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EFFICIENCY OF DIAZOTROPHIC BACTERIA UNDER DIFFERENT LEVLS OF MINERAL NITROGEN AND MOLYBDENUM ON YIELD AND QUALITY OF SUGAR BEET

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ABSTRACT

To study the effect of inoculation with N_2-fixing bacteria on sugar beet, a green house experiment was carried out in the season of 2004/2005 using four nitrogen fixing bacteria to select the bacteria that sutable for oscar boly variety .Based on the results obtained from this experiment *Rhizobium* st. ICARDA 441 and *Azotobacter chroococum* were chosen for the field experiment. The field experiment was conducted at Kafr El-Sheikh Governorate season (2005/2006) using both chosen bacteria strains each individually as biofertilizer under two levels of nitrogen (45 kg N /fed and 90 kg N /fed) and three levels of molybdenum (0 , 0.5 kg Mo / fed and 0.75 kg Mo /fed).

Results indicated that increasing N-dose up to 90 kg N/fed caused a significant reduction in the values of leaves dry weight , leaf N-content ,nitrogenase and dehydrogenase activities, this reduction was more pronounced especially in nitrogenase activity under both tested bacteria strains ,meanwhile , both bacteria strains attained significant increases in the values of leaves dry weight and N-content under the low dose of nitrogen (45 kg N / fed).

Application the low dose of nitrogen (45 kg N / fed) combined with *Azotobacter* inoculation produced the highest significant yields of tops, roots and sugar. Moreover, the combination between the three studied factors gave a positive significant effect on TSS% and purity %.

Rhizobia inoculation combined with the higher dose of Molybdenum (0.75 kg /fed) decreased the enzyme activities and plant parameters. It could be concluded that using low dose of nitrogen (45kg/fed) and low dose of molybdenum (0.5kg/fed) combined with bacteria inoculation for sugar beet crop to obtain the highest yield and quality.

INTRODUCTION

Sugar beet crop provides about 40% of the total production not only in Egypt but also in the world. The cultivated area of sugar beet increased from 17 thousand feddan in1982 to reach to 168 thousand in the growing season 2005. The continuous application of mineral nitrogen to the agricultural soils resulted in the environmental pollution and caused ground water toxicity (Shrestha and Ladha, 1998).

Many investigators have a much great concern to find out a solution to reduce mineral nitrogen application by using diazotrophs bacteria (Biological nitrogen fixation (BNF) technology) to supply the plant with N-fixed from the air (Jeyahal and Kuppuswamy, 2001).

Treated sugar beet seeds with *Azotobacter chroococcum*, *Bacillus megatherium* and *Bacillus circulants* resulted in high significant vegetative growth characters, root length and diameter, root fresh weight and top weight compared to the untreated plants (Afify *et al.*, 1994)

The major of nitrogenase enzyme is catalyzed by the molybdenum Nitrogenase, although some N_2 fixing bacteria additionally caution altemative vanadium or iron – only, nitrogenase that are expressed when molybdenum is not available (Eady, 1996).

The most improved part of biological nitrogen fixation is nitrogenase enzyme which converted N_2 to NH_3 that converted to amino acids transported to plant in an essential process for supporting growth (Waters *et al.*, 1998).

Markova and Milic (2001) inoculated sugar beet with *Azotobacter chroococcum* in field experiments over 10 years, it could be concluded that bacteria improved the yield of sugar beet. *Azotobacter* is a nitrogen fixing bacteria and in the same time they produce PGPR, and auxins, which are quantitavely the most abundant phytohormones secreted by *Azotobacter chroococcum*, that proved by Guido and Lugtenberg(2001).

Khalil (2002) cited that inoculation with *Azotobacter chroococcum* and *Bacillus megatherium* saved about 25 kg N/ fed of mineral nitrogen , which reduced the cost of production and the environmental pollution, in addition to the increase of sugar yield and the recoverable sugar .

Rhizobia, which form root nodules and fix nitrogen (N_2) are symbiotic legumes. Extending the ability of these bacteria to fix N_2, they are also have excellent potential to be used with non legumes due to that rhizobia naturally produce molecules (auxins, cytokinins, abscici acids, and vitamins) that promote plant growth, their colonization and infection of cereal roots would be expected to increase plant development. (Matiru and Dakora, 2004.

Neamat-Alla (2004) mentioned that inoculation of sugar beet seeds with *Azospirillum* and phosphorine (a biofertilizer consists of phosphate dissolving bacteria) had insignificantly affected root length and diameter.

Abou – Zeid and Osman (2005) found that inoculation of sugar beet seeds with *Azotobacter chroococcum* and Bacillus polymyxa under different levels of mineral nitrogen led to insignificant increase in root fresh weight and root dimension at harvest, however, bacterial inoculation caused significant increases in root and sugar yields. They added that *Bacillus* inoculation combined with 40 kg N / fed gave root and sugar yields as equal as those of 80 kg N / fed.

This study was conducted to evaluate the efficiency of molybdenum and bacterial inoculation on mineral nitrogen needed and their effect on yield and quality of sugar beet crop.

