

Effect of Nitrogen Fertilizer Application and Rhizobium Bacteria Inoculation on Growth and Yield of Soybean (*Glycine Max* (L.) Merrill) in Nangarhar Province, Afghanistan.

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ABSTRACT

One of the major advancements in modern agriculture from biological nitrogen fixation (BNF) research is the development of *Rhizobium* inoculants. Soybean, a legume with high nitrogen demand, forms an effective symbiosis with *Rhizobium* species, making it highly responsive to BNF. In Afghanistan, however, limited information exists on the combined effects of nitrogen fertilizer and *Rhizobium* inoculation on soybean productivity. This study evaluated the effects of *Rhizobium japonicum* inoculation, nitrogen fertilizer at two rates (25 and 50 kg N ha⁻¹), and their integration on soybean growth and yield. A field experiment was conducted during the 2024 summer season in Behsud district, Nangarhar province, using a randomized complete block design with three replications. Treatments included control (no inoculation or nitrogen), *Rhizo-Power* inoculation, 25 kg N ha⁻¹, and 50 kg N ha⁻¹. Growth, reproductive, and yield parameters were measured, and data were analyzed using ANOVA at $p < 0.05$. Results showed significant treatment effects. The highest grain yield (1135 kg ha⁻¹), hay yield (821 kg ha⁻¹), and biological yield (1956 kg ha⁻¹) were obtained under full nitrogen application. *Rhizobium* inoculation alone and 25 kg N ha⁻¹ produced comparable grain yields (1025 and 992 kg ha⁻¹, respectively), while the control yielded the lowest (650 kg ha⁻¹). The findings demonstrate that integrating *Rhizobium* inoculation with reduced nitrogen fertilizer sustains high soybean yields, offering a cost-effective and environmentally friendly nutrient management strategy for Afghanistan's agro-ecological conditions.

Keywords: Biological nitrogen fixation, Growth, Nitrogen fertilizer, *Rhizobium* inoculation, Soybean, yield

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a globally important leguminous crop, valued for its high protein content (≈40%), oil content (≈20%), and its diverse applications in food, feed, and industry (1). In Afghanistan, soybean was first introduced in the 1970s and later revitalized through international initiatives aimed at improving nutrition and soil fertility. Despite these efforts, soybean productivity in the country remains low, primarily due to limited knowledge and adoption of improved agronomic practices, particularly nitrogen management (2). Nitrogen is a critical nutrient for soybean growth and yield. Unlike cereals, soybean can meet a substantial portion of its nitrogen requirement through biological nitrogen fixation (BNF) in

symbiosis with *Rhizobium* species (3). The efficiency of this process depends on soil fertility, strain compatibility, and environmental conditions (4). Previous studies indicate that small starter doses of nitrogen fertilizer can stimulate early plant growth and nodulation, whereas excessive nitrogen often suppresses BNF and reduces the benefits of inoculation (5). Research conducted in other regions has demonstrated the potential of integrating moderate nitrogen fertilization with effective *Rhizobium* inoculation. For example, (6) reported that inoculation with efficient strains significantly enhanced nodulation, nitrogen uptake, and yield compared with non-inoculated controls. Similarly, (7) found that applying 20–30 kg N ha⁻¹ as a starter dose improved early

plant vigor while maintaining efficient BNF. In contrast, higher nitrogen levels ($>60 \text{ kg N ha}^{-1}$) were associated with reduced nodule formation and limited yield advantage (8).

In Afghanistan, however, research on soybean nitrogen management remains scarce. Farmers often lack access to quality inoculants and either apply no fertilizer or rely on excessive urea application, both of which limit yield potential and threaten long-term soil health. These challenges highlight the need to evaluate integrated approaches that combine *Rhizobium* inoculation with balanced nitrogen fertilization under local agro-ecological conditions.

Therefore, this study was conducted to investigate the effects of nitrogen fertilizer and *Rhizobium* inoculation on soybean growth and yield in Nangarhar Province, Afghanistan. The results are expected to provide evidence-based recommendations for improving nitrogen management in soybean production systems, thereby enhancing productivity and contributing to sustainable soil fertility.

