Plant Archives Volume 21, No 1, 2021 pp. 1130-1137 *e-ISSN:2581-6063 (online), ISSN:0972-5210*

Plant Archives

Journal home page: www.plantarchives.org

DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.149

THE EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA IN REDUCING NITROGEN AND PHOSPHORUS FERTILIZERS APPLICATION IN SUGARCANE

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(Date of receiving -16-12-2020; Date of acceptance -19-02-2021)

ABSTRACT

This study was performed in order to investigate the effect of Plant growth-promoting rhizobacteria in reducing nitrogen and phosphorus Fertilizers Application in Sugarcane. The field experiment of this study was in the form of Split–block design with subplots in stips with four replications and three factors, including bacterial factor at four levels (control, *Enterobacter cloaca*, *Pseudomonas putida* and a combination of two types of bacteria), nitrogen factor at three levels (50, 75 and 100% recommended nitrogen for sugarcane (and phosphorus factor at three levels (50, 75 and 100% recommended phosphorus for sugarcane), was carried out in 2016-2017crop year in DC_{7-10} research farm of Dehkhoda sugarcane agro-industryin Ahvaz, in the southwest of Iran, on CP_{73-21} sugarcane variety. According to the analysis of variance tables, simple and interaction effects of the tested treatments, in the case of quantitative traits, including stalk yield, height, diameter, stalk density, percentage of nitrogen and phosphorus of leaves, chlorophyll content, LAI and HI in sugarcane were significant at the level of 1% probability. Comparison of means showed that the application of simultaneous application of growth-promoting bacteria along with the application of 75% recommended nitrogen and phosphorus for sugarcane, compared with the control treatment (application of 100% recommended nitrogen and phosphorus for sugarcane, without the use of bacteria), Was able to succeed in these traits 96.9%, 98.1%, 95.7%, 96.3%, 100.2% ,101.9%, 91.2% and 94.8%, respectively and Provide 21/9, 23/1, 20/7, 21/3, 25, 25, 16.2 and 19.8% of the nutrients of nitrogen and phosphorus for sugarcane, respectively, and is saved the same amount of nitrogen and phosphorus consumption for sugarcane. Also, regarding the sugarcane yield, the simultaneous application treatment of the tested bacteria along with the application of 100% recommended phosphorus and nitrogen for sugarcane, Compared to the control treatment, achieved a success of 110.15%, and increased the sugarcane yield by 10.15%

Keywords*:* Chlorophyll, Density, LAI, Nitrogen, Phosphorus, Rhizobacteria, Sugarcane , Yield

INTRODUCTION

The world's population will increase from 7.2 billion in 2015 to 8.3 billion by 2030, and the world's per capita sugar consumption in 2015 was 21 kg per year, which is projected to reach 25 kg per year in 2030. Therefore, with the increasing global population, increasing yield growth indicators in sugarcane (*Saccharum officinarum*) as a strategic product in order to achieve food security, is inevitable. Recent developments, although able to help humans meet the food they need, but the study and efforts to increase productivity and healthy food production, the need for a revolution with emphasis on environmental principles and sustainable conservation of resources is more tangible(Kan *et al.,* 2009) and in this regard, the latest research and studies are moving to a period called(green-micro revolution) in which the efficient use of plant growth-promoting bacteria has been proven And these microorganisms cane Provide essential nutrients for the crop, including nitrogen and phosphorus , and reduce production costs and environmental challenges by increasing the efficiency of chemical fertilizer use (Leonel Roza, 2020). Therefore, the application of biological fertilizers, especially plant growth-promoting bacteria in combination with the use of chemical fertilizers, is the most important strategy of integrated plant nutrition for sustainable management of agricultural ecosystems and increasing their production in sustainable agricultural systems with sufficient inputA group of researchers isolated Pseudomonas from the sugarcane rhizosphere and, following their study, confirmed the function of the bacterium in solubilizing phosphorus stabilized in soil and biologically nitrogen fixation in the air, and proved that it improves growth and nitrogen content in Sugarcane and it is important and they also stated that the use of plant growth promoting bacteria in biological agricultural trade is one of the pillars of sustainable agriculture and is effective in reducing environmental pollution (Li *et al.,* 2017).*Pseudomonas putida* and *Enterobacter cloacae* are the most well-known growth-promoting bacteria in crops, including sugarcane, and agricultural researchers have reported that those bacteriahave been isolated from sugarcane stems, roots, leaves and rhizospheres, and its ability to Biological fixation of nitrogen, and Solubilization of phosphorus stabilized in soil during coexistence with sugarcane have been proven(SaminaMehnaz, 2009,2011). Other researchers used *Enterobacter cloacae* to inoculate sugarcane and to investigate its effect on solubilization of