MATERIALS AND METHODS

Two experiments were carried out to study the influence of molybdenum and bacterial inoculation on the quantity of mineral nitrogen needed and their effect on qualitative and quantitative parameters of sugar beet crop.

The first experiment was in pots (greenhouse) season 2004/2005 by using soil collected from Kafr El-Sheikh Governorate using four N-fixing bacteria, two of them were rhizobia strains, *Rhizobium leguminosarum* biovar vicea strains (ICARDA 441 and ARC 207) and the others were *Azotobacter*

chroococcum and *Azospirillum brasilense*. Sugar beet variety, oscar poly was used with 15% of mineral nitrogen fertilizer as encourage dose. Plant growth and nitrogen content of seedlings were determined after 90 days as well.

Bacterial preparation and inoculation method:

In greenhouse experiment, two groups of bacteria were used; *Rhizobium leguminosarum* biovar vicea strains, (ICARDA 441) and (ARC 207) as a symbiotic. *Azotobacter chroococcum* and *Azospirllum brasilense* as free living bacteria (non-symbiotic). Bacterial strains were supplied with the unit of Biofertilizers, Department of Microbiology, Soils, Water and Environ. Res. Institute, ARC, Giza. Each individual bacterium was grown in a broth culture contains 10^8cell mL^{-1} on yeast extract manitol agar medium (Vincent, 1970); (Hegazi and Neimila, 1976) and (Döbereiner *et al.*, 1976) for *Rhizobia, Azotobacter* and *Azospirillum,* respectively, then added to each pot at planting over the seeds with repeating after one and two weeks of planting. The most efficient two bacteria in N_2 . fixation were chosen to be used in the field by mixing the individual broth culture with neutralized fine peat then mixed with sugar beet seeds using an adhesive agent and air dried in shadow before sowing.

Based on the results obtained from this experiment *Rhizobium* st. ICARDA 441 and *Azotobacter* were selected to execute the field experiment

The field experiment was carried out at Sakha Res. Station (ARC), Kafr El-Sheikh Governorate, season, 2005/2006.

The present work included 18 treatments, which were the combination between three biofertilizers treatments (control, *Azotobacter chroococcum* and R*hizobium leguminosarum* biovar vicea strain, (ICARDA 441), two mineral nitrogen levels (45 kg N/fed and 90 kg N/fed) and three levels of molybdenum (control, 500 g Mo/fed and 750 g Mo/fed) in form of ammonium molybdate, applied to soil after thinning.

Nitrogen fertilizer was added in form of urea (46%N) in two split equal doses, the first was applied after thinning (at 4-leaf stage) to have one plant per hill, while the second dose was added one month later. Phosphorus fertilizer was applied at the level of 30 kg P_2O_5 /fed as calcium super phosphate (15.5% P_2O_5) at seedbed preparation, meanwhile potassium fertilizer was added with the first dose of nitrogen at the level of 48 kg K_2O /fed as potassium sulphate (50% K_2O).

Plot area was 21 m^2 consisting of six rows, 7 m long and 50 cm apart, spacing between hills was 20 cm. A multigrm sugar beet variety viz Oscar poly was sown on September 27^{th}.

A split plot design with three replications was used, where mineral nitrogen fertilizer occupied the main plots, while the combination between bacterial inoculation and molybdenum were randomly allocated in the sub-plots. The normal agronomic practices in sugar beet fields were carried out as recommended by SCRI, ARC, Ministry of Agriculture.

Physical and chemical properties of the experimental soil (Table1) were determined according to Jackson (1973).

Table (1): Physical and chemical properties of the experimental soil in Kafr El- Sheikh Governorate (2005/2006)

Mechanical analysis (%)												
Coarse sand		Fine sand		Silt		Clay		Soil Texture				
1.4		23.07		20.08		55.45		Clay				
Chemical analysis												
Available nutrients (ppm)			pH 1:2.5	EC (dS/m)	Anions(meq/L)				Cations (meq/L)			
N	P	K			$CO_3^{=}$	HCO_3^-	Cl-	$SO_4^{=}$	Na+	K^+	Ca^{++}	Mg^{++}
17.15	6.39	284.34	8.1	20.0	–	1.0	14.28	4.93	11.5	0.28	1.17	7.26

Data recorded:

1-Pot experiment

a. Seedlings fresh weight (g)
b. Seedlings length (cm)
c. Leaves number
d. Leaf area (cm^2)
e. Total chlorophyll according to Dainel (1949).
f. Seedlings dry weight (g)
g. Seedlings nitrogen content (mg/plant)

2. Field experiment:

2-1- Growth characters:

a. Leaf area (cm^2)
b. Root length (cm).
c. Root diameter (cm)
d. Root fresh weight (g/plant)
e. Leaves dry weight (g/plant)
f. Leaf nitrogen content (mg/plant)

2-2 Enzyme activity:

a- Dehydrogenase activity (DHA) as mL H_2 100 g^{-1} $soil^{-1}h$. (Casida *et al.*, 1964)

B- Nitrogenase activity as µ mole C_2H_4 g^{-1} day^{-1}(Leth Bridage *et al.*, 1982)

enumerated for tested the microbial activity at the first period of plants (4 months).

