EFFECT OF N_2 FIXERS AND N-FERTILIZATION ON SUGAR BEET YIELD AND QUALITY

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Abstract

Two field experiments were carried out at Sakha Agric. Res. Station, Kafr EL-Sheikh governorate during the two successive seasons 1999/2000 and 2000/2001. to study the effect of *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus megatherium* under different levels of nitrogen fertilizer (0, ½, full of recommended doses of N. fertilizer 70 Kg. per / feddan. on sugar beet growth, leaves and roots (fresh and dry weight). Inoculating sugar beet plant with bio fertilizer increased the fresh weight of sugar beet leaves and roots in the two seasons. The highest increase was recorded with bio fertilizer and recommended dose of nitrogen fertilizer. Data also, cleared that bio fertilizer and mineral nitrogen had significant effect on dry weight of leaves and roots of sugar beet plants., the highest increase was recorded at 150 and 210 days after planting in two seasons and the percentage of total soluble solid were significantly increased when plants inoculated with biofertilizer only, other treatments had insignificant increase. The sucrose percentage increased in the sugar beet plants while the purity percent did not affect in all treatments. Inoculation sugar beet with nitrogen fixing bacteria and mineral nitrogen increased the root and sugar yield at harvest. In general inoculation sugar beet plants with *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus megatherium* increased root yield, sucrose and sugar yield by 11.10, 11.26 and 10.61% respectively as compared with control.

INTRODUCTION

Sugar beet rhizosphere is an important zone for the growth of bacteria especially biological nitrogen fixing bacteria (BNF) and phosphate dissolving bacteria (PDB). Biological (BNF) and (PDB) play an important role in the global nitrogen and phosphorus cycle.

Recently, the beneficial effect of N2 fixers Azospirillum and Azotoacter may by due to enhancing of phytohormons, auxins, gibberelin and cytokinin like substance to the culture medium (Tien *et al.,* 1979). El-Badry and El-Bassel (1993) found that inoculation with (Azospirillum + Azotobacter) on sugar beet when received nitrogen fertilizer at rates 45 Kg N/fed, gave the best results for crop yield, total soluble compounds and sugar amount /Fed. This was about equal to these obtained with higher rates of 60 Kg and 75 Kg N/Fed without bacterial inoculation. They also added that this represents saving about 40% in N-fertilizers. Favilli *et al.*(1993) reported that the effect of inoculation with Azospirillum on sugar beet given 60 kg N/ha as ammonium sulfate produced a root yield of 159 kg/plot (30m2) compared with the yield of 143 and 125 kg/plot without seed inoculation where 100 or 60 kg N/ha were applied. Aly (1996) reported that inoculation with Azospirillum increased the dry weight of sugar beet leaves in 60,120, 180 days and resulted in low significant effect on nitrogen uptake of leaves and roots and significant result on sucrose content and photosynthetic pigments in leaves. Stajner *et al.* (1997) reported that inoculation of sugar beet seeds with Azotobacter chroococcum increased in chlorophyll, carotenoids, soluble proteins and dry matter of leaves. Sukhovitskaya (1998) reported that inoculation the seeds of sugar beet with bacillus megaterium increased crop yields by 23%. Cakmakci *et al.* (1999) reported that seed inoculation of sugar beet with Bacillus polymyxa and phosphate dissolving (Bacillus megatherium var. phosphaticum) bacteria was investigated in comparison to control and mineral fertilizer application in the green house and in the field. In the green house, bacterial inoculation of seed increased sugar beet root and dual inoculation gave increase of 19.0 and 25.9% respectively and increased by 12.0% and 16.5% in the field respectively. Bacillus megaterium var. phosphaticum alone substitute casting N P fertilizers in sugar beet. Maareg and Sohir (2001) Studied the effect of three bio-fertilizers i.e. Rhizobactrine, phosphatine and Cerealine on sugar beet growth. They found that Cerealine caused an increase in weight of root and foliage and increased the quality of sugar beet characters, i.e. T.S.S%, sucrose %, purity % and sugar yield. Khalil (2002) found that inoculation with Azotobacter chroococcum and Bacillus megatherum to provide sugar beet with nitrogen and available phosphorus increased the root yield, sugar yield and sugar percentage. The objectives of this study was to investigate the effect of Azospirillum brasilense, (Azo1) Azotobacter chroococcum (Azo2),

Bacillus megatherium (B3) as well as their interaction at different levels of nitrogen fertilizer of the recommended rate of (70 kg N/ fed) on sugarbeet growth, root yield and quality.

MATERIALS AND METHODS

Isolation and purification of *Azospirillum, Azotobacter* and phosphate dissolving bacteria in rhizosphere of sugar beet plants. The soil samples with their growing sugar beet plants were collected from Sakha Agric.Res. station at Kafr El-Sheikh Governorate. Isolates from the rhizosphere was carried out according to Bilal *et al.* (1990). Identification and characterization of Azospirillum, Azotobacter and phosphate dissolving bacteria. Azospirillum: From root region the enrichment cultured technique was adapted by using the nitrogen deficient Simi solid malate (NFM) recommended by Dobereiner (1978) and Dobereiner and Day (1976), Holm and Jensen (1972), and Eid (1978).Isolated Azotobacter and Bacillus were inoculated in modified Ashby*s medium for Azotobacter (Higazi and Niemela, 1976) and Modified Bunt and Rovera medium (Modified by Abdel Hafez, 1966). Species of Azospirillum, Azotobacter and Bacillus were kindly identified in Microbiological Resource Center (MIRCEN) in faculty of Agriculture, Ain Shams University.