Stabilized phosphorus in the soil of cultivated sugarcane in Khuzestan and in their study, they found that in soils with low available phosphorus, the activity of *Enterobacter cloaca* in the sugarcane rhizosphere increased the efficiency of phosphorus uptake, so that inoculated sugarcane with *Enterobacter cloaca* was able to absorb up to 76% of its phosphorus. By stimulating the growth of growth-promoting bacteria, which reduced the dependence of phosphorus uptake on sugarcane root development (Safirzadeh *et al.,* 2019).Since in Khuzestan climate, in the southwest of Iran, with more than one hundred thousand hectares of sugarcane fields, the percentage of soil organic matter as the main source of soil nitrogen is less than one percent and on the other hand due to calcareous soil, a significant amount of soil phosphorus in the form of insoluble calcareous compounds, therefore, the sugarcane agricultural ecosystem, as a monocultural and ratoon-capable system, has low self-reliance in terms of providing important nutrients such as nitrogen and phosphorus and needs a significant amount of phosphorus and nitrogen chemical fertilizers to show its yield potential (Hong Minh Tam and Cao Ngoc Dief, 2020).And in the soil conditions of Khuzestan, according to the soil test, at least 300 and 350 $kg.ha^{-1}$ require nitrogen and phosphorus chemical fertilizers, respectively,which both increases production costs and in the case of nitrogen chemical fertilizer, increases environmental challenge. Therefore, this study was conducted in order to investigate the effect of plant growth-promoting bacteria on yield and yield components in sugarcane, by providing part of itsFertilizer required for nitrogen and phosphorus and saving the amount of its recommended chemical fertilizers and reducing production costs.

MATERIALS AND METHODS

Plant Growth-promoting Rhizobacteria under test

In this experiment, two type of PGPR[1],included *Pseudomonas putida* and *Enterobacter cloacae* were used that were isolated from sugarcane roots and its rhizosphere soil in the south of Khuzestan and are fully compatible with the microclimatic conditions of Khuzestan soil. Both types of bacteria propagated in the laboratory of the research site and in Nutrient Broth culture medium and with a density of 10^7cfu/ml were used to inoculate sugarcane cuttings in this experiment.

Field studies

This experiment was carried out in 2016-2017 crop year, with the participation of Khuzestan University of Agriculture and Natural Resources and Agro-industrial Sugarcane of Dehkhoda, in DC7-10 research farm at Sugarcane Agro-industry of Dehkhoda with geo coding of 31° and 31´north latitude and 48° and 43´east longitude with 18 meters above sea level, in an area of 5270 m^2, was applied on CP73 -21 sugarcane variety.In this regard, in order to determine the physical and chemical properties of the soil of the test site, the soil of each replicate was sampled before planting and implementation of the design and the results of its analysis in Table (1) show that the soil had a clay-loam texture andits acidity is in the range of alkaline soils and also the amount of absorbable phosphorus is less than the critical level for many crops such as sugarcane (Khavazi *et al.,* 2014).

Experimental factors

This study was conducted in the form of a split-block design (strip) and in the form of a randomized complete block design with four replications.According to Table (2), three factors including, nitrogen fertilizer factor at three levels (50, 75 and 100%recommended nitrogenfor sugarcane) in the main plots, bacterial factor in four levels (without bacteria, *Pseudomonas putida*, *Enterobacter cloaca* and simultaneous use of two types of bacteria) in sub-strip plots and phosphorus factor in three fertilizer levels (50, 75 and 100%recommended phosphorusfor sugarcane) which were randomly placed in sub-sub plots and in experimental blocks.

Specifications of experimental plots

The main plots

Levels of nitrogen treatment, including(N_{50}, N_{75}, $N_{100\%}$Recommended nitrogen for sugarcane(from the source of urea fertilizer were applied in the main plots (Table 2) and applied in three installments on 2017/04/04, 2017/05/05 and 2017/06/05, in solution form and during irrigation operations of the experimental field.Each main plot of this study consisted of 48 furrow and the length of each furrow was 5 meters and the distance between center to center two adjacent furrow was 183 cm. The area of each main plot was 439.2 m^2 and its distance from the next main plot was 2.5 m.

Sub-plots

Levels related to PGPRfactor according to Table (2) were placed in sub-plots and each sub-plot of 12 furrow and each furrow with a length of 5 m and an area of 109.8 m^2 and the distance between two adjacent sub-plots wastwo furrow.

Sub-sub plots

Levels related to phosphorus factor from triple superphosphate fertilizer source, at three levels (P_{50}, P_{75}, $P_{100\%}$ Recommended phosphorus for sugarcane) were applied to the sub-sub plots on 2016/08/31(Table 2), According to the plan of design.

Statistical analysis

The results of measurements related to field experiments, grouping of means and drawing of charts were performed by using SAS(9.3),version(9.1), MSTATC and EXCEL software, respectively.

