

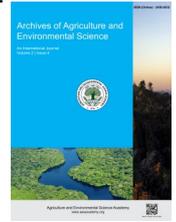


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ORIGINAL RESEARCH ARTICLE



Impact of various *Rhizobium* application methods on production and nodule formation in cowpea

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ABSTRACT

The study aimed to determine the effect of various *Rhizobium* inoculation methods on the yield, growth, and reproductive characteristics of two cowpea varieties under field conditions in the tropical region of Tikapur, Kailali. With four replications, the study was carried out in a two-factorial Randomized Complete Block Design from April to August of 2024. Four different *Rhizobium* inoculation methods were tested on two cowpea varieties, Aakash and Prakash: T₁ (Control), T₂ (seed inoculation), T₃ (seed + soil inoculation), and T₄ (soil inoculation). The majority of parameters were significantly influenced by the variety, inoculation methods and their interaction. While Aakash showed better physiological growth, the Prakash variety performed better in reproductive attributes and had a greater grain yield (1.39 t/ha). With the highest plant height (92.74 cm), number of leaves (52.75), branches (5.54), effective nodules (84.69), pods per plant (21.29), seeds per pod (12.10), thousand-grain weight (138.95 g), and grain yield (1.98 t/ha), T₃ (seed + soil inoculation) regularly outperformed the other treatments. Correlation analysis revealed a significant positive relationship between effective nodulation and yield-related traits. These results indicate that integrated *Rhizobium* treatment may greatly increase cowpea productivity, especially by combining both seed and soil inoculation. The study offers a cost-effective, promising method for enhancing the performance of legumes, which is particularly advantageous for smallholder farmers. To confirm the scalability and consistency of these findings, the study suggests more extensive long-term research in other agro-ecological zones.

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INTRODUCTION

Cowpea (*Vigna unguiculata* (Linn.) Walp.) belongs to the Leguminaceae family and is a prominent grain legume that is grown in tropical and subtropical areas and has a diploid chromosome number of $2n=2x=22$ (Tan *et al.*, 2012). It is a significant staple food crop in sub-Saharan Africa, especially in the arid savanna regions of West Africa, where it is an essential source of plant proteins and vitamins for humans when its tender leaves and immature pods are consumed as vegetable. Moreover, it is used as animal feed to generate monetary income (Dugje *et al.*,

2009). Africa is believed to be the origin of this particular legume, specifically in Nigeria, where weedy and wild variants are still widely used today (Guimarães *et al.*, 2023). With an annual global production of over 4.5 million metric tons, cowpeas are a staple food for millions of people, mostly in developing nations (Animasaun *et al.*, 2015). Consumers and researchers throughout the world have recently become more interested in cowpea because of its anti-diabetic, anti-cancer, anti-hyperlipidemic, anti-inflammatory, and anti-hypertensive qualities (Jayathilake *et al.*, 2018).

Cowpea's capacity to fix atmospheric nitrogen allows it to flour-

ish on sandy, nutrient-depleted soils. Its complex nutritional makeup, which includes high levels of protein, and energy, also helps to maintain dietary balance (Singh & Dubey, 2024). Its primary use is as an intercrop with different food crops, such as sorghum and maize. In comparison to many legume crops, it can withstand higher levels of heat and drought (Carvalho et al., 2017). It is frequently referred to as a "hungry-season crop" due to its early maturity and ability to sustain cropping systems when cultivated in rotation with cereal crops (Namakka et al., 2017). Nepalese agriculture relies heavily on legumes since they enhance food security, soil health, and sustainable farming practices. However, the production of legumes is limited by problems such as low soil nitrogen levels in soil and inadequate fertilizer management (Joshi et al., 2024). Pulses have higher rates of storage loss and less production stability than grains. Despite their importance in Nepalese farming systems, pulses are just a subsidiary of the larger farming systems since cereals are regarded as more significant as staple food crops (Dahal et al., 2022).

