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RESEARCH ARTICLE

Growth and Yield Responses of Mungbean to *Rhizobium phaseoli* Inoculation and Urea Fertilizer Application

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ABSTRACT

Mungbean (*Vigna radiata* L.) is an important legume for food and nutritional security, but its productivity is often constrained by poor nodulation and soil fertility challenges. Integrating rhizobial inoculation with mineral nitrogen inputs has been proposed as a strategy to improve growth and yield while reducing fertilizer dependency. This study examined the independent and combined effects of *Rhizobium phaseoli* inoculation and urea fertilization on mungbean growth, nodulation, and yield under acidic soil conditions. A split plot design was used with three levels of *Rhizobium* inoculation (0, 10, and 15 g/kg seed) and four levels of urea fertilizer (0, 25, 37.5, and 50 kg/ha), resulting in 12 treatment combinations replicated three times. Parameters measured included nodule number, effective nodules, leaf area, plant height, number of filled pods, seeds per pod, hundred-seed weight, and seed yield per plant, per plot, and per hectare. Data were analyzed using ANOVA followed by Duncan's Multiple Range Test at the 5% significance level. No significant interaction between inoculation and urea was detected for any parameter. Urea application significantly reduced nodulation, with unfertilized plants forming the most total (57.1 nodules/plant) and effective nodules (28.0 nodules/plant). Inoculation did not improve growth or yield, likely due to soil acidity limiting rhizobial effectiveness. Most yield components were unaffected by treatments, but grain yield per plot was highest without urea (604.6 g/plot; 1.61 t/ha), declining with fertilizer application. High urea inputs suppressed nodulation and failed to improve yield under acidic soil conditions. In contrast, unfertilized plants achieved the best balance of nodulation and yield. These findings highlight the need for soil amelioration and the use of adapted rhizobial strains rather than reliance on mineral nitrogen fertilization. Sustainable mungbean productivity will depend on integrating biological nitrogen fixation with improved soil fertility management.

Key words: *Vigna radiata*, *Rhizobium phaseoli*, Urea fertilization, Nodulation, Nitrogen management, Yield.

INTRODUCTION

Mungbean (*Vigna radiata* L.) is an important short-duration legume crop widely cultivated in Asia and increasingly recognized for its nutritional and agronomic value. It provides a rich source of protein, micronutrients, and essential amino acids, making it a key component of food security strategies in regions with limited access to animal protein (Suharno et al., 2023). Beyond its role in human nutrition, mungbean offers agronomic benefits through crop diversification, soil fertility improvement, and compatibility within cereal-based cropping systems (Rahman et al., 2024). Its short growth cycle allows it to fit well into intercropping or rotation systems, particularly with rice and maize, thereby improving land use efficiency (Singh et al., 2023).

Nitrogen (N) is one of the most limiting nutrients in mungbean production, influencing vegetative growth, nodule formation and ultimately seed yield. While legumes have the inherent capacity to fix atmospheric nitrogen through symbiosis with rhizobia, the efficiency of biological nitrogen fixation (BNF) depends on several factors including rhizobial strain, soil fertility, and agronomic practices (Zhou et al., 2022). In many tropical soils, low native rhizobial populations or poor effectiveness of indigenous strains often constrain nodulation and nitrogen fixation in mungbean (Haque et al., 2023). To overcome this, inoculation with effective *Rhizobium* strains has been promoted as a low-cost and environmentally friendly practice that can enhance nodulation, N fixation, and yield (Zhang et al., 2024).

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Despite this, farmers in many regions continue to rely heavily on chemical fertilizers, particularly urea, to meet nitrogen requirements of mungbean and other legumes. Urea application can increase early vegetative growth, stimulate photosynthesis, and improve yield components when applied at optimal rates (Li et al., 2022). However, excessive or poorly managed urea fertilization may inhibit nodulation and symbiotic nitrogen fixation due to reduced plant dependence on rhizobia, while also contributing to environmental problems such as nitrate leaching, ammonia volatilization, and greenhouse gas emissions (Wang et al., 2023). Thus, the integration of biological inoculants with reduced levels of urea fertilizer has emerged as a promising strategy to sustain productivity while lowering external nitrogen inputs (Ali et al., 2023).