2-3 - Chemical constituents:

a. Photosynthetic pigments i.e. chlorophyll a, b and carotenoids (mg/g fresh weight) were determined according to Wettstein (1957).
b. Total soluble solids (TSS%) was detrmined using handle refractometer.

c. Sucrose percentage was determined using Sacchrimeter apparatus according to procedure out lined by Le-Docte (1972).
d. Purity % was calculated according the following equation

Purity percentage = Sucrose percentage x 100/TSS%

2-4- Yield and its components:

a. Top yield (Ton / fed)
b. Root yield (Ton / fed)
c. Sugar yield (Ton /fed) was estimated according the following equation:

Sugar yield (Ton /fed) = root yield (Ton/fed) x Succrose %

Statistical analysis:

The collected data were subjected to statistical analysis for the split plot design according to Snedecor and Cochran (1980). The comparison between means is done as least significant difference (L.S.D) at 5% level was used.

RESULTS AND DISCUSSION

Results given in Table (2) show the effect of the examined bacterial inoculation on some morphological and chemical traits of sugar beet crop under greenhouse condition. Results showed that the inoculation with *Rhizobia st.* ICARD 441 attained a superior effect on the most of the examined parameters compared to *Rhizobia st.* ARC 207.This influence was significant with dry weight, nitrogen content and chlorophyll, meanwhile insignificant with the other parameters. These results are in agreement with those obtained by Matiru and Dakora (2004) who reported that *Rhizobium leguminosarum* bv. Viceae infected cereal plant (sorgum and millet) because of PGPR produced and released into cropping system promotes plant growth and possibly led to increase yield. Also, it could be mentioned that the strain (ICARDA 441) is a foreign strain and have a good performance with the plant. Also, it could be noted a positive and significant differences were recorded due to inoculation with *Azotobacter* in dry weight and nitrogen content compared to inoculation with *Azospirillum* . In this point. Pandey *et al.* (1998) proved that *Azotobacter* exhibited the best performance of the plant than *Azospirillum.*

Table (2): Effect of inoculation with some nitrogen fixing bacteria on seedling parameters of sugar beet at 90 days in greenhouse (2004/2005)

Treatments	Seedling fresh weight (g)	Seedling length (cm)	Leaves number	Leaf area (cm^2)	Total Chlorophyll (mL/ g fwt.)	Seedling Dry weight (g)	Nitrogen content mg/plant)
Rhizobia (441)	16.19	17.0	8.0	23.50	1.71	1.64	130.14
Rhizobia (207)	14.18	16.0	8.0	17.38	1.55	1.10	71.20
Azospirillum	7.27	16.5	7.0	20.82	1.35	1.09	68.10
Azotobacter	13.62	16.0	8.0	24.30	1.50	1.32	91.90
L.S.D at 0.05	7.907	N.S	N.S	N.S	0.2249	0.228	3.209

Field experiment:

1- Plant growth parameters:

Data in Table (3) show that there was a positive effect on leaf dry weight and nitrogen content due to inoculation treatments. Inoculation with *Azotobacter* surpassed *Rhizobia* and uninoculated treatment. However, *Rhizobia* inoculation attained a significant increase in leaf N content followed by *Azotobacter* treatment.

Table (3): Effect of bacterial inoculation, molybdenum and mineral nitrogen on dry weight and N- content of sugar beet leaves at harvest (2005 / 2006)

Treatments		dry weight (g/plant)				N- content (mg/plant)			
N- fertilizer	Biofertilizers	Kg Mo/fed.			Mean	Kg Mo/fed.			Mean
		Zero	0.5	0.75		Zero	0.5	0.75	
0kg/fed.	Without	87.48	54.12	108.06	83.22	1455.80	1297.96	1144.96	1299.57
	Azotobacter	80.52	71.38	70.16	74.02	2198.20	1626.17	1297.96	1707.44
	Rhizobia	62.91	69.18	55.40	62.50	1144.96	1632.65	1296.36	1357.99
Mean		**76.97**	**64.89**	**77.87**	**73.25**	**1599.65**	**1518.93**	**1246.43**	**1455.00**
45kg/fed.	Without	41.94	37.49	61.92	47.12	599.74	478.04	1603.75	893.84
	Azotobacter	88.33	89.83	82.50	86.89	2614.57	2102.02	306.75	2592.45
	Rhizobia	89.19	100.39	62.91	84.16	2729.20	2842.00	2113.78	2561.66
Mean		**73.15**	**75.90**	**69.11**	**72.72**	**1981.17**	**1807.35**	**2259.42**	**2015.98**
Without		64.71	45.80	84.99	65.17	1027.77	888.00	1374.35	1096.70
Azotobacter		84.43	80.60	76.33	80.45	2406.38	1864.09	802.35	1690.94
Rhizobia		76.05	84.78	59.15	73.33	1937.08	2237.32	1705.07	1959.82
Whole mean		**75.05**	**70.39**	**73.49**	**72.98**	**1790.41**	**1663.14**	**1293.92**	**1582.49**

L.S.D. at 0.05

Nitrogen (N)	12.33	452.72
Molybdenum(Mo)	N.S	210.85
Inoculation (In)	9.53	240.66
Mo x In	N.S	N.S
Mo x N	N.S	603.28
N x In	21.04	603.28
Mo x In x N	N.S	281.05

Regarding to the effect of molybdenum, results revealed that there was insignificant effect on leaves dry weight, meanwhile, N-content of leaves had negatively responded to Mo application.