Results and Discussion

Results of analysis of variance

The results of analysis of variance show that the simple and interaction effects of plant growth promoting rhizobacteria, nitrogen and phosphorus treatments on sugarcane quantitative traits including stem density,

Table 1. Result of field soil analysis of experimental site

Year	soil texture	%OC	%OM	%N	P(mg/kg)	K^+(mg/lkg)	Mg^{2+} (Meq/L)	Ca^{2+} (Meq/L)	pH	Ec(ds/m)
2016	**Clay loam**	0.49	0.85	0.08	7.6	183.63	9.38	10.34	8	2.9

Table 2. Experimental factors and their levels

Factor	Explanation of Factor	Levels of Factor			
A	Nitrogen	N_{50}	N_{75}	N_{100}	
B	Bacteria	Control(No bacteria)	B_P	B_E	$B_{E+}B_P=B_{EP}$
C	Phosphorus	P_{50}	P_{75}	P_{100}	

N_{50}=50% recommended nitrogen, N_{75}=75% recommended nitrogen, N_{100}=100% recommended nitrogen

P_{50}=50% recommended phosphorus, P_{75}=75% recommended phosphorus, P_{100}=100% recommended phosphorus

B=Bacteri, B_0=No bacteria, B_P = *Pseudomonas putida,* B_E=*Enterobacter cloacae,* B_P+B_E =B_{EP}

Table 3. Analysis of variance of treatments of nitrogen, phosphorus and PGPR on the measured quantitative traits

		M.S.								
S.O.V.	**df**	**Height (cm)**	**diameter (mm)**	**density (m^2)**	**Yield (ton/ha)**	**Leaf nitrogen (%)**	**Leaf chlorophyll (SPAD)**	**Leaf phosphorus (%)**	**HI (%)**	**LAI**
Block	3	582.4*	9.94**	1.15**	835.57**	0.007**	48.11**	0.0045**	606*	2.23*
Nitrogen	2	13171.5**	27**	162.8**	4111**	2.36**	190.9**	0.1247**	1862.1**	51.56**
Error	6	42.37ns	0.48ns	1.1ns	34.88ns	0.005ns	1.98 ns	0.003ns	53.7ns	0.19ns
Bacteria	3	4550.2**	220**	19**	718.2**	0.093**	41.47**	0.015**	182.6**	4.62**
Error	9	9.14ns	0.16ns	0.14ns	5.81 ns	0.001ns	0.63 ns	0.004ns	11.2ns	0.03ns
Nitrogen × Bacteria	6	360.78*	0.45*	0.62**	61.94**	0.02*	10.11**	0.003**	14.5**	0.36**
Phosphorus	2	2652.92**	648**	8.37**	488.37**	0.06**	49.38**	0.0138**	565.9**	3.71**
Error	6	18.37ns	0.24ns	0.13ns	2.38ns	0.006ns	0.91 ns	0.001ns	5.3ns	0.01ns
Nitrogen × Phosphorus	4	215.34**	0.52**	0.26**	19.88**	0.03**	1.29**	0.0017**	70.9**	0.38**
Bacteria × Phosphorus	6	52.61**	45.3*	0.14**	26.87**	0.01**	1.45**	0.002**	15.7**	0.02**
Nitrogen × Bacteria × Phosphorus	12	73.44**	51**	0.25**	18.76**	0.009**	1.68**	0.003**	24.0**	0.12**
C.V.(%)		9	10	13	12	2	6	2.5	12	10
n.s, * and **: non-significant, significant at the %5 and %1 probability levels, respectively										

Table 4. Comparison of means of interaction effects PGPR, nitrogen and phosphorus factors on measured quantitative traits of sugarcane

Treatment	Tillering	Diameter	Height	Yield	Leaf nitrogen	Leaf chlorophyll	Leaf Phosphorus	HI	LAI
	(m2)	(mm)	(cm)	(ton/ha)	(%)	)SPAD (	(%)	(%)	
$N_{50}B_0P_{50}$	9.09g	18.6r	206.25s	76.84q	1.673i	35.35g	0.132r	62.2q	4.1x
$N_{50}B_0P_{75}$	9.34g	20.87op	209.26s	84.8p	1.687i	36.32fg	0.140r	74.76mn	4.12x
$N_{50}B_0P_{100}$	9.55g	21.4no	219.25r	88.59o	1.731h	39.58ef	0.155q	75.86 ln	4.12x
$N_{50}B_PP_{50}$	10.07f	20.6pq	226.24q	91.93n	1.735h	40.22de	0.165o-q	67.75p	4.32v-x
$N_{50}B_PP_{75}$	10.31f	22.9kl	253j-m	98.2l	1.734h	41.60a-e	0.177m-o	75.23 ln	4.52t-w
$N_{50}B_PP_{100}$	10.74f	23.53i-k	260.75e-i	96.91lm	1.742h	42.56a-e	0.185lm	74.29 mn	4.72r-u
$N_{50}B_EP_{50}$	9.92g	20.12q	222qr	93.72mn	1.735h	39.60ef	0.157pq	70.41 op	4.22wx
$N_{50}B_EP_{75}$	10.20f	22.5lm	234.25p	94.85mn	1.741h	41.72a-e	0.170n-p	71.96 no	4.42u-x
$N_{50}B_EP_{100}$	10.47f	23.14j-l	240.25no	98.67l	1.740h	41.12b-e	0.182mn	76.12 k-m	4.62s-v
$N_{50}B_{PE}P_{50}$	11.03e	21.85mn	244.23n	98.89l	1.770h	40.93c-e	0.187lm	75.71 l-n	4.92p-s
$N_{50}B_{PE}P_{75}$	11.43e	23.80h-j	250.50m	99.32l	1.761h	41.39b-e	0.197kl	76.48 k-m	5.12n-q
$N_{50}B_{PE}P_{100}$	11.68e	24.70fg	251lm	99.78l	1.760h	42.08a-e	0.210jk	76.75 l-n	5.32 l-o
$N_{75}B_0P_{50}$	10.93f	21.80mn	235.75op	93.62mn	1.775h	40.73c-e	0.240hi	76.15 k-m	4.82q-t

