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Effect of Biofertilizers and Phosphorus Levels on Soil Fertility, Yield and Nodulation in Chickpea (*Cicer arietinum* L.)

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The field experiment was conducted to evaluate the effect of biofertilizers and phosphorus (P) levels on soil fertility, yield and nodulation in chickpea at Instructional Farm of Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh, India during *rabi* season 2015-16. The nine treatments comprised of biofertilizers (0, PSB and AM) and three P levels (0, 40 and 60 kg P_2O_5 ha^{-1}) were tested in randomized block design replicated as thrice. The experimental soil having silty loam in texture, pH (1:2.5) 8.24, EC 0.34 dS m^{-1}, organic carbon 3.5 g kg^{-1}, available nitrogen (N) 180, P 18.2 and potassium (K) 226 kg ha^{-1}. The plant spacing was row to row 30 cm and plant to plant 10 cm. The variety PG-186 was taken as a test crop. The yield, dry matter accumulation, number, fresh and dry weight of nodule and availability of N, P and K were increased with the increasing levels of P and with inoculation of biofertilizers. The maximum was received with the application of 60 kg P_2O_5 ha^{-1} followed by 40 kg P_2O_5 ha^{-1}. Among the application of biofertilizers (PSB and AM), the maximum were recorded with the application of AM which was statistically at par with PSB and significantly superior over the without inoculation.

Key words: Phosphorus levels, PSB, AM, fertility, nodulation, yield, chickpea

Pulses play a pivotal role and occupy a unique position in Indian agriculture. It provides protein rich diet to vegetarian mass of the country. Chickpea (*Cicer arietinum* L.) seed contain about 20-22% of protein. Phosphorus (P) is one of the macronutrients for growth and developments of the plants, and it is the second in importance next to nitrogen (N). The P plays a vital role in the metabolism of plants. It is also known as a structural component of nucleic acid, co-enzymes, phosphoproteins and phospholipids present in plants. Phosphorus fertilization is a major input in crop production (Blackshaw *et al.* 2004). It involves in metabolic activities as constituents of nucleoprotein and nucleotide and also imparts a key role in the formation of energy rich bond like adenosine diphosphate (ADP) and adenosine triphosphate (ATP). However, its concentration and solubilization in soil is low due to its interacting nature and hence P is a critical nutrient limiting for plant growth. The P reaction in the soil has important implication for crop growth and fertilizers efficiency. The status and fixation capacity of P in soil strongly influences the availability of P. Pulses are heavy feeders of P because it is constituent of all living organism. It plays vital role in the energy transformation in living tissues. Phosphorus is called mineral of life. Especially in the early stages of plant development, adequate supply of P is required for development of the reproductive parts and has a P positive effect on root growth, early maturity and reduced disease incidence. In many soil types, P is the most limiting nutrient for the production of crops, especially legumes like chickpea which generally having higher P requirement because the process of symbiotic nitrogen fixation consumes a lot of energy.

Arbuscular mycorrizae, more commonly known as AM fungi, has a more appropriate meaning. The term refers to the presence of intracellular structure arbuscles that formed in the roots during various phases of development. The AM are found in root system of most flower plants. The AM fungi increased P uptake by plants in three ways (i) absorption of P from soil by hyphae, (ii) translocation of P along with hyphae, and (iii) the transfer of P to cortical root cells, which is readily used by plant. The AM fungi also increased availability to zinc (Zn), copper (Cu), iron (Fe) and K and N nutrition. The combination of

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AM fungi and P greatly increased the nodulation and N fixation in addition to P uptake and growth. Thus, the increased P uptake by AM stimulates the activity of rhizobium which is depending on an adequate supply of P. Production of growth promoters, tolerance to pathogens and boosting synergistic interaction with beneficial soil microorganisms such as N-fixer and P-solubilizers which are the other advantages associated with the use of AM. This culture is limited because it is an obligate symbiotic and has to be maintained and multiplied on live plants.