Biofertilizers are biological materials that contain either dormant or active microbe cells (algae, fungi, bacteria, or actinomycetes) that help fix atmospheric nitrogen or solubilize/mobilize soil nutrients while secreting compounds that promote growth and yield. Biofertilizers are made in Nepal using strains of *Rhizobium* and *Azotobacter* spp. (Kandel et al., 2023). *Rhizobia* are gram-negative bacteria that coexist with a variety of grain legumes as their host plants (Fahde et al., 2023). *Rhizobium* inoculation, which involves introducing specific elite strains of *Rhizobium* bacteria to legume roots, is one technique that appears to have promise for resolving these problems. A promising strategy for increasing legume output in Nepalese agriculture is *Rhizobium* inoculation, which provides long-term fixes for nutrient deficits and enhances soil health (Joshi et al., 2024). Globally, symbiotic nitrogen fixation is thought to fix 70 million metric tons of nitrogen yearly (Chhetri et al., 2019). Future studies and sustainable farming methods could benefit from *Rhizobium* inoculation as a cost-effective and ecologically friendly alternative to chemical fertilizers (Thilakarathna et al., 2019). Sustainable nutrient management in pulses has been increasingly important in the modern world. Adopting cutting-edge technologies that can increase pulse crop yield and production is essential to balancing the necessity of environmental sustainability with the growing demand for food (Khanna et al., 2019).

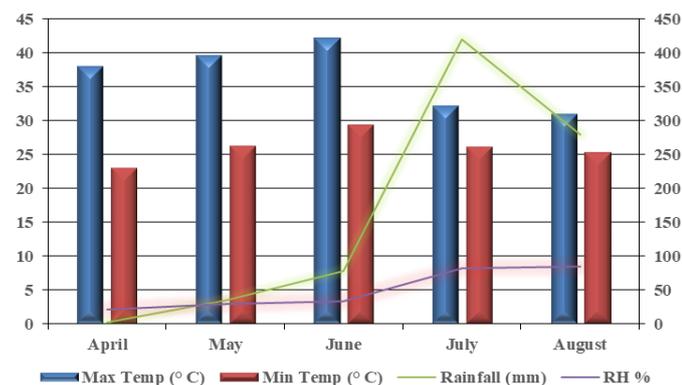


Figure 1. Weather parameters recorded at Tikapur, Kailali during April–August, 2024. Source: NASA POWER.

According to Brazilian research, cowpea reacted well to inoculation, and grain yields increased by 30% (Boddey et al., 2017). Inoculating the soil with *Rhizobium* increases the number of nodules (Aminu et al., 2015). Despite extensive national research highlighting the benefits of *Rhizobium* inoculation for enhancing legume yields, limited information exists under Nepalese field conditions for its utilization and different methods of *Rhizobium* application (Seed coating, soil inoculation, or combined approach) for cowpea cultivation.

This research gap highlights the need to identify the most effective method of *Rhizobium* inoculation to enhance cowpea productivity. Previous studies mainly focus on the effect of inoculation versus no inoculation, with little emphasis on how the different methods of inoculation influence nodulation and yield. This study, therefore, provides a novel comparison among various *Rhizobium* application methods: on seed, on soil, seed + soil and control to determine which approach maximizes nodulation and yield under tropical condition of Nepal. The aim of this study is to evaluate the impact of different *Rhizobium* application methods on the nodulation, growth, and yield of cowpea. The findings are expected to provide practical insights for farmers and researchers, offering a cost-effective, eco-friendly strategy to enhance soil fertility and pulse production, contributing to the sustainability of Nepalese agriculture.

MATERIALS AND METHODS

Selection of site

The experiment was conducted in the research field of the College of Natural Resource Management in Tikapur, Kailali, located at 28°31'30 N latitude and 81°07'15 E longitude, at a height of 140 masl, from April 15 to August 19, 2024. It is situated in the tropical region of Nepal's Sudurpashchim Province, 609 kilometers southwest of Kathmandu. Rainfall in the study area ranged from 2.38 to 420.28 mm, with the sowing season in April recording the least amount and the flowering stage recording the most (Figure 1). The soil in the experimental site was clay loam with a pH of 6.4. The specific agro-meteorological data recorded throughout the study period have been presented in Figure 1.

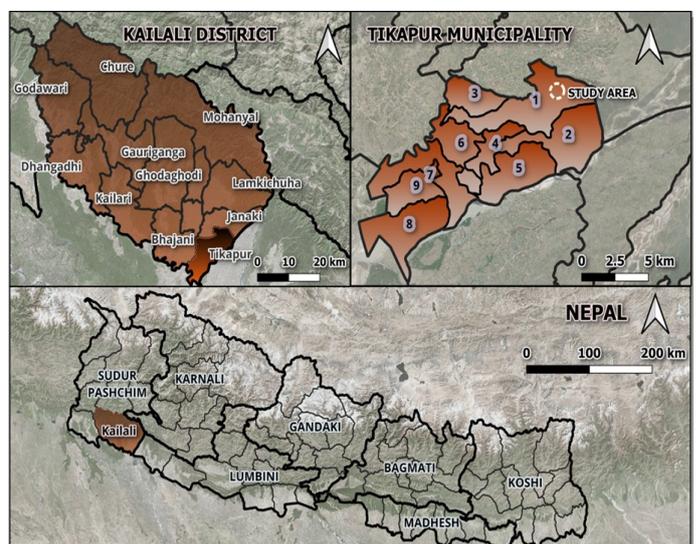


Figure 2. Map showing the study area.