Effect of N-fertilizer illustrated that full nitrogen dose (90 kg N/fed) significantly increased leaves dry weight against insignificant effect on N-content.

Concerning the interaction between the studied factors, results exhibited insignificant influence on the leaves dry weight as well as leaf N-content due to the first order interaction between molybdenum and inoculation treatments.

The most effective interaction was observed between nitrogen levels and inoculation treatments. Increasing N-dose up to 90 kg N/fed caused significant reductions in the values of leaves dry weight and leaf N-content under the effect of both tested bacterial strains , meanwhile ,

both bacteria strains attained significant increases in the values of leaves dry weight and leaf N-content under the effect of low nitrogen dose (45 kg N / fed). This finding may indicate that inoculation combined with the low nitrogen dose has promoted bacterial activity. These results are in agreement with those obtained by Markova and Milic (2001) who reported that *Azotobacter* synthesizes auxins; cytokinins and the growth materials are the primary substances controlling the enhanced growth. Also, Dakora *et al.* (2002) reported that rhizobia produce phytohormones that released into cropping systems, which in turn promotes plant growth. In addition Badr (2004) found that the use of biofertilizers significantly increased sugar beet growth.

2-Root characters:

Results in Table (4) showed that application of molybdenum had no significant effect on root parameters in terms of root dimension and root fresh weight / plant, whereas bacterial inoculation, which affected root fresh weight significantly was also affected by the combination between nitrogen dose and inoculation treatment that exhibited the most effective interaction. Fertilizing sugar beet crop with 45 kg N /fed enhanced root growth parameters. This effect was significantly with respect to root length and root fresh weight only.

These results are confirmed by those observed by Neamat-Ala (2004) who showed, the inoculation of sugar beet with some N_2-fixing bacteria such as *Azospirillum* and phosphate dissolving bacteria recorded insignificant effects on root length and diameter at either growth or harvest stages. Sultan *et al.*(1999) found that inoculation of sugar beet seeds with *Azotobacter* increased, root length and diameter. Also, Maareg and Badr (2001) showed that inoculation of sugar beet with *Bacillus polymyxa* caused an increase in length, diameter and weight of root and foliage weight.

3-Microbial nitrogenase and dehydrogenase activities:

Data in Table (5) show nitrogenase and dehydrogenase activities measured in sugar beet rhizosphere area that go side by side in both enzymes except for little differences. Results showed that molybdenum application in a rate of 500 g fed^{-1} significantly increased both enzyme activities. On the contrary the higher dose of molybdenum (750 g Mo fed^{-1}) decreased both enzyme activities.

Regarding the effect of inoculation, both *Azotobacter* and *Rhizobia* inoculation cleared significant increases in the enzymes activities compared to uninoculated treatments .Application of N-fertilizer at a rate of 90 kg N / fed, strongly decreased both enzyme activities with a highly significant response, especially in N_2-ase activity, which was obvious.

Regarding the interaction effect between the studied factors, results revealed that except for the interaction between nitrogen application and both of molybdenum and/or inoculation treatments, the various combinations between the studied factors were insignificant with respect to their effect on dehydrogenase activity. However, the values of nitrogenase enzyme activity exhibited a significant response to the various combinations between the studied factors.

Table (4): Effect of bacterial inoculation, molybdenum and mineral nitrogen on some growth characters of sugar beet roots at harvest (2005/2006)

