



Original Research Article

Response of Sugar Beet Plant (*Beta vulgaris* L.) to Mineral Nitrogen Fertilization and Bio-Fertilizers

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ABSTRACT

Keywords

Sugar beet (*Beta vulgaris* L.), Mineral Nitrogen, Bio-fertilizers, root yield

Two field experiments were conducted at Nubaria area, Alexandria Governorate, during 2012/2013 and 2013/2014 seasons, to study the effect of biofertilizers and mineral nitrogen fertilizer treatments on anatomy, yield and quality of sugar beet. Biofertilizers were microbin, rhizobacterin, phosphorin and their interactions, however, mineral nitrogen fertilizer levels were 0, 35, 70 and 105 kg N/fed. A split plot design with four replications was used. The results revealed that application the mixture of Microbeen + Rhizobacterin + Phosphorien produced the highest values of most studied characters in both growing seasons as compared with using each bio-fertilizer alone. The highest values of root length and diameter, foliage and root fresh weights, TSS % and root yield/fed in the two seasons produced with adding 105 kg N/fed. However, the highest means of sucrose % and apparent purity % were resulted from control treatment (0 kg N/fed) in the two growing seasons. Generally, it could be concluded that application the mixture of Microbeen + Rhizobacterin + Phosphorien as biofertilizers and adding 105 kg N/fed as a mineral fertilization led to improve most characters of sugar beet plant.

Introduction

It is well known that sugar beet (*Beta vulgaris* L.) is the second source of sucrose all over the world and in Egypt as well. The importance of sugar beet crop to agriculture is not confined only to sugar production, but also it is adapted to saline, sodic and calcareous soils. Moreover, sugar beet is specialized as a short duration crop, where its growth period is about half that of sugar cane. Furthermore, sugar beet requires less water, which a kilogram of sugar requires about 1.4m³ and 4.0 m³ water to be produced by sugar beet and sugar cane,

respectively (Sohier, Ouda, 2001). Fertilizers play an important role in increasing crop production. The main macronutrients present in inorganic fertilizers are nitrogen, phosphorus, and potassium which influence vegetative and reproductive phase of plant growth (Patil, 2010). In recent years, many investigators apply biofertilizers to minimize the environmental pollution which resulted from mineral fertilizers and also to reduce its costs, (Abu EL-Fotoh *et al.*, 2000 and Cakmakci *et al.*, 2001). Application of

Azotobacter spp. caused solubilization of mineral nutrients and synthesis of vitamins, amino acids, auxins as well as gibberellins, which stimulate plant growth and gave the highest yields, (Sprenat, 1990). EL-Badry and EL-Bassel (1993) and Favilli *et al.*, (1993) found that inoculation sugar beet with bio-fertilizers caused a significant saving in nitrogen fertilizer (about 25-40 %) and significant increase in root and sugar yields. Sultan *et al.*, (1999) and Bassal *et al.*, (2001) recorded that inoculation of sugar beet seeds with Azotobacterin significantly increased TSS %, sucrose % and purity % as well as root and sugar yields/fed. Cakmaki *et al.*, (2001) and Maareg and Badr (2001) confirmed that Cerialine caused an increase TSS %, sucrose %, purity % and sugar yield/fed. Kandil *et al.*, (2002) and Gehan, Amin *et al.*, (2013) reported that inoculation seeds of sugar beet with biofertilizers significantly increased root, top and sugar yields/fed. Ramadan *et al.*, (2003) showed that biofertilization treatments had significant effect on root, top and sugar yields/fed. Badawi *et al.*, (2004) found that biofertilization treatments caused a significant effect on TSS %, sucrose %, purity %, root, top and sugar yields/fed. Rhizobacterin treatment produced the highest values of yield quality parameters, excluding TSS% (in the first and third seasons) and purity % (in the second season) as well as all yield characters in both seasons. Concerning application of the mixture of Rhizobacterin + Cerialine and Cerialine biofertilizer, its ranked after Rhizobacterin treatment, respectively with respecting their effect on quality and yield traits in both seasons. While, control treatment resulted in the lowest means ones. Aly *et al.*, (2009) recorded that inoculation with *Azotobacter chroococcum* and *Bacillus megatherium* saved about 25 kg N/fed of mineral fertilizer, which decreased the costs and the environmental pollution, in addition

to the increase of sugar yield and recoverable sugar/fed. Furthermore, inoculation with *Azospirillum* increased sucrose content in sugar beet roots.