The experimental design and cowpea cultivation method

A two-factorial Randomized Complete Block Design (RCBD) including eight treatments and four replications was used in the experiment, resulting in 32 plots in total (8 treatments × 4 replications), each with a total area of 2.7 m² and dimensions of 1.8 m × 1.5 m (Figure 3). The experiment consisted of two cowpea varieties (V₁: Aakash and V₂: Prakash) combined with four *Rhizobium* inoculation methods; T₁: control (no inoculation), T₂: seed inoculation, T₃: seed + soil inoculation, and T₄: soil inoculation. Three days before planting, the experimental field was leveled, clods were broken up, weeds and crop residues were removed, and the field was finely tilled using a power tiller. A basal dose of 20:40:20 NPK (Nitrogen, Phosphorus, and Potassium) kg/ha was applied using urea, DAP (Di-ammonium Phosphate), and MOP (Muriate of Potash) over the entire experimental area (145.35 m²), in addition to well-decomposed FYM at 15 kg/plot (Darai et al., 2023). For *Rhizobium* treatment, 153.6 g of seeds from the Aakash and Prakash varieties were treated with 1.536 g of *Rhizobium* using a sugar solution (100 g sugar in 100 ml water) and then allowed to dry in the shade for five hours (Ayalew et al., 2021). For soil inoculation, *Rhizobium* was thoroughly mixed with well-decomposed FYM and applied in a line along the sowing row just before seed sowing, ensuring a depth of 3–5 cm. The *Rhizobium* and seeds were obtained from NARC (Nepal Agricultural Research Council), Khumaltar, Lalitpur, Nepal. On April 15, 2024, the plants were manually sown by dibbling with a 45 cm row-to-row and 30 cm plant-to-plant spacing. At 25 and 40 DAS (Days After Sowing), manual weeding was done twice. Light irrigation was used both before and after planting, as well as during thinning and flowering.

Sampling and data collection

There were 20 plants altogether in each plot, which had five rows with four plants in each row. Three rows were utilized for observation, and two of the five rows were designated as boundary rows. In order to measure the different attributes, five plants were chosen at random from these observational rows. Plant architectural characteristics include germination %, plant height, number of leaves, and number of branches. Reproductive and pod characteristics include number of flowers, number of effective nodules and number of non-effective nodules and yield characteristics like pods per plant, pod length, seeds per pod, thousand-grain weight, and grain yield were recorded. Data

on plant height, number of leaves, and number of branches were recorded at 30 DAS, 45 DAS, and 60 DAS, while the number of flowers was assessed at 51 DAS and 54 DAS. To compute grain yield (kg ha⁻¹), the seed weight per plot was first adjusted to a standard moisture content of 12%, using the formula recommended by Emmanuel et al. (2025).

$$\text{Grain Yield (kg/ha)} = \frac{\text{Seeds weight per plot (g)}}{1000} \times \frac{100 - \text{moisture \%}}{88\%} \times \frac{10000\text{m}^2}{\text{Plot area (m}^2\text{)}}$$

Statistical analysis

The data were analyzed using RStudio version 4.4.1, Analysis of Variance (ANOVA) was employed to evaluate the effects of treatments and their interactions, along with correlation analysis using the packages “agricolae” and “ggplot2”. Fischer's least significant difference (LSD) test was used to test the significance of differences among the treatment means at P ≤ 0.05 probability levels.

RESULTS AND DISCUSSION

Plant architectural characteristics

The ANOVA showed that the effect of variety and *Rhizobium* treatments on germination % was non-significant (NS) (Table 1). The germination percentage demonstrated by Aakash and Prakash were 92.50% and 91.56% respectively, suggesting consistent seed vitality among the varieties. The low R² value of 0.157 suggests that other uncontrolled factors, like seed quality, moisture conditions at sowing, or micro environmental differences played a more significant role, as only 15.7% of the variability in germination was explained by the treatment and variety factors. All treatments exhibited a considerable increase in plant height, as assessed at 30, 45, and 60 days after sowing (Table 1). The ANOVA revealed a highly significant difference (P ≤ 0.001) for both variety and *Rhizobium* treatments at 30 DAS, with T₃: seed + soil inoculation and V₂: Prakash recorded the highest plant height (34.31 cm and 30.97 cm, respectively). Varieties and *Rhizobium* were not significantly affected plant height at 45 and 60 DAS, while T₃ continuously displayed numerically larger values. These results suggest that *Rhizobium* inoculation, especially seed + soil treatment, promotes early vegetative growth, declining over time as the crop matures. Bam et al. (2022) and Khanna et al. (2019) found that *Rhizobium* inoculation has a favorable effect on plant height of mung bean and cowpea, respectively. These results corroborate the findings of our research outcomes. There may be enough nitrogen-fixing bacteria to support plant development, explaining the rise in plant height brought on by *Rhizobium* inoculation (Shrestha et al., 2023).