Treatments		Root length (cm)				Root diameter (cm)				Root fresh weight (g)			Mean
N-fertilizer	Biofertilizers	Kg Mo/fed.			Mean	Kg Mo/fed.			Mean	Kg Mo/fed.			
		Zero	0.5	0.75		Zero	0.5	0.75		Zero	0.5	0.75	
90kg/fed.	Without	21.00	25.00	26.50	24.17	10.00	10.75	11.00	10.58	595.0	887.0	1215.0	899.0
	Azotobacter	23.00	20.00	19.75	20.92	10.00	9.75	9.50	9.75	997.0	807.0	805.0	869.67
	Rhizobia	22.00	24.00	20.00	22.00	9.00	9.75	9.25	9.33	665.0	790.0	540.0	665.00
Mean		**22.00**	**23.00**	**22.08**	**22.36**	**9.67**	**10.08**	**9.92**	**9.89**	**752.3**	**828.0**	**853.3**	**811.20**
45kg/fed.	Without	20.50	22.25	22.25	21.67	9.00	9.50	9.75	9.42	567.5	622.0	792.0	660.50
	Azotobacter	25.00	24.75	24.75	24.83	10.75	10.25	10.00	10.33	1320.0	1227.0	885.0	1144.00
	Rhizobia	22.75	25.00	24.25	24.00	10.00	10.25	10.00	10.08	815.0	937.5	882.5	878.33
Mean		**22.75**	**24.00**	**23.75**	**23.50**	**9.92**	**10.00**	**9.92**	**9.94**	**900.85**	**928.8**	**853.17**	**894.28**
Without		20.75	23.62	24.37	22.92	9.50	10.12	10.37	10.00	581.25	754.50	1003.5	779.75
Azotobacter		24.00	22.37	22.25	22.87	10.37	10.00	9.75	10.04	1158.5	1017.0	845.00	1006.83
Rhizobia		22.37	24.50	22.12	23.00	9.50	10.00	9.62	9.70	740.00	863.75	711.25	771.66
Whole mean		**22.37**	**23.50**	**22.91**	**22.93**	**9.79**	**10.04**	**9.92**	**9.91**	**826.58**	**878.41**	**853.23**	**852.74**

L.S.D. at 0.05			
Nitrogen (N)	N.S	N.S	N.S
Molybdenum(Mo)	N.S	N.S	N.S
Inoculation (In)	N.S	N.S	135.44
Mo x In	N.S	N.S	N.S
Mo x N	N.S	N.S	224.06
N x In	4.56	N.S	224.06
Mo x In x N	N.S	N.S	228.15

Table (5): Effect of bacterial inoculation, molybdenum and mineral nitrogen on nitrogenase and dehydrogenase enzyme activities in sugar beet rhizosphere at 120 days (2005 / 2006)

Treatments		Nitrogenase µ moleC_2H_4/g soil/day				Dehydrogenase mL H_2/ 100g soil/h			
N-fertilizer	Biofertilizers	Kg Mo/fed.			Mean	Kg Mo/fed.			Mean
		Zero	0.5	0.75		Zero	0.5	0.75	
90 kg/fed.	Without	11.52	12.48	15.84	13.28	59.79	96.25	112.58	89.54
	Azotobacter	26.64	33.12	28.32	29.36	127.74	121.08	119.58	122.5
	Rhizobia	24.48	36.0	27.12	29.20	113.16	123.08	118.41	113.21
Mean		**20.88**	**27.20**	**29.76**	**35.82**	**100.23**	**113.47**	**116.85**	**116.18**
45kg/fed.	Without	18.48	54.72	45.36	39.52	112.58	124.83	131.83	123.08
	Azotobacter	95.36	81.36	82.80	86.50	163.91	146.41	127.74	146.02
	Rhizobia	91.92	98.88	51.36	80.72	148.75	175.58	121.91	148.74
Mean		**68.58**	**78.32**	**59.84**	**68.91**	**141.74**	**148.94**	**127.16**	**139.28**
Without		15.00	33.60	30.60	26.40	86.18	110.54	122.20	106.31
Azotobacter		61.00	57.24	55.56	57.93	145.82	133.74	123.66	134.26
Rhizobia		58.20	67.44	39.24	54.96	130.95	149.33	120.16	133.48
Whole mean		**44.73**	**52.76**	**41.80**	**46.43**	**120.98**	**131.20**	**122.04**	**123.85**

L.S.D. at 0.05		
Nitrogen (N)	7.76	N.S
Molybdenum(Mo)	5.95	12.82
Inoculation (In)	5.67	N .S
Mo x In	15.52	N .S
Mo x N	15.89	32.65
N x In	15.89	32.65
Mo x In x N	5.40	N .S

Concerning the interaction between molybdenum and inoculation, it could be noted that the application of molybdenum attained a positive response towards the values of nitrogenase activity under the effect of various biofertilizer treatments. The middle dose of molybdenum (0.5 kg /fed) surpassed the other dose (0.75 kg Mo /fed). Also, the interaction between Mo and N recorded similar effect on both enzyme activities, where the middle dose of molybdenum (0.5 kg /fed) produced the highest values of enzyme activities with remarkable increases in these values under the effect of low dose of N(45 kg/fed). Concerning the interaction between inoculation treatments and nitrogen fertilizer, data pointed out that inoculation treatments showed a positive response in the values of enzyme activities under the effect of various nitrogen levels with a relative increases in these values under the effect of low level of nitrogen (45 kg N / fed) .

