Journal of Food Legumes
37(1): 84-89, 2024
DOI: 10.59797/jfl.v37.i1.180

Research Paper

Effect of phosphorus levels and bio-fertilizers on nutrient uptake, quality and yield of chickpea under sub-tropical conditions of Madhya Pradesh

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***Received:** 08 February 2024*
***Accepted:** 01 March 2024*

Handling Editor:
Dr. Narendra Kumar, ICAR-Indian Institute of Pulses Research, Kanpur, India

ABSTRACT

In the present investigation, the effect of phosphorus (P) levels and bio-fertilizers on nutrient uptake, quality and yield of chickpea was studied over two years during Rabi 2021–22 and 2022–23. The study revealed significantly higher uptake of N, P and potassium (K) by seed and stover, higher protein content, grain, and stover yield on the application of 50 kg ha^{-1} P. Similarly application of 50 kg ha^{-1} P along with bio-fertilizers (PSB + *Rhizobium*) also showed higher N, P and K uptake by seed and stover, higher protein content and protein yield, grain and stover yield and this combination was identified the most promising treatment for enhancing chickpea yield.

Key words: Biofertilizers, Chickpea, Nitrogen, Nutrient uptake, Phosphorus, Protein, PSB, *Rhizobium*, Yield.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is commonly known as gram or Bengal gram. It is the most important protein-rich *Rabi* pulse crop in India. It is of two types: *desi* and *kabuli.* The seed size of the *Kabuli* chickpea is larger than *desi* chickpea. In India, chickpea was cultivated in an area of 10.74 million ha with a production of 13.54 million tonnes and average productivity was 1261 kg ha^{-1} in 2021-22 (AIRCP Report 2023). Madhya Pradesh, Rajasthan, Maharashtra, Karnataka and Uttar Pradesh are the main chickpea-growing states in the country (AIRCP Report 2023).

Chickpea can fix 140 kg of atmospheric nitrogen per hectare with its ability of symbiotic nitrogen fixation. However, excessive cultivation and growing of nutrient-exhausting crops have caused the soil deficient in macro- as well as in micronutrients due to nutrient mining. Nowadays, the use of only nitrogenous fertilizers has contributed to soil nutrient imbalance, notably in terms of the availability of micronutrients. The nutrient application must be adjusted for agricultural production because unbalanced use lowers crop yields. Nonetheless, a little amount of nitrogenous fertilizer is needed during the early stages of crop establishment because grams is a leguminous crop and can meet a large portion of its nitrogen needs by fixing atmospheric nitrogen. Phosphorus (P) is known to be one of the important elements involved in plant nutrition because of its role in several functions related to the growth, development, and metabolism of the plant (Nosheen and Shafique 2006). It is also known as the "energy currency" of the plants (Dey *et al*. 2017) as it controls many metabolic activities within the plants. It plays an important role in root growth and development, stalk strength, formation of flower and seed, hastens crop maturity, and stimulates biological activities like nodulation and N_2 fixation, thereby improving the yield of crops (Singh and Singh 2016). Phosphorus aids in healthy root growth, which increases root nodules and then speeds up the organic process. The deficiency of P is one of the major limitations in crop production (Lynch and Brown 2008). In India, the majority of the soils are low to medium in available P status (Pathak 2010) and most of the P applied to the soil in the form of chemical fertilizers gets fixed and becomes unavailable to the plants for utilization (Dey *et al.* 2017). Hence, the crop response to the application of P is highly profitable for crop production (Tiwari 2001). Accordingly, 80% of India's soils require the use of P at recommended amounts (Motsara 2002), and the application of some P fertilizer would be necessary to stop P mining from the soils to maintain high agricultural yields.