The number of leaves at 30 DAS was significantly impacted by *Rhizobium* treatments (p≤0.01), with seed inoculation (T₂) exhibiting the maximum leaf number (9.52), while the control (T₁), and seed + soil inoculation (T₃) displayed the lowest (7.03) (Table 1). While early vegetative growth was sensitive to inoculation, the effect diminished over time, as no apparent changes were observed at 45 and 60 DAS. Also, Shrestha et al. (2023) found that

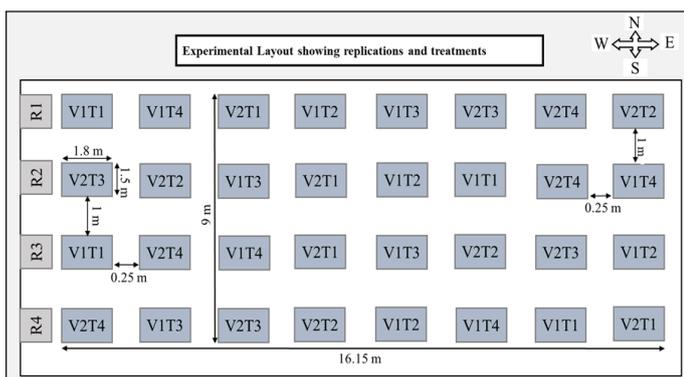


Figure 3. Experimental layout of research site.

Table 1. Overall performance of plant architectural characteristics.

Treatments	Germination %	Plant height (m)			Number of leaves			Number of branches		
		30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
Variety										
V1: Aakash	92.5	29.87 ^b	53.32	89.65	8.4	35.45	53.88	3.45	4.26 ^a	4.98 ^a
V2: Prakash	91.56	30.97 ^a	50.51	83.42	7.81	34.41	53.28	3.13	3.67 ^b	4.78 ^b
LSD (0.05)	5.19	0.37	6.08	7.81	0.88	5.08	6.51	0.32	0.14	0.13
F- test	NS	***	NS	NS	NS	NS	NS	NS	***	**
C.V.%	7.68	1.64	15.94	12.27	14.89	19.77	16.52	13.4	5.02	3.86
Rhizobium										
T1: Control	91.88a	26.43 ^d	48.4	80.45 ^b	8.48 ^{ab}	38.85	57.48	2.78 ^b	3.35 ^d	4.69 ^b
T2: seed inoculation	90.63a	31.98 ^b	52.82	89.19 ^{ab}	9.52 ^a	36.03	51.9	3.11 ^b	3.86 ^b	4.82 ^b
T3: seed + soil inoculation	95.00	34.31 ^a	56.65	92.74 ^a	7.03 ^c	32.67	52.75	4.29 ^a	5.05 ^a	5.54 ^a
T4: soil inoculation	90.63	28.96 ^c	49.79	83.77 ^{ab}	7.38 ^{bc}	32.16	52.2	2.99 ^a	3.61 ^c	4.48 ^c
LSD	7.34	1.42	8.6	11.04	1.77	7.18	9.21	0.45	0.21	0.19
F- test	NS	***	NS	NS	**	NS	NS	***	***	***
C.V.%	7.68	1.64	15.94	12.27	14.89	19.77	16.52	13.41	5.02	3.86
Grand mean	92.03	30.12	51.92	86.53	8.1	34.93	53.58	3.29	3.97	4.88
R ² - Value	0.157	0.98	0.24	0.53	0.59	0.35	0.215	0.776	0.954	0.912

CV: Coefficient of variance, LSD: Least significant difference, R²-Value: coefficient of determination; ** indicates P ≤ 0.01; *** indicates P ≤ 0.001; NS indicates non-significant difference, DAS: Days after sowing; Values sharing a common letter or without letter within the same column do not differ

Table 2. Overall performance of plant reproductive characteristics.

