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EFFECT OF BACTERIAL BIOFERTILIZATION AND N-LEVELS ON YIELD AND QUALITY OF SUGAR BEET (VARIETY LOLA)

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ABSTRACT

Two field experiments were conduced in Sakha Agricultural Research Station farm to investigate response of sugar beet plant var. Lola to nitrogen levels (100%, 75% and 50% of recommended dose) and/or combined microbial inoculation (*Azotobacter chroococcum* + *Bacillus megatherium*). Results showed that N-application significantly increased top and root yields, root fresh weight and sugar yield (ton/fed.), but did not significantly affect the percentages of N, P, K, α-amino nitrogen, Na, sucrose, extractability, sugar extractability/plant and purity.

Microbial inoculation significantly increased top and root yields and root fresh weight, while did not significantly influence percentages of N, P, K, sucrose, sugar extracted/P or extractability, α-amino nitrogen and Na.

The treatment, inoculation + 75% N gave the highest economic net return without exerting a bad effect on yield quality. Therefore, the study recommended the application of this treatment as an agricultural process for sugar beet, where it showed a positive effect on the yield and resulted in saving a lot amounts of chemical N fertilizer. The matter which is important in decreasing the deleterious effect of nitrogenous fertilizers residue on the environment.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is a complementary sugar crop to narrow the gap between the consumed and produced sugar. In addition sugar beet is the second source of sugar production after sugar cane, where about 40% of sugar production all over the world is produced annually from sugar beet (El-Sayed *et al.,* 2007).

In Egypt, the cultivated area of sugar beet increased from 17000 feddan in 1982 to 168000 in 2005 (Tantawy *et al.,* 2006).

In recent years, agricultural sustainability has emerged as a worldwide is largely because of the increasing pressure on the limited supply of land for food production and the irrelevance of present-day conventional agriculture on non-renewable fossil fuel. A considerable interest exists in adopting alternative agricultural practices and low input systems with the belief that present conventional agricultural systems using soluble fertilizers have detrimental effects on soil physical, chemical and biological properties, plants, farm animals and the environment (Murata and Goh, 1997).

In Egyptian soils total phosphorus content is present in unavailable inorganic or organic forms. Increasing alkalinity of soil increases unavailability of phosphorus (Balba 1981).

Many investigators have much greet concern to find out a solution to reduce mineral nitrogen and phosphorus application by using diazotrophs bacteria, mycorrhyza and phosphate-dissolving bacteria.

N_2-fixing and phosphate-dissolving bacteria play a significant role as plant growth-promoting rhizobacteria in the biofertilization of crops (Sahin *et al.,* 2004). They studied the effect of biofertilization with some strains of N_2-fixing and others of phosphate solubilizing bacteria in relation to chemical fertilization on sugar beet yield. They concluded that dual inoculation with N_2-fixing bacteria and P-solubilizing bacteria significantly increased root and sugar yield of sugar beet plant.

Therefore, the present investigation aimed to study the influence of biofertilization with a combined inoculum contained, N_2-fixing and P-solubilizing bacteria as well as dressing with gradual levels of mineral nitrogen on yield and quality parameters of sugar beet plants.

MATERIAL AND METHODS

Two field experiments were conducted at Sakha Agricultural Research Station farm (seasons 2007/2008 and 2008/2009) to investigate the effect of biofertilization with combined microbial inoculum (*Azotobacter chroococcum* + *Bathillus megatherium*) and N-levels (50, 75 and 100% of recommended dose) on yield, yield components and chemical characteristics of *Beta vulgaris* plants.

Seeds: *B. vulgaris* vr. Lola were kindly provided from Sugar Crops research Institute.

***Azotobacter* sp**.:Isolated and purified in Sakha Agricultural Research Station-Lab. of Bacteriology.

Bacillus megatherium*:* Kindly obtained from Institute of Soil, Water and Environment, Department of Agricultural Microbiology, Cairo, Giza.

Medium 1: (Vancura and Mucura 1990) used for isolating and culturing of *Azotobacter* sp., composed of: sucrose, 30 g; K_2HPO_4, 0.16 g; NaCl, 0.2 g; $MgSO_4$-$7H_2O$, 0.2 g; $CaCO_3$, 2.0 g; $FeSO_4$, 0.05 g; Na_2MO_4, 0.005 g; $NaBO_4$, 0.005 g and distilled water, 1 liter.