Preparation of bacteria for inoculation: Each of isolate *Azospirillum brasilense* (Azo1), *Azotobacter chroococcum* (Azo2), and *Bacillus megatherium* (B3) was grown according to (Hino and Wilson, 1959). Sugar beet seeds Inoculated *individual* and interaction with the isolates by socking over night in large basin in contact with the bacterial suspension. The Arabic gum (5% w/v) was added to the bacteria. Mineral fertilization: Nitrogen fertilizer was added as urea (46.5%N) at the rates of 0 , 35 , 70 , Kg N /fed., which represent 0, ½, and full recommended rate for the field. They were added in two equal doses at one month after planting for the first and three weeks later for the second dose. Moreover, 15 P_2O_5/fed. and 24 kg. K_2O/fed as potassium sulfate (48% K_2O) were added prior to plantation.

Soil samples were and air dried, mixed, grinned and sieved through 2 mm before analysis their Mechanical and chemical analysis are shown in Table 1.

Table 1. Mechanical and chemical analysis of the experimental fields During 1999/2000 and 2000/2001seasons.

Soil properties	1999/2000	2000/2001
*Mechanical analysis		
Sand %	39	40
Silt %	27	29
Clay %	34	31
PH	7.50	7.78
*Chemical analysis		
Ec m. mhos/cm	1.00	1.25
Ca++ (meq/L	3.00	2.40
Mg++ "	2.00	4.80
Na+ "	7.00	6.50
K+ "	0.2	0.40
So4- "	3.00	2.00
HCO3- "	3.50	4.00
CL- "	5.50	8.00
T.N %	0.11	0.11
Available P (ppm)	13.50	12.25
Available K (ppm)	280.00	310.00

Two field Experiments were carried out at Sakha Agric. Res. Station, Kafr El-Sheikh Governorate, during the two successive seasons 1999/2000 and 2000/2001. Each experiment included 21 treatments with three replicates arranged in complete randomized block design. The plot area was 21 m^2 (7 X 3) = 1/200 feddan. Plant samplings were collected after 75, 150 and at 210 days. The obtained data were subjected to analysis of variance according to procedures outlined by Snedecor and Cochran (1980).

Experimental treatments:

1-Control without nitrogen and without bacterial inoculation.

2-Seeds + 1/2 recommended dose of nitrogen and without bacterial inoculation.

3-Seeds + full recommended dose of nitrogen and without bacterial inoculation.

4-Seeds + *Azospirillum brasilense* and without nitrogen fertilizer.

5-Seeds + *Azospirillum brasilense* and with 1/2 recommended dose of nitrogen.

6-Seeds + *Azospirillum brasilense* and with full recommended dose of nitrogen.

7-Seeds + *Azotobacter chroococcum* and without nitrogen fertilizer.

8-Seeds + *Azotobacter chroococcum* and with 1/2 recommended dose of nitrogen.

9-*Seeds + Azotobacter chroococcum and with full recommended dose of nitrogen.*

10-*Seeds + Bacillus megatherium and without nitrogen fertilizer.*

11-Seeds + Bacillus *megatherium* and 1/2 recommended dose of nitrogen.

12- Seeds + Bacillus *megatherium* and full recommended dose of nitrogen.

13-Seeds + *Azospirillum brasilense + Bacillus megatherium* and without nitrogen fertilizer.

14-Seeds + *Azospirillum brasilense + Bacillus megatherium* and 1/2 recommended dose of nitrogen.

15-Seeds + *Azospirillum brasilense + Bacillus megatherium* and full recommended dose of nitrogen.

16-Seeds + *Azotobacter chroococcum +Bacillus megatherium* and without nitrogen fertilizer

17-Seeds + *Azotobacter chroococcum +Bacillus megatherium* and 1/2 recommended dose of nitrogen.

18-Seeds + *Azotobacter chroococcum +Bacillus megatherium* and full recommended dose of nitrogen.

19-Seeds + *Azospirillum brasilense + Azotobacter chroococcum +Bacillus megatherium* and without nitrogen fertilizer.

20-Seeds + *Azospirillum brasilense + Azotobacter chroococcum +Bacillus megatherium* and 1/2 recommended dose of nitrogen.

21-Seeds + *Azospirillum brasilense + Azotobacter chroococcum+Bacillus megatherium* and full recommended dose of nitrogen.

Determination of sucrose: Sucrose content was evaluated using Saccharimeter apparatus by method of Le Doct (1927).

Determination of juice purity: Purity was obtained according to Sapronova *et al.* (1979) using the following equation:

$$\text{Purity} = {}^{\text{sucrose}}/_{\text{T.S.S.}} \times 100.$$

Total soluble solids percentage (T.S.S) in the fresh roots was determined by hand refract meter at harvest.

RESULTS AND DISCUSSION

Effect of bio fertilization on fresh weight of leaves in the two seasons.

