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INFLUENCE OF SULPHUR, BIOFERTILIZERS AND MINERAL NITROGEN ON SUGAR BEET YIELD, YIELD COMPONENTS, TECHNOLOGICAL CHARACTERISTICS AND SOME SOIL ELEMENTAL CONTENT.

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ABSTRACT

Two field experiments were conducted during successive seasons 2004/2005 and 2005/2006 at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate to study the effect of sulphur, seed inoculation with two types of bacteria *Thiobacillus thioparus* and *Azospirillum brasilense* as biofertilizers and their combinations with elemental sulphur under two levels of mineral N, 70 and 90 kg/fed. on sugar beet yield, technological characteristics and plant and soil elemental content. The design of the experiment was split plot with four replicates, sulphur and biofertilizers were distributed in the main plots while nitrogen rates were assigned in the sub- plots. Sulfur oxidizing bacteria *Thiobacillus* and free living nitrogen fixing bacteria Azospirillum, isolated from Egyptian soil were added with Arabic Gum mixture and mixed with sugar beet seeds before sowing immediately in different treatments to compensate amount of nitrogen request to plant by this bacteria. The important results could be summarized as follows:-

Inoculation with bacteria *Thiobacillus* under different periods increased the concentration of sulfates of the culture. While inoculation with *A. brasilense* increased nitrogen content.

Biofertilizers, sulphur addition, mineral N fertilizer and the interaction had a high significant effect on sugar beet yield, yield components and the technological properties.

Sulphur application and seeds inoculation by sulfur oxidizing bacteria *Thiobacillus* or *A. brasilense* N_2 fixing bacteria led to a high significant increase in root yield, top yield, sugar yield, root dimensions, sucrose, purity and extractability .

Increasing nitrogen fertilizer rate from 70 kg/fed. to 90 kg/fed. resulted in increasing root , top, sugar yields , juice impurities (Na % and alpha amino N %), sugar loss to molasses % .On the other hand, sucrose %, , potassium %, purity %, extractability % and extracted sugar % were decreased in the two studied seasons.

Inoculation of sugar beet seeds with *A. brasilense* increased available N contents in leaves and soil.

Application of sulphur and inoculation of beet seeds with *Thiobacillus* resulted in increasing plant growth and consequently, decreased soil salinity, pH and increased P, K, Zn, Fe and Mn in leaves and its availability in the soil.

Increasing N fertilizer rate up to 90 kg/ fed. increased N P K contents of sugar beet leaves and soil available N, P, Zn, Fe and Mn.

It could be concluded that application of elemental sulphur, sugar beet seeds inoculation with sulfur oxidation bacteria ,*Thiobacillus* and free living nitrogen fixing bacteria *Azospirillum* as a biofertilizers and 90 kg mineral nitrogen achieved the highest yield and proper technological characteristics of sugar beet.

INTRODUCTION

Sugar beet one of sugar crops important for production of sugar and other products .In recent years the world beginning return to biofertilization and organic agriculture. In this trend biofertilization was used by application sulfur oxidizing bacteria *Thiobacillus thioparus* to oxidation sulphur to SO_4 ,(**Mc Cready and Krause (1982)**. Sulfur oxidation plays an important role in reducing soil PH and thus increases the availability of phosphorus and micronutrients, **Abd El-Fattah *et al.*,(1990)**. **El-Leboudi *et al.* (1985)**, **Shata *et al.* (1990)** and **El-Kammah and Ali (1996)** concluded that sugar beet yield , sucrose content and total soluble salts were significantly increased with increasing levels of applied sulphur. **Hashem *et al.* (1997)** concluded that soil pH and EC values associated with residual sulfur treatment were decreased compared with control .Root and sugar yields were significantly increased with the higher rate of residual sulfur and/ or organic matter. The N2 fixing bacteria *Azotobacter clostridia* and *Azospirillum brasilense*, sulfur oxidizing bacteria were sharply influenced by sulfur content in the soil. **Hussein (2002)** found that boron fertilization and sulfur application to soil at rate 0.6 % increased root and sugar yields, TSS , juice purity and decreased soil pH. **Ouda (2002)** showed that application of S at rate 6, 8 and 10 kg/fed. for sugar beet in sandy soil decreased the most studied characters and concluded that addition of 100 kg N/fed. with 6 kg S/fed. Produced the highest sugar yield. **Thomas *et al.* (2003)** revealed that application of sulfur (25 kg/ha) resulted in 25 % increase in root yield and significant increase in shoot and root dry matter in the low sulfur soil content. Root quality was also increased due to the reduction of alpha- amino N concentration. **Hoffmann *et al.* (2004)** found that sugar beet yield and sucrose concentration were decreased, while K, Na and alpha – amino N considerably increased with extremely low sulfur supply. With sulfur concentration 0.3 % in young leaf blades no change in yield or quality occurred as a result of S application .**El-Gala *et al.* (1989) , Abd El-Fattah *et al.* (1990) and Hilal *et al.* (1990)** concluded that application of sulphur to the soil increased crop yield , plant content of N, P, K, Zn, Fe and Mn.