Treatments	No. of flowers		Effective Nodules	Non-effective Nodules
	51 DAS	54 DAS		
Variety				
V1: Aakash	23.31 ^b	40.87	61.46 ^b	6.18 ^a
V2: Prakash	27.68 ^a	42.13	66.88 ^a	5.59 ^b
LSD (0.05)	2.89	5.94	5.20	1.31
F- test	**	NS	*	*
C.V.%	15.42	19.47	11.03	10.62
Rhizobium				
T1: Control	24.50	35.62	44.56 ^d	5.31 ^b
T2: seed inoculation	28.37	45.00	70.00 ^b	5.43 ^b
T3: seed + soil inoculation	25.12	41.87	84.69 ^a	7.25 ^a
T4: soil inoculation	24.00	43.5	67.44 ^c	5.56 ^b
LSD (0.05)	4.08	8.52	7.36	1.85
F- test	NS	NS	***	***
C.V.%	15.42	19.47	11.03	10.62
Grand mean	25.5	41.50	64.17	5.89
R ² - Value	0.825	0.403	0.879	0.815

CV: Coefficient of variance, LSD: Least significant difference, R²-Value: coefficient of determination; * indicates P ≤ 0.05; ** indicates P ≤ 0.01; *** indicates P ≤ 0.001; NS indicates non-significant difference; DAS: Days After Sowing; Values sharing a common letter or without letter within the same column do not differ significantly.

Rhizobium treatment had a substantial effect on the number of leaves in Chinese long bean at 30 days after sowing (DAS), but not at 45 or 60 DAS. Early treatment effects that decreased with time are shown in a moderate R² (0.59) at 30 DAS. Overall, early leaf growth was enhanced by seed inoculation, but as plants became older, the differences decreased. Branching at every stage was significantly affected by *Rhizobium* treatments (P ≤ 0.001). Consistently, the control (T₁) recorded the fewest branches, whereas the seed + soil inoculation (T₃) recorded the most. By 45 and 60 DAS, variety V₁ (Aakash) had a much higher number of branches than variety V₂ (Prakash). All things considered, T₃ and V₁ were the best at encouraging branching. In accordance with this, Chaulagain et al. (2024) found that the inoculation of *Rhizobium* significantly increased the number of effective branches in mung bean. The cell division of the apical meri-

stem is affected by the nitrogen supplied by *Rhizobium* inoculation in seeds, leading to the development of taller plants and bigger leaves with more branches (Sun et al., 2020).

Plant reproductive characteristics

The varieties had a significant effect on the number of flowers produced at 51 DAS (P ≤ 0.01), but by 54 DAS, the difference between the two varieties was non-significant (Table 2). V₂ (Prakash) produced the largest number of flowers (27.68) at 51 DAS and had a significantly higher number of effective nodules (66.88) than V₁ (Aakash). V₁ had more ineffective nodules (6.18) than V₂ (5.59), indicating that V₂ is significantly better not only at producing flowers but also at efficient nodulation than V₁. Nodulation was significantly impacted by *Rhizobium* treatments (P ≤ 0.001), while flowering attributes were unaffected (NS). The

result showed increased symbiotic activity with the largest number of effective nodules (84.69) from seed + soil inoculation (T₃), followed by seed inoculation (T₄) (70.00). Additionally, T₃ had the largest number of non-effective nodules (7.25), indicating vigorous nodulation with some inefficiency (Table 2). The inoculation was beneficial, as evidenced by the control (T₁) recording the lowest nodulation (44.56 effective, 5.31 non-effective). *Rhizobium* inoculation resulted in substantially higher total and effective nodule numbers, most likely because of greater nutrient availability and a better root environment (Bam et al., 2022). This is consistent with our findings, which show that all *Rhizobium* administration methods resulted in much higher effective nodulation than the control, indicating that *Rhizobium* plays an important role in fostering strong root development and nitrogen fixation in cowpeas.

Effective (0.879) and non-effective (0.815) nodules had high R² values, suggesting that the treatments accounted for a significant portion of the variance in nodulation. *Rhizobium* promotes efficient nodulation by releasing *Nod* factors in response to root (iso) flavonoids, which cause root hair curling, infection thread creation, and nodule growth, resulting in the generation of nitrogen-fixing bacteria within the plant (Abd-Alla et al., 2023). The augmented root nodulation in cowpea can be attributable to *Rhizobium*'s ability to produce indole-3-acetic acid (IAA) and solubilize phosphate, hence promoting the growth of roots and nodule formation (Belhiba et al., 2020). *Rhizobium* protects plants from ethylene stress by converting the ethylene precursor, 1-aminocyclopropane-1-carboxylate (ACC), into ammonia and α -ketobutyrate using the enzyme ACC deaminase (Verma et al., 2020). This process lowers ethylene production under stressful circumstances, and *Rhizobium* strains that produce ACC deaminase are more efficient at nitrogen fixation and nodulation (Kandil & Ünlü, 2023).

