg. N /fed. is still uninjured dose of nitrogen to N-fixation bacteria action

Sucrose percentage

Effect of nitrogen levels and N-fixing bacteria on the values of sucrose percentages at the various growth stages is presented in Table 7.

Data cleared in Table 7 pointed out that at early growth stage the influence of N-dose was not defined, however, in older age i.e. 180 days after sowing the pronounced effect of N-dose on this measurement was clear and the highest values of sucrose percentages were attained with 80 kg N/fed. The fruitful influence of nitrogen on sucrose has been mentioned by Azzazy (2004).

Table 6. Effect of nitrogen fertilizer levels and bio-fertilization and their interaction on root fresh weight (g./plant) of sugar beet plants.

Nitrogen fertilizing dose	Biological treatment	Days after sowing			
		2006/07		2007/08	
		120	180	120	180
	Control	330.000	444.000	365.000	517.667
	Azotobacter(A)	443.001	570.333	362.667	578.000
20 Kg/Fed	*Bacillus* (B)	397.003	542.000	303.667	534.667
	Azospirillum(C)	539.333	641.667	378.000	546.000
	A+B+C	652.333	803.000	425.000	658.000
Mean		472.334	600.200	366.867	577.667
	Control	380.333	579.667	276.333	424.000
	Azotobacter(A)	526.333	769.667	388.667	619.667
40 KgN/Fed	*Bacillus* (B)	463.335	646.333	351.667	565.000
	Azospirillum(C)	588.667	879.333	378.333	621.333
	A+B+C	730.334	1002.667	518.667	754.333
Mean		537.801	775.533	382.733	596.867
	Control	532.333	692.333	378.000	521.333
	Azotobacter(A)	625.668	917.667	457.00	665.333
80 KgN/Fed	*Bacillus* (B)	591.667	757.667	422.667	621.667
	Azospirillum(C)	671.335	1043.667	531.667	828.000
	A+B+C	728.333	1139.333	614.333	947.000
Mean		629.867	910.133	480.733	716.667
	Control	414.222	572.000	339.778	505.667
	Azotobacter(A)	531.668	752.556	402.778	621.000
	Bacillus (B)	484.002	648.667	359.333	573.778
	Azospirillum(C)	599.778	854.889	429.333	665.111
	A+ B + C	703.667	981.667	519.333	786.444
LSD at 0.05 level of significance:					
Nitrogen fertilizer (N)		9.834	12.996	12.055	8.354
Nitrogen fixation (F)		23.364	22.39	11.152	17.78
N x F		N.S	N.S	17.878	28.5

The available data indicated that the mixture of the three studied bacteria i.e. *Azotobacter chroococcum (A)*, *Bacillus polymyxa* (B), *and Azospirillum brasilense(c)* surpassed the other treatments with respect to its influence on the values of sucrose percentages. Moreover, it is clearly showed that the lowest sucrose percentages were produced in case of control treatment. This finding was fairly true in the two growing seasons as well as at the different growth stages. Also it could be noted that effect of N-fixing bacteria on sucrose percentages was statistically significant in both seasons and their growth stages. This result is in a good line with those obtained by Hilal (2005).

Table7. Effect of nitrogen fertilizer levels and bio-fertilization and their interaction on sucrose (%) of sugar beet plants.

Nitrogen fertilizing dose	Biological treatment	Days after sowing			
		2006/07		2007/08	
		120	180	120	180
	Control	11.067	13.633	11.767	14.900
	Azotobacter(A)	11.533	15.400	12.033	15.800
20 KgN/Fed	*Bacillus* (B)	11.300	14.500	11.500	14.903
	Azospirillum(C)	12.367	15.700	12.700	15.767
	A+B+C	13.533	16.733	14.233	16.967
	Control	12.167	14.600	10.467	14.633
	Azotobacter(A)	13.167	15.600	13.500	15.967
40 KgN/Fed	*Bacillus* (B)	12.700	15.100	12.833	15.100
	Azospirillum(C)	13.533	16.00	15.133	16.433
	A+B+C	14.900	17.333	10.467	17.500
	Control	11.800	14.633	11.667	14.833
	Azotobacter(A)	13.100	16.00	13.133	16.600
80 KgN/Fed	*Bacillus* (B)	12.333	15.600	12.503	15.967
	Azospirillum(C)	13.167	16.267	13.333	17.000
	A+B+C	14.767	17.367	14.733	17.933
	Control	11.678	14.289	11.300	14.789
	Azotobacter(A)	12.600	15.667	12.889	16.122
	Bacillus (B)	12.111	15.067	12.279	15.323
	Azospirillum(C)	13.022	15.989	13.367	16.400
	A+ B + C	14.400	17.144	14.700	17.467
LSD at 0.05 level of significance:					
Nitrogen fertilizer (N)		0.06225	0.1287	0.143	0.150
Nitrogen fixation (F)		0.092	0.1413	.118	0.142
N x F		N.S	0.2265	0.189	0..227