Data presented in Tables 2 and 3 represent The interaction effect between the studied treatments on fresh weight of sugar beet leaves increased in two seasons, the highest value was oblained by addation of Azospirillum +Azotobacter +Bacillus and recommended dose of nitrogen the increase was 10.54% from fresh weight of leaves than control. Similar results have been achieved by Afify *et al.* (1994), Maareg and Sohir (2001), they reported that inoculation with *Azotobacter chroococum, Bacillus megatherum,* in combination with mineral NPK fertilizer resulted in significant higher fresh weight of leaves. Baddy and Doberener (1982), Okon *et al.* (1988), Han and New (1998), Subba Rao, (1982). The ability of Azospirillum and Azotobacter for mitrogen fixation and Bacillus for mineralization that release phosphorus to rhizosphere of sugar beet plants. In addition Brown *et al.* (1968), Tien *et al.* (1979), Vlassak and Reynders (1980), Malik *et al.* (1993) and Fiorelli *et al.* (1996) reported that these bacteria produce growth promoters such as gibberlin, cytokinin and indole acetic acid which enhance growth of fresh weight of sugar beet leaves.

Effect of bio fertilization on fresh weight of roots in the two seasons.

Tables 4 and 5 represent The significant and/or the insignificant influence on fresh weight of root it could be to ability of Azospirillum and Azotobacter for nitrogen fixation and *Bacillus megatherum* to release available phosphorus to sugar beet plants and produce growth promoting from these bacteria Brown *et al.* (1968), Tien *et al.* (1979), Vlassak and Reynders (1980), Malik *et al.* (1993), Fiorelli *et al.* (1996). This results is in agreement with Khalil (2002) who reported that the effect Azotobacter and *Bacillus megatherum* on sugar beet increased the fresh weight of sugar beet roots.

Effect of biofertilization on dry weight of leaves in the two seasons.

Stages 75, 150 and 210 days, except the first sample at 75 days in the first season, where that the highest value of dry weight in the first season (43.02 and 95.32 g/plant) and (0.68, 45.89 and 101.22 g/plant) in the second season in Table 6 and 7. This

result is in agreement with Aly (1996) who studied the effect of *Azospirillum brasilense* on nitrogen at all stages, he found that an increase on dry weight of sugar beet leaves.

Effect of biofertilization on dry weight of roots in the two seasons.

Data given in tables (8&9), cleared that effect of inoculation with biofertilizers and nitrogen rates and their interactions on dry weight of roots that there were no significant effect of different treatments as compared to control throughout the first sample at 75 days in two seasons. Regardless the significant and / or the non-significant influence of this trail, it could be noticed that varietals response to nitrogen fixation, release to phosphorus from bacteria and enhance of growth plants. similar results were obtained by El-Badry and El-Bassel (1993) who found that treatment in the all tested of Azospirillum and Azotobacter showed increased in weight of sugar beet plants. Also, these effects were similar to results of Aly (1996) who found that inoculation with Azospirillum at 60 days, did not affect significantly on dry weight root of sugar beet plants.

Effect of *Azospirillum brasilense, Azotobacter chroococcum, Bacillus megatherium* under different levels of nitrogen fertilizer on yield quality:

Total soluble solid (T.S.S%):

Data presented in tables (10&11) showed that inoculation with bacteria without nitrogen fertilizer had no significant effect in roots at harvest in all treatments in the first season except Azo1xAzo2xB3, which showed significant effect. On the other hand bacteria with nitrogen fertilizer had no significant effect in 1/2 and full-recommended dose. In the second season all treatments had no significant effect on T.S.S%.

Sucrose %:

The results obtained in the first season revealed that inoculation without nitrogen fertilizer gave significant effect with Azo1xB3 and Azo1xAzo2xB3 (14.98 and 15.18% of sucrose), while inoculation with nitrogen fertilizer had no significant effect. In the second season all treatments showed no significant effect in comparison with control.

Purity %:

In the first season all treatments had no significant effect in comparison with control. In the second season the inoculation only and with nitrogen fertilizer had significant effect with Azo 1 x B3 only, the results was (81.10 and 84.27% of purity).

Results obtained, proved that interaction between inoculations under different levels of nitrogen fertilizer enhanced the efficiency of sugar beet to absorb more nitrogen and phosphorus from acid producing and phosphate dissolving bacteria which were stimulated in the rhizosphere by *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus megatherium*. Such obtained results are in agreement with many investigators. (Abd-el-Hafez (1966), Cakmakci *et al.* (1999) Maareg and Sohir (2001). Who found that biofertilizers increased the quality of sugar beet.

Effect of *Azospirillum brasilense*, *Azotobacter chroococcum*, *Bacillus megatherium* under different levels of nitrogen fertilizer on root and sugar yield

Tables 12&13, present root and sugar yield (ton/fed) at harvest.The inoculation only and with nitrogen fertilizer increased root yield with Azo1xAzo2xB3, the average of results was 15.10 ton/fed. incomparison with control (14.00 ton/fed) in the first season. In the second season, the inoculation without nitrogen and with nitrogen fertilizer increased root yield with Azo1xB3 and Azo1xAzo2xB3, the means were: 15.39 and 16.04 ton/fed and 14.74 ton/fed for control.

