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Effect of phosphorus, Sulphur and biofertilizers on growth attributes and yield of chickpea (C*icer arietinum* L.)

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Abstract
Phosphorous, sulphur and biofertilizers are able to increase the root development, growth, yields and quality of crop plants. In this contests to assess the effect of phosphorus, sulphur and biofertilizers on growth attributes and yield of chickpea (*Cicer arietinum* L.) a field experiment was conducted at the Agronomy Research Farm of Narendra Deva University of Agriculture & Technology, Faizabad (U.P.) during *rabi* season of two consecutive years of 2014-15 and 2015-16. The experiment was layout in SPD having twenty-four treatment combinations consisted of three phosphorus levels (0, 30, 60 kg P_2O_5 ha^{-1}), two sulphur levels (0, 20 kg ha^{-1}) and four seed inoculation with biofertilizers (un-inoculated, PSB, Rhizobium and PSB + Rhizobium). The application of phosphorus at 60 kg ha^{-1}, sulphur at 20 kg ha^{-1} and seed inoculation with PSB + Rhizobium significantly increased the growth, dry weight, number of nodules $plant^{-1}$ and yields (grain & straw yield) of chickpea over the control /un-inoculated. Use of optimum fertilizer level along biofertilizers could able to enhance the performance of crop in terms of growth and yield.

Keywords: Phosphorous, Sulphur, biofertilizers- PSB & *Rhizobium*

Introduction
Pulses are the important source of dietary protein and have exclusive properties of sustaining and restoring soil fertility along with it, consumption of pulses along with cereals increase biological value of protein consumed. For majority of vegetarian in India, pulses are the major source of protein. Chickpea (*Cicer arietimum L.*) belongs to family *fabaceae* originated in southeastern turkey and derived from the greek word ˈ*kikus*ˈ meaning force or strength. Chickpea is known by various name in different countries like- *garbanzo* (Spanish), *pois chiche* (French), *kichar* or *chicher* (German), *chana* (Hindi) and gram or bengal gram (English). In some countries of world (Turkey, Romania, Bulgaria, Afghanistan) it is also called ˈ*nakhut*ˈ or ˈ*nohut*ˈ. Gram is mostly consumed in the form of processed whole seed and *dal* but also used for preparing a variety of snacks, sweets and condiments, which are very useful for stomach ailments and blood purification. Pulses and their crop residues are major source of high quality and nutritive value of livestock feed. Chickpea contains 18-22 per cent protein, 52-70 per cent carbohydrate, 4-10 per cent fat and sufficient quantity of minerals, calcium, phosphorus, iron and vitamins. Besides, it is also important for sustainable agriculture as it improves the physico-chemical and biological properties of the soil. Its deep roots also open the soil, which ensure better aeration and heavy leaf drop increases the organic matter in the soil. It can fix about 25-30 kg N ha^{-1} through symbiosis (Reddy and Reddy, 2005) [19] and these minimize dependency on chemical fertilizers. Thus, chickpea plays a vital role in improving the soil health. In India, Madhya Pradesh (39%), Maharashtra (14%), Rajasthan (14%), Uttar Pradesh (7%), Karnataka (6%), and Gujarat (5%) are the major chickpea growing states which together account for more than 85 per cent of the production.

Production is limited by lack of plant nutrient available in the soil because majority of our farmers hardly use any manure or fertilizer for legume cultivation. However, there is a possibility to enhance the productivity through optimum fertilization and management, as there is a wide gap between the average yield and yield potential of crop. Plant nutrient, suitable cultivars and correct fertilizer have significant effect on yield and yield component (Khan *et al*. 2005) [10]. Generally, Indian soils are lacking in effective and specific strains of Rhizobium which are responsible for symbiotic nitrogen fixation. Phosphorus is regarded as the pioneer plant nutrient needed by the leguminous crops for rapid and proper root development, which later on becomes helpful for better nodulation by *Rhizobium* bacteria.

Sufficient supply of phosphorus to plant, hastens the maturity and increases the rate of nodulation and pod development. It is also an important constituent of vital substances like phospholipids and phosphoprotein. Since legume is heavy feeder of phosphorus, therefore, application of phosphatic fertilizer to chickpea promotes the growth, nodulation and the yield. Phosphorus also imparts hardiness to shoot, improves the quality and regulates the photosynthesis and covers other physico- biochemical process. Most of the phosphorus present in the soil is unavailable to plants which are made available through the activities of efficient micro- organism like bacteria, fungi and even cyanobacteria with production of organic acid and increasing phosphatase enzyme activity.