$N_{75}B_0P_{75}$	11.18e	22.67l	240.70n	98.72l	1.767h	41.25b-e	0.232l	76.8j-m	5.02 o-r
$N_{75}B_0P_{100}$	11.53e	25.50e	242.55n	105.16jk	1.776h	41.80a-e	0.255f-h	78.43g-m	5.22 m-p
$N_{75}B_PP_{50}$	12.26d	24.50gh	250.75lm	104.96jk	1.770h	41.24b-e	0.238h-i	77.61h-m	5.62 i-l
$N_{75}B_PP_{75}$	12.70d	26.50d	254.25j-m	107.16h-j	1.870g	42.05a-e	0.268d-f	80.28f-k	5.92 f-i
$N_{75}B_PP_{100}$	13.12c	26.60d	256.75h-k	107.51g-j	1.911f	42.64a-e	0.258e-g	80.83f-j	6.12d-g
$N_{75}B_EP_{50}$	12.07ed	24.35gh	250.75lm	104.70jk	1.767h	41.40b-e	0.231i	77.42h-m	5.52j-m
$N_{75}B_EP_{75}$	12.56d	26.21d	253.25j-m	106.09h-k	1.868g	41.84a-e	0.230i	79.25f-l	5.82g-j
$N_{75}B_EP_{100}$	12.95d	26.77d	256o-k	106.93h-k	1.865g	42.67a-e	0.243g-i	80.29f-k	6.02e-h
$N_{75}B_{PE}P_{50}$	12.41de	25.30ef	252k-m	105.75i-k	1.765h	41.65a-e	0.252f-h	78.49g-m	5.72h-k
$N_{75}B_{PE}P_{75}$	13.32c	26.40cd	257g-k	107.66g-j	1.985de	42.51a-e	0.272cd	80.87f-j	6.22d-f
$N_{75}B_{PE}P_{100}$	13.42c	26.94cd	259.75e-i	108.23f-j	1.990c-e	43.47a-e	0.263d-f	81.61e-h	6.32d-f
$N_{100}B_0P_{50}$	11.97de	23.40jk	241.65n	103.52k	1.748h	41.15b-e	0.250f-h	76.15k-m	5.42k-n
$N_{100}B_0P_{75}$	13.63c	25.50e	257.75f-j	109.63d-h	1.981c-e	44.09a-d	0.255f-h	83.14c-f	6.72a-c
$N_{100}B_0P_{100}$	13.83c	27.60bc	262e-h	111.07d-g	1.982c-e	44.75a-c	0.267d-f	85.22b-e	6.82ab
$N_{100}B_PP_{50}$	13.94c	24.30gh	262.25e-g	108.84f-i	1.963de	43.84a-e	0.252f-h	81.37e-i	6.4 c-e
$N_{100}B_PP_{75}$	14.17b	27.42bc	273cd	112.39c-e	1.952e	44.42a-d	0.282c	85.93a-d	6.9a
$N_{100}B_PP_{100}$	14.69b	31.30a	283.5a	116.52b	2.038ab	45.30ab	0.307b	86.86a-c	7.01a
$N_{100}B_EP_{50}$	13.84c	24.20g-i	262.75ef	109.02e-i	1.962de	42.66a-e	0.247gh	81.94e-g	6.26 d-f
$N_{100}B_EP_{75}$	14.00b	27.55bc	269.5d	111.45d-f	1.986c-e	44.10a-d	0.275cd	85.24b-e	6.72a-c
$N_{100}B_EP_{100}$	14.47b	31.50a	278.75b	115.25bc	2.010bc	45.21ab	0.286c	89.812a	7.02a
$N_{100}B_{PE}P_{50}$	13.94c	25.41e	263.5e	109.3e-i	1.970c-e	44.06a-d	0.257e-g	82.26d-g	6.46b-d
$N_{100}B_{PE}P_{75}$	14.44b	27.70bc	275.25bc	112.94cd	1.998cd	44.7a-c	0.285c	86.92a-c	6.86 a
$N_{100}B_{PE}P_{100}$	15.01a	31.70a	284.5a	122.35a	2.062a	45.95a	0.347a	87.3ab	7.1 a

a1=N_{50}=50% recommended nitrogen, a2=N_{75}=75% recommended nitrogen, a3= N_{100}=100% recommended nitrogen

c1=P_{50}=50% recommended phosphorus, c2=P_{75}=75% recommended phosphorus, c3= P_{100}=100% recommended phosphorus