The effectiveness of *Rhizobium* inoculation in mungbean varies widely depending on host genotype, inoculant quality, and soil conditions (Kumar et al., 2024). Some studies report significant increases in nodulation, biomass, and grain yield with inoculation (Rahman et al., 2022), whereas others show limited or no response, especially in soils with high nitrogen availability (Hu et al., 2023). Likewise, the response to urea fertilization in mungbean has been inconsistent, with low to moderate rates often proving beneficial, but higher rates suppressing nodulation and failing to increase yield (Tahir et al., 2024). These contrasting outcomes suggest the need for research that directly compares the relative and interactive effects of *Rhizobium* inoculation and urea fertilization under specific agroecological conditions.

Recent advances in sustainable agriculture emphasize integrated nutrient management approaches, where the judicious combination of organic amendments, biofertilizers, and chemical fertilizers improves nutrient use efficiency and soil health (Zhou et al., 2022; Sahoo et al., 2024). For legumes such as mungbean, striking the right balance between symbiotic nitrogen fixation and supplemental nitrogen supply is crucial. Enhancing BNF through inoculation while reducing chemical fertilizer inputs aligns with global climate-smart agriculture goals, ensuring that productivity gains do not come at the expense of soil and environmental sustainability (FAO, 2023).

In Indonesia and other tropical countries, mungbean production is often constrained by poor soil fertility, particularly low nitrogen levels. Farmers typically apply urea as the main nitrogen source, but its efficiency is limited by leaching and volatilization losses under high rainfall conditions (Putra et al., 2023). Inoculation with effective *Rhizobium phaseoli* strains may offer a practical solution to improve nodulation and reduce the need for external N fertilizers. However, evidence on the combined use of inoculants and reduced urea rates in local mungbean production systems remains limited.

Given these considerations, this study was conducted to evaluate the effects of *Rhizobium phaseoli* inoculation and urea fertilization on the growth and yield of mungbean. Specifically, it aimed to determine whether inoculation could reduce the need for chemical nitrogen inputs while maintaining or improving yield performance. The outcomes are expected to contribute to the development of sustainable nitrogen management practices in mungbean cultivation and provide insights for optimizing legume productivity in smallholder systems.

MATERIALS AND METHODS

The experiment was conducted in a field trial using mungbean (*Vigna radiata* L.) cultivar Vima-2 as the plant material. Seeds were obtained from a certified source, and inoculation was carried out using a mungbean-specific *Rhizobium phaseoli* inoculant provided by a microbiology laboratory. Fertilizers applied during the study included urea, SP-36, and KCl, while crop protection employed a commercial insecticide as needed to control pests. Standard field tools such as hoes, sprayers, measuring devices, and analytical balances were used to facilitate land preparation, crop management, and data collection.

The study followed an experimental approach based on a split plot design. The main plot factor was the level of *Rhizobium phaseoli* inoculation, which consisted of three treatments: no inoculation, inoculation at 10 g/kg seed, and inoculation at 15 g/kg seed. The subplot factor was urea fertilization, which comprised four application rates: 0, 25, 37.5, and 50 kg/ha. This factorial arrangement resulted in 12 treatment combinations, each replicated three times, yielding a total of 36 experimental units. Treatments were applied at sowing and managed according to recommended agronomic practices for mungbean cultivation.

Observations were recorded on several growth and yield parameters to assess treatment effects. Growth parameters included plant height and leaf area, while symbiotic performance was evaluated by counting the total number of root nodules and the number of effective nodules per plant. Yield-related traits measured were the number of filled pods per plant, number of seeds per pod, dry seed weight per plant, 100-seed weight, and total dry seed yield per plot. Data collected from these observations were subjected to analysis of variance (ANOVA). Where significant differences were detected, mean separation was performed using Duncan's Multiple Range Test (DMRT) at the 5% level of significance.

RESULTS

The combined analysis of variance revealed that there was no significant interaction between *Rhizobium phaseoli* inoculation and urea fertilization across all measured parameters. Nevertheless, the independent effect of urea dosage was evident in several variables, particularly in root nodulation and overall seed yield. The results are presented in detail below.

