33

Egypt. J. Soil. Sci. Vol. **60**, No. 4, pp. 469-482 (2020)

Egyptian Journal of Soil Science

http://ejss.journals.ekb.eg/

Impact of Biofertilizers on Maize (*Zea mays* L.) Growth and Yield under Calcareous Soil Conditions

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THE AGRICULTURAL expansion in Egypt is of great importance to face the shortage in cereal crop production. Biofertilizers recently are used to overcome the deficiency of some nutrients due to their fixation in the soil and consequently increase its fertility especially in calcareous soil. The current work is aiming to assess the use of phosphate solubilizing bacteria (PSB) and cyanobacteria (Cyan.) for maize production in calcareous soil. A field experiment was conducted to achieve this goal in a split-plot type in three replicates over two years. The obtained results were promising, where all growth parameters; leaf chlorophyll content, plant height, ear length, ear diameter and ear weight, showed a significant increase. Yield parameters; grain weight, weight of 100 grains, grain yield and straw yield were increased by 56, 27, 86 and 26%, respectively compared to control. Effect of the phosphate solubilizing bacteria on yield exceeded that of cyanobacteria but the effect of their combination was the highest. Nutrient uptake by grain and straw was also remarkably increased, where N, P and K uptake by grain showed an increase of about 112, 192 and 198%, respectively of their values of control treatment. Grain components; carbohydrate and protein contents increased by 8.8 and 112%, respectively as compared to control. Carbohydrate content under PSB treatment was higher than its value under Cyan. while protein content showed the opposite. Finally, available N, P and K in soil increased by about 55, 94 and 39%, respectively at the end of the experiment.

Keywords: Calcareous soil, Maize, Biofertilizers, Phosphate solubilizing bacteria, Cyanobacteria, Wadi Al-Arish

Introduction

Many countries are facing the challenge of narrowing the gap between the demand and consumption of the agricultural stuff, particularly food, and consequently achieving self-sufficiency. Realizing this goal requires applying some measures commonly known as horizontal and vertical expansion in agriculture. However, in some cases, the horizontal expansion is either limited or costly as in Egypt. Therefore, it is obligatory to use both problematic and marginal soils as well as following suitable management processes for these types of soils in order to achieve the most agricultural production per land unit (United Nations and FAO, 2017).

Mineral fertilizers are the most exclusive mean to increase agricultural production. Environmentally, the excessive use of these fertilizers, in the long term causes soil degradation besides being, economically, cost-effective (Iwuagwu et al., 2013). Scientists, consequently, directed their researches towards the use of biofertilizers besides the mineral fertilizers to achieve greater agricultural production, lesser environmental pollution and, at the same time, to preserve the soil quality (Bashan et al., 2004; Sharma et al., 2007). El-Naim et al. (2017) affirmed that the use of different microbial strains as biofertilizers has led to a decrease in the use of chemical fertilizers and has provided high harvests quality free of harmful agrochemicals. Beyranvand et al. (2013) and El-Zemrany et

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DOI: 10.21608/ejss.2020.45922.1392
Received: 11/10/2020; Accepted: 2/12/2020

al. (2016) expounded that the positive effect of biofertilizer may result from its potency to increase the availability of phosphorus, nitrogen and other nutrients especially under the calcareous nature of the soil which commonly diminishes the availability of nutrients. Moreover, many researches proved that nitrogen and phosphate biofertilizers application increases maize growth, yield and yield components due to root growth promotion which consequently lead to enhance nutrients and water uptake from the soil, (Beyranvand et al., 2013; Amin and Hamidreaza, 2015 and Kumar et al., 2019).

Maize (*Zea mays* L.) is one of the major cereal crops for many countries in the world (Ashraf et al., 2016). It is a very versatile grain that benefits mankind in many ways. FAO ranked it the third crop following wheat and rice in world production (FAO, 2013). Maize is a staple human food for some people, a feed for livestock and raw material for many industrial products such as corn oil, corn flour and starch. In Egypt, maize is considered the most second important cereal crop after wheat however, its consumption is greater than the local production. Therefore, increasing its unit area production as well as the cultivated area is highly recommended (Amer, 2016). Maize production is dependent on fulfilling the nutrient requirements particularly nitrogen, phosphorus and potassium as well as fertilizer application management (Arunkumar, 2007; Rafique et al., 2017 and Kumar et al., 2019). Nitrogen plays a key role in the vegetative growth and grain production of maize plant, and also improves its protein contents (Gustavo et al., 2016; Ljubica et al., 2018; Abd El-Hafeez and Bashandy, 2019). It is reported that N application to maize increase fodder nutritive value by increasing crude protein and reducing ash and fiber contents (Khan et al., 2014; Hafez and Abdelaal, 2015). Phosphorus, as a main plant nutrient, is essential for plant growth where is involved in several key plant functions; such as transferring energy inside the plant (Hameeda et al., 2008 and Viruel et al., 2014). However, due to its crucial fixation as insoluble phosphates of iron, aluminum, and calcium especially in calcareous soil, plants may suffer from its deficiency in most soils especially calcareous soils (Cordell et al., 2011; Sharma et al., 2013 and Hellal et al., 2019).

Phosphate solubilizing bacteria (PSB) improve plant growth, yield and phosphorus content of several crops, and may be used as bioinoculant to enhance sustainable production (Hameeda et al., 2008 and Viruel et al., 2014). Amanullah and Adil Khan (2015) confirmed that sowing with inoculated maize seeds with PSB had resulted in higher yield and yield components over uninoculated seeds. PSB is identified to be belonging primarily to the Pseudomonas, Bacillus, Mycobacterium, and Enterobacter genera, among others (Hanif et al., 2015; Li et al., 2015 and Wang et al., 2017). Cyanobacteria (Cyan.) are well known as atmospheric nitrogen fixing microorganisms for field crops and important agronomic biofertilizers (Singh et al. 2013; Singh et al., 2014; Abd EL-Kader, 2018). Iwuagwu et al. (2013) applied biofertilizer treatments to the maize experiment 10 days after planting. They got a significant increase in plant height, root length, stem diameter, fresh and dry weight of seedlings on the application of the microbial inoculants when compared to control. However, Abou EL-Nour et al. (2019) stated that addition of NPK with biofertilizers significantly increased maize growth parameters as compared to the separated application of NPK and biofertilizers. Schütz et al. (2018) and Taha et al. (2018) illustrated based on previous studies that yield response due to biofertilizer application was generally small at low soil phosphate levels, efficacy increased along higher soil P levels in the order P solubilizers, and N fixers.

The aim of the current study is to assess the use of phosphate solubilizing bacteria and cyanobacteria along with mineral phosphate fertilizer for maize production in calcareous soil. The work also tests the availability of P to ensure the ability of the biofertilizers to afford soluble form.

