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Enhancing Yield and Quality of Sugar Beet through Combining of N and P Fertilizers with Application of Biofertilizer

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ABSTRACT
A field experiment was carried out at Mallawi Agriculture Research Station, Minia Governorate, Egypt, during the two successive seasons (2018/2019 and 2019/2020). The experiments were allocated in a spilt plot design with three replicates. The main plots assigned to four biofertilization treatments i.e. control, *Azospirllium ssp*, *Pseudomonas ssp* and mixture of *Azospirllium ssp* and *Pseudomonas ssp*). The sub plots were devoted for three levels of nitrogen and phosphorus fertilizers (50, 75, and 100% of RD "(90 kg N) and 31 kg P_2O_5 (15.5%)". Sugar beet plant growth, microbial densities in the rhizosphere, root yield, Physical and chemical properties of sugar beet roots and recoverable sugar yield (tonfed) were studied. Generally application of the above mentioned fertilizers individually or interaction resulted in a significant effect on all the studied traits in both seasons as compared with the control. On one hand the biofertilizer consisted of a mixture of *Azospirllium ssp* and *Pseudomonas ssp* recorded the highest values of microbial densities in the rhizosphere, plant growth parameters root yield, recoverable sugar yield (ton/fed), physical and chemical properties of roots and obtained sugar compare with other treatments?. On the other hand, treatment of 100% of nitrogen and phosphorus fertilizers gave the highest values of all the studied characters. The interactions between biofertilizers, nitrogen and phosphorus fertilizers significantly affected the studied traits in both seasons. Under the conditions of present work, the results suggest that sugar beet inoculated with a mixture of *Azospirllium ssp*, *Pseudomonas ssp* combined with 75 % RD of nitrogen and phosphorus fertilizers (204.5 &150 kg/fed) is highly recommended to obtain the highest yield of sugar beet.

Keywords: *Azospirllium ssp*, *Pseudomonas* ssp, nitrogen and phosphorus fertilizers, Sugarbeet yield and quality.

1. Introduction

Sugar beet (*Beta vulgaris* L.) a member of the family Chenopodiaceae, grown as a feed stock for the production of pure sugar, it is one of the most important cash crops in the world (Thalooth *et al.,* 2019). Sugar beet represents the first main sugar crop for sugar production in Egypt. About 35% of total world sugar production and 57.62% of the total Egyptian local sugar production (2.2 million ton in 2016), while sugar cane produced the rest (42.38%). Improvement of sucrose yield in sugar beet has been a slow process (Ferweez and Abd El-Monem, 2018). Ministry of Agriculture and Land Reclamation, in Egypt, encourages the growers to grow sugar beet over sugar cane as a water-saving measure. Accordingly, sugar beet has become, since 2013, the first source of sugar in Egypt, while the sugar cane ranks second.

It is well known that mineral fertilizers are important factors for vigorous growth and consequently higher yield of different plant species. However, repeated application of mineral fertilizers caused environmental pollution. Alternative biological fertilization of non-legume crops by N_2-fixing bacteria had a great importance in recent years (Santi and Franche, 2013). Bio-fertilizers are formulations of beneficial microorganisms, which upon application can increase the availability of nutrients by their biological activity and help to improve the soil health (Itelima *et al.,* 2018). Microorganisms secrete various plant growth and health promoting substances bio-fertilizers are

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considered as a low cost, effective and renewable source of plant nutrients to supplement chemical fertilizers (Nemeat Alla, 2016). These organisms exist either as free living in nature or as latent cells of efficient strains of microbes belonging to many taxa of bacteria, fungi, protozoa and cynanobacteria Kingdom (Lucy *et al.,* 2004; Smith and Read, 2010).

Aboshady *et al.* (2009) found that N_2-fixing and phosphate-dissolving bacteria play a significant role as plant growth-promoting rhizobacteria in the biofertilization of crops. They studied the effect of biofertilization with some strains of N_2-fixing and others of phosphate solubilizing bacteria in relation to chemical fertilization on sugar beet yield. They concluded that dual inoculation with N_2-fixing bacteria and P-solubilizing bacteria significantly increased root and sugar yield of sugar beet plant. The use of N_2-fixing bacteria has economic importance to modern agriculture as they can partially replace the cost of mineral N fertilizers, lowering production costs and reducing environmental pollution ensuring high yields.