Root Nodules

The number of nodules formed was significantly influenced by urea fertilization, whereas inoculation with *Rhizobium phaseoli* alone did not alter nodule production. As summarized in Fig. 1, the absence of urea (N0) resulted in the highest average nodule formation (57.06 nodules per plant). By contrast, plants receiving 50 kg/ha of urea (N1) had markedly fewer nodules (29.67 per plant), representing almost a 50% reduction compared to the unfertilized control. Intermediate urea rates of 37.5 and 25 kg/ha (N2 and N3, respectively) also produced relatively low nodule counts, averaging 24.67 and 25.61 nodules per plant. Inoculation with either 10 g/kg or 15 g/kg seed inoculant did not improve nodulation relative to the uninoculated control. This pattern indicates that nitrogen supplied from urea diminished the plant's reliance on symbiotic fixation, thereby reducing the initiation and development of nodules.

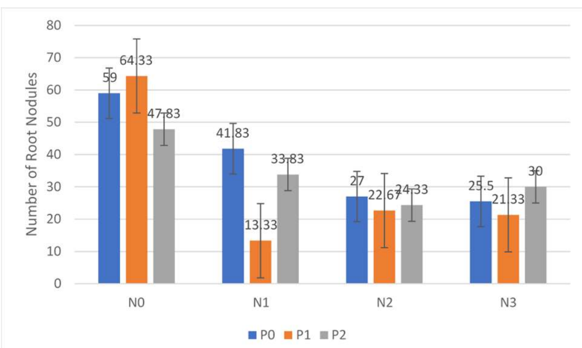

Fig. 1: Number of root nodules formed in mungbean under *Rhizobium phaseoli* inoculation and urea fertilizer treatments (31 days after planting).

A similar response was observed for the number of effective nodules, defined as those capable of active nitrogen fixation. As shown in Fig. 2, plants without urea fertilization (N0) had the greatest number of effective nodules (28 per plant on average). At 50 kg/ha urea (N1), effective nodules were drastically reduced to fewer than 10 per plant. Inoculation treatments did not show consistent improvements, suggesting that soil conditions limited the establishment of the inoculant strain.

These findings highlight that high levels of available nitrogen from urea suppress both total and effective nodulation, while inoculation benefits were not expressed under the acidic soil conditions of the experimental site.

Leaf Area

Leaf expansion was not significantly affected by either *Rhizobium* inoculation or urea application. As shown in Fig. 3, mean leaf area ranged between 607 and 937 cm² per plant. While numerical differences existed, particularly with larger leaf areas observed in treatments with moderate urea input, statistical analysis confirmed no significant variation. This indicates that leaf expansion in mungbean was constrained more by soil fertility and environmental factors than by the experimental treatments.

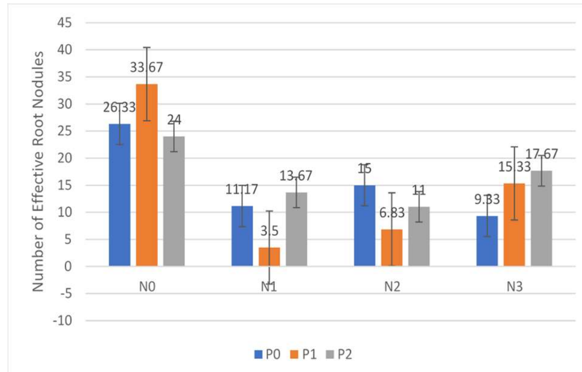

Fig. 2: Number of effective root nodules in mungbean under *Rhizobium phaseoli* inoculation and urea fertilizer treatments (31 days after planting).

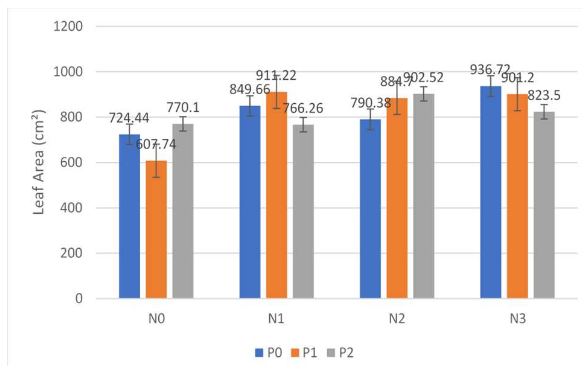

Fig. 3: Leaf area (cm²) of mungbean under *Rhizobium phaseoli* inoculation and urea fertilizer treatments.

Plant Height

Similar to leaf area, plant height was unaffected by inoculation or urea fertilization. Mean values ranged from 45.43 to 55.69 cm (Fig. 4). Although plants in some fertilized treatments tended to be slightly taller, no treatment effect reached statistical significance. This suggests that under the prevailing acidic soil conditions, neither inoculation nor urea application substantially improved vertical growth.

