J. of Plant Production, Mansoura Univ.,Vol 12 (1):87 - 96, 2021

Journal of Plant Production

Journal homepage: www.jpp.mans.edu.eg
Available online at: www.jpp.journals.ekb.eg

Effect of N-fixing Bacteria on Nitrogen Fertilizer Requirements for some Sugar Beet Varieties

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ABSTRACT

Two field trials were carried out at Tamiya Agricultural Research Station, (latitude of 29.58°N and longitude of 30.96°E), Fayoum Governorate,Egypt, during 2018/2019 and 2019/2020 seasons to study the relative importance of nitrogen-fixing bacteria *i.e. Bacillus polymyxa* (BC)and *Azospirillum brasilense*(AZS) as a soil drench under mineral nitrogen fertilizer levels and their effects on some sugar beet varieties. A split-plot design was used. Four treatments of nitrogen fertilization (80 kg N/fed ”control”, 60 kg N/fed + soil drench with BC, 60 kg N/fed + soil drench with AZS and 60 kg N/fed + soil drench with a mixture of BC and AZS) were occupied the main plots, whereas, six varieties (Beta303, Sirona, Panther,Des9003, Athospoly and Maximus) were distributed randomly in the sub-plots. Adding 60 kg N/fed + soil drench with a mixture of AZS and BC was adequate to produce the economical values of extractable sugar%, root and sugar yields/fed. Varieties Sirona and Maximus had the highest values of root and sugar yields/fed. Significant interactions effects among nitrogen treatments and varieties on the studied traits were discussed. Root and sugar yields/fed were significantly and positively correlated with leaf area index and crop growth rate, root diameter and its fresh weight/plant, in both seasons. Under the environmental conditions of this study, it was found that sowing Sirona and/or Maximus varieties under fertilization of 60 kg N/fed in combination with a mixture of BC and AZS at 5L/fed twice as a soil drench could be recommended to achieve the economical root and sugar yields/fed.

***Keywords**: Azospirillum brasiliense, Bacillus polymyxa*, fertilization, nitrogen, sugar beet, varieties.

INTRODUCTION

Fertilization plays a great and important role in the growth, yield and quality of sugar beet, for this reason many studies were made to find out the optimum level of nutritional elements to induce the highest yield and the best quality. Due to the relationship between root production, crop quality and market, sugar beet producers face unique challenges in N- fertilizer management. Top sugar beet yields require high N-rates, but juice quality is decreased by excess N-supply. The use of chemical fertilizers and pesticides has caused enormous environmental destruction and has even had an indirect impact on humans and animal. In most nations, bio-fertilizer, an environmentally sustainable fertiliser, is used. Therefore, bio fertilizers are important if we are to ensure a healthy future for generations (Edugreen, 2007). Several reports showed that, Azospirillum sp. proved to be more efficient and effective as far as growth and hence root and sugar yields/fed. In this connection, the bio-fertilizer in different a combination with mineral fertilizers increased chlorophyll and carotenoids (Medani et al. 2000) root length and diameter and sugar yield (Bassal et al. 2001 and El- Hosary et al. 2010); root and top yields (Kandil et al. 2002). Also, Zhang et al. (2009), El-Sarag (2009) and Awad et al. (2013) found that there was a substantial rise in root and sugar yields/fed using bacterial inoculation of beet seeds. Elfadaly et al. (2013) found that B. polymyxa and A. brasilense in a mixed form was more distinguished than A. brasilense followed by B. polymyxa in achieving higher values of root dimensions and leaves and root fresh weight/plant and sucrose%. Abdelaal and Tawfik (2015)found that a sharp increment in root dimensions and its fresh weight as same as root yield/fed when beets were fed with combination of bio-fertilizers + 105 kg N/fed. Rashed et al. (2016) cleared that, the highest values of root fresh weight/plant and its diameter, sucrose%, purity%, top, root and sugar yields were recorded, using a foliar application of the mixture with Azospirillum brasiliense and Bacillus sp., as compared to foliar application separately with each. Sarhan and El-Zeny (2020) demonstrated that, fertilizing beets with 110 kg N/fed + seeds inoculation with a mixture of bio-fertilizers (Cerialin + Rhizobacterin), recorded the maximum values of root yield/fed. However, the same mixture of bio-fertilizers along with 90 kg N/fed attained the maximum sugar yield/fed.

Sugar beet seeds sown in Egypt are imported and thus, under the Egyptian conditions, beet varieties should be evaluated to select the best varieties with respect to yield and quality characteristics. Variety is regarded as the corner stone for the production process, the key objective of the breeder is to pick the superior varieties from the imported ones, in addition to the recommended set of agronomical practices. Badawi et al. (2002) and Osman et al. (2003) in Egypt found that, Kawemira variety was superior to Top, Lola, and Pleno sugar beet varieties in sucrose%, root, top and sugar yields fed. Azzazy et al. (2007) and El-Sheikh et al. (2009) indicated that sugar beet varieties markedly differed in root fresh weight/plant, root and sugar yields, while root length and diameter as well as sucrose% and purity% were not significant variances. Safina et al. (2012) stated that cultivars of sugar beet considerably differed in productivity and quality. Hozayn et al. (2013) clarified a

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DOI: 10.21608/jpp.2021.152023

significant difference among the examined varieties in all the studied traits of sugar beet. Ahmed et al. (2017) showed that, root length, root and sugar yields/fed, in addition to sucrose%, purity% and impurities content differed markedly for different sugar beet cultivars. Thalooth et al. (2019) reported that the highest values of root length, diameter, and fresh weight/plant as well as root, top and sugar yields/fed were reported by Heba variety, while Sirona variety was ranked the second.

The presented work was carried out to study the influence of plant growth promoting rhizobacteria (PGPR) *i.e. Baciluus polymyxa* and *Azospirillum brasilense* under mineral nitrogen fertilizer levels on yield and quality properties of some sugar beet varieties grown in a clay loam soil at Fayoum Governorate, Egypt.

MATERIALS AND METHODS

Two field trials were carried out at Tamiya Agricultural Research Station, (latitude of 29.58° N and longitude of 30.96° E), Fayoum Governorate, Egypt, during 2018/2019 and 2019/2020 seasons to study the relative importance of nitrogen-fixing bacteria *i.e. Bacillus polymyxa* and *Azospirillum brasilense* as a soil drench with mineral nitrogen fertilizer and their effects on productivity and quality of some sugar beet varieties. A split-plot design was used with four replications. The treatments of N fertilization were occupied in the main plots, whereas varieties were distributed randomly in the sub-plots.