B=Bacteri, b1= B_0=No bacteria, b2= B_P = *Pseudomonas putida*, b3=B_E=*Enterobacter cloacae*, b4= B_P+B_E =B_{PE}

The numbers inside each column that have a common letter are not statistically significant at the 5% level

Table 5. Comparison of the mean treatment of the simultaneous application of the tested bacteria in addition to the application of 75% nitrogen and phosphorus recommended for sugarcane (N75BPEP75), compared to the mean control treatment (N100B0P100), in terms of quantitative traits measured

Trait	Unit	Control($N_{100}B_0P_{100}$)	$N_{75}B_{EP}P_{75}$	Success compared to control(%)	Nitrogen saving(%)	Phosphorus saving(%)
Yield	t/ha	111.07d-g	107.66 g-j	96.9	21.9	21.9
Height	cm	262 e-h	257 g-k	98.1	23.1	23.1
Diameter	mm	27.6 bc	26.4 cd	95.65	20.7	20.7
Tillering	m^2	13.83 c	13.32c	96.3	21.3	21.3
Leaf nitrogen	%	1.982c-e	1.985 c	100.2	25.2	25.2
Leaf Phosphorus	%	0.267d-f	0.272 d	101.9	26.9	26.9
LAI		6.82ab	6.22d-f	91.2	16.2	16.2
HI	%	85.22b-e	80.87f-i	94.8	19.8	19.8

N_{50}=50% recommended nitrogen, N_{75}=75% recommended nitrogen, N_{100}=100% recommended nitrogen

P_{50}=50% recommended phosphorus, P_{75}=75% recommended phosphorus, P_{100}=100% recommended phosphorus

B=Bacteri, B_0=No bacteria, B_P = *Pseudomonas putida*, B_E=*Enterobacter cloacae*, B_P+B_E =B_{PE}

The numbers within each row that have a common letter are not statistically significant at the 5% level

height, diameter, stalk yield, percentage of nitrogen and phosphorus in leaves, leaf chlorophyll content, LAI and HI have been significant at the level of 1% probability(Tables 3).

Sugarcane yield and its components

Stalk yield of sugarcane

In this regard, the comparison of the mean of interaction effects shows that the treatment of simultaneous application of *Pseudomonas putida* and *Enterobacter cloaca*, along with the application of 75% of recommended phosphorus and nitrogen for sugarcane ($N_{75}B_{PE}P_{75}$), with a yield of

Table 6. Comparison of the mean of simultaneous application of the tested bacteria with the application of 100% nitrogen and phosphorus recommended for sugarcane (N100BPEP100), with the mean of the control treatment (N100B0P100), regarding the measured quantitative and qualitative traits

Trait	Unit	Control ($N_{100}B_0P_{100}$)	$N_{100}B_{EP}P_{100}$	Success compared to control(%)	Increase compared to control (%)
Yield	t/ha	111.07d-g	122.35a	110.2	10.2
Height	cm	262 e-h	284.5a	108.6	8.6
Diameter	mm	27.6 bc	31.7a	114.9	14.9
Tillering	m2	13.83 c	15.01a	108.5	8.5
Leaf nitrogen	%	1.982c-e	2.062a	104	4
Leaf chlorophyll	SPAD	44.75a-c	45.95a	102.68	2.68
Leaf Phosphorus	%	0.267d-f	0.347a	130	30

N_{50}=50% recommended nitrogen, N_{75}=75% recommended nitrogen, N_{100}=100% recommended nitrogen

P_{50}=50% recommended phosphorus, P_{75}=75% recommended phosphorus, P_{100}=100% recommended phosphorus

B=Bacteri, B_0=No bacteria, B_P = *Pseudomonas putida*, B_E=*Enterobacter cloacae*, B_P+B_E =B_{EP}

The numbers within each row that have a common letter are not statistically significant at the 5% level.

