J Agric. Res., 2021, Vol. 59(3):287-293
www.jar.com.pk
Agriculture Department
Government of Punjab

AGRONOMY

PHOSPHORUS SOLUBILIZING BACTERIA (PSB) IN COMBINATION WITH DIFFERENT FERTILIZER SOURCES ENHANCE YIELD PERFORM ANCE OF CHICKPEA (*CICER ARIETINUM* L.)

Nawal Zafar * [1], Siraj Ahmed [2], Muhammad Kashif Munir [3], Muhammad Saqib [4], Muhammad Zafar [5], Babar Hussain Babar [6], Tariq Mahmood [7] and Fayyaz Ahmed [8]

ABSTRACT

A two-year (2018-19 and 2019-20) field trial was conducted at research area of Cereals and Pulses section, Agronomic Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan to appraise the effect of seed treatment and nutrient sources on yield and yield components of chickpea. Trial was laid out in a randomized complete block design (RCBD) and replicated thrice. Treatments as included were (T_1) NPK @ 32:85:0 kg/ha (recommended), (T_2) seed treatment with PSB + recommended dose of synthetic fertilizer, (T_3) farm yard manure @ 3.58 t/ha, (T_4) seed treatment with PSB + farm yard manure @ 3.5 t/ha, (T_5) FYM @ 3.5 t/ha remaining P from SSP. Data regarding yield and yield contributing factors were recorded by using standard procedures and were analyzed statistically using fisher's technique of analysis of variance and treatment means were compared by using least significant difference (LSD) test at 5% level of probability. The results revealed that seed inoculation in T_2 significantly enhanced the yield by 17.55% (average of both years) over recommended NPK. Seed inoculation with PSB + FYM @ 3.5 t/ha (T_3) enhanced the yield by 16.97% (average of both years). Seed treatment with PSB + recommended NPK resulted in significant increase in number of pods per plant, number of grains per pod and 100- seed weight by 11.58, 12.35 and 10.15%, respectively over recommended NPK. While seed treatment with PSB+FYM @ 3.5 t/ha resulted in 10.58, 13.36 and 7.87% increase in number of pods per plant, number of grains per pod and 100- seed weight, respectively over FYM @ 3.5 t/ha alone. However, application of FYM @ 3.5 t/ha and remaining P from SSP did not shown response as much as did PSB with synthetic and organic fertilizer source. It was found that seed treatment using PSB along with application of synthetic fertilizer or FYM at the time of sowing enhances yield and yield contributing factors of chickpea.

KEYWORDS: *Cicer arietinum*; farm yard manure; inoculant; PSB; seed treatment; fertilizer; Pakistan

[1,4,6] Scientific Officer, [2] Research Associate, [3,8] Senior Scientist, [5] Principal Scientist, Cereals and Pulses Section, [7] Principal Scientist, Vegetable and Oilseed Section, Agronomic Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan

*Corresponding author's email: nawzaf20@gmail.com

Article received on: 27/11/2020
Accepted for publication: 16/03/2021

Copyright: @ 2021 by authors

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop of rabi season. It is a widely grown edible grain legume with high protein contents; hence it is also referred to as "the peoples' protein" (Namvar and Sharifi, 2011). During 2018-19 chickpea was grown on an area of 943 thousand hectares with total production of 447 thousand tonnes and average production of 0.474 tonnes per hectare (GoP, 2019). In Pakistan, this crop is usually grown on marginal lands and unlike other major field crops, no proper attention is given to its proper nutrient management. Several types of organic materials have been reported to be added as source of suitable soil amendments and as an alternate mean for soil fertilization which in turn increase crop production and improve nutrient availability and uptake from the soil (Makinde and Ayoola, 2010). Among different fertilizer sources, microbial inoculants have engrossed attention due to their eco-friendliness and cost effectiveness by decreasing consumption of unrenewable inputs from various resources all over the world (Gopalakrishnan *et al.,* 2015).