Materials and Methods

Experimental location

Climate

The climate of Al-Arish region is arid characterized by relatively rainy, cold winters and dry hot summer (Abdel Ghaffar et al., 2015). The rainfall occurs mostly during the period November-March with mean annual ranges between 25 and 118 mm/year with an average about 100.7 mm. The monthly evaporation varied from 1.5 to 10.3mm / day in December to July, respectively (Hassan, 2002). The mean temperature ranges from 27.8 to 14.4 °C and 25.9 to 13.4 °C (World Bank, 2013).

Soil

In general, the soils of Wadi Al-Arish are lacking of pedological features that characterize the soil development under arid conditions. Also, the soils are developed on fluvial deposits influenced by limestone of the upstream and consequently are calcareous having coarse texture. The soils that are developed on dunes generally have no sign of soil forming processes. On the other hand, the coastal zone soils are exclusively saline. While in the desert region the gravel plain soils are dominantly calcareous and classified as Haplic Calcisols (Hegazi et al., 2005 and Gad, 2016). Therefore, the soil resources of Wadi Al-Arish area are mostly limited.

Irrigation water

The available irrigation water is groundwater where, water resources of the area are limited and in general of low quality (Abdel Ghaffar et al., 2015) due to over pumping as well as seawater intrusion (Gad et al., 2015).

Field Experiment

A field experiment was conducted at the Agricultural Experimental Station of Agricultural Research Center at Al-Arish, North Sinai Governorate, Egypt located at latitudes 31° 06′ 47.43″N and longitude 33° 49 ′33.81″E. The experiment was carried out in three replicates and designed in a split-plot type. Each plot was 4.0 × 2.5 m, the seeds were sowed at depth of 5-6 cm in rows having 50 cm distance in between and 40 cm among the plants. The soil was plowed and 20 m^3/fed. of compost was added and mixed with the soil. Potassium sulphate (48% K_2O) fertilizer of 50 kg/ fed. was added before cultivation and ammonium nitrate (33.5% N) fertilizer of 120 kg/ fed. was added in four branches. The maize cultivar tribal hybrid 352 yellow seeds were inoculated overnight with phosphate solubilizing bacteria (PSB), cyanobacteria (Cyan.) and with mixture of both (PSB+Cyan.) before sowing. The used biofertilizers were provided by the Department of Soil Microbiology; Soil, Water and Environment Research Institute (SWERI), Agriculture Research Center (ARC), Egypt. The experimental field was irrigated by drip irrigation system and was executed over two successive summer seasons; from 28/5 to 22/9/2017 and from 18/5 to 18/9/2018.

The experimental treatments

- Biofertilizer: (Phosphate solubilizing bacteria and cyanobacteria)
  - Control: Without biofertilizer
  - PSB: Phosphate solubilizing bacteria
  - Cyan.: Cyanobacteria
  - (PSB+Cyan.): Phosphate solubilizing bacteria + cyanobacteria
- Mineral fertilizer: (Phosphate fertilizer form of calcium superphosphate 15% P_2O_5)
  - P0: Control without mineral P fertilizer
  - P1: 10 kg P_2O_5 /fed.
  - P2: 15 kg P_2O_5 /fed.
  - P3: 20 kg P_2O_5 /fed.

1	Control + P_0	5	PSB + P_0	9	Cyan. + P_0	13	(PSB+Cyan.) + P_0
2	Control + P_1	6	PSB + P_1	10	Cyan. + P_1	14	(PSB+Cyan.) + P_1
3	Control + P_2	7	PSB + P_2	11	Cyan. + P_2	15	(PSB+Cyan.) + P_2
4	Control + P_3	8	PSB + P_3	12	Cyan. + P_3	16	(PSB+Cyan.) + P_3

The experimental plots

Laboratory analyses

Soil analyses

Surface soil samples (0-30 cm) were collected from the experiment plots in each season and were air-dried, gently ground and sieved through 2 mm sieve. Then laboratory analyses were carried out to determine the main soil physicochemical properties, as follows: particle size distribution, soluble cations (Ca^{2+}, Mg^{2+}, Na^+ and K^+) and anions (Cl, HCO_3^- and CO_3^{2-}), pH, EC, $CaCO_3$ and OM contents were determined following the methods described by Soil Survey Staff (2014). The extracted available N was determined by Kjeldahl method and the extracted available K was determined using the Flame Photometer (Page et al., 1982). Available P was extracted according to Olsen et al. (1954) and determined using ascorbic acid (Van Reeuwijk, 1993).

Water analysis

The pH, EC, cations and anions were determined according to USDA (2004).

Plant analyses

The plant samples were collected at physiological maturity at 90 days from sowing for yield components and after harvesting at 120 days from sowing for grain yield. Samples of 8 plants each were taken from every plot for testing chlorophyll a and b chlorophyll contents spectrophotometric determination in the leaves as described by Moran (1982), and the total chlorophyll (a+b) was calculated as the sum of a and b contents. At harvesting stage, random representative plants at each plot were collected for growth and yield parameters; plant height (cm),ear length (cm), ear weight (g $plant^{-1}$), grain weight per ear (g), plant dry weight (g), 100-grain weight (g), grain yield (kg fed^{-1}) and stalk yield (kg fed^{-1}) measurements. Samples of grain and leaves were taken randomly from each plot then dried at 70° C, ground and wet digested to determine their N, P and K concentrations and uptakes were calculated. N was determined using Kjeldahl Method, P was spectrophotometrically determined and K was determined using Flame Photometer. Also, a sample of 50 g grains was taken from grain yield of each plot, ground and crude protein and carbohydrates contents were determined according to A.O.A.C. (1990).

Statistical analyses

The variable means among different treatments were compared using the least significant differences (fisher LSD) at $p \leq 0.05$ using InfoStat modeling software (Version, 2014) in according to Di Rienzo et al. (2012).

Results and Discussion

Soil characterization

The soils characteristics at the beginning of the current field experiment are recorded in Table 1. They had sandy loam texture of about 66.32% sand, 32.9% silt and 0.78%clay. They were non-saline (EC 3.10 dSm^{-1}), non-sodic (SAR 7.04), soil reaction is alkaline (pH 8.40), and total $CaCO_3$ content was high (188.5 g kg^{-1}). The soluble cations were Ca^{2+},Mg^{2+}, Na^+ and K^+ of 6.26, 7.72, 15.83 and 0.23mmolc L^{-1} and soluble anions Cl^-, HCO_3^- and SO_4^{2-} of 18.64, 2.50 and 8.90 mmolc L^{-1}, respectively. It is worth to mention that the dominant soluble cation was Na^+ followed by Mg^{2+}, Ca^{2+} and K^+ while the dominant anion was Cl^- followed by SO_4^{2-} and HCO_3^-. In addition, these soils were characterized by very poor OM content (0.7 g kg^{-1}) and N, P, K were 11.80, 3.15 and 25.60 mg kg^{-1}, respectively.