Bio-fertilizer has emerged as a promising component of integrating nutrient supply system in intensive agriculture. Therefore, attempts have been paid to the use of bio-fertilizer as being most cheap and safe for agricultural application. Aly *et al.* (2009) recorded that inoculation with *Azotobacter chroococcum* and *Bacillus megatherium* saved about 25 kg N/fed. of mineral fertilizer, which reduced the cost of plant production and the environmental pollution, in addition to the increase of sugar yield and recoverable sugar/fed. Furthermore, inoculation with Azospirlilum spp increased sucrose content in sugar beet roots. Species of *Pseudomonas*, *Bacillus*, *Rhizobium* bacterial genera have the ability to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite (Richardson *et al.,* 2009), as well as rock phosphate (Owen *et al.,* 2015) and make them available to crops.

Nour El-Din *et al.* (2010) concluded that, at the application of EM or PGPR alone, the plant did not attain a consistent in yield. The combined application of 75% N + 50% P + EM + PGPR increased crop yield, quality and economic net profit and also saved the used mineral fertilizers of about 25% N and 50% P. EM treatment with 75%N and 50% P had consistent positive effect on sugar beet production, moreover, when the level of applied nitrogen decreased to 50% of recommended with 100% P and EM inoculation, the productivity negatively affected. So, when EM was used alone as an inoculant, they should be accompanied with the full dose of N and P addition.

Nosheen *et al.* (2021) *Pseudomonas* spp. produce hormones that promote root growth, improve nutrient availability and improve crop. Moreover, solubilize the insoluble forms of P in the soil into soluble forms by secreting organic acids and lowering soil pH to dissolve bound phosphates.

Abdelaal *et al.* (2015) revealed that application of a 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. Generally, it could be concluded that application of 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.

This study aimed to investigate response of sugar beet to bio and mineral fertilization on regarding yield of sugar beet and its attributes as well as quality under conditions of El-Minia Governorate.

2. Material and Methods

2.1. Microbial strains

Twenty isolates of *Azospirllium ssp*, *Pseudomonas ssp* were isolated at random from rhizosphere of sugar beet cultivars of Minia governorate. After purification, the isolates were tested towards their efficiency for nitrogen fixation using the ambient assay of nitrogenase activity according to Postage (1972). The isolates were maintained on semi-solid medium (Dobereiner and Day, 1976) for *Azospirllium ssp* isolates (King *et al.,* 1954) for *Pseudomonas ssp.*

2.2. Determination of bacteria

In vivo study to determine count of *Azospirllium ssp*, *Pseudomonas ssp* and total count of bacteria was estimated at 30, 45 and 60 days after cultivation in rhizosphere soils of sugar beet plants. To determine numbers of *Azospirllium ssp* dilution frequency method and was used (Haskin's 1934). The total count of bacteria was determined as described by (Allen, 1959). The Bunt and Rovira medium (Bunt and Rovira 1955) modified by (Abdel-Hafez, 1966) was used to determine the presence of phosphate solubilizing bacteria.

The nitrogenase enzyme activity in rhizosphere of plant was measured as acetylene reduction activity (ARA) by GC analysis according to Somasegaran and Hoben (1994). Specific species of *Azospirllium ssp* and *Pseudomonas ssp* were measured using the most probable number (x10^6 cfu /g dry soil) as described by (Cochran 1950) and (Dobereiner and Day 1976).

2.3. Design of the experiments

Two field experiments were carried out at Mallawi Agricultural Research Station, El-Minia Governorate, Egypt, during two winter seasons of 2018/2019 and 2019/2020. The experiments were allocated in a spilt plot design with three replicates. The main plots assigned to three biofertilization treatments i.e. *Azospirllium ssp*, *Pseudomonas ssp* and mixture with *Azospirllium ssp*, *Pseudomonas ssp*). As well as the sub plots were devoted for three levels of nitrogen and phosphorus fertilizer (50, 75 and 100 % RD).

Prior to planting, Sugar beet cultivar namely Montebianco was sown on 15th and 10th October in both seasons (2018/2019 and 2019/2020), respectively. Sub plot area was 21 m^2, each consisting of five line, 60 cm wide, 7 m long. Sugar beet was sown in hills 15 cm apart on rows.

Nitrogen fertilizer was applied in the form of Ammonium nitrate (33.5 % N), was added as a side dressing at the recommended rate of 90 kg N/fed (100 % RD) into three equal doses, the first was applied after thinning and the others were applied at 2-weeks interval after the first application. Phosphorus fertilizer was applied in the form of calcium super- phosphate (15.5% P_2O_5) at rate of 200 kg fed^{-1} during soil preparation. All treatments were treated with potassium sulphate 150 kg (48% K_2O) according to the recommendations of the Ministry of Agriculture, Egypt. Beet plants were thinned to one plant/hill at the age of 35 days from sowing.