Response of sugar beet to nitrogen fertilizer has been studied by many scientists. They concluded that sugar beet produce maximum sucrose if nitrogen is available in the proper soil. **Nemeat- Alla (2005)** found that root length and diameter, root and top yields were increased with increasing N rate from 60 to 100 kg/fed. while sugar yield, sucrose, and juice purity were not affected. Nitrogen fixation by chemolithotrophic was used for reducing the application of mineral nitrogen fertilizer, (**Rai and Sreenivasa (1994)**. He added that *Azospirillum brasilens* was used as biofertilization (atmospheric nitrogen fixer), which increased root yield of sugar beet. **Parvatham *et al.* (1989)** showed that the highest shoot girth, root length and root volume of okra cultivars were obtained from soil treated with *Azospirillum* inoculums at 25 kg/ha. **Sundravelu and Muthurishnan (1993)** concluded that radish plants from seeds treated with *Azospirillum* and / or GA_3 had more and longer leaves, longer and thicker roots and a greater total weight root than control. **Cakmakci *et al.* (2001)** revealed that sugar beet seed inoculation with N_2 - fixing bacteria significantly increased yield, yield components and quality parameters .Mineral N, P and N P fertilizers application also increased root yield. They added that N_2 - fixing bacteria had yields equal to mineral N application. **Shafika *et al.* (2005)** concluded that increasing nitrogen fertilizer level up to 110 kg N /fed. increased root, top, and sugar yields, juice impurities (Na % and alpha amino N %), sugar loss to molasses % and concentrations of N, P , Na. On the other hand , K % and alkalinity coefficient were decreased. Increasing the applied sulphur dose up to 8 cm^3 S / l led to additional increase in root, top, and sugar yields, sucrose, juice purity, extractable sugar, alkalinity coefficient and N P K elements.

MATERIALS AND METHODS

Two field trials were carried out at Sakha Agricultural Research Station (Kafr El-Sheikh Governorate) during successive seasons 2004/2005 and 2005/2006 to study the effect of biofertilization with *Thiobacillus thioparus* , *Azospirillum brasilense* and sulphur under two levels of nitrogen on sugar beet yield, roots quality and plant and soil elemental contents . The treatments were inoculation of sugar beet seeds with oxidation bacteria *Thiobacillus* , free living ,nitrogen fixing bacteria *Azospirillum* as biofertilizers , elemental sulphur at the rate of 50 kg /fed. added before sowing and the combinations under two levels of mineral nitrogen 70 and 90 kg N / fed.

The design of the experiment was split plot with four replicates as follows:

Main plots:

1-Control.

2 - Elemental sulpher, 50 kg/fed. (S).

3 – *Thiobacillus* as sulphur oxidizing bacteria.

4 –Azospirillum as nitrogen fixing bacteria.

5 - S + *Thiobacillus.*

6 - S + *Azospirillum.*

7- S + *Thiobacillus* + *Azospirillum.*

Sub plots:

1- N 70: application of 70 kg N/fed.

2- N 90: application of 90-kg N/fed.

Biofertilizer treatments were allocated in the main plots and nitrogen levels were randomly distributed in the sub-plots. Phosphorus fertilizer was applied in the form of calcium super phosphate (15.5% P_2O_5) at the rate of 30 kg P_2O_5 /fed at seed bed preparation. Nitrogen fertilizer was applied as Urea (46% N) at the rate of 70 and 90 kg N/fed in two equal doses (after thinning and one month later). A multigerm sugar beet variety Corola was sown during the 1st week of October while harvest was done 7 months later in both seasons. Plot size was 21 m^2. Sugar beet was sown on ridges of 7 m long, 6 ridges, and 50-cm apart with 20 cm between hills. The previous crop was maize in both seasons. The soil of the experimental site was non saline non alkaline and clayey in texture. Some physical and chemical properties of the upper 30-cm of soil of the experimental site are illustrated in Table (1). Other agricultural practices were done as recommended by Sugar Crops Research Institute.

Table (1) Some chemical and physical properties of the experimental site.

Soil Properties	First season	Second seadon
pH , 1:2:5	7.82	8.21
ECe, dS /m	2.12	2.79
Soluble Cations and Anions, meq/l		
Ca^{2+}	5.8	5.0
Mg^{2+}	3.4	4.0
Na^{+}	11.5	18.1
K^{+}	0.58	0.7
CO_3^{2-}	0.0	0.0
HCO_3^{-}	3.46	3.5
Cl^{-}	4.30	6.6
SO_4^{2-}	13.52	17.7
ESP	8.00	10.2
Total carbonates, %	2.35	2.63
Organic matter, %	1.52	1.36
Available nitrogen, ppm	27.8	23.3
Available-P (extracted by NaHCO3), ppm	5.95	5.2
Available-K (extracted by ammonium acetate) ppm	396	416
DTPA extracted Zn, PPm	2.5	2.4
DTPA extracted Fe, PPm	16.1	14.6
DTPA extracted Mn, PPm	21.8	19.6
Particle Size Distribution		
Sand, %	17.64	18.87
Silt, %	33.52	32.73
Clay, %	48.84	48.40
Texture class	Clayey	Clayey