Medium 2: Nutrient broth (Nour El-Dein and Younis, 2005). The medium composed of beef extract, 1.0 g; yeast extract, 2.0 g; peptone, 5.0 g, sodium chloride 5.0 g and distilled water, 1 liter, it use din preservation and culturing of *B. megatherium*.

A split plot design with four replicates was used, where nitrogen levels incorporated main plots and inoculation treatments occupied sub-plots. The following treatments were considered:

1. Inoculated and fertilized with 50% N.
2. Not inoculated and fertilized with 75% N.
3. Inoculated and fertilized with 75% N.
4. Not inoculated but fertilized with 75% N.
5. Microbial inoculation and fertilized by 100% N.
6. Traditional control: Chemical fertilized with 100% N.

Inoculation process was performed by mixing *B. vulgaris* seeds with the combined inoculum as beat based (200 g/fed.) using appropriate sticking material as Arabic gum prior to sowing, then followed by irrigation. The inoculum consists of *Azotobacter chroococcum* with 2.5 x 10^8 cells/g, *B. megatherium* counted 2 x 10^8 cells/g. Seeds were planted at 15th and 19th of October in 2007/2008 an 2008/2009 seasons.

The average soil mechanical and chemical properties of the experimental site were determined according to Jackson (1973) and presented in Table 1.

Table (1): Some chemical and physical properties of the experimental soil at the two seasons.

Soil properties										Available nutrients (ppm)					
Seasons	Coarse sand %	Fine sand %	Silty %	Clay %	Textural class	$CaCO_3$ %	E.C (1: 5 dSm^{-1})	pH	Organic matter %	N	P	K	Fe	Zn	Mn
2005/06	5.24	14.4	32.0	45.1	Silty clay	3.14	2.85	8.0	1.87	27.3	8.50	414	9.42	7.52	124
2006/07	4.95	15.2	31.1	46.2		2.67	3.14	8.14	1.81	28.7	8.98	387	11.6	6.32	136

Nitrogen fertilizer was applied as urea (46% N) in two equal doses, the first was added after thinning (45 days from sowing) and the second was added 30 days later. Potassium fertilizer was applied in the form of potassium sulphate 48% K_2O at a rate of 100 kg/fed. was fully added after thinning, while phosphorus fertilizer was added during land preparation at the rate of 30 kg P_2O_5/fed.

Plot size of 14 m^2 consisted of 4 rows 7 m long and 0.5 m width. Sowing date of the two experiments was attempted during September. The normal agronomic practices were carried out as recommended. Soil chemical properties of the experimental sites were determined according to Jackson (1973) and are presented in Table 1.

Yield parameters:

Yield parameters included top yield (ton/fed.), root fresh weight (ton/fed.) and sugar yield (ton/fed.) of plants were recorded.

Chemical constituents:

N, P and K analysis:

Nitrogen concentration (%) was determined in roots by micro-Kjeldahl method reported by A.O.A.C. (1990). Phosphorus was measured colouremtrically according to Snell and Snell (1967). A flame photometer model E.E.L. was used to estimate potassium as reported by Richard (1945).

Juice quality characteristics were determined in the fresh roots using an automatic French systems (HYCE):

1. Sucrose percentage was determined using polarimeter on a lead acetate extract of fresh macerate root according to the method of Le-Doct (1927).
2. Sodium percentage was determined using flame photometer and α-amino nitrogen was determined using ninhydrin and hydrindantin method according to Carruthers *et al.* (1962).

3. Percentage of purity was calculated according to the following formula purity % = 99.36 [14.24 (V_1 + V_2 + V_3)/V_4] (Devillers, 1988).

Where:

V_1 = sodium % V_2 – Potassium %.

V_3 = α-amino N% V_4 = sucrose % (pol %).

4. Sugar loss of molasses (SM), sugar extractable and extractability % were calculated according to the following formula:

Sugar loss to molasses = (V_1 + V_2) 0.14 + V_1 x 0.25 + 0.5 Devillers (1988).

Extractable sugar % = V_4-SM-0.6, Dexter *et al.* (1967).

Extractability % = extractable sugar/sucrose %.