Phosphorus solubilizing bacteria (PSB) has been proved as the cheapest source to increase P availability particularly in legumes which enhance productivity of crops. The PSB possess the ability to bring sparingly soluble inorganic or organic phosphates into soluble form by secreting organic acids. Organic acid may chelate Ca, Al, Fe and Mg resulting in effective availability of these elements along with soil P and hence it's higher utilization by plants. Phosphobacterin is found in soils rich in organic matter and low in available P. The ability of PSB to convert insoluble forms of P to an accessible form is an important trait in sustainable farming for increasing plant yields. In particular, PSB is capable of increasing availability of P to plants either by mineralization of organic P or by solubilization of inorganic P by production of acids. The PSB has been considered to have potential use as inoculants or biofertilizer to improve the plant growth and yield. Although, the beneficial effects of PSB on crop productivity have been widely described, but the use of PSB as biofertilizer is scarcely documented in chickpea. Keeping in view the importance of AM and PSB on solubilization of native and added P the present study was taken to evaluate the effect of biofertilizers and P levels on soil fertility, yield and nodulation in chickpea crop.

Materials and Methods

The field experiment was conducted to evaluate the effect of biofertilizers and P levels on soil fertility, yield and nodulation in chickpea crop at Instructional Farm of Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh, India during *rabi* season 2015-16. The experimental soil having silty loam in texture, pH (1:2.5) 8.24, electrical conductivity (EC) 0.34 dS m^{-1}, organic carbon 3.4 g kg^{-1}, available N 180, P 18.2 and K 226 kg ha^{-1}. Nine treatments comprised of three levels P (0, 40 and 60 kg P_2O_5 ha^{-1}) and inoculated with PSB and AM. The recommended dose of N and K was applied through urea and muriate of potash, respectively. The seed treatment was done by PSB @ 25 g kg^{-1} seeds. The treated seeds were kept in shade approximately for two h to get dry; thereafter the seeds were sown in plots as per treatment. The AM fungi were used as soil inoculants at the time of seed sowing. Soil based AM spore populations of soil inoculum of AM fungi were also determined and it was found 175 spores g^{-1} soil. The chickpea variety PG-186 was taken as a test crop.

To assess the various treatment effects, soil sample were collected after harvest of the crop from each plots. Soil pH and EC were determined by following Chopra and Kanwar (1991). Soil organic carbon was determined by Walkley and Black (1934) rapid titration procedure. Soil available N was determined following Subbiah and Asija (1956). Available P was determined by Olsen *et al.* (1954) method. Available K was determined by following Jackson (1973). Plants from 1 m row length were uprooted from sample rows of each plot at 45, 60 75 days after sowing (DAS) and at harvest. After removal of root portion, the samples were first air dried for some days and finally dried in an electric oven at 70 °C and recorded the dry matter yield. The number of nodules per plant was counted at 45, 60 and 75 DAS. For this, five plants were selected randomly in sample rows of each plot and uprooted carefully. The effective root nodules were counted to record average number of nodules per plant. The fresh weight of nodules was weighed just after the removal from the plant root and dry weight were recorded after oven dried root nodules. The data recorded on various parameters were subjected to statistical analysis following analysis of variance technique and were tested at 5% level of significance to interpret the significant differences.

Results and Discussion

Grain and straw yield

The perusal of data (Table 1) revealed that the application of different levels of P recorded significantly higher grain and straw yield. The maximum seed (1.89 t ha^{-1}) and straw yield (2.96 t ha^{-1}) were recorded with application of 60 kg P_2O_5 ha^{-1} which was significantly superior over control and recorded an increase of 17.3 per cent in seed and 14.3 per cent in straw yield, respectively over control. The increase in yield with P application could be ascribed to the overall improvement in plant growth as it plays an important role in plant metabolism and uptake

Table 1. Effect of biofertilizers and phosphorus levels on yield and dry matter accumulation by chickpea

Treatments	Yield (t ha^{-1})		Dry matter accumulation (g $plant^{-1}$)			
	Seed	Straw	45 DAS	60 DAS	75 DAS	At harvest
Phosphorus levels (kg P_2O_5 ha^{-1})						
0	1.61	2.59	1.13	3.85	7.96	13.53
40	1.81	2.84	1.30	4.51	8.97	14.36
60	1.89	2.96	1.44	5.22	10.25	14.96
SEm±	0.04	0.07	0.09	0.35	0.62	0.35
CD (P=0.05)	0.11	0.21	0.29	1.07	1.88	1.06
Biofertilizers						
Un-inoculated	1.67	2.68	1.22	3.93	7.98	13.85
PSB	1.79	2.81	1.29	4.45	8.87	14.17
AM	1.85	2.90	1.37	5.20	10.18	14.83
SEm±	0.04	0.07	0.09	0.35	0.62	0.35
CD (P=0.05)	0.11	0.21	0.29	1.07	1.88	1.06

resulting in better yield (Yadav *et al.* 2002). These results are very close to the findings of Shivakumar *et al.* (2004).