Irrigation water characterization

The analytical results of the well water used to irrigate the experiment (Table 2) revealed that it was severely saline (EC= 5.35dSm^{-1}) non alkaline (SAR 7.11) and contained NaCl, $CaCl_2$, Na_2SO_4 and $CaSO_4$ salts in descending order. Due to the high salinity of irrigation water, the soil moisture content was ensured to be kept at the field capacity during the course of the experiment in order to avoid any salt accumulation within the plant root zone.

Maize growth parameters

Leaf chlorophyll content

Chlorophyll (Ch) is the major leaf constituent of photosynthesis process in plants through its ability of light absorption to generate the needed energy for the completion of this basic biological process. The presented data in Figure (1) revealed that leaf chlorophyll a, b and a+b contents increased as they were influenced by mineral phosphate fertilizer and biofertilizers and their combinations. They showed the minimum values under control treatment Control+P0 and maximum values under (PSB+Cyan.)+P3.

TABLE 1. Physicochemical properties and available macronutrients of the experiment soil

Particle Size Distribution (%)				
Sand	**Silt**	**Clay**	**Texture class**	
66.32	32.90	0.78	Sandy loam	
Chemical Properties				
ECe (dSm^{-1})	pH (1:2.5)	OM g kg^{-1}	Total $CaCO_3$ g kg^{-1}	
3.10	8.40	0.7	188.5	
Soluble Caions (mmolc L^{-1})				
Ca^{2+}	Mg^{2+}	Na^+	K^+	
6.26	7.72	15.83	0.23	
Soluble Anions (mmolc L^{-1})				
Cl^-	HCO_3^-	CO_3^{2-}	SO_4^{2-}	SAR
18.64	2.50	---	8.90	7.04
Available Macronutrients (mg kg^{-1})				
N	P	K		
11.80	3.15	25.60		

TABLE 2. Chemical analysis of well water used for irrigation for experimental area

ECe (dSm^{-1})	pH	Soluble cations (mmolc L^{-1})				Soluble anions (mmolc L^{-1})				SAR %
		Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-	HCO_3^-	CO_3^{2-}	SO_4^{2-}	
5.35	7.50	16.17	5.80	31.07	0.10	43.60	1.20	---	8.34	7.11

The analysis of variance (Fig. 1) pointed out that Ch a content was significant under mineral fertilizer level but very highly significant under biofertilizer. While both Ch b and Ch a+b contents were highly significant under mineral fertilizer levels and very highly significant under biofertilizer types. It could be concluded that chlorophyll was comparatively affected by biofertilizer types more than by mineral P-fertilizers. LSD at 0.05 was illustrated in Fig.1 by small letter attached to the variable mean values. Wherever an uncommon letter is appearing beside a value in each column and in each row then, this means that it is significantly different from the others. It was observed that chlorophyll content under PSB treatment was higher than under Cyan. treatment. In this respect many authors reported similar findings where, Iwuagwu et al. (2013) stated that growing microbial inoculated maize seedlings showed a significant increase in the chlorophyll content of the maize plants when compared to control. Also, Wu et al. (2019) found that chlorophyll content was significantly increased due to the interaction between mineral phosphate and PSB treatments. They stated that the co-inoculated plants had higher chlorophyll content than that of either plants inoculated with single PSB strains or non-inoculated plants.

Plant height

The highest height of maize plant was 183.6 cm found under (PSB+Cyan.)+P3 treatment while the lowest was 153.4 cm recorded under Control+P0 treatment (Fig. 1) achieving a significant difference. The analysis of variance showed highly significant differences of plant height under mineral phosphate levels and very highly significant differences under biofertilizer types. The LSD at 0.05 of plant height under the phosphate mineral fertilizer revealed significant differences between P0, P1and both P3 and P4 treatments which they were nonsignificant. The biofertlizer treatment Control had no significant differences in plant height with Cyan. treatment but had significant difference with PSB and (PSB+Cyan.) treatments. The interaction of mineral phosphate fertilizer with biofertilizers showed nonsignificant difference of plant height under Control+P0 treatment and that under Control+P1, Cyan.+P0 and PSB+P0 treatments however, it was significantly different under all other treatments. On the other hand, the plant height under (PSB+Cyan.)+P3 treatment was found nonsignificant with that under PSB+P2, (PSB+Cyan.)+P1, PSB+P3 and (PSB+Cyan.)+P2 treatments, but significant with the plant height under the rest of treatments.

Ear length

The maize ear length responded to biofertilizers differently than to mineral phosphate fertilizer. Data in Fig. 1 showed that ear length was 10.45 cm under Control+P0 treatment and it reached 19.97 cm under (PSB+Cyan.)+P3 treatment. There was nonsignificant difference for ear length under mineral phosphate treatments but was found significantly different under biofertilizer treatments according to the analysis of variance. LSD at 0.05 resulted in significant difference between P0 from one side and P2, P3 from the other side. However, under biofertilizers significant difference was noticed only between Control and (PSB+Cyan,) treatments. As the interaction between mineral phosphate fertilizer and biofertilizer treatments is concerned, incorporation of P0 treatment had resulted in significant difference with those were treated with (PSB+Cyan.) treatment and vice versa.

Ear diameter

The minimum ear diameter was 3.51 cm observed under Control+P0 treatment and the maximum was 5.78 cm under (PSB+Cyan.)+P3 treatment (Fig. 1). Based on the analysis of variance nonsignificant difference for ear diameter under both mineral-P and biofertilizers treatments was observed. The LSD clearly confirmed the slight effect of mineral phosphate fertilizer where significant difference was only detected between P0 and P3 while there was no effect of biofertilizers on ear diameter. The treatments interaction also showed only significant differences between Control+P0 from one side and Control, PSB, PSB+P3, (PSB+Cyan.)+P2 and (PSB+Cyan.)+P3 from the other side.

Ear weight

Ear weight was 284.51 and 532.83 g/plant under Contrl+P0 and (PSB+Cyan.)+P3 treatments, respectively. The analysis of variance resulted in highly significant difference among ear weight under mineral phosphate applications, very highly difference under biofertilizer types and highly significant under the interaction of both mineral and biofertilizers. The LSD at 0.05 showed that the biofertilizers had more effect on ear weight than mineral phosphate fertilizer where all biofertilizer treatments gave significant differences between each other at 0.05. While in the case of mineral fertilizer the ear weight under both P2 and P3 were significant as compared to P0. The interaction of mineral and biofertilizer treatments showed distinct significant differences between groups of treatments promoted basically by the biofertilizers and their combination.

