



## Growth and Yield of Cowpea (*Vigna unguiculata* L.) Under Different Levels of Phosphorous and Seed Inoculation with Rhizobium

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Received: May 15, 2023  
Revised: June 27, 2023  
Published: July 10, 2023

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The authors declare that there is no conflict of interest.

### ABSTRACT

Cowpea (*Vigna unguiculata* L.) is a major source of protein and an important component of Nepal's cropping systems. However, yields are very low due to a lack of improved cultivars, poor management practices, and limited input use. The objective of this study was to evaluate the effects of rhizobia inoculant and P on cowpea growth and yield. A field study was carried out in Bharatpur-11, Chitwan, Nepal, during the spring season of 2022, using a randomized complete block design with three replications and eight treatments. The treatments included four different phosphorus doses (20, 40, 60, and 80 kg ha<sup>-1</sup>) and seed inoculation (un-inoculated and inoculated). The rhizobium-inoculated plants produced significantly higher grain yield (1.27 t ha<sup>-1</sup>) and various yield-attributing parameters than un-inoculated plants. Phosphorus fertilizer at 40 kg ha<sup>-1</sup> produced a higher grain yield (1.41 t ha<sup>-1</sup>) than other phosphorus doses. The combination of rhizobium inoculation and application of phosphorus at the rate 40 kg ha<sup>-1</sup> yielded the highest grain yield (1.53 t ha<sup>-1</sup>). The results show that phosphorus dose of 40 kg ha<sup>-1</sup> combined with Rhizobium inoculation with seed has the potential to improve cowpea grain yield.

**Keywords:** Cowpea, growth, yield traits, *Rhizobium leguminosarum*

### How to cite this article:

Poudel A, BB Adhikari and J Shrestha. 2023. Growth and Yield of Cowpea (*Vigna unguiculata* L.) under different levels of Phosphorous and Seed Inoculation with Rhizobium. Agronomy Journal of Nepal. 7(1): 9-15.  
DOI: <https://doi.org/10.3126/aj.n.v7i1.62056>

### INTRODUCTION

Cowpea (*Vigna unguiculata* L.) is a grain legume that is widely grown in tropical and subtropical areas. Cowpea grains contain protein content of 21-33%, a carbohydrate content of 57%, a fat content of 1.3%, and a mineral content of 3.5% (Li et al 2001), which is also economically important crop with various beneficial properties such as anti-diabetic, anti-cancer, anti-hyperlipidemic, anti-inflammatory and anti-hypertensive properties (Jayathilake et al 2018). Cowpea production and productivity in Nepal are 3632 tons and 0.99 t ha<sup>-1</sup>, respectively which covers land area of 3657 ha (MoALD 2018). It grows from the terai to the mid-hills, river basins, and low hills (NGLRP 2000). It is primarily used as an intercrop with various food crops like maize, sorghum and its N-fixing ability helps to increase soil fertility. It also has the ability to tolerate more heat and drought compared to many legume-related crops (Carvalho et al 2017). During the growth and development of

cowpea, the average nitrogen fixation ability in the soil ranges from 40 to 80 kg N ha<sup>-1</sup>, sometimes reaching 200 kg N ha<sup>-1</sup> (Meena et al 2015).

The use of superior exotic rhizobia strains as inoculants has been proposed as a common strategy for improving the efficiency of symbiotic nitrogen fixation and legume productivity (Zahid et al 2015). *Rhizobium leguminosarum*, as a legume partner, is potentially a more suitable species for genetic studies and the vital symbiotic nitrogen-fixing bacteria (Berlinger 1974). Rhizobium inoculation in legume seed is an efficient and cost-effective method of increasing rhizobia in soil. As a result, there is an increase in the rhizosphere of legume crops (Deaker et al 2004). It is an effective alternative form to the various hazardous synthetic fertilizers that have now emerged as an alternative form of inorganic fertilizers that also provide an economically appealing and environmentally sound source of nutrient supply while increasing crop productivity in cowpea. Rhizobium is a gram-negative soil bacteria that is well-known for its symbiotic relationships with a variety of leguminous plants. They are used as bio-fertilizers, an environment friendly technology in place of urea. The use of inoculants in conjunction with phosphorus increases grain yield, dry matter, and a variety of other growth and yield parameters when compared to the use of only inoculants or phosphorus, indicating that phosphorus deficiency limits cowpea growth and yield (Boahen et al 2017).

