

Original Research Article

Influence of different doses and methods of Phosphorous Solubilizing Bacteria and phosphorus levels on the Phosphorus solubilizing bacteria population and phosphorous use efficiency in sunflower and chickpea

Comment [P1]: We advise authors to improve the title of the article, there are too many words repeated unnecessarily.

ABSTRACT

A field experiment was conducted to study the effect of different doses and method of phosphate solubilizing bacteria (PSB) application and the phosphorus levels on sunflower during Rabi, 2020 at College of Agriculture, PJTSAU, Rajendranagar, Hyderabad. The experiment was laid out in Randomized Block Design, comprising eleven treatments with three replications. Initial soil parameters of experimental site indicated that the soil belongs to sandy loam texture, with alkaline in soil reaction, non-saline, low in O.C, low in available nitrogen, medium in available phosphorus, available potassium and available sulphur. The results showed that due to addition of PSB in powdered and liquid form there was an increase in PSB population in the soil, the increase in population is with the application of 75% phosphorus + PSB @ 6 kg/ha in both crops. The increase in population is more with the soil application than the Drenching. Due to this there was an increase in the phosphorous use efficiency also. The above study indicated that combined application of PSB with reduced P levels could help in saving fertilizer dose to the extent of 25% in sunflower and 50% in chickpea.

Comment [P2]: The effect of the application method and different doses

Key words : PSB population, phosphorous use efficiency, Soil application , Drenching

INTRODUCTION

Sunflower (*Helianthus annus* L.) belongs to the family *Asteraceae* and is characterized by considerable decor ability, and colour of the flower from cream to yellow. It is a potential source of high quality edible oil, ranges second next to soybean as an oil crop in the world (FAO). As of January 2016, the total world area under sunflower was 24.7 m ha with an average yield of 1.67 tones per ha (NSA, 2016). Sunflower seed contain 48 - 52 % of good quality edible

oil. The global sunflower seed and oil production in the year 2018-19 was estimated at 51.41 mt and 19.45 mt. In India during *Rabi* 2018-19 sunflower crop has occupied an area of 1.145 lakh hectares and in Telangana sunflower crop covers 0.020 lakh hectare or 2030 hectare. In Telangana Siddipet (1226 ha) is the major sunflower producing district.

Chickpea (*Cicer arietinum* L.) is a multipurpose pulse crop consumed by the people in different forms. Chickpea is one of the major *rabi* pulse crop. Among, the pulses chickpea is known as “King of pulses”. In India, it occupies about 9.18 million hectare area with production of 8.22 million tones and an average productivity of 900 kg ha⁻¹. In India 2017-18, chickpea was cultivated in about 106 lakh hectare with productivity of 1056 kg ha⁻¹. In Telangana the area contributed for chickpea cultivation was 1.03 lakh hectare and production of 1.50 lakh tones.

Phosphorus positively affects the sunflower growth and productivity by increasing photosynthetic rate and the radiation use efficiency and consequently the availability of assimilates (Rodriguez *et al.*, 1998). Phosphorus nutrient in legumes stimulates a greater attention in increasing the productivity, as it encourages healthy root growth and promotes rhizobial activity resulting in increased nodulation that exemplify nitrogen fixation. Phosphate solubilizing bacteria (PSB) enhances phosphorus availability to plants by lowering soil pH by microbial production of organic acids and mineralization of organic phosphorus. Introduction of PSB in the rhizosphere of crop also increases the efficiency of phosphate fertilizers (Gaur, 1990). Most of the soils in Telangana are low in available phosphorus status, farmers are using high amount of DAP fertilizer, to reduce the cost on fertilizer and also to increase the availability of phosphorus, present study has been investigated.

Comment [P3]: Authors should improve the introduction and cite up-to-date literature that represents the current, state-of-the-art context.

MATERIALS AND METHODS

The experiment was conducted during *Rabi*, 2020 and the geographical location of the experimental site was 17° 32' N Latitude, 78° 40' E Longitude with an altitude of 477 m above mean sea level. Agro-climatologically the area is classified as Southern Telangana Agro Climatic Zone of Telangana state.