III- Microbial Enumeration:

Azotobacter chroococcum(A),Bacillus polymyxa(B) and *Azospirillum brasilense (C)* counts in the rhizosphere of sugar beet plants as affected by bacterial inoculation and nitrogen application are presented in Tables 8 and 9 for1st and 2nd season, respectively. It was observed from the data that inoculation with N_2-fixing bacteria stimulated the counts of the total bacteria in plants rhizosphere particularly in the presence of N fertilizer compared to the un inoculated plants. Furthermore, N-application induced a favorable effect on the total bacterial counts. The addition of N-fertilizer alone caused a pronounced increase in the total counts of bacteria (data not shown) compared to untreated control. Data demonstrated that inoculation of sugar beet seeds with used N_2-fixing bacteria individually or in a mixed form led to the higher numbers of (A) or, (B) at 120 and 180 days after sowing as a result of applying half dose of N-fertilizer of 40 kg N/fed in the first season (Table 8) while in the second

season (Table 9), the higher count of N-fixing bacteria were obtained when the lowest dose of N-fertilizer of 20 kg N/fed was applied. Also, inoculation of sugar beet seeds with used N_2-fixing bacteria either individually or in a mixed form gave higher numbers of A or B or C than un inoculated control. This was due to inoculation with N_2-fixing bacteria that stimulated the counts of other bacteria in plant rhizosphere via not only for providing nitrogen, but also for producing a variety of growth-promoting substances Hilal (2005). These substances stimulate the production of root exudates which in turn affect their numbers and increased with increasing the plant growth Soudi *et. al.,* (2008).The results may be indicated that the introduced inoculums has the ability to survive and colonize in the root zone of plants. Similar results were obtained by *Saleh* (1998). Also these data are in agreement with those of *Abotaleb et. al.,* (2002), who reported that inoculation with diazotrophic bacteria had an activation effect on the population of both bacteria and actinomycetes in plant rhizosphere. Results also showed that, in the first season inoculation with the mixed bacteria was the superior treatment in increasing the log numbers of the tested bacteria (A, B and C) which represented 6.23, 5.78 , 5.45 and 5.63, 5.52 , 5.75 after 120 days and after 180 days, respectively. The corresponding values of the untreated control were 5.28, 4.64, 4.86, 4.56, 4.64, and 4.95. While in the second season, the superior treatment wasn't definite. Generally, listed results in Table 8. revealed that applying of 40 Kg N/fed led to the maximum log number of the tested bacterial strains which was significant only for *Azospirillum spp* count in the first period. The results shown in Table 9 demonstrated that the interaction between the studied factors appeared a pronounced response, however this response was statistically significant in the 2nd season for tops yield only. Meanwhile the differences between the various combinations of the studied factors were not enough to be significant with respect to its effect on root yield of both seasons. Regardless the significant effect, it could be noted that the use of bacterial mixture treatment attained the highest values of tops and roots yield under the different nitrogen levels in the two growing seasons.

Table 8. Effect of nitrogen fertilizer levels and bio-fertilizers on log numbers of the tested N-fixing bacteria in sugar beet rhizospher at two growth stages of the first cultivated season (2006-2007).

Nitrogen fertilizing dose	Biological treatments	Log number of bacterial count					
		120 days after sowing			180 days after sowing		
		Azotobacter	Bacillus	Azospirillum	Azotobacter	Bacillus	Azospirillum
20 Kg.N/Fad	Control	5.11	4.04	5.29	4.52	4.98	5.53
	Azotobacter(A)	6.36	4.88	6.6	5.21	4.22	5.17
	Bacillus (B)	5.8	5.37	5.41	5.81	4.35	5.51
	Azospirillum(C)	4.85	4.80	6.4	4.77	5.76	5.58
	A+B+C	6.08	5.69	6.06	5.80	5.69	5.82
Mean		5.64	4.95	5.84	4.26	5.00	4.35
40 Kg.N/Fad	Control	5.82	5.11	4.90	4.90	4.56	4.64
	Azotobacter(A)	6.16	4.90	5.67	6.06	4.91	4.98
	Bacillus (B)	5.93	6.36	5.05	5.93	6.33	5.04
	Azospirillum(C)	5.90	5.04	5.78	5.01	4.74	5.61
	A+B+C	6.41	6.27	5.28	5.41	5.23	5.73
Mean		6.04	5.53	5.33	5.46	5.15	5.20
80 Kg.N/Fad)	Control	5.03	4.77	4.27	5.25	5.39	4.67
	Azotobacter(A)	5.73	4.71	4.92	5.57	5.76	4.65
	Bacillus (B)	5.67	6.08	5.44	4.93	4.41	5.01
	Azospirillum(C)	6.04	5.23	5.35	4.94	4.96	5.05
	A+B+C	6.17	5.35	6.23	5.77	5.48	5.07
Mean		5.72	5.22	5.28	5.27	5.20	4.89
Control		5.28	4.64	4.86	4.89	4.89	4.95
Azotobacter(A)		6.31	4.84	5.55	5.61	4.95	4.94
Bacillus (B)		5.81	5.94	5.40	5.56	4.99	5.19
Azospirillum(C)		5.60	5.03	5.92	4.91	5.13	5.42
A+B+C		6.23	5.78	5.45	5.63	5.52	5.75
L.S.D at 5%	N.fertelizaer(N)	N.S	N.S	0.206	N.S	N.S	N.S
	N.fixation(F)	0.487	0.449	0.429	0.571	0.402	N.S
	NxF	N.S	N.S	N.S	N.S	0.697	N.S