Data recorded:

A-Isolation and purification of bacteria:

1- *Azospirillum*:

Egyptian fertile soil sample was used to isolation of N_2 fixing ***Azospirillum*** by enrichment method by using Erlenmeyer flask (lap Full) containing 25 ml. of semi solid by adding 0.5 % agar of Dobrinier medium free of any nitrogen sources and inoculated with 0.10 mg fertile soil and incubated at 30C° for 72 hours. Purification was done by streaking on agar medium. The growth of colony was selected and tested microscopically and stain reaction , then applied of ***Azospirillum*** characters in **Holt (1994)** to be insure belonging to ***Azospirillum brasilense*** pure culture ,and tested to capable of atmosphere nitrogen ,by growing in flasks 250 ml containing 100 ml of Dobriniere with addition of bromthymol blue and determination of total nitrogen at 2, 4, 6, 8 and 10 days from start of inoculation process using microKjldahl method.

2- *Thiobacillus*:

Sulfur oxidizing *Thiobacillus* was isolated from Egyptian soil by enrichment technique, by using flasks containing Starkey's broth medium, bacteria were amplified and purified by streaking on plate of Starkey's agar medium, and examinated by microscope using gram staining and applied of criterions of Thiobacillus bacteria in **Starkey (1966) and Holt(1994).**To be insure belonging to *Thiobacillus* the culture was tested for efficiency of sulfur oxidation and free nitrogen fixation using flasks containing of Starkey's broth medium and total nitrogen was determined according to microKjldahl and sulfate formation according to **(Page et al. , 1982)** at 2,4,6,8 and 10 days from start of inoculation.

B-Preparation of bacterial inoculums:

1 - *Azospirillum*:

Mother culture of local isolate bacteria was propagated by inoculation of 250 ml flasks containing 100 ml of Dobriniere medium. Flasks incubated for 72 h under appropriate growth condition. Count of ***Azospirillum*** cells was about 3.5 x 10^7 CFU /ml of culture. Sugar beet seeds were inoculated with the inoculum using 100 ml of the culture and 80 g Arabic gum per feddan. Doberniere medium composition according to **Doberniere (1978)** is Malic acid 5.0 g , KH_2PO_4 0.5 g , $MgSO_4 . 7H_2O$ 0.2 g, NaCl 0.1 g, $CaCl_2$ 0.02 g,

$FeSO_4.7H_2O$ 0.5 g, Sodium molybdate 0.002 g, $MnSO_4.4H_2O$ 0.01 g, KOH 4.0 g, Agar 1.75 g, Distilled water 1000 ml, 0.5 % alcoholic solution of bromothymol blus , pH 6.8

2 - *Thiobacillus*:

Purified culture were inoculated in 250 ml flasks containing 50 ml, of Starkey's broth medium and incubated at 30^C for six days. Medium composition, g/l is $Na_2HPO_4.7H_2O$ 2.27, KH_2PO_4 1.8, $MgCl_2$ $.7H_2O$ 0.1, $(NH_4)_2SO_4$ 1.098, $MnCl_2.2H_2O$ 0.023, $CaCl_2$ 0.003, $FeCl_3.6H_2O$ 0.033, Na_2CO_3 1.0 and $Na_2S_2O_3.5H_2O$.Count of *Thiobacillus* was $5.2x10^6$ CFU /ml. Inoculation of seeds by *Thiobacillus* or dual inoculation with *Thiobacillus* and *A. brasilense* were as mentioned for inoculation with *Azospirillum*.

Test organism isolation and inoculums production:

Two purified isolates of bacteria were used in different treatments, the first was sulfur oxidizing bacteria chemolithotrophic *Thiobacillus* and the second was free living atmosphere nitrogen fixing bacteria *Azospirillum*.

1. *Azospirillum*: It was isolated from Egyptian soil by the Staff of Microbiology Lab.,Bot.,Dept.,Fac.,Agric.,Al-Azhar Univ., after that pass with different stages as purification, capable on nitrogen fixation and identification according to microbiology protocol , Holt 1994 and Shokry 2006.Finally inoculum production under microaerophilic condition by added agar 0.2 % of growth medium viable cells up to 275 x 10^6/ml.
2. *Thiobacillus*: It was local isolated, purified, identified and tested on sulfur oxidation partially as well as capable on atmosphere nitrogen fixation as oligonitrophilic according to Starkey (1966) and Holt 1994 ; American Public Health Association,

C- Sugar beet yield, yield components and technological characteristics:

At harvest, a sample of ten guarded plants was taken at random to determine the following characters:

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

1. Sucrose percentage (Pol. %) was determined using polarimeter on a lead acetate extract of fresh macerate root according to the method of (Le-Doct ,1927).