5. Root fresh weight (kg/plant).
6. Root yield (tons/fed.) was determined on the whole plot basis.
7. Sugar yield (tons/fed.) was calculated according to following equation: Sugar yield = root yield x sucrose % x purity %.
8. Top yield (ton/fed.).

Statistical analysis:

Analysis of variance (ANOVA) was performed on all experimental data according to Snedecor and Cochran (1980) and means were compared using the student Newman-Keuls test with Sigma State software. The significance level was $P < 0.05$.

RESULTS

The data presented in Table 2 illustrated some important sugar beet yield components as response to application of N-levels and/or seed bacterial inoculation. Regardless inoculation, increase of nitrogen fertilizer level from 50% to 100% of recommended dose generally increased all studied yield parameters, neversless, the differences between levels 75% and 100% mostly were not significant ($P < 0.05$).

Table (2): Effect of bacterial inoculation and N-levels application on some sugar beet yield parameters.

N% of recommended		Top yield (ton/fed.)		Root yield (ton/fed.)		Root fresh weight (kg/plant)		Sugar yield (ton/fed.)	
		2008	2009	2008	2009	2008	2009	2008	2009
50	NI	7.43 a	7.17 d	17.18 c	18.69 c	0.71 c	0.67 c	2.90 b	3.07 c
	I	8.11 cd	13.15 b	21.25 b	24.23 b	0.93 b	0.81 b	3.15 b	3.82 bc
75	NI	9.07 bc	10.00 c	24.61 a	23.65 b	0.92 b	0.91 ab	3.15 b	3.02 c
	I	10.16 b	15.54 a	25.96 a	27.59 a	.04 ab	1.01 a	3.95 a	4.58 ab
100	NI	9.20 bc	11.89 b	18.31 c	25.37 b	0.99 ab	0.97 a	3.99 a	4.03 bc
	I	11.42 a	15.58 a	24.70 a	28.43 a	1.06 a	1.01 a	4.45 a	5.17 a

Means with different letters within the same column differ significantly at $P < 0.05$.
NI = Not inoculated sugar beet plants.
I = Bacterial inoculated sugar beet plants.

The inoculation with *Azotobacter chroococcum* and *Bacillus megatherium* bacteria as combined inoculum consistently increased all studied plant yield parameters, these increases were mostly significant. The

increase in yield parameter values for inoculation over uninoculation gave mostly the highest records at 50% N, whereas, it recorded 23.7 and 29.7% for root yield and 8.7 and 24.7% for sugar yield at seasons 2008 and 2009, respectively. Furthermore treatment of 50% N with inoculation mostly did not exhibit significant variations than those of 75% N and 100% N but not inoculated. However, the best treatment attained increase in yield parameters was 100% N with inoculation which gave the highest consistent at both consequent seasons (11.42 and 15.58 ton top yield/fed.; 24.70 and 28.43 ton root/fed., 1.06 and 1.01 kg fresh root/plant; 4.45 and 5.17 ton sugar/fed.).

Table 3 illustrated the influence of N-levels and/or bacteria inoculation on some quality parameters for sugar beet plant. The data exhibited that decrease of N-levels lower than recommended dose (100% N) to 50% did not significantly affect the studied quality characters of the plant at both seasons. Similarly, bacterial inoculation for plant seeds of both seasons had no significant positive effects on the studied quality characters. However, the bacterial inoculation with 75% N gave sucrose percentage as similar as these of 100% N treatment.

Table (3): The effect of bacterial inoculation and N-levels application on some sugar quality characteristics.

N% of recommended		Sucrose (%)		Sugar loss to molasses (%)		Purity (%)		Extractable sugar(%)		Extractability (%)	
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
50	NI	17.03ab	16.99 a	1.22 a	1.32 a	94.92 a	95.16 a	15.01 a	15.52ab	88.14 a	89.06 a
	I	15.89 b	16.29 a	1.25 a	1.24 a	94.7 a	95.10 a	144.21a	14.30 b	84.92 b	88.23 a
75	NI	13.86 c	15.05 b	1.3 a	1.21 a	94.6 a	94.68 a	11.98 b	13.58 b	86.24ab	87.55 a
	I	16.69ab	17.50 a	1.38 a	1.37 a	94.5 a	94.85 a	14.60 a	15.62ab	87.62ab	88.34 a
100	NI	16.71ab	17.11 a	1.27 a	1.31 a	94.7 a	94.93 a	14.58 a	15.36ab	88.12 a	88.23 a
	I	18.64 a	18.91 a	1.38 a	1.38 a	94.9 a	95.06 a	16.55 a	17.27 a	88.8 a	88.87 a

Means with different letters within the same column differ significantly at P < 0.05.
NI = Not inoculated sugar beet plants.
I = Bacterial inoculated sugar beet plants.