Generally, all examined growth parameters; plant height, ear length, ear diameter and ear weight had shown increment of their mean values (Fig. 1) due to the impact of biofertilizer types and mineral phosphate fertilizer applications. The biofertilizers provided the plant roots with plenty of available nutrients due to their solubilizing capacity releasing the fixed ions and consequently improve the vegetative plant growth.

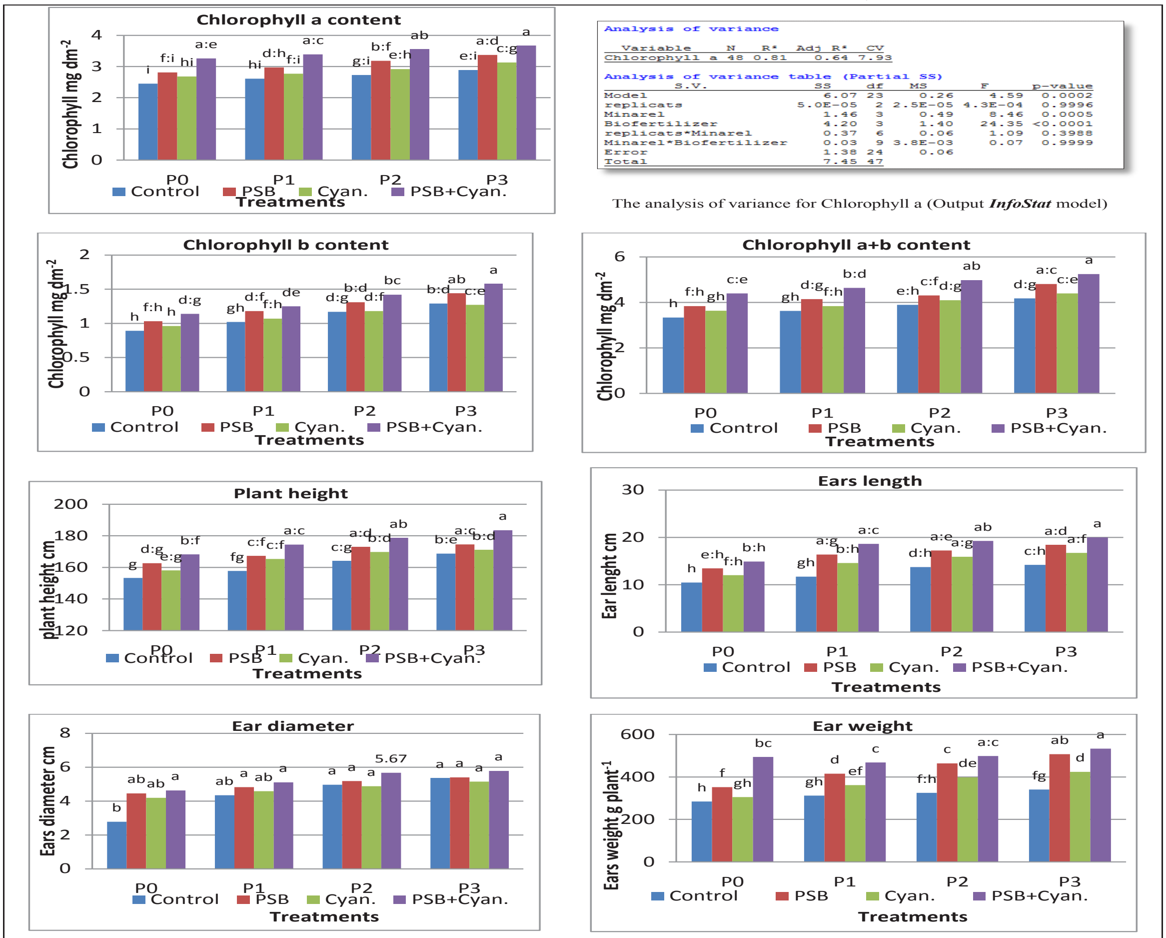
The analysis of variance revealed that plant height and ear weight/plant were highly significant under mineral phosphate fertilizer levels and very highly significant under biofertilizer types. The ear length was highly significant under biofertilizer types only while ear diameter was nonsignificant under both mineral and biofertilizers.

The obtained results could be in agreement with those of Iwuagwu et al. (2013) and El-Azab and El-Dewiny (2018) who found that the application of biofertilizer supported the maize roots and vegetation growth and improved the biological functions of the plant.

Yield parameters

Grain weight

The lowest maize grain weight was 113.57 g/plant obtained under Control+P0 treatment and the highest was 176.93 g/plant achieved under (PSB+Cyan.)+P3 treatment (Table 3), gaining an increase of about 56%. The analysis of variance revealed that grain weight was highly significant under mineral fertilizer application but very highly significant under biofertlizer types while nonsignificant under the interaction between both. The LSD at 0.05 showed that there were significant differences between the grain weights under mineral fertilizer doses. However, under Control and Cyan. treatments there were



Analysis of variance

Variable	N	R²	Adj R²	CV
Chlorophyll a	48	0.81	0.64	7.93

Analysis of variance table (Partial SS)

S.V.	SS	df	MS	F	p-value
Model	6.07	23	0.26	4.59	0.0002
replicats	5.0E-05	2	2.5E-05	4.3E-04	0.9996
Minarel	1.46	3	0.49	8.46	0.0005
Biofertilizer	4.20	3	1.40	24.35	<0.0001
replicats*Minarel	0.37	6	0.06	1.09	0.3988
Minarel*Biofertilizer	0.03	9	3.8E-03	0.07	0.9999
Error	1.38	24	0.06		
Total	7.45	47			

The analysis of variance for Chlorophyll a (Output *InfoStat* model)

Control = without bio fertilizer, P0= without phosphate fertilizer, P1= 10 kg P_2O_5 fed^{-1}, P2= 15 kg P_2O_5 fed^{-1}, P3=20 kg P_2O_5 fed^{-1}, PSB = phosphate solubilizing bacteria and Cyan.= cyanobacteria. Different lowercase letters within the same column indicate significant differences at 0.05.

Fig. 1. Maize growth parameters as influenced by mineral phosphate and biofertilizer

nonsignificant differences at 0.05 but significantly different with PSB and (PSB+Cyan.) treatments. The effect of both mineral phosphate and biofertilizers was significant between Control+P0 and (PSB+Cyan.)+P3 treatments.

100 Grain weight

One of the yield parameters is the weight of 100 grains which in the current experiment acquired almost 27% increment where it increased from 21.18 g under Control+P0 application to 26.98 g under the (PSB+Cyan.)+P3 treatment (Table 3). The analysis of variance showed that this parameter was only significant for biofertilizer. The LSD at 0.05 supported this result where the only significant difference for the effect of mineral fertilizer levels was obtained between P0 and P3 application. The same was obtained for biofertlizer types as the only significant difference was between Control and Cyan. from one side and the combination of PSB and Cyan. from the other side. On the other hand, interaction of mineral phosphate and biofertilizers gave significant difference of the 100 grain weight only between (PSB+Cyan.)+P3 and the other treatments which were not incorporated with either P3, PSB or (PSB+Cyan.).