The experimental soil was sandy loam in texture, alkaline in soil reaction, non - saline, low in O.C and available nitrogen, medium status of available phosphorus, available potassium

and available sulphur. The experiment was laid out in RBD comprising eleven treatments with three replications. The experimental details is given in below table

Table 1. Experimental details

Technical details	Experiment
Season	Rabi, 2020
Design	Simple RBD
Replication	03
Treatments	11
Varities	KBSH – 78
Seed rate	5 kg ha ⁻¹
Spacing	60 x 30 cm
Duration	98 days
RDF	60:90:30 kg ha ⁻¹ NPK
Gross plot size	4.8m x 3 m
Net plot size	3.6m X 2.4 m

Table 2. Treatment details

Treatment	Treatment detail
T ₁	100% NPK , (RDF)
T ₂	No P
T ₃	No P + PSB-D
T ₄	No P +PSB-SA ₁
T ₅	No P + PSB-SA ₂
T ₆	75 % P + PSB-D
T ₇	75 % P+ PSB-SA ₁
T ₈	75 % P + PSB-SA ₂
T ₉	50% P + PSB-D
T ₁₀	50% P+ PSB-SA ₁
T ₁₁	50% P + PSB-SA ₂

D = Drenching @ 50 ml L⁻¹ or 8 L ha⁻¹.

SA₁ = Soil application of PSB @ 3 kg ha⁻¹

SA₂ = Soil application of PSB @ 6 kg ha⁻¹

PSB was applied as soil application and drenching at the time of sowing. Lignite based powder form with two doses @ 3 and 6 kg per hectare was properly mixed with vermicompost @ 1 t ha⁻¹ was applied to soil in the sowing line. The liquid PSB @ 8 L per hectare was drenched in the sowing line.

Comment [P4]: PSB was applied to the soil

Microbial analysis

Soil microbial population were enumerated from the samples collected at 0-15cm. The serial dilution was made to determine the microbial population in different treatments. One gram of soil was suspended in 10 ml of sterile 0.85% saline solution and swirled for 5 minutes. The dilutions were made by transferring 1 ml of this suspension to subsequent 9 ml of sterile solution which shows 10^{-1} dilution. The dilutions were made up to 10^{-4} . The enumeration of PSB was done after culturing the organisms on a Pikovskaya media. The media was prepared and sterilized in autoclave at temperature of 121°C and pressure at 15 psi for 15 minutes. After that, the media was poured in to petri plates under sterile condition in Laminar air flow and kept undisturbed for 5-6 hours to get solidify. After solidification one milliliter of sterile suspension was transferred to petri plates on pikovskaya medium and the suspension was spread all over the petri plate with the L- shaped glass rod by rotating the petri plate in clock wise and anticlockwise to attain uniform spread. After that plates were kept inverted position and incubated at 30°C for 7-10 days in an incubator. After the specified period, the PSB colonies were counted by observing the production of clearing zones around the colonies. It was an indication of the presence of PSB (Sundararao and Sinha, 1963). The colonies were counted and enumerated by using the formula by Schmidt and Cadwell, 1967. No. of cfu x dilution Number of PSB in 1 gram of soil = Dry weight of 1 gram moist soil x aliquot take

$$\text{Number of PSB in 1 gram of soil} = \frac{\text{No. of cfu x dilution}}{\text{Dry weight of 1 gram moist soil x aliquot taken}}$$

Recovery Efficiency of P (REp)

It refers to the increase in P uptake by plant (above ground parts) per unit of P applied. The recovery efficiency is generally expressed in percentage terms.

$$\text{REp} = \frac{\text{P uptake by the crop with P application} - \text{P uptake by the crop without P application} \times 100}{\text{Amount of P applied}}$$

The data recorded for various parameters during the course of investigation in sunflower and chickpea experiments were tabulated and statistically analyzed by following the standard

methods for Randomized block design as suggested by Panse and Sukhatme (1978) with the help of computer software (CVSTAT).