The inoculation and nitrogen fertilizer increased sugar yield in comparison with control in all treatments with Azo2, B3, Azo2xB3, Azo1, Azo1xB3 and Azo1xAzo2xB3 (2.32, 2.35,2.73,2.41,2.44 and 2.56 ton/fed of sugar) respectively, but the control was 2.28 ton/fed of sugar in the first season. In the second season, the sugar yield increased with B3, Azo1, Azo2xB3, Azo1xB3 and Azo1xAzo2xB3 (2.27, 2.31, 2.36, 2.47 and 2.69 ton/fed of sugar) respectively, but the control was 2.25 ton/fed of sugar.Similar conclusions were obtained by Maareg and Sohir (2001) and Khalil *et al.* (2002).

REFERENCES

1. AbdeL Hafez, A. M. 1966. Some studies on acid producing microorganisms in soil and rhizosphere with special reference to phosphate dissolvers.Ph.D. Thesis, Faculty of Agric., Ain Shams Univ.

2. Afify, Aida H., Hammouda, F. M., Basyouni, A. M. and F. I. A. Hauka. 1994. Effect of bacterization and mineral fertilization on the main characters and major insect infestations of sugar beet plant. J. Agric. Sci. Mansoura Univ.,19 (1):201-208.

3. Aly, M. E. H. 1996. Effect of inoculation with atmospheric nitrogen fixing Azospirillum on growth of some sugar crops .M. Sc. Thesis, Fac. of Agric., Al-Azhar Univ.

4. Bilal, R., Rasul. G., Gureshi, Javed A. and Malik A. Kauser. 1990. Characterization of Azospirillum and related diazotrophs associated with roots of plants growing in saline soils. World J. Microbiotech. 6:46-52.

5. Boddy, R.M. and J. Dobereiner. 1982. Association of Azospirillum and other diazotrophs with topical graminae. In: Non symbiotic nitrogen fixation and organic matter in the tropics symposia papers 1. Transactions of the 12th International Cong. of Soil Science. Held in India, 8-16 Feb. 1982. Indian Soc. Of Soil Sci, New Delhi, pp. 28-47.

6. Brown, M.E., Jackson, R. M. and S. R. Burlinghan. 1968. Effects produced on tomato plants, *Lycoperaicum esculontum* by seed or root treatment with gibberellic and indol-3-acetic. J. Expt. Bot., 19: 544-552.

7. Cakmakci, R., Kanter, F. and O. F. Algar. 1999. Sugar beet and barley yield in relation to *Bacillus polymyxa* and *Bacillus megaterium* var. *phosphoticum* inoculation. J. of Plant Nutrition and Soil Science 162:4, 437-442, 29

8. Dobereiner, J. 1978. Proc. Isotops in Biological Dinitrogen Fixation, pp. 51-69. IAEA, Vienna.

9. Dobereiner,J. and J. M. Day. 1976. Associative symbioses in tropical grasses characerization of microorganisms and dinitrogen fixing sites. In Proceeding of First International Symposium on Nitrogen Fixation. ED. W. E. Newton and C.J.Nyman. Ed. Vol.2. Washington State University press.Pullman, vol. 2,pp 518-538.

10. Eid, M. A. 1978. Studies on the free N_2-Fixing bacteria in the rhizosphere of sugar cane. M.Sc. Thesis, Fac. Agric., Cairo Univ.

11. El-Badry, M. and A. El-Bassel. 1993. Improving sugar beet crop by inoculation with free-living nitrogen fixing bacteria. 6th Internat. Symp. On Nitrogen Fixation with Non-legumes., Ismailia-Egypt, 1993.

12. Favilli, R., Pastorelli, R and A. Gori. 1993. Response of sugar beet to Azospirillum bacterization in field experiments. (BIBLIOGRAPHIC CITATION): Agricoltura-Mediterranea. 1993, 123: 4, 281-285, 26 ref.

13. Fiorelli, F., Pasetti, L. and E. Galli. 1996. Fertility-promoting metabolites produced by *Azotobacter vinelandii* grown on olive-mill wastewater. International Bio detegradation. 38 (3/4): 165-167.

14. Han, S. O. and P. B. New. 1998. Variation in nitrogen fixing ability among natural isolates of Azospirillim. Microbial Ecology.36,2:193-201.

15. Higazi, N.A. and S. Niemela. 1976. A note on the estimation of Azotobacter densities by membrane filter technique. J. Appl. Bacteriol. 41:311.

16. Hino, S. and P. W. Wilson. 1959. Nitrogen fixation by a facultative *Bacillus*. J. Bacteriol. 75:403-404.

17. Holm, E. and V. Jensen. 1972. Aerobic chemo organotrophic bacteria of a Denish bech forest Microbiology of a Danish beech forest. 23: 248-260. Copenhagen.

18. Khalil, R., Soha. 2002. Response of some sugar beet (*Beta vulgaris* L.) varieties to mineral and bio-fertilization under Egyptian environmental conditions. M.sc. Thesis, Institute of Environmental studies and Research, Ain Shams Univ.

19. Le Doct, A. 1927. Commercial determination of sugar beet roots using the sacks le doct process. Int. Sugar J., 29: 488-492.

20. Maareg, M. F. and Sohir, T. Badr. 2001. Impact of three soil bio-fertilizers applied separately and in combination with a nematicide on *Meloidogyne incognita* infecting sugar beet. Egyptian J. of Agronematology 4 (1/2): 1-9.