Grain yield

The maize grain yield increased almost 86%where the minimum was 1.22 ton/fed. under Control+P0 treatment and the maximum was 2.27 ton/fed. under (PSB+Cyan.)+P3 treatment (Table 3). Analysis of variance declared that grain yield was very highly significant between mineral phosphate applications but highly significant with the biofertilizer types and nonsignificant difference with the interaction of both mineral and biofertilizers. LSD at 0.05 also showed that mineral phosphate promoted the grain yield where there were significant differences between its levels. However, Control had nonsignificant difference with Cyan. but was significant with both PSB and (PSB+Cyan.) types. The interaction of mineral and biofertilizer resulted in significant differences between the treatments incorporated with P0 and all other treatments which showed nonsignificant differences between them.

Straw yield

Straw yield (Table 3) recorded the least amount of 6.47 ton/fed. under Control+P0 treatment and reached the highest of 8.15 ton/fed. under (PSB+Cyan.)+P3 treatment, achieving an increase of about 26%. According to the analysis of variance, the straw yield was found highly significant between mineral levels while it was very highly significant between biofertilizer types indicating that biofertilizer was more effective than mineral phosphate for producing straw. The LSD at 0.05 of straw yield under mineral phosphate applications was nonsignificant between P0 and P1 as well as between P2 and P3 but significant between those two groups. Biofertilizer types had significant differences between Control and Cyan. as well as both PSB and (PSB+Cyan.) treatments which had nonsignificant differences. The interaction of mineral phosphate and biofertilizer resulted in gradual significant differences between all other treatments.

TABLE 3. Maize yield parameters as influenced by mineral phosphate fertilizer and biofertilizer

Treatments		Grain weight (g plant^{-1})	100- Grain weight (g)	Grain yield (Mg fed^{-1})	Straw yield (Mg fed^{-1})
Control	P0	113.57 k	21.18 c	1.22 f	6.47 g
	P1	117.45 jk	21.97 bc	1.47 d:f	6.86 fg
	P2	128.04 h:j	22.16 bc	1.64 c:e	7.24 ef
	P3	143.45 d:g	22.84 a:c	1.75 cd	7.48 c:f
PSB	P0	123.66 ik	22.93 a:c	1.26 f	7.42 d:f
	P1	136.88 e:h	23.71 a:c	1.69 cd	7.56 c:f
	P2	147.06 d:f	24.46 a:c	1.90 bc	8.20 a:c
	P3	168.60 ab	24.94 a:c	2.10 ab	8.68 fg
Cyan.	P0	116.43 jk	22.17 bc	1.24 f	6.88 fg
	P1	125.16 h:k	22.63 bc	1.62 c:e	7.15 e:g
	P2	135.10 g:i	22.86 a:c	1.75 cd	7.88 b:e
	P3	149.36 cd	23.36 a:c	1.87 bc	8.13 e:d
(PSB+ Cyan.)	P0	135.84 f:h	23.43 a:c	1.28 f	7.77 b:e
	P1	147.94 de	24.77 a:c	1.93 bc	8.33 ab
	P2	159.98 bc	25.65 ab	2.11 ab	8.72 a
	P3	176.93 a	26.98 a	2.27 a	8.15 a:d
LSD 0.05		12.01	4.25	0.31	0.75

Control = without bio fertilizer, P0= without phosphate fertilizer, P1= 10 kg P_2O_5 fed^{-1}, P2= 15 kg P_2O_5 fed^{-1}, P3=20 kg P_2O_5 fed^{-1}, PSB = phosphate solubilizing bacteria and Cyan.= cyanobacteria.
Different lowercase letters within the same column indicate significant differences at 0.05.

Thus the obtained results showed increasing of the yield parameter values as maize plants had undergone the different applied treatments. This increase could be attributed to the redundancy of the essential nutrients in the root zone as a result of the biological activity of the applied biofertilizers. It was clearly noticed that the effect of the phosphate solubilizing bacteria on yield exceeded that of cyanobacteria but their combination gave the highest yield. These results were in agreement with the findings of Hameeda et al. (2008) and Viruel et al. (2014) who stated that phosphate solubilizing bacteria (PSB) improves plant growth and crop yield. Also, Singh et al. (2013), Singh et al. (2014) and Abd EL-Kader (2018) declared that cyanobacteria are atmospheric nitrogen fixing microorganisms and therefore they improve the field crop yields as biofertilizers.

Nutrient uptake

N-uptake by grains

Table 4 showed that the minimum N uptake by grains was 22.92 kg/fed. recorded under Control+P0 treatment while the maximum was 48.58 kg/fed. detected under (PSB+Cyan.)+P3 treatment. Thus, the amount of N uptake by grain increased by about 112% of its value of control treatment. The analysis of variance clearly showed very highly significant effect of both mineral phosphate applications and biofertilizer types on N uptake by grain. However, the interaction of mineral phosphate levels and biofertilizer types was found nonsignificant. The LSD at 0.05 of N uptake by grain as affected by mineral phosphate applications resulted in significant differences between levels. While the biofertilizer types showed nonsignificant differences between Control and Cyan. treatments and between PSB and Cyan. treatments. However, (PSB+Cyan.) treatment was significant with all other treatments. The interaction between mineral phosphate fertilizer and biofertilizer types confirmed that high levels of mineral fertilizer promoted the significant differences between the experimental treatments. The P0 incorporation into various treatments created the most significant differences of N uptake with the other treatment. The (PSB+Cyan.)+P3 treatment had nonsignificant difference with PSB+P3 and (PSB+Cyan.)+P2 treatments but significant difference with all other treatments.

P-uptake by grains

The minimum P uptake by grains was 4.14 kg/fed. found under the Control+P0 treatment and the maximum amount was 12.03 kg/fed. achieved under the (PSB+Cyan.)+P3 treatment (Table 4). This increase, then, represented almost 192% of the corresponding value of the maize planted in the initial soil condition. The analysis of variance showed very highly significant P uptake by grain under both mineral phosphate fertilizer levels and biofertilizer types while the interaction between them resulted in nonsignificant differences. The LSD at 0.05 showed significant differences of P uptake by grain under mineral phosphate fertilizer levels. The Control and Cyan. gave nonsignificant differences of P uptake by grain while PSB and (PSB+Cyan.) showed significant differences between them and with other treatments. The interaction between mineral phosphate fertilizer and biofertilizer types showed that incorporation of levels of mineral fertilizer directed the significant differences between the experimental treatments. The P0 incorporation into various treatments showed the most significant differences of P uptake by the other treatment. The (PSB+Cyan.)+P3 treatment had nonsignificant difference with PSB+P2 treatment but was significantly different with all other treatments.