107.66 tons per hectare, was able to achieve 96.9% success, compared to the control treatment($N_{100}B_0P_{100}$ or using 100% of recommended phosphorus and nitrogen, without using PGPR) with a yield of 111.77 tons per hectare and in this way, it provided 21.9% of the sugarcane's nutritional needs for nitrogen and phosphorusand the same amount of savings in the use of nitrogen and phosphorus fertilizers for sugarcane is created.Regarding sugarcane yield, also comparing the mean interaction effect of the treatment of two types of tested bacteria and the use of 100% nitrogen and phosphorus ($N_{100}B_{PE}P_{100}$), with a yield of 122.35 tons per hectare, compared with the control treatment ($N_{100}B_0P_{100}$), with a yield of 111.7 tons Per hectare, showed 110.15% success and in the case of sugarcane yield, 10.15% improved compared to the control(Tables 4, 5 and 6). In this regard, Roberta, M. Santos and et al(2018) reported that the treatment of sugarcane growth-promoting bacteria with chemical fertilizers increased the dry matter of sugarcane by 13% compared to the control treatment (without the use of bacteria). Govidaranjan, M., Hossain, A. and *et al.,* (2020) reported that the application of growth-promoting bacteria along with 50% of the recommended nitrogen fertilizer in sugarcane, compared to the positive control (application of 100% of the recommended nitrogen without bacteria), regarding Biomass production was 114% successful, and more than 50% savings in nitrogen fertilizer for sugarcane established. Poliana, A., Leonel R. and et al(2020) also reported that the application of two types of sugarcane growth-promoting bacteria with 25% of recommended phosphorus fertilizer, compared with the positive control treatment, in the case of the yield of sugarcane stalk was 108% successful and resulted in 75% savings in phosphorus fertilizer consumption for sugarcane. . K.S.Shukla (2020) obtained similar results and reported that the treatment of bacterial application with 50% nitrogen, phosphorus and potassium recommended for sugarcane, compared to the positive control treatment (application of 100% nitrogen, phosphorus and potassium recommended, without PGPR), achieve a 104% success rate for sugarcane and lead to a 50% reduction in the consumption of nitrogen, phosphorus and potassium for sugarcane.

Height, diameter and density of sugarcane

Comparison of the mean of interactions shows that $N_{75}B_{PE}P_{75}$ treatment, in terms of height, diameter and density of sugarcane (257 cm, 26.4 mm and 13.32 stems per m^2, respectively), compared to the control treatment ($N_{100}B_0P_{100}$) with 262 cm in height, 27.4 mm in diameter and 13.83 density per m^2, in the case of the mentioned traits, was able to achieve 98.1%, 95.7% and 96.3% success, respectively, and in this way, 23.1%, 20.7% and 21.3%, respectively, save on the use of nitrogen and phosphorus fertilizers for sugarcane (Tables 4, 5 and 6).Also, regarding the mentioned traits, comparison of the mean interaction effect of the treatment of the two types of bacteria tested and the application of 100% nitrogen and phosphorus ($N_{100}B_{PE}P_{100}$), with 284.5 cm in height, 31.7 mm in diameter and 15.01 density per m^2, In comparison withthe control treatment, 108.6%, 114.9% and 108.5% showed success, respectivelyand improved the height, diameter and density of stem in sugarcane in comparison with the control treatment, 8.6, 14.9 and 8.5%, respectively(Tables 4, 5 and 6).Studies by other researchers also confirm the results of this study. Antonio Morgado González *et al.,* (2015) when evaluating PGPR on sugarcane, reported that these bacteria in comparison with the control treatment (treatment without bacterial application) increased height, diameter, density and dry matter content of sugarcane by 27.55%, 20.75, 38.5% and 59.5%, respectively.S.K. Shukla(2020) obtained similar results and reported that the bacterial application treatment with 50% NPK recommended for sugarcane, compared to the positive control treatment (application of 100% recommended nitrogen, phosphorus and potassium, without sugarcane growth stimulant bacteria), In terms of height and diameter of sugarcane stalks, it was achieved

101 and 103% success, respectively, and saved more than 50% in the use of chemical fertilizers nitrogen and phosphorus recommended for sugarcane.

Sugarcane leaf nitrogen

Regarding the measured trait of sugarcane leaf nitrogen, a comparison of the mean of the interactions shows that between the mean of $N_{75}B_{PE}P_{75}$ treatment (with 1.985% leaf nitrogen) and the mean control treatment ($N_{100}B_0P_{100}$) with 1.982% leaf nitrogenwas no significant difference at 5% probability leveland N75BPEP75 treatment was able to achieve 100.2% success compared to the control treatmentand provide 25.2% of the nutrient nitrogen for the sugarcane cropand save the same amount of nitrogen fertilizer recommended for sugarcane. Also, regarding the mentioned trait, comparing the average interaction of N100BPEP100 treatment with the amount of 2.062% of leaf nitrogen, in comparison with the control treatment ($N_{100}B_0P_{100}$) with the values of 1.982% of leaf nitrogen, showed 104% successand improved the nitrogen uptake in sugarcane leaves by 4% compared to the control treatment(Tables 4, 5 and 6).Research by other researchers also confirms the results of this study. In this regard, Roberta, M.Santos*et al.,* (2018) reported that PGPR are a suitable alternative to nitrogen and phosphorus chemical fertilizers by performing biological nitrogen fixation and phosphorus fixation stabilized in soil without increasing the adverse environmental effects or reducing the yield of sugarcane. Also Nivaldo Schultz *et al.,* (2014) reported that in the case of mean nitrogen content of sugarcane stem, there was no statistically significant difference between the treatment of PGPR and the control treatment (full application of Recommended nitrogen, without bacteria) and PGPR was able to supply up to 120 kg.ha^{-1} of sugarcane nitrogen. Also Govidaranjan, M., Hossain, A. and *et al.,* (2020) reported that the application of PGPR along with 50% of the recommended nitrogen fertilizer in sugarcane, compared with the positive control treatment (full application of recommended nitrogen without bacteria), regarding Leaf nitrogen percentage and stem nitrogen content achieved success 102 and 116%, respectively and in both cases, more than 50% compared to the control treatment, in the use of nitrogen fertilizer recommended for sugarcane was saved.