Grain yield and pod characteristics

ANOVA showed moderate significant difference ($P \leq 0.01$) for yield between varieties, and highly significant difference ($P \leq 0.001$) among *Rhizobium* treatments. Also, pods per plant, pod length, seeds per pod, and thousand-grain weight (TGW) were all highly significant ($P \leq 0.001$) across both varieties and *Rhizobium* treatments (Table 3). V₂ (Prakash) performed better in terms of pod length (15.26 cm), seeds per pod (11.66), and yield (1.39 t/ha); V₁ (Aakash) recorded significantly higher values for pods per plant (17.91) and TGW (137.52 g), indicating different varietal responses in terms of seed filling and reproductive efficiency. With the highest number of pods per plant (21.29), maximum pod length (16.45 cm), and highest seed count per pod (12.10), the combined seed + soil inoculation (T₃) consistently surpassed all other treatments, followed by seed inoculation (T₂), while the control (T₁) had the lowest values for all traits. Also, TGW and yield were highest for soil + seed inoculated treatment (138.95 g and 1.98 t/ha), followed by seed inoculated treatment (135.8 g and 1.51 t/ha), and soil inoculated treatment (133.84 g and 1.06 t/ha). Anjum et al. (2006) revealed that inoculation greatly enhanced mung bean growth and yield components, and concluded that seed inoculation was more successful than soil inoculation, which supports our finding. Carranca et al. (2018) reported that nitrogen is essential for plant growth and development and has a favorable effect on fruit weight and size. *Rhizobium* inoculation improves plant growth and development by increasing the biological fixation of atmospheric nitrogen and by protecting plants from disease and extreme stress, which in turn increases yield (Kebede, 2021 and Singh et al., 2020). All pod and yield characteristics had R² values between 0.97 and 0.995, meaning that 97% to 99.5% of the variance in pods per plant, pod length, seeds per pod, thousand-grain weight, and grain yield could be explained by the treatments used. These high R² values show a persistent and significant impact of *Rhizobium* and varietal inoculation treatments on cowpea productivity, indicating good model reliability and treatment effects.

Table 3. Analysis of variance and mean performance of yield and pod characteristics.

Treatments	Pods Per Plant	Pod Length	Seeds Per Pod	TGW	Yield
Variety					
V1: Aakash	17.91 ^a	13.93 ^a	9.29 ^b	137.52 ^a	1.28
V2: Prakash	16.29 ^b	15.26 ^b	11.66 ^a	130.85 ^b	1.39
LSD (0.05)	0.20	0.15	0.14	0.38	0.068
F- test	***	***	***	***	**
C.V.%	1.61	1.43	1.82	0.39	6.94
Rhizobium					
T1: Control	13.76 ^d	11.55 ^c	8.35 ^d	128.15 ^d	0.79 ^d
T2: seed inoculation	17.91 ^b	16.36 ^a	11.81 ^b	135.80 ^b	1.51 ^b
T3: seed + soil inoculation	21.29 ^a	16.45 ^a	12.10 ^a	138.95 ^a	1.98 ^a
T4: soil inoculation	15.44 ^c	14.01 ^b	9.63 ^c	133.84 ^c	1.06 ^c
LSD (0.05)	0.28	0.22	0.19	0.54	0.096
F-test	***	***	***	***	***
C.V.%	1.61	1.43	1.82	0.39	6.94
Grand mean	17.11	14.59	10.47	134.18	1.33
R ² - Value	0.994	0.995	0.994	0.993	0.97

CV: Coefficient of variance, LSD: Least significant difference, R²-Value: coefficient of determination; ** indicates $P \leq 0.01$; *** indicates $P \leq 0.001$.

