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An Analysis of the Effects of Phosphorus Solubilizing Bacteria (PSB) on Phosphorus Intake to Increase Groundnut Productivity

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ABSTRACT

Background: Groundnut (*Arachis hypogaea* L.) is a self-pollinated, auto-tetraploid legume crop with 2n=40 chromosomes and belongs to the family Fabaceae. Microbial inoculant *Azotobacter* (*Azotobacter chroococcum*), *Bacillus* (*Bacillus subtilus*) and *Pseudomonas* (*Pseudomonas sps*.) were applied @ 250 g/acre. Consortium comprised of 3 microbial inoculants/strain @ 166.0 each for 500 g per acre

Methods: For in depth studies field experiment on PSBs along with two doses of P_2O_5 were carried out during two consecutive *Kharif* season in 2020 and 2021 on SG99.The use of phosphate solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield. Strains from the genera *Pseudomonas*, *Bacillus* and *Azotobacter* are among the most powerful phosphate solubilizes.

Result: Results revealed that the different treatments of recommended dose and double dose of PSBs exerted their significant effect on initiation of flowering, 50% flowering, completion of flowering and days to maturity.

Key words: Flowering, Groundnut, Microbial inoculants, PSBs, SG99.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a self-pollinated, auto-tetraploid legume crop with 2n=40 chromosomes and belongs to the family Fabaceae. Groundnut is an important food and oilseed crop. Groundnut, also known as “the poor man’s nut” or “the king of vegetable oilseeds” appears to have originated in South America, *i.e*. the northwest of Brazil andits secondary cultivation centre is in Africa. Groundnut is an important oilseed crop in India, where it ranks first in terms of area and second in terms of output after soybean. It covered around 26.4 million ha worldwide with a total production of 37.1 million metric tonnes (Faldu *et al*., 2018). China produces the most groundnuts (17.57 million metric tonnes), followed by India (6.73 million metric tonnes), Nigeria (4.45 lakh metric tonnes), Sudan (2.83 million metric tonnes) and the United States of America (2.49 million metric tonnes), which accounted for 36.01, 13.79, 9.12, 5.80 and 5.11 percent of total world production (48.80 million metric tonnes) in 2019-20. Groundnut is grown mainly in Hoshiarpur district in the Punjab state and is grown in a very small area, *i.e.* nearly 1.2 thousand hectares. The productivity of groundnut in Punjab was 816 kg per hectare in 1990-91, which increased to 1739 kg per hectare in 2012-13 and then to 1920 kg per hectare in 2016-17 (Anonymous, 2022).

Phosphorus, a macronutrient, ranks second in plant requirements after nitrogen and is involved in a variety of plant metabolic processes. Because phosphorus is fixed in soil layers, it is sometimes lacking in the soil. (Khan *et al*., 2009). Phosphorus-solubilizing soil microbes gaining significance due to their ability to mineralize complex compounds (Wani *et al*., 2005). The release of various organic acids by these microorganisms causes acidification of microenvironments (Maliha *et al*., 2004) and, as a result, the substitution of P ions with cations, which is referred to as “phosphorus solubilization” (Trivedi and Sa, 2008). Phosphorus is important in plants because it is a component of nucleoproteins, phytins andphospholipids, as well as an essential component of a number of enzymes and a key component in energy transfer.

The influence of interactions with phosphorus and rhizobium inoculation helps plant establishment. The plant with the highest flower count was where the application of phosphorus was balanced. According to Praveen *et al.* (2012) and later, as cited by Ganesh *et al.* (2015) meaningfully improved flower count was observed with the application of phosphorus fertilizer, which also speeds up the meristem tip activity. As reported by Keba *et al.* (2014) for the flower count per plant, phosphorus was very important for the essential reproductive organs, like the flowers or sinks, on which yield is mainly dependent and was responsible for higher yields. By applying phosphorus fertilizer, the vegetative growth of the plant is influenced in a positive way. Shiyam (2010) reported that different levels of

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phosphorous had a negative effect on plant height. According to Slave and Gunjal (2011), with the change in phosphorus concentration, a significant change in plant height was found. According to Zafar *et al.* (2013), with improved root arrangement, phosphorus provides better contact between roots and soil, resulting in the absorption of phosphorus and other important nutrients. It has been reported that by a few specified times, phosphorus remaining in the soil as a key constituent and poorly resolvable mineral phosphate were formed andthey were basically not available to the plant (Marschner and Marschner, 2012). Phosphorus is crucial for various aspects of plant growth, including nitrogen fixation, nutrient absorption androot development. Phosphorus fertilizers are essential for promoting concentrated, fibrous root growth and enhancing the overall health of plants, as highlighted by Niu *et al*. (2012).