K-uptake by grains

The minimum K uptake by grains was 3.33 kg/fed. obtained under the Control+P0 treatment and the maximum amount was 9.91 kg/fed. detected under the (PSB+Cyan.)+P3 (Table 4). Consequently, K uptake by grain under mineral phosphate and biofertilizer applications was higher than under the control treatment of almost 198%. The analysis of variance revealed a very highly significant differences of K uptake by grain between the mineral phosphate fertilizer levels and highly significant differences between biofertilizer types. The LSD at 0.05 showed significantly differences of K uptake by grain under P0, P1 and both P2 and P3 mineral phosphate fertilizer applications. While it resulted in nonsignificant differences between K uptake by grain under Control, PSB and Cyan. types but significantly different under (PSB+Cyan.) treatment. As the interaction between mineral and biofertilizers is concerned, the mineral fertilizer levels was directing the significant differences between the experimental treatments. Incorporating P0 into the treatments made significant differences of K uptake by grain with the other treatments. The PSB+P3, (PSB+Cyan.)+P2 and (PSB+Cyan.)+P3 treatments had nonsignificant difference between each other but they were significantly different with all other treatments. Generally it could be concluded that K content in grain was less than P and N contents.

N-uptake by straw

The N uptake by straw was at minimum as 12.86 kg/fed. under Control+P0 treatment and at maximum as 31.09 kg/fed. under (PSB+Cyan.)+P3 treatment (Table 4), achieving an increase of nearly 142%. Based on the analysis of variance, there were very highly significant differences of N uptake by straw between the mineral phosphate fertilizer levels as well as between biofertilizer types but nonsignificant differences with the interaction of both. The LSD at 0.05 revealed nonsignificant differences of N uptake by straw under mineral phosphate fertilizer levels P2 and P3 but significantly different under P0, P1 and both P2 and P3. However, under biofertilizer types N uptake was nonsignificant between Control and Cyan. types as well as between PSB and (PSB+Cyan.) but significantly different between these two groups. The interaction between mineral and biofertilizer showed that N uptake was significantly different.

P-uptake by straw

The P uptake by straw was at minimum as 9.63 kg/fed. under Control+P0 treatment and at maximum as 24.60 kg/fed. under (PSB+Cyan.)+P3 treatment (Table 4) realizing an increase of about 155%. The analysis of variance showed very highly significant P uptake by grain under both mineral phosphate fertilizer levels and biofertilizer types while the interaction between them resulted in nonsignificant differences. The LSD at 0.05 revealed nonsignificant differences of P uptake by straw under mineral phosphate fertilizer levels P2 and P3 but significantly different under P0, P1 and both P2 and P3.It is worth to mention that it was the same response of N uptake by straw under mineral phosphate fertilizer levels. P uptake under biofertilizer types was nonsignificant between Control and Cyan. treatments as well as between PSB and Cyan. But (PSB+Cyan.) treatment was significantly different with all others. The interaction between mineral and biofertilizer showed that P uptake was significantly different.

K-uptake by straw

The K uptake by straw was at minimum as 84.10 kg/fed. under Control+P0 treatment and at maximum as 119.80 kg/fed. under (PSB+Cyan.)+P3 treatment (Table 4), with an increase of almost 42%. The analysis of variance showed very highly significant K uptake by grain under both mineral phosphate fertilizer levels and biofertilizer types while the interaction between them resulted in nonsignificant differences. The LSD at 0.05 of K uptake by straw revealed nonsignificant differences under mineral phosphate fertilizer levels P2 and P3 but significantly different under P0, P1 and both P2 and P3, showing the same trend of N and P uptake by straw. However, biofertilizer types effect on K uptake by straw presented completely different trend from those of N and P where the results gave significant differences between all types. While the interaction of mineral and biofertilizer treatments on K uptake by straw showed nonsignificant differences confirming that the treatments had similar effect. It was noticed that K content in straw exceeded both N and P contents.

Grain components

Carbohydrates

The grain carbohydrate content (Table 4) was ranging between 72.48 and 78.88 % under Control+P0 and (PSB+Cyan.)+P3 treatments, respectively gaining an increase of about 8.8%. The analysis of variance showed no significant differences of carbohydrate content under mineral phosphate fertilizer levels but high significant with biofertilizer types. LSD at 0.05 revealed that the significant effect of mineral phosphate fertilizer on carbohydrate content occurred only between P0 and P3levels. While the significant effect of biofertilizer types was noticed between Control, PSB and (PSB+Cyan.)+P3 treatments. The interaction of mineral fertilizer levels and biofertilizer type shad resulted in significant differences between carbohydrate content under P0, P1or P2 with either Control or Cyan. Treatments and under P2 and P3 with either PSB or (PSB+Cyan.) treatments.

Maize grain carbohydrate content was incrementally increased as the mineral phosphate fertilizer levels increased as well as applying biofertilizers and as applying the combination of mineral phosphate and biofertilizers. However, the carbohydrate content under PSB treatment exceeded the correspondent value under Cyan. treatment.

Protein

The grain protein content (Table 4) was ranging between 130.67 and 276.92 kg/fed. under Control+P0 and (PSB+Cyan.)+P3 treatments, respectively with an increase of almost 112%. The grain protein content was very highly significantly different under either mineral phosphate fertilizer levels or biofertilizer types and their combination according to the analysis of variance. The LSD at 0.05 indicated that there were significant differences of protein content under mineral phosphate fertilizer levels. However, there were nonsignificant differences of protein under Control and PSB treatments and under PSB and Cyan. treatments. On the other hand, (PSB+Cyan.) treatment was significant with all other treatments. Interaction of both mineral phosphate fertilizer levels and biofertilizer types showed that grain protein content was guided by mineral phosphate fertilizer levels where P0 level with biofertilizer types were significantly different from all other treatments.

It was clear that protein content followed the N uptake by grain trend which could be due to the fact that nitrogen is the main protein constituent. Moreover, the protein content under Cyan. treatment exceeded the correspondent value under PSB treatment which is mainly the opposite trend of other measured variables of the current work. Currently, the experiment results proofed that biofertilizers improved the grain components of maize crop planted in calcareous soil and overcame the essential nutrients deficiency. Also they showed that applying PSB+Cyan. combination with the high level of mineral phosphate fertilizer (P3) realized the highest grain components under the present experimental conditions.