Interaction effect of cowpea cultivars and *Rhizobium* treatments

ANOVA revealed a significant relationship between *Rhizobium* treatments and cowpea varieties for all measured traits. With the most branches (5.97), pods per plant (22.72), non-effective nodules (22.75), and TGW (142.14 g), the $V_1 \times T_3$ combination continuously performed better (Table 4). Additionally, $V_2 \times T_3$ produced the highest plant height at 60 DAS (100.60 cm), longest pods (18.22 cm), the most effective nodules (86.87), and the maximum number of seeds per pod (13.85). On the other hand, $V_1 \times T_1$ and $V_2 \times T_1$ often had the lowest values for the majority of attributes. Overall, the best combinations for improving cowpea growth and yield characteristics were $V_1 \times T_3$ and $V_2 \times T_3$ (Table 4). Although there was no significant interaction between variety and *Rhizobium* in terms of grain yield, $V_2 \times T_3$ had the greatest yield (2.08 t/ha), followed by $V_1 \times T_3$ (1.87 t/ha). The control treatments, notably $V_2 \times T_1$ and $V_1 \times T_1$, recorded significantly lower plant height, number of pods per plant, seeds per pod, and total grain weight, further supporting the beneficial effects of *Rhizobium* inoculation on cowpea growth and yield. The combined soil + seed *Rhizobium* treatment consistently produced the highest number of effective nodules, yield, and thousand-grain weight in both cowpea varieties (Figure 4). The variety,

Prakash showed better nodulation and yield performance across treatments, particularly under soil + seed, while the variety Aakash showed lower yield overall but relatively more thousand-grain weight (Figure 4). This suggests that genetic nitrogen fixation and productivity traits are significantly influenced by the variety and *Rhizobium* application method.

Pearson's correlation coefficient

The results of a correlation analysis showed that yield was strongly and positively correlated with the number of pods per plant ($r = 0.88^{***}$), number of branches at 60 DAS ($r = 0.61^{***}$), pod length ($r = 0.80^{***}$), number of seeds per pod ($r = 0.79^{***}$), number of effective nodules ($r = 0.69^{***}$), and thousand-grain weight ($r = 0.62^{***}$) (Table 5). This suggests that these traits play a significant role in increasing yield. Shrestha et al. (2023) revealed similar findings, indicating that the correlation between Chinese long bean variables, such as plant height, number of branches, number of leaves, and number of nodules, was strongly correlated with yield. Except for the number of leaves, which in our case did not significantly correlate with yield. Significant positive correlations were found between the number of branches at 60 DAS and yield ($r = 0.61^{***}$), thousand-grain weight ($r = 0.51^{**}$), and pods per plant ($r = 0.72^{***}$) (Table 5).

Table 4. Interaction effect of cowpea cultivars and different *Rhizobium* treatments on morphological and yield parameters.

Interaction	Plant height at 60 DAS	Number of branches at 60 DAS	Number of effective nodules	Number of non-effective nodules	Pods per plant	Pod length (m)	Seeds per pod	TGW
V1×T3	84.87abcd	5.97a	82.5a	22.75a	22.72a	14.67c	10.35b	142.14a
V2×T3	100.60a	5.10b	86.87a	19.85b	19.85b	18.22a	13.85a	135.75d
V1×T2	97.76ab	4.62c	51.25c	18.97c	18.97c	14.95c	10.05c	139.88b
V2×T1	78.60cd	4.67c	48.50c	13.00g	13.00g	12.87d	8.95d	125.45g
V1×T1	82.30bcd	4.70c	40.62c	14.52f	14.52f	10.22e	7.75e	130.84f
V1×T4	93.7abc	4.62c	71.50b	15.42e	15.42e	13.17d	9.00d	137.19c
V2×T4	73.84d	4.32d	68.50b	15.45e	14.45e	14.85c	10.25bc	130.48f
V2×T2	80.62bcd	5.02b	63.62b	16.85d	16.85d	17.77c	13.57a	131.72e
LSD (0.05)	15.62	0.28	10.40	0.40	0.40	0.31	0.27	0.76
F-test	*	***	***	***	***	***	***	***

CV: Coefficient of variance, LSD: Least significant difference; * indicates $P \leq 0.05$; *** indicates $P \leq 0.001$; Values sharing a common letter within the same column do not differ significantly.

Table 5. Pearson's correlation coefficient illustrating the relationship between several Cowpea parameters

	Plant height (m) at 60 DAS	Number of branches at 60 DAS	Number of leaves at 60 DAS	Effective nodules	Pods per Plant	Pod Length (m)	Seed per Pod	TGW (kg)	Yield (t/ha)
Plant height at 60 DAS	1.00	0.06 Ns	-0.02 Ns	0.29 Ns	0.35 *	0.22 Ns	0.20 Ns	0.45 *	0.36 *
Number of branches at 60 DAS		1.00	0.08 Ns	0.47 **	0.72 ***	0.27 Ns	0.30 Ns	0.51 **	0.61 ***
Number of leaves at 60 DAS			1.00	-0.09 Ns	-0.15 Ns	-0.20 Ns	-0.23 Ns	-0.14 Ns	-0.18 Ns
Effective nodules				1.00	0.62 ***	0.61 ***	0.57 ***	0.47 **	0.69 ***
Pods per Plant					1.00	0.52 **	0.47 **	0.85 ***	0.88 ***
Pod Length						1.00	0.97 ***	0.25 Ns	0.80 ***
Seed per Pod							1.00	0.15 Ns	0.79 ***
TGW								1.00	0.62 ***
Yield									1.00