Sugarcane leaf chlorophyll

Regarding the measured trait of sugarcane leaf chlorophyll with SPAD device, comparison of the mean of interactions shows that between the mean chlorophyll of $N_{75}B_{PE}P_{75}$ treatment (with SPAD number 42.51) and the mean chlorophyll of control treatment ($N_{100}B_0P_{100}$) with SPAD number equal to 44.75, statistically at 5% probability level, there is no significant difference and this treatment was able to achieve 94.92% success compared to the control treatmentand it provided 19.92% of the sugarcane's nutritional needs for nitrogen and phosphorusand the same amount of savings in the use of nitrogen and phosphorus fertilizers for sugarcane is created (Tables 4 and 6).Also, regarding the mentioned trait, the comparison of the average interaction of $N_{100}B_{PE}P_{100}$ treatment with SPAD number of 45.95, in comparison with the control treatment with SPAD number of 44.75, achieved 102.68% success(Table4, 6).The study of other researchers also confirms the results of this study. Including Govidaranjan, M. and et al(2020) reported that the application of plant growth-promoting bacteria with 50% of the recommended nitrogen fertilizer in sugarcane, compared to the positive control treatment (full application of the recommended nitrogen, without bacteria), regarding the chlorophyll content of sugarcane leaves, Achieved 101% success.

Sugarcane leaves Phosphorus

Regarding the measured trait of sugarcane leaf phosphorus, the comparison of the mean of interactions shows that between the mean leaf phosphorus of $N_{75}B_{PE}P_{75}$ treatment (with an mean of 0.272%) and the mean leaf phosphorus of the control treatment ($N_{100}B_0P_{100}$) with 0.267% phosphorus, there is no significant difference statistically, at 5% probability and this treatment was able to achieve 101.9% success compared to the control treatment and 26.9% to save the recommended phosphorus fertilizer consumption for sugarcane. Regarding the mentioned trait, also comparing the average interaction of N100BPEP100 treatment with an average of 0.347% of leaf phosphorus, in comparison with the control treatment with an average of 0.267% of leaf phosphorus, was able to achieve up to 130% successand increase the phosphorus uptake of sugarcane leaves by 30% during the period of its rapid vegetative growth, compared to the control(Tables 4, 5 and 6).Studies by other researchers have confirmed this research. B.Sundara, V.Natarajan and K.Hari(2002) showed that in the case of percentage of sugarcane leaf phosphorus, between the mean of treatment of simultaneous application of two types of growth-promoting bacteria along with the application of 75% of recommended phosphorus for sugarcane and control treatment (full application of Recommended phosphorus for sugarcane, without the use of bacteria), there is no significant difference in the 5% probability level.Also, Poliana and Leonel Roza)2020(following their research, found that the treatment of simultaneous application of two types of sugarcane growth-promoting bacteria along with 75% of the recommended phosphorus for sugarcane, compared to the control treatment (full aplication of the recommended phosphorus for sugarcane, without the use of bacteria) In addition to saving 25% of the recommended phosphorus consumption for sugarcane, it increased the accumulation of phosphorus in its stem by 38%.

Leaf Area Index (LAI)

Comparison of the mean effects of bacterial and nitrogen factors on leaf area index trait shows that there is no statistically significant difference between control treatments ($N_{100}B_0$) and $N_{75}B_{PE}$ treatment at 5% probability level and also, comparing the mean effects of two factors, bacterium and phosphorus, on leaf area index trait shows that there is no significant difference between $B_{PE}P_{50}$ treatment and control treatments(B_0P_{100}) at 5% probability level and the mentioned treatment was 98.9% successful compared to the control treatment.Regarding the measured trait of sugarcane leaf area index, comparison of the mean of the interactions of the three factors of bacteria, nitrogen and phosphorus shows that between the mean of treatments, $N_{100}B_{PE}P_{50}$ (with an average of 6.46), $N_{100}B_{PE}P_{75}$ (with an average of6.86) and the mean control treatment ($N_{100}B_{0}P_{100}$) there is no statistically significant difference at the level of 5% probability and these two treatments were able to achieve 94.7% and 100.5% success, respectively, compared to the control treatment.However, there is a statistical difference between $N_{75}B_{PE}P_{75}$ treatment (with an average of 6.22) and control treatment (with an average of 6.82) at the level of 5% probability and this treatment was 91.2% successful compared to the control treatment(table 4 and 5). Govidaranjan, M. *et al.,* 2020 in their research showed that the application of growth-promoting bacterial treatment with 50% recommended nitrogen for sugarcane, compared to the control treatment (application of 100% recommended nitrogen without bacteria), achieved 103% success andalso, Liliana, S. *et al.,* 2011 in their greenhouse experiments, showed that the bacterial application treatment on sugarcane produced more leaves than the control treatment and in this case, Antonio, M.G. *et al.,* 2016, when evaluating plant growth-promoting bacteria on sugarcane, found that these bacteria increased the leaf area of sugarcane by 49% compared to the control treatment.