2. Potassium and sodium were determined using flame photometer and α-amino nitrogen was determined using ninhydrin and hydrindantin method according to (**Carruthers *et al.* ,1962**).
3. Purity % was calculated according to the following formulas: Purity % = 99.36 - [14.27(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 to molasses (SM), sugar extractable and extractability % were calculated according to the following formulas:

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 %.

Root fresh weight, root yield, sugar yield and top yield were determined as follows:

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 the following equation:

   Sugar yield = root yield x sucrose % x purity %.
8. Alkalinity coefficient = v1 + v2 /v3
9. Top yield (tons/fed).

D- Plant analysis:

Leaves of sugar beet samples were dried , milled and wet digested for determination of N, P, K, Zn, Fe and Mn according to(**Chapman and Pratt ,1967**).

E-Soil analysis:

Soil available N was extracted by K_2SO_4 solution 0.5 N and determined using Automatic Micro – Kjeldahl. Soil available P was extracted by $NaHCO_3$ 0.5 M and, determined according to (**Murphy and Riley ,1962**).Soil available K was extracted by ammonium acetate 1 N and determined using the Flame – photometer according to (**Page et al. , 1982**). Soil available Zn, Fe and Mn were extracted by DTPA solution **Lindsay and Norvell, (1978)**.

The collected data were statistically analized according to the method described by (**Snedecor and Cochran ,1981**).

RSULTS AND DISCUSSION

1-Effect of incubation with *A. brasilense* and *Thiobacillus* bacteria on N_2 - fixation and sulphur oxidation:

The obtained results in Table (2) showed that inoculation with *A. rasilense* fixes the atmospheric nitrogen whereas N content increased from 40 ppm up to 45 ppm after 8 days. On the other hand, inoculation with sulphur oxidizing bacteria *Azospirillum* oxidized elemental sulphur whereas SO_4 increased from 300 ppm after 2 days to 650 ppm after 10 days.

Table (2) Nitrogen and sulfate concentrations(ppm) in the culture as affected by inoculation with *Azospirillum and Thiobacillus.*

Incubation period days	*Azospirillum brasilins*	*Thiobacillus thioparus*	
	N	$SO4^{2-}$	N
2	40	300	22
4	42	620	24
6	45	640	24
8	45	645	24
10	42	650	23

2- Sugar beet yield and yield components:

The obtained results in Table (3) showed that the biofertilizer treatments , mineral nitrogen levels and the interaction had high significant effect on root yield in the 1st and 2nd seasons. Seed inoculation by sulfur oxidizing *Thiobacillus* bacteria or *A. brasilense* N_2 - fixing bacteria led to a high significant increase in root, shoot, sugar yields, root diameter and root length in the 1st and 2nd seasons. *Thiobacillus* oxidizing S element to SO_4 causing decrease in soil pH, leading to increase availability of most plant nutrients (**Mc Cready and Krause ,1982**). There are different mechanisms responsible for the effective role of *A. brasilense* in enhancing plant growth, the main effect represented in N2 – fixation performed by this microb (**Subba Rao, 1984**). *A. brasilense* is also active producers to phytohormonse and vitamins of group B (**Rodelas et al., 1993**). The ability of this microorganism in producing plant growth phytohormonse explains growth promotion of the inoculated plants. Also, it has been established that growth regulators like cytokines inhibit the loss of

Table (3) Sugar beet yield and yield components as influenced by sulphur ,biofertilizers and nitrogen rates:

Treatments	First season					Second season				
	Root yield, ton/fed.	Top yield, ton/fed.	Sugar yield, ton/fed.	Root diameter, Cm	Root Length , cm	Root yield, ton/fed.	Top yield, ton/fed.	Sugar yield, ton/fed.	Root Diameter , cm	Root length, cm
Sulphur and biofertilizers										
Control	23.30	8.91	4.38	11.33	33.67	23.09	8.96	3.75	13.67	38.38
Sulphur (S)	23.78	10.06	4.27	12.18	30.34	24.32	9.63	4.06	11.64	37.00
Thiobacillus (T)	23.88	10.15	4.35	12.01	31.83	24.68	8.66	4.10	13.18	39.56
A. brasilense (A)	23.48	9.86	4.22	12.36	30.51	24.81	8.56	3.94	14.69	40.84
S + T	24.30	10.82	4.46	13.68	33.84	25.61	9.64	4.16	15.14	41.59
S + A	24.40	10.25	4.71	12.01	30.84	25.31	9.30	4.03	13.34	40.19
S + T + A	25.39	10.12	4.57	11.86	33.19	25.77	9.12	4.37	13.30	39.00
F-test	**	**	**	**	**	**	**	**	**	**
L.S.D 0.05	0.613	0.6	0.375	1.483	2.567	0.984	0.619	0.235	1.810	3.758
L.S.D 0.01	0.886	0.868	0.442	2.146	3.414	1.424	0.895	0.239	2.618	4.435
Nitrogen										
N 1	23.52	9.35	4.32	12.01	31.58	23.56	8.29	3.90	13.75	39.43
N 2	25.20	10.70	4.53	12.39	32.49	26.04	9.96	4.22	13.38	39.59
F-test	**	**	**	**	**	**	**	**	**	ns
L.S.D 0.05	0.265	0.282	0.131	0.216	0.492	0.238	0.226	0.128	0.702	0.00
L.S.D 0.01	0.360	0.383	0.179	0.375	0.669	0 .325	0.308	0.174	0.956	0.00
Interaction										
B x N	**	**	**	**	**	**	**	**	**	**

