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ORIGINAL RESEARCH

Synergistic Effects of *Rhizobium* and Micronutrients (Molybdenum and Boron) on the Growth, Nodulation, and Yield of Blackgram (*Vigna mungo* L.)

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ABSTRACT: Blackgram (*Vigna mungo* L.) is a vital pulse crop for food and nutritional security in the mid-hills of Nepal, but its productivity remains constrained by poor nutrient management and limited use of *Rhizobium* inoculants. In particular, deficiencies of essential micronutrients such as boron (B) and molybdenum (Mo), together with underutilization of beneficial *Rhizobium*, restrict nodulation, nitrogen fixation, and yield potential. To address this issue, a field experiment was conducted at the Agronomy Farm, Lamjung Campus, to assess the combined effects of *Rhizobium* inoculation and micronutrient supplementation on local blackgram. A split-plot design with three replications was used, where *Rhizobium* inoculation (inoculated vs. non-inoculated) formed the main plot factor, and seven nutrient treatments were assigned to subplots: control, 250 ppm B, 500 ppm B, 250 ppm Mo, 500 ppm Mo, 250 ppm B + 250 ppm Mo, and 500 ppm B + 500 ppm Mo. Results showed that *Rhizobium* inoculation significantly improved nodulation, biomass, and yield attributes compared with non-inoculated plots. The combined application of 500 ppm B and 500 ppm Mo with *Rhizobium* inoculation achieved the highest nodulation, biomass, and seed yield (120.73 g per 2.1 m^2). These findings suggest that integrated nutrient management using *Rhizobium* inoculation with balanced B and Mo supplementation can substantially enhance blackgram productivity, reduce dependence on chemical fertilizers, and contribute to sustainable pulse-based farming systems in Nepal's mid-hills.

KEYWORDS: Blackgram, grain yield, integrated nutrient management, nodulation, *Rhizobium* inoculation

1. Introduction

Blackgram (*Vigna mungo* L.), also known as mash, urd bean, black matpe bean, or black lentil, is a widely grown, self-pollinating grain legume. This annual herbaceous crop grows upright and rapidly (30–100 cm tall), bearing trifoliate leaves, yellow papilionaceous flowers, and hairy pods containing black, oblong seeds. Blackgram can be cultivated twice a year, in both spring and autumn, and integrates easily into diverse crop rotation systems (Tahir, 2014). It is a rich source of protein (24%), starch (67%), fiber (3.5%), fat (1.74%), and lysine, making it especially valuable in vegetarian diets (Chaudhary et al., 2020). Beyond its nutritional importance, it also has medicinal applications in managing diabetes, digestive disorders, mental health issues, hair diseases, sexual dysfunction, rheumatic ailments, and systemic conditions (Tahir et al., 2014). Its palatability, high nutritive value, and digestibility enable it to command a premium price over other pulses in the country.

Blackgram is cultivated across many regions of Asia and Africa, with India, Nepal, Pakistan, Afghanistan, Sri Lanka, Bangladesh, Burma, China, Laos, Vietnam, and Japan being the main centers for its cultivation and genetic diversity. In Nepal, it holds importance as the fourth major pulse crop, following lentil, chickpea, and pigeon pea. In addition to the important source of protein in the human diet, it is also an important source as livestock feed. It is an important cash crop in the subsistence farming system of mid-hills, playing a vital role in maintaining soil fertility as it fixes nitrogen in soil.

The Nepal Agricultural Research Council (NARC) has recommended improved varieties such as Rampur Mash and Khajura Mash 1 for cultivation in Nepal. However, it has been observed that Nepalese farmers primarily grow this crop using non-inoculated seeds from the previous season and do not use micronutrients or other inputs, largely due to their remote locations and limited resources (Thilakarathna et al., 2019). In present scenario blackgram is cultivated in about 28,383 ha, yielding 26,114 tons with a productivity of 880 kg ha^{-1} (MoALD, 2023). While cereal crop production is associated with significant greenhouse gas emissions, legume production systems like blackgram are viewed as effective and essential for sustainable agriculture and environmental health (Goyal et al., 2021).