MATERIALS AND METHODS

Study Area

The study was conducted in Joi-10 village, Behsud district, Nangarhar province, Afghanistan ($34^{\circ}15'13''\text{N}$, $70^{\circ}58'33''\text{E}$; 546 m above sea level) during the summer of 2024. The region experiences a semi-arid to arid climate, with average annual rainfall between 250–400 mm, and hot summer temperatures reaching 48–49°C. Irrigation was supplied biweekly using local canals to supplement the low rainfall.

Meteorological data including temperature, rainfall, humidity, and wind speed were recorded from the Shesham-Bagh station, Jalalabad. A field experiment was conducted using a randomized complete block design (RCBD) comprising ten treatments, each replicated three times, totaling 30 plots. Each plot measured $2 \times 3 \text{ m}$ (6 m^2), with row and plant spacing of 40 cm and 10 cm, respectively. The treatments included combinations of nitrogen (as urea), farmyard manure (12 t ha^{-1}), and *Rhizobium japonicum* inoculation. Soybean variety 'Dewon' was selected and inoculated with *Rhizobium japonicum* at 14 g kg^{-1} seed using a brown sugar (gur) solution. Inoculation was done under shade immediately before sowing to maintain bacterial viability. Seeds were sown manually at a depth of 2–3 cm on

June 22, 2024. The conducted experiment comprised ten treatments: T_1 (control), T_2 (*Rhizobium* at 14 g kg^{-1} seed), T_3 (50% recommended N), T_4 (100% recommended N), T_5 (FYM 12 t ha^{-1}), T_6 (50% N + *Rhizobium*), T_7 (100% N + *Rhizobium*), T_8 (FYM + *Rhizobium*), T_9 (50% N + FYM + *Rhizobium*), and T_{10} (100% N + FYM + *Rhizobium*).

Soil samples (0–30 cm) depth were collected before sowing and analyzed for physicochemical properties at the Shesham-Bagh Agricultural Institute-Nangarhar, Afghanistan. The soil was sandy loam in texture with a pH of 8.15, EC of 0.149 mmhos/cm, organic carbon 1.80%, total N 0.155%, available N $393.88 \text{ kg ha}^{-1}$, available P 42.12 kg ha^{-1} , and available K $344.00 \text{ kg ha}^{-1}$. Fertilizers included urea (46% N) and tri-super phosphate (17% P_2O_5). Nitrogen was applied during sowing and after the first weeding, away from the seed to prevent injury, following standard procedures (Jackson, 1973; (13) Walkley & Black, 1934; (14) Subbiah & Asija, 1956) (15).

Land was plowed and properly harrowed on June 18, 2024 and the field was laid out into 30 plots followed by manual sowing of seeds on June 22. The seedling emergence was observed by July 1. Nitrogen fertilizer and FYM was applied before sowing. Weeding was done manually at 3 and 6 weeks after sowing. A total of eight irrigations were applied during the growing season, with irrigation scheduled twice per month based on field conditions. Pest control involved the use of Cypermethrin (for cutworm) and Acetamiprid + Abamectin (for aphids), applied monthly post-emergence.

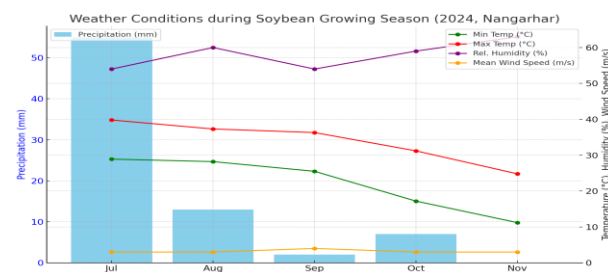


Figure 1: Combined figure of weather conditions (precipitation, temperature, humidity, and wind speed) during the soybean growing season in Nangarhar (Jul–Nov 2024)

Statistical Analysis

All collected data were subjected to one-way analysis of variance (ANOVA) using SPSS software (version 25). Treatment means were separated using Tukey's Honest Significant Difference (HSD) test at a significance level of $p < 0.05$. Economic returns were evaluated based on gross income, net income, and the benefit-cost ratio (CIMMYT, 1988). The benefit-cost ratio (BCR) was calculated by dividing the net return by the total cost of cultivation for each treatment, following the method outlined by CIMMYT (1988). Total costs included inputs such as seed, fertilizers, Rhizobium inoculants, farmyard manure, labor, irrigation, and pest control. Gross income was derived from grain and hay yields based on local market prices, and net return was obtained by subtracting the total cost from the gross income.