Nitrogen fertilizer levels caused significant differences in all yield and quality of sugar beet. This results was confirming by El-Shafai (2000), El-Harriri and Gobarh (2001), Kandil *et al.*, (2002), Seadh (2004), Gomaa *et al.*, (2005), Ibrahim *et al.*, (2005), Leilah *et al.*, (2005), Ramadan (2005), El-Geddawy *et al.*, (2006), Nemeat Alla *et al.*, (2007), Monreala *et al.*, (2007), Seadh *et al.*, (2007), Seadh (2008), Shewate *et al.*, (2008), Zhang *et al.*, (2009), El-Sarag (2009) and Attia *et al.*, (2011). Abou-Amou *et al.*, (1996) stated that the the highest values of purity (78.75 %) were obtained by 80 kg N/fed. El-Hawary (1999) reported that fertilizing sugar beet with 90 kg N/fed recorded the highest values of sucrose %. El-Harriri and Gobarh (2001) pointed out that application of 110 kg N/fed markedly increased TSS %. The optimum means of sucrose and purity percentages were obtained from using 75 kg N/fed. in both seasons, (Seadh, 2008). Monreala *et al.* (2007) stated that the highest values of quality parameters were obtained from the lowest level of nitrogen (30 kg N/ha). Also, Abou Zeid and Osman (2005); Amal *et al.*, (2008); Seadh (2008); Shewate *et al.*, (2008); Zhang *et al.*, (2009); El-Sarag (2009); Alaa *et al.*, (2009) and Attia *et al.*, (2011) found that bacterial inoculation of sugar beet seeds though caused insignificant increases in root quality and growth parameters but it significantly increased root and sugar yields/fed. *Bacillus* inoculation along with 40 kg N/fed gave root and sugar yields as those obtained by addition of 80 kg N/fed. Furthermore, *Bacillus* inoculation along with the addition of the full N dose 80 kg/fed gave a significant increase which amounted to 18 and 39% in root and sugar

yields, respectively compared to application of 80 kg/fed alone.

The aim of this investigation was to study the effects of biofertilization treatments and mineral nitrogen fertilizer levels to improve yield and yield characters of sugar beet plant.

Materials and Methods

Two field experiments were carried out at Nubaria area, Alexandria Governorate, during 2012/2013 and 2013/2014 seasons to study the effect of biofertilization treatments and mineral nitrogen fertilizer levels on sugar beet. Seeds of sugar beet cv. Lola were sown on October, 10 in the 1st and 2nd growing seasons, respectively. The experiments were laid-out in a split plot design with four replications. In both seasons, each experiment included thirty-two treatments, eight biofertilization treatments and four nitrogen levels. The main plots were assigned to the following eight biofertilization treatments:

- 1- Without biofertilization (control).
- 2- Microbin.
- 3- Rhizobacterin.
- 4- Phosphorin.
- 5- Microbin + Rhizobacterin.
- 6- Microbin + Phosphorin.
- 7- Rhizobacterin + Phosphorin.
- 8- Microbin + Rhizobacterin + Phosphorin.

Microbin, Rhizobacterin and Phosphorin as commercial products were produced by Biofertilizer Unit, Agriculture Research Center (ARC), Giza, Egypt, which included free-living bacteria able to fix atmospheric nitrogen and phosphorus in the rhizosphere of soil. Microbin and Rhizobacterin treatments were done before first irrigation directly by mixing the recommended dose of each biofertilizer with fine clay as side-dress

near from hills. Phosphorin treatment was carried out by slightly wet seeds by little quantity of water and mixed by phosphorin biofertilizer and then directly sown. The sub-plots were occupied with the following four mineral nitrogen fertilizer levels {0 kg N/fed. (Control), 35 kg N/fed, 70 kg N/fed and 105 kg N/fed}. Ammonium nitrate fertilizer in the forms (33.5 %N) were applied as a side-dressing in two equal doses, one half after thinning (35 days from sowing) and the other before the third watering (70 days from sowing). Each experimental basic unit included 5 ridges, each 60 cm apart and 3.5 m length, resulted an area of 10.5 m² (1/400 fed). The preceding summer crop was maize (*Zea mays* L.) in both seasons. Soil samples were taken for conducting some physical and chemical analysis according to A.O.A.C. (2005) and all data were shown in table 1.