Cowpea requires more phosphorus than nitrogen in the form of single superphosphate or superphosphate (FAO 2005). Legumes require phosphorus in every step of growth and development of plants. Phosphorus is required to initiate nodulation, growth stimulation, and the efficiency of the rhizobium-legume symbiosis (Haruna et al 2012), as well as flower initiation, seed and fruit development, and the formation of young cells such as shoot and root tips, where maximum metabolism and rapid cell division occur (Ndakemi et al 2007). The increased grain yield due to P addition may be attributed to increased root and shoot ratio, root elongation, plant height and increased root branching (Shen et al 2011). With an increase in phosphorus dose up to 12 mg kg<sup>-1</sup>, seed yield enhanced from 2.6 to 5.4 g per plant (Dey et al 2019).

The purpose of this study was to identify how seed inoculation and phosphorus fertilizers affected cowpea growth and yield.

## **MATERIALS AND METHODS**

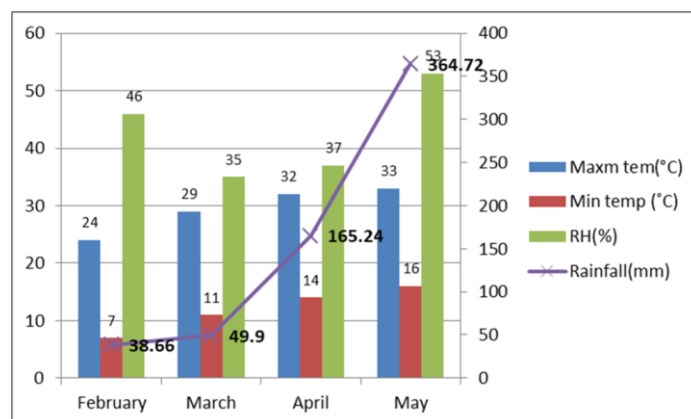
### **Experimental site**

A field experiment was conducted during February to May, 2022 to investigate the effect of rhizobium seed inoculation and different levels of phosphorus doses on cowpea productivity at an agronomy farm located at Bharatpur-11, Chitwan, Nepal. The research plot was located at 27.6931° N latitude and 84.4498° E longitude, 208m above the mean sea level. The soil characteristics of the experiment field was sandy loamy with organic carbon 4.72% (medium), nitrogen 0.61% (low), phosphorus 17.41 kg (low), potassium 70.08 kgha<sup>-1</sup> (low), and pH 5.65 (acidic). Nitrogen was identified by Kjeldahl method, phosphorus by Spectro photometer, potassium by Flame photometer method, organic carbon by Walkey and Black method and pH by pH meter.

### **Meteorological data**

During the experiment, the mean temperature ranged from 7 to 16 °C. In May, the maximum temperature ranged from 24°C to 33°C. During the research time interval, the relative humidity ranged from 46% to 53%. During the first stage of the experiment, 38.66 mm of rainfall fell, but May received the most, 364.72 mm, during the experimental period (Figure 1).

Meteorological data such as maximum-minimum temperature, rainfall, and relative humidity were collected from meteorological stations and then uploaded to the world weather online page.



**Figure 1: Weather condition during experimentation period at Bharatpur, Chitwan, 2022 (Source: World weather online)**

### Description of experimental materials

In this experiment, a suitable cowpea variety "Prakash" was grown which was brought from Grain Legumes Research Program, Khajura, Banke. Dawadi Agrovet, Narayangarh, Chitwan, Nepal provided the urea, single super phosphate (SSP), muriate of potash (MOP), and *Rhizobium leguminosarum*.

