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Effect of bio-fertilizer and phosphorus on growth and yield of cowpea

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Abstract
A field experiment was conducted during the *kharif* season 2023, at the Crop Research Farm, Department of Agronomy, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj, (U.P.) to determine the "Effect of Bio-fertilizers and Phosphorus on Growth and Yield of Cowpea". The results revealed that application of Rhizobium + Phosphorus 60kg/ha (Treatment 3) recorded significantly higher plant height (59.27 cm), maximum number of branches/plant (8.87), maximum number of nodules/plant (16.53), higher plant dry weight (65.87g), higher crop growth rate (8.28 g/m^2/day), higher relative growth rate (0.005 g/g/day), maximum number of pods/plant (34.33), maximum number of seeds/pod (10.73), higher test weight (g) (34.48), higher grain yield (1.55 t/ha), higher stover yield (2.97 t/ha) higher harvest index (34.27%) were recorded in treatment 3 (Rhizobium + Phosphorus 60 kg/ha). The maximum gross return, net return and benefit cost ratio were also recorded with application of Rhizobium + Phosphorus 60 kg/ha (treatment 3) was found to be productive as well as economically feasible.

Keywords: Biofertilizers, cowpea, growth, phosphorus, rhizobium, yield

1. Introduction
Cowpea (*Vigna unguiculata* L.) commonly as lobia known in India is one of the important kharif pulse crops grown for vegetable, grain, forage and green manuring. This crop has great importance because of availability of short duration, high yielding and quick growing variety. Green tender pods are used as vegetable; the vegetable cowpea pods contain moisture 84.6%, protein 4.3%, carbohydrate 8.0% and fat 0.2%. Cowpea is commonly grown in sub- tropical regions that are moderately humid and warm. It is more drought resilient however it is not tolerant to frost and waterlogging. Seeds of cowpea are nutritious and cheap source of quality protein, vitamins, iron, phosphorus as well as an excellent substitute for eggs, meat and other protein rich foods thus they are significant part of human diet.

Globally cowpea is grown across the world on an area of 14.5 mha of land planted each year and the total annual production is 6.2 mt with a productivity of about 6.9 mt (USDA, 2024) [33]. In India, pulses are grown nearly in 25.43 m ha with an annual production of 17.28 mt and productivity of 679 kg/ha. The per capita availability of pulses in India is 35.5g/day as against the minimum requirement of 70g/day/capita as advocated by the Indian Council of Medical Research. In India during 2022-23 cowpea grew about 13.3 mha with an annual production of 8.06 m t and productivity of 596 kg/ha. Some of the states like Uttar Pradesh cowpea is cultivated in about 2.38 m ha with an annual production of 2.56 and productivity of 1079 kg/ha major producer of cowpea in India (GOI, 2023) [12].

The available phosphorus and nitrogen condition of Indian soils are classified as low to medium. Phosphorus deficiency is usually the key factor for the seed yield of pulse crops on all soil types. Applying phosphorus to pulse crops can result in a residual impact of 20–35 kg/ha of phosphorus, which can significantly increase the production of pulses (Khanna *et al.* 2019) [18]. In the current situation, when farmers would rather not use artificial fertilizers, concentrating on locally accessible sources of nutrients is a simple solution. At the moment, one of the biggest challenges facing agricultural experts is cutting back on the usage of costly chemical fertilizers, which have detrimental effects on both the environment and human health.

Large-scale soil N replenishment is frequently achieved through the use of chemical fertilizers, which are expensive and seriously pollute the environment (Barakzai *et al*. 2020) [6].