Bio-fertilizers, also known as microbial inoculants, are substances used to treat soil and seeds that contain living or dormant cells of beneficial microorganism strains (Mishra *et al*. 2010). These organisms aid crop plants in absorbing nutrients through their interaction in the rhizosphere and can biologically change nutritionally important elements from an unavailable to an available form. The biofertilizers that reside in the root zone encourage plant development and nutrient uptake by releasing auxins and gibberellins. They also raise the levels of the powerful plant growth hormones gibberellic acid and indole acetic acid in plants. For plants to use atmospheric nitrogen, it must be altered or fixed. In addition to promoting plant growth and development, biofertilizers lower production costs because they tend to utilize fewer chemical fertilizer doses. They can be utilized for leguminous crops, vegetables, food, and fodder (Ali *et al*. 2006). Three regularly used microorganisms as biofertilizers (PGPR) are rhizobia, plant growth-promoting rhizobacteria, and phosphate-solubilizing bacteria (PSB). Pulses have similar nutritional needs as cereal, however because of their special biological nitrogen fixation ability, just 20 kg ha^{-1} of nitrogen is advised. Rhizobium and phosphate solubilizing bacteria (PSB) play a crucial part in N_2 fixation and P solubilizations, which gives them a significant amount of significance. Rhizobium and PSB use have been beneficial in raising chickpea productivity (Rudresh *et al*. 2005). Hence, applying phosphatic fertilizers coupled with PSB enhances the uptake of phosphorus by plants as well as significantly raising crop production (Kachave *et al*. 2018). To increase yield and economic return from chickpea, it is essential to apply sufficient fertilizers and the right strain of biofertilizers. Thus, keeping the above fact in view, an experiment was conducted to assess the productivity and profitability of chickpea to study the effect of phosphorus levels and bio-fertilizers on nutrient uptake, quality, and yield of chickpea under sub-tropical conditions of Madhya Pradesh.

MATERIALS AND METHODS

Weather and climate details

The experimental site, in general, is bestowed with sub-tropical climate which experiences a mean annual rainfall of 900 mm and has the hot and dry early summers followed by a hot and humid monsoon season and cold winters. Of the total rainfall received in the Gwalior outskirts, only 70-75% of rainfall is received from June to early October, whereas the remaining 25-30% of rain is received throughout the year with a major portion of rains during the winters.

Experimental site

The experiment was conducted at the Crop Research Centre of School of Agriculture, ITM University, Gwalior (M.P.) in the *Rabi* 2021-22 and 2022-23. Geographically, the experimental site is located at an elevation of 206 meters above mean sea level (AMSL) situated at 26^{0} 14' N latitude and 78^{0} 14' E longitude which falls in the sub-tropical areas on the outskirts of Gwalior, Madhya Pradesh. The soil of the experimental field was sandy loam in texture, which comes under inseptisol soil order. The soil is slightly alkaline in reaction (pH 7.57), low in organic carbon (4.0 g/kg), available nitrogen (183.50 kg/ha) and potassium (243.0 kg/ha) but medium in available phosphorus (14.40 kg/ha) with electrical conductivity in the safer range.

Experimental design and treatment details

The field experiment comprised sixteen treatment combinations, which were laid out in factorial randomized block design with three replications. The treatments comprised of two factors (i) phosphorus levels *viz*., 0 kg P_2O_5 ha^{-1}, 30 kg P_2O_5 ha^{-1}, 40 kg P_2O_5 ha^{-1}, 0 kg P_2O_5 ha^{-1} and (ii) bio-fertilizers *viz*., no inoculation, PSB, *Rhizobium*, and PSB + *Rhizobium*

Methodology and crop management

The chickpea crop variety, JG-16 was sown in lines at a spacing of 30 cm between the rows × 10 cm between the plants. The chickpea crop was fertilized with a recommended dose of nitrogen through urea at 20 kg ha^{-1} which was broadcasted in the field a day before the sowing. However, phosphorus application was made as per the requirement of the treatment and application of biofertilizers *i.e.* *Rhizobium* (*Mesorhizobium ciceri*) and Phosphate solubilizing bacteria (PSB) were applied through the seed treatment method just before sowing as per the treatment. Further, the crop was managed as per regional recommendations of the crop.