Samples Collection

Prior to sowing, composite soil samples were collected from the 0–30 cm depth across the experimental site. The samples were dried at room temperature, sieved through a 2 mm mesh, and analyzed for physicochemical properties at the Shesham-Bagh Agricultural Research Laboratory. Particle size distribution (sand, silt, clay) was determined by the hydrometer method, and soil textural class was identified using the USDA triangle. Soil pH and electrical conductivity (EC) were measured in a 1:2 soil-water suspension. Organic carbon and organic matter were analyzed using the Walkley and Black (1934) method, and total nitrogen was estimated using the Kjeldahl method. Available nitrogen was determined by the alkaline permanganate method (Subbiah & Asija, 1956), available phosphorus by the Olsen method (Bremner & Mulvaney, 1982), and available potassium by flame photometry. Results of the analysis are presented in Table 1.

Table1: Soil Physicochemical Properties of the Experimental Site.

Property	Mean Value
Sand (%)	54.00
Silt (%)	36.00
Clay (%)	10.00
Textural Class	Sandy loam
pH (1:2 Soil: Water)	8.15
Electrical Conductivity (dSm ⁻¹)	0.149

Calcium Carbonate (%)	7.50
Organic Carbon (%)	1.80 (medium)
Available Nitrogen (kg ha ⁻¹)	94.08 (low)
Available Phosphorus (kg ha ⁻¹)	35.4 (Optimum)
Available Potassium (kg ha ⁻¹)	170 (Optimum)

RESULTS

Growth Parameters

Data was recorded 30 days after sowing and at harvest, on germination, plant height, number of branches and leaves, nodulation, pod and seed characteristics, Chlorophyll content was measured using a SPAD-502Plus chlorophyll meter, and yield parameters. Five plants per plot were randomly sampled per plot. Nodules were carefully extracted and counted after washing. Samples were dried at room temperature at 49°C to determine dry matter. Grain and hay yields were converted to kg ha⁻¹. Net returns and benefit-cost ratios were also calculated.

Table 2 presents the effects of various treatments on plant height, number of branches, and number of leaves per plant at harvest. The tallest plants and the highest number of branches were observed in T₄ (100% Nitrogen) with 65.00 cm plant height and 7.55 branches per plant, demonstrating nitrogen's critical role in enhancing vegetative growth. T₂ (Only Rhizobium) and T₃ (50% Nitrogen) also showed strong vegetative performance, with plant heights of 60.89 cm and 59.11 cm, and 7.44 and 7.11 branches per plant, respectively. This indicates the effectiveness of both biological nitrogen fixation and moderate nitrogen application in promoting shoot growth and plant vigor. In contrast, lower plant height and branch numbers were recorded in T₈, T₉, T₁₀, and the control (T₁). The shortest plants were found in T₁₀ (46.55 cm), while the least number of branches (4.89) was recorded in T₁, suggesting that the absence of nitrogen or ineffective treatments leads to reduced vegetative development. Furthermore, the highest number of leaves per plant was observed in T₈ (FYM only) with 102.22 leaves, followed by T₁₀ (97.00) and T₉ (93.44), indicating the positive effect of organic matter on leaf proliferation. However, these treatments did not perform as

well in terms of plant height and branching, likely due to the slow nutrient release from FYM and less availability of readily accessible nitrogen.

These results highlight the importance of nitrogen, especially chemical and biological sources, for maximizing plant height and branching. Although FYM enhanced leaf production, its slower nutrient availability may limit other vegetative traits, suggesting that integrated approaches may be needed to balance growth characteristics.