### Experimental design, treatment details and crop management

The treatments were distributed in a two-factor randomized complete block design (RCBD) with three replications of twenty-four plots, each with a plot size of 3m × 2m. The experiment includes two treatments: inoculated seed and uninoculated seed, as well as various phosphorus doses of 20, 40, 60, and 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Seeds were sown manually dibbling two seeds per hill at 4-5 cm depth Row-to-row spacing was kept at 60 cm, and plant-to-plant spacing was kept at 20 cm. Six tons of FYM per acre were used as the base manure dose. Similarly, varying doses of P<sub>2</sub>O<sub>5</sub>, 20 kg ha<sup>-1</sup> N, and 20 kg ha<sup>-1</sup> K<sub>2</sub>O were applied according to the treatment. As a base dose, a half dose of nitrogen and a full dose of P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O in the form of urea, single super phosphate, and muriate of potash, respectively were used. The remaining half dose of nitrogen was applied as a top dressing after 30 days of seed sowing. In half of the treatments, seed inoculation was accomplished through the use of a bio-fertilizer (*Rhizobium leguminosarum*), which was applied to the seed just prior to seed sowing at a rate of 80g per kg seed. Hand weeding was used to get rid weeds 25 days after sowing and another weeding at 20 days after the first weeding. First surface irrigation was given after the emergence of seedlings. After that, surface irrigation was provided every three days during the early stages of the crop.

Harvesting was done after pod reached its physiological maturity. Three harvesting at 15 days interval was done. First harvesting was done at 74 DAS followed by second harvest at 90 DAS and last harvest at 102 DAS.

### Data collection

To eliminate border effects, the data was collected only from the net plot by excluding the outer one row and one column of the border. From each experimental plot, ten plants were chosen at random. Plant height, leaf count (three leaflets are considered one leaf), leaf area index (using the formula (leaf area/canopy coverage area) (m<sup>2</sup>)), pod length, grain count per pod, test weight (total weight in grams of 1000 seeds), were measured from the given formula. After 70 days, leaf parameters were measured. At harvest, yield and yield-related traits were recorded.

$$\text{Grain yield (kg ha}^{-1}\text{) at 10\% moisture} = \frac{(100 - \text{MC}) \times \text{Plot yield (kg)}}{(100 - 10) \times \text{Plot area (m}^2\text{)}} \times 10000\text{m}^2$$

$$\text{Harvest Index} = \frac{\text{Grain yield (kg ha}^{-1}\text{)}}{\text{Biological yield (kg ha}^{-1}\text{)}} \times 100.$$

### Data analysis

Data were recorded and entered into MS-Excel 2016. The data were analyzed using R-Studio (version 1.2.5033). Randomized complete block design (RCBD) two-way ANOVA was used to analyze data. The

treatment means were compared by the Least Significant Difference (LSD) test at 5% level (Steel & Torrie, 1984).

### Statistical analysis

The data were subjected to analysis of variance, and Duncan's multiple range test at  $\alpha$  level 0.05 (DMRT) for mean separations (Gomez and Gomez 1984). Data were arranged in MS-Excel 2007. Dependent variables were subjected to analysis of variance using the RStudio (version 1.2.5033) for split-plot design.

## RESULTS

### Growth parameters

The Rhizobium inoculation resulted in the tallest plant (50.79 cm), the most leaves (24.28), and the highest leaf area index (0.169) (Table 1). The phosphorus levels caused significant ( $p < 0.001$ ) differences in plant height, leaf number, and leaf area index. The application of 40 kg  $P_2O_5$   $ha^{-1}$  resulted in the highest plant height (57.38 cm). Similarly, the application of 40 kg  $P_2O_5$   $ha^{-1}$  resulted in the highest number of leaves (24.76) and leaf area index (0.173) (Table 1). The interaction of Rhizobium inoculation and phosphorous levels resulted in significant changes in plant height and number of leaves, but not in leaf area index. The interaction effect of Rhizobium inoculation and phosphorous level @ 40 kg  $P_2O_5$   $ha^{-1}$  resulted in the greatest plant height (59.76 cm) and number of leaves (26.66) (Table 1).