Sulphur is now recognized as major plant nutrient along with nitrogen (N), phosphorus (P) and potassium (K). It is a key element of higher pulse production, is required in the formation of proteins, vitamins and enzymes. Besides, it is involved in biological nitrogen fixation. Deficiencies of sulphur in Indian soil is widespread due to extensive use of sulphur free fertilizer coupled with extensive cultivation of high sulphur demanding crop, Moreover, sulphur requirement of crop plants is quite high, with high yielding varieties and increased cropping intensity large amounts of nutrients are removed from the soil gradually. Sulphur deficiency is becoming more critical with each passing year which is severely restricting crop yield, produce quality, nutrient use efficiency and economic returns on millions farms. Like any essential nutrient, sulphur also has certain specific function to perform in the plant. thus, sulphur deficiencies can only be corrected by the application of sulphur fertilizer (Tendon & Messick 2007) [37]. Application of fertilizer to alkaline soils has been reported to reduce the pH of soil (Taalab *et al.* 2008) [33].

Biofertilizers may colonizes the rhizosphere and promotes growth by increasing the availability and supply of nutrients and/or growth stimulus to crop. Nitrogen fixer and phosphate solubilizing microorganisms play an important role in supplementing nitrogen and phosphorus to the plant, allowing a sustainable use of nitrogen and phosphate fertilizers (Tambekar *et al*., 2009) [34]. Some important strains are mentioned as plant growth promoting rhizobacteria (PGPR) and that can be used as biofertilizers (Kennedy *et al*., 2004) [9] i.e. *Rhizobium, Pseudomonas, Azospirillum, Azotobacter, Bacillus, Burkholdaria, Erwinia, Mycobacterium, Flavobacterium*, etc. Multi-location trials had indicated that inoculation of seed with phospho-bacterial increased the yield of rice by 10-20 per cent, wheat by 10-40 per cent, bengal gram by 10-30 per cent, and potato by 30-35 per cent over control (Gaur, 1985).

Materials and Methods

Experimental Site

A field experiment was conducted at the Agronomy Research Farm of Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad (U.P.) situated at sub-tropical climate zone of indo-gangetic plains and 26.47^0N latitude and 82.12^0E longitude at an altitude of 113 metres from mean sea level receiving 1200 mm annual rainfall, during *rabi* season of two consecutive years of 2014-15 and 2015-16 to assess the effects of phosphorus, sulphur and biofertilizers on performance of chickpea. The soil of the experimental field was silty loam in texture with low organic carbon (0.31%) and nitrogen (175.40 kg ha^{-1}) and medium in phosphorus (16.30 kg ha^{-1}) and potassium (238 kg ha^{-1}).

Experimental Treatments

The chickpea variety ‘Avrodhi’ was treated with biofertilizers and sown at the rate of 80 kg seed ha^{-1}. In experiment there was twenty-four treatment combinations involved in which three phosphorus levels (0, 30 & 60 kg P_2O_5 ha^{-1}), two sulphur levels (0 & 20 kg ha^{-1}) and four seed inoculation with biofertilizers (un-inoculated, PSB, Rhizobium and PSB + Rhizobium), followed in split plot design with three replications. The experimental plot area was 18 m^2 and inoculated seeds were sown (below 8 cm.) in the plot at row spacing of 30 cm. while, plant to plant spacing was 10 cm. Nitrogen and potash were applied uniformly to each treatment @ 30 and 40 kg ha^{-1} through urea, DAP & MOP. To evaluate the effects of different treatments observation were taken at growing period and harvesting. The grain yields were recorded on plot basis and then converted in to q ha^{-1}.

Results and Discussion

Plant Population

Plant population is directly related to yield of crop, both more and less population than optimum is harmful for production point of view. The data presented in Table 1 (pooled data of 2 years) revealed that no-significant variation was found due to application of different levels of phosphorus & sulphur and inoculation of biofertilizers on plant population of chickpea at 20 DAS but at harvesting stage it affects significantly during both the years. At harvest maximum plant population (29.50) m^{-2} was recorded with application of 60 kg P_2O_5 ha^{-1} and 20 kg sulphur ha^{-1} but inoculation of biofertilizers gave the maximum plant population (29.67) m^{-2}. At harvest stage the plant population increased significantly with the increase in phosphorus levels, it may due to the pronounced effect of higher levels of phosphorus on root growth, which in turn increased the nutrient and moisture extraction from the soil which reduces the mortality and ultimately gave the better plant population at harvest stage.