21. Malik, K.A, Rasual, G, Hassan, V., Mahnez, S. and M. Ashraf. 1993. Role of N_2-fixing and growth hormone producing bacteria improving growth of wheat and rice. Sixth Internal. Symp. Of Nitrogen Fixation with Non-legumes, 6-10 Sept., 1993, Ismailia, Egypt.

22. Okon, Y., Fallik, E., Saring, S., Yahalom, E. and S. Tal. 1988. Plant growth promoting effects of Azospirillum. In: H. Both, F. J. de Bruijn and J. W. Newton (eds.). "Nitrogen Fixation: Hundred years after" Gustv Fischer, Stuttgart, West Germany.

23. Sapronova, A.R, Joshman, A. E. and V. A. Loseava. 1979. General technology of sugar and sugar substances. Pischchevaya Promyshlennost. Pub. Moscow, 464p.

24. Snedecor, G. W. and W. G. Cochran. 1980. Statistical Methods. Seventh ed., Iowa State Univ. Press, Ames, Iowa, USA, pp.255-269.

25. Stajner, D., Kerresan, S., Gasic, O., Mimica-Dukic, N. and H. Zongli. 1997. Nitrogen and *Azotobacter chroococcum* enhance oxidative stress tolerance in sugar beet. (BIBLIOGRAPHIC CITATION): Biologia Plantarum. 1997, 39: 3, 441-445, 19 ref.

26. Subba N. S. Rao. 1982. Bio fertilizers in Agriculture. Oxford and IBH Publishing Co., New Delhi, Bombay, Calcutta. P. 82.

27. Sukhovitskaya, L. A. 1998. Survival Retest and growth stimulating affects of *Bucillus megatherium* and *Agrobacterium radiobacter* strains. Introduced into soil. (BIBLIOGRAPHIC CITATION): Applied Biochemistry and Microbiology [Appl.-Biochem. Microbiol.]1998 vol. 34, no. 1, pp. 81-83.

28. Tien, T. M, Gashins, M.R. and D. H. Hybbell. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on Pearl millet (*Pennisetum americium.*). Appl. Environ. Microbiol, 37: 6101-1024

29. Vlassak, K. and L. Reynders. 1980. *Azospirillum rhizocoenses* in agriculture practicen IV Int. Symp. On N_2- fixation, Canberra.

Table 2. Effect of inoculation with *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus megatherium* under different Levels of nitrogen fertilizer on fresh weight (g/plant) of sugar beet leaves, season 1999 / 2000.

N-fertilizer	Season 1999 - 2000								
	75 days			150 days			210 days		
Treatments	0	1/2	1	0	1/2	1	0	1/2	1
Control	4.79	4.91	5.11	286.41	334.52	363.39	573.19	625.21	671.39
Azo 1	4.91	5.00	5.02	361.1	382.10	402.19	561.43	573.25	692.56
Azo 2	4.88	4.50	5.00	279.33	325.65	388.71	544.00	581.39	688.81
B3	4.77	4.62	4.92	299.12	379.11	421.37	570.68	573.84	677.00
Azo1 x B3	5.12	5.00	4.88	389.41	409.30	443.19	590.91	609.34	601.56
Azo2 x B3	4.83	4.96	5.09	393.79	381.51	409.37	553.69	549.71	583.11
Azo1xAzo2 x B3	4.90	4.79	5.01	379.00	399.44	440.69	599.27	640.50	701.91
L.S.D at 0.05%:									
Inoculation (I)		N.S			25.33			13.23	
N-fertilizer (N)		0.13			16.58			8.66	
(I) X (N)		N.S			43.88			22.92	

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3: *Bacillus megatherium*

Table 3. Effect of inoculation with *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus megatherium* under different Levels of nitrogen fertilizer on fresh weight (g/plant) of sugar beet leaves, season 2000 / 2001.

N-fertilizer Treatments	Season 2000 - 2001								
	75 days			150 days			210 days		
	0	1/2	1	0	1/2	1	0	1/2	1
Control	4.74	4.89	5.05	280.11	340.25	380.33	581.19	630.20	678.33
Azo 1	4.86	4.92	4.99	391.02	390.93	409.87	601.41	647.90	699.39
Azo 2	4.82	4.51	5.01	299.11	334.31	381.43	592.17	659.29	671.44
B3	4.72	4.57	4.91	293.61	373.00	415.71	601.51	591.11	681.37
Azo1 x B3	5.01	5.06	4.97	384.39	403.19	439.56	620.91	652.31	719.13
Azo2 x B3	4.87	4.91	5.00	379.29	381.14	401.91	611.49	641.69	688.37
Azo1xAzo2 x B3	4.89	4.92	5.10	399.39	389.53	422.93	650.99	711.70	749.87

L.S.D at 0.05%:			
Inoculation (I)	0.17	31.03	24.89
N-fertilizer (N)	0.11	20.31	16.29
(I) X (N)	N.S	53.42	43.1

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3: *Bacillus megatherium*

Table 4. Effect of inoculation with *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus megatherium* under different Levels of nitrogen fertilizer on fresh weight (g/plant) of sugar beet roots, season 1999 / 2000.