Decrease of N-level not significantly affected percentages of Na, K, P, N and α-amino-nitrogen (Table 4). Inoculation also did not exhibit significant variations in these constituents. The obtained results of the two studied seasons approximately had similar trends.

Table (4): Effect of bacterial inoculation and N-levels application on percentages of some sugar beet chemical constituents.

N% of recommended		Na		K		P		N		α-amino-nitrogen (%)	
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
50	NI	0.99ab	0.9 a	3.26 a	3.1 a	0.15 a	0.15 a	1.04 a	1.06 a	0.81 a	0.68 a
	I	1.01ab	0.96 a	3.43 a	3.14 a	0.17 a	0.16 a	1.23 a	1.21 a	0.62 a	0.66 a
75	NI	0.91 b	0.93 a	2.91 a	3.24 a	0.16 a	0.16 a	1.29 a	11.05a	0.69 a	0.72 a
	I	1.10ab	1.07 a	3.84 a	3.72 a	0.15 a	0.15 a	1.34 a	1.33 a	0.69 a	0.79 a
100	NI	0.93ab	1.05 a	3.48 a	3.54 a	0.16 a	0.16 a	1.05 a	1.24 a	0.81 a	0.74 a
	I	1.16 a	1.05 a	3.83 a	3.83 a	0.14 a	0.15 a	1.05 a	1.15 a	0.74 a	0.89a

Means with different letters within the same column differ significantly at P < 0.05.
NI = Not inoculated sugar beet plants.
I = Bacterial inoculated sugar beet plants.

The present study indicated that increasing N level increased sugar beet yield parameters, however, there were no significant differences between 75 and 100% levels. These results were in accordance with those of Tantawy *et al.* (2006) and El-Sayed *et al.* (2007). The same authors confirm, also, that N-level did not significantly affect other quality and nutrition characteristics, our findings were in agreement with their results. Quality characteristics did not show significant influence in the present investigation. Study of El-Sayed *et al.* (2007) was in the same line with our results.

The combined inoculation revealed encouragement results which raized sugar beet yield parameters over uninoculated treatments under all N-levels, the best applicable and economic treatment was inoculation + 75% N. Afify *et al.* (1994) concluded that application of *Azotobacter chroococcum*, *Bacillus megatherium* and *B. circulance* increased all traits of sugar beet. Results of Mahfouz and Sharaf Eldin (2007) indicated that biofertilization of *Foeniculum vulgare* with a mixture of *A. chroococcum, Azospirillum liboferum* and *B. megaterhium* applied with chemical fertilizers (only 50% of the recommended dosage of NPK) increased vegetative growth (plant height, number of branches, and herb fresh and dry weight per plant) compared to chemical fertilizer treatment only.

The application of effective soil microorganisms increases the soil biological activity and quality of field and vegetable crops (Glick, 2003). It provides plants with an easier intake of phosphorus and potassium absorption of active growth substances and vitamins, auxin, gibberellins produced by biofertilizers, hence it is of more advantage over chemical fertilizers (Mantelin and Touraine, 2004). Bacteria of the genera *Azotobacter* and *Azospriillum* ar free living N_2-fixing organisms which live in association with plant roots in the rhizosphere. Under appropriate conditions, these bacteria can enhance plant development and promote the yield of several agriculturally important crops in different soils and climatic regions (Jagnow, 1987; Becking, 1992; Okon and Labandera-Gonzalez, 1994). These beneficial effects of *Azotobacter* and *Azospirillum* on plants are attributed mainly to an improvement in root development, an increase in the rate of water and mineral uptake by roots (Brown, 1974; Okon and Itzisohn, 1995). Plant inoculation with associative nitrogen fixing bacteria and phosphorus significantly increases yields and biomass of field crops (Govedarica *et al.,* 1997) and productivity and quality of sugar beet (Mrkovacki *et al.,* 2007).