Growth Attributes

On the basis of data (Table 1) we can say that the maximum plant height was recorded with the highest dose of phosphorus (60 kg P_2O_5 ha^{-1}) and sulpur (20 kg ha^{-1}) which was significantly higher over rest of the doses of P_2O_5 at all the stages during both the years. The application of 60 kg P_2O_5 ha^{-1} and sulphur at 20 kg ha^{-1} increased the plant height about 23.31 & 10.72 percent respectively over control. At harvest stage over un-inoculated treatment 12.03 per cent plant height was increased due to inoculation with PSB + Rhizobium, which was significantly higher than the other but interaction effect of all factors was found non-significant. Increment in plant height may be due to increased uptake of nitrogen and phosphorus by the plants, which was made available through N fixation and P solubilisation by the beneficial microorganisms (Singh *et al*., 2016) [28]. Above results are also in agreement with the findings of Raju and Verma (1984) [17]. Arya *et al*. (2002) [1], Singh *et al*. (2005) [25].

The number of leaves $plant^{-1}$ at various stages of crop growth was influenced significantly (Table 1) by increasing levels of phosphorus at all the stage of crop growth during both years. Application of 60 kg P_2O_5 ha^{-1} recorded higher number of leaves $plant^{-1}$ as compared to other levels of phosphorus. The application of 60 kg P_2O_5 ha^{-1} recorded 8.12 per cent more leaves $plant^{-1}$ over 30 kg P_2O_5 ha^{-1} & 27.28 per cent more over control at 90 DAS while, 30 kg P_2O_5 ha^{-1} was found at par. Sulphur (20 kg ha^{-1}) and PSB + Rhizobium seed inoculation was recorded significantly higher number of leaves plant^{-1} over rest of treatment while non-significant variation was found with interaction of all factors.

Phosphorus (60 kg P_2O_5 ha^{-1}), sulphur (20 kg P_2O_5 ha^{-1}) & inoculation of seed with PSB + Rhizobium was significantly affect the number of branches plant^{-1} while, interaction was non-significant (Table 1). Highest number of branches plant^{-1} was observed with 60 kg P_2O_5 ha^{-1} (29.04 percent) which was at par with 30 kg P_2O_5 ha^{-1}, Sulphur 20 kg ha^{-1} (8.37 percent) and PSB + Rhizobium seed inoculation (20.46 percent) more over control during both years. Phosphorus promotes root growth, cell formation, leaf development, seed formation and accelerates early maturity of crop which may result to increment in branches of chickpea plant.

Analogous to other growth characters the dry weight plant^{-1} increased with increase in age of crop up to harvest stage but maximum increase in dry weight was noticed between 90 DAS and at harvest stage of the crop. Application of 60 kg P_2O_5 ha^{-1}, sulphur 20 kg ha^{-1} and inoculation of chickpea with PSB + Rhizobium recorded significantly higher dry weight over the control while, interaction effect of all factors was found non-significant (Table 1). The maximum dry weight plant^{-1} was recorded with the application of phosphorus 60 kg ha^{-1} (19.38 g at 90 DAS and 33.00 g at harvest), sulphur 20 kg ha^{-1} (17.07 g at 90 DAS and 21.56 g at harvest) and seed was inoculated with the PSB + Rhizobium (18.00, 31.56 g at 90 DAS & harvest stages respectively) during both the years. This might be due to the fact that phosphorus being an energy bond compound and its major role is transformation of energy essential for almost all metabolic processes *viz*., photosynthesis, respiration, cell elongation and cell division, activation of amino acids for synthesis of protein and carbohydrate metabolism which ultimately increase all the growth attributes and dry weight of plants. Similar results have also been reported by Saraf *et al.* (1997) [21] Singh and Singh (1997) and Singh *et al.* (2010) [26, 30].

Above raise in growth attributes may be due to action of different factors, phosphorus increases the resistance in plant against diseases and offsets injurious effect of other nutrients. In the absence of phosphorus, the vital metabolic processes like phosphorylation and carbohydrate transformation are adversely affected (Stewart and Williams, 1942; Dean and Fried, 1952) [6]. Growth increment was also reported by Kharche *et al.* (2006) [11] and Tiwari *et al*. (2005) [35] due to use of sulphur. Application of PSB + Rhizobium increase the availability of nutrients like N and P and more availability of nutrients resulted an increase in physiological processes *viz*., cell elongation, cell division and formation of meristematic tissues which ultimately enhanced growth attributes and dry matter production. Similar results have also been reported by Karwasra (2007), Chaudhary *et al.* (2005) [8, 1].