In groundnuts (peanuts), phosphorus plays a significant role in supporting root and lateral root formation, which is essential for optimal vegetative growth. This aspect has been noted in the findings of Ganesh *et al*. (2015), who emphasized the acceleration of meristem tip activity.

According to Bajya *et al.* (2023), Mepiquat chloride (MC) is an important growth retardant inhibits vegetative growth and accelerates the development of reproductive parts by reducing plant height, thereby decreasing the distance between the source and sink, resulting in better translocation of photosynthetic into developing pods, which is expected to improve groundnut harvest index. Hence the present study, was conducted on three groundnut genotypes *viz*. TG37A, J87 and SG99 to observed the difference of growth retardant mepiquat chloride, water spray.

According to Bajaya *et al.* (2022), During survey, maximum collar rot incidence was observed in Jaipur (28.85%) and minimum in Bikaner (21.04%) district while overall mean disease incidence of eight districts was 22.99 per cent covering 200 fields of major groundnut growing districts of Rajasthan. Among six levels of seed rate (80, 85, 90, 95, 100 and 105 kg/ha), higher disease reduction with increased pod yield was observed with seed rate of 105 kg/ha as compared to standard recommended seed rate (80 kg/ha). As reported by Meena *et al.* (2021), On the basis of experimental finding, it can be concluded that the application of PEC @4 t/ha + Zn @ 4 kg/ha alongwith the recommended dose of fertilizer results in significantly higher yield and protein content of blackgram under Typic Haplustepts soil of sub-humid southern plain of Rajasthan.

MATERIALS AND METHODS

Field experiments were conducted at the research farm of oilseeds section, Department of Plant Breeding and Genetics, Punjab Agricultural University Ludhiana in design during *Kharif* season 2020-2021. The experimental site is located at 30°54′N latitude and 75°48′E longitude and at an altitude of 247 meters above the mean sea level. Microbial inoculant *Azotobacter* (*Azotobacter chroococcum*), *Bacillus* (*Bacillus subtilus*) and *Pseudomonas* (*Pseudomonas sps*.) were applied @ 250 g/acre. Consortium comprised of 3 microbial inoculants/strain @ 166.0 each for 500 g per acre.

Phenological traits

The data consisted of the number of days from the date of sowing the crop to the appearance of the first flower. Number of days elapsed from the date of sowing to the date of first flower opening in 50 per cent plant was recorded on the plot basis. Completion of flowering was recorded by counting the number of days required for physiological maturity *i.e.* when plants under go leaf senescence and all pods from the plants turn green to yellow.

Physiological parameters

Chlorophyll content was estimated in 4th leaf from the top (fully expended leaflet) with the help of chlorophyll meter (SPAD502 plus). Readings measured in 10 plants per plot at flowering and podding stages. Chlorophyll content is expressed in terms of Soil Plant Analysis Development (SPAD) units.

Yield and yield attributes

Pods from the net plot area were washed and cleaned to remove the soil adhering to the pods, Impurities and immature pods. The developed pods were dried completely (up to 8% moisture level) and weighed. On the basis of pod yield net plot^{-1} the pod yield ha^{-1} was calculated.

Statistical analysis

The data were analysed by method of Analysis of Variance obtained by Panse and Sukhatme (1978). The recorded data in the experiment was statistically analysed by two factorial randomized block design. Significance was tested by F value at 5 per cent level of probability. Critical differences were worked out for the effects which were significant.

RESULTS AND DISCUSSION

Effect on microbial count in soil

The total rhizospheric bacterial, fungal, actinomycetes, phosphate-solubilizing and diazotrophic bacterial population was enumerated of before and after sowing soil samples of groundnut. The soil adhering to the plant roots was collected, brought to the laboratory, serially

Table 1: Initial count of microorganisms (CFU/g) in the soil samples before sowing.

Microorganisms	CFU/g soil	
	Soil depth (cm)	
	0-15	15-30
Bacteria	2.5×10^{7} (7.39)	2.6×10^{6} (6.41)
Fungi	3.4×10^{3} (3.53)	3.3×10^{2} (2.52)
Actinomycetes	2.5×10^{5} (5.39)	1.8×10^{4} (4.25)
PSB	2.5×10^{5} (5.39)	2.8×10^{4} (4.45)
Diazotrophs	1.3×10^{6} (6.11)	1.6×10^{4} (4.20)

Table 2: Microbial counts (CFU/g) in the soil samples after harvest.