**Table 1. Effect of seed inoculation and different doses of phosphorus fertilizer on growth and yield attributes of cowpea**

Treatment	Plant height (cm)	No. of leaves	Leaf area index (LAI)
Main effects of Rhizobium inoculation (RI)			
RI <sub>0</sub> : Non-Inoculated	47.59 <sup>b</sup>	22.03 <sup>b</sup>	0.144 <sup>b</sup>
RI <sub>1</sub> : Inoculated	50.79 <sup>a</sup>	24.28 <sup>a</sup>	0.169 <sup>a</sup>
LSD (0.05)	1.92	0.763	0.016
F test	*	**	*
CV (%)	4.218	1.88	5.77
Main effects of Phosphorus (P)			
P1: 20 kg $P_2O_5$ $ha^{-1}$	45.85 <sup>c</sup>	20.91 <sup>c</sup>	0.133 <sup>c</sup>
P2: 40 kg $P_2O_5$ $ha^{-1}$	57.38 <sup>a</sup>	24.76 <sup>a</sup>	0.173 <sup>a</sup>
P3: 60 kg $P_2O_5$ $ha^{-1}$	49.76 <sup>b</sup>	23.88 <sup>ab</sup>	0.166 <sup>a</sup>
P4: 80 kg $P_2O_5$ $ha^{-1}$	43.79 <sup>c</sup>	23.09 <sup>b</sup>	0.154 <sup>b</sup>
LSD (0.05)	2.52	0.89	0.009
F test	***	***	***
CV (%)	4.065	3.05	4.68
Interaction effects (RI $\times$ P)			
RI <sub>0</sub> $\times$ P1	43.41 <sup>d</sup>	20.40 <sup>f</sup>	0.12f
RI <sub>0</sub> $\times$ P2	54.99 <sup>b</sup>	22.86 <sup>cd</sup>	0.16cd
RI <sub>0</sub> $\times$ P3	48.71 <sup>c</sup>	22.51 <sup>cde</sup>	0.157cde
RI <sub>0</sub> $\times$ P4	43.27 <sup>d</sup>	22.36 <sup>de</sup>	0.14e
RI <sub>1</sub> $\times$ P1	48.29 <sup>c</sup>	21.41 <sup>ef</sup>	0.15de
RI <sub>1</sub> $\times$ P2	59.76 <sup>a</sup>	26.66 <sup>a</sup>	0.19a
RI <sub>1</sub> $\times$ P3	50.80 <sup>c</sup>	25.44 <sup>b</sup>	0.17ab
RI <sub>1</sub> $\times$ P4	44.31 <sup>d</sup>	23.82 <sup>c</sup>	0.16bc
LSD (0.05)	3.56	1.26	0.014
F test	*	*	NS
CV (%)	4.07	3.05	4.68

Means followed by the same letter(s) in a column do not differ at 5% level of significance by DMRT; CV= Coefficient of variance, LSD= Least significant difference. \*\*\*= Significant at 0.001 level of significance, \*\* = Significant at 0.01 level of significance, \*= Significant at 0.05 level of significance, NS=Not significant at 0.05 level of significance

### Yield and yield related parameters

Rhizobium inoculation resulted in the longest pod length (18.81 cm), most grains per pod (15.88), highest grain yield (1.27 t  $ha^{-1}$ ), highest biomass (5.91 t  $ha^{-1}$ ), highest test weight (148.35 g) and highest harvest index (21.32) (Table 2). The significant differences were observed in pod length, number of grains, grain yield, test weight, biomass and harvest index when phosphorus levels were varied. The application of 40 kg  $P_2O_5$   $ha^{-1}$  resulted in the greatest pod length (18.84 cm), number of grains/pod (15.78), grain yield (1.41 t  $ha^{-1}$ ), test weight (151.57 g), biomass (6.33t  $ha^{-1}$ ) and harvest index (22.36) (Table 2). The interaction of Rhizobium inoculation and phosphorous levels resulted in significant changes in pod length, grain yield, test weight, biomass and harvest

index. The interaction effect between Rhizobium inoculation and phosphorous level @ 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> produced the highest pod length (18.97cm), number of grains/pod (16.10), grain yield (1.53 t ha<sup>-1</sup>), test weight (154.31g), biomass (6.56 t ha<sup>-1</sup>) and harvest index (23.51) (Table 2).