Phosphorus (P) is an important macronutrient required by the plants. Plant use P during ATP synthesis, cell division and in cell membranes. Its deficiency in plants causes stunted growth and causes leaves to turn purple. The status of soil P is influenced by a number of factors which include soil type, temperature and

other environmental conditions as well as several crop management practices (Funga *et al.,* 2016). Most of the P becomes insoluble as it is adsorbed by soil particles. Only 10-20% of the added P is available to plants, whereas remaining P remains insoluble in the form of tri-calcium phosphate and as rock phosphate in soil (Gupta *et al.,* 2014). When P_2O_5 containing fertilizers are added in calcareous and alkaline soils they are converted into tri-calcium phosphate and in acidic soil they form ferric hydroxyl phosphate, aluminium phosphate or ferrous phosphate.

Biofertilizers play a significant role in minimizing the use of synthetic fertilizer by fixing atmospheric nitrogen and increasing P availability to the crop (Selvakumar *et al.,* 2012). They show a vital and positive effect on crop yield and nutrient uptake by the plant from the soil. The activity of such inoculants is influenced by different factors such as climate, soil biodiversity, soil fertility and soil carbon content. Phosphorus solubilizing bacteria (PSB) assist in the release of inorganic P and enhance its availability to growing plants. PSB being a group of bacteria enhance the organic and inorganic P from insoluble compounds after hydrolyzing. PSB release organic acids having low molecular weight which chelate the cations bond to phosphate through their hydroxyl and carboxyl groups and thus convert inorganic P into available P. Bacteria belonging to *Bacillus, Rhizobium* and *Pseudomonas* genera have enhanced role in phosphorus solubilisation (Gupta *et al.,* 2014). Panhwar *et al.* (2012) suggested the use of PSBs in crop production as they have potential to manage phosphorus in P-Deficient soils. Role of PSB has been well reported in enhancing P availability to plants and it has been tested in many crops but a significant knowledge is still lacking for the use of PSB for treating chickpea. The aim of this experiment was to examine the role of PSB in combination with inorganic and organic nutrient sources on yield and yield components of chickpea.

MATERIALS AND METHODS

This study was conducted at research area of Cereals and Pulses Section, Agronomic Research Institute, Faisalabad during rabi season 2018-19 and 2019-20. The experiment was designed in randomized complete block design with 3 replications. The net plot size was 9 m x 3 m. The study was comprised of five treatments in which microbial inoculant Phosphorus Solubilizing Bacteria was tested under the same input level of synthetic fertilizer (Urea, SSP 18%) and organic fertilizers (farm yard manure @ 3.5 t/ha) along with the non-inoculated control.

Treatment plan was considered as under:-

T_1 = NPK @ 32:85:0 kg/ha (recommended)

T_2 = Seed treatment with PSB + recommended dose of synthetic fertilizer

T_3 = Farm yard manure @ 3.5 t/ha

T_4 = Seed treatment with PSB + farm yard manure @ 3.5 t/ha

T_5 = FYM @ 3.5 t/ha remaining P from SSP

Chickpea variety Noor-2013 was sown maintaining R-R and P-P distance of 60 cm and 15 cm, respectively. Soil was analyzed for its various physiochemical properties (Table 1). Seedbed was prepared by cultivating the soil twice upto the depth of 12 cm followed by one planking. Kabuli chickpea cultivar Noor-2013 was used @ 75 kg/ha. Seed was treated with fungicide before sowing. In treatments where bio-fertilizer was to be applied, seed was treated @ 4 packets phosphorus solubilizing bacteria inoculant per 10 kg seed prior to sowing. Farm yard manure @ 3.5 t/ha was incorporated in the field 20 days before sowing after getting it analyzed for NPK (0.96%, 0.54% and 0.8%, respectively). Whole fertilizer dose was applied at sowing. First irrigation was applied 50 days after sowing. Hand weeding was done twice (at 30 and 70 days after sowing) to keep the crop weed-free. Crop was maintained following other standard agronomic practices.

Prior to harvesting the crop, at maturity data regarding yield contributing parameters i.e. plant height, No. of branches, number of pods per plant and number of grains per pod of 10 randomly selected plants from each experimental unit plot were recorded. 100-grain weight was recorded as well using a digital balance. The crop was then harvested, tied into bundles and sun dried properly before threshing it manually. Grain yield was calculated separately from each plot and was converted into t/ha. The data thus collected were analyzed using fisher's analysis of variance technique and the difference among treatment means was compared using least significant difference (LSD) test at 5% probability level (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

Chickpea crop performed better during 2019-20 than 2018-19. The better performance of chickpea during 2019-20 can partly be attributed to improved physio-chemical conditions and nutrient status of the experimental soil (Table 1).