* B = biofertilizers

some micronutrients e.g. Cu, B, Zn, and Mo from leaves (**Mauk and Nooden, 1992)**. All these aforementioned effects of A. brasilense inoculation leads together to enhancement of plant growth and yield, and decrease the extensive use of chemical fertilizers leading to a clean food and environment. Application of sulphur and seed inoculation by Thiobacillus or Azospirillum encouraged the increase of the abovementioned yield parameters. The highest average root yields 25.39 and 25.77 ton/fed. were resulted under sulphur application and seed inoculation by *Thiobacilus* and *Azospirillum* treatment in the 1st and 2nd seasons, respectively. However **El-Adl (2002)** revealed that increasing the rate of elemental sulphur increased fresh and dry weights of sugar beet leaves and roots, root and sugar yields The highest sugar yield in the 1st season, 4.71 ton/fed. was obtained under S + *Azospirillum* treatment, while in the 2nd season the highest sugar yield 4.37 ton/fed. was resulted under S + *Thiobacilus* bacteria + *Azospirillum* treatment. Data also revealed that application of sulphur and inoculation with *Thiobacillus* gave the highest average shoot yield , root diameter and root length in the 1st and 2nd seasons. Regarding the effect of mineral nitrogen rate data in Table (3) cleared that increasing N rate from 70 kg/fed. to 90 kg/fed. resulted in increased root, top, and sugar yields, root diameter and root length in the two successive seasons. The same results were found by **Hilal et al (1992) ; Zeinab et al (2000) and Hanaa (2001)**.The interaction between biofertilizers and mineral nitrogen had a high significant effect on sugar beet yield and yield components as shown in Table (3). obtained results are in agreement with those of; **Cakmakci et al. (2001); Ghazy et al. (2002)**; and **Shafika et al. (2005)**

3-Technological characteristics:

Data in Table (4) revealed that root quality parameters except for extractability in the 1st season were significantly affected by biofertilizer treatments in the two studied seasons. Application of elemental sulphur increased sucrose %, alkalinity %, purity %, extractability % and extracted sugar % in the two studied seasons. In contrast, sulphur addition decreased juice impurities (Na % and alpha amino N %), sugar loss to molasses.

Inoculation of sugar beet seeds with *Thiobacilus* bacteria or *Azospirillum* resulted in the same preferable effect of sulphur on the technological characteristics. The combinations between sulfur , *Thiobacilus* bacteria and *Azospirillum* almost increased sucrose %,

Table (4) Some technological characteristics of sugar beet roots as influenced by added sulphur, biofertilizers and nitrogen rates.

Treatments	Sucrose, %	Na,%	K,%	Alpha- amino N,%	Alkalinity, %	Purity, %	Extractability, %	Extracted sugar,%	Loss to molasses,%
Sulphur and biofertilizers	**First season**								
Control	18.40	0.47	2.44	0.37	7.36	96.85	91.30	16.81	1.01
Sulphur(S)	18.51	0.43	2.28	0.33	8.40	97.00	91.36	16.90	0.99
T.thioparus (T)	18.90	0.42	2.16	0.35	7.33	97.15	91.69	17.33	0.97
A. brasilense (A)	18.36	0.43	2.06	0.36	7.78	97.14	91.50	16.80	0.96
S + T	18.71	0.43	2.23	0.35	7.95	97.09	91.61	17.14	0.97
S + A	18.55	0.44	2.20	0.34	8.18	97.07	91.48	16.97	0.98
S + T + A	19.47	0.40	2.14	0.34	7.50	97.21	91.88	17.89	0.98
F-test	**	**	**	**	**	**	Ns	**	**
L.S.D 0.05	1.071	0.042	0.228	0.023	1.011	0.336	0.00	0.851	0.003
L.S.D 0.01	1.126	0.056	0.329	0.031	1.041	0.354	0.00	1.031	0.045
Nitrogen									
N 1	18.9	0.42	2.22	0.33	7.98	97.09	91.84	17.35	0.97
N 2	18.5	0.45	2.15	0.36	7.59	97.05	91.31	16.9	0.98
F-test	**	**	*	**	*	**	*	**	ns
L.S.D 0.05	0.241	0.015	0.006	0.01	0.387	0.0261	0.453	0.314	0.00
L.S.D 0.01	0.373	0.02	0.00	0.014	0.00	0.038	0.00	0.428	0.00
Interaction									
B x N	**	**	*	**	**	**	**	**	ns
Treatments	Sucrose,%	Na,%	K,%	Alpha-amino N,%	Alkalinity,%	Purity,%	Extractability,%	Extracted sugar,%	Loss to molasses,%
Sulphur and biofertilizers	**Second season**								
Control	16.70	0.56	2.06	0.35	7.06	96.82	90.36	15.09	1.01
Sulphur (S)	17.03	0.46	2.24	0.26	10.60	96.88	90.66	15.44	0.99
Thiobacillus (T)	17.10	0.40	2.15	0.34	7.51	96.95	90.88	15.54	0.96
A. brasilense (A)	16.33	0.40	2.22	0.24	11.03	96.86	90.39	14.76	0.97
S + T	16.94	0.42	2.31	0.31	7.78	96.80	90.61	15.35	0.99
S + A	16.40	0.41	2.09	0.32	8.06	96.91	90.55	14.85	0.95
S + T + A	17.70	0.36	2.00	0.31	9.16	97.21	91.41	16.18	0.92
F-test	**	**	**	**	**	**	**	**	**
L.S.D 0.05	0.244	0.011	0.118	0.009	0.545	0.315	0.935	0.673	0.006
L.S.D 0.01	0.332	0.019	0.171	0.011	0.788	0.337	1.05	0.974	0.008
Nitrogen									
N 1	17.08	0.40	2.17	0.29	8.91	96.95	90.89	15.50	0.96
N 2	16.70	0.46	2.14	0.31	8.57	96.90	90.51	15.13	0.98
F-test	**	**	**	**	**	**	**	**	**
L.S.D 0.05	0.207	0.01	0.05	0.004	0.103	0.033	0.188	0.164	0.009
L.S.D 0.01	0.281	0.014	0.068	0.006	0.14	0.048	0.256	0.224	0.012
Interaction									
B x N	**	**	**	**	**	**	**	**	**