Biofertilizers may mobilize the nutrients needed by plants through natural processes including atmospheric N fixation and P solubilization. They can also be obtained from plant nodules or the rhizosphere and stimulate plant development by releasing hormones and anti-metabolites (Bhardwaj *et al*. 2014) [8]. It also increases the phosphorus availability to the plants through reducing P fixation, thus enhancing soil fertility (Arbad and Ismail, 2011) [5]. It has been used to promote sustainable agriculture through replacing chemical fertilizers and enhanced shelf life without affecting the ecosystem adversely (Sahoo *et al.* 2014) [26]. The symbiotic biological nitrogen fixation bacterium Rhizobium needs phosphorus for growth and survival in the soil, colonization of the rhizosphere, infection and nodule development, and energy transformation during nitrogen fixation in root nodules (O, Hara *et al.* 1988) [14]. Rhizobium is predominantly used for inoculating pulses seeds which increases the N availability to plants and boost the growth as well as yield of the crop (Korir *et al.* 2017) [20] since it establishes symbiotic relationship with legume crops through nodulation and N fixation (Khuntia *et al.* 2022) [19].

Phosphate Solubilizing Bacteria (PSB) plays a vital role in supplementing P required by the crop. It helps in dissolving the interlocked phosphates by bringing out more amounts of unavailable and fixed phosphates in soil into soluble form and makes it available to plants (Elhaissoufi *et al*. 2022) [11]. The use of phosphatic fertilizers in combination with PSB has been observed to solubilize phosphate in the soil, enhance plant absorption of phosphorus, and thus improve crop output (Kalayu, 2019) [16]. Phosphate Solublizing Bacteria (PSB) have the consistent capacity to increase the availability of phosphate to plant by mineralizing organic phosphorus compounds (Beg and Singh 2009) [7]. Dual inoculation of Rhizobium and phosphate solubilizing bacteria (PSB) may help the plant to acquire both N and P.

Azotobacter directly stimulates plant development by secreting large quantities of physiologically active compounds, such as biotin, gibberellic acid, nicotinic acid, pentothenic acid, B vitamins, indole-3-acetic acid (IAA), and cytokinin (Oskar *et al.* 2014) [23] and ammonia (Narula and Gupta, 1986) [22] or indirectly by protecting the plant from diseases. Additionally, Azotobacter stimulates the synthesis of phytohormones, which improves stress tolerance, N_2 fixation, nutrient uptake stimulation, and pathogen biocontrol. These actions increase the host plant's availability and supply of primary nutrients.

Application of phosphorus has been found very effective altogether soil types and called as vital element for increasing the yield. Aside from its essential role in growth and development of roots, phosphorus is important for growth of Rhizobium bacteria liable for biological N fixation to extend the efficiency of pulses as soil renovator and serves the twin purpose of accelerating yield of main also as succeeding crop. It also improves the standard of grain. It plays a vital role in energy storage and transfer. Phosphorus may be a constituent of nucleic acids (DNA and RNA) and majority of enzymes which are of great importance within the transformation of energy in carbohydrate metabolism and respiration of plants. Phosphorus stimulates the symbiotic organic process because in presence of phosphorus bacterial cell becomes mobile which is pre requisite for migration of bacterial cell to plant organ for nodulation (Charel, 2006) [9]. Phosphorus helps in proper root development which increases root nodules and consequently increases organic process. It develops anion adsorption and releases sulphate ions into the soil solution (Tiwari and Gupta, 2006) [30]. Thus, it's going to be subjected to leaching if not haunted by plant roots. Keeping in view of the above fact, the experiment was conducted to find out "Effect of bio-fertilizer and phosphorus on growth and yield of cowpea*"*.