Despite its high potential, blackgram production in Nepal remains low. This is attributed to poor cultivation techniques, lack of knowledge about micronutrient and high-quality fertilizers, pest and disease pressures, and acidic soils (Jat et al., 2017). Nematodes like; *Rotylenchulus reniformis* (reniform nematodes) have been observed to disrupt the symbiotic relationship between soybeans and Rhizobia, leading to reductions in both nodule number and size (Hussey & Barker, 1976). In acidic soils, the availability of molybdate ions is negligible due to adsorption onto positively charged metal oxides, and their concentration increases with soil pH (Rutkowska et al., 2017). Acidic soils with a pH less than 5.5 are considered poor quality, and increased metal ion toxicity is a concern. Acidic pH reduces the availability of B and Mo, as reported by Rutkowska et al. (2017). These factors negatively impact the growth and symbiotic traits of rhizobia, affecting their survival, nodulation capacity, and spread (Ferguson et al., 2013). These issues contribute to black gram being one of the most underutilized crops in Nepal. Given the moderate acidity and micronutrient deficiencies in Nepalese soils, and the high cost and inaccessibility of soil-applied nutrients for remote farmers, seed priming with micronutrients and *rhizobium* inoculation offers a simple and effective approach to improve plant establishment, growth, and yield.

Mo was applied as a seed treatment to lengthen the pods in blackgram due to increase in cell division and enzymatic reaction (Tahir, 2014). When boron is added, it becomes quickly available to the crop throughout its growth and promotes tissue differentiation and cell proliferation, which may be the reason for pod length (Sharmila et al., 2020). Through the manufacture of chlorophyll and the integrity of the photosynthetic system, Mo also directly affects the photochemical phase of photosynthesis, growth of seeds, the ovary, the metabolism of carbohydrates, and the production of pollen (Liu et al., 2005). Therefore, this study was undertaken to evaluate the combined effects of *Rhizobium* inoculation and micronutrient (B and Mo) supplementation on the growth, nodulation, and yield of a local blackgram variety (Kalu) under Sundarbazar agro-climatic conditions. The objectives of the current study were (1) To assess the individual and combined effects of *Rhizobium* inoculation and B and Mo supplementation on growth, nodulation, and yield of local blackgram and (2) To evaluate the adaptability and performance of the local cultivar under mid-hill conditions. We hypothesized that *Rhizobium* inoculation together with boron and molybdenum supplementation will have a synergistic effect, significantly improving nodulation, growth, and yield of blackgram compared with single-factor treatments or control.

2. Materials and methods

2.1 Description of the study site

The field experiment was conducted at the Agronomy Farm of Lamjung Campus, Nepal, located in the sub-tropical agro-ecological zone at 28.126255°N latitude and 84.417299°E longitude, with an altitude of 857 meters above sea level. Soil testing performed at the Soil and Fertilizer Testing Laboratory, Pokhara, showed the soil was slightly acidic with a pH of 5.1.