Nutrient uptake

For the purpose of estimating the concentrations of nitrogen, phosphorus, and potassium, plant samples were collected at harvest time. Additionally, the chickpea seeds from each plot were harvested for uptake studies. After being oven-dried, the grain, and stover samples were crushed into a fine powder using an electric grinder, and the concentrations of nitrogen, phosphorus, and potassium in each sample were then determined.

By multiplying the corresponding dry matter accumulation by the percent nutrient content, the uptake of N, P, and K in grain and straw samples was calculated using the following formula:

$$\text{Nutrient uptake (kg/ha)} = \frac{\text{Nutrient content (\%)} \times \text{dry matter accumulation (kg/ha)}}{100}$$

**Note: Nutrient is either N, P or K.*

Quality analysis

The nitrogen content was analyzed for each treatment sample by the modified micro Kjeldhal method. The protein content in the chickpea grain which was expressed in percentage was calculated by multiplying nitrogen content (percent) in the grain by factor 6.25 (AOAC, 1970). The protein yield was calculated by multiplying protein content in the grain by their respective grain yield as per the formula given below:

$$\text{Protein yield (kg/ha)} = \frac{\text{Protein content (\%)} \times \text{Grain yield (kg/ha)}}{100}$$

Yield

With respect to the grain and straw yield, from the individual plot, the net plot was harvested and subsequently, the grain and straw yield thus obtained were weighed and expressed in kg ha^{-1}.

Statistical analysis

Statistical analysis of yield and yield attributes was performed to examine the effect of different treatments. The analysis of variance was conducted using OP-Stat developed by CCSHAU, Hisar for all observations recorded during the years. Fisher's test of significance was used to compare the difference between means at a 5 % probability level. Standard errors along with critical differences at 5 % of significance were computed for discriminating the treatment effects for chance effects.

RESULTS AND DISCUSSION

Nutrient Uptake

The data related to nitrogen, phosphorus, and potassium uptake by seed and stover uptake (Table 1, 2 & 3) by chickpea was significantly affected by varying levels of phosphorus and bio-fertilizers. Among the varying levels of phosphorus, the maximum values of nitrogen, phosphorus, and potassium uptake by seed and stover uptake were recorded with the application of 50 kg P ha^{-1} followed by the application of 40 kg P ha^{-1}. This may be due to the fact that nitrogen, phosphorus, and potassium marked improvement in N uptake by grain and stover of plants seems to be on account of its greater availability in soil environment and enhanced translocation in plant system. The nutrients N, P, K might have been utilized in greater quantities due to their abundant availability. An improved metabolism to greater translocation of the crop and ultimately increases the content in seed and straw. These results are in close conformity with earlier studies (Das *et al.* 2013, Balai *et al.* 2017). Further, among the biofertilizers, nitrogen, phosphorus, and potassium uptake by seed and stover was also significantly affected due to the application of bio-fertilizers in chickpea as compared to no inoculation. The highest values of nitrogen, phosphorus, and potassium by seed and stover uptake were recorded under the application of PSB + *Rhizobium* and the lowest nitrogen, phosphorus, and potassium were recorded with no inoculation respectively. The nutrient uptake by crops was enhanced due to seed inoculation before sowing which can be explained by the increased specific activities of isocitric and malic dehydrogenase, the source of electrons for fixation, creating a better nutritional environment (Deshwal *et al.* (2003). The interaction effect of different levels of phosphorus and bio-fertilizers showed statistically nonsignificant results on the nitrogen, phosphorus, and potassium uptake by grain and stover.