Biofertilizer (*Azospirllium ssp*, *Pseudomonas ssp* and mixture of *Azospirllium ssp* and *Pseudomonas ssp* were added by dipping the seeds of sugar beet in cell suspensions enriched with nutrient broth medium (Difco, 1985) for 48 hours at 28°C.

Treatments: The main plot for four treatments were as follow:

1- Control (without adding biofertilizers)
2- Inoculation with the biofertilizer *Azospirllium ssp*.
3- Inoculation with the biofertilizer *Pseudomonas ssp***.**
4- Inoculation with a mixture of *Azospirllium ssp* and *Pseudomonas ssp*.

The sub plots were devoted for three levels of nitrogen and phosphorus fertilizer at the following concentrations:

1- 50% NP RD (45 kg N) and 15.5 kg P_2O_5 (15.5%).
2- 75% NP RD (67.5 kg N & 23.25 kg kg P_2O_5).
3- 100% NP RD (90 kg N &31 kg P_2O_5).

2.4. Soil analysis

Some chemical and physical properties of the experimental soil are shown in Table (1) as determined according to the procedures outlined by Jackson (1967).

2.5. Data recorded

At harvest (after 195 days from sowing date), samples of five roots were taken at random from the three middle rows of each plot to record: roots yield (t/fed), Root length (cm). 2- Root diameter (cm).

Samples were taken at random, cleaned with running tap water and dried. Each sample was grated separately with grater into cassettes and mixed thoroughly to determine the quality characteristics. Total soluble solids (TSS %) in roots was measured in juice of fresh roots by using Hand Refract meter. Purity, %: was determined as a ratio between sucrose % and TSS % of roots. Sugar loss percent and Quality index were calculated using the following equations according to Cooke and Scott (1993):

1. Sugar recovery % = Pol% - [0.29 + 0.343 (K +Na) + a - N (0.094)].
2. Sugar loss percent = [0.29+0.343 (K+ Na) + a- N (0.094)] where, K, Na and a - N determined as milliequivalent/100 g beet.

1. Alpha amino nitrogen, sodium and potassium contents were estimated according to the procedure of sugar company by Auto Analyzer. The results were calculated as milliequivalent per 100 g beet.
3.-Recoverable sugar yield (ton/fad) was calculated from the following equation:
Recoverable sugar yield (ton/fad) = Roots yield (ton /fad) x Sugar recovery %.

Concentrations of N, P, K were analyzed from matured roots. Five clean sample roots from each plot were collected randomly. The finely ground and dried tissues were wet digested using sulphuric-perchloric acid mixture (1:1) as described by A.O.A.C (2005). Total nitrogen percentage was determined by Kjeldahl method according to Jackson (1967). Total phosphorus percentage was estimated calorimetrically using the chlorostannus-reduce molybdo phosphoric blue color method and measured at the wave length of 640 nm using spectrophotometer as described by Jackson (1967). Total Potassium percentage was determined using the flame photometer as described by Jackson (1967).

Data collected were subjected to analysis of variance (ANOVA) for obtained data in each season performed. The measured variables were analyzed using MSTATC. Differences among treatments were evaluated by LSD test at 5% according to procedure outlined by Elias and Karim (1984).

Table 1: Some physical and chemical properties of the experimental soil

Property	Values
Particle size distribution (%):	
Sand	13.20
Silt	37.20
Clay	49.60
Texture grade	Silty Clay loam
$CaCO_3$	0.90
Saturation percent (SP)	41.00
pH (soil paste 1: 2.5)	8.11
E.C (dSm^{-1}, at 25°C)	1.20
Organic matter%	1.18
Soluble cations and anions (meq L^{-1}):	
Ca^{++}	1.60
Mg^{++}	2.30
Na^{+}	1.50
K^{+}	0.24
$CO^{=}_3$	0.00
HCO^{-}_3	2.88
Cl^{-}	2.12
$SO^{=}_4$	0.64
Total-N (%)	0.15
Total soluble- N (mg kg^{-1})	18.40
Available- P (mg kg^{-1})	12.80
Available-K (mg kg^{-1})	215.20

3. Results and Discussion

3.1. Microbial densities in the rhizosphere of sugar beet plants

The results in (Table 2) denoted the nitrogenase activity of twenty isolates of *Azospirllium ssp* and *Pseudomonas ssp*. It is clear that the best isolate of *Azospirllium ssp* with the highest recorded values was the isolate (Azo. 1) with sugar beet which attained (25.03 and 25.11 n moles / C_2H_4 / 1ml culture/ hr.) while the best isolates of *Pseudomonas ssp* with sugar beet which attained (37.77 and 37.97 n moles / C_2H_4 / 1ml culture/ hr.) was (*Psu.* 1) in the two growing seasons, respectively.