Filled Pods per Plant

The number of filled pods per plant, defined as pods containing at least 50% seeds, was not significantly affected by either factor. Mean pod number ranged from 49 to 52 across treatments (Fig. 5).

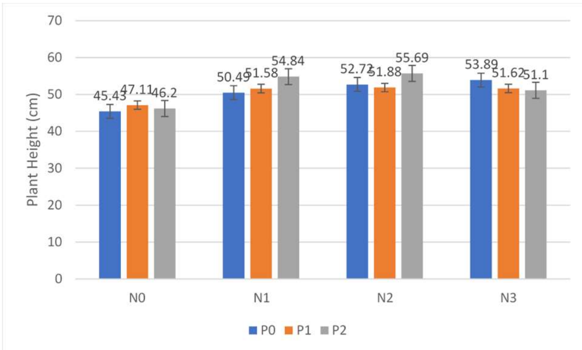

Fig. 4: Plant height (cm) of mungbean under *Rhizobium phaseoli* inoculation and urea fertilizer treatments.

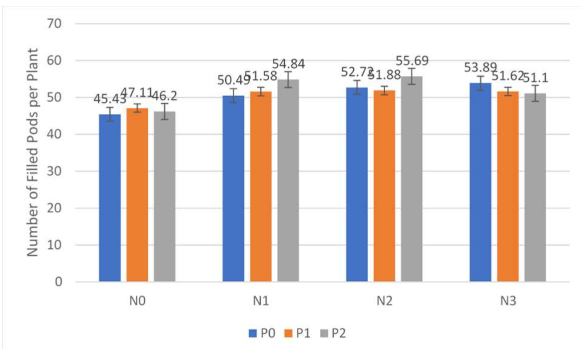

Fig. 5: Number of filled pods per mungbean plant under *Rhizobium phaseoli* inoculation and urea fertilizer treatments.

Number of Seeds per Pod

The number of seeds per pod did not differ significantly among inoculation or urea treatments. As presented in Fig. 6, values were remarkably sFigure across treatments, ranging from 9.04 to 11.09 seeds per pod. The overall average was approximately 10–11 seeds per pod, consistent with varietal characteristics of Vima-2 mungbean. Although minor numerical variation was observed, neither *Rhizobium* inoculation nor urea application provided a significant advantage. This indicates that seed set within pods was primarily governed by genetic potential rather than external nitrogen supply.

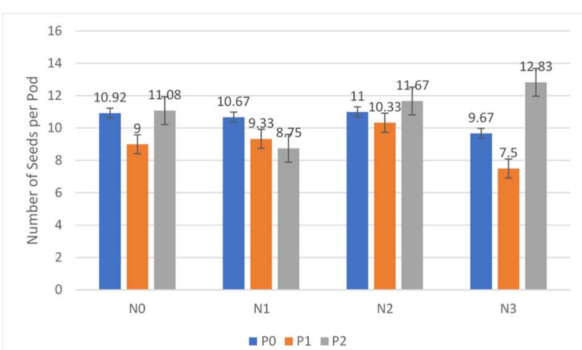

Fig. 6: Number of seeds per pod in mungbean under *Rhizobium phaseoli* inoculation and urea fertilizer treatments.

Seed Weight per Plant

Dry seed weight per plant was not significantly affected by inoculation or nitrogen application. Across all treatments, individual plant yield ranged narrowly, without clear trends associated with either factor (Fig. 7). This suggests that although nodulation was suppressed by high urea levels, compensatory effects in photosynthesis and assimilate partitioning may have maintained seed weight at the plant level.

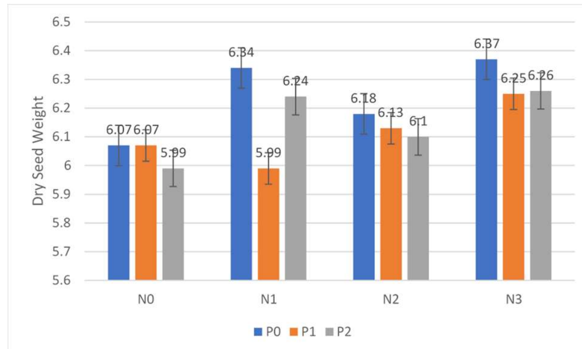

Fig. 7: Dry seed weight per mungbean plant (g) under *Rhizobium phaseoli* inoculation and urea fertilizer treatments.