The experimental field well prepared and then divided into the experimental units. Calcium super phosphate (15.5 % P₂O₅) at the rate of 200 kg/fed was applied during soil preparation. Potassium fertilizer in the form of potassium sulphate (48 % K₂O) at the rate of 48 kg K₂O/fed was applied before the first irrigation. Sugar beet was hand sown 3-5 balls/hill using dry sowing method on one side of the ridge in hills 20 cm apart at the first week of October in both seasons. Plants were thinned at the age of 35 days from planting to obtain one plant/hill (35000 plants/fed). Plants were kept free from weeds, which were manually controlled by hand hoeing at two times. The common agricultural practices for growing sugar beet according to the recommendations of Ministry of Agriculture were followed, except the factors under study.

At maturity (age of 210 days), the three middle rows of each plot were harvested to determine the following characters:

Samples of twenty roots were taken randomly, send to the laboratory, cleaned with running tap water, dried, each sample was grated separately with grater into cassettes and mixed thoroughly to determine the quality characteristics as described in Cooke and Scott (1993).

- 1- Root length (cm).
- 2- Root diameter (cm).
- 3- Root fresh weight (g/plant).
- 4- Shoot fresh weight (g/ plant).
- 5- Total soluble solids (TSS %) in roots was measured in juice of fresh roots by using Hand Refractometer.
- 6- Sucrose percentage was determined according to Le Docte (1927).
- 7- Purity percentage: It was estimated according to the following equation:-

$$\text{Purity \%} = \frac{\text{Sucrose \%}}{\text{TSS \%}}$$

8. Root yield and top yield (ton/fed.):- Plants of sugar beet from each plot were harvested topped to determine root yield and top yield as ton/fed. on fresh weight basis.

Data collected were subjected to the proper analysis of variance (ANOVA) to Gomez and Gomez (1984). Differences among treatments were evaluated by the least significant difference (LSD) at 5% level. Homogeneity of variance was examined before combined analysis.

Results and Discussion

Effect of biofertilization treatments

Biofertilization treatments caused a significant effect on root length and diameter, root and shoot fresh weights as shown in table 2. Application the mixture of Microbeen + Rhizobacterin+ Phosphorien produced the highest values of yield attributes (root length and diameter, root and

shoot fresh weights) in both growing seasons. It was followed by application the mixture of Microbeen + Rhizobacterin then application the mixture of Rhizobacterin + Phosphorien with regard its effect on yield attributes in the two growing seasons. From obtained results, it could be observed that using of Rhizobacterin biofertilizer either alone or in the mixture with Microbeen or Phosphorien surpassed other treatment during both seasons. However, the lowest values of root length, root diameter, root and shoot fresh weights were resulted from control treatment (without biofertilization) in both seasons. This increase in yield attributes as a result of application biofertilizers particularly Rhizobacterin may be due to its role in nitrogen fixation via free living bacteria which reduce the soil pH especially in the rhizosphere which led to increase the availability of most essential macro and micro-nutrients as well as excretion some growth substances such as IAA and GA₃ which plays an important roles in formation a large and active root system and therefore increasing nutrient uptake, which stimulating establishment and vegetative growth, hence increasing root and shoot fresh weights and also root length and diameter. Favilli *et al.*, (1993) found that inoculation sugar beet seeds with Azosperillum accelerated the germination, seedling growth and optimum plant growth and increased root and sugar yield and reduce nitrogen fertilizer requirement during the growth season. Many investigators confirming this conclusion *i.e.* Badawi *et al.*, (2004), Kandil *et al.*, (2004) and Gehan, A. Amin *et al.*, (2013).