The results in (Table 3) clearly indicated that total count of bacteria were much higher in rhizosphere samples soil of sugar beet plants. The results also showed that the total count of bacteria was affected with the age of sugar beet that decreased at the late stages at the ages of 60 days after sowing. The highest numbers were found in samples of rhizosphere of 75% NP + Mix (18.2 and 18.4 x 10^6 C.F.U.) at 45 days after sowing in the two growing seasons, respectively.

The results in (Table 4) clearly indicated that counts of*Azospirillum* were much higher in rhizosphere samples soil of sugar beet plants at the age of 30 to 60 days which inoculated with *Azospirillum* as compared with those uninoculated. The results also indicated that the count of *Azospirillum* was affected with the age of sugar beet plants more than 45 days after sowing. The highest numbers were found in samples of rhizosphere of sugar beet plants 75% NP + Mix (38.2 and 38.4 x 10^4 C.F.U.) at 45 days after sowing in the two growing seasons, respectively.

Table 2: Nitrogenase activity of *Azospirillum* and *Pseudomonas* in the rhizosphere of sugar beet plants

No.	Nitrogenase activity n moles/C_2H_4/1 ml culture/hr			
	First season		**Second season**	
	Pseudomonas	***Azospirillum***	***Pseudomonas***	***Azospirillum***
1	37.77	25.03	37.97	25.11
2	25.00	19.55	25.20	19.75
3	13.66	16.77	13.86	16.97
4	17.34	14.01	17.54	14.31
5	18.32	11.98	18.52	12.33
6	15.96	15.60	16.30	5.90
7	13.80	18.34	13.95	8.64
8	10.15	17.66	10.33	7.86
9	13.88	14.67	13.98	14.87
10	17.02	12.45	17.22	12.55
11	15.28	11.85	15.48	11.91
12	22.12	17.99	22.32	18.22
13	24.23	18.60	24.43	8.80
14	17.55	21.62	17.75	21.82
15	13.61	17.74	13.71	17.84
16	18.66	11.22	18.76	11.42
17	13.36	19.82	13.46	9.91
18	17.33	13.76	17.53	13.86
19	10.20	17.67	10.40	17.77
20	24.90	11.40	24.98	11.60

Table 3: Total count of bacteria x 10^6(C.F.U.)/g in the rhizosphere of sugar beet plants

Treatments	First season			Second season		
	30 days	**45 days**	**60 days**	**30 days**	**45 days**	**60 days**
50% NP	5.7	7.2	6.4	5.8	7.4	6.6
75% NP	6.6	7.7	7.1	6.7	7.8	7.2
100% NP	5.2	6.5	6	5.4	6.7	6.2
50% NP + *Azospirillum*	11.4	13.5	12.6	11.5	13.6	12.7
75% NP + *Azospirillum*	12.3	15	14.4	12.5	15.2	14.6
100% NP + *Azospirillum*	10.7	11.4	11	10.8	11.6	11.2
50% NP + *Pseudomonas*	12.9	14.5	14	13	14.6	14.1
75% NP + *Pseudomonas*	13.7	15.4	14.8	13.8	15.5	14.9
100% NP + *Pseudomonas*	11.5	12.6	11.9	11.7	12.8	12
50% NP + Mix	14.6	16.4	15.3	14.7	16.5	15.4
75% NP + Mix	16.5	18.2	17	16.7	18.4	17.2
100% NP + Mix	13.7	15.4	14.3	13.9	15.6	14.5

Table 4: Counts of *Azospirillum* 10^4 (M.P.N./g) in the rhizosphere of sugar beet plants