Table 1. Pre - sowing soil analyses of the experimental site (0-15 cm)

Soil character	2018-19	2019-20
EC (mS/cm)	1.49	1.50
Soil pH	7.42	7.35
Organic matter (%)	0.63	0.73
Available N (%)	0.03	0.05
Available P (ppm)	8.7	9.1
Available K (ppm)	220	240
Saturation	32	33
Texture	Sandy loam	Sandy loam

Plant population of chickpea during both years of study was found non-significant. Different nutrient sources exerted significant influence on number of branches per plant in the first year (2018-19) however; this effect was non-significant during second year of study. Different nutrient sources exerted a significant influence on plant height. Seed treatment with phosphorus solubilizing bacteria (PSB) and adding recommended dose of synthetic fertilizer to the soil resulted in significant increase in plant height (PH) during both years and branches per plant (BPP) in first year of study (2018-19). Slightly less response of yield and BPP was observed when seed was treated with PSB + 3.5 t/ha FYM. Adding of FYM @ 3.5 t/ha either alone or with SSP reduced the plant height in comparison to control (Table 2 & Fig. 1, 2). Moreover, association of these parameters among themselves was found non-significant during both years of study (Tables 3 & 4).

Different nutrient sources significantly affected the pods per plant (PPP), grains per pod (GPP), 100- seed weight (TW) and grain yield (GY). In comparison to control (sole application of recommend fertilizer), seed treatment with PSB + recommended dose of synthetic fertilizer significantly increased PPP, GPP, TW and GY by 17, 13, 11 and 15%, respectively during 2018-19 and 7, 5, 9 and 19%, respectively during year 2019-20. Likewise, in comparison to sole application of FYM, seed treatment with PSB + addition of FYM @ 3.5 t/ha increased the afore mentioned parameters by 9, 17, 9 and 17%, respectively during 2018-19 and 18, 4, 6 and 17% during 2019-20. The addition of 3.5 t ha^{-1} FYM and remaining P from SSP did not result in any significant increase in these attributes. Moreover, association of these attributes among themselves was found significant during year 2018-19, while association of TW with GY was non-significant during 2019-20 (Tables 3 & 4).

Plant population was not influenced by seed treatment or application of different nutrient sources as the sowing was done at proper R x R and P x P distance. Plant height was significantly affected by nutrient sources during both the years. Treatments where PSB was applied showed a remarkable increase in all recorded parameters over other treatments (Table 2 & Fig. 1, 2). This can indirectly be attributed to the positive and admirable effects of PSB on soil and plant. Application of PSB in soil enhanced the bioavailability of phosphorus (P) and activity of several enzymes; thus in turn influence the physico-chemical properties of soil. Shi *et al.* (2017) found that application of PSB alone in soil enhanced the concentration and activity of several enzymes viz. urease, invertase and phosphatase by 2.32, 1.31 and 1.18-fold. Application of PSB has also been reported to decrease the soil's capacity to adsorb P. Moreover, enhanced concentration and availability of Olsen-P and other inorganic P forms like Ca_2-P, Ca_8-P, Ca_{10}-P, Fe-P, Al-P and occluded-P was also found where PSB was applied along with inorganic P source (tri calcium phosphorus (TCP). PSB also blocked the conversion of Olsen-P to Ca_{10}-P to increase its accessibility to plant. Hu *et al.* (2012) reported that PSB can add to the available phosphorous pool of the soil by transforming the unavailable P to its available form; thereby increasing its levels and ultimately improving the crop performance. P being a macro-nutrient plays a critical role in root and shoot growth of plants and of ATP, RNA, DNA and phospholipids. Increase in PH and BPP of chickpea in our study are a result of the critical role of PSB in enhancing P concentration and availability in soil when PSB was applied with inorganic fertilizers and FYM (Table 2 & Fig. 1, 2) which was an organic source of nutrients.