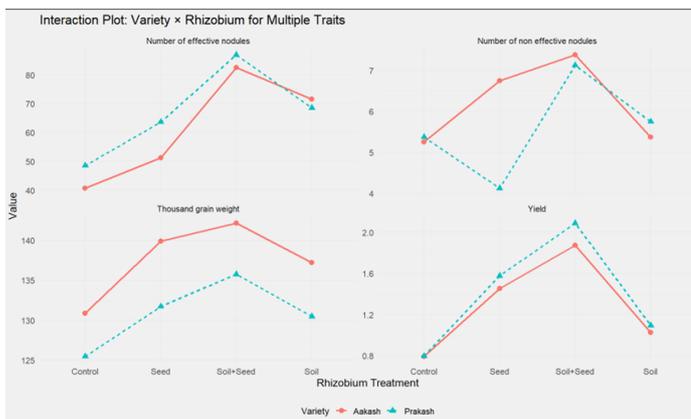


Figure 4. Interaction plot of Variety \times Rhizobium inoculation method for multiple traits.

In legume plants, having more branches greatly increases the potential output since each branch provides additional spaces for the development of flowers, which in turn produce more pods and increase the overall yield (Abayomi et al., 2008). Strong positive correlations were found between effective nodules and pods per plant ($r = 0.62^{***}$), pod length ($r = 0.61^{***}$), seeds per pod ($r = 0.57^{***}$), TGW ($r = 0.47^{**}$), and yield ($r = 0.69^{***}$). Leguminous plants have nodules that house nitrogen-fixing bacteria that transform atmospheric nitrogen into vital nutrients. The more nodules there are, the more nitrogen is available, which increases reproductive performance and yield (Darini & Astuti, 2023). Likewise, there was a strong correlation between the number of pods per plant and TGW ($r = 0.85^{***}$), pod length ($r = 0.52^{**}$), and effective nodules ($r = 0.62^{***}$), indicating that it is associated with increased reproductive output. Both yield and pod-related features were positively correlated with effective nodules, highlighting the contribution of symbiotic nitrogen fixation to increased production. On the other hand, the number of leaves had no effect on yield and only weak and non-significant associations with the majority of attributes. Overall, the findings point to nodulation, grain weight, number of branches, and pod-related characteristics as the main factors influencing cowpea output.

Conclusion

This study showed that, the key growth, and yield parameters were strongly influenced by variety and *Rhizobium* application methods. Within the varieties, Aakash showed the most desirable physiological qualities, while Prakash was superior in reproductive parameters and ultimately produced a higher yield (1.39 t/ha) than Aakash (1.27 t/ha). The ability of seed + soil inoculation (T_3) to improve biological nitrogen fixation and overall plant productivity was demonstrated by its constant superior performance across the majority of evaluated variables, especially in effective nodulation and pod characteristics. Results showed that traits such as effective nodules, pod length, seeds per pod, and thousand-grain weight strongly correlated with yield, highlighting their significance as selection criteria in breeding programs. Also, these results demonstrate the possibility of integrated *Rhizobium* application, particularly T_3 , as an economical and environmentally friendly substitute for chemical fertilizers

for farmers with limited resources. The consistent patterns observed across different parameters strengthen the reliability of our results, even though the study included only a limited number of varieties and was conducted under specific agro climatic conditions. Future studies are required to investigate multi-location trials and wider varietal screening to confirm these results and improve cowpea productivity in various environments.

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DECLARATIONS

Author's contribution statement: Conceptualization: A.D.; Methodology: A.D., R.C.B. and L.D.B.; Software and validation: A.D., R.C.B. and L.D.B.; Formal analysis: A.D.; Investigation: R.C.B.; Resources: A.D. and L.D.B.; Data curation: R.C.B. and A.R.; Writing—original draft preparation: A.D., R.C.B., L.D.B. and A.R.; Writing—review and editing: A.D., L.D.B., R.C.B.; Visualization: A.D. and R.C.B.; Supervision: A.D. and L.D.B. All authors have read and agreed to the published version of the manuscript.

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