Table 2: Shows the Effects of Nitrogen Fertilizer and Rhizobium Bacteria on Plant Height, Number of Branches, and Number of Leaves

Treatment	Plant Height (cm) at harvest	Number of Branches (Plant ⁻¹) at harvest	Number of Leaves (Plant ⁻¹) at harvest
T1	56.77	4.89	57.89
T2	60.89	7.44	87.78
T3	59.11	7.11	88.0
T4	65.0	7.55	89.22
T5	54.44	6.22	71.44
T6	51.55	7.0	85.89
T7	57.89	7.33	85.67
T8	53.44	6.66	102.22
T9	51.66	7.11	93.44
T10	46.55	6.89	97.0
LSD	8.97	2.41	23.36
Esm±	3.02	0.81	7.86
C.V%	9.39	20.58	15.86

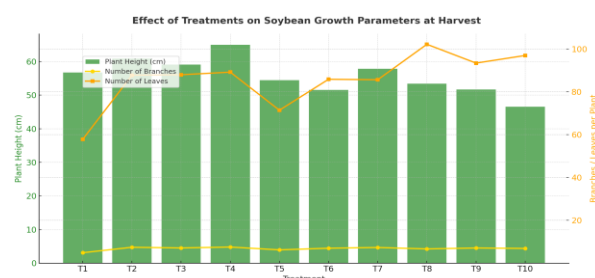


Figure 2: Effect of Treatments on Soybean Growth Parameters at Harvest

Yield Performance

Table 3 presents the number of pods per plant⁻¹, 100-grain weight, percentage of nonstandard grains per pod, and percentage of grains per pod across the ten treatments. These parameters are critical indicators of reproductive efficiency and seed quality in soybean production.

The highest number of pods per plant was observed in T₁₀ (100% N + Rhizobium + FYM) with 57.99 pods, closely followed by T₂ (Rhizobium only) at 55.11 pods, and T₄ (100% N) with 51.44 pods. This suggests that both full nitrogen application and Rhizobium inoculation independently or in combination significantly enhance flowering and pod-setting in soybean. The lowest pod count was recorded in T₁ (Control) with only 30.11 pods, indicating the critical role of nutrient supplementation.

Despite a slight variation in seed weight, the weight of 100 grains remained relatively consistent across treatments, ranging between 15.00 g (T₅) and 17.00 g (T₁, T₃). Treatments receiving Rhizobium and nitrogen (T₃, T₄, T₇) maintained a high grain weight (≥ 16.33 g), which contributes to overall grain yield. Lower grain weights in FYM-only treatment (T₅: 15 g) could be attributed to delayed or suboptimal nutrient availability during seed development.

The percentage of nonstandard grains—an indicator of poor seed development was lowest in T₉ (50% N + Rhizobium + FYM) at 0%, followed by T₂ (1%), T₃ and T₄ (1.33%), and T₇ (1.67%). These results indicate that integrated treatments and Rhizobium alone effectively minimize grain deformities, likely by supporting more consistent nutrient uptake and nodulation. The control (T₁) and FYM-only (T₅) treatments had the highest percentage of nonstandard grains (2.67% and 5%, respectively), underscoring the inadequacy of no or slow-releasing nutrient sources in maintaining seed quality.

Regarding the percentage of grains per pod, the best result was obtained with T₇ (100% N + Rhizobium) at 88.67%, followed by T₄ (86%) and T₂ (81.33%). These treatments also had high pod numbers and low nonstandard grain percentages, suggesting strong reproductive success. On the other hand, the control (T₁) and T₉ recorded the lowest grain percentages per pod (37.33% and 51.67%, respectively), which contributed to lower total grain yield.