Table 1: Plant population, plant height, number of leaves per plant, number of branch per plant and dry weight of chickpea as influenced by different levels phosphorus & sulphur and inoculation of biofertilizers (pooled data of 2 years)

Treatment	**Plant population(m^{-2})**		**Plant Height (cm)**	**Number of leavesplant^{-1}**	**Number of branch plant^{-1}**	**Dry weightplant^{-1}(g)**	
	20 DAS	**At harvest**	**At harvest**	**90 DAS**	**90 DAS**	**90 DAS**	**At harvest**
Phosphorus (P_2O_5) levels (kg ha^{-1})							
0	30.38	27.52	33.54	834.37	11.65	14.54	26.12
30	30.75	29.00	38.41	1054.21	14.71	17.15	31.94
60	30.50	29.50	43.74	1147.42	16.42	19.38	33.00
SEm±	0.54	0.47	0.73	17.02	0.24	0.28	0.43
CD (P=0.05)	NS	1.50	2.30	53.64	0.75	0.88	1.37
Sulphur levels (kg ha^{-1})							
0	30.50	28.01	36.38	971.52	13.83	16.34	29.14
20	30.58	29.50	40.75	1052.48	14.69	17.70	31.56
SEm±	0.44	0.39	0.59	13.89	0.19	0.23	0.35
CD (P=0.05)	NS	1.22	1.88	43.80	0.61	0.72	1.11
Bio-fertilizers							
Un-inoculation	29.83	27.33	35.73	950.16	12.40	16.04	28.75
PSB	30.83	29.33	38.21	1002.97	14.20	16.97	29.53
Rhizobium	30.67	29.50	39.69	1026.54	14.85	17.06	31.56
PSB+ Rhizobium	30.83	29.67	40.62	1068.32	15.59	18.01	31.56
SEm±	0.51	0.42	0.75	17.02	0.24	0.28	0.45
CD (P=0.05)	NS	1.21	2.15	48.82	0.69	0.81	1.30
Intractions	NS	NS	NS	NS	NS	NS	NS

Nodulation

Phosphorus is an important nutrient which directly affect the nodulation of legume and pulses. It is clear from the data given in Table 2 that the number of nodules plant^{-1} increased consistently with increasing doses of phosphorus upto 60 kg P_2O_5 ha^{-1}. The number of nodules plant^{-1} increased with the advancement in the age of crop plant upto 60 DAS. The data revealed that the maximum number of nodules plant^{-1} (36.61 & 36.89 at 60 DAS and 90 DAS, respectively) was counted under the effect of 60 kg P_2O_5 ha^{-1}. More number of nodulation was found (33.07 & 29.95 at 60 DAS and 90 DAS, respectively) where the application of 20 kg S ha^{-1} and (35.24 & 32.29 at 60 DAS and 90 DAS, respectively) was counted under inoculation of PSB + Rhizobium. On the basis of data, we can say that both sulphur and biofertilizers increase nodules in plant roots significantly while, interaction effect of all factors was found non-significant. Fresh weight & dry weight of nodules plant^{-1} was also improved significantly due to increased level of phosphorus (Sinde and Sarfi, 1992; Tomar and Raghu, 1994; Sarkar *et al*., 1995, sulphur (Saeed *et al*., 2004) [36, 22, 8] and biofertilizers inoculation.

This might be due to the fact that application of phosphorus results profuse growth of roots which ultimately resulted formation of more number of nodules of large size. PSB + Rhizobium inoculation significantly increased the number of nodules and fresh & dry weight of nodules plant^{-1} mainly due to the fact that the nitrogenase enzyme present in the bacteria gets introduced through infection causes nodule formation. Application of PSB facilitates the root development *vis-à-vis* nodule formation and proper development of nodules by increasing the availability of phosphorus through the mobilizing the unavailable phosphorus present in the soil.

Similar findings have also been reported by Yadav *et al.* (1994), Bhuiyan *et al.* (1998) and Swarnkar *et al.* (2010) [38, 32].

Yields

Different doses of phosphorus, sulphur and seed inoculation with biofertilizers significantly influenced the grain yield of chickpea during both the years but non-significant variation was found due to interaction of all factors. It is evident from the data (pooled) given in Table 2 that the maximum grain yield (21.76 q ha^{-1}) was recorded with the application of 60 kg P_2O_5 ha^{-1}, (21.51 q ha^{-1}) was with the application of 20 kg S ha^{-1} and (21.53 q ha^{-1}) with inoculation of PSB + Rhizobium which was significantly higher over control.