Data in table 3 clear that application of biofertilization treatments were associated with significant effect on total soluble solids (TSS), sucrose and apparent purity percentages in the two growing seasons. Application the mixture of three studied

biofertilizers (Microbeen + Rhizobacterin + Phosphorien) significantly improved quality traits of sugar beet and induced the highest values of them in the two growing seasons, with exception apparent purity percentage in the second season which resulted from using the mixture of Rhizobacterin+ phosphorien. Generally, it can be observed that biofertilization treatments especially that included Rhizobacterin biofertilizers led to gradual tendency to improve all quality determinations as compared with control treatment in both seasons. This increase in quality determinations due to biofertilization treatments especially Rhizobacterin may be due to its role in improving growth and dry matter accumulation by increasing the uptake and availability of most nutrients, consequently enhancement sucrose content in roots. Similar results were reported by many investigators *i.e.* Maareg and Badr (2001); Badr (2004) and Gehan A. Amin *et al.*, (2013).

Data in table 3 show that root yield/fed was significantly responded due to biofertilization treatments in both seasons. Noteworthy, application the mixture of Microbeen + Rhizobacterin + Phosphorien biofertilizers yielded the highest values of root yield (24.83 and 24.88 ton/fed) in the first and second seasons, respectively. Concerning application the mixture of Microbeen + Rhizobacterin and Rhizobacterin + Phosphorien, its ranked after aforementioned treatment, respectively with respecting their effect on root and sugar yields/fed in the two seasons. On the other hand, control treatment (without biofertilization) resulted in the lowest means of these yield traits. This effect of biofertilization treatments may be ascribed to its role in improving plant growth, vigor of plant and yields through fixing atmospheric nitrogen as well as release of certain growth regulators, stimulatory

compounds and nutrients in soil by the introduced organisms. Similar results were obtained by Gehan, A. Amin *et al.*, (2013).

Effect of mineral nitrogen levels

All yield attributes (root length and diameter as well as root and shoot fresh weights) significantly increased as a result of increasing nitrogen fertilizer levels from 0 to 35, 70 and 105 kg N/fed in both seasons (Table 2). Fertilizing sugar beet plants with 105 kg N/fed produced the highest values of all studied yield attributes in the two seasons. Application of 70 kg N/fed resulted in the best findings after the highest level of nitrogen fertilizer with significant differences comparison with other levels. While, the lowest ones were obtained due to plant did not received any amount of nitrogen fertilizer (0 kg N/fed) in both seasons. Such effect of nitrogen on these characteristics may be returned to its role in building up metabolites and activation of enzymes that associate with accumulation of carbohydrates, which translated from leaves to developing roots as well as increasing division and elongation of cells, consequently increasing root size. The present results are in line with those obtained by Ramadan (2005); El-Geddawy *et al.*, (2006); Alaa *et al.*, (2009); Awad *et al.*, (2012); Awad *et al.*, (2013 a and b). and Gehan A. Amin *et al.*, (2013).

Data presented in table 3 showed that significant differences in all yield quality determinations were noticed due nitrogen fertilizer levels in both growing seasons. The highest values of TSS % were obtained by application of 105 kg N/fed in the first and second seasons. However, the highest means of sucrose % and purity % were resulted from control treatment (0 kg N/fed.) in the two growing seasons.

Table.1 Physical and chemical analysis of the experimental site

Seasons	Partial size %			Soil Textu ral %	Soil pH 1:2.5	E.C *. ds/m	CaC O ₃ %	Organi c matte r%	Available contents %			
	Clay	Silt	Sand						N	P	K	
2012/2013	3.0	3.3	93.7	Sandy	7.7	1.6	10.6 %	0.75	4.4	3.21	132	
2013/2014	3.6	4.7	91.7	Sandy	7.8	1.9	9.9 %	0.90	6.5	3.01	120	
Seasons	Soluble cations (meq/l)				Soluble anions (meq/l)				Available contents (ppm)			
	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	B	Fe	Zn	Mn
2012/2013	2.00	3.02	3.24	0.25	2.50	1.10	3.02	2.17	0.31	4.2	2.6	3.8
2013/2014	2.05	3.00	3.14	0.35	2.60	1.09	3.00	2.10	0.35	4.1	3.5	2.4

*In the soil paste extract.