alkalinity %, purity %, extractability % and extracted sugar % in the 1st and 2nd seasons. The highest average values (19.47 and 17.7 %) for sucrose, (97.21 and 97.21 %) for purity. (91.88 and 91.41%) for extractability and (17.89 and 16.18 %) for extracted sugar were recorded under sulphur + *Thiobacilus* bacteria + *Azospirillum* treatment in the 1st and 2nd seasons ,respectively.

Regarding to the mineral nitrogen treatments the obtained results showed that increasing nitrogen fertilizer rate from 70 kg/fed. to 90 kg/fed. resulted in increasing juice impurities (Na % and alpha amino N %), sugar loss to molasses % .On the other hand, sucrose %, , potassium %, purity %, extractability % and extracted sugar % were decreased in the two studied seasons. Respecting to the interaction between biofertilizers and nitrogen fertilizer rates the analysis of variance indicated that the aforementioned quality parameters were high significantly influenced except loss to molasses in the 1st season. The obtained results are in agreement with those of; **Zeinab et al (2000); Hanaa (2001); El-Adl (2002) ; Ghazy et al. (2002); Cakmakci et al. (2001) and Shafika et al. (2005).**

4- Plant elemental content:

Data in Table (5) indicated that biofertilizers, sulphur and mineral nitrogen fertilizer treatments increased the concentrations of macro and micronutrients in leaves of sugar beet compared with control during the two growing seasons. Nitrogen content in leaves of sugar beet was more influenced and increased by seed inoculation with *A. brasilense* and nitrogen fertilizer(**Subba Rao, 1984).** While phosphorus, zinc, iron and manganese contents in leaves were increased by sulphur and *Thiobacillus* treatments. Whoever **El-Gala et al. (1989) , Abd El-Fattah et al. (1990) and Hilal et al. (1990)** found that application of sulphur increased plant content of N, P, K, Zn, Fe and Mn. It should be take in consideration that inoculation with *Thiobacillus* for oxidation sulphur to SO_4 resulted in reducing soil reaction and consequently increased the availability of phosphorus and micronutrients for plant uptake, **(Mc Cready and Krause (1982)**The obtained results also showed that increasing nitrogen fertilizer from 70 to 90 kg N/fed. increased beet leaves content of N, P, K, Zn, Fe and Mn in the 1st and 2nd seasons. The highest N, P and K concentrations in leaves 3.1, 0.3 and 2.0% in the 1st season and 3.05, 0.29 and 2.15 % in the 2nd season, respectively were obtained under sulphur + *Thiobacilus* and *Azospirillum* treatment. While the highest

Table (5) Elemental content of sugar beet leaves as affected by sulphur, biofertilizers and nitrogen rate.