Table 1. Effect of varying levels of phosphorus and bio-fertilizers on average nitrogen uptake by grain and stover of chickpea

Treatments	Nitrogen uptake (kg ha^{-1})					
	Grain			Stover		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
Phosphorus level						
P_0 = 0 kg P_2O_5 ha^{-1}	25.77	29.48	27.59	25.12	27.88	26.48
P_1 = 30 kg P_2O_5 ha^{-1}	27.72	32.07	29.85	26.95	29.84	28.36
P_2 = 40 kg P_2O_5 ha^{-1}	30.90	35.42	33.12	28.64	31.62	30.12
P_3 = 50 kg P_2O_5 ha^{-1}	33.21	37.51	35.31	30.34	33.91	32.26
SEm±	1.11	1.18	1.23	0.79	0.93	0.81
CD (p=0.05)	3.20	3.41	3.54	2.28	2.68	2.34
Biofertilizers						
B_0 = No inoculation	25.00	28.88	26.90	24.56	27.43	25.97
B_1 = PSB	30.48	34.73	32.57	28.33	31.00	29.62
B_2 = *Rhizobium*	28.06	32.36	30.17	27.20	30.28	28.71
B_3= PSB + *Rhizobium*	34.05	38.51	36.23	30.97	34.53	32.92
SEm±	1.11	1.18	1.23	0.79	0.93	0.81
CD (p=0.05)	3.20	3.41	3.54	2.28	2.68	2.34
Phosphorus level × Biofertilizers						
CD (p=0.05)	NS	NS	NS	NS	NS	NS

Table 2. Effect of varying levels of phosphorus and bio-fertilizers on average phosphorus uptake by grain and stover of chickpea

Treatments	Phosphorus uptake (kg ha^{-1})					
	Grain			Stover		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
Phosphorus level						
P_0 = 0 kg P_2O_5 ha^{-1}	4.69	5.24	4.96	3.93	3.93	3.82
P_1 = 30 kg P_2O_5 ha^{-1}	5.19	5.86	5.52	4.16	4.16	4.03
P_2 = 40 kg P_2O_5 ha^{-1}	5.81	6.50	6.15	4.40	4.40	4.27
P_3 = 50 kg P_2O_5 ha^{-1}	6.27	6.95	6.62	4.62	4.62	4.54
SEm±	0.17	0.21	0.19	0.12	0.12	0.14
CD (p=0.05)	0.50	0.60	0.55	0.33	0.34	0.41
Biofertilizers						
B_0 = No inoculation (Control)	4.55	5.13	4.83	3.84	3.84	3.75
B_1 = PSB	5.73	6.37	6.04	4.35	4.35	4.20
B_2 = *Rhizobium*	5.25	5.90	5.57	4.20	4.20	4.09
B_3= PSB + *Rhizobium*	6.44	7.15	6.80	4.71	4.71	4.63
SEm±	0.17	0.21	0.19	0.12	0.12	0.14
CD (p=0.05)	0.50	0.60	0.55	0.33	0.34	0.41
Phosphorus level × Biofertilizers						
CD (p=0.05)	NS	NS	NS	NS	NS	NS

Table 3. Effect of varying levels of phosphorus and bio-fertilizers on average potassium uptake by grain and stover of chickpea

Treatments	Potassium uptake (kg ha^{-1})					
	Grain			Stover		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
Phosphorus level						
P_0 = 0 kg P_2O_5 ha^{-1}	8.84	9.70	9.27	25.93	28.45	27.18
P_1 = 30 kg P_2O_5 ha^{-1}	9.49	10.54	10.01	27.79	30.44	29.09
P_2 = 40 kg P_2O_5 ha^{-1}	10.57	11.64	11.10	29.53	32.26	30.88
P_3 = 50 kg P_2O_5 ha^{-1}	11.27	12.32	11.78	31.27	34.59	33.12
SEm±	0.26	0.28	0.26	0.82	0.85	0.85
CD (p=0.05)	0.74	0.81	0.76	2.37	2.45	2.46
Biofertilizers						
B_0 = No inoculation (Control)	8.58	9.50	9.04	25.34	28.00	26.65
B_1 = PSB	10.42	11.42	10.92	29.20	31.63	30.37
B_2 = *Rhizobium*	9.61	10.64	10.12	28.05	30.89	29.44
B_3= PSB + *Rhizobium*	11.55	12.65	12.09	31.92	35.23	33.80
SEm±	0.26	0.28	0.26	0.82	0.85	0.85
CD (p=0.05)	0.74	0.81	0.76	2.37	2.45	2.46
Phosphorus level × Biofertilizers						
CD (p=0.05)	NS	NS	NS	NS	NS	NS