Hundred-Seed Weight

Seed size, expressed as 100-seed weight, also showed no significant treatment effects. As presented in Fig. 8, values exceeded 6 g across all treatments, with little variation among inoculation or urea levels. This stability implies that seed development was largely unaffected by external nitrogen sources, and that pod filling in mungbean is relatively resilient under suboptimal soil conditions.

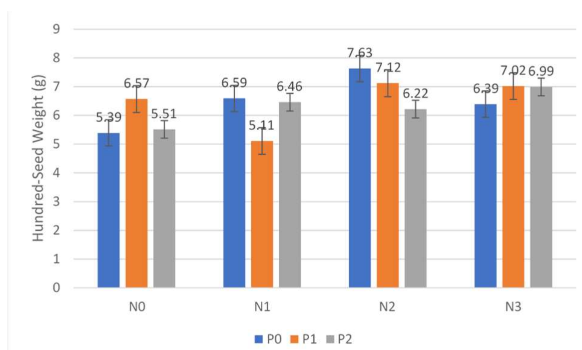

Fig. 8: Hundred-seed weight (g) of mungbean under *Rhizobium phaseoli* inoculation and urea fertilizer treatments.

Seed Yield per Plot

Unlike most yield components, seed yield per plot was significantly affected by urea fertilization. As shown in Fig. 9, unfertilized plants (N0) produced the highest mean plot yield (604.62 g), whereas plots receiving urea (25–50 kg/ha) yielded slightly less, averaging between 581.52 and 589.53 g. Inoculation did not significantly alter yield within any urea level. These results confirm that under the acidic soil conditions of the trial, high urea application reduced the effectiveness of symbiotic nitrogen fixation without conferring additional yield benefits.

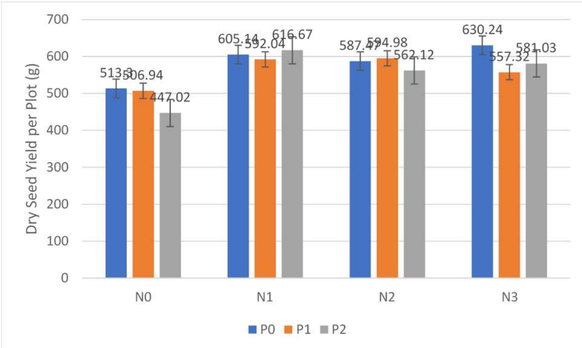

Fig. 9: Dry seed yield per mungbean plot (g) under *Rhizobium phaseoli* inoculation and urea fertilizer treatments.

Seed Yield per Hectare

When yields were extrapolated to a hectare basis (Fig. 10), the same pattern was observed. The highest yield (1.61 t/ha) was obtained in plots without urea (N0), while yields in urea-fertilized plots (25–50 kg/ha) ranged between 1.55 and 1.57 t/ha. These values are substantially below the potential yield of the Vima-2 variety (2.4 t/ha), reflecting the limitations imposed by soil acidity and nutrient availability.

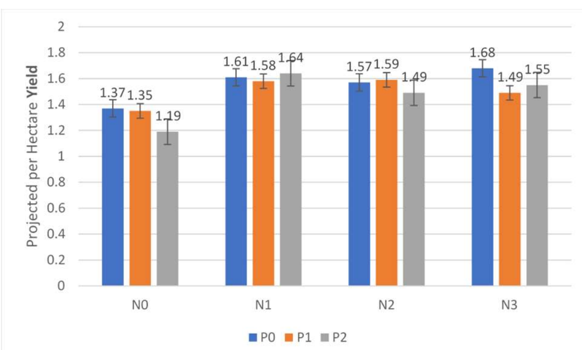

Fig. 10: Projected per hectare dry seed yield of mungbean (t/ha) under *Rhizobium phaseoli* inoculation and urea fertilizer treatments.

Overall, the results demonstrate that while inoculation with *Rhizobium phaseoli* did not significantly improve nodulation or yield under the given soil conditions, urea fertilization strongly influenced nodulation and yield outcomes. Nodulation (both total and effective) was highest in the absence of urea, confirming that external nitrogen reduced the plant's reliance on symbiotic fixation. Plot and hectare yields, however, were maximized under unfertilized conditions, suggesting that under acidic soils, additional nitrogen inputs were not efficiently utilized and may even have suppressed yield potential. Most other growth and yield parameters, including leaf area, plant height, filled pods, seeds per pod, and seed size, were unaffected by either treatment.