Table 2. Analysis of Variance (ANOVA) table of yield and yield contributing factors of chickpea

SOV	d.f	PH (cm)		PP/m²		BPP		GPP	
		2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
Treatments	4	67.83**	70.77**	0.77 ^{NS}	0.93^{NS}	0.077**	0.146^{NS}	0.034**	0.111**
Replication	2	9.8	2.87	0.47	0.80	0.008	0.041	0.004	0.051
Error	8	1.89	8.87	0.97	0.88	0.009	0.069	0.003	0.011
Total	14								
				PPP		TW		GY (Kg/ha)	
				2018-19	2019-20	2018-19	2019-20	2018-19	2018-19
Treatments	4			20.27**	10.57**	2.36**	1.58*	85871**	85871**
Replication	2			0.87	0.20	0.13	0.22	26699	26699
Error	8			1.37	1.37	0.082	0.27	11403	11403
Total	14								

Note: SOV= Source of Verification, d.f= degree of freedom, *= Significant (p≤0.05), **= Significant (p≤0.01), NS= Non-significant,

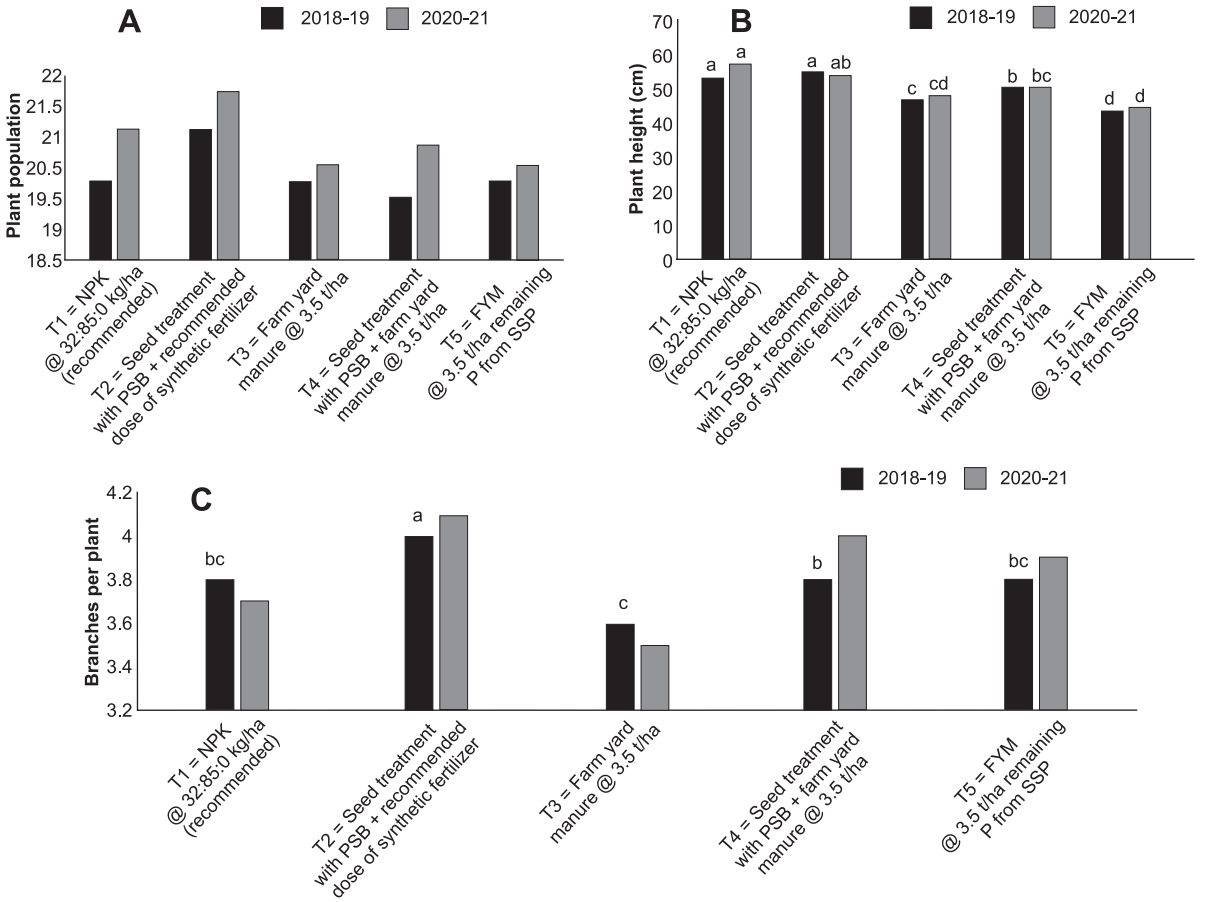


Fig. 1. Effect of different nutrients sources on (A) plant population/m2, (B) plant height (cm) and (C) number of branches per plant of chickpea