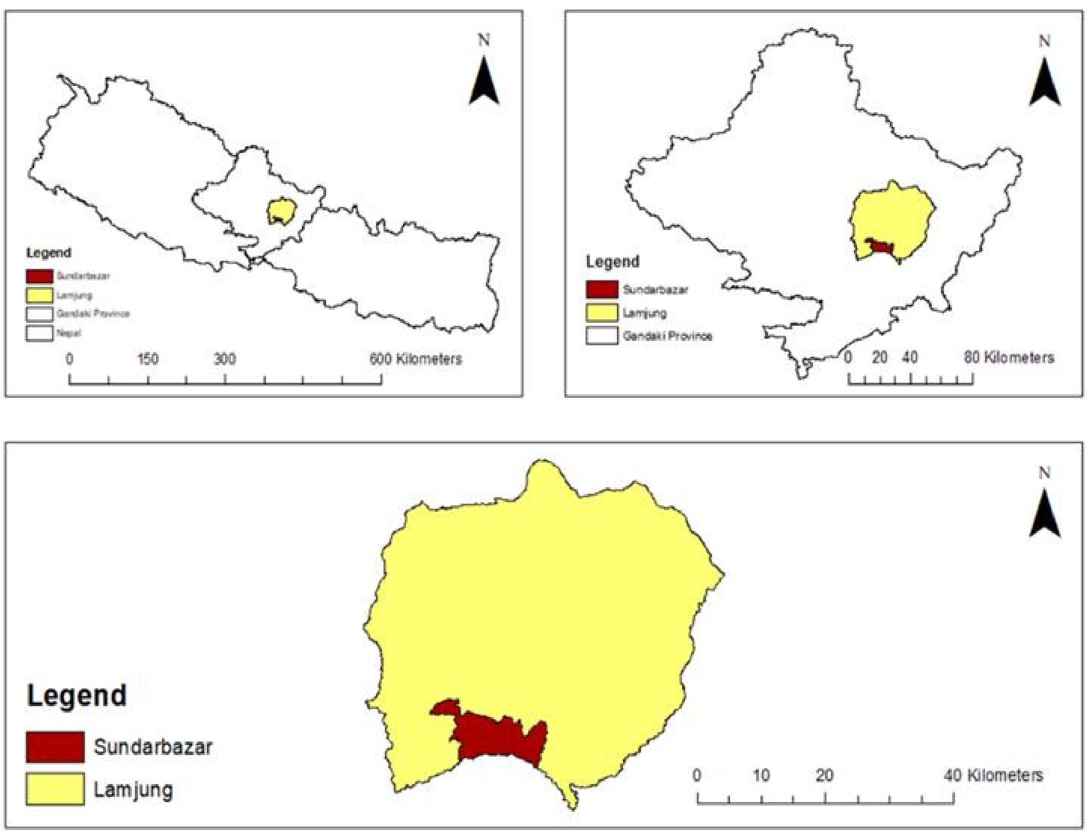

Figure 1. Site of experiment of Agronomy Farm of Lamjung Campus, Nepal.

The soil texture was loamy, which generally provides good water retention and aeration. Organic matter content was 2.37%, nitrogen was low at 0.12%, phosphorus was moderately available at 26.51 kg/ha, and potassium was relatively high at 265.6 kg/ha. These results indicate that nitrogen supplementation may be necessary to improve soil fertility, while phosphorus and potassium levels are sufficient for crop growth. The experimental site experiences sub-humid weather conditions, characterized by cold winters, hot summers, and a distinct rainy season. Minimum temperatures range from 6 to 10°C, while maximum temperatures can reach up to 39°C. The area receives ample rainfall during the rainy season, which lasts from June to September

2.2 Experimental design and treatments

The experiment was arranged in a split-plot design with three replicates. The local black gram cultivar Kalu was used as the test crop. The main plot treatments consisted of two inoculation levels: (1) *Rhizobium* inoculated and (2) *Rhizobium* non-inoculated (control). Seven micronutrient seed priming treatments were applied to the sub-plots as detailed in Table 1.

Boric acid and sodium molybdate were used as sources of boron (B) and molybdenum (Mo) for seed priming. Seeds were soaked in micronutrient solutions for 3 hours and then shade-dried for 8 hours before *Rhizobium* inoculation. The inoculant, *Bradyrhizobium*, obtained from the Nepal Agricultural Research Council (NARC), was mixed with cow dung as a carrier and applied to the primed seeds. Seeds were sown manually by line sowing, with spacing of 30 cm between rows and 10 cm within rows, sowing two seeds per hill at 2–3 cm depth. Each experimental plot measured 2.1 m² and contained approximately 70 plants.