Treatment/Doses of P_2O_5		Microorganisms									
		Bacteria		Fungi		Actinomycetes		PSB		Diazotrophs	
		0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
Control recommended dose	EP1	2.8×10^{8}	2.4×10^{6}	2.0×10^{4}	3.7×10^{2}	4.2×10^{6}	3.3×10^{4}	3.8×10^{6}	3.0×10^{4}	1.2×10^{7}	1.2×10^{4}
		(8.45)	(6.38)	(4.30)	(2.57)	(6.62)	(4.51)	(6.58)	(4.48)	(7.08)	(4.08)
Control double dose	EP2	3.4×10^{8}	3.1×10^{6}	2.2×10^{4}	3.2×10^{2}	4.4×10^{6}	3.4×10^{4}	4.0×10^{6}	3.9×10^{4}	3.7×10^{7}	2.0×10^{4}
		(8.53)	(6.49)	(4.34)	(2.51)	(6.64)	(4.53)	(6.60)	(4.59)	(7.57)	(4.30)
Azotobacter recommended dose	AP1	1.7×10^{8}	2.7×10^{6}	3.0×10^{4}	4.5×10^{2}	6.3×10^{6}	4.3×10^{4}	5.2×10^{6}	5.1×10^{4}	8.9×10^{7}	6.7×10^{4}
		(8.23)	(6.43)	(4.48)	(2.65)	(6.79)	(4.63)	(6.72)	(4.71)	(7.95)	(4.83)
Azotobacter double dose	AP2	3.7×10^{8}	3.1×10^{6}	3.4×10^{4}	4.4×10^{2}	6.6×10^{6}	4.6×10^{4}	6.3×10^{6}	5.9×10^{4}	9.2×10^{7}	6.5×10^{4}
		(8.57)	(6.49)	(4.53)	(2.64)	(6.82)	(4.66)	(6.79)	(4.77)	(7.96)	(4.81)
Bacillus recommended dose	BP1	2.4×10^{8}	2.1×10^{6}	3.5×10^{4}	4.5×10^{2}	5.3×10^{6}	4.3×10^{4}	7.6×10^{6}	5.2×10^{4}	5.4×10^{7}	2.4×10^{4}
		(8.38)	(6.32)	(4.54)	(2.65)	(6.72)	(4.63)	(6.88)	(4.71)	(7.73)	(4.38)
Bacillus double dose	BP2	3.8×10^{8}	2.8×10^{6}	3.6×10^{4}	4.9×10^{2}	5.8×10^{6}	4.5×10^{4}	8.2×10^{6}	6.0×10^{4}	6.1×10^{7}	2.6×10^{4}
		(8.58)	(6.45)	(4.56)	(2.69)	(6.76)	(4.65)	(6.91)	(4.78)	(7.78)	(4.41)
Pseudomonas recommended dose	CP1	2.7×10^{8}	2.8×10^{6}	3.1×10^{4}	5.5×10^{2}	6.2×10^{6}	5.2×10^{4}	6.8×10^{6}	3.8×10^{4}	3.8×10^{7}	1.3×10^{4}
		(8.43)	(6.45)	(4.49)	(2.74)	(6.79)	(4.71)	(6.83)	(4.58)	(7.58)	(4.11)
Pseudomonas double dose	CP2	3.5×10^{8}	3.3×10^{6}	3.3×10^{4}	5.8×10^{2}	6.6×10^{6}	5.0×10^{4}	7.0×10^{6}	7.0×10^{4}	4.0×10^{7}	3.0×10^{4}
		(8.54)	(6.52)	(4.51)	(2.76)	(6.82)	(4.69)	(6.84)	(4.84)	(7.60)	(4.48)
Consortium recommended dose	DP1	4.7×10^{8}	3.3×10^{6}	3.6×10^{4}	6.2×10^{2}	6.9×10^{6}	5.6×10^{4}	7.8×10^{6}	7.1×10^{4}	9.2×10^{7}	6.7×10^{4}
		(8.67)	(6.52)	(4.56)	(2.79)	(6.84)	(4.75)	(6.89)	(4.85)	(7.96)	(4.82)
Consortium double dose	DP2	5.2×10^{8}	3.6×10^{6}	3.4×10^{4}	6.5×10^{2}	7.2×10^{6}	5.7×10^{4}	8.3×10^{6}	7.0×10^{4}	9.6×10^{7}	6.8×10^{4}
		(8.72)	(6.56)	(4.53)	(2.81)	(6.86)	(4.76)	(6.91)	(4.84)	(7.98)	(4.83)