Treatments	First season			Second season		
	30 days	**45 days**	**60 days**	**30 days**	**45 days**	**60 days**
50% NP	8.2	10.4	9.5	8.3	10.6	9.7
75% NP	9.4	11.2	10	9.5	11.4	10.2
100% NP	8.6	10	9.2	8.7	10.2	9.4
50% NP + *Azospirillum*	28.3	33.4	30.2	28.4	33.6	30.4
75% NP + *Azospirillum*	32.4	35.9	33.2	32.5	36.1	33.3
100% NP + *Azospirillum*	22.6	24.4	23.2	22.7	24.6	23.3
50% NP + *Pseudomonas*	18.9	22.8	19.4	19.0	23.0	19.5
75% NP + *Pseudomonas*	21.3	24.7	22.4	21.4	24.9	22.5
100% NP + *Pseudomonas*	17.9	20.4	18.6	18.0	20.6	18.7
50% NP + Mix	34.6	36.4	352	34.7	36.6	35.3
75% NP + Mix	36.5	38.2	37.3	36.6	38.4	37.4
100% NP + Mix	33.4	35.5	34.2	33.5	35.7	34.3

The data presented in (Table 5) show that counts of *Pseudomonas* were high in rhizosphere of sugar beet plants. However, the highest number of *Pseudomonus* rhizosphere samples of sugar beet plants 75% NP + Mix was (25.2 and 25.3 × 10^4) was found at the age of 45 days after sowing in the two growing seasons.

Table 5: Counts of *Pseudomonas* 10^4 (M.P.N./g) in the rhizosphere of sugar beet plants.

Treatments	First season			Second season		
	30 days	45 days	60 days	30 days	45 days	60 days
50% NP	7.4	9.2	8.4	7.6	9.3	8.5
75% NP	9.2	10.7	8.9	9.4	10.8	9.0
100% NP	6.9	8.5	6.2	7.1	8.6	6.3
50% NP + *Azospirillum*	12.2	14.5	13.6	12.4	14.6	13.7
75% NP + *Azospirillum*	16.4	18	17.4	16.6	18.1	17.5
100% NP + *Azospirillum*	14.3	16.4	15.3	14.5	16.5	15.4
50% NP + *Pseudomonas*	17.9	19.5	18.5	18.1	19.6	18.6
75% NP + *Pseudomonas*	20.9	23.4	21.8	21.1	23.5	21.9
100% NP + *Pseudomonas*	18.6	20.6	19.9	18.8	20.7	20.0
50% NP + Mix	19.6	21.4	20.4	19.8	21.5	20.5
75% NP + Mix	23.5	25.2	24	23.7	25.3	24.1
100% NP + Mix	18.2	20.6	19.8	18.4	20.7	19.9

3.2. Growth parameters

The obtained results in this work (Table 6) reveal that the studied growth parameters of sugar beet, i.e., root length, diameter (cm), responded significantly to inoculation with *Azospirllium ssp and Pseudomonas* alone and mixture of *Azospirllium ssp and Pseudomonas* during the two growing seasons. Application of the mixture of *Azospirllium ssp and Pseudomonas* caused an increase in root length (cm) by 5.23% and 5.20%, root diameter by 3.54% and 3.50% compared to inoculation with *Azospirllium ssp and Pseudomonas* alone during the two growing seasons, This increase in yield attributes to the application of biofertilizers that may be due to their role in nitrogen fixation, and reduce the soil pH by secretion organic acids especially in the rhizosphere which led to increase the availability of most essential macro and micro-nutrients as well as excretion of some growth substances such as IAA and GA3 which plays an important roles in increasing root and shoot fresh weights and also root length and diameter (Abdelaal *et al.,* 2015)

The results tabulated in the same table indicate that NP-fertilizer levels exhibited a significant effect on root length, diameter (cm), in the two growing seasons. It was observed from results that applying NP had a significant increase in root length by 4.45 and 4.43 %, root diameter 3.08 and 3.04% was recorded with adding 100 % RD of NP-fertilizer compared to 50 and 75 % of nitrogen and phosphorus fertilizers, respectively. 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 Gehan *et al.* (2013) and Abdelaal *et al.* (2015).

The interaction between additions of bio fertilizers under NP-fertilization levels was significant on root length and root diameter (cm) in the two growing seasons. The highest values (44.24 and 44.49 cm) of root length, (11.86 and 12.01 cm) of root diameter, was recorded with treated application of the mixture of *Azospirllium ssp and Pseudomonas* with 75% nitrogen and phosphorus fertilizer respectively. These results were agreed with those of Nour, (2010).