**Table 2. Effect of seed inoculation and different doses of phosphorus fertilizer on yield parameters of cowpea**

Treatment	Pod Length	No. of Grains per pod	Grain yield (t ha <sup>-1</sup> )	Test wt. (1000 seed)	Biomass (t ha <sup>-1</sup> )	Harvest index (%)
Main effects of Rhizobium inoculation (RI)						
RI <sub>0</sub> : Non-Inoculated	18.13 <sup>b</sup>	14.85 <sup>b</sup>	1.09 <sup>b</sup>	140.09 <sup>b</sup>	5.33 <sup>b</sup>	20.34 <sup>b</sup>
RI <sub>1</sub> : Inoculated	18.81 <sup>a</sup>	15.88 <sup>a</sup>	1.27 <sup>a</sup>	148.35 <sup>a</sup>	5.91 <sup>a</sup>	21.32 <sup>a</sup>
LSD (0.05)	0.58	0.43	0.097	3.66	0.35	0.96
F test	*	**	*	*	*	*
CV (%)	1.81	1.59	4.68	1.45	3.53	2.62
Main effects of Phosphorus (P)						
P1: 20 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	18.14 <sup>b</sup>	14.8 <sup>c</sup>	0.92 <sup>d</sup>	131.86 <sup>c</sup>	4.81 <sup>d</sup>	19.29 <sup>b</sup>
P2: 40 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	18.84 <sup>a</sup>	15.78 <sup>a</sup>	1.41 <sup>a</sup>	151.57 <sup>a</sup>	6.33 <sup>a</sup>	22.36 <sup>a</sup>
P3: 60 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	18.75 <sup>a</sup>	15.67 <sup>a</sup>	1.31 <sup>b</sup>	150.31 <sup>a</sup>	6.01 <sup>b</sup>	21.77 <sup>a</sup>
P4: 80 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	18.16 <sup>b</sup>	15.23 <sup>b</sup>	1.06 <sup>c</sup>	143.15 <sup>b</sup>	5.34 <sup>c</sup>	19.88 <sup>b</sup>
LSD (0.05)	0.33	0.26	0.064	2.18	0.32	0.69
F test	***	***	***	***	***	***
CV (%)	1.43	1.33	4.32	1.21	4.47	2.67
Interaction effects (RI x P)						
RI <sub>0</sub> × P1	17.59 <sup>b</sup>	14.07 <sup>e</sup>	0.85 <sup>e</sup>	121.95 <sup>d</sup>	4.34 <sup>d</sup>	19.54 <sup>d</sup>
RI <sub>0</sub> × P2	18.73 <sup>a</sup>	15.47 <sup>bc</sup>	1.30 <sup>b</sup>	148.84 <sup>b</sup>	6.11 <sup>ab</sup>	21.20 <sup>b</sup>
RI <sub>0</sub> × P3	18.53 <sup>a</sup>	15.27 <sup>c</sup>	1.17 <sup>c</sup>	147.81 <sup>b</sup>	5.67 <sup>bc</sup>	20.62 <sup>bc</sup>
RI <sub>0</sub> × P4	17.68 <sup>b</sup>	14.63 <sup>d</sup>	1.04 <sup>d</sup>	141.79 <sup>c</sup>	5.21 <sup>c</sup>	19.99 <sup>cd</sup>
RI <sub>1</sub> × P1	18.68 <sup>a</sup>	15.53 <sup>bc</sup>	1.01 <sup>d</sup>	141.77 <sup>c</sup>	5.29 <sup>c</sup>	19.06 <sup>d</sup>
RI <sub>1</sub> × P2	18.97 <sup>a</sup>	16.10 <sup>a</sup>	1.53 <sup>a</sup>	154.31 <sup>a</sup>	6.56 <sup>a</sup>	23.51 <sup>a</sup>
RI <sub>1</sub> × P3	18.96 <sup>a</sup>	16.07 <sup>a</sup>	1.45 <sup>a</sup>	152.82 <sup>a</sup>	6.34 <sup>a</sup>	22.91 <sup>a</sup>
RI <sub>1</sub> × P4	18.63 <sup>a</sup>	15.83 <sup>ab</sup>	1.08 <sup>cd</sup>	144.51 <sup>c</sup>	5.46 <sup>c</sup>	19.79 <sup>cd</sup>
LSD (0.05)	0.47	0.36	0.091	3.09	0.45	0.99
F test	*	*	**	***	NS	***
CV (%)	1.43	1.33	4.32	1.21	4.47	2.67