diluted up to 10^{-6} and plated on nutrient agar, potato dextrose agar, yeast malt extract agar, modified Pikovskayas agar and Jensen's agar for enumerating bacterial, fungal, actinomycetes, phosphate-solubilizing and diazotrophic bacterial populations, respectively. The Petri plates were incubated in triplicates at 28°C for 48 h for total bacterial and diazotrophic bacterial count, 3-5 days for fungal and phosphate-solubilizing bacterial count and for 5-7 days for actinomycetes count. The results were expressed as colony forming units (CFU) per gram of soil. CFU: Colony Forming Units Value in parenthesis indicate $\log_{10}$ CFU/g.

Soil sample collected before sowing consisted of 2.5×10^5 CFU of *Actinomycetes* and 2.5×10^5 CFU of PSB and 3.4×10^3 CFU of fungi from 0-15 cm soil depth however variation existed.

Soil sample collected before sowing from 15-30 cm soil depth consisted of 1.8×10^5 CFU of *Actinomycetes* and 2.8×10^3 CFU of PSBs and 3.3×10^2 CFU of fungi however variation existed.

Soil sample collected after harvesting, control recommended dose of only P_2O_5 consisted 2.83×10^8, 2.0×10^4, 4.2×10^6, 3.8×10^6 and 1.20×10^7 CFU of Bactria, fungi, *Actinomycetes,* PSB and diazotrophs from 0-15 cm soil depth however variation existed. Whereas with control double dose consisted 3.38×10^8, 2.2×10^4, 4.4×10^6, 4.0 10^6 and 3.67 10^7 CFU of Bactria, fungi, *Actinomycetes,* PSBs and diazotrophs.

Similarly, from soil sample collected at 15-30 cm depth control recommended dose of only P_2O_5 consisted of 2.43×10^6, 3.75×10^2, 3.3×10^4, 3.0×10^4 and 1.2×10^4 CFU of Bactria, fungi, *Actinomycetes,* PSB and diazotrophs from 0-15 cm soil depth however variation existed. Whereas with control double dose consisted 3.12×10^6, 3.2×10^2, 3.4×10^4, 3.9×10^4 and 2.0×10^4 CFU of bacteria, fungi, *Actinomycetes,* PSBs and diazotrophs. Whereas with application of PSBs, bacteria, fungi, *Actinomycetes,* PSBs and diazotrophs ranged from 1.67×10^8 to 9.2×10^7 with recommended dose and bacteria, fungi, *Actinomycetes,* PSB and diazotrophs with double dose ranged from 2.11×10^6 to 9.2×10^7 (Table 1).

An increase in microbial count was observed with the treatments at 0-15 cm depth (Table 1 and 2). The bacterial count increased from the initial count of 2.5×10^7 (taken at the start of the experiment) to 1.67-5.17$\times10^8$ with the highest count observed with the treatment *Consortium double dose*. Likewise, the fungal count increased from an initial count of 3.4×10^3 to 2.0-3.6$\times10^4$ CFU/g. Similarly, count of actinomycetes increased from 2.5×10^5 to 7.2×10^7, PSBs from 2.5×10^5 to 8.3×10^6 and diazotrophs from 1.3×10^6 to 9.6×10^7. Microbial count decreased with increase in soil depth. However, different treatments had no effect on the microbial count in the soil samples taken from 15-30 cm.

For in depth studies field experiment on PSBs along with two doses of P_2O_5 were carried out during two consecutive *Kharif* season in 2020 and 2021 on SG99.The

Table 3: Effect of seed treatment (PSB) and phosphorous on flowering behaviour in SG99.