DISCUSSION

The present study demonstrated that neither *Rhizobium phaseoli* inoculation nor urea fertilization produced significant interaction effects on mungbean growth and yield parameters. This outcome suggests that under the conditions of the trial, particularly the acidic soil environment, the contributions of inoculated rhizobia and applied nitrogen operated independently, without synergistic enhancement. The suppression of nodulation at higher urea levels and the absence of significant benefits from inoculation reflect the challenges of managing nitrogen in legume-based systems when soil constraints limit microbial activity and nutrient uptake.

One of the clearest findings was the decline in both total and effective nodules as urea dosage increased. Plants without nitrogen fertilization formed the highest number of nodules, whereas the application of 50 kg/ha of urea reduced nodulation by more than half. This inverse relationship between mineral nitrogen availability and biological nitrogen fixation is well established. When readily available nitrogen is abundant, plants rely less on symbiotic associations, resulting in reduced nodule initiation and function (Hu et al., 2023; Tahir et al., 2024). Several studies with mungbean and other legumes confirm that excessive urea suppresses nodulation and nitrogen fixation (Rahman et al., 2022; Haque et al., 2023). Thus, the results of this study reinforce the concept that optimal nitrogen management in legumes requires balancing external supply with symbiotic contributions.

Interestingly, inoculation with *Rhizobium phaseoli* did not significantly improve nodulation or yield relative to the uninoculated control. This may be attributed to the acidic soil conditions of the experimental site, with pH near 5.0. Acidic soils are known to limit the survival, proliferation, and infection efficiency of rhizobia (Zhou et al., 2022). High concentrations of exchangeable aluminum and manganese in such soils can be toxic to both rhizobia and host root systems (Singh et al., 2023). Previous work has shown that rhizobial inoculants often fail in acidic environments unless lime or other amendments are used to raise soil pH. This constraint likely explains why inoculation effects were negligible, despite the use of a recognized inoculant source.

Leaf area and plant height were unaffected by treatments, highlighting that vegetative growth was limited by environmental conditions rather than nitrogen management. The low responsiveness of these parameters suggests that mungbean growth potential was constrained by multiple soil fertility factors beyond nitrogen, such as phosphorus or micronutrients, which are often deficient in highly weathered soils (Sahoo et al., 2024). Moreover, photosynthetic capacity and leaf expansion are closely tied to soil moisture and root health, which may have been restricted under the prevailing field conditions (Ali et al., 2023).

Yield-related traits, including the number of filled pods per plant, seeds per pod, and hundred-seed weight, were also unaffected by inoculation or urea fertilization. This stability reflects the strong genetic determination of reproductive traits in mungbean. Several studies report that while nodulation and biomass may vary, seed size and seed number per pod tend to remain constant across environments (Li et al., 2022; Singh et al., 2023). Thus, the lack of treatment effects on these parameters is consistent with the conservative nature of yield components in short-duration legumes.

Seed yield per plot and per hectare provided the most striking results. Yields were significantly higher in unfertilized plots compared to those receiving urea. This suggests that the addition of mineral nitrogen not only failed to improve productivity but also suppressed potential yields. Such outcomes are plausible in acidic soils where nitrogen fertilization can exacerbate soil acidity and reduce nutrient availability (Putra et al., 2023). Furthermore, the suppression of nodulation by urea may have deprived plants of symbiotically fixed nitrogen during critical reproductive stages, limiting final yields despite the presence of applied fertilizer. This pattern aligns with findings in other legume systems where low to moderate nitrogen input enhances growth, but excessive application reduces biological fixation and depresses yields (Zhang et al., 2024; Kumar et al., 2024).

The yields recorded in this study, averaging 1.3–1.6 t/ha, were below the potential yield of the Vima-2 variety (2.4 t/ha). This yield gap underscores the role of environmental constraints, particularly soil acidity, in limiting the effectiveness of both inoculation and fertilization. Similar yield gaps have been reported in other field studies where adverse soil conditions constrained the responsiveness of mungbean to agronomic interventions (Rahman et al., 2024). Overcoming such barriers requires integrated soil fertility management, including liming, organic amendments, and balanced fertilization, in order to create conditions conducive to both plant growth and microbial symbiosis.