2. Materials and Methods

The experiment was conducted during *Kharif* season 2023 at Crop Research Farm, Department of Agronomy, SHUATS, Prayagraj (U.P). The soil of the experimental field was sandy loam in texture, nearly neutral in soil reaction pH 7.6, low level of organic carbon (0.63%), available N (224 Kg/ha), P (38.2 kg/ha), K (240.7 kg/ha) and zinc (2.32 mg/kg). The treatment consisted of seed treatment with Rhizobium (20 g/kg seed), PSB (Phosphate Solubilizing Bacteria) – 20 g/kg seed and Azotobacter – 20 g/kg seed and three levels of phosphorus (40, 50 and 60 kg/ha). The experiment was laid out in RBD with 10 treatments each replicated thrice. The treatment combinations are T_1- Rhizobium + Phosphorus (40 kg/ha), T_2- Rhizobium + Phosphorus (50 kg/ha), T_3- Rhizobium + Phosphorus (60 kg/ha), T_4- PSB + Phosphorus (40 kg/ha), T_5- PSB + Phosphorus (50 kg/ha), T_6- PSB + Phosphorus (60 kg/ha), T_7- Azotobacter + Phosphorus (40 kg/ha), T_8- Azotobacter + Phosphorus (50 kg/ha), T_9- Azotobacter + Phosphorus (60 kg/ha), T_{10}- Control NPK (25:50:25 kg). Data recorded on different aspects of crop, *viz*., plant height, number of branches/plant, number of nodules/plant, plant dry weight, CGR, RGR, yield attributes and yield were subjected to statistically analysed by analysis of variance method as described by (Gomez and Gomez, 1976) [13].

3. Results and Discussion

3.1 Growth Attributes

3.1.1 Plant height (cm)

The data showed that treatment 3 [Rhizobium + Phosphorus (60 kg/ha)] recorded significant and higher plant height (59.27 cm). However, treatment 6 [PSB + Phosphorus (60 kg/ha)] was found statistically at par with treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. Significant and higher plant height was observed with application of Rhizobium might be due to its ability to fix atmospheric nitrogen, enhanced availability and transportation of nitrogen, which influences photosynthetic organs development of photosynthetic organs. These results are in conformity with findings of Khanna *et al.* (2019) [18]. Further, significantly increased plant height was recorded with application of phosphorus (60 kg/ha) might be due to increased carbohydrate accumulation and their remobilization to reproductive parts of the plants, being the closest sink and hence, resulted in increased plant growth. Similar result was reported by Khandelwal *et al*. (2012) [17].

3.1.2 Number of branches/plant

The results showed that significant and maximum number of branches/plant (8.87) was recorded in treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. However, treatment 6 [PSB + Phosphorus (60 kg/ha)] was found to be statistically at par with treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. Significant and higher plant height was observed with application of rhizobium might be due to it preserve a suitable ratio between the nutrients given to the plant for maximum growth along with chlorophyll production and elongation. These results were collaborated with Tripathi *et al.* (2021) [32]. Further, significantly increased in number of branches/plant was recorded with application of phosphorus (60 kg/ha) might be due to phosphorus plays an important role in photosynthetic reactions in plant which resulted in increased growth and development, involvement in cell division, elongation, multiplication and development. These results are in conformity with findings of Khanna *et al.* (2019) [18].

3.1.3 Number of nodules/plant

The results revealed that significant and maximum number of nodules/plant (16.53) was recorded in treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. However, treatment 6 [PSB + Phosphorus (60 kg/ha)] was found to be statistically at par with treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. Significant and higher plant height was observed with application of rhizobium might be due to emphasized longer roots that enhanced mungbean nodulation which aids in atmospheric nitrogen fixation by means of Rhizobium nitrogenase activity. These results were similar to Ahmad *et al.* (2013) [2]. Further, significantly increased in number of nodules/plant was with the application of phosphorus (60 kg/ha) might be due to phosphorus initiates the nodule formation as well as influence the efficiency of Rhizobium and legume symbiosis and thereby enhancing the nitrogen fixation. These results are in conformity with findings of Khanna *et al.* (2019) [18].

3.1.4 Plant dry weight (g)

The results showed that significant and higher plant dry weight (75.06 g) was recorded treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. However, treatment 6 [PSB + Phosphorus (60 kg/ha)] was found to be statistically at par with treatment 3 [Rhizobium + Phosphorus (60 kg/ha)].