The data obtained in (Table 7) reveal that the studied productivity traits, root yield and recoverable sugar yield (ton/fed.) of sugar beet significantly responded to inoculation with *Azospirllium ssp and Pseudomonas* alone and a mixture of *Azospirllium ssp and Pseudomonas* during the two growing seasons. It could be noted that inoculation with the mixture of *Azospirllium ssp and Pseudomonas* caused an increase in root yield ton fed^{-1} 9.5 and 9.44% compared with other treatments, respectively. While, recoverable sugar yield of sugar beet increased by 14.05 and 13.58 % compared to without addition, respectively. These results might be attributed to biofertilizers that showed improvement in specific gravity (lower bulk density),

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Table 6: Effect of biofertilizer on root length (cm) and root diameter (cm) of sugar beet at different NP fertilizers level during 2018/2019 and 2019/2020 seasons

Bio fertilizer (A)	Root length (cm)								Root diameter (cm)							
	First season				Second season				First season				Second season			
	Nitrogen and phosphorus fertilizers levels															
	50	75	100	Mean	50	75	100	Mean	50	75	100	mean	50	75	100	Mean
Without	39.85	40.27	42.67	40.93	40.10	40.52	42.92	41.18	10.82	11.41	11.68	11.30	10.97	11.56	11.98	11.45
Azospirllium	40.82	43.02	41.87	42.02	41.07	43.64	42.12	42.27	11.51	11.61	11.73	11.62	11.66	11.86	11.80	11.78
Pseudomonas	41.69	43.63	43.38	42.56	41.94	43.88	42.63	42.81	11.53	11.57	11.56	11.55	11.68	11.90	11.72	11.79
Mixed	41.84	44.24	43.12	43.07	42.09	44.49	43.37	43.32	11.56	11.87	11.81	11.75	11.69	12.01	11.88	11.86
Mean	41.05	42.51	42.78	42.12	41.30	42.76	43.13	42.40	11.36	11.62	11.70	11.56	11.50	11.83	11.85	11.71
LSD 0.05 (A)	0.311				0.321				0.193				0.203			
LSD (B)	0.159				0.169				0.144				0.154			
LSD (AB)	0.317				0.327				0.288				0.298			

Table 7: Effect of bio fertilizer on root yield and recoverable sugar yield (ton/fad) of sugar beet at different NP fertilizer level during 2018/2019 and 2019/2020 seasons

Bio fertilizer (A)	Root yield ton fed^{-1}								Recoverable sugar yield(ton/fed)							
	First season				Second season				First season				Second season			
	Nitrogen and P fertilizers levels (B)															
	50	75	100	Mean	50	75	100	Mean	50	75	100	mean	50	75	100	Mean
Without	25.93	27.24	32.00	28.39	26.11	27.42	32.12	28.55	3.61	3.68	4.65	3.98	3.67	3.73	4.71	4.04
Azospirllium	28.13	30.05	29.62	29.27	28.31	30.23	29.60	29.38	3.68	4.41	4.15	4.8	3.74	4.33	4.11	4.06
Pseudomonas	28.84	31.24	29.81	29.96	29.02	31.42	29.79	30.08	3.74	4.38	4.09	4.07	3.80	4.31	4.15	4.09
Mixed	28.94	32.03	31.02	30.66	29.12	32.21	30.12	30.48	3.88	4.69	4.39	4.32	3.94	4.75	4.31	4.33
Mean	27.96	30.14	30.61	29.27	28.14	30.32	30.41	29.62	3.73	4.29	4.32	4.11	3.79	4.28	4.32	4.13
LSD0.05 A	0.050				0.055				0.016				0.018			
LSD B	0.037				0.047				0.012				0.014			
LSD AB	0.054				0.064				0.014				0.016			

Data in (Table 7) clarified that applying NP-fertilizers levels exhibited a remarkable and significant effect on root yield and recoverable sugar yield (ton/fed.) of sugar beet in both seasons. It was observed from both seasons that there was an increase in root yield of sugar beet by 7.80 and 7.75% and recoverable sugar yield of beet roots by 15.82 and 15.57% with applying NP-fertilizers levels at 100 % NP compared with (50% and75 %NP-fertilizer levels), respectively. 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 enhancing root length, diameter as well as root fresh weight and finally root yield. Similar results were recorded by Awad *et al.* (2013a and b) and Abdelaal *et al.* (2015).

Significant interactions were recorded between addition of biofertilizer and NP-fertilizer level on root yield and recoverable sugar yield of beet roots during the two growing seasons as shown in Table 7. Therefore, the highest value (32.03 and 32.21 ton/fed) for root yield of sugar beet was obtained with adding the mixture of *Azospirllium ssp and Pseudomonas* with 75 % NP. While, the highest values for recoverable sugar yield (4.69 and 4.75 ton/fed) was recorded with adding the mixture of *Azospirllium ssp and Pseudomonas* and 75 % NP, respectively. It was found that bacterial inoculation of sugar beet seeds though caused insignificant increases in root quality and growth parameters it significantly increased root and sugar yields/fed.