Table 3. Correlation (Pearson) analysis of different recorded parameters of chickpea showing strength of association during 2018-19

	BPP	GPP	PH	PP	PPP	TW
GPP	0.88*	*				
PH	0.61 NS	0.51 NS	*			
PP	0.71 NS	0.66 NS	0.49 NS	*		
PPP	0.95*	0.96 **	0.44 NS	0.62 NS	*	
TW	0.87 NS	0.97**	0.51 NS	0.49 NS	0.97**	*
G.Y	0.95*	0.88*	0.80 NS	0.66 NS	0.88*	0.88*

* = Significant (p≤0.05), ** = Significant (p ≤ 0.01), NS = Non-significant, BPP = Branches per plant, GPP = Grains per pod, PH= Plant height, PP = Plant population, PPP = Pod per plant, TW = Test-weight (100-grain weight)

Table 4. Correlation (Pearson) analysis of different recorded parameters of chickpea showing strength of association during 2019-20

	BPP	GPP	PH	PP	PPP	TW
GPP	0.67 NS	*				
PH	0.15 NS	0.82 NS	*			
PP	0.57 NS	0.95*	0.76 NS	*		
PPP	0.50 NS	0.92*	0.81 NS	0.95*	*	
TW	0.91*	0.69 NS	0.18 NS	0.70 NS	0.67 NS	
G.Y	0.76 NS	0.95*	0.63 NS	0.96**	0.93*	0.86 NS

* = Significant (p ≤ 0.05), ** = Significant (p ≤ 0.01), NS = Non-significant, BPP = Branches per plant, GPP = Grains per pod, PH = Plant height, PP = Plant population, PPP = Pod per plant, TW = Test-weight (100-grain weight)