RESULTS AND DISCUSSION

PSB population in the rhizosphere soil of sunflower at different growth stages

Effect of different doses and methods of PSB application and P levels on the PSB population in the rhizosphere soil of sunflower crop has been presented in Table 3 and depicted by the Figure 1. The data indicated that there was an increase in PSB population from flower initiation to grain filling stage and there after decline in PSB population from grain filling to maturity stage. Among, all the stages highest population was seen at grain filling stage.

At flower initiation, the maximum population (43.9×10^4 cfu /g of soil) was noticed in treatment T₈, which had received 75% of phosphorus levels and soil application of PSB @ 6 kg ha⁻¹ followed by T₇ (39.0×10^4 cfu /g of soil) *i.e.*, 75% P + PSB- SA₁ and the lowest population (13.8×10^4 cfu/g of soil) was noticed in T₂ *i.e.*, No P. There was a significant increase in PSB population over the control T₂. More number of colony count was recorded due to soil application of PSB than drenching.

The data showed there was increase in PSB population from flower initiation to grain filling stage. At grain filling stage, highest population (44.6×10^4 cfu/g of soil) was seen in T₈, 75% P+ PSB-SA₂ and lowest population of PSB (16.6×10^4 cfu/g of soil) was seen in T₂ treatment. There was a fall in PSB population from grain filling to maturity stage. Among all the treatments, at maturity stage highest population (33.7×10^4 cfu/g of soil) was seen in T₈ and lowest population (15.7×10^4 cfu/g of soil) was seen in T₂ *i.e.*, No P .

The highest population was seen in T₈, 75% P+ PSB- SA₂ in all stages. The population of PSB in T₆, 75% P + PSB-SA₂ which was on par with T₁₁ *i.e.*, 50% P+ PSB-SA₂. Whereas, lower PSB population were found in the uninoculated treatment without P levels which was on par with the treatment T₃, No P+ PSB-D where only efficient PSB was inoculated without any phosphorus levels.

Soil microbial biomass appears to increase with both mineral fertilization and bacterial inoculation. The beneficial effect of inoculation on PSB population may be direct, due to an

increased supply of available P and N, K or indirect, through changes in the growth rate and metabolic activities of crop.

Table 3: Effect of different doses and methods of PSB application and P levels on PSB population during growth stages of sunflower

Treatment	PSB population X 10 ⁴ cfu/g of soil		
	Flower initiation	Grain filling	Maturity
T-1	14.3	18.07	15.8
T-2	13.8	16.60	15.7
T-3	16.5	20.84	20.6
T-4	18.6	24.04	20.9
T-5	20.8	25.82	21.4
T-6	35.8	34.69	31.7
T-7	39.0	41.80	33.1
T-8	43.9	44.60	33.7
T-9	28.1	30.67	28.8
T-10	32.0	33.79	29.4
T-11	34.3	34.01	28.5
SEM±	0.8	1.07	1.7
CD (0.05)	2.3	3.15	5.1
CV%	5.1	6.26	11.7

Comment [P6]: In no part of the work was the statistical treatment described, which should be indicated by letters next to each result in the tables and graphs.

PSB population in the rhizosphere soil of chickpea at different growth stages

Effect of different doses and methods of PSB application and P levels on PSB population of Chickpea at different growth stages was presented in Table 4 and depicted by Figure 2. The data indicated that there was increase in PSB population from flower initiation to pod filling stage and there after reduction in population of PSB from pod filling to maturity stage.

At flower initiation stage, the PSB population ranged from 19.94 to 38.27 X 10⁴cfu/g of soil. Among all the treatments, highest population of PSB (38.27 x 10⁴cfu/g of soil) was seen in T₈, 75% P + PSB- SA₂ and lowest population of PSB (19.94 X 10⁴cfu/g of soil) was seen in T₂,

No P same trend was followed at grain filling and maturity stage. More number of PSB was seen with soil application than drenching due to application of vermicompost along with PSB in soil application.

The data showed with the increase in phosphorus levels, the PSB population increased significantly. More number of PSB colony counts was seen with 75% P level and lowest population was seen in uninoculated treatment plus with out phosphorus levels.