Table 1. Sub-plot treatment details

Treatments	Details
T1	Control
T2	250 ppm of Mo
T3	250 ppm of B
T4	500 ppm of B
T5	500 ppm of Mo
T6	250 ppm of B + 250 ppm of Mo
T7	500 ppm of B + 500 ppm of Mo

Field preparation included deep plowing twice with a power tiller, incorporation of well-decomposed farmyard manure 27 days before sowing, and thorough clearing of debris and weeds. Balanced fertilizer (20:20:20 NPK) was applied at 20 kg/ha for each nutrient, with nitrogen applied both basally and in split doses during sowing and the vegetative growth phase.

Crop management involved gap filling and thinning at 15 days after sowing to maintain uniform plant population, two rounds of hand weeding at 25 and 40 days after sowing, and insecticide applications (mancozeb and metalaxyl) at 45 days after sowing to manage pests.

2.3 Sampling and measurements

Random sampling was employed to ensure unbiased and representative data collection across all treatments and replicates. For each measured parameter, samples were selected randomly, typically from 10 plants per plot. Black gram, being an indeterminate crop, was harvested twice at full pod maturity to maximize yield accumulation. Harvested crop bundles were sun-dried, manually threshed, and samples were stored plot-wise for analysis.

Plant height was measured in the field at maturity from the base of the stem at ground level to the tallest plant tip. Root length was recorded after carefully uprooting and washing the roots, measuring from the stem base to the tip of the longest root using a measuring scale. The number of branches per plant was counted on 10 randomly selected plants per plot at maturity to assess branching. Nodulation efficiency was estimated by counting the number of nodules on the roots of 10 uprooted plants per plot. The total number of pods per plant was counted from 10 randomly selected plants per plot, and the number of seeds per pod was determined by counting seeds in 10 randomly selected pods in each plot. The weight of 1000 seeds was measured using a precision balance to evaluate seed size and quality. Grain yield was obtained by harvesting all mature grains from each plot, drying them to a standard moisture content of 9%, and weighing to express the yield in kg/ha.

2.4 Statistical analysis

Data were entered into Microsoft Excel for organization and graphical presentation. Analysis of variance (ANOVA), least significant difference (LSD), coefficient of variation (CV), standard error of the mean (SEM), and grand mean calculations were performed using R Studio to determine treatment effects and statistical significance.

3. Results and discussions

3.1 Plant height and number of branches

Rhizobium inoculation significantly increased plant height and the number of branches compared to non-inoculated plants (Table 2). The average plant height at maturity was 94.23 cm with inoculation versus 81.95 cm without, while branching increased from 8.48 to 9.41 branches per plant. This improvement can be attributed to *Rhizobium's* role in enhancing nitrogen fixation, which supports better nutrient availability and promotes vegetative growth (Chen et al., 2024). Additionally, *Rhizobium* influences the synthesis of plant hormones such as auxins and cytokinins, which regulate cell division and lateral branch development, leading to a bushier morphology (Sharmila et al., 2020; Tripathi et al., 2021).

Table 2. Effect of *Rhizobium* inoculation on plant height and branch number

Treatment	Plant height at maturity	Branching at maturity (Number)
Inoculation	94.23^{a}	9.41^{a}
Non-inoculation	81.95^{a}	8.48^{b}
SEM±	4.601	0.1472
LSD0.05	28.00	0.896
p-value	***	*
CV%	23.9	7.5

Note: SEM – Standard Error of the Mean, LSD – Least Significant Difference, CV- Coefficient of Variation, P value *** indicates significance at the 0.001 level.

Table 3. Effect of B and Mo on plant height and branch number per plant.

Treatment	Plant height at maturity	Branching at maturity
Control	73.15^{f}	7.12^{f}
250 ppm B	76.16^{e}	7.73^{e}
250 ppm Mo	81.998^{d}	8.25^{e}
500 ppm B	84.78^{d}	8.93^{d}
500 ppm Mo	90.42^{c}	9.53^{c}
250 ppm B +250 ppm Mo	98.02^{b}	10.15^{b}
500 ppm B +500 ppm Mo	112.12^{a}	10.9^{a}
Grand mean	88.09	8.95
SEM±	1.012	0.20
$LSD_{0.05}$	2.97	0.597
P value	***	***

Note: SEM – Standard Error of the Mean, LSD – Least Significant Difference, P value *** indicates significance at the 0.001 level.