It is clear from the data given in Table 2 that the maximum straw yield (25.06 q ha^{-1}) was recorded with application of 60 kg P_2O_5 ha^{-1} which was significantly higher over 30 kg P_2O_5 ha^{-1} and control. Straw yield was also found significant due to application of 20 kg S ha^{-1} and seed inoculation with PSB + Rhizobium. The maximum straw yield by the application of S (24.82 q ha^{-1}) was found with 20 kg S ha^{-1} and PSB + Rhizobium seed inoculation.

Phosphorous application accelerated the production of photosynthates and their translocation from source to sink, which ultimately gave the higher values of yield contributing characters. Increase in yield contributing characters has also been reported by Raju *et al*. (1991); Tomar and Raghu (1994); Bahadur *et al*. (2002); Khan *et al.* (2005); Meena *et al.* (2006) and Kumar *et al.* (2007) [18, 36, 2, 10, 13, 12] with increasing levels of phosphorus and sulphur (Chaudhary and Goswami, 2005) [5]. The increase in yields with biofertilizers was mainly due to the increase in almost all growth and yield contributing characters, which ultimately resulted a significant increase in grain and straw yields. The results of present investigation are in close conformity with those of Bhuiyan *et al.* (1998), Singh *et al.* (1983) [29] and Sarna *et al.* (2008) [23]. This was mainly due to fact that the better availability of N and P caused well developed root system having higher nitrogen fixing capacity resulting better growth and development of plants and better diversion of photosynthates towards sink, even use of single or combination of biofertilizers might be much advantageous for farmers (Singh *et al*., 2017) [27]. The findings of Bahadur *et al.* (2002) [2]; Meena *et al.* (2002) [13] and Parmanik and Singh (2003) [16] also confirm the results obtained during the study on yield contributing characters of chickpea.

Table 2. Number of nodules, fresh weight of nodules, dry weight of nodules and yields (grain and straw) of chickpea as influenced by different levels phosphorus & sulphur and inoculation of biofertilizers (pooled data of 2 years)

Treatment	**Number of nodules plant^{-1}**		**Fresh weight of nodules plant^{-1} (g)**		**Dry weight of nodules plant^{-1} (g)**		**Grain yield (q ha-1)**	**Straw Yield (q ha-1)**
	60 DAS	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS	90 DAS	90 DAS
Phosphorus levels (kg ha^{-1})								
0	25.43	20.34	2.95	2.47	0.53	0.41	18.75	21.58
30	33.36	29.17	3.45	2.96	0.67	0.50	20.10	22.97
60	36.61	36.89	4.12	3.28	0.79	0.59	21.76	25.06
SEm±	0.45	0.52	0.05	0.04	0.01	0.009	0.34	0.37
CD (P=0.05)	1.42	1.65	0.16	0.15	0.03	0.027	1.10	1.18
Sulphur levels (kg ha^{-1})								
0	30.53	27.65	3.37	2.79	0.63	0.48	18.89	21.58
20	33.07	29.95	3.65	3.0	0.69	0.52	21.51	24.82
SEm±	0.36	0.42	0.04	0.04	0.008	0.007	0.28	0.30
CD (P=0.05)	1.16	1.34	0.16	0.12	0.02	0.022	0.89	0.96
Bio-fertilizers								
Un-inoculation	27.94	24.35	3.21	2.37	0.59	0.39	19.07	21.81
PSB	30.45	28.61	3.43	2.94	0.65	0.50	19.70	22.50
Rhizobium	33.57	29.95	3.62	3.11	0.68	0.54	20.50	23.66
PSB+ Rhizobium	35.24	32.29	3.77	3.20	0.72	0.57	21.53	24.82
SEm±	0.47	0.47	0.05	0.05	0.01	0.024	0.34	0.39
CD (P=0.05)	1.35	1.36	0.15	0.14	0.02	0.009	1.00	1.12
Intractions	NS	NS	NS	NS	NS	NS	NS	NS

Conclusion

Pulses are most important crop for nutritional security because of its high protein content. Along with this they also play an important role in soil health improvement by nutrient fixation and increment of microbial activity but due to low productivity farmers do not want to grow pulse crops. On the basis of present investigation, it may conclude that with the application of adequate and balance plant nutrients along with suitable biofertilizers can enhance the growth and yield of crop. Most of the Indian soils are poor in phosphorus status because of higher rate of fixation of phosphorus, PSB solubilize these unavailable P and make it available for plants and rhizobium fix atmospheric N in to the root for plant use. So, use of biofertilizers may reduce the application of chemical fertilizers and we get healthy, pollution free production for better future of our increasing populations.

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