Significant and higher plant dry weight was with the application of Rhizobium inoculation might be due to increased nodulation and a faster rate of nitrogen fixation, which may have aided in plant development through symbiotic processes. Similar results were noticed by Rajib *et al*. (2013) [25]. Further, significantly increased in plant dry weight was with the application of phosphorus (60 kg/ha) might be due to its essentiality for the symbiotic biological N-fixation process, enhanced microbial activity in nodules, early root development, and root proliferation which additionally resulted in more effective and efficient metabolite partitioning and sufficient photosynthate and nutrient translocation for the development of reproductive structures. These results are in close conformity with Verma *et al.* (2015) [34].

3.1.5 Crop Growth Rate (g/m^2/day)

The results revealed that during 60-75 interval DAS, no significant difference was recorded among all the treatments. Statistically highest crop growth rate was recorded in treatment 3 [Rhizobium + Phosphorus (60 kg/ha)].

3.1.6 Relative Growth Rate (g/g/day)

The results showed that during 60-75 interval DAS, no significant difference was recorded among all the treatments. Statistically highest relative growth rate was recorded in treatment 3 [Rhizobium + Phosphorus (60 kg/ha)].

3.2 Yield and Yield Attributes

3.2.1 Number of pods/plant

The results showed that treatment 3 [Rhizobium + Phosphorus (60 kg/ha)] recorded significant and maximum number of pods/plant (34.33). However, treatment 6 [PSB + Phosphorus (60 kg/ha)] and treatment 9 [Azotobacter + Phosphorus (60 kg/ha)] were found to be statistically at par with treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. The significant and maximum number of pods/plant was observed with the application of rhizobium inoculation might be due to enhanced photosynthate generation, which will enhance flower viability and lead to the formation of more number of pods. Similar results were noticed by Khanna *et al.* (2019) [18]. Further, significantly increased in number of pods/plant was recorded with the application of phosphorus (60 kg/ha) increased root growth, improved nutrient availability and absorption, energy conversion, improved root development, and various kinds of metabolic processes. These results are in close conformity with Abraham *et al.* (2021) [1].

3.2.2 Number of seeds/pod

The results revealed that treatment 3 [Rhizobium + Phosphorus (60 kg/ha)] recorded significant and maximum number of seeds/pod (34.33). However, treatment 6 [PSB + Phosphorus (60 kg/ha)] was found to be statistically at par with treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. The significant and higher seeds/pod was observed with the application of rhizobium might be due to Rhizobium may have produced more metabolites and transported them to various sinks, especially the productive structures like pods and seeds, as well as having an increased broad root system and higher nodulation. Similar results were noticed by Verma *et.al.* (2022) [35]. Further, significantly increased in number of seeds/pod was recorded with the application of phosphorus (60 kg/ha) might be due to its necessity for the formation of new shoots, branches, and pods on plants since it controls the processes of photosynthesis and carbohydrate metabolism, which are thought to be the primary factors limiting growth, particularly during the reproductive season. These results are in close conformity with Tripathi and Parashar (2020) [31].

3.2.3 Test weight (g)

The results showed that treatment 3 [Rhizobium + Phosphorus (60 kg/ha)] recorded significant and higher test weight (34.48g). However, treatment 6 [PSB + Phosphorus (60 kg/ha)] was found to be statistically at par with treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. The significant and higher test weight was observed with the application of rhizobium inoculation might be due to more productive characteristics, which enhance the absorption of more photosynthates in plants in a limited number of sinks (seeds), increasing the size of the seeds and therefore influencing the test weight. Similar results were noticed by Meera *et al.* (2022) [21]. Further, significantly increased in test weight was recorded with the application of phosphorus (60 kg/ha) improved crops plant development and growth, which may have enhanced the amount of assimilates available for seed, which eventually grew greater weight. These results are in close conformity with Ali *et al.* (2004) [3].