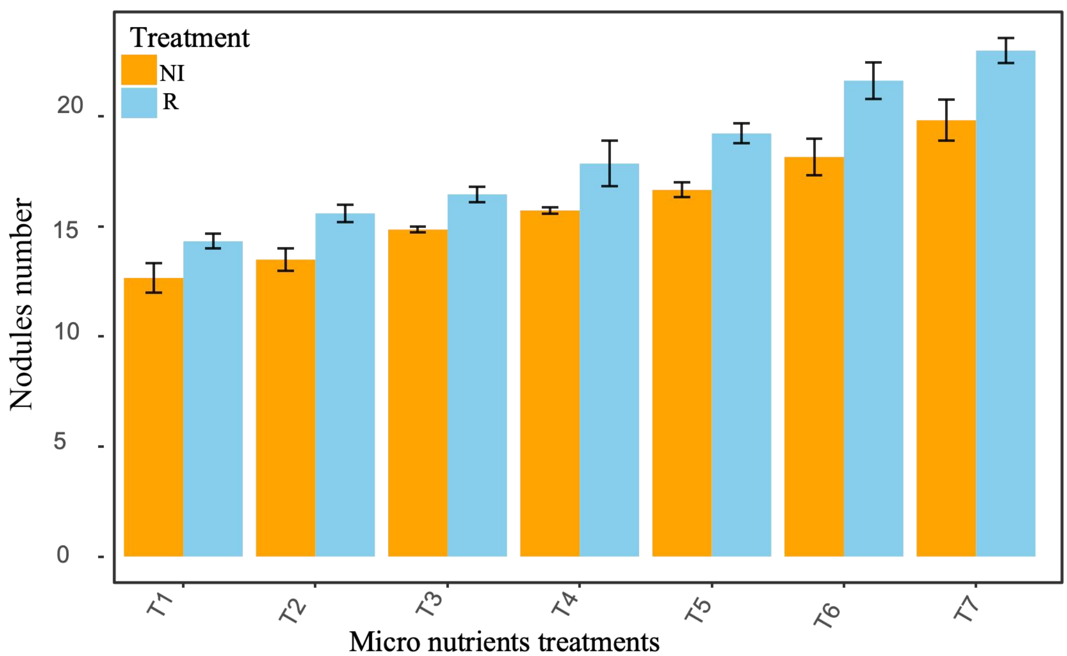

Figure 2. Effect of *Rhizobium* inoculation and micronutrient loading on nodules per plant. Bars represent standard error. NI = Non-inoculated; R = *Rhizobium* inoculated. Treatments: T1 = Control, T2 = 250 ppm Mo, T3 = 250 ppm B, T4 = 500 ppm B, T5 = 500 ppm Mo, T6 = 250 ppm B + 250 ppm Mo, T7 = 500 ppm B + 500 ppm Mo.

Micronutrient treatments with boron (B) and molybdenum (Mo) significantly affected plant height and branching (Table 3). The combined application of 500 ppm B and 500 ppm Mo resulted in the tallest plants (112.12 cm) and highest branch numbers (10.9 branches/plant), while the control treatment recorded the lowest values. Boron and molybdenum improve enzyme function, chlorophyll synthesis, and photosynthate production, which enhance growth and branching (Singh, 2017; Pazhanisamy et al., 2023). Their synergistic effect supports improved nutrient uptake and chloroplast development, promoting overall plant vigor.

3.2 Nodulation parameters

Rhizobium inoculation significantly increased root nodule number, demonstrating its critical role in biological nitrogen fixation (Figure 2). The combined seed priming with B and Mo further enhanced nodulation compared to single or no micronutrient treatments. Molybdenum is essential for the synthesis and activity of nitrogenase, the key enzyme in nitrogen fixation, which explains the increased nodulation and improved nitrogen availability to plants (Mahilane et al., 2024). This increase in nodules and their mass likely facilitated better nitrogen assimilation, promoting plant growth and yield (Vijila & Jebaraj, 2008).