Treatments of N fertilization were as follows:

TN_1: 80 kg N/fed "$fed^{-1} = 0.42\ ha^{-1}$" (the recommended rate of mineral nitrogen fertilizer).

TN_2: 60 kg N/fed + soil drench with *Bacillus polymyxa*

TN_3: 60 kg N/fed + soil drench with *Azospirillum brasilense.*

TN_4: 60 kg N/fed + soil drench with a mixture of *Bacillus polymyxa*.and *Azospirillum brasilense*

Varieties: The six examined varieties of sugar beet, type of seeds and origin country are presented in Table 1.

Table 1. Sugar beet varieties, type of seeds and origin country.

Sugar beet varieties	Type of Seeds	Origin country
Beta 303	Multi-germ	Germany
Sirona	Multi-germ	France
Panther	Multi-germ	Germany
Des 9003	Multi-germ	Germany
Athos poly	Multi-germ	Netherland
Maximus	Multi-germ	Germany

Inoculant microorganisms *i.e. Bacillus polymyxa* and *Azospirillum brasiliense* were kindly provided from the Bio-fertilizers Production Unit of Agric. Microbiol. Res. Dept., Soil, Water, and Environ. Res. Inst., ARC, Giza, Egypt. *Azospirillum braselience* was grown on Dobereiner medium (Dobereiner and Baldini 1979), while nutrient agar medium was prepared for *Bacillus polymyxa* (Wrieght, 1934). The account of *Azospirillum brasiliense* and *Bacillus polymyxa* were 10^9 cells/ml. The bacterial suspension was added at a rate of 5 L/300 liters of water/fed. Soil drench with N-fixing bacteria was done twice with the same concentration: at 45 and 75 days after sowing.

Physical properties of the experimental soil were analyzed using the procedure described by Black *et al.* (1981). Soil chemical analysis was determined according to the method of Jackson (1973). Physical and chemical analyses of the experimental soil (at 30-cm depth) are presented in Table 2.

Table 2. Soil physical and chemical properties of the experimental sites.

2018/2019 season							
Particle size distribution %			**Texture class**	**Available nutrients (mg/kg soil)**			**pH**
Sand	**Silt**	**Clay**		**N**	**P**	**K**	
24.1	36.6	39.3	Clay loam	52.5	5.19	141	8.27
EC	Soluble cations and anions (meq/l)						
dS/cm^{-1}	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	HCO_3^-	SO_4^{-2}	Cl^-
3.41	9.61	5.51	18.32	0.67	2.50	5.41	26.20
2019/2020 season							
Particle size distribution %			Texture class	Available nutrients (mg/kg soil)			pH
Sand	Silt	Clay		N	P	K	
25.3	36.1	38.6	Clay loam	54.7	5.4	147	8.31
EC	Soluble cations and anions (meq/l)						
dS/cm^{-1}	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	HCO_3^-	SO_4^{-2}	Cl^-
3.72	11.10	5.61	20.10	0.42	2.71	5.32	29.20

Overall dose of 30 kg P_2O_5/fed was added in the form of calcium super-phosphate (15% P_2O_5) during seedbed preparation. Mineral nitrogen fertilizer was applied in the form of urea (46% N) in two equal doses, the 1st after thinning (4-6 true-leaf stage) and the 2nd dose one month later. Potassium fertilizer was added at 24 kg K_2O/fed as potassium sulfate "48% K_2O" with the 1st dose of nitrogen. The sub plot area was 21 m^2 and consisted of 6 ridges 0.5 m apart and 7 m in length with 20 cm between hills. Sugar beet varieties were sown in the 2nd week of October, while harvesting was done at age of 210 days, in both seasons. The preceding crop was clover in both seasons. Other agricultural field practices were done as recommended by Sugar Crops Research Institute.

The recorded data:

After 100 (T_1) and 130 (T_2) days from sowing, ten plants were randomly sampled from the guarded ridges of each sub-plot to determine the following traits:

1. **LAI = leaf area per plant (cm^2) / plant ground area (cm^2)** ……… (Watson, 1958)
2. **Net assimilation rate "NAR" ($g/m^2/day$) = $(W_2 - W_1)(\log_e A_2 - \log_e A_1) / (A_2 - A_1)(T_2 - T_1)$** (Radfords, 1967).
3. **Crop growth rate "CGR" (g/day) = $(W_2 - W_1) / (T_2 - T_1)$**

Where,

W_1, A_1 and W_2, A_2, refer to dry weight per plant and leaf area at time T_1 and T_2, respectively.

At harvest, a sample of ten plants was randomly taken from the middle ridges of each sub-plot to determine the following characteristics:

1. Root length and diameter/plant (cm).
2. Root and foliage fresh weights/plant (g).
3. Potassium "K", sodium "Na" and alpha amino nitrogen concentrations (meq/100 g beet) in roots were estimated as shown by Cooke and Scott (1993).
4. Sugar lost to molasses% (SLM) was calculated according to Deviller (1988) as follows:

$$\mathbf{SLM = 0.14\ (Na + K) + 0.25\ (\alpha\text{–amino N}) + 0.5}$$

5. Sucrose% was estimated using "Saccharometer" according to the method described in A.O.A.C. (2005).

The impurities contents (K, Na and α-amino N) and sucrose% were determined in the Quality Control Laboratory at Fayoum Sugar Company, Egypt.

6. Extractable sugar% (ES%) was calculated using the following equation of Dexter *et al.* (1967):

ES% = sucrose% – SLM% – 0.6

7. Quality index (QI) was calculated according to Cooke and Scott (1993) equation:

QI = (extractable sugar% x 100) / sucrose %

8. Top and root yields/fed (ton), which were determined on sub-plot weight (kg) and converted to tons/fed.

9. Sugar yield/fed (ton) was calculated according to the following equation:

Sugar yield/fed (ton) = root yield/fed (ton) x extractable sugar%.

Statistical analysis:

The collected data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the split-plot design published by Gomez and Gomez (1984) by using "MSTAT-C" computer software package. Least significant of difference (LSD) method was used to test differences between means at 5% level of probability to compare between means as described by Snedecor and Cochran (1980). Also, simple correlation coefficient was computed among some studied traits according to Steel and Torrie (1980).