The second order interaction cleared that *Rhizobia* inoculation treatments combined with low dose of nitrogen (45 kg N / fed) and the middle dose of molybdenum (0.5 kg /fed) achieved the highest values of both enzyme activities. These results are in agreement with those obtained by Luis and Ludden (2005) who proved that the essential conversion of atmospheric nitrogen to ammonia is catalyzed by N_2-ase system and substrate reduction occurs within the Mo-Fe protein. But plants need Mo in a rare concentration (0.1 ppm) and also bacteria need Mo in a low level and that explain a reduction in N_2-ase activity as a results of toxic effect at Mo higher concentration (750 g mo fed^{-1}). While dehydrognease decreased when bacterial activity reduced. Abd El-Rasoul *et al.* (2004) in wheat, El-Zeky *et al.* (2005) in rice and Abo El-Eyoun (2005) in Maize who found that inoculation with *Azotobacter* increased significantly both N_2-ase and dehydrogenase activity over the control as a result of microorganisms count increasing. El-Komey *et al.* (1998) and El- Mohandes (2000) explained that the higher of organic matter mineralization and high level of N-fertilizer caused an opposite trend of N_2-fixation as a result N_2-ase activity inhibition. Also, dehydrogenase activity increased with bacterial inoculation and this was in agreement with Seagnozzi *et al.* (1995) who reported that there is a significant relationship between (DHA) activity and microbial count in soil. Da silva *et al.* (1993) reported that the inorganic nitrogen accumulation inhibited the N_2-ase activity.

4- Chlorophyll a, b, carotenoids and leaf area :

Results given in Table (6) revealed that pigment values in terms of Chl. a, b and carotenoids content as well as leaf area responded positively to Mo application , the middle dose (0.5 kg Mo /fed) recorded the highest values of these parameters , these effects were statistically positive except for tChl.a which was not enough to reach the level of the positive significance.

For, inoculation effect on the pigment content ,it could be noticed that Chl. a affected significantly by *Azotobacter* inoculation , meanwhile Chl. b attained the highest value due to *Rhizobia* inoculation .On the contrary the highest value of carotenoids was recorded with the untreated treatments.

Table (6): Effect of bacterial inoculation, molybdenum and mineral nitrogen on photosynthetic pigments and leaf area of sugar beet at harvest(2005 / 2006)

Treatments		Chlorophyll a (mL/g fwt.)				Chlorophyll b (mL/g fwt.)				Carotenoides (mL/g fwt.)			Mean	Leaf area (cm^2)			Mean
N-fertilizer	Biofertilizers	Kg Mo/fed.			Mean	Kg Mo/fed.			Mean	Kg Mo/fed.				Kg Mo/fed			
		Zero	0.5	0.75		Zero	0.5	0.75		Zero	0.5	0.75		Zero	0.5	0.75	
30 kg/fed.	Without	3.00	3.80	3.95	3.58	2.06	2.17	2.20	2.14	1.59	1.62	1.65	1.62	193.13	212.83	217.10	207.69
	Azotobacter	3.85	3.70	3.25	3.60	2.80	2.65	2.65	2.70	1.40	1.37	1.00	1.26	229.90	210.55	199.03	213.16
	Rhizobia	3.65	3.80	3.75	3.73	2.90	2.92	2.85	2.89	1.10	1.30	1.25	1.22	191.83	219.53	211.33	207.56
Mean		**3.50**	**3.77**	**3.65**	**3.64**	**2.59**	**2.58**	**2.57**	**2.58**	**1.36**	**1.43**	**1.30**	**1.36**	**204.95**	**214.30**	**209.15**	**209.47**
45kg/fed.	Without	2.92	2.96	3.00	2.96	1.90	1.94	1.96	1.93	0.90	0.94	1.00	0.95	146.93	200.05	203.13	183.37
	Azotobacter	3.96	3.85	3.80	3.87	2.84	1.90	1.85	2.20	1.50	1.25	1.00	1.25	236.18	184.18	175.83	198.73
	Rhizobia	3.70	3.80	3.53	3.68	2.75	2.94	2.50	2.73	1.20	1.32	0.90	1.14	195.93	225.43	190.88	204.08
Mean		**3.53**	**3.54**	**3.44**	**3.50**	**2.50**	**2.26**	**2.10**	**2.29**	**1.20**	**1.17**	**0.97**	**1.11**	**193.01**	**203.22**	**189.95**	**195.39**
Without		2.96	3.38	3.47	3.27	1.98	2.05	2.08	2.03	1.24	1.28	1.32	1.28	170.03	206.44	210.11	195.53
Azotobacter		3.90	3.77	3.52	3.73	2.82	2.27	2.25	2.45	1.45	1.31	1.00	1.25	233.04	197.36	187.43	205.94
Rhizobia		3.67	3.80	3.64	3.70	2.82	2.93	2.67	2.81	1.15	1.31	1.07	1.18	193.88	222.48	201.10	205.82
Whole mean		**3.51**	**3.65**	**3.54**	**3.57**	**2.54**	**2.42**	**2.33**	**2.43**	**1.28**	**1.30**	**1.13**	**1.23**	**198.98**	**208.76**	**199.55**	**202.43**

L.S.D. at 0.05

Nitrogen (N)	0.36	0.17	0.380	N.S
Molybdenum(Mo)	N.S	0.11	0.098	18.81
Inoculation (In)	0.05	0.10	0.130	N .S
Mo x In	0.46	0.31	0.560	N .S
Mo x N	0.27	0.30	0.290	N .S
N x In	0.27	0.30	0.290	41.81
Mo x In x N	0.18	0.11	0.230	N .S

Concerning nitrogen fertilizer effect on pigment content, results obtained showed that pigment content was positively responded to the higher dose of nitrogen (90 kg N / fed).