Available nutrients in soil

Available N

Nitrogen availability in soil (Fig. 2) was increased by increasing the mineral phosphate fertilizer levels and biofertilizer types as well as their combinations under the current experimental conditions. It is worthy to mention that soil available N amount was higher under Cyan. type than under PSB which could be simply explained by the nitrogen fixation action of the cyanobacteria. The current experiment recorded a minimum available N in the soil at 11.98 mg kg^{-1} under Control+P0 treatment and maximum at 18.55 mg kg^{-1} under (PSB+Cyan.)+P3 treatment. Thus, the available N in soil was increased by about 55% due to the use of biofertilizers. According to the analysis of variance, available N amount in soil showed clear distinction variations between mineral phosphate fertilizer levels as well as between biofertilizer types. While the interaction of mineral and bio fertilizers resulted in nonsignificant differences of available N in soil. LSD at 0.05 confirmed significantly different amounts of available N in soil under both mineral phosphate fertilizer levels and biofertilizer types. The combination of different mineral fertilizer levels and biofertilizer types showed that P0 or P1 with Control or PSB treatments as well as P2 or P3 with Cyan. or (PSB+Cyan.) treatments were significantly different from the rest of the current experiment combinations.

TABLE 4. Maize grain and straw contents as influenced by mineral phosphate fertilizer and biofertilizer

Treatments		N-uptake (kg fed^{-1})		P-uptake (kg fed^{-1})		K-uptake (kg fed^{-1})		Grain component	
		Grain	Straw	Grain	Straw	Grain	Straw	Carbohydrate (%)	Protein (kg fed^{-1})
Control	P0	22.92 g	12.86 g	4.14 h	9.63 g	3.33 g	84.10 h	72.48 d	130.67 g
	P1	27.64 e:g	17.72 d:g	5.19 gh	12.15 fg	4.26 e:g	91.38 f:h	72.95 cd	157.53 e:g
	P2	31.68 de	20.36 c:e	6.38 fg	14.21 e:g	5.48 d:f	99.28 d:g	73.86 b:d	180.56 de
	P3	34.94 cd	23.14 bc	7.88 d:f	17.89 c:e	6.30 c:e	103.29 c:f	75.43 a:d	199.16 cd
PSB	P0	23.50 g	17.01 e:g	4.76 gh	11.99 fg	3.29 g	98.00 d:g	76.15 a:d	133.97 g
	P1	33.24 c:e	22.67 bc	7.60 d:f	15.86 d:f	4.88 e:g	103.60 c:f	76.25 a:d	176.05 d:f
	P2	38.80 bc	27.93 ab	9.15 b:d	19.68 b:d	6.59 cd	114.83 a:c	77.10 a:c	196.29 c:e
	P3	43.72 ab	30.36 a	10.53 ab	22.66 a:c	7.80 a:c	124.10 a	78.02 ab	215.72 b:d
Cyan.	P0	23.89 fg	14.59 fg	4.49 h	10.47 g	3.80 fg	90.24 gh	74.42 b:d	136.17 fg
	P1	30.89 d:f	19.34 c:f	6.45 fg	14.33 e:g	5.71 c:f	95.86 e:h	75.05 a:d	189.46 c:e
	P2	34.44 c:e	23.80 bc	7.31 ef	17.30 de	6.52 cd	109.68 b:d	75.68 a:d	221.19 bc
	P3	37.85 b:d	26.82 ab	8.75 b:e	18.71 b:e	7.08 b:d	113.86 a:c	76.06 a:d	249.19 ab
(PSB+ Cyan.)	P0	24.48 fg	18.69 c:f	5.02 gh	14.02 e:g	4.11 fg	104.08 c:e	76.72 a:c	139.56 fg
	P1	39.63 bc	27.53 ab	8.35 c:e	19.97 a:d	6.75 b:d	117.46 ab	77.07 a:c	225.87 bc
	P2	44.42 ab	31.45 a	9.89 bc	23.49 ab	8.72 ab	126.43 a	77.78 ab	253.21 ab
	P3	48.58 a	31.09 a	12.03 a	24.60 a	9.91 a	119.80 ab	78.88 a	276.92 a
LSD 0.05		7.07	5.58	1.81	4.91	2.13	12.59	4.19	40.31

Control = without bio fertilizer, P0= without phosphate fertilizer, P1= 10 kg P_2O_5 fed^{-1}, P2= 15 kg P_2O_5 fed^{-1}, P3=20 kg P_2O_5 fed^{-1}, PSB = phosphate solubilizing bacteria and Cyan.= cyanobacteria.

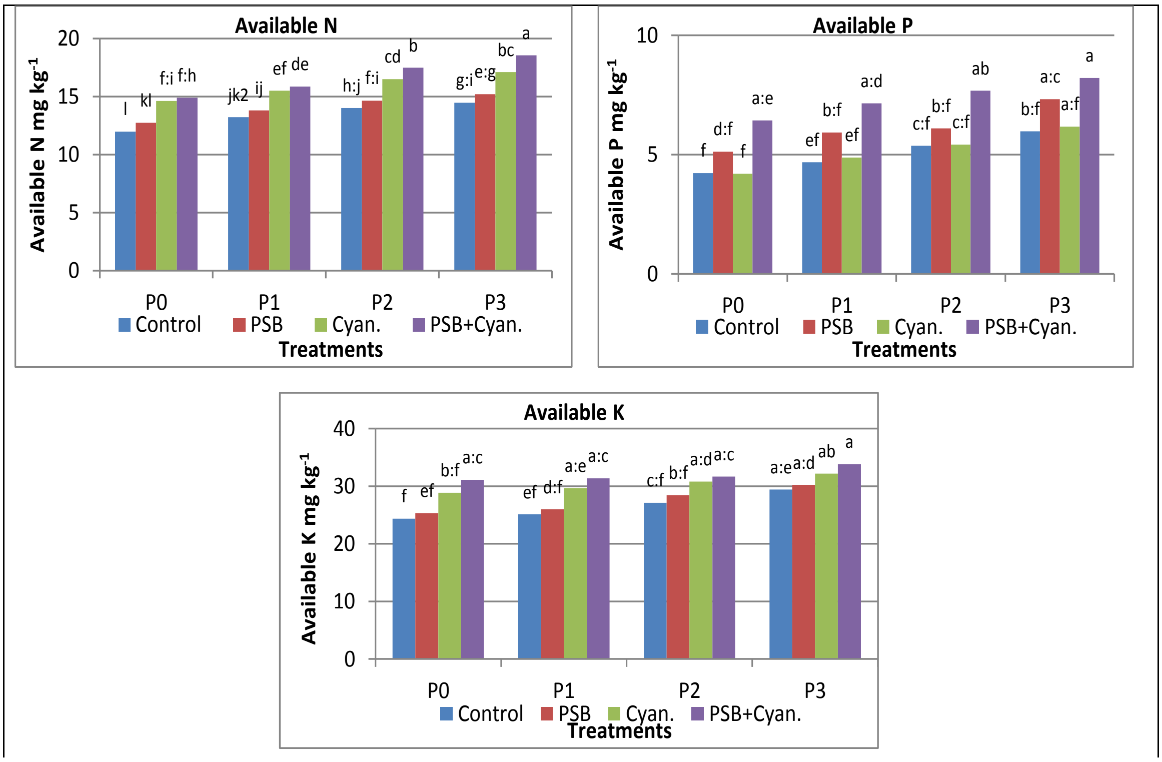
Different lowercase letters within the same column indicate significant differences at 0.05.