N-fertilizer Treatments	Season 1999 - 2000								
	75 days			150 days			210 days		
	0	1/2	1	0	1/2	1	0	1/2	1
Control	3.10	3.19	3.20	183.19	199.72	204.10	878.35	939.75	1009.41
Azo 1	3.32	3.39	3.52	188.78	199.13	210.53	892.25	981.50	1019.39
Azo 2	3.00	3.42	3.41	182.39	192.14	201.40	883.49	980.00	1002.40
B3	3.19	3.39	3.49	189.71	198.97	207.11	992.19	1152.32	1193.55
Azo1 x B3	3.63	3.81	3.75	200.38	209.27	215.49	998.37	949.92	1215.50
Azo2 x B3	3.52	3.69	3.43	208.10	217.92	220.02	892.44	999.15	1075.14
Azo1xAzo2 x B3	4.01	4.34	4.59	212.56	231.42	239.17	991.52	1159.11	1201.99

L.S.D at 0.05%:			
Inoculation (I)	0.16	6.41	26.29
N-fertilizer (N)	0.10	4.20	17.21
(I) X (N)	0.29	11.11	45.5

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3 : *Bacillus megatherium*

Table 5. Effect of inoculation with *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus megatherium* under different Levels of nitrogen fertilizer on fresh weight (g/plant) of sugar beet roots, Season 2000 / 2001.

N-fertilizer	Season 2000 - 2001								
	75 days			150 days			210 days		
Treatments	0	1/2	1	0	½	1	0	1/2	1
Control	3.17	3.52	3.79	193.14	210.39	229.43	899.31	943.79	997.15
Azo 1	3.69	3.82	3.79	211.91	226.01	238.33	1010.71	1101.82	1009.53
Azo 2	3.29	3.44	3.52	201.53	214.90	231.11	979.53	999.40	969.98
B3	3.33	3.29	3.63	199.41	210.27	223.53	982.33	1019.47	1000.39
Azo1 x B3	3.49	3.82	3.99	219.44	238.10	247.96	1250.14	1293.55	1310.14
Azo2 x B3	3.55	3.73	3.79	209.58	231.00	238.93	1051.10	1191.00	1122.92
Azo1xAzo2 x B3	3.82	4.00	4.38	232.51	253.47	241.19	1283.55	1299.37	1352.93

L.S.D at 0.05%:			
Inoculation (I)	0.18	4.25	70.94
N-fertilizer (N)	0.11	6.05	N.S
(I) X (N)	0.31	16.02	122.88

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3 : *Bacillus megatherium*

Table 6. Effect of inoculation with *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus megatherium* under different Levels of nitrogen fertilizer on dry weight (g/plant) of sugar beet leaves, Season 1999 / 2000.

N-fertilizer / Treatments	Season 1999 - 2000								
	75 days			150 days			210 days		
	0	1/2	1	0	1/2	1	0	1/2	1
Control	0.56	0.55	0.59	33.48	37.53	39.81	79.00	83.71	99.55
Azo 1	0.58	0.60	0.64	38.31	40.11	43.79	81.33	86.63	102.36
Azo 2	0.51	0.53	0.61	34.91	39.43	40.33	79.39	84.09	100.01
B3	0.55	0.58	0.69	31.19	38.11	42.01	80.11	83.31	101.15
Azo1 x B3	0.59	0.57	0.74	39.13	41.19	42.53	84.00	88.73	104.91
Azo2 x B3	0.49	0.53	0.73	38.29	40.31	43.01	83.15	87.11	103.39
Azo1xAzo2 x B3	0.62	0.63	0.76	40.01	43.39	45.67	87.19	91.99	106.78

L.S.D at 0.05%:			
Inoculation (I)	N.S	2.57	3.75
N-fertilizer (N)	0.05	1.68	2.45
(I) X (N)	N.S	4.46	6.50

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3 : *Bacillus megatherium*

Table 7. Effect of inoculation with *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus megatherium* under different Levels of nitrogen fertilizer on dry weight (g/plant) of sugar beet leaves, season 2000 / 2001.

N-fertilizer Treatments	Season 2000 - 2001								
	75 days			150 days			210 days		
	0	1/2	1	0	1/2	1	0	1/2	1
Control	0.59	0.57	0.59	37.41	39.11	41.73	82.21	84.91	93.79
Azo 1	0.60	0.63	0.65	39.91	43.11	45.39	81.00	91.19	95.39
Azo 2	0.51	0.53	0.59	43.19	41.79	43.15	79.41	87.34	92.18
B3	0.56	0.53	0.69	35.00	41.12	43.93	84.09	95.00	99.19
Azo1 x B3	0.59	0.60	0.74	39.11	45.37	47.11	93.73	99.87	100.09
Azo2 x B3	0.48	0.55	0.73	39.97	43.11	45.99	83.30	89.41	94.59
Azo1xAzo2 x B3	0.61	0.66	0.79	43.38	46.11	48.19	95.22	102.12	106.33
L.S.D at 0.05%:									
Inoculation (I)			0.04			2.25			3.59
N-fertilizer (N)			0.02			1.47			2.35
(I) X (N)			0.07			3.91			6.23

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3 : *Bacillus megatherium*

Table 8. Effect of inoculation with *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus megatherium* under different Levels of nitrogen fertilizer on dry weight (g/plant) of sugar beet roots, season 1999 / 2000.