Treatments	First season						Second season					
	Available macronutrients,%			Available micronutrients, ppm			Available macronutrients,%			Available micronutrients, ppm		
	N	P	K	Zn	Fe	Mn	N	P	K	Zn	Fe	Mn
Sulphur and biofertilizers												
Control	2.00	0.22	1.15	29.50	276.0	32.5	1.95	0.21	1.25	28.50	254.0	30.5
Sulphur (S)	2.35	0.26	1.40	38.50	306.5	41.0	2.35	0.25	1.50	38.00	282.0	38.5
Thiobacillus (T)	2.25	0.25	1.40	36.50	295.5	37.5	2.20	0.24	1.50	35.50	271.5	35.5
A. brasilense (A)	2.85	0.24	1.55	33.00	296.0	36.5	2.80	0.23	1.65	32.50	272.5	34.5
S + T	2.30	0.28	1.60	44.00	330.0	46.0	2.35	0.27	1.75	43.00	303.5	43.0
S + A	3.00	0.28	1.90	42.00	326.5	44.5	2.90	0.27	2.00	41.00	300.0	42.0
S + T + A	3.10	0.30	2.00	48.00	354.5	50.0	3.05	0.29	2.15	47.00	326.5	47.0
Nitrogen												
N 1	2.36	0.24	1.44	35.71	287.3	37.9	2.33	0.23	1.54	34.71	264.3	35.6
N 2	2.74	0.28	1.70	41.86	337.0	44.4	2.70	0.27	1.83	41.14	310.0	41.9

concentrations of Zn, Fe and Mn 48.0, 354.5 and 50.0 ppm in the first season, respectively and, 47.0, 326.5 and 47.0 ppm in the second season , respectively were obtained under sulphur + *Thiobacilus* and *Azospirillum* treatment. Increasing the rate of mineral nitrogen fertilizer from 70 to 90 kg/fed. increased the contents of macro and micro – nutrients in leaves of sugar beet during the two growing seasons.

5-Soil content of available macro and micronutrients after harvesting:

Data in Table(6) show the effect of sulphur, biofertilizes and mineral N rates on the soil salinity , soil pH ,soil available N, P and K and diamin triethyline penta acetic acid (DTPA) extractable Zn, Fe and Mn in the surface (0-30) soil layer. The obtained results clear that sulphur application decreased soil salinity and pH values and increased the DTPA extractable Zn, Fe and Mn compared to control. The reduction in soil salinity could be attributed to the improvement of soil physical properties which encouraged leaching of soluble ions. While formation of sulfuric acid in the soil solution due to sulphur application and inoculation with sulfur oxidizing bacteria *Thiobacillus* resulted in reducing soil reaction and consequently increased the availability of phosphorus and micronutrients. These results are in agreement with those of **El-Leboudi et al. (1985), Shata et al.** (1990) **, El-Kammah and Ali (1996) Hashem et al.(1997) and Abd Allah (1998)**.Inoculation of seeds by *Thiobacillus* exhibited the reduction of soil ECe and pH values and increased the concentrations of DTPA extractable Zn, Fe and Mn .The average values of ECe and pH decreased from (2.33 dS/m and 7.81) for control to (1.87 dS/m and 7.56) in the 1st season and from (2.84 dS/m and 8.2) for control to (2.28 dS/m and 7.94) in the 2nd season, respectively by application of sulphur and seed inoculation with *Thiobacillus* and *A. brasilense*. Regarding the N2 fixing Azospirillum it is important to notice that in the case of sugar beet (as non – legume) nodules did not developed. The habitat of these bacteria in the root surface and intercellular spaces of cortex cells In these rhizosphere associations the host plant provides root exudates as energy source for N2 fixation. The highest average values of soil available N, P and K and DTPA - extractable Zn, Fe and Mn were recorded under S + T+ A treatment during the two studied seasons. Data also revealed that soil macro and micronutrients were increased with increasing the rate of mineral

Table (6) Soil salinity, pH and macro and micronutrients as affected by sulphur, biofertilizers and nitrogen rates.

Treatments	First season								Second season							
	EC, dS / m	pH, 1:2.5	Available macronutrients ,ppm			Available micronutrients ,ppm			EC ,dS / m	pH, 1:2.5	Available macronutrients ,ppm			Available micronutrients ,ppm		
			N	P	K	Zn	Fe	Mn			N	P	K	Zn	Fe	Mn
Sulphur and biofertilizers																
Control	2.33	7.81	27.0	5.6	401.0	2.6	16.4	22.0	2.84	8.20	24.6	5.1	409.0	2.5	14.6	20.0
Sulphur (S)	1.93	7.72	27.6	9.5	420.5	4.4	25.3	28.5	2.35	8.11	25.1	8.6	429.0	4.1	22.5	26.0
Thiobacillus (T)	2.25	7.64	27.2	7.7	415.0	3.5	20.0	26.0	2.74	8.02	24.8	6.9	423.0	3.3	17.8	23.6
A. brasilense (A)	2.29	7.82	33.3	6.2	421.0	3.0	18.1	23.9	2.79	8.21	30.3	5.6	429.0	2.8	16.1	21.8
S + T	1.90	7.61	27.6	11.8	425.5	5.0	28.0	34.0	2.32	7.99	25.1	10.6	431.2	4.7	24.9	31.0
S + A	1.93	7.72	34.9	10.0	423.0	4.6	25.5	30.1	2.35	8.11	31.8	9.0	434.7	4.3	22.7	27.4
S + T + A	1.87	7.56	37.4	12.3	432.6	5.3	29.2	35.1	2.28	7.94	34.1	11.1	437.4	5.0	26.0	31.9
Nitrogen																
N 1	2.06	7.70	29.5	8.9	406.6	3.9	22.7	28.1	2.52	8.09	26.9	8.0	414.9	3.6	20.2	25.6
N 2	2.07	7.69	31.9	9.1	450.4	4.2	23.6	28.9	2.53	8.07	29.0	8.2	459.1	3.9	21.0	26.3

nitrogen fertilizer from 70 to 90 kg N / fed. in the two growing seasons of sugar beet. These results are in good agreement with those obtained by **Sonbol et al. (2007)**.