Sugarcane Harvest Index (HI)

Comparison of the mean effects of bacterial and nitrogen factors on sugarcane harvest index shows that there is no significant difference between $N_{75}B_{PE}$ treatment and control treatment ($N_{100}B_{0}$) at a statistical level of 5%and the mentioned treatment was able to achieve 98.5% success compared to the control treatmentand also, comparing the mean effects of two factors, bacterium and phosphorus, in the case of mentioned trait shows that there is no significant difference between $B_{PE}P_{50}$ treatment and control treatment($B_{0}P_{100}$) at 5% probability leveland the mentioned treatment was 98.6% successful compared to the control treatment.Also, regarding the sugarcane harvest index, comparison of the average interaction effects of the three factors of bacteria, nitrogen and phosphorus shows that is no statistically significant difference between the mean of treatments, $N_{100}B_{PE}P_{50}$ (with a mean of 82.26), $N_{75}B_{PE}P_{100}$ (with a mean of 81.61) and the mean of control treatment ($N_{100}B_{0}P_{100}$) at the 5% probability leveland these two treatments were able to achieve 96.52% and 95.7% success of the control treatment, respectively but, there is a statistical difference between $N_{75}B_{PE}P_{75}$ treatment (with an average of 80.87) and control treatment (with an average of 85.22) at the level of 5% probability and this treatment was 94.89% successful compared to the control treatment(table 4 and 5).According to Raman K., 2013 and Fageria *et al.,* 2006 because the harvest index in all crops is the ratio of economic yield to biological yield and on the other hand as the main components of sugarcane economic yield including stem density per unit area, height and The diameter of the stem isand research Roberta, M. S. *et al.,* 2020; Aidin, H., 2005; Silezio, F. *et al.,* 2017; Govidaranjan, M., *et al.,* 2020 and Antonio, M.G. *et al.,* 2016 have proven the role and effect of growth-promoting bacteria in improving economic yield and its components in sugarcane. Therefore, it is concluded that the effect of growth-promoting bacteria in sugarcane improves the harvest index and the results of this experiment confirm this.

CONCLUSION

According to the results of this study, between the mean treatment of simultaneous application of *Pseudomonas putida* and *Enterobacter cloaca* with 75% of recommended nitrogen and phosphorus for sugarcane($N_{75}B_{PE}P_{75}$), and the mean of control treatment($N_{100}B_{0}P_{100}$)(application of 100% of recommended nitrogen and phosphorus for sugarcane, without the use of PGPR), Regarding leaf nitrogen percentage, leaf phosphorus percentage, sugarcane yield and sugar yield, there is no statistically significant difference at the level of 5% probabilityand this treatment, compared to the control treatment, was 100.2, 101.9, 96.9 and 98% successful for the mentioned traits, respectivelyand provided 25.15, 26.9, 21.9 and 23% of the nutrients of phosphorus and nitrogen for sugarcane and saved the same amount of recommended nitrogen and phosphorus for sugarcane.Also, about leaf nitrogen percentage, leaf phosphorus percentage, sugarcane yield and sugar yield, the simultaneous application of the tested bacteria along with the use of 100% of nitrogen and phosphorus recommended for sugarcane ($N_{100}B_{PE}P_{100}$), in comparison with the control treatment, respectively 104, 130, 110/15 and 116% was successful. This treatment, compared to the control treatment, increased the percentage of leaf nitrogen and phosphorus of sugarcane by 4 and 30%, respectively, and also improved sugarcane yield and sugar yield per unit area by 10.15 and 16%, respectively.So according to the above results, in sugarcane agronomy in Khuzestan region in southwestern Iran, which has calcareous soils with low organic matter, the potential of PGPR of sugarcane soils can be used to provide 21.9 to 26.9% of the recommended nitrogen and phosphorus for sugarcane and their application along with the full use of recommended nitrogen and phosphorus fertilizers increased stalk yield and sugar yield of sugarcane, compared to the control, by 10.15 and 16%, respectivelyand this strategy, in addition to ensuring the stability of quantitative and qualitative yield of sugarcane and increasing its self-reliance coefficient for Nutrients of nitrogen and phosphorus, both reduces the cost of sugarcane production in terms of saving on nitrogen and phosphorus fertilizers and reduces the environmental challenge associated with nitrogen fertilizer. Also in this study, the simultaneous application of *Pseudomonas putida* and *Enterobacter cloaca* in all treatments in terms of improving the traits measured in the experiment, showed better results than their individual application.

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