The inoculation of biofertilizer significantly improved the yield of chickpea, among the inoculation of biofertilizer AM fungi was found superior over PSB. The maximum seed (1.85 t ha^{-1}) and straw yield (2.90 t ha^{-1}) were recorded with inoculation of AM which was significantly superior over control and statistically at par with the inoculation of PSB. Soil inoculation with AM and PSB recorded 10.1 and 7.2 per cent in grain and 8.4 and 5.0 per cent in straw yield, respectively over no inoculation. This might be because of more solubility of P and other nutrients which increased the nutrient availability resulted in sufficient formation of photosynthates which promotes the metabolic activities, accelerates cell division and formation of meristem. Similar findings were reported by Chandra and Pareek (2002), Tiwari *et al.* (2005) and Jarande *et al.* (2006).

Dry matter accumulation

The data regarding dry matter accumulation (Table 1) revealed that the dry matter accumulation increased with increasing levels of P up to 60 kg P_2O_5 ha^{-1} with successive increment of days of sowing 45, 60, 75 and at harvest. The maximum dry matter accumulation of 1.44, 5.22, 10.25 and 14.96 g $plant^{-1}$ were recorded at 45, 60, 75 DAS and at harvest, respectively with the application of 60 kg P_2O_5 ha^{-1} which was significantly superior over control and statistically at par with the application of 40 kg P_2O_5 ha^{-1}.

The inoculation of biofertilizers *viz*., PSB and AM influenced in the increment of dry matter accumulation. The maximum dry matter accumulation were received with the inoculation of AM which was significantly higher over uninoculated but statistically at par with PSB. Enhanced vegetative growth in the term of dry matter accumulation $plant^{-1}$ provided more sites for the translocation of photosynthesis and ultimately resulted in increased number of yield attributes. These results are in agreement with the findings of Ram and Dixit (2000) and Arya *et al.* (2002).

Nodulation

It is apparent from the data presented in table 2 that the number of nodules, fresh and dry weight of nodule increased with increasing P levels up to 60 kg P_2O_5 ha^{-1}. The maximum number, fresh and dry weight of nodule were found (8.66 at 45, 10.66 at 60 and 8.66 at 75 DAS), (376 mg at 45, 671 mg at 60 and 383 mg at 75 DAS) and (86.2 mg at 45, 174 mg at 60 and 95.3 mg at 75 DAS), which increased significantly over control and statistically at par with application of 40 kg P_2O_5 ha^{-1}. It was also observed that the significant enhancement in number, fresh and dry weight of nodule due to application of 40 kg P_2O_5 ha^{-1} at 45 DAS over 60 DAS. The reduction were found with the advancement in days after sowing. The increased number of nodules with increasing levels of P may be attributed to the supply of P to the plant roots at various growth stages specially at the time of nodule formation and the application of P with microbial association and its activity improved the number of nodules. The increase in nodulation was highest with 60 kg P_2O_5 ha^{1} when applied with AM. These results are in conformity with those reported by Meena *et al.* (2004) and Das *et al.* (2013). With the inoculation of biofertilizer (PSB and AM), the maximum response in number, fresh and dry weight

Table 2. Effect of biofertilizers and phosphorus levels on nodulation of chickpea