NS: Not Significant

Table 9. Effect of nitrogen fertilizer levels and bio fertilizers on log numbers of the tested N-fixing bacteria in sugar beet rhizospher at two growth stages of the second cultivation season (2007-2008)

Nitrogen fertilizing dose	Biological treatments	Log number of bacterial count					
		120 days after sowing			180 days after sowing		
		Azotobacter	Bacillus	Azospirillum	Azotobacter	Bacillus	Azospirillum
20 Kg.N/Fed	Control	4.99	4.62	5.04	5.91	4.99	5.58
	Azotobacter(A)	6.22	5.11	5.82	5.34	5.60	5.20
	Bacillus (B)	5.10	7.46	5.99	5.85	6.58	6.97
	Azospirillum(C)	6.54	5.99	6.23	5.83	5.88	6.82
	A+B+C	4.99	6.48	7.09	6.33	6.21	5.91
40 Kg.N/Fed	Control	5.04	6.51	5.04	4.91	4.90	4.99
	Azotobacter(A)	6.07	4.72	5.66	5.01	4.60	5.10
	Bacillus (B)	4.80	5.50	4.77	5.22	6.45	5.78
	Azospirillum(C)	4.99	4.79	5.57	5.46	4.74	6.12
	A+B+C	5.90	5.76	5.22	5.70	5.18	5.95
80 Kg.N/Fed)	Control	4.87	4.98	4.85	4.49	4.70	4.71
	Azotobacter(A)	5.60	4.54	4.95	4.64	4.43	4.94
	Bacillus (B)	4.58	5.48	4.68	4.72	6.41	4.93
	Azospirillum(C)	4.58	4.85	5.30	4.48	4.55	5.60
	A+B+C	5.28	5.05	4.96	4.66	5.18	5.90
Control		4.97	4.95	4.96	5.11	4.87	5.09
Azotobacter(A)		5.97	4.95	5.48	5.00	4.65	5.08
Bacillus (B)		4.85	6.15	5.14	5.27	6.48	5.90
Azospirillum(C)		4.88	5.22	5.70	5.26	4.95	6.18
A+B+C		5.90	5.77	5.76	5.56	5.53	5.72
L.S.D at 5%	N.fertelizaer(N)	0.195	0.464	0.195	0.254	N.S	0.531
	N.fixation(F)	0.528	0.528	0.624	0.499	0.463	0.619
	NxF	N.S	0.914	N.S	N.S	N.S	N.S

NS: Not Significant

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التعداد البكتيري في الريزوسفير وخصائص نموبنجر السكرنتيجة للتسميد الحيوي

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أقيمت تجربتين حقليتين فى محطه البحوث الزراعيه بسخا بمحافظه كفر الشيخ بنجاح خلال موسمى (2006–2007) (2007–2008) لزراعة نبات بنجر السكربأستخدام التسميد الحيوى مع التسميد المعدنى. تمت التجربه لدراسه تاثير التلقيح ببعض بكتيريا المثبته للنيتروجين الجوى مثل (أزوتوبكتر كروكوم– باسليس بلوميكسا– أزوسبريليم براسلينس) تحت مستويات مختلفة من التسميد المعدنى الازوتى (25–50–100%) من الجرعه الموصى بها وهي80كجم ن/فدان مضافة في صورة يوريا. أوضحت النتائج أنه:

بزياده مستويات النيتروجين يزداد (معنويا) طول وقطر الجذور – وزن العرش والجذر الطازج (جم/نبات).

سجلت نتائج إستخدام الخليط من البكتيريا المثبه للنيتروجين الجوىمع 80كجم ن/فدان اعلى القيم لطول وقطر الجذر ووزن الاوراق والجذر الطازج (جم/نبات).

لم يكن للنيتروجين المعدنى والبكتيريا المثبته للازوت الجوى تاثير معنوى على شوائب العصيرالمستخلص.