Quality analysis

Protein percentage in seed and protein yield

The data revealed that the highest protein percentage and protein yield was recorded with 50 kg P_2O_5 ha^{-1} which was statistically at par with 40 kg P_2O_5 ha^{-1} (Table 4). This increase in protein content may be due to an increase in N content in the grains with the application of higher doses of phosphorus which ultimately led to more protein content. These findings are in line with those of Meena *et al.* (2006) in chickpea. Among the biofertilizers, the highest values of protein percentage were recorded under the application of PSB+ *Rhizobium* followed by the application of PSB and the lowest protein percentage and protein yield were recorded under no inoculation respectively. These findings are in close conformity with those of Singh and Singh (2012), Balai *et al.* (2017), Bhattacharjya and Chandra (2013) in chickpea. The improvement in protein content might be due to enhancement in symbiotic parameters which result in higher N fixation by the crop plants leading to more transport of N to the grain. The interaction effect of phosphorus levels and biofertilizers for protein percentage and protein yield was found to be non-significant during both years of study.

Table 4. Effect of varying levels of phosphorus and bio-fertilizers on average protein content and protein yield in the grain of chickpea

Treatments	Protein content (%)			Protein yield (kg ha^{-1})		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
Phosphorus level						
P_0 = 0 kg P_2O_5 ha^{-1}	18.33	19.49	18.91	161.04	184.26	172.65
P_1 = 30 kg P_2O_5 ha^{-1}	19.12	20.28	19.70	173.24	200.44	186.84
P_2 = 40 kg P_2O_5 ha^{-1}	19.38	20.54	19.96	193.11	221.37	207.24
P_3 = 50 kg P_2O_5 ha^{-1}	20.16	21.23	20.69	207.59	234.46	221.03
SEm±	0.30	0.26	0.28	5.84	5.34	5.12
CD (p=0.05)	0.87	0.75	0.80	16.87	15.42	14.78
Biofertilizers						
B_0 = No inoculation (Control)	18.34	19.50	18.92	156.24	180.51	168.38
B_1 = PSB	19.37	20.53	19.95	190.52	217.08	203.80
B_2 = *Rhizobium*	18.99	20.15	19.57	175.38	202.25	188.82
B_3= PSB + *Rhizobium*	20.30	21.36	20.83	212.83	240.69	226.76
SEm±	0.30	0.26	0.28	5.84	5.34	5.12
CD (p=0.05)	0.87	0.75	0.80	16.87	15.42	14.78
Phosphorus level × Biofertilizers						
CD (p=0.05)	N.S	N.S	N.S	N.S	N.S	N.S

Yield (kg ha^{-1})

The data on seed and stover yield of chickpea

Table 5. Effect of varying levels of phosphorous and biofertilizers on yield and harvest index of chickpea