3.2.4 Grain yield (t/ha)

The results showed that treatment 3 [Rhizobium + Phosphorus (60 kg/ha)] recorded significant and higher grain yield (1.55 t/ha). However, treatment 6 [PSB + Phosphorus (60 kg/ha)] and treatment 9 [Azotobacter + Phosphorus (60 kg/ha)] were found to be statistically at par with treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. increased soil nitrogen availability during crop growth and increased nitrogen fixation from the environment, as well as by growth-regulating compounds produced by biofertilizer. Similar results were noticed by Singh and Singh (2017) [28]. Further, significantly increased in grain yield was recorded with the application of phosphorus (60 kg/ha) might be due to its necessity for root development, which leads to enhanced glucose synthesis, photosynthetic translocation, and nitrogen fixing for higher crop yields. These results are in close conformity with Abraham *et al.* (2021) [1].

3.2.5 Stover yield (t/ha)

The results revealed that treatment 3 [Rhizobium + Phosphorus (60 kg/ha)] recorded significant and higher stover yield (2.97 t/ha). However, treatment 6 [PSB + Phosphorus (60 kg/ha)] was found to be statistically at par with treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. better nutrient absorption, which facilitates the transport of photosynthates from source to sink and increases leaf photosynthetic activity. Similar results were noticed by Singh *et al.* (2024) [29]. Further, significantly increased in stover yield was recorded with the application of phosphorus (60 kg/ha) might be due to phosphorus increased plant biomass output, nodule weight and number, and leaf chlorophyll content all strongly positively correlated with stover yield. These results are in close conformity with Prajapati *et al.* (2013) [24].

3.2.6 Harvest index (%)

The results showed that treatment 3 [Rhizobium + Phosphorus (60 kg/ha)] recorded significant and higher harvest index (34.27%). However, treatment 4 [PSB + Phosphorus (40 kg/ha)], treatment 6 [PSB + Phosphorus (60 kg/ha)], treatment 7 [Azotobacter + Phosphorus (40 kg/ha)] and treatment 9 [Azotobacter + Phosphorus (60 kg/ha)] were found to be statistically at par with treatment 3 [Rhizobium + Phosphorus (60 kg/ha)]. The significant and higher harvest index was observed with the application of rhizobium inoculation might be due to photosynthesis is enhanced by physiological activity, which is then transferred to different regions of the plant where it provides economic advantages. Similar results were noticed by Jamir *et al.* (2022) [15]. Further, significantly increased in harvest index was recorded with the application of phosphorus (60 kg/ha) enhanced cell activity, enhanced cell growth and multiplication, luxurious growth, and yield characteristics of the crops; however, because of enhanced nutrient absorption and utilization, enhanced crop growth overall and mirrored source-sink relationships, ultimately enhancing seed and stover yield. These results are in close conformity with Anand *et al.* (2022) [4].

3.3 Economics

The results revealed that maximum gross returns (174457.00), net returns (113077.70) and B:C ratio (1.84) was recorded in treatment 3 [Rhizobium + Phosphorus (60 kg/ha)] as compared to other treatments. Higher B:C ratio was observed with the application of Rhizobium along with Phosphorus (60 kg/ha) might be attributed to greater increase in grain and straw yield as compared to cost of cultivation with increasing levels of phosphorus. These results are in close conformity with the findings of Khanna *et al.* (2019) [18].

Table 1: Effect of biofertilizers and phosphorus on growth attributes of cowpea.

Sr. No.	Treatment Combinations	Plant height (cm)	Number of branches/plant	Number of nodules/plant	Plant dry weight (g)	Crop Growth Rate ($g/m^2/day$)	Relative Growth Rate (g/g/day)
1	Rhizobium + Phosphorus (40 kg/ha)	44.20	5.87	12.73	62.52	11.09	0.0113
2	Rhizobium + Phosphorus (50 kg/ha)	45.60	6.73	8.27	63.00	17.91	0.0091
3	Rhizobium + Phosphorus (60 kg/ha)	59.27	8.87	16.53	75.06	17.60	0.0132
4	PSB + Phosphorus (40 kg/ha)	47.27	6.87	10.27	65.82	10.57	0.0118
5	PSB + Phosphorus (50 kg/ha)	42.40	7.07	10.07	64.23	16.36	0.0094
6	PSB + Phosphorus (60 kg/ha)	57.80	8.71	16.00	74.51	11.27	0.0113
7	Azotobacter + Phosphorus (40 kg/ha)	44.80	6.20	9.27	65.07	15.09	0.0102
8	Azotobacter + Phosphorus (50 kg/ha)	46.47	7.27	11.07	63.54	10.79	0.0126
9	Azotobacter + Phosphorus (60 kg/ha)	52.87	7.93	13.67	67.64	14.45	0.0090
10	Control NPK (25:50:25 kg/ha)	37.73	5.13	7.27	45.89	10.82	0.0074
F Test		S	S	S	S	NS	NS
S.Em (±)		0.35	1.10	0.25	0.77	0.97	0.001
CD (p=0.5)		1.05	2.32	0.74	2.30	-	-