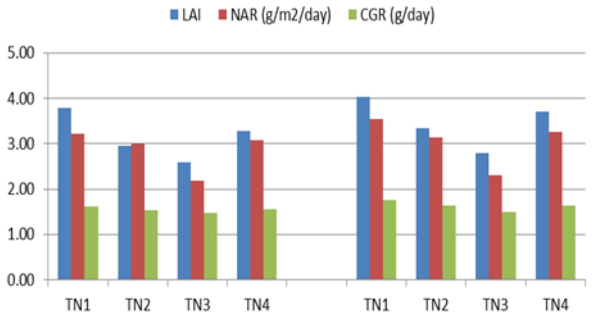
RESULTS AND DISCUSSIONS

A. Growth and physiological characteristics:

As shown in Fig. 1 leaf area index (LAI), net assimilation rate (NAR) and crop growth rate (CGR) significantly affected by feeding sugar beet plants with N-fertilizer dose and its combinations with N-fixing bacteria *i.e. Bacillus polymyxa* and *Azospirillum brasilense*, in both seasons.

Feeding beet plants with the recommended rate of N-fertilizer as an individual dose (80 kg N/fed) produced an insignificant increase in the above-mentioned traits, as compared to that gained by 60 kg N/fed in combination with *Bacillus* and *Azospirillum* as a mixture, in both seasons, except for LAI in the 1st one and CGR in the 2nd one. Therefore, addition of 60 kg N/fed in combination associated with a mixture of *Bacillus* and *Azospirillum* was adequate to produce the best values of NAR (3.07 and 3.26 g/m^2/day) in the 1st and 2nd season, respectively, CGR (1.56 g/day) in the 1st one and LAI (3.70) in the 2nd one. These results may be due to overlap between the large leaves of adjacent plants at higher nitrogen rate, which decreased dry matter accumulation efficiency/unit leaf area because of competition for light and in turn raised NAR values but without significant effect, when nitrogen level was increased to 80 kg N/fed. In this respect, Gharib and EL-Henawy (2011) found that, ,raising nitrogen rate from 75 to 90 kg/fed substantially increased LAI and CGR, while leading to a decrease in NAR.

Concerning the performance of sugar beet varieties, Fig. 2 shows that, the examined varieties substantially differed in LAI and NAR in both seasons, in addition to CGR in the 2nd one. The varieties Panther and Des9003 were the most distinguished varieties; as it is show a sharp increment in term of NAR of 0.35 and 0.31 g/m^2/day in the 1st season, corresponding to 0.22 and 0.20 g/m^2/day in the 2nd one, consecutively, as compared to Sirona variety. While, the highest increases in LAI values were obtained with Sirona and Maximus varieties. These results referred to the superior of cultivated variety than others might be due to genetic variability (Abu-Ellail *et al.* 2019).



TN$_1$: 80 kg N/fed "the recommended rate of mineral N-fertilizer", TN$_2$: 60 kg N/fed + soil drench with *Bacillus*, TN$_3$: 60 kg N/fed + soil drench with *Azospirillum*, and TN$_4$: 60 kg N/fed + soil drench with a mixture of *Bacillus* and *Azospirillum*.

Fig. 1. Leaf area index (LAI), net assimilation rate (NAR) and crop growth rate (CGR) as affected by mineral N-fertilizer and its combinations with N-fixing bacteria in 2018/2019 and 2019/2020 seasons

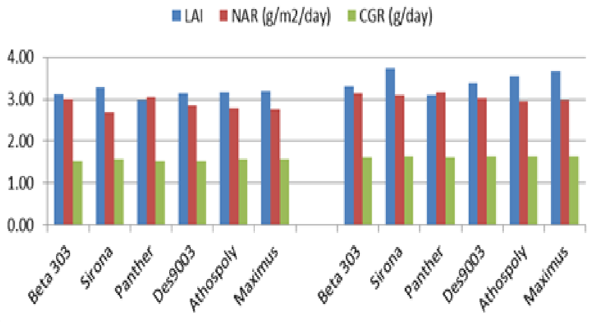


Fig. 2. Leaf area index (LAI), net assimilation rate (NAR) and crop growth rate (CGR) as affected by performance of sugar beet varieties in 2018/2019 and 2019/2020 seasons.

Data in the Table 3 elucidate that soil application of the recommended rate of N-fertilizer gave the highest averages of root dimensions and its fresh weight/plant in both seasons, but it did not reach the level of significance as compared to that gained by 60 kg N/fed with a mixture of N-fixing bacteria. Insignificant variances were found between applying combination of 60 kg N/fed + application of *Bacillus* and combination of the same N-level + soil drench with *Azospirillum*, in their influence on root length in the 1st season, in addition to root diameter and root and top fresh weights/plant, in the 2nd one. Supplying beets with 60 kg N/fed in combination with bio-fertilizer mixture resulted in a substantial increment reached 0.86 cm in root diameter, corresponding to 162 g in root fresh weight/plant, in the 2nd season, as compared to that given 60 kg N/fed + soil drench with *Bacillus* bacteria, separately. These results are in line with those obtained by Bassal *et al.* (2001) and El-Hosary *et al.* (2010). These results may be due to the role of nitrogen element as an essential component in proteins, enzymes, chlorophyll and atomic acids in the plant, which enhance plant growth and cell division. In addition, the beneficial effects of bacteria, especially root weight, can be due to the improvement of root growth parameters (Ghosh and Mohiuddin, 2000). The same trend was also recorded by Elfadaly *et al.* (2013) and Abdelaal and Tawfik (2015).

As for the varietal differences of sugar beet varieties, the results in Table 3 demonstrated that the tested varieties considerably varied in their effect on root length in the 1st season and top fresh weight/plant in the 2nd one, as well as root diameter and its fresh weight/plant, in both seasons. The longest and thickest roots, in addition to the heaviest leaves and roots/plants were obtained from Sirona variety with superiority over the other examined ones. The most distinguished variety was Sirona, having the thickest roots (10.94 and 12.83 cm), and producing the greatest root fresh weigh/plant (906 and 1010 g), in the 1st and 2nd season, respectively. However, the lowest values for root dimensions and its fresh weight/plant were obtained from Panther and Des 9003 varieties. The variations in these traits among beet varieties may be due to differences in gene make-up and its response to environmental conditions. The differences among sugar beet varieties were found by Osman *et al.* (2003), Azzazy *et al.* (2007) and Abu-Ellail *et al.* (2019).