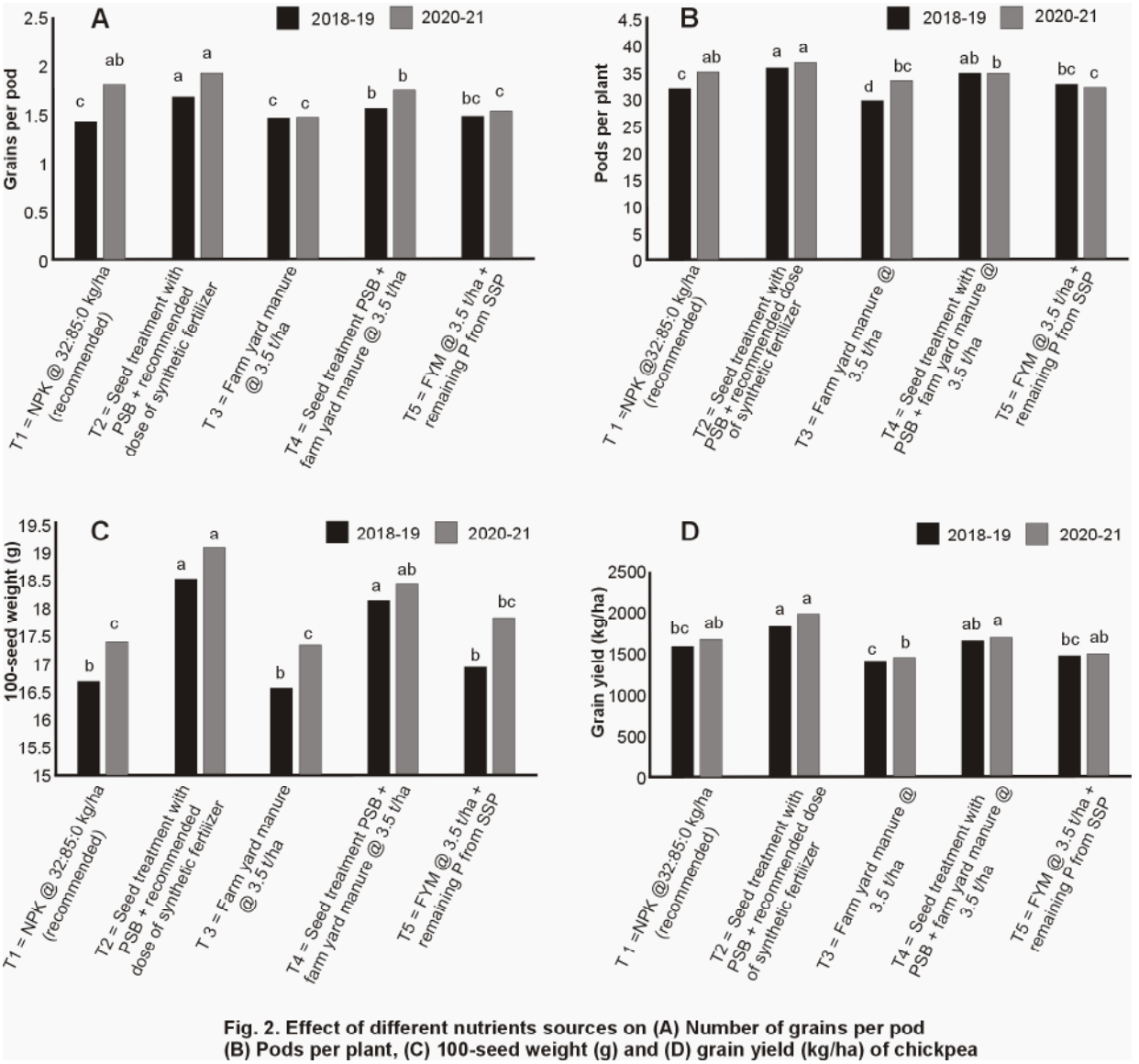


Fig. 2. Effect of different nutrients sources on (A) Number of grains per pod (B) Pods per plant, (C) 100-seed weight (g) and (D) grain yield (kg/ha) of chickpea

PSB are able to synthesize indole acetic acid and gibberellic acid which are phytohormones (Ramkumar and Kannapiran, 2011) and are responsible for cell elongation, leaf expansion, apical dominance, vascular development, flower initiation and fruit formation in plants (Wang *et al.,* 2001; Yamaguchi, 2008). Moreover, gibberellic acid improves the nutrient use efficiency, photosynthetic efficiency, light interception, leaf area index, growth and developmental processes of the plants. More number of PPP, GPP, TW and GY were enhanced in the study where PSB was applied with synthetic fertilizer and FYM; these findings are thus in line with the aforementioned indirect role of phosphorus (Table 2 & Fig. 1, 2). Singh *et al.* (2018) found that an ample supply of P to crops accelerates root development, nodulation and pod development. Legumes need an ample amount of P for development and better performance, therefore the addition of P lead to better crop performance regarding yield. In our study, addition of nutrients either from synthetic or inorganic source along with PSB enhanced the chickpea performance. Yield of chickpea showed a remarkable increase when PSB was applied with synthetic and organic nutrient source (Table 2 & Fig. 1, 2) which might be attributed to the increase of yield contributing factors i.e. PPP, GPP and TW. Yasmin and Bano (2011) reported the role of PSBs in enhancing growth, yield and quality of crops and reduction in use of synthetic and organic fertilizers. Moreover, Ponmurugan and Gopi (2006) also reported the role of PSBs in improving crop the growth by enhancing biological nitrogen fixation and micronutrients such as

Iron and Zinc availability. A strong correlation was found between yield and yield contributing factors during both year of study except in test weight (100- seed weight) with grain yield where correlation was non-significant during second year of study (Tables 3, 4).

CONCLUSION

In accordance with the above results it was inferred that PSB should be applied with recommended dose of fertilizer for better and enhanced yield. PSB can also be added with organic fertilizer for better yield while producing organically.

REFERENCES

Funga, A., O. Chris, O.C. Ojiewo, L. Turoop, S.G. Githiri and S. Mwangi. 2016. Symbiotic effectiveness of elite rhizobia strains nodulating Desi type chickpea (*Cicer arietinum* L.) varieties. J. Plant Sci. 4(4): 88-94.