Table.2 Root length and diameter, root and shoot fresh weights as affected by bio-fertilization treatments, nitrogen fertilizer levels and their interaction during 2012/2013 and 2013/2014

Characters Treatments	Root length (cm)		Root diameter (cm)		Root fresh weight (g)		Shoot fresh weight (g)	
	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014
A- Bio-fertilization treatments:								
1- Without	17.12	17.33	9.95	10.10	519.3	330.3	232.6	244.6
2- Microbeen	18.36	18.45	10.65	10.80	591.4	38.75	274.15	268.9
3- Rhizobacterin	20.14	20.46	11.23	11.20	856.5	666.4	389.6	384.5
4- Phosphorien	19.65	19.85	10.95	11.05	587.3	621.5	267.8	277.8
5- Microbeen+ rhizobacterin	20.17	20.54	12.65	12.65	866.8	871.4	398.7	397.4
6- Microbeen+ phosphorien	19.04	19.15	12.85	12.90	865.4	865.2	388.5	391.2
7-Rhizobacterin+ phosphorien	19.44	19.68	13.05	13.00	871.5	871.3	392.3	387.9
8- Microbeen+ rhizobacterin+phosphorien	27.84	27.95	13.48	13.62	900.1.	945.6	425.6	413.2
F. test	*	*	*	*	*	*	*	*
LSD at 5 %	0.22	0.58	0.19	0.99	18.8	15.7	20.06	9.6
B- Nitrogen fertilizer levels:								
0 kg N/fed.	17.87	17.11	11.22	10.95	423.2	447.2	194.3	201.3
35 kg N/fed.	18.84	18.45	11.66	11.76	633.5	655.6	297.1	300.4
70 kg N/fed.	20.33	20.53	12.58	12.54	855.7	871.6	390.8	401.3
105 kg N/fed.	22.01	22.42	14.92	14.64	1097.4	1100.4	497.4	500.2
F. test	*	*	*	*	*	*	*	*
LSD at 5 %	0.18	0.29	0.14	0.53	16.9	14.7	15.2	6.7
C- Interaction:								
A X B	*	*	*	*	*	*	*	*

Table.3 Total soluble solids (TSS), sucrose and purity percentages as well as root yield as affected by bio-fertilization treatments, nitrogen fertilizer levels and their interaction during 2012/2013 and 2013/2014 seasons

Characters Treatments	TSS (%)		Sucrose (%)		Purity (%)		Root yield (ton/fed)	
	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014
A- Bio-fertilization treatments:								
1- Without	21.54	21.44	16.28	16.27	75.61	75.96	15.31	15.34
2- Microbeen	22.26	22.29	16.57	16.57	74.44	74.35	17.33	17.37
3-	22.29	22.43	16.57	16.55	74.34	73.80	22.88	22.90
4- Phosphorien	22.95	22.97	16.61	16.63	72.42	72.43	16.84	16.86
5- Microbeen+ rhizobacterin	22.88	22.91	16.75	16.81	73.19	73.38	23.76	23.78
6- Microbeen+ phosphorien	23.68	23.68	16.85	16.88	71.17	71.33	23.51	23.57
7- Rhizobacterin+	23.67	23.67	16.84	16.85	71.21	71.25	23.65	23.70
8- Microbeen+ rhizobacterin+ phosphorien	23.77	23.80	17.18	17.24	72.33	72.48	24.83	24.88
F. test	**	**	**	**	**	**	*	*
LSD at 5 %	0.052	0.062	0.038	0.022	0.34	0.34	0.427	0.429
B- Nitrogen fertilizer levels:								
0 kg N/fed.	21.86	21.93	16.30	16.34	74.62	74.57	12.763	12.056
35 kg N/fed.	22.89	22.85	16.57	16.58	72.49	72.69	15.797	18.493
70 kg N/fed.	23.22	23.27	16.85	16.86	72.60	72.48	24.617	27.026
105 kg N/fed.	23.55	23.55	17.1	17.12	72.49	72.74	28.228	25.138
F. test	**	**	**	**	**	**	*	*
LSD at 5 %	.054	0.101	0.021	0.035	.201	0.27	0.468	0.471
C- Interaction:								
A X B	**	**	**	**	**	**	*	*