3.3 Yield and yield components

Although *Rhizobium* inoculation and micronutrient priming did not show statistically significant effects on the number of pods per plant (Figure 3), the highest pod counts were recorded in the combined 500 ppm B and Mo treatment.

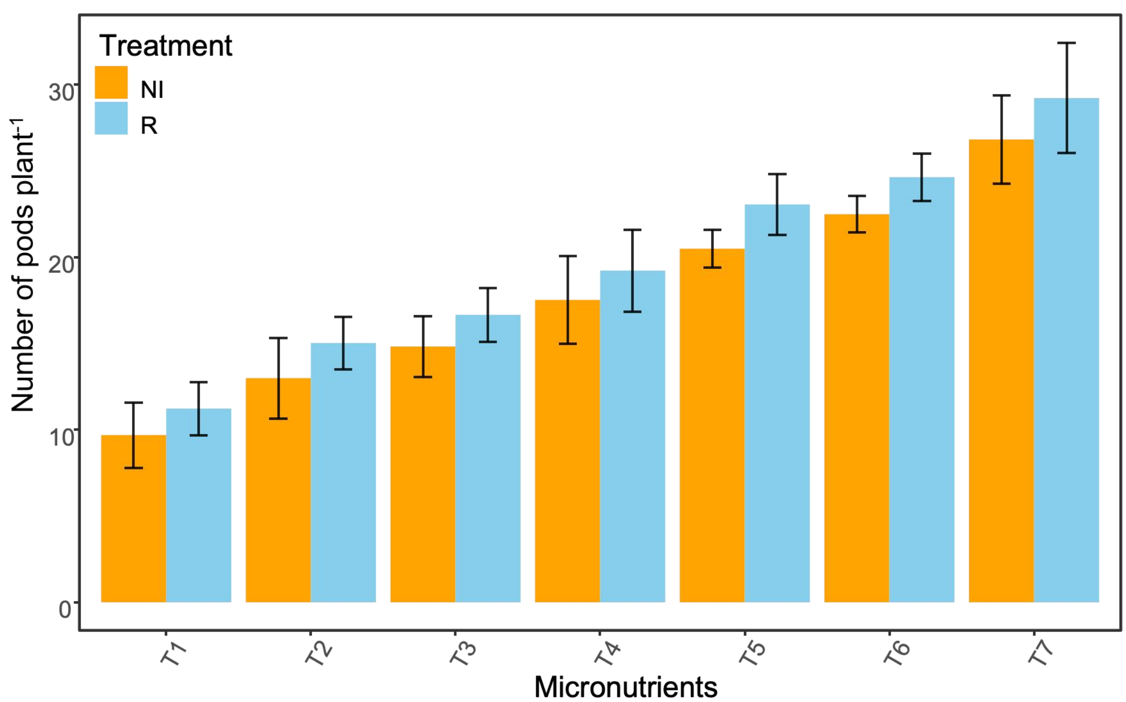

Figure 3. Number of pods per plant of blackgram as influenced by *Rhizobium* inoculation and micronutrient supplementation. Bars represent standard error. NI = Non-inoculated; R = *Rhizobium* inoculated. Treatments: T1 = Control, T2 = 250 ppm Mo, T3 = 250 ppm B, T4 = 500 ppm B, T5 = 500 ppm Mo, T6 = 250 ppm B + 250 ppm Mo, T7 = 500 ppm B + 500 ppm Mo.

Table 4. Effect of *Rhizobium* inoculation on various yield parameters.