Addition of bio fertilizer exhibited a significant effect on physical properties of beet roots, i.e., Na (milliequivalents /100g beet), α - N (milliequivalents/100g beet), purity and sugar loss (SL%) of sugar beet in both seasons as shown in Table 8. As a result of adding bio fertilizers *Azospirllium* ssp, *Pseudomonas* and as a mixture*,* Na, α - N and loss sugar content of sugar beet was decreased. The minimum values 1.40 and 1.41 mg $100g^{-1}$, 1.51 and 1.52 mg $100g^{-1}$ and 2.58 and 2.59 % were recorded at application of the mixture of *Azospirllium ssp and Pseudomonas* for Na, ɑ - N and loss sugar content of sugar beet during the two growing seasons, respectively as compared the other treatments. While purity of sugar beet increased. The maximum value 83.03 and 83.58 % was obtained at application of the mixture of *Azospirllium ssp and Pseudomonas.*

The present results in (Table 8) indicate the effect of NP-fertilizers levels on Na (milliequivalents /100g beet), α - N (milliequivalents/100g beet), purity and sugar loss (SL%) of sugar beet in both seasons. The minimum values 1.34 and 1.36 mg $100g^{-1}$, 1.52 and 1.54 mg $100g^{-1}$ and 2.59 and 2.60 % were recorded at 100% NP for Na, α - N and loss sugar content of sugar beet during the two growing seasons, respectively. While purity of sugar beet increased. The maximum values were 82.84 and 83.39 % at application of 100% NP.

The results in (Table 8) show that the interaction between bio fertilizers and NP-fertilizers levels. The minimum values were 1.29 and 1.30, 1.22 and 1.23 and 2.44 and 2.46 %*,* at interaction of application of the mixture of *Azospirllium ssp and Pseudomonas* with75% NP-fertilizers for Na, α - N and loss sugar content of sugar beet during the two growing seasons, respectively. While purity of sugar beet increased. The maximum value 84.65 and 85.20 % at application of the mixture of *Azospirllium ssp and Pseudomonas* with75% NP-fertilizer for Na, ɑ - N and loss sugar.

Results in Table (9) showed the effect of addition of biofertilizer (*Azospirllium ssp, Pseudomonas* and Mix) and NP-fertilizers levels on N P K content and total soluble solids (TSS) of sugar beet. The interaction between biofertilizer (*Azospirllium ssp, Pseudomonas* and Mix) and NP levels indicated that the application of the mixture of *Azospirllium ssp and Pseudomonas* with75% NP-fertilizer recorded an increase (41.89 and 36.05 %, 218 and 218% and 33.03 and 32.96%) for N, P and K content compared with another treatment in 1^{st} and 2^{nd} seasons respectively. But, TSS values indicated that the application of the mixture of *Azospirllium ssp and Pseudomonas* with75% NP-fertilizer recorded a decrease by 24.11 and 23.56 % compared with another treatment in 1^{st} and 2^{nd} seasons, respectively. Using nitrogen fixing bacteria and phosphate dissolving bacteria, are important in plant nutrition by increasing N and P uptake by the plants (Gobarah *et al.,* 2011).

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Table 8: Effect of bio fertilizer on α-N, Na content (milliequivalents/100g beet), purity and sugar loss % of sugar beet at different NP fertilizer level during 2018/2019 and 2019/2020 seasons