Treatment/Doses of P_2O_5		Days to initiation flowering			Days to 50% flowering			Completion of flowering			Days to maturity		
		2020	2021	Pooled mean	2020	2021	Pooled mean	2020	2021	Pooled mean	2020	2021	Pooled mean
Control recommended dose of only P_2O_5	EP1	26.7	26.0	26.3	35.0	35.9	35.5	42.0	41.4	41.7	121.2	121.5	121.4
Control double dose	EP2	26.7	26.0	26.3	37.0	36.7	36.8	43.0	44.0	43.5	120.3	121.0	120.7
Azotobacter recommended dose	AP1	27.2	27.1	27.2	34.0	33.7	33.8	45.0	46.3	45.7	121.0	119.8	120.4
Azotobacter double dose	AP2	26.6	27.0	26.8	36.0	36.3	36.2	44.0	43.2	43.6	120.0	120.2	120.1
Bacillus recommended dose	BP1	26.7	27.1	26.9	32.0	31.0	31.5	45.0	44.7	44.9	121.5	120.7	121.1
Bacillus double dose	BP2	26.3	27.0	26.7	37.0	36.2	36.6	44.5	45.1	44.8	121.3	120.6	121.0
Pseudomonas recommended dose	CP1	26.3	26.0	26.2	33.0	32.8	32.9	45.2	44.7	45.0	119.0	122.0	120.5
Pseudomonas double dose	CP2	26.7	27.5	27.1	35.0	34.1	34.5	43.6	42.4	43.0	121.0	119.8	120.4
Consortium recommended dose	DP1	26.3	27.0	26.7	30.0	30.8	30.4	43.0	43.8	43.4	120.1	122.2	121.2
Consortium double dose	DP2	27.0	27.3	27.2	32.0	32.1	36.8	47.0	46.9	47.0	121.0	120.7	120.9
Average		26.7	26.8	26.7	34.1	34.0	35.0	44.2	44.3	44.2	120.6	120.9	120.7
C.D. (p=0.05)		NS	NS	Y=NS, T=NS, Y×T=NS	1.657	1.356	Y=NS, T=1.004, Y×T=NS	2.05	2.131	Y=NS, T=1.386, Y×T=NS	5.742	5.036	Y=NS, T=3.662, Y×T=5.179

data pertaining to the effect of different treatments on days to initiation flowering, 50% flowering, completion of flowering and days to maturity are presented in Table 3. Results revealed that the different treatments of recommended dose and double dose of PSBs exerted their significant effect on initiation of flowering, 50% flowering, completion of flowering and days to maturity (Table 3). Days to initiation of flowering was non-significant during *kharif* 2020-2021 and for interaction of the year. Whereas days to 50% flowering significantly varied from 30.8 to 37.0 days and 30.8 to 36.7 during *kharif* 2020 and *kharif* 2021, respectively. The results showed that days to 50% flowering significantly declined after PSBs treatment and maximum reduction observed with *Consortium* recommended dose (DP1) (30.0 days and 30.8 days) during *kharif* 2020 and *kharif* 2021, respectively. Pooled mean showed that day to 50% flowering varied from 30.4 to 36.8 days and minimum days to 50% flowering recorded with *Consortium* recommended dose (DP1) *i.e.* 30.4 days (Table 3).

Completion of flowering differed significantly among the recommended dose and double dose of PSBs during *kharif* 2020 and *kharif* 2021 than the recommended dose and double dose of only P_2O_5. During *kharif* 2020, completion of flowering varied from 42.0 to 47.0 days and during *kharif* 2021 ranged from 41.4 to 46.9 days. Maximum days to completion of flowering observed with *Consortium double dose* (DP2) (47.0 days) during *kharif* 2020 and *kharif* 2021, respectively. Pooled means showed that among the different PSBs maximum days to completion observed with *Consortium double dose* (DP2) *i.e*. 47.0 days (Table 3). A previous study documented that effect of phosphorous fertilizer on groundnut and showed the significant effect of on plant height, leaf count, number of branches, days to 50% flowering, number of pods/plant, 100 seeds weight, total yield and biomass with the application of 60 kg P ha^{-1} (Ibrahim *et al.,* 2019).

Days to maturity significantly varied from 119.0 to 121.5 days and 119.8 to 122.2 days) during *kharif* 2020 to *kharif* 2021 respectively. The minimum days to maturity observed with *Pseudomonas* recommended dose (CP1) (119.0 days) during 2020 and *Pseudomonas* double dose (CP2) *i.e.* 119.8 days during 2021. Pooled mean showed that *Azotobacter* double dose (AP2) showed minimum days to maturity *i.e.*120.8 days (Table 3).

CONCLUSION

SG99 treated with microbial inoculants at the time of sowing to test their ability to mobilize phosphorous uptake in order to enhance yield. Treatments comprising of recommended and double dose of PSB significantly reduced days to initiation of flowering, 50%, completion flowering and days to maturity. SPAD values enhanced by 19.2% and RWC content by 15.6 % with *Azotobacter* double dose over control.

Conflict of interest

There is no conflict of interest in this article.

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