Table 3. Growth characteristics of sugar beet varieties as affected by mineral N-fertilizer and its combinations with N-fixing bacteria in 2018/2019 and 2019/2020 seasons.

Treatments	2018/2019 (1st season)				2019/2020 (2nd season)			
	Root length(cm)	Root diameter(cm)	Root fresh weight/plant (g)	Top fresh weight/plant (g)	Root length(cm)	Root diameter(cm)	Root fresh weight/plant(g)	Top fresh weight/plant(g)
N-fertilizer treatments (N)								
TN_1	26.42	11.74	939	367	28.82	12.45	1008	385
TN_2	23.10	10.08	803	247	24.53	10.91	793	251
TN_3	21.89	8.28	692	189	23.09	10.28	732	227
TN_4	24.90	10.60	871	303	27.69	11.77	955	347
LSD at 0.05	1.64	1.19	76	48	1.20	0.81	64	41
Varieties (V)								
Beta 303	23.54	10.10	820	273	25.96	10.98	825	296
Sirona	26.32	10.94	906	287	26.61	12.83	1010	321
Panther	22.81	9.54	730	264	25.35	10.55	755	292
Des9003	23.30	9.75	781	269	25.75	10.92	823	292
Athospoly	24.09	10.28	838	281	26.07	11.33	892	306
Maximus	24.41	10.43	882	286	26.45	11.50	927	307
LSD at 0.05	2.15	0.33	43	NS	NS	1.12	74	21
Interaction: N x V	NS	*	*	*	NS	NS	NS	*

TN1: 80 kg N/fed "the recommended rate of mineral N-fertilizer ", TN2: 60 kg N/fed + soil drench with *Bacillus*, TN3: 60 kg N/fed + soil drench with *Azospirillum*, and TN4: 60 kg N/fed + soil drench with a mixture of *Bacillus* and *Azospirillum*. NS: insignificant differences.

B. Quality parameters:

Results in Table 4 indicated that, application of N-fertilizer and its combinations with bio-fertilizer bacteria to the soil appreciably affected sucrose%, potassium and α-amino N contents in sugar beet root and sugar lost to molasses% (SLM), in both seasons, in addition to quality index, in the 2nd one, meanwhile, sodium content was not affected. The reduction in nitrogen level from 80 kg N/fed as an individual dose to 60 kg N/fed in combination with bio-fertilizer sources led to decreases in the values of SLM, in both seasons.

Application of Bacillus and Azospirillum as a mixture + 60 kg N/fed resulted in a significant increase in sucrose%, as compared to the same N level in combination with both of *Bacillus* or *Azospirillum,* separately, in both seasons. On reverse, it could be observed that the difference between fertilization with 80 kg N/fed individually and 60 kg N/fed in combination with a mixture of bio fertilizers, in their effect on this trait was insignificant, in both seasons. Fertilizing sugar beet with 60 kg N/fed in combination with a mixture of bio nitrogen fertilizer sources increased sucrose% significantly by 1.34 and 1.58 in the 1st and 2nd season, consecutively, compared to plants treated with *Bacillus* alone. These results are consistent with those stated by El-Hosary *et al.* (2010) and Rashed *et al.* (2016). These results may be explained on the fact that higher N rates depressed sugar accumulation in beet roots as a result to increase top fresh weight as mentioned before therefore more metabolites were consumed in building up leaves. The depressive effect of high N rates on sucrose accumulation was reported by Stevens *et al.* (2011), Al-Mekdad (2012) and Okasha (2013).

In the same table, the differences among the studied sugar beet varieties in their impact on K and Na contents, SLM, sucrose% and quality index, in both seasons as same as α-amino N in the 2nd one, were significant. The highest values of sucrose% and quality index were obtained from Panther variety, superior to the other ones. Panther variety achieved a marked increment in sucrose% by 1.08 and 1.42, as compared to Maximus variety, corresponding to 1.31 and 1.69, as compared to Sirona variety, in the 1st and 2nd season, successively. Likewise, the same variety *i.e.* Panther led to a marked increment in quality index by 1.56 and 1.69, as compared to Maximus variety, corresponding to 2.21 and 2.37, as compared to Sirona variety, in the 1st and 2nd season, successively. These results are in harmony with those reviewed by El-Sheikh *et al.* (2009), Safina *et al.* (2012) and Sarhan and El-Zeny (2020). The results obtained revealed that sugar beet varieties obviously varied with respect to their content of sucrose consequently their quality index and theirs difference mainly due to their different in maturity states which attributed by gene-make up influence.

Table 4. Quality parameters of sugar beet varieties as affected by mineral N-fertilizer and its combinations with N-fixing bacteria in 2018/2019 and 2019/2020 seasons.

Treatments	2018/2019 (1st season)						2019/2020 (2nd season)					
	Impurities of juice (meq/100 g beet)			Sugar lost to molasses %	Sucrose %	Quality index	Impurities of juice (meq/100 g beet)			Sugar lost to molasses %	Sucrose %	Quality index
	K	Na	α-amino N				K	Na	α-amino N			
N-fertilizer treatments (N)												
TN_1	4.20	2.36	1.40	1.77	18.38	87.11	4.52	2.12	1.48	1.80	18.37	86.94
TN_2	3.54	1.84	1.12	1.53	16.87	87.37	3.94	1.68	1.24	1.60	16.62	86.78
TN_3	3.37	1.80	1.11	1.50	16.44	87.17	3.68	1.65	1.21	1.55	15.04	85.71
TN_4	3.85	2.14	1.27	1.66	18.21	87.64	4.07	2.01	1.34	1.69	18.20	87.44
LSD at 0.05	0.28	NS	0.14	0.11	1.02	NS	0.25	NS	0.09	0.07	0.54	0.66
Varieties (V)												
Beta 303	3.76	1.97	1.21	1.60	17.65	87.48	4.05	1.80	1.29	1.64	17.51	87.20
Sirona	3.93	2.40	1.36	1.73	16.82	86.15	4.26	2.13	1.39	1.74	16.00	85.36
Panther	3.44	1.72	1.13	1.50	18.13	88.36	3.88	1.54	1.25	1.57	17.69	87.73
Des9003	3.72	1.92	1.18	1.58	17.81	87.76	3.99	1.85	1.28	1.64	17.62	87.30
Athospoly	3.78	2.03	1.21	1.62	17.39	87.29	4.06	1.98	1.33	1.68	17.27	86.81
Maximus	3.82	2.16	1.25	1.65	17.05	86.8	4.06	1.88	1.36	1.67	16.27	86.04
LSD at 0.05	0.21	0.40	NS	0.08	0.69	0.68	0.23	0.30	0.09	0.04	0.93	0.72
Interaction: N x V	NS	*	*	*	NS	NS	*	NS	NS	*	NS	*