Table 2: Effect of biofertilizers and phosphorus on yield and yield attributes of cowpea.

Treatment No.	Treatment Details	Yield and yield attributes					
		Number of pods/plant	Number of seeds/ pod	Test weight (g)	Grain yield (t/ha)	Stover yield (t/ha)	Harvest index (%)
1	Rhizobium + Phosphorus (40 kg/ha)	26.13	7.87	28.91	1.24	2.76	31.05
2	Rhizobium + Phosphorus (50 kg/ha)	27.93	7.20	28.77	1.29	2.81	31.53
3	Rhizobium + Phosphorus (60 kg/ha)	34.33	10.73	34.48	1.55	2.97	34.27
4	PSB + Phosphorus (40 kg/ha)	30.40	7.73	30.67	1.41	2.82	33.31
5	PSB + Phosphorus (50 kg/ha)	29.20	7.80	30.79	1.27	2.83	31.00
6	PSB + Phosphorus (60 kg/ha)	33.60	10.50	33.97	1.51	2.91	34.14
7	Azotobacter + Phosphorus (40 kg/ha)	29.87	8.07	28.60	1.34	2.76	32.75
8	Azotobacter + Phosphorus (50 kg/ha)	30.87	8.40	29.10	1.29	2.76	31.77
9	Azotobacter + Phosphorus (60 kg/ha)	31.07	9.40	29.81	1.47	2.84	34.08
10	Control NPK (25:50:25 kg/ha)	22.40	6.07	25.32	1.07	2.55	29.53
F. Test		S	S	S	S	S	S
S.Em		1.13	0.33	0.54	3.49	0.04	0.59
CD (p=0.5)		3.36	0.99	1.62	0.08	0.12	1.75

Table 3: Effect of biofertilizers and phosphorus on economics of cowpea.

Treatments No.	Treatment Details	Economics			
		Total cost of cultivation (INR/ha)	Gross return (INR/ha)	Net returns (INR/ha)	B:C ratio
1	Rhizobium + Phosphorus (40 kg/ha)	57879.00	141188.00	83309.33	1.44
2	Rhizobium + Phosphorus (50 kg/ha)	59629.00	146702.00	87072.67	1.46
3	Rhizobium + Phosphorus (60 kg/ha)	61379.00	174457.00	113077.70	1.84
4	PSB + Phosphorus (40 kg/ha)	57832.60	158962.00	101129.10	1.75
5	PSB + Phosphorus (50 kg/ha)	59582.60	144227.00	84644.07	1.42
6	PSB + Phosphorus (60 kg/ha)	61332.60	169987.00	108654.10	1.77
7	Azotobacter + Phosphorus (40 kg/ha)	57927.00	151980.00	94053.00	1.62
8	Azotobacter + Phosphorus (50 kg/ha)	59677.00	145860.00	86183.00	1.44
9	Azotobacter + Phosphorus (60 kg/ha)	61427.00	165868.00	104441.30	1.70
10	Control NPK (25:50:25 kg/ha)	60063.80	121943.00	61879.53	1.03

4. Conclusion
It is concluded that with the application of Rhizobium along with Phosphorus 60 kg/ha (Treatment 3), was observed with higher growth attributes, yield attributes and benefit-cost ratio in cowpea.

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