The increase in PSB population may be due to increased availability of P in all inoculated treatments over uninoculated. Another probable reason may be the availability of N and P which appears to have encouraged the multiplication of the organisms. Solubilization of rock phosphate release more available P into soil by PSB and this has probably increased the vigorous root growth resulted in the increased root metabolic activity and the availability of nutrients to plants.

Table 4. Effect of different doses and methods of PSB application and P levels on PSB population at different growth stages of chickpea

Treatment	PSB population X 10 ⁴ cfu/g of soil		
	Flower initiation	Pod filling	Maturity
T-1	20.24	22.97	19.13
T-2	19.94	21.27	17.50
T-3	21.61	24.70	21.30
T-4	25.43	27.20	22.37
T-5	28.92	29.93	23.33
T-6	33.63	35.90	32.70
T-7	35.96	38.30	33.87
T-8	38.27	39.47	38.03
T-9	30.23	31.80	30.40
T-10	32.00	32.80	31.53
T-11	34.87	35.83	32.83
SEM±	1.45	2.6	1.1
CD (0.05)	4.27	7.8	3.1

CV%	8.59	14.8	6.7
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Comment [P7]: In no part of the work was the statistical treatment described, which should be indicated by letters next to each result in the tables and graphs

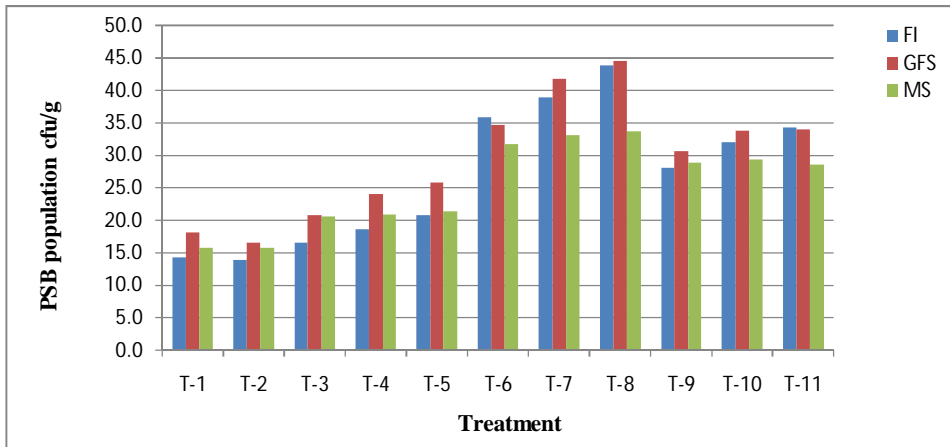


Figure 1 PSB population (x10⁴cfu/g of soil) of sunflower with different doses and methods of PSB application with varying levels of P levels.

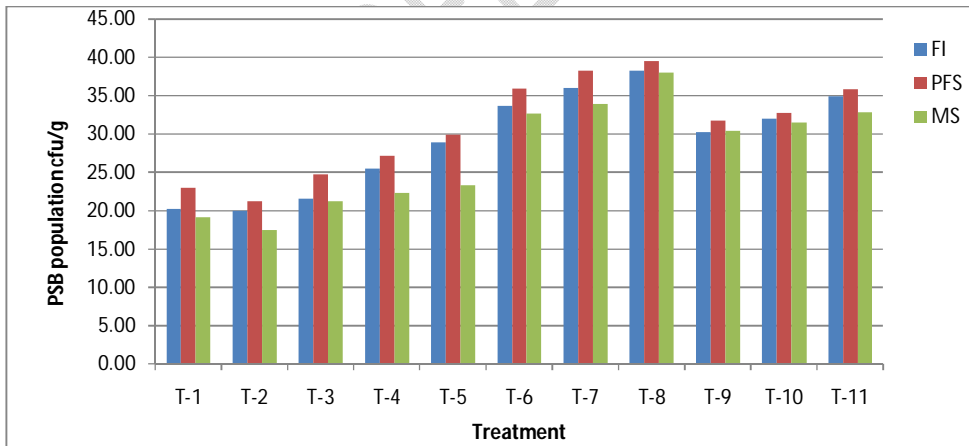


Figure 2 PSB population (x10⁴cfu/g of soil) of chickpea with different doses and methods of PSB application with varying levels of P levels.