TN1: 80 kg N/fed "the recommended rate of mineral N-fertilizer ", TN2: 60 kg N/fed + soil drench with *Bacillus*, TN3: 60 kg N/fed + soil drench with *Azospirillum*, and TN4: 60 kg N/fed + soil drench with a mixture of *Bacillus* and *Azospirillum*. NS: insignificant differences.

C.Extractable sugar% and top, root and sugar yields/fed

Data in Table (5) pointed out that adding 80 kg N/fed was as equal as the applying of 60 kg N/fed with the mixture of *Bacillus* and *Azospirillum* compound in their influence on top yield in the 2nd season and root yield, extractable sugar% and sugar yield/fed, in both seasons. The distinctions between the two treatments were not significant. These results may be throw some light on the relative importance of bio-fertilizer compound which helps in the reduction of the used N-element consequently share in pollution reduction and economical values of production. Feeding beets with 60 kg N/fed + a combination of *Bacillus* and *Azospirillum* resulted in a substantial increment in extractable sugar% reached 1.22 and 1.49, corresponding to 0.53 ton (17.61%) and 0.62 ton (19.50%) in sugar yield/fed, in the 1st and 2nd season, successively, as well as 1.75 tons (8.54%) for root yield/fed in 1st one, as compared to that gained with the combination of 60 N/fed + soil drench with *Bacillus polymyxa*, separately, meanwhile, adding the same N level with *Azospirillum* gave the lowest values of the above mentioned traits. These results may be back to the relative influence of the studied bio nitrogen fertilizer sources in fixing more N-element and make available for beet plants to absorb it and make use of it in all biotic processes in the plants, which reflected on plant growth criteria in terms of root dimensions, in addition to root and top fresh weights/plant (Table 3), which reflected on the final products such as root, top and sugar yields/fed. The same trends were obtained by Awad *et al*. (2013), Abdelaal and Tawfik (2015) and Sarhan and El-Zeny (2020).

Table 5. Top, root and sugar yields/fed and extractable sugar% of sugar beet varieties as affected by mineral N-fertilizer and its combinations with N-fixing bacteria in 2018/2019 and 2019/2020 seasons.

Treatments	2018/2019 (1st season)				2019/2020 (2nd season)			
	Top yield/fed(ton)	Root yield/fed(ton)	Extractable sugar%	Sugar yield/fed(ton)	Top yield/fed(ton)	Root yield/fed(ton)	Extractable sugar%	Sugar yield/fed(ton)
N-fertilizer treatments (N)								
TN_1	11.50	23.07	16.01	3.68	12.41	24.28	15.97	3.87
TN_2	9.34	20.48	14.74	3.01	10.75	22.15	14.42	3.18
TN_3	7.94	19.67	14.33	2.81	9.38	19.81	12.89	2.55
TN_4	9.99	22.23	15.96	3.54	12.24	23.96	15.91	3.80
LSD at 0.05	1.32	1.68	1.10	0.34	2.21	2.13	0.56	0.38
Varieties (V)								
Beta 303	9.40	20.08	15.44	3.11	11.09	21.51	15.27	3.32
Sirona	10.17	24.63	14.49	3.57	11.07	25.54	13.66	3.51
Panther	8.96	18.41	16.02	2.97	11.02	20.51	15.51	3.19
Des9003	9.11	19.50	15.63	3.06	10.87	20.97	15.38	3.23
Athospoly	10.33	22.26	15.18	3.39	11.80	21.83	14.99	3.31
Maximus	10.17	23.31	14.8	3.46	11.32	24.96	13.99	3.53
LSD at 0.05	0.50	0.70	0.68	0.16	0.56	0.92	0.92	0.22
Interaction: N x V	NS	*	NS	*	*	*	NS	NS

TN_1: 80 kg N/fed "the recommended rate of mineral N-fertilizer ", TN_2: 60 kg N/fed + soil drench with *Bacillus*, TN_3: 60 kg N/fed + soil drench with *Azospirillum*, and TN_4: 60 kg N/fed + soil drench with a mixture of *Bacillus* and *Azospirillum*. NS: insignificant differences.

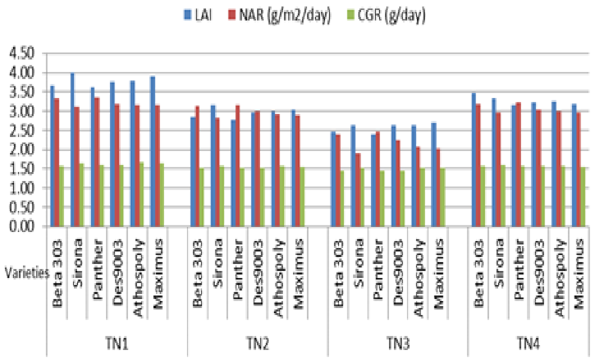
Concerning the behavior of sugar beet varieties, the results showed that the examined varieties differed appreciably in extractable sugar% and root and sugar yields/fed, in both seasons, as well as, top yield/fed in the 1st one. The varieties Sirona and Maximus were the most distinguished varieties in root and sugar yields/fed; as it showed a sharp increase in term of root yield/fed over passed the other tested varieties. Meanwhile, Panther variety came in the last rank. The superiority of Sirona and Maximus varieties might refer to better root traits (Table 3). In this regard, it could be noticed that the real increase in the values of root yield/fed at harvest compensated the decrease in the sucrose% (Table 4) in turn let to a marked increment among varieties in sugar yield. This observation may be referred to the gene make-up among varieties and their response to the environmental conditions. Similar tendency was observed by Ahmed *et al.* (2017), Thalooth *et al.* (2019) and Abu-Ellail and El-Mansoub (2020).