The lack of inoculation response may also reflect the presence of indigenous rhizobia in the soil. If native populations are abundant, introduced strains may not outcompete them, especially under unfavorable soil conditions (Haque et al., 2023). Studies have shown that the effectiveness of inoculation depends heavily on strain competitiveness and adaptation to local soils (Kovács et al., 2024). The negligible difference between inoculated and uninoculated plants in this study suggests that either the indigenous rhizobia were already functional, or that inoculant strains could not establish dominance. Future research should focus on evaluating strain compatibility under acidic soils and exploring co-inoculation strategies with acid-tolerant rhizobia or mycorrhizal fungi.

The environmental implications of the findings are important. Overreliance on urea not only reduced nodulation but also offered no yield advantage. This highlights the potential for reducing nitrogen fertilizer inputs in mungbean production without compromising yield. Lowering fertilizer use is consistent with sustainable intensification goals and can reduce greenhouse gas emissions and nutrient losses (Wang et al., 2023). Encouraging biological nitrogen fixation through appropriate inoculation and soil management may offer a more sustainable pathway.

From a practical standpoint, the results suggest that under acidic soil conditions similar to those of the study, applying urea beyond minimal levels is not beneficial for mungbean production. Instead, emphasis should be placed on improving soil conditions through liming and organic amendments, coupled with the use of adapted inoculants. This approach would enhance nodulation, support sustainable nitrogen supply, and potentially close the yield gap. Additionally, integrated nutrient management practices that combine small amounts of fertilizer with biological inputs have been shown to improve efficiency and resilience in legume systems (Sahoo et al., 2024; Ali et al., 2023).

In conclusion, the findings of this study reinforce the complex relationship between mineral nitrogen fertilization and symbiotic nitrogen fixation in mungbean. While external nitrogen supply can stimulate growth under some conditions, excessive reliance on urea suppresses nodulation and fails to improve yield, particularly in acidic soils. The absence of inoculation effects underscores the need for locally adapted strains and supportive soil management practices. Future efforts should focus on integrating rhizobial inoculation with soil amelioration and balanced fertilization to maximize the dual benefits of biological and chemical nitrogen sources in mungbean production.

Conclusion

This study evaluated the effects of *Rhizobium phaseoli* inoculation and urea fertilization on the growth and yield performance of mungbean (*Vigna radiata* L.). The results clearly indicated that there was no significant interaction between inoculation and fertilizer application across measured parameters, reflecting the independent nature of their effects under the experimental conditions. Urea fertilization significantly influenced root nodulation, with the highest number of both total and effective nodules recorded in unfertilized plants, while high nitrogen doses strongly suppressed nodulation. This confirms that external nitrogen supply diminishes the plant's reliance on symbiotic nitrogen fixation.

Inoculation with *Rhizobium phaseoli* did not produce significant improvements in nodulation, vegetative growth, or yield compared with the uninoculated control. The lack of responsiveness is likely linked to the acidic soil environment of the trial, which limited rhizobial survival, competitiveness, and infection efficiency. Yield components including leaf area, plant height, filled pods per plant, seeds per pod, and hundred-seed weight were largely unaffected by either factor, reflecting the strong genetic control of reproductive traits in mungbean.

Grain yield per plot and per hectare was significantly affected by urea fertilization, but unexpectedly, the highest yields were recorded in unfertilized plots. This suggests that in acid soils, high mineral nitrogen inputs are not efficiently utilized and may even constrain yield by suppressing nodulation. Average yields ranged from 1.3–1.6 t/ha, considerably below the potential of the Vima-2 variety (2.4 t/ha), indicating a persistent yield gap driven by soil limitations.

Overall, the findings emphasize the need for integrated soil and nutrient management strategies in mungbean production. Rather than relying on high doses of urea, improving soil conditions through liming and organic amendments, together with the use of acid-tolerant and competitive rhizobial strains, may enhance biological nitrogen fixation and yield performance. Reducing fertilizer dependency while strengthening biological inputs aligns with sustainable intensification goals, offering pathways to improve mungbean productivity in smallholder systems across the tropics.

DECLARATIONS

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Data Availability: The data will be available upon request to the corresponding authors.

Ethics Statement: All animal studies have been approved by the relevant ethics committee or institutional supervisory board and have been conducted in accordance with the ethical standards.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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