Treatments	Number of nodules plant^{-1}			Fresh weight of nodules (mg)			Dry weight of nodules (mg)		
	45 DAS	60 DAS	75 DAS	45 DAS	60 DAS	75 DAS	45 DAS	60 DAS	75 DAS
Phosphorus levels (kg P_2O_5 ha^{-1})									
0	5.66	6.33	5.66	333.2	620.6	329.8	81.4	128.7	84.4
40	7.33	8.66	7.00	358.7	654.4	359.0	84.0	143.7	87.7
60	8.66	10.66	8.66	376.7	671.4	383.9	86.2	174.2	95.3
SEm±	0.52	0.38	0.57	9.92	7.24	8.16	0.88	1.61	0.61
CD (P=0.05)	1.56	1.16	1.71	29.7	21.7	24.4	2.64	4.84	1.84
Biofertilizers									
Un-inoculated	6.00	6.66	6.33	346.8	637.4	345.2	82.0	142.3	87.6
PSB	7.33	8.66	7.33	357.4	646.1	358.0	83.5	148.0	88.5
AM	8.33	9.66	8.33	384.3	662.9	370.4	85.0	156.3	91.3
SEm±	0.52	0.38	0.57	9.92	7.24	8.16	0.88	1.61	0.61
CD (P=0.05)	1.56	1.16	1.71	29.7	21.7	24.4	2.64	4.84	1.84

of nodule were recorded with the inoculation of AM at 45, 60 and 75 DAS. The perusal of data clearly revealed that the early stage of crop growth at 45 DAS the significant differences were found than without inoculation. However, at later stages of the crop growth at 60 and 75 DAS there was significant differences with soil inoculation of AM over the seed inoculation of PSB. The increased fresh and dry weight of nodules under higher doses of P may be because of beneficial effects of P on number of nodules assuming that P has direct role in biological N fixation in legumes which ultimately increases the weight of nodules. Sufficient amount of P also enhanced the activities of rhizobia and increased the formation of nodules. Phosphorus appears essential for both nodulation and N_2 fixation (Ssali and Kiya 1983). Nodules are strong sinks for P (Hart 1989); N_2 fixation-dependent plants require more P than those supplied with combined N. Nodulation, N_2 fixation, and specific nodule activity are directly related to the P supply (Singleton *et. al* 1985; Jakobson 1985). The increase in fresh and dry weight of nodules was highest with 60 kg P_2O_5 ha ^{1} when applied with AM. Similar findings were also reported by Chandra and Pareek (2002) and Jarande *et al.* (2006).

Available nutrients after harvest of the crop

The data regarding the available plant nutrient after the harvest of the crop (Table 3) revealed that the build-up of available N, P and K increased with the application in increasing levels of P up to 60 kg ha^{-1}. In general, N status increased with increase in the levels of P and biofertilizers (PSB and AM). This might be attributed to the application of P and biofertilizers which enhanced and established better root system. Nutrients possibly stimulate the nodulating bacteria for more fixation of atmosphere N_2 resulting in increase (9.74%) of its contents in the soil over control. Available P content of soil increased after harvesting of chickpea crop by 12.0 per cent over control with increasing the levels of P and biofertilizers application which might be due to favourable condition for availability of nutrients in the soil. Available K content of soil increased (5.36% over control) after harvesting of chickpea with increasing levels of P and biofertilizers application might be due to better establishment of crop which improved the availability of most of the nutrients including K. These results are also in agreement with those obtained by Suri *et al.* (2006).

Table 3. Effect of biofertilizers and phosphorus levels on available nutrients after harvest of chickpea

Treatments	Available nutrient (kg ha^{-1})		
	Nitrogen	Phosphorus	Potassium
Phosphorus levels (kg P_2O_5 ha^{-1})			
0	210.1	19.3	226.3
40	226.6	20.4	232.7
60	230.5	21.6	238.5
SEm±	2.36	0.04	1.02
CD (P=0.05)	7.08	0.13	3.05
Biofertilizers			
Un-inoculated	220.4	20.2	230.4
PSB	222.1	20.4	232.9
AM	224.7	20.6	233.0
SEm±	2.36	0.04	1.02
CD (P=0.05)	7.08	0.13	3.05

Conclusions

On the basis of above discussion it may concluded that the application of 60 kg P_2O_5 ha^{-1} as well as inoculation with AM fungi produced higher yield, dry matter accumulation, number, fresh and dry weight of nodule and build-up of available nutrient status soil. Thus, application 60 kg P_2O_5 ha^{-1} as well as inoculation with AM fungi may be recommended to enhance the profitable cultivation of chickpea for the farmers of eastern Uttar Pradesh.

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