The various combination between the studied factors showed significant effects on the pigment contents of sugar beet leaves. With regard to the second order interaction between the studied factors, it could be deduced that the effective combination between the studied factors towards pigments differed due to the pigment type. The highest values of Chl.a and b were attained by the use of 0.5 kg Mo / fed + 45 kg N / fed without inoculation and 0.5 kg Mo/fed+45 Kg N/fed combined with *Rhizobia* inoculation respectively. Whereas application of 0.75 kg Mo / fed + 90 kg N / fed without inoculation was necessary to attain the highest value of carotenoids.

However, the effect of inoculation treatment or nitrogen fertilizer as well as the various interactions was insignificant with respect to their effect on leaf area.

These results are in agreement with those recorded by Stajner *et al.* (1997) who stated that sugar beet inoculation with *Azotobacte chroococcin* increased the content of chlorophyll and carotenoids. Leaf area recorded insignificant difference between treatments, while an increase was observed with the use of 90kg/N-fertilizer combined with *Azotobacter* inoculation and /or rhizobia + 0.5 kg Mo/ fed treatments. Sultan *et al.* (1999) found that inoculation of sugar beet with *Azotobacter* increased the leaf area index.

5- Yield and yield components:

Results given in Table (7) cleared that the examined levels of molybdenum had no significant influence on yields of tops, roots and sugar.

Once more, data in Table (7) revealed that inoculation with *Azotobacter* distinctly and significantly surpassed the other biofertilizer treatment with respect to top and root yields as well as sugar yield.

These findings were similar to those obtained by Markova and Milic (2001) who reported that *Azotobacter chroococcum* improveved the yield of sugar beet. Kennedy and Islam (2001), mentioned that *Azotobacter* can supplement the use of urea-N in wheat production either by BNF or growth promotion, they added that Azotobacter synthesizes auxins, cytokinins and these growth materials are the primary substances controlling the enhanced growth and necessary to find a good association plant genotype, particularly *Azotobacter* that forms a good association.

Regarding nitrogen effect on sugar beet yield and its components, results showed that there was insignificant effect on these traits.

Due to the influence of the interactions between the studied factors, the results showed that the interaction between Mo and bacterial inoculation was significantly with respect to roots and sugar yield. Inoculation with *Azotobacter* + without molybdenum application attained the highest values of root and sugar yields. Moreover, the most effective interaction was observed between nitrogen levels and inoculation treatments. Application of low nitrogen dose (45 kg N / fed) combined with *Azotobacter* inoculation produced the highest significant yields of tops, roots and sugar. Sultan *et al*

(1999), Ali (2003) and Badr (2004) showed that inoculation of sugar beet seed with nitrogen fixing bacteria increased significantly root yield per fed Attention was paid for N-fertilizer that gave high results in range of 50% N combined with inoculation and these results are in conformity with those obtained by Basham and Holguin (1997) who found that associative diazotrophs are beneficial at low levels of N-fertilizer, where the yields of cereal plants increased significantly by inoculation with associative N_2-fixers in the presence of half dose of recommended N-fertilizer. Increasing of top and root yields of sugar beet treated with 500 g molybdenum /fed explained that molybdenum is very important for plant and microorganisms. These results were previously confirmed by Luis and Ludden (2005) who reported that most of the information known about the maturation of nitrogenase components comes from studies done on the Mo-containing nitrogenase.

6- Sugar quality:

Data presented in Table (8) showed that juice quality in terms of TSS %, sucrose % and purity % affected insignificantly by Molybdenum application. Similar results were recorded for inoculation treatments with respect to the percentage of TSS and sucrose. However, purity % increased significantly due to inoculation with *Azotobacter*, which recorded the highest purity %.

Regarding the influence of nitrogen fertilizer on juice quality ,results pointed out that decreasing the applied dose of nitrogen (45 kg N/fed) significantly increased both of TSS% and sucrose %, however , the difference between nitrogen level did not reach the level of significance with respect to its effect on purity % .

Concerning the interaction effects between the studied factors , data exhibited that the interaction between molybdenum and nitrogen levels affected significantly the sucrose and purity percentages .Moreover , the combination between the three studied factors gave significant effect on TSS% and purity % .

These results are agreement with those obtained by Badr (2004) who reported that biological and mineral N-fertilizer had slightly positive effect on sugar percentage and purity percentage. Markova and Milic (2001) proved over 10 years experiments that inoculation by *Azotobacter chroococcum* increased the sugar beet root yields from 4-26% and increased the sugar content from 2.5 to 5.39% and crystallization of sugar from 7 to 24%.