D. Significant interaction effects:

1. Significant interaction effect between N treatments and varieties on growth characteristics:

Fig. 3 showed that, the interaction between nitrogen treatments and sugar beet cultivars significantly affected net assimilation rate (NAR), leaf area index (LAI) and crop growth rate (CGR) in the 2nd season. The best interaction was obtained by Sirona variety under application of 80 kg N/fed, where it gave the highest values amounted by 4.58 and 1.76 g day-1 for LAI and CGR, respectively.

Insignificant differences were observed among the averages of leaf area index, when nitrogen level was raised from 60 kg N/fed in combination with a mixture of N-fixing bacteria to 80 kg N/fed individually, with all cultivars, except for variety Athospoly. Substantial variances in the values of net assimilation rate were found between variety Panther and both of Sirona or Maximus, when sugar beet plants were fed with 60 kg N/fed in combination with a mixture of N-fixers, while the differences between the same varieties, in this respect, failed to reach the level of significance under 80 kg N/fed.



TN1: 80 kg N/fed "the recommended rate of mineral N-fertilizer ", TN2: 60 kg N/fed + soil drench with *Bacillus*, TN3: 60 kg N/fed + soil drench with *Azospirillum*, and TN4: 60 kg N/fed + soil drench with a mixture of *Bacillus* and *Azospirillum*.

Fig. 3. Significant interaction effect between N treatments and varieties on leaf area index (LAI), net assimilation rate (NAR) and crop growth rate (CGR) in 2019/2020 season.

Data in Table 6 illustrated that the interaction between the six examined varieties and soil application with nitrogen treatments sharply affected top fresh weight/plant, in both seasons, in addition to root thickness and its fresh weight/plant, in the 1st one.

Table 6. Effect of the interaction between sugar beet varieties and N-fertilizer treatments on root diameter, root fresh weight/plant and top fresh weight/plant in 2018/2019 and/or 2019/2020 seasons.

Varieties	**Root diameter (cm)**				**Root fresh weight/plant (g)**			
	2018/2019 (1st season)				**2018/2019 (1st season)**			
	N-fertilizer treatments							
	TN_1	**TN_2**	**TN_3**	**TN_4**	**TN_1**	**TN_2**	**TN_3**	**TN_4**
Beta 303	11.64	9.98	8.28	10.49	923	810	673	873
Sirona	13.31	10.34	9.16	10.96	1040	894	740	950
Panther	10.84	9.74	7.22	10.35	850	744	527	800
Des 9003	10.85	9.92	7.78	10.46	890	708	687	840
Athospoly	11.86	10.18	8.53	10.55	906	850	713	883
Maximus	11.92	10.31	8.73	10.77	1025	810	810	882
LSD at 0.05	0.66				0.87			

Varieties	Top fresh weight/plant (g)							
	2018/2019 (1st season)				2019/2020 (2nd season)			
	N-fertilizer treatments							
	TN_1	TN_2	TN_3	TN_4	TN_1	TN_2	TN_3	TN_4
Beta 303	367	249	167	310	404	229	222	330
Sirona	390	251	202	307	374	277	264	370
Panther	383	234	152	287	394	242	195	337
Des9003	330	238	213	293	384	236	197	350
Athospoly	383	246	195	300	354	273	257	340
Maximus	347	267	207	323	401	246	227	353
LSD at 0.05	0.36				0.41			

TN1: 80 kg N/fed "the recommended rate of mineral N-fertilizer ", TN2: 60 kg N/fed + soil drench with *Bacillus*, TN3: 60 kg N/fed + soil drench with *Azospirillum*, and TN4: 60 kg N/fed + soil drench with a mixture of *Bacillus* and *Azospirillum*.

Varieties Maximus and Sirona significantly increased in root fresh weight/plant by 143 and 90 g, consecutively, when nitrogen fertilizer level was raised from 60 kg/fed in combination with a mixture of *Bacillus* and *Azospirillum* to 80 kg N/fed individually, in the 1st season, whilst the other varieties were not affected. The highest and statistical values of root fresh weight/plant were observed with Maximus and/or Sirona under the N-level of 80 kg/fed; meantime, Panther and/or Des9003 recorded the lowest one under the same conditions. Except for varieties Panther and Beta303, which showed a substantial decrease in top fresh weight/plant by decreasing nitrogen level from 80 kg N/fed individually to 60 kg N/fed in combination with a mixture of the two bacteria species, in both seasons, there were fluctuating differences in the above-mentioned trait from season to another with the other varieties under the same conditions of nitrogen treatments.

2. Significant interaction effects between N treatments and varieties on quality parameters:

In Table 7, the interaction between the examined varieties and nitrogen treatments either mineral individually or with bio fertilizers had a substantial effect on sugar lost to molasses% (SLM) in both seasons, root contents of sodium and α-amino N, in the 1st one, in addition to quality index and K content, in the 2nd one.

The varieties Sirona and Athospoly showed a statistical increase in SLM, when beets were fed with 60 kg N/fed + soil drench with *Bacillus* and *Azospirillum* as a mixture, as compared to that gained from adding the same N-level + soil drench with *Bacillus*, in both seasons.

Not all varieties were markedly affected in alpha amino nitrogen and sodium contents with raising nitrogen level from 60 kg/fed in combination with a mixture of *Bacillus* and *Azospirillum* to 80 kg N/fed as an individual dose, except for Des9003 in sodium content, in the 1st season. The superiority of all varieties in achieving the highest quality index in the 2nd season, when sugar beet plants were fed with 60 kg N/fed + soil drench with the *Bacillus* and *Azospirillum* in a mixture, may be due to the differences among varieties and the ability of N-fixing bacteria to provide approximately 25% of the recommended dose of mineral nitrogen necessary for the needs of the plant and without appreciably increasing in juice content of impurities.

Table 7. Effect of the interaction between sugar beet varieties and N-fertilizer treatments on quality parameters in 2018/2019 and/or 2019/2020 seasons.