Means followed by the same letter(s) in a column do not differ at 5% level of significance by DMRT; CV= Coefficient of variance, LSD= Least significant difference. \*\*\*= Significant at 0.001 level of significance, \*\* = Significant at 0.01 level of significance, \*= Significant at 0.05 level of significance, NS: Non-significant

## DISCUSSION

Cowpea plants inoculated with Rhizobium strains yielded more seed than uninoculated plants. Ali et al 2004 reported on the effects of seed inoculation on various growth and yield-attributing parameters. Growth parameters including leaf area, plant height, number of leaves increase significantly with the application of phosphorus (Krasilnikoff et al 2003). The significant difference due to response of phosphorus doses was obtained in the yield of cowpea (Okeleye and Okelana 2013). Nkaa et al (2014) and Ndor et al (2012) discovered that 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> produced the highest values in all parameters when compared to other phosphorus doses in cowpea. The combined treatment of phosphorus and inoculation resulted in maximum growth and development of chickpea plants when compared to only phosphorus or inoculation, which was a similar achievement reported by (Meskel et al 2018). Similarly, according to (Choudhary et al 2011) explained that biological yield of cowpea was significantly increased with increasing levels of fertility of 20 kg N along with 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. (Namakka et al 2017) reported that out of various doses of phosphorus, the highest yield was obtained by application of 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> in cowpea, while Haruna et al 2013 reported the highest yield of 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Cowpea's highest test weight was found to be 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> by Singh et al 2011.

Our findings suggested that combining the Rhizobium inoculant with P fertilizer could increase cowpea grain yield. According to (Boahen et al 2017), inoculants combined with P increased grain yield and other yield components in cowpea compared to P alone or inoculants. Similarly, Patel et al (2010) also noticed that 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> along with *Rhizobium* seed inoculation gives significantly higher growth, maximum nodulation and seed yield of cowpea. (Jadhav et al 2011) also discovered that 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> with seed inoculation significantly increased growth, seed yield and quality of cowpea which is similar to the finding of this research.

## CONCLUSION

The study revealed that different levels of phosphorous and Rhizobium inoculation influenced cowpea growth and yield. Cowpea growth and yield were increased by using Rhizobium inoculation and phosphorous @ 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. The combination of 40 kg ha<sup>-1</sup> phosphorus and Rhizobium inoculation 80g kg<sup>-1</sup> resulted in the highest cowpea yield. The finding concluded that neither higher nor lower applications of fertilizer are effective, but the appropriate applications are required to give maximum output. This result will benefit the cowpea farmers in Bharatpur, Chitwan, and other similar areas in increasing cowpea production.

## ACKNOWLEDGEMENT

The author would like to thank Nepal Polytechnic Institute and its faculties for continuous support and guidance during course of study.

## AUTHORS' CONTRIBUTION

Ankita Poudel: Designed the experiment, implemented the experiment, analyzed and interpreted the data, wrote the paper.

Bishnu Bilas Adhikari: Regular constructive comments and support during this research work; guidance during preparation of manuscript.

Jiban Shrestha: Reviewed draft of the manuscript.

## CONFLICTS OF INTEREST

There is no conflict of interest regarding this manuscript.

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