Treatment	Pod length (cm)	Seed per pod	1000 seed weight(gram)
Inoculation	4.18[a]	5.74[a]	56.35[a]
Non-inoculation	4.068[b]	5.41[b]	50.88[b]
SEM±	0.013	0.027	0.319
LSD0.05	0.0847	0.16433	1.94
p-value	*	*	**
CV%	1.5	2.2	2.7

Note: SEM–Standard Error of the Mean, LSD–Least Significant Difference, P value *** indicates significance at the 0.001 level.

Table 5. Effect of molybdenum (Mo) and boron (B) application on grain yield components of blackgram.

Treatment	Pod length (cm)	Seed per pod	1000 seed weight (gram)
Control	3.75[f]	5.2e	44.54[f]
250 ppm B	3.86[e]	5.30[e]	48.16[e]
250 ppm Mo	3.99[d]	5.45[d]	51.345[d]
500 ppm B	4.17[c]	5.58[c]	54.405[cd]
500 ppm Mo	4.28[b]	5.67[c]	55.99bc
250 ppm B +250 ppm Mo	4.39[a]	5.79[b]	58.508[b]
500 ppm B +500 ppm Mo	4.44[a]	6.01[a]	62.35[a]
Grand mean	4.125	5.574	53.61
SEM±	0.023	0.037	1.09
$LSD_{0.05}$	0.069	0.109	3.18
p-value	***	***	***
CV%	1.4	1.6	5

Note: SEM – Standard Error of the Mean, LSD – Least Significant Difference, P value *** indicates significance at the 0.001 level.

3.4 Yield and yield components

Although *Rhizobium* inoculation and micronutrient priming did not show statistically significant effects on the number of pods per plant (Figure 3), the highest pod counts were recorded in the combined 500 ppm B and Mo treatment. This suggests that while the treatments generally improve vegetative growth, pod development may also depend on other factors such as genetic potential and environmental conditions (Kuzbakova et al., 2022). Enhanced meristematic activity due to *Rhizobium* inoculation could stimulate floral development, leading to more pods (Tripathi et al., 2021; Sadiq et al., 2023)

Rhizobium inoculation significantly increased pod length, number of seeds per pod, and 1000 seed weight compared to non-inoculated plants (Table 4). Increased nitrogen fixation fosters improved nutrient availability, enhancing pod growth and seed development. This symbiotic relationship results in larger pods, more seeds, and heavier seeds, reflecting higher crop productivity (Kavitha and Srimathi, 2022).

Seed priming with B and Mo significantly improved pod length, seeds per pod, and 1000 seed weight (Table 5). The combination of 500 ppm B and 500 ppm Mo showed the highest pod length (4.44 cm), seed number per pod (6.01), and seed weight (62.35 g). These micronutrients enhance nitrogen fixation, nutrient uptake, and carbohydrate metabolism, thereby promoting reproductive development and seed filling (Hossain et al., 2021; Veer et al., 2022). Improved photosynthate translocation to reproductive organs supports increased seed size and quality (Myageri & Dawson, 2022).

The highest grain yield (120.73 g per 2.1 m^2) was obtained from the combined treatment of *Rhizobium* inoculation with 500 ppm B and 500 ppm Mo (Figure 4). This yield improvement is attributed to enhanced nitrogen fixation by *Bradyrhizobium*, better nutrient use efficiency, increased photosynthesis, and improved growth parameters such as height, branching, and biomass accumulation (Fazil et al., 2024). Boron and molybdenum also contributed to stress tolerance and efficient nutrient assimilation, leading to increased yield (Thilakarathna et al., 2019; Thomas & Vincent, 2012).