Available P

Considering that the current experiment was implemented in calcareous soil, it obviously revealed gradual increment of available P in soil with the increase of applied phosphate fertilizer levels and /or applied biofertlizer types. The obtained results (Fig. 2) showed that the minimum available P in the soil was at 4.23 mg kg^{-1} under Control+P0 treatment and maximum at 8.22 mg kg^{-1} under (PSB+Cyan.)+P3 treatment, achieving an increase of almost 94%. However, the available P in the soil was lower under Cyan. biofertilizer type than under PSB or PSB+Cyan. types. Analysis of variance results indicated highly significant amounts of available P in the soil under both mineral phosphate fertilizer levels and biofertlizer types. LSD at 0.05 revealed that the effect of mineral phosphate fertilizer levels on available P in soil was significantly different from P0 under P2 and P3 but nonsignificant between P0 and P1. The Control, PSB and (PSB+Cyan.) biofertilizer types had significant different effect on available P in soil. However, Control and Cyan. treatments showed nonsignificant difference of their resultant available P in soil. The interaction between both mineral fertilizer levels and biofertilizer types revealed that the (PSB+Cyan.)+P3 combination resulted in significant different effect on available P in soil from all other combinations.

Available K

The minimum amount of available K in soil was 24.37 mg kg^{-1} under Control+P0 treatment and maximum at 33.83 mg kg^{-1} under (PSB+Cyan.)+P3 treatment (Fig. 2) gaining an increase of about 39%. The analysis of variance r showed that mineral phosphate fertilizer levels affected significantly the available K in soil but under biofertilizer types it was highly significant. However, the interaction of mineral phosphate fertilizer levels with biofertilizer types had resulted in nonsignificant available K amount in soil. LSD at 0.05 revealed that available K amount in soil under P0, P1 and P2 treatments was nonsignificant as well as under P2 and P3 while under P3 was significantly different from that under P0 and P1. Available K amount in soil was nonsignificant under Control and PSB type group as well as under Cyan. and (PSB+Cyan.) type group but significant between the two groups.



Control = without bio fertilizer, P0= without phosphate fertilizer, P1= 10 kg P_2O_5 fed^{-1}, P2= 15 kg P_2O_5 fed^{-1}, P3=20 kg P_2O_5 fed^{-1}, PSB = phosphate solubilizing bacteria and Cyan.= cyanobacteria. Different lowercase letters within the same column indicate significant differences at 0.05.

Fig. 2. Available N, P and K in soil as influenced by mineral phosphate fertilizer and biofertilizer at the end of the experiment

Available nutrients in soil

Generally, the soil contents of available nutrients; NPK increased at the end of the experiment as the soil was influenced by the biofertilizer applications.

The data in Fig. 2 indicated that biofertilizers represented by cyanobacteria caused an increase in soil available N more than that under PSB however, the combination of both gave higher amount. On the other hand, PSB increased the soil available P more than that under Cyan., but the combination of both biofertilizers achieved higher content. Concerning the soil available K, the obtained results referred to higher content under Cyan. than PSB while the combination of both had resulted in the highest. Ali et al. (2019) and WU et al. (2019) got similar results as they stated that available of N, P and K was significantly ($P<0.05$) increased in soil due to biofertilizers treatments when compared to the untreated one.

Conclusion

The use of phosphate solubilizing bacteria and cyanobacteria as biofertilizers in calcareous soil substantially significantly improved both maize plant growth and yield. They consistently increased the nutrient uptake by grain and straw as well as the carbohydrate and protein contents in grain as compared to the corresponding amounts of the control treatment. Moreover, the available nutrients; NPK in the calcareous soil increased due to applying biofertilizers. Further investigations would be carried out by applying biofertilizers in the presence of elemental sulphur as amendment for calcareous and alkaline soils to improve the soil physicochemical properties.

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تأثير الأسمدة الحيوية على نمو وإنتاجية الذرة (*Zea mays* L.) تحت ظروف التربة الجيرية

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يعتبر التوسع الزراعي في مصر ذو أهمية كبيرة لمواجهة النقص في إنتاج محاصيل الحبوب. وحديثا تم استخدام الأسمدة الحيوية للتغلب على نقص بعض العناصر الغذائية بسبب تثبيتها في التربة خاصة في الأراضي الجيرية وبالتالي زيادة خصوبتها.

يهدف العمل الحالي إلى تقييم استخدام البكتيريا المذيبة للفوسفات والبكتيريا المثبتة للأزوت في إنتاج الذرة تحت ظروف التربة الجيرية. وكانت النتائج المتحصل عليها من التجربة الحقلية التي أجريت على مدى عامين واعدة. حيث زاد تجميع مدلولات النمو المتمثلة في محتوى الأوراق من الكلوروفيل وطول النبات وطول وقطرو وزن الكوز زيادة معنوية. وكذلك تمت زيادة مدلولات المحصول المتضمنة وزن الحبوب ووزن 100 حبة ومحصول الحبوب ومحصول القش بنسبة 56 و 27 و 86 و 26٪ على التوالي. ولقد فاق تأثير البكتيريا المذيبة للفوسفات على تأثير البكتيريا المثبتة للأزوت فيما يخص كمية المحصول. بينما كان تأثير مزيجهما هو الأعلى. كما ارتفعت كمية العناصر الغذائية في الحبوب والقش. على السواء. بشكل ملحوظ حيث أظهرت النتائج زيادة امتصاص الحبوب للنيتروجين والفوسفور والبوتاسيوم بنحو 112 و 192 و 198٪ على التوالي عن القيم المماثلة لمعاملة الكنترول. وأيضا زيادة محتوى الحبوب لكل امن الكربوهيدرات والبروتين بنسبة 8.8٪ و 112٪ على التوالي مقارنة بنتائج معاملة الكنترول. وكان محتوى الكربوهيدرات تحت تأثير البكتيريا المذيبة للفوسفات أعلى من قيمته تحت تأثير البكتريا المثبتة للأزوت بينما أظهر محتوى البروتين العكس من ذلك. كذلك أرتفع محتوى التربة من النيتروجين والفوسفور والبوتاسيوم الصالح بالنسب 55 و 94 و 39٪ على التوالي في نهاية التجربة.