Table 3: Shows the Effect of Nitrogen fertilizer and Rhizobium Bacteria on Number of Pods, Weight of 100 Grains, Percentage of

Nonstandard Grains and Percentage of Grains in Pod⁻¹

Treatment	Number of Pods Plant ⁻¹	Weight of 100 Grains (g)	Percentage of Nonstandard Grains in Pod ⁻¹	Percentage of Grains in Pod ⁻¹
T ₁	30.11	17.00	2.67	37.33
T ₂	55.11	16.67	1.00	81.33
T ₃	46.66	17.00	1.33	58.67
T ₄	51.44	16.67	1.33	86.00
T ₅	31.66	15.00	5.00	59.33
T ₆	41.00	16.00	3.67	73.33
T ₇	47.89	16.33	1.67	88.67
T ₈	47.78	15.67	2.33	67.33
T ₉	51.00	16.00	0.00	51.67
T ₁₀	57.99	15.67	2.00	80.33
LSD	21.27	1.349	2.98	12.12
Esm±	7.16	0.454	1.00	4.08
C.V%	26.92	4.855	82.63	10.33

Table 4 presents the effects of various nutrient management treatments on grain yield, hay yield, biological yield, and net return in soybean production. Among all treatments, T₄ (100% Nitrogen) recorded the highest grain yield (1135.00 kg ha⁻¹), hay yield (821.11 kg ha⁻¹), and biological yield (1956.11 kg ha⁻¹), resulting in the maximum net return of 72,227.78 AFN ha⁻¹. This clearly demonstrates the critical role of full nitrogen fertilization in boosting both productivity and profitability. Closely to T₄, T₂ (Only Rhizobium) and T₃ (50% Nitrogen) also showed competitive results, with grain yields of 1025.00 kg ha⁻¹ and 991.67 kg ha⁻¹, and net returns of 64,844.44 AFN ha⁻¹ and 62,577.78 AFN ha⁻¹, respectively. These findings highlight that Rhizobium inoculation alone or in combination with moderate nitrogen application can effectively enhance grain production and offer good economic returns, likely due to improved biological nitrogen fixation. T₇ (100% N + Rhizobium) and T₆ (50% N + Rhizobium) yielded 991.67 kg ha⁻¹ and 870.00 kg ha⁻¹ of grain, respectively, with corresponding net returns of 60,875.00 AFN ha⁻¹ and 51,941.67 AFN ha⁻¹. These results suggest that the combination of Rhizobium with chemical nitrogen, especially at full dose can contribute to improved yield performance and profitability, although the insignificant gains over T₄.

In contrast, treatments involving farmyard manure (FYM) such as T₅ (FYM 12 t/ha-1), T₈ (Rhizobium + FYM), T₉ (50% N + Rhizobium + FYM), and T₁₀ (100% N + Rhizobium + FYM) resulted in lower grain yields (ranging from 697.78 to 805.56 kg ha⁻¹) and net returns (ranging from 38,558.33 to 46,219.44 AFN ha⁻¹). These findings indicate that FYM alone or in complex combinations with other inputs may not release nutrients rapidly enough to meet the crop's demands during critical growth stages, leading to reduced economic efficiency.

The control treatment (T₁), which received no input, produced a lowest grain yield of 650 kg ha⁻¹ and net return of 35500.00 AFN ha⁻¹. The lowest than all treatments, serves as a baseline indicator of soil fertility and productivity without intervention. Surprisingly, this outperformed several FYM-based treatments, further suggesting that FYM, even when combined with Rhizobium or nitrogen, does not significantly enhance yields without timely and sufficient nutrient availability.

The results demonstrate that 100% nitrogen application (T₄) is agronomically and economically the most effective strategy. However, biological approaches such as Rhizobium inoculation (T₂) or partial nitrogen use (T₃) also offer sustainable and profitable alternatives with lower input costs. Conversely, FYM-based treatments, though environmentally friendly, require further optimization to compete with chemical and biological nitrogen sources in terms of yield and profitability.

Table 4: Shows the Effects of Nitrogen Fertilizer and Rhizobium Bacteria on Yield and Economic Performance of Different Treatments.