Varieties	**Potassium (meq/100 g beet) 2019/2020 (2nd season)**				**Sodium (meq/100 g beet) 2018/2019 (1st season)**				**α-amino nitrogen (meq/100 g beet) 2018/2019 (1st season)**			
	N-fertilizer treatments											
	TN1	**TN2**	**TN3**	**TN4**	**TN1**	**TN2**	**TN3**	**TN4**	**TN1**	**TN2**	**TN3**	**TN4**
Beta 303	4.86	3.76	3.45	4.12	2.52	1.69	1.17	2.51	1.00	1.45	1.16	1.24
Sirona	4.33	4.22	4.25	4.26	2.57	1.89	2.39	2.75	1.66	1.21	1.17	1.41
Panther	4.36	3.79	3.41	3.96	1.84	1.96	1.47	1.61	1.32	1.04	1.11	1.06
Des9003	4.28	4.10	3.61	3.98	2.45	2.00	1.91	1.32	1.29	1.11	1.15	1.17
Athospoly	5.00	3.81	3.50	3.94	2.43	1.28	2.14	2.25	1.33	1.14	1.14	1.23
Maximus	4.28	3.95	3.85	4.14	2.36	2.19	1.70	2.38	1.80	0.77	0.90	1.52
LSD at 0.05	0.46				0.80				0.45			

Varieties	Sugar lost to molasses% 2018/2019 (1st season)				Sugar lost to molasses% 2019/2020 (2nd season)				Quality index 2019/2020 (2nd season)			
	TN1	TN2	TN3	TN4	TN1	TN2	TN3	TN4	TN1	TN2	TN3	TN4
Beta 303	1.69	1.62	1.41	1.71	1.84	1.56	1.46	1.69	86.77	87.87	86.19	87.79
Sirona	1.88	1.59	1.61	1.83	1.79	1.68	1.71	1.79	85.48	84.98	84.17	86.54
Panther	1.61	1.50	1.44	1.48	1.71	1.50	1.46	1.62	87.70	87.40	87.56	88.16
Des9003	1.76	1.55	1.52	1.51	1.77	1.61	1.51	1.65	87.66	86.70	86.67	87.99
Athospoly	1.79	1.45	1.56	1.66	1.87	1.61	1.55	1.69	86.74	87.16	85.04	87.92
Maximus	1.88	1.49	1.46	1.76	1.80	1.61	1.59	1.68	86.11	86.35	84.31	87.02
LSD at 0.05	0.15				0.08				1.44			

TN1: 80 kg N/fed "the recommended rate of mineral N-fertilizer ", TN2: 60 kg N/fed + soil drench with *Bacillus*, TN3: 60 kg N/fed + soil drench with *Azospirillum*, and TN4: 60 kg N/fed + soil drench with a mixture of *Bacillus* and *Azospirillum*.

3. Significant interaction effects between N treatments and varieties on yields/fed

Data in Table 8 confirmed that the interaction between the examined varieties and soil application with nitrogen levels either individually or in combinations with N-fixing bacteria significantly affected root yield/fed in both seasons, sugar yield/fed in the 1st season and top yield/fed in the 2nd one.

Table 8. Effect of the interaction between sugar beet varieties and N-fertilizer treatments on yields/fed and extractable sugar%, in 2018/2019 and/or 2019/2020 seasons.

Varieties	**Root yield/fed (ton) 2018/2019 (1st season)**				**Root yield/fed (ton) 2019/2020 (2nd season)**			
	N-fertilizer treatments							
	TN1	**TN2**	**TN3**	**TN4**	**TN1**	**TN2**	**TN3**	**TN4**
Beta 303	21.67	19.37	19.33	19.94	23.80	21.24	18.10	22.88
Sirona	25.30	24.50	23.86	24.87	27.53	24.88	22.67	27.08
Panther	20.89	16.76	15.38	20.61	21.38	20.70	18.44	21.51
Des9003	21.77	18.68	17.07	20.47	21.73	20.70	19.47	21.97
Athospoly	23.92	21.71	20.86	22.53	24.05	20.85	18.85	23.55
Maximus	24.89	21.88	21.49	24.96	27.20	24.55	21.34	26.75
LSD at 0.05	1.41				1.46			

Varieties	Top yield/fed (ton) 2019/2020 (2nd season)				Sugar yield/fed (ton) 2019/2020 (1st season)			
	N-fertilizer treatments							
	TN1	TN2	TN3	TN4	TN1	TN2	TN3	TN4
Beta 303	12.56	10.72	9.13	11.95	3.66	2.85	2.71	3.21
Sirona	11.51	10.58	10.38	11.81	3.84	3.44	3.26	3.75
Panther	12.45	10.81	8.40	12.43	3.55	2.59	2.31	3.42
Des9003	12.16	10.28	9.19	11.85	3.55	2.80	2.51	3.37
Athospoly	12.82	11.36	9.78	13.23	3.73	3.25	2.91	3.66
Maximus	12.95	10.77	9.40	12.15	3.78	3.14	3.15	3.78
LSD at 0.05	1.12				0.32			

TN1: 80 kg N/fed "the recommended rate of mineral N-fertilizer ", TN2: 60 kg N/fed + soil drench with *Bacillus*, TN3: 60 kg N/fed + soil drench with *Azospirillum*, and TN4: 60 kg N/fed + soil drench with a mixture of *Bacillus* and *Azospirillum*.

There was a statistical variance between application of 60 kg N/fed in combination with *Bacillus* and *Azospirillum* as a mixture and the same of N-level with *Bacillus* and/or *Azospirillum*, separately, in their impact on sugar yield in the 1st season and top yield in the 2nd one for all the examined varieties. Meanwhile, insignificant differences were observed in these traits when beets were fertilized with 60 kg N/fed + a mixture of N-fixers bacteria and 80 kg N/fed individually with the same varieties, except for sugar yield in the 1st season with Beta303 variety.

Significant variances were detected in root yield/fed in both seasons for varieties Panther, Des9003, Athospoly and Maximus when plants were fertilized with 60 kg N/fed + soil drench with N-fixers bacteria and *Azospirillum* under the same N-level

Raising N-level from 60 kg N/fed in combination with a mixture of N-fixers bacteria to 80 kg N/fed individually led to an insignificant difference in the values of top and root yields/fed with all varieties, in the 2nd season. Sirona and/or Maximus varieties achieved the best and economical values of root yield in both seasons as well as sugar yield in the 1st season under fertilization with 60 kg N/fed in combination with a mixture of N-fixing bacteria, as compared to the other examined varieties. These results may be due to the positive effect of the real increase in the values of root yield/fed at harvest compensated the decrease in the sucrose% (Table 4), which positively affected the sugar yield.