Effect of different methods and doses of PSB and P levels on the PUE (%) and removal of Phosphorus by Sunflower and chickpea

PUE (%)

The data pertaining to the effect of different doses and methods of PSB application and P levels on PUE (%) of sunflower and chickpea was presented in Table 5.

The PUE (%) of sunflower ranged from 7.52 to 18.71 % and use efficiency of Phosphorus for sunflower was recorded highest in T₁₁ (18.71 %), treated with 75% P with soil application of PSB @ 6 kg/ha. Lowest was recorded with T₁ (7.52%) *i.e.* 100% NPK.

The PUE (%) of chickpea ranged from 15.00 to 20.86 % and phosphorus use efficiency of chickpea was recorded highest in T₁₁ (25.43%), treated with 50% P with soil application of PSB @ 6 kg/ha. Lowest was recorded in with T₁ (8.45%) *i.e.* 100% NPK.

Highest PUE of was recorded in treatment with combined application of PSB and RDF. These results were also supported by the findings of Laharia *et al.*, (2014) .

Removal of phosphorus (kg ha⁻¹)

The data pertaining to the effect of different doses and methods of PSB application and P levels on P removal was shown in Table 6..

The P uptake of sunflower was ranged from 15.00 to 20.86 kg ha⁻¹. The highest P uptake was recorded in T₈ (20.84 kg ha⁻¹) with application of 75% phosphorus along with soil application of PSB @ 6 kg ha⁻¹. The lowest uptake was seen in T₄ (15.00 kg ha⁻¹) treated with 100% NPK.

The P uptake of chickpea was ranged from 8.52 to 13.16 kg ha⁻¹. Highest P uptake was recorded in treatment T₁₁ (13.16 kg ha⁻¹) received 50% phosphorus with soil application @ 6 kg ha⁻¹ and lowest uptake (8.52 kg ha⁻¹) was seen in T₂ received No P.

Highest P recovery was observed with combined application of PSB and P level. From the results highest P recovery was obtained with increase in P level with soil application of PSB. These results were supported by Gabbane *et al.*, (2016).

Table 5 : Effect of different doses and methods of PSB and levels of P levels on PUE (%)

Treatment	Treatment detail	PUE (%)	
		Sunflower	Chickpea
T1	100% NPK	7.52	8.95
T6	75 % P + PSB-D	13.09	6.64
T7	75 % P+ PSB-SA ₁	15.85	9.06
T8	75 % P + PSB-SA ₂	18.71	11.64
T9	50% P + PSB-D	13.73	19.56
T10	50% P+ PSB-SA ₁	16.06	22.86
T11	50% P + PSB-SA ₂	17.44	25.43

Table 6 :Effect of different doses and methods of PSB application levels of Plevels on removal of phosphorus.

Treatment	Treatment detail	Removal of phosphorus (kg ha ⁻¹)	
		Sunflower	Chickpea
T1	100% NPK	15.00	10.90
T6	75 % P + PSB-D	17.07	8.52
T7	75 % P+ PSB-SA ₁	18.93	9.61
T8	75 % P + PSB-SA ₂	20.86	10.77
T4	50% P + PSB-D	14.41	11.40
T10	50% P+ PSB-SA ₁	15.46	12.39
T11	50% P + PSB-SA ₂	16.08	13.16

Conclusion

The PSB population at different stages of sunflower and chickpea has a significant increase over the 100% NPK. There was an increase in PSB population over the initial soil

population due to external application of PSB. There was an increase in PSB population from flower initiation to grain filling stage and decrease in population from grain filling stage to maturity stage. The PUE (%) has a positive effect with different doses and methods of PSB application and P levels. Highest uptake of the phosphorus by sunflower crop was observed with 75% P level and PSB application. The uptake of the Phosphorus by chickpea crop increased with the soil application of PSB and P level.

Comment [P8]: The conclusion needs to be improved.

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