Treatment	Grain Yield (kg ha ⁻¹)	Hay Yield (kg ha ⁻¹)	Biological Yield (kg ha ⁻¹)	Net Return (AFN/ ha ⁻¹)
T ₁	650.00	500.00	1150.00	35500.00
T ₂	1025.0	777.78	1802.78	64844.44
T ₃	991.67	771.11	1762.78	62577.78
T ₄	1135.0	821.11	1956.11	72227.78
T ₅	756.11	547.22	1303.33	42447.22
T ₆	870.0	641.67	1511.67	51941.67
T ₇	991.67	715.0	1706.67	60875.0
T ₈	697.78	531.67	1229.45	38558.33
T ₉	805.56	579.44	1385.0	46219.44
T ₁₀	772.22	570.0	1342.22	43583.33
LSD	222.7	147.9	267.27	
Esm	74.96	49.77	89.99	
C.V%	14.6	13.03	10.06	

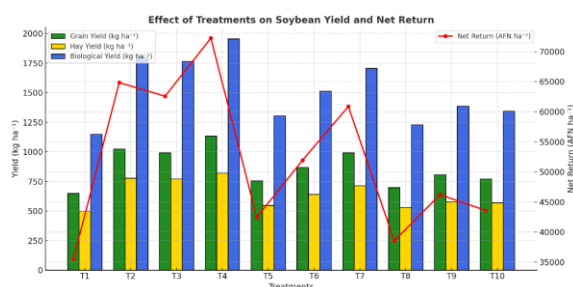


Figure 3: Grain Yield, Hay Yield, Biological Yield.

DISCUSSION

The present study demonstrated that nitrogen fertilization and Rhizobium inoculation significantly influenced soybean (*Glycine max* (L.) Merrill) yield attributes, including grain yield, hay yield, and biological yield. Among all treatments, T₄ (100% nitrogen) produced the highest grain yield (1135.00 kg ha⁻¹), hay yield (821.11 kg ha⁻¹), and biological yield (1956.11 kg ha⁻¹), which were significantly higher ($p < 0.05$) than all other treatments. These results indicate that adequate nitrogen enhances both vegetative and reproductive growth. Similar observations have been reported by Ramesh *et al.* (2018), who found increased biomass and leaf area with nitrogen application, and Verma *et al.* (2019), who highlighted positive effects of nitrogen on soybean growth and yield.

Treatments T₂ (Rhizobium inoculation alone) and T₃ (50% nitrogen) also showed significant improvements in yield attributes, with grain yields of 980.00 kg ha⁻¹ and 1025.00 kg ha⁻¹, and biological yields of 1780.00 kg ha⁻¹ and 1825.00 kg ha⁻¹, respectively. These increases were significant compared to the control ($p < 0.05$), suggesting that either biological nitrogen fixation or moderate nitrogen supplementation can effectively improve soybean productivity. Similar outcomes were reported by Singh *et al.* (2012) and Chaudhary *et al.* (2017), demonstrating that Rhizobium inoculation improves nitrogen availability, resulting in enhanced plant growth and yield.

The combination treatment T₇ (100% nitrogen + Rhizobium) also performed strongly, with grain yield of 1100.00 kg ha⁻¹, hay yield of 800.00 kg ha⁻¹, and biological yield of 1900.00 kg ha⁻¹, which were significantly higher than moderate nitrogen or Rhizobium alone ($p < 0.05$). This indicates a synergistic effect between synthetic nitrogen and biological inputs, consistent with previous studies emphasizing balanced nutrient management for optimal soybean growth (Ramesh *et al.*, 2018).

Conversely, treatments containing farmyard manure (FYM), particularly T₈, T₉, and T₁₀,

resulted in lower yields, with grain yields ranging from 772.22–805.56 kg ha⁻¹ and biological yields of 1342.22–1385.00 kg ha⁻¹, significantly lower than nitrogen and Rhizobium treatments ($p < 0.05$). This underperformance may be attributed to slower nutrient release from organic inputs and microbial competition, as noted by Patel *et al.* (2019). These findings align with previous reports indicating that FYM alone or in combination may not consistently enhance yield unless nutrient release is synchronized with crop demand.

Overall, the study indicates that nitrogen application and Rhizobium inoculation, either alone or in combination, are more effective than slow-release organic inputs in improving soybean productivity. These findings highlight the importance of integrated nutrient management strategies that combine synthetic and