Table.4 Root yield/fed as affected by the interaction between bio-fertilization treatments and nitrogen fertilizer levels during 2012/2013 and 2013/2014 seasons

Characters N-levels Bio-fertilization	Root yield (ton/fed)							
	2012/2013				2013/2014			
	0 kg N/fed.	35 kg N/fed.	70 kg N/fed.	105 kg N/fed.	0 kg N/fed.	35 kg N/fed.	70 kg N/fed.	105 kg N/fed.
1- Without	8.820	12.741	17.394	22.288	8.874	12.756	17.432	22.314
2- Microbeen	10.009	14.360	19.696	25.268	10.022	14.412	19.741	25.311
3- Rhizobacterin	14.033	20.543	27.003	29.974	14.045	20.584	27.023	29.987
4- Phosphorien	9.570	13.845	18.977	24.974	9.611	13.887	18.988	24.987
5- Microbeen+ rhizobacterin	14.500	21.275	28.488	30.788	14.513	21.289	28.511	30.814
6- Microbeen+ phosphorien	14.220	21.043	28.105	30.677	14.256	21.100	28.184	30.745
7- Rhizobacterin+ phosphorien	14.411	21.066	28.394	30.733	14.517	21.102	28.423	30.765
8- Microbeen+ rhizobacterin+ phosphorien	16.542	22.776	28.877	31.123	16.612	22.814	28.918	31.165
F. test	*				*			
LSD at 5 %	1.420				1.414			

The decrease in quality parameters due to excessive nitrogen application can be ascribed to its role in increasing root weight and diameter, tissue water content as well as increasing non-sucrose substances such as proteins and alpha amino acid, and hence decreasing sucrose content in roots. Confirming this conclusion Monreala *et al.*, (2007); Seadh (2008) and Awad *et al.*, (2013 a).

Nitrogen fertilizer levels caused significant effect on root yield in the two growing seasons (Table 3). The highest values of root (28.228 and 25.138 ton/fed) were produced from fertilizing beet plants with 105 kg N/fed in the first and second seasons, respectively. However, application of 70 kg N/fed induced the best root yield/fed after formerly nitrogen level in both seasons. The lowest values of root yield/fed were

obtained from control treatment (0 kg N/fed) in the two growing seasons. The increase in root yield due to application of nitrogen fertilization can be explained through the fact that nitrogen has a vital role in building up metabolites, activating enzymes and enhanced root length, diameter as well as root fresh weight and finally root yield. Similar results were recorded by Alaa *et al.*, (2009); El-Sarag (2009); Attia *et al.*, (2011); Awad *et al.*, (2012) and Awad *et al.*, (2013a and b).

Effect of interaction

The interaction between both studied factors (biofertilization treatments and nitrogen fertilizer levels) had a significant effect on all studied characters in the two growing seasons. The effect of the interaction between biofertilization treatments x

nitrogen fertilizer levels on root and was significant in the two growing seasons (Table 4). The optimum treatment that produced the highest values of root yield was utilization the mixture of Microbeen + Rhizobacterin + Phosphorien beside mineral fertilizing beets plants with 105 kg N/fed, where its results were 31.123 and 31.165 ton/fed in the first and second seasons, respectively. It was followed by the treatment of using the mixture of Microbeen + Rhizobacterin and 105 kg N/fed with without any significant differences in both growing seasons. Whereas, the lowest values of root yield (8.820 and 8.874 ton/fed) and (1.312 and 1.331 ton/fed) were resulted from control treatment of both factors (without biofertilization and nitrogen fertilizer) in the first and second seasons, respectively.

Generally, it could be recommended that fertilizing sugar beet with mixture of Microbeen + Rhizobacterin + Phosphorien and fertilizing with 105 kg N/fed increased yield and yield component of sugar beet plants.

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