Conclusion and Recommendation:

It could be concluded that application of elemental sulphur, sugar beet seeds inoculation with sulfur oxidation bacteria *Thiobacillus* and free living nitrogen fixing bacteria *Azospirillum* as a biofertilizers and 90 kg mineral nitrogen achieved the highest yield and proper technological characteristics of sugar beet.

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الملخص العربي

تأثير إضافة الكبريت ، المخصبات الحيوية و التسميد النتروجين المعدني على إنتاج بنجر السكر ، الصفات التكنولوجية و المحتوى العنصري للنبات و التربة.

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أقيمت تجربتان حقليتان خلال موسمين زراعيين متتاليين (٢٠٠٤-٢٠٠٥) و (٢٠٠٥-٢٠٠٦) بمزرعة محطة البحوث الزراعية بسخا – محافظة كفر الشيخ، لدراسة مدى استجابة محصول بنجر السكر لإضافة الكبريت الزراعي ، الأسمدة الحيوية و التسميد النيتروجيني المعدني على إنتاج بنجر السكر و مكوناته ، الصفات التكنولوجية و المحتوى العنصري للنبات و التربة. استخدم التصميم الإحصائي قطع منشقة حيث وزعت معاملات الكنترول، الكبريت ، التلقيح ببكتيريا الثيوباثيلس المؤكسدة للكبريت ، بكتيريا الازوسبيريلم حرة التثبيت للنتروجين الجوى ، الكبريت + التلقيح ببكتيريا الثيوباثيلس، الكبريت + بكتيريا الازوسبيريلم و الكبريت + التلقيح ببكتيريا الثيوباثيلس + بكتيريا الازوسبيريلم في القطع الرئيسية. و قد وزعت معاملات السماد النتروجينى المعدني في القطع المنشقة بمستويين ٧٠ و ٩٠ كجم نتروجين / فدان.

و يمكن تلخيص أهم النتائج المتحصل عليها كما يلي :-

* أوضحت تجربة التحضين للتلقيح ببكتيريا الثيوباثيلس زيادة تركيز الكبريتات دليلا على نشاط اللقاح فى أكسدة الكبريت في حين زاد تركيز النتروجين في حالة التلقيح ببكتيريا الازوسبيريلم.
* أظهرت النتائج أن معاملات الكبريت و التسميد الحيوي و النتروجين المعدني و التفاعل بينهما كان لها تاثيرعالى المعنوية على إنتاج بنجر السكر و مكوناته و الصفات التكنولوجية.
* أدت إضافة الكبريت و تلقيح بذور بنجر السكر ببكتيريا الثيوباثيلس + بكتيريا الازوسبيريلم إلى زيادة عالية المعنوية في إنتاج الجذور ، العرش ، أبعاد الجذر ، السكروز، النقاوة ، نسبة الاستخلاص و السكر المستخلص.

* زيادة التسميد النيتروجيني المعدني من ٧٠ إلى ٩٠ كجم نتروجين / فدان أدى إلى زيادة عالية المعنوية في إنتاج الجذور ، إنتاج العرش ، إنتاج السـكر ، تركيز الصوديوم النتروجين الأميني و الفقد مع المولاس في حيـن انخفـض السـكروز، الـنقاوة ، نسـبة الاستخلاص و السكر المستخلص.

تـلقيح بـذور بنجر السكر ببكتيريا الازوسبيريلم نتج عنة زيادة تركيز النتروجين الميسر فى النبات و التربة.

أدت إضـافة الكبريت و تلقيح بذور بنجر السكر ببكتيريا الثيوباثيلس إلى خفض قيم الملوحة بالتربة و الرقم الهيدروجيني و كذلك زيادة تركيزات عناصر الفوسفور ، البوتاسيوم ، الزنك ، الحديد و المنجنيز في أوراق النبات و كذلك الميسر منها في التربة.

و أخيرا يمكن التوصية بإضافة الكبريت الزراعي بمعدل ٥٠ كجم / فـدان و تلقيح بذور بنجر السكر ببكتيريا الثيوباثيلس + بكتيريا الازوسبيريلم كمخصـبات حيويـة مع إضافة السماد النتروجين المعدني بمعدل ٩٠ كجم نتروجين للحصول على أعلى إنتاج و انسب صفات تكنولوجية.