E. Correlation coefficient analysis of some studied traits:

It is clearly shows from the correlation study (Table, 9) that there are positive and highly significant ($P \leq 0.01$) correlation coefficients were detected between top, root and sugar yields with leaf area index, in both seasons. Positive and highly significant ($P \leq 0.01$) correlation coefficients were obtained between top and root yields in both seasons and sugar yield in the 2nd one with each of CGR, root diameter and root fresh weight/plant. in addition, positive and substantial correlations were observed at 5% probability level between sugar yield and each of root diameter and its fresh weight/plant and sucrose%, in the 1st season, as well as between root yield and net assimilation rate, in the 2nd one.

Table 9. Correlation coefficient analysis of root, top and sugar yields/fed and some studied traits for the examined varieties under different nitrogen treatments in 2018/2019 and 2019/2020 seasons.

Characteristics	Top yield/fed (ton)		Root yield/fed (ton)		Sugar yield/fed (ton)	
	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020
Leaf area index	0.883**	0.794**	0.625**	0.793**	0.850**	0.868**
Net assimilation rate (g/m^2/day)	0.464*	0.820**	0.154	0.515*	0.483*	0.851**
Crop growth rate (g/day)	0.909**	0.860**	0.768**	0.684**	0.880**	0.853**
Root diameter (cm)	0.881**	0.605**	0.702**	0.835**	0.855*	0.724**
Root fresh weight/plant (g)	0.866**	0.686**	0.809**	0.874**	0.901*	0.812**
Sucrose%	0.445*	0.781**	0.440	0.265	0.495*	0.772**
Quality index	-0.380	0.403	-0.618	-0.148	-0.292	0.411*
Top yield/fed (ton)	1.000	1.000	0.763**	0.626**	0.867**	0.877**
Root yield/fed (ton)	0.763**	0.626**	1.000	1.000	0.889**	0.816**

*** and ** denote significance at 0.05 and 0.01 levels of probability, respectively.**

The results showed that top and sugar yields exhibited positive and high significant correlations with sucrose%, in the 2nd season, while, positive and insignificant correlation was found between sucrose% and root yield, in the two seasonal. It was observed that root yield in both seasons as well as top and sugar yields in the 1st one showed a negative and non-significant correlation with quality index. On the contrary, sugar yield had a positive and significant ($P \leq 0.05$) correlation with quality index in the 2nd season. In this respect, many investigators studied the association between top, root and sugar yield with juice quality. Ibrahim (2011) and Nemeat Alla et al. (2019) confirmed that top, root and sugar yields were sharply and positively correlated with sucrose %. Conversely, top and root yields were negatively correlated with quality index. Results showed that top and root yields in both seasons were significantly ($P \leq 0.01$) contributed to variations in white sugar yield. Root circumference is correlated positively with root yield and can be used as a selection criterion for high yield (Campbell and Cole, 1986). Also, Sklenar et al. (1997) exhibited positive correlation between root weight and yield.

CONCLUSION

Under the environmental conditions of this study at Tamya, Fayoum Governorate, Egypt, there was no significant differences between fertilizing sugar beet with 80 kg N/fed individually and 60 kg N/fed in combination with N-fixing bacteria as a mixture at 5 L/fed twice. in most studied traits. Sirona and/or Maximus sugar beet varieties were more distinguished in achieving economical root and sugar yields/fed under fertilization of 60 kg N/fed in combination with a mixture of *Bacillus polymyxa* and *Azospirillum brasilense* bacteria at 5 L/fed twice .

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تأثير البكتيريا المثبتة للنيتروجين علي الاحتياجات من الأسمدة النيتروجينية لبعض أصناف بنجر السكر

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أُجريت تجربتان حقليتان في محطة بحوث طامية للبحوث الزراعية بمحافظة الفيوم (دائرة عرض 29.58° شمالاً وخط طول 30.96° شرقاً) خلال موسمي 2019/2018 و 2020/2019 لدراسة أثر استخدام البكتريا المثبتة للنيتروجين على الاحتياجات السمادية من النيتروجين لبعض أصناف بنجر السكر. اشتملت الدراسة علي أربعة معاملات من التسميد النيتروجيني كإضافة أرضية (80 كجم نيتروجين/فدان "كنترول" ، 60 كجم نيتروجين/فدان + اضافة بكتريا الباسيلس بولي ميكسا ، 60 كجم نيتروجين/فدان + اضافة بكتريا الازوسبيريليوم برازيلينس ، 60 كجم نيتروجين/فدان + اضافة خليط من بكتريا الباسيلس بولي ميكسا والازوسبيريليوم برازيلينس) ، وستة أصناف من بنجر السكر(Beta303 ، Sirona، Panther، Des9003 ، Athospolyو Maximus) ، وذلك في تصميم قطع منشقة مرة واحدة حيث وُضعت معاملات التسميد النيتروجيني في القطع الرئيسية في حين وُزعت الأصناف عشوائياً في القطع الشقية. أظهرت النتائج أن اضافة 60 كجم نيتروجين/فدان + اضافة خليط من بكتريا الباسيلس و الازوسبيريليوم كانت كافية للحصول علي الحاصل الامثل للجذور والسُكَّر/فدان وأفضل قيم للسكر المستخلص%. سجل الصنفان Sironaو Maximus اعلي القيم لحاصل الجذور والسكر/فدان. تمت مُناقشة التفاعلات المعنوية بين معاملات التسميد النيتروجيني والأصناف على الصفات المدروسة. أوضحت الدراسة ارتباطاً موجباً ومعنوياً بين صفات حاصل الجذور والسكر/فدان وكلا من صفات دليل مساحة الاوراق ومعدل نمو المحصول وقطر الجذر والوزن الغض للجذر/نبات، وذلك في كلا الموسمين. تحت الظروف البيئية لهذه الدراسة يُمكن التوصية بزراعة صنف بنجر السكر Sironaأو Maximus مع التسميد بالتوليفة (60 كجم نيتروجين/فدان + اضافة خليط من بكتريا باسيلس بولي ميكسا والازوسبيريليوم برازيلينس بمعدل 5 لتر/فدان مرتين) كإضافة أرضية للحصول علي الحاصل الاقتصادي للجذور والسُكَّر/فدان.

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