Soil and rhizosphere bacteria can, also, affect the mineral nutrition of plants by changing root-uptake characteristics, due to modification of root morphology or alteration of uptake mechanisms and relative growth rate or internal composition of plants (Tinker, 1984). The ability of *Azotobacter* to change root morphology and plant growth rates has been widely described and commonly related to the production of biologically active substances by this genus (Bashan and Levanony, 1990; Becking, 1992).

Data of Table 5 showed that the lowest net return (L.E/fed.) resulted from application of 50% of recommended dose of nitrogen without microbial inoculation (929.9 L.E/fed.). In contrast, it was indicated that the highest value (3896.3 L.E/fed.) obtained due to microbial inoculation plus 75% of

recommended nitrogen followed by treatment of microbial inoculation plus, full dose of nitrogen (100%) which attained net return reached 3661.0 (L.E/fed.). Therefore, the present study recommends the application of 75% N + microbial inoculation treatment for its beneficial role in saving nitrogen fertilizer and increasing economic net return of feddan in addition to keeping the agroenvironment clean.

Table (5): Economic evaluation of average of the two studying seasons of sugar beet plants treated with N-levels and/or microbial inoculation.

N% of recommended		Average root yield (ton/fed.)	Fixed costs (L.E/fed.)	Changed costs (L.E./fed.)	Total costs (L.E./fed.)	Value (L.E./fed.)	Net return (L.E./fed.)
50	NI	17.94	3930	1150	5080	6009.9	929.9
	I	22.74	3930	975	4905	7617.9	2712.9
75	NI	24.13	3930	1320	5250	8083.6	2833.6
	I	26.78	3930	1145	5075	8971.3	3896.3
100	NI	21.84	3930	1485	5415	7316.4	1901.4
	I	26.57	3930	1310	5240	8901.0	3661.0

Fixed costs (L.E/fed.): Include costs of seeds (100), irrigation (80), howing (300), harvest (450) and rent of feddan (3000).
Changed costs (L.E/fed.): Include chemical fertilizers (urea, 160 L.E/50 kg; super phosphate, 95 L.E/50 kg and potassium sulphate, 300 L.E/50 kg) and inoculum (15).
Value include price of root yield (L.E/fed.)
Average root yield comprise average yield of the two studying seasons
NI = Not inoculated sugar beet plant.
I = Bacterial inoculated sugar beet plant

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تـأثير التسـميد الحيـوى البكتيـرى مـع مسـتويات مختلفـة مـن الأزوت علـى إنتاجيـة ونوعية نبات بنجر السكر (صنف لولا)

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أقيمت تجربتان حقليتان فى مزرعة محطة البحوث الزراعية بسخا لدراسة استجابة نبات بنجر السكر صنف لولا لمستويات مختلفة من التسميد النيتروجينى (١٠٠% ، ٧٥% ، ٥٠% من الجرعة الموصى بها) و/أو التسميد بلقاح حيوى مركب (آزوتوباكر + باسلس ميجاثيريم). أظهرت النتائج أن زيادة التسميد النيتروجينى زاد معنويا من إنتاجية المجموع الخضرى وإنتاجية الجذور ووزن الجذر الطازج ونسب الفوسفور والبوتاسيوم والفا أمينونيتروجين والصوديوم والسكروز وقابلية الاستخلاص وقابلية الاستخلاص/نبات والنقاوة للجذور.

زاد التلقيح الميكروبى بدرجة معنوية من وزن المجموع الخضرى ومحصول الجذور والوزن الطازج للجذر ، بينما لم يؤثر معنويا فى نسب كل من النيتروجين والفوسفور والبوتاسيوم والسكروز ومعدل الاستخلاص ومعدل الاستخلاص/نبات والفا أمينونيتروجين والصوديوم بالجذور.

أعطت معاملة التلقيح + ٧٥% أزوت أعلى صافى عائد اقتصادى ولم تسبب تأثيرا سيئا على نوعية الإنتاج. لذلك فإننا نوصى بتطبيق هذه المعاملة ضمن العمليات الزراعية لبنجر السكر نظرا لأهميتها الكبيرة فى زيادة الإنتاجية وتوفير كمية لا بأس بها من السماد الأزوتى مما يؤدى إلى التقليل من الأثر السيئ لبقايا الأسمدة الأزوتية على البيئة.

قام بتحكيم البحث

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