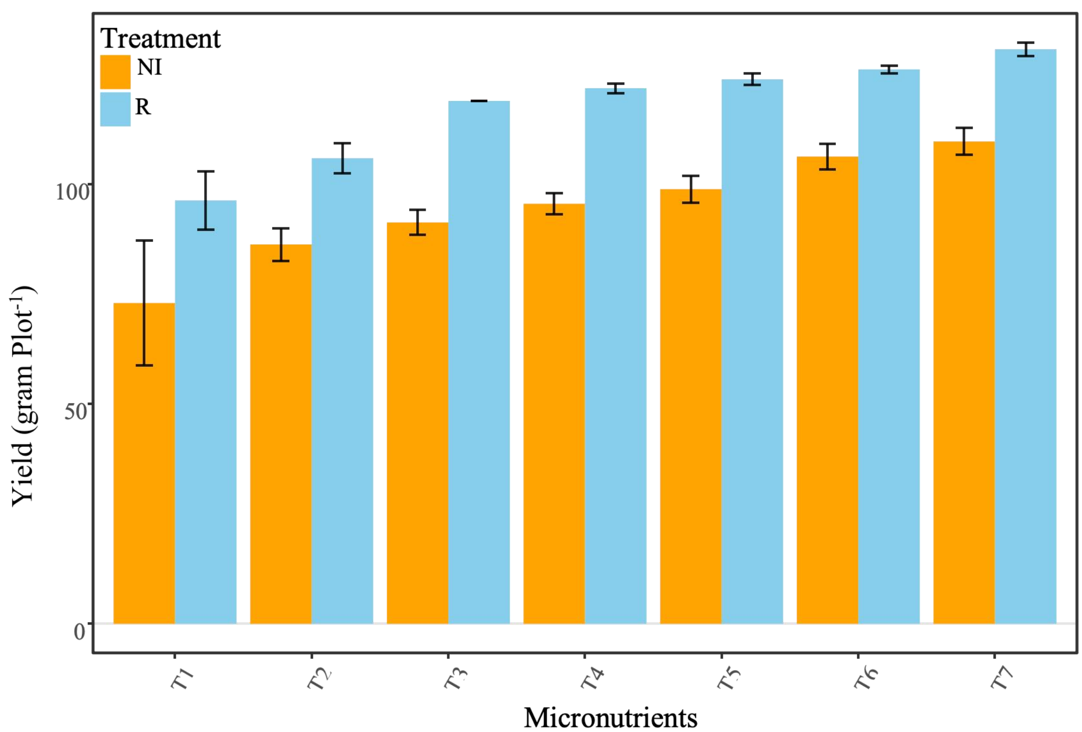

Figure 4. Grain yield of blackgram per plot (2.1 m²) as influenced by *Rhizobium* inoculation and micronutrient supplementation. Both factors had a highly significant effect ($p < 0.001$).

The observed synergistic effect underscores the importance of combined micronutrient fertilization and microbial inoculation for optimizing black gram productivity

4. Conclusion

The combined application of micronutrients-specifically 500 ppm boron (B) and 500 ppm molybdenum (Mo) produced significant positive effects on multiple growth and yield parameters. These included increased plant height, more branches, higher nodulation counts, longer and more numerous pods, improved 1000-seed weight, and ultimately, increased grain yield. This study shows that *Rhizobium* inoculation significantly improves black gram growth and yield, with inoculated plants displaying increased height, branches, nodulation, and seeds per plant. These benefits, along with micronutrient supplementation, are especially important for boosting productivity in micronutrient-deficient soils. However, the study also identified environmental challenges that may affect these benefits. Heavy rainfall during the cultivation period likely caused considerable leaching of essential nutrients and rhizobia, which can reduce nutrient availability and negatively impact the symbiotic relationship necessary for nitrogen fixation. To maximize inoculation and micronutrient effectiveness, managing environmental factors like rainfall is essential. Future research should explore rainfall impacts on nutrient retention and develop strategies to minimize leaching, supporting sustainable black gram cultivation and food security in vulnerable regions.

Author contributions

Shankar Bhandari: Designed and conducted experiment, data collection, data analysis, wrote original manuscript. Gaman Sharma: Data analysis and wrote original manuscript. Sudikshya Shrestha: Review and editing and supervised experiment. All authors have read and approved the final version of the manuscript

Ethical Approval

Not applicable

Acknowledgments

Not applicable

Conflicts of interest

The authors declare no conflict of interest.

Availability of data and materials

Data will be available on a formal request from the corresponding authors.

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Not applicable

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