N-fertilizer	Season 1999 - 2000								
	75 days			150 days			210 days		
Treatments	0	1/2	1	0	1/2	1	0	1/2	1
Control	0.25	0.26	0.28	29.79	32.17	33.13	168.19	177.32	181.88
Azo 1	0.26	0.27	0.29	33.59	34.97	34.99	159.99	181.91	189.73
Azo 2	0.25	0.25	0.28	31.92	30.16	33.76	172.31	177.10	181.15
B3	0.26	0.27	0.30	30.79	32.14	33.97	169.57	172.99	176.31
Azo1 x B3	0.25	0.27	0.28	35.37	37.46	37.83	163.83	183.33	192.49
Azo2 x B3	0.26	0.25	0.28	33.19	34.34	38.10	171.74	178.00	191.39
Azo1xAzo2 x B3	0.27	0.26	0.29	32.49	35.41	37.42	173.11	185.56	197.76

L.S.D at 0.05%:			
Inoculation (I)	N.S	2.22	7.45
N-fertilizer (N)	0.01	1.45	4.88
(I) X (N)	N.S	3.85	12.91

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3 : *Bacillus megatherium*

Table 9. Effect of inoculation with *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus megatherium* under different Levels of nitrogen fertilizer on dry weight (g/plant) of sugar beet roots, Season 2000 / 2001.

N-fertilizer	Season 2000 - 2001								
	75 days			150 days			210 days		
Treatments	0	1/2	1	0	1/2	1	0	1/2	1
Control	0.27	0.27	0.29	33.42	34.55	36.10	148.39	162.04	163.94
Azo 1	0.26	0.27	0.30	33.92	36.11	42.18	153.15	169.92	177.37
Azo 2	0.28	0.29	0.30	32.19	34.14	37.91	157.72	166.31	170.10
B3	0.29	0.27	0.31	33.15	35.91	39.72	160.00	165.55	170.39
Azo1 x B3	0.27	0.28	0.32	32.92	36.47	43.55	159.11	167.57	176.15
Azo2 x B3	0.28	0.29	0.31	33.11	35.81	40.94	161.29	172.31	177.99
Azo1xAzo2 x B3	0.29	0.31	0.32	32.77	35.56	44.71	153.97	170.31	179.93

L.S.D at 0.05%:			
Inoculation (I)	N.S	3.01	N.S
N-fertilizer (N)	0.01	2.03	5.98
(I) X (N)	N.S	N.S	N.S

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3: *Bacillus megatherium*

Table 10. Effect of inoculation with *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus megatherium* under different Levels of nitrogen fertilizer on quality yield at harvest, season 1999 / 2000.

N-fertilizer Treatments	Season 1999 - 2000								
	T.S.S%			Sucrose%			Purity%		
	0	1/2	1	0	1/2	1	0	1/2	1
Control	19.03	19.69	19.89	14.35	16.00	17.69	75.41	81.26	88.94
Azo 1	19.12	20.01	20.42	14.55	16.42	17.98	76.10	82.06	88.05
Azo 2	19.07	20.19	20.00	14.71	16.00	16.82	77.14	79.24	84.10
B3	19.00	18.92	20.00	15.00	16.10	17.00	78.95	85.10	85.00
Azo1 x B3	20.21	20.83	19.92	15.53	16.92	17.22	76.84	81.23	86.45
Azo2 x B3	19.71	19.92	19.81	15.44	15.96	17.00	78.34	80.16	85.81
Azo1xAzo2 x B3	20.09	20.23	20.08	15.21	16.96	17.98	75.71	83.84	89.54
L.S.D at 0.05%:									
Inoculation (I)			N.S			N.S			N.S
N-fertilizer (N)			0.51			0.86			2.87
(I) X (N)			N.S			N.S			N.S

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3 : *Bacillus megatherium*

Table 11. Effect of inoculation with *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus megatherium* under different Levels of nitrogen fertilizer on quality yield at harvest, season 2000 / 2001.

N-fertilizer Treatments	Season 2000 - 2001								
	T.S.S%			Sucrose%			Purity%		
	0	1/2	1	0	1/2	1	0	1/2	1
Control	18.92	19.80	20.22	13.92	15.40	16.02	73.57	77.78	79.23
Azo 1	18.99	19.87	20.19	14.22	15.02	16.44	74.88	75.59	81.43
Azo 2	19.08	19.39	21.11	14.00	14.98	16.00	73.38	77.26	75.79
B3	19.22	19.54	20.69	14.40	15.14	15.90	74.92	77.48	76.85
Azo1 x B3	18.47	19.14	19.00	14.98	15.52	17.22	81.10	81.09	90.63
Azo2 x B3	19.39	19.99	18.23	14.36	15.92	16.51	74.06	79.64	90.57
Azo1xAzo2 x B3	20.00	19.92	21.18	15.18	16.42	17.98	75.90	82.43	84.89

L.S.D at 0.05%:

Inoculation (I)	0.64	0.94	3.22
N-fertilizer (N)	0.42	0.61	2.11
(I) X (N)	1.12	N.S	5.59

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3 : *Bacillus megatherium*

Table 12. Effect of inoculation with *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus megatherium* under different levels of nitrogen fertilizer on root and sugar yield (ton/fed), sason 1999 / 2000.