Gopalakrishnan, S., V. Srinivas, G. Alekhya, B. Prakash, H. Kudapa, A. Rathore and R.K. Varshney. 2015. The extent of grain yield and plant growth enhancement by plant growth-promoting broad-spectrum *Streptomyces* sp. in chickpea**.** Springer Plus. 31(4):228-233.

GoP. 2019. Pakistan Economic Survey: Agriculture. Islamabad, Pakistan.

Gupta, G.N., S. Srivastava, S.K. Khare and V. Prakash. 2014. Role of phosphate solubilizing bacteria in crop growth and disease management. J. Pure Appl. Micro. 8(1):461-474.

Hu, X.F., Y.S. He and N. Yue. 2012. Effects of different phosphate solubilizing bacteria bio-fertilizers on growth of maize seedling and available phosphorus concentration in soil. Hunan Agric. Sci. 42:74–77.

Makinde, E.A. and C.T. Ayoola. 2010. Growth, yield and NPK uptake by maize with complementary organic and inorganic fertilizers. African J. Food Agric. Nutr. Develop. 10:1-15.

Namvar, A. and R.S. Sharifi. 2011. Phenological and morphological response of chickpea (*Cicer arietinum* L.) to symbiotic and mineral nitrogen fertilization. Zemdirbyste. Agric. 98(2):121-130.

Panhwar, Q.A., R. Othman, Z.A. Rahman, S. Meon and M. Ismail. 2012. Isolation and characterization of phosphate solubilizing bacteria from aerobic rice. African J. Biotech. 11 :2711-2719.

Ponmurugan, P.C. and C. Gopi. 2006. In vitro production of growth regulators and phosphates activity by phosphate solubilizing bacteria. African J. Biotech. 5:348-350.

Ramkumar, V.S. and E. Kannapiran. 2011. Isolation of total heterotrophic bacteria and phosphate solubilizing bacteria and in vitro study of phosphatase activity and production of phytohormones by PSB. Arch. Appl. Sci. Res. 3:581-586.

Selvakumar, G., S. Reetha and P. Thamizhiniyan. 2012. Response of biofertilizers on growth, yield attributes and associated protein profiling changes of blackgram (*Vigna mungo* L. Hepper). World appli. Sci. J. 16(10):1368-1374.

Shi, X.K., J.J. Ma and L.J. Liu. 2017. Effects of phosphate-solubilizing bacteria application on soil phosphorus availability in coal mining subsidence area in Shanxi. J. Plant Interact. 12(1): 137–142.

Singh, R., T.P.D. Singh, G. Singh and A.K. Singh. 2018. Effect of phosphorus, Sulphur and biofertilizers on growth attributes and yield of chickpea (*Cicer arietinum* L.). J. Pharmacogn. Phytochem. 7(2):3871-3878.

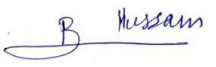
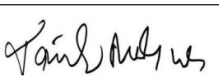
Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Edn. McGraw Hill Book Co. Inc. New York.

Wang, Y., S. Mopper and K.H. Hasentein. 2001. Effects of salinity on endogenous ABA, IAA, JA, and SA in Iris hexagona. J. Chem. Ecol., 27:327–342.

Yamaguchi, S. 2008. Gibberellin metabolism and its regulation. Annu. Rev. Plant Physiol. 59:225–251.

Yasmin, H. and A. Bano. 2011. Isolation and characterization of phosphate solublizing bacteria from Rhizosphere Soil of Khewra Salt Range and Attock. Pak. J. Bot. 43:1663-1668.

CONTRIBUTION OF AUTHORS

Sr. No.	Author's name	Contribution	Signature
1	Nawal Zafar	Conducted the experiment and wrote the manuscript	
2	Siraj Ahmed	Statistically analysed the data	
3	Muhammad Kashif Munir	Supervised the experiment	
4	Muhammad Saqib	Helped in data collection	
5	Muhammad Zafar	Reviewed the manuscript	
6	Babar Hussain Babar	Assisted in on farm management practices of research experiment	
7	Tariq Mahmood	Proof read the manuscript	
8	Fayyaz Ahmed	Assisted in results and discussion writeup	