Bio fertilizer(A)	Na (milliequivalents /100g beet)								α - N (milliequivalents /100g beet)							
	First season				Second season				First season				Second season			
	Nitrogen and P fertilizers levels (B)															
	50	75	100	Mean	50	75	100	Mean	50	75	100	Mean	50	75	100	Mean
Without	1.54	1.33	1.61	1.49	1.55	1.34	1.62	1.50	2.20	1.71	2.38	2.04	2.39	1.72	2.03	2.05
Azospirllium	1.44	1.39	1.54	1.46	1.45	1.40	1.56	1.47	1.77	1.53	1.78	1.69	1.78	1.54	1.78	1.70
Pseudomonas	1.45	1.37	1.56	1.46	1.46	1.38	1.57	1.47	1.80	1.64	1.89	1.78	1.90	1.65	1.81	1.79
Mixed	1.37	1.29	1.52	1.40	1.39	1.30	1.54	1.41	1.49	1.22	1.82	1.51	1.83	1.23	1.50	1.52
Mean	1.45	1.34	1.56	1.45	1.46	1.36	1.57	1.46	1.77	1.52	1.97	1.76	1.98	1.54	1.78	1.77
$LSD_{0.05}$ A	0.001				0.011				0.002				0.009			
LSD B	0.001				0.012				0.003				0.010			
LSD AB	0.002				0.022				0.005				0.015			
	Purity								Sugar loss %							
Without	79.35	80.40	82.25	80.67	79.90	80.95	82.80	81.22	3.03	2.68	2.85	2.86	3.04	2.69	2.86	2.87
Azospirllium	81.00	82.95	81.80	81.92	81.55	83.50	82.35	82.47	2.91	2.55	2.75	2.74	2.92	2.56	2.77	2.75
Pseudomonas	81.40	83.35	81.55	82.10	81.95	83.90	82.10	82.65	2.90	2.67	2.82	2.80	2.91	2.69	2.83	2.81
Mixed	81.75	84.65	82.70	83.03	82.30	85.20	83.25	83.58	2.46	2.44	2.83	2.58	2.48	2.46	2.84	2.59
Mean	80.88	82.84	82.08	81.93	81.43	83.39	82.63	82.48	2.83	2.59	2.81	2.74	2.48	2.60	2.82	2.75
$LSD_{0.05}$ A	0.263				0.273				0.120				0.125			
LSD B	0.146				0.156				0.112				0.119			
LSD AB	0.291				0.301				0.124				0.129			

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Table 9: Effect of bio fertilizer on N P K content and total soluble solids (TSS) of sugar beet at different NP fertilizer level during 2018/2019 and 2019/2020 season

Bio fertilizer (A)	N%								P%							
	First season				Second season				First season				Second season			
	Nitrogen and P fertilizers levels (B)															
	50	75	100	Mean	50	75	100	Mean	50	75	100	Mean	50	75	100	Mean
Without	0.74	0.80	1.03	0.86	0.86	0.92	1.12	0.97	0.17	0.21	0.45	0.28	0.19	0.23	0.47	0.30
Azospirllium	0.82	0.97	0.92	0.90	0.94	1.10	1.06	1.03	0.28	0.48	0.40	0.39	0.30	0.50	0.42	0.41
Pseudomonas	0.87	1.02	0.96	0.95	0.99	1.14	1.08	1.07	0.31	0.50	0.43	0.41	0.33	0.52	0.45	0.43
Mixed	0.91	1.05	1.00	0.99	1.03	1.17	1.10	1.10	0.38	0.54	0.47	0.46	0.40	0.56	0.49	0.48
Mean	0.84	0.96	0.98	0.93	0.98	1.08	1.09	1.05	0.29	0.43	0.44	0.38	0.30	0.45	0.46	0.40
LSD0.05 A	0.025				0.041				0.004				0.005			
LSD B	0.017				0.27				0.003				0.004			
LSD AB	0.024				0.044				0.00				0.006			
	K%								TSS%							
Without	4.45	4.65	5.11	4.74	4.46	4.67	5.13	4.75	24.15	23.84	21.44	23.14	24.70	24.39	21.99	23.69
Azospirllium	4.78	5.50	4.92	5.07	4.80	5.51	4.94	5.08	23.53	21.90	20.33	21.92	24.08	22.45	20.88	22.47
Pseudomonas	4.80	5.89	5.02	5.24	4.82	5.91	5.03	5.25	21.81	19.25	22.31	21.12	22.36	19.80	22.86	21.67
Mixed	4.93	5.92	5.25	5.37	4.94	5.93	5.26	5.38	22.86	18.33	20.62	20.60	23.41	18.88	21.17	21.15
Mean	5.49	5.08	5.10	4.75	5.50	5.09	5.12	4.74	23.09	20.83	21.17	21.70	23.64	21.38	21.42	22.25
LSD 0.05 A	0.011				0.012				0.129				0.139			
LSD B	0.013				0.014				0.167				0.177			
LSD AB	0.016				0.017				0.336				0.346			

3. Conclusion

Under the conditions of present study, it can be concluded from the results suggest that sugar beet inoculated with a mixture of *Azospirllium ssp* and *Pseudomonas ssp* combined with 75% NP RD (67.5 kg N) /fed of nitrogen and & 23.25 kg kg P_2O_5 kg /fed phosphorus fertilizer is highly recommended to obtain the highest yield of sugar beet.

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