N-fertilizer / Treatments	Season 1999 - 2000					
	Root yield (ton/fed)			Sugar yield (ton/fed)		
	0	1/2	1	0	1/2	1
Control	10.29	14.31	17.42	1.47	2.29	3.08
Azo 1	10.54	15.02	18.00	1.53	2.47	3.24
Azo 2	10.37	14.92	18.12	1.54	2.39	3.05
B3	10.79	14.87	18.01	1.62	2.39	3.06
Azo1 x B3	10.02	15.00	18.73	1.56	2.54	3.23
Azo2 x B3	10.87	15.01	17.99	1.67	2.40	3.06
Azo1 x Azo2 x B3	10.18	15.73	19.41	1.55	2.67	3.47

L.S.D at 0.05%:		
Inoculation (I)	N.S	0.25
N-fertilizer (N)	1.88	0.16
(I) X (N)	N.S	N.S

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3 : *Bacillus megatherium*

Table 13. Effect of inoculation with *Azospirillum brasilense,Azotobacter chroococcum* and *Bacillus megatherium* under different levels of nitrogen fertilizer on root and sugar yield (ton/fed), sason 2000 / 2001.

N-fertilizer / Treatment	Season 2000 - 2001					
	Root yield (ton/fed			Sugar yield (ton/fed)		
	0	1/2	1	0	1/2	1
Control	10.82	15.07	18.33	1.51	2.32	2.94
Azo 1	10.99	15.41	18.69	1.56	2.31	3.07
Azo 2	10.56	15.34	18.00	1.48	2.30	2.88
B3	10.69	15.57	18.44	1.54	2.36	2.93
Azo1 x B3	10.96	16.01	19.22	1.64	2.48	3.31
Azo2 x B3	10.88	16.00	18.15	1.53	2.55	3.00
Azo1 x Azo2 x B3	11.04	16.92	20.16	1.68	2.78	3.62
L.S.D at 0.05%:						
Inoculation (I)		N.S			N.S	
N-fertilizer (N)		1.88			0.54	
(I) X (N)		N.S			N.S	

Azo1: *Azospirillum brasilense*
Azo2: *Azotobacter chroococcum*
B3 : *Bacillus megatherium*

تأثير النتروجين الحيوى المثبت و التسميد الآزوتي على بنجر السكر

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أقيمت تجربتان حقليتان بمحطة البحوث الزراعية بسخا بمحافظة كفر الشيخ لدراسة تأثير التسميد الحيوي و المعدنى على محصول وجودة بنجر السكر خلال موسمي الزراعة ١٩٩٩/٢٠٠٠، ٢٠٠٠/٢٠٠١ وقد لقحت تقاوي بنجر السكر بميكروبات *Azospirillum brasilense* (Azo1) *Azotobacter chroococcum*, (Azo2) ،*Bacillus megatherium* (B3) ، وزرعت تحت مستويات مختلفة من التسميد الآزوتي: صفر ، ٢/١ ، و جرعة كاملة حسب التوصيات الفنية (٧٠ وحدة أزوت في صورة يوريا ٥و٤٦ %) بإجمالي ٢١ معاملة وقد تم أخذ العينات النباتية بعد ٧٥ ، ١٥٠ ، ٢١٠ يوم من الزراعة – تم تقدير الوزن الطازج و الجاف للأوراق و الجذور و جودة المحصول وقد أدي التلقيح الحيوي إلي زيادة الوزن الطازج للأوراق ولجذور نباتات بنجر السكر وكانت أعلي زيادة عند استخدام المخصبات الحيوية مع التسميد المعدني عند المستوى الموصى به. كذلك أدي التلقيح بالمخصبات الحيوية مع التسميد المعدني الي زيادة معنوية في الوزن الجاف لأوراق وجذور نباتات بنجر السكر وكانت أعلي زيادة عند ١٥٠ و ٢١٠ يوم من عمر النبات.وكذلك أدي التلقيح الحيوي الي زيادة النسبة المئوية للجوامد الذائبة الكلية زيادة معنوية عند التلقيح بالمخصبات الحيوية فقط و لم تكن الزيادة معنوية في باقي المعاملات. وقد ازدادت النسبة المئوية للسكروز في جذور بنجر السكر وكانت هذه الزيادة معنوية عند التلقيح بالبكتريا المثبتة للأزوت و البكتريا المذيبة للفوسفات. – لم تتأثر النسبة المئوية لنقاوة السكر في جميع المعاملات المختلفة. أظهر التلقيح لبنجر السكر بمثبتات الأزوت الحيوية مع التسميد بالنتروجين المعدني زيادة محصول الجذور و كذلك محصول السكر بصفة عامة. أدى استخدام خليط من الأسمدة الحيوية *Azospirillu brasilense*, (Azo1) *Azotobacter chroococcum* (Azo2) +*Bacillus megatherium* (B3), الي زيادة انتاجية محصول بنجر السكر بنسبة ١٠و١٦ % ونسبة السكروز في الجذور بمقدار ٦١و ١٠% وزيادة محصول السكر بنسبة ٢٦و١١% مقارنة بالكنترول (بدون معاملة).