Table (7): Effect of bacterial inoculation, molybdenum and mineral nitrogen on sugar beet yield (ton/fed) at harvest (2005 / 2006)

Treatments		Top yield (ton fed^{-1})				Root yield (ton fed^{-1})				Sugar yield (ton fed^{-1})			
N-fertilizer	Biofertilizers	Kg Mo/fed			Mean	Kg Mo/fed			Mean	Kg Mo/fed			Mean
		Zero	0.5	0.75		Zero	0.5	0.75		Zero	0.5	0.75	
90kg/fed.	Without	15.50	20.77	22.87	19.71	29.54	30.08	30.23	29.95	4.86	5.63	5.06	5.18
	Azotobacter	19.49	17.58	13.05	16.71	30.10	29.94	26.97	29.00	5.07	5.71	4.66	5.15
	Rhizobia	17.77	21.18	15.80	18.25	26.63	26.74	23.43	25.60	4.36	4.56	3.97	4.30
Mean		**17.58**	**19.84**	**17.24**	**18.22**	**28.76**	**28.92**	**26.88**	**28.18**	**4.76**	**5.30**	**4.56**	**4.88**
45kg/fed.	Without	12.21	12.88	18.73	14.61	20.20	22.93	29.15	24.09	3.47	4.32	5.15	4.31
	Azotobacter	23.34	22.95	22.70	23.00	34.66	29.55	29.52	31.24	6.96	4.82	5.79	5.86
	Rhizobia	20.51	21.32	15.18	19.00	34.13	35.45	19.90	29.83	5.41	6.18	3.61	5.07
Mean		**18.68**	**19.05**	**18.87**	**18.87**	**29.66**	**29.31**	**26.19**	**28.39**	**5.28**	**5.10**	**4.85**	**5.08**
Without		13.85	16.82	20.80	17.16	24.87	26.50	29.69	27.02	4.16	4.97	5.10	4.74
Azotobacter		21.41	20.26	17.87	19.85	32.38	29.74	28.24	30.12	6.01	5.26	5.22	5.50
Rhizobia		19.14	21.25	15.49	18.62	30.38	31.09	21.66	27.71	4.88	5.37	3.79	4.68
Whole mean		**18.13**	**19.44**	**18.05**	**18.54**	**29.21**	**29.11**	**26.53**	**28.28**	**5.02**	**5.20**	**4.70**	**4.98**

L.S.D. at 0.05			
Nitrogen (N)	N .S	N.S	N .S
Molybdenum(Mo)	N.S	N.S	N.S
Inoculation (In)	0.77	1.84	0.525
Mo x In	N .S	7.20	0.794
Mo x N	N .S	N .S	N .S
N x In	5.10	6.26	0.732
Mo x In x N	N .S	7.84	0.931

Table (8): Effect of bacterial inoculation, molybdenum and mineral nitrogen on quality characters of sugar beet roots at harvest (2005 / 2006)

Treatments		TSS %			Mean	Sucrose %			Mean	Purity %			Mean
N-fertilizer	Biofertilizers	Kg Mo/fed.				Kg Mo/fed.				Kg Mo/fed.			
		Zero	0.5	0.75		Zero	0.5	0.75		Zero	0.5	0.75	
90 kg/fed.	Without	23.0	24.0	22.7	23.23	16.46	18.72	16.73	17.30	71.57	78.0	73.70	74.42
	Azotobacter	23.3	22.0	21.5	22.27	16.86	19.06	17.28	17.73	72.36	86.64	80.37	79.79
	Rhizobia	21.2	21.5	23.4	22.03	16.36	17.06	16.96	16.79	77.17	79.35	72.48	76.33
Mean		**22.5**	**22.5**	**22.53**	**22.51**	**16.56**	**18.28**	**16.99**	**17.27**	**73.70**	**81.33**	**75.52**	**76.85**
45kg/fed.	Without	22.7	23.0	24.2	23.30	17.20	18.86	17.66	17.91	75.77	82.0	72.98	76.92
	Azotobacter	22.3	23.2	24.3	23.27	20.08	16.32	19.60	18.67	90.04	70.34	80.66	80.35
	Rhizobia	23.3	24.0	23.3	23.53	15.86	17.42	18.14	17.14	68.07	72.58	77.87	72.84
Mean		**22.77**	**23.4**	**23.93**	**23.37**	**17.71**	**17.53**	**18.47**	**17.90**	**77.96**	**74.97**	**77.17**	**76.70**
Without		22.8	23.5	23.4	23.3	16.83	18.79	17.19	17.60	73.67	80.00	73.34	75.67
Azotobacter		22.8	22.6	22.9	22.77	18.47	17.69	18.44	18.20	81.20	78.49	80.51	80.07
Rhizobia		22.2	22.7	23.3	22.78	16.11	17.24	17.55	16.96	72.62	75.96	75.17	74.58
Whole mean		**22.63**	**22.95**	**23.23**	**22.93**	**17.14**	**17.90**	**17.73**	**17.58**	**75.83**	**78.15**	**76.34**	**76.78**

L.S.D. at 0.05			
Nitrogen (N)	0.0552	0.0552	N .S
Molybdenum(Mo)	N .S	N .S	N.S
Inoculation (In)	N.S	N.S	4.366
Mo x In	N .S	N .S	N .S
Mo x N	N .S	0.063	6.853
N x In	N .S	N .S	N .S
Mo x In x N	0.095	N .S	8.462

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