Treatments	Seed yield (kg ha^{-1})			Stover yield (kg ha^{-1})			Biological yield (kg ha^{-1})		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
Phosphorus level									
P_0 = 0 kg P_2O_5 ha^{-1}	877.41	943.51	910.46	2266.58	2454.92	2360.75	3143.99	3398.43	3271.21
P_1 = 30 kg P_2O_5 ha^{-1}	905.68	987.34	946.51	2322.67	2514.33	2418.50	3230.01	3501.68	3365.84
P_2 = 40 kg P_2O_5 ha^{-1}	995.37	1075.37	1035.37	2439.67	2631.33	2535.50	3435.04	3706.70	3570.87
P_3 = 50 kg P_2O_5 ha^{-1}	1066.56	1149.65	1108.10	2513.08	2718.08	2615.58	3579.64	3851.06	3715.35
SEm±	27.78	27.77	31.28	57.10	60.09	58.36	77.32	86.39	80.67
CD (p=0.05)	80.24	80.20	90.33	164.91	173.54	168.56	223.30	249.50	232.99
Biofertilizers									
B_0 = No inoculation (Control)	852.39	925.16	888.78	2217.67	2417.67	2317.67	3071.72	3342.83	3207.28
B_1 = PSB	993.75	1073.75	1033.75	2409.33	2581.00	2495.17	3403.08	3654.75	3528.91
B_2 = *Rhizobium*	921.64	1001.64	961.64	2358.17	2566.50	2462.33	3279.81	3559.81	3419.81
B_3= PSB + *Rhizobium*	1077.24	1155.32	1116.28	2556.83	2753.50	2655.17	3634.07	3900.48	3767.28
SEm±	27.78	27.77	31.28	57.10	60.09	58.36	77.32	86.39	80.67
CD (p=0.05)	80.24	80.20	90.33	164.91	173.54	168.56	223.30	249.50	232.99
Phosphorus level × Biofertilizers									
CD (p=0.05)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

was influenced by varying levels of phosphorus and bio-fertilizers (Table 5) Among the varying levels of phosphorus, the application of 50 kg P ha^{-1} recorded the highest grain yield, stover yield, and ultimately the biological yield. On the other hand, the minimum grain yield, stover yield, and ultimately the biological yield observed with the application of phosphorus at 0 kg/ha. The maximum seed yield per plant as well as per hectare with optimum phosphorus rates was attributable to better nodulation and efficient functioning of nodule bacteria for fixation of nitrogen to be utilized by plants during the seed development stage inthe synthesis of protein which in turn led to increase in seed yield. Khan *et al.* (2004) and Tomar *et al*. (2023) found that the increase in phosphorus levels significantly increased the seed yield of chickpea and mungbean, respectively. This could be attributed to a better supply of phosphorus resulting in higher branches and pods and there by higher yields. It is a fact that phosphorus plays an important role in the formation of new shoots and the number of branches/plants. In addition, it regulates the photosynthetic and carbohydrate metabolism which can be considered to be one of the major factors limiting growth, particularly during the reproductive phase.

Among the bio-fertilizers, the maximum grain yield, stover yield, and biological yield were recorded with the application of PSB+ *Rhizobium* followed by the application of PSB while the lowest grain yield, stover yield, and biological yield were observed with no inoculation. This is due to the Bulk inoculation of beneficial microbes plays an important role in improving three major aspects of determination i.e. strong sink development of reproductive structure, formation of vegetative higher photosynthesis, and production of assimilates to fill economically to sink (Kumar *et al.* 2015, Saket and Rahi 2019, Sai *et al*. 2022).

CONCLUSION

It can be concluded that among the phosphorus levels, application of 50 kg ha^{-1} phosphorus recorded significantly highest nitrogen, phosphorus, and potassium uptake by seed and stover, higher protein content and protein yield, grain and stover yield. Among the biofertilizers, application of phosphate solubilizing bacteria (PSB) + *Rhizobium* recorded treatment combination, and application of 50 kg ha^{-1} phosphorus and bio-fertilizers (PSB + *Rhizobium*) recorded the highest nitrogen, phosphorus, and potassium uptake by seed and stover, higher protein content and protein yield, grain and stover yield. Thus, for chickpea cultivation, the applicationof 50 kg ha^{-1} phosphorus and the application of phosphate solubilizing bacteria + Rhizobium was found to be the most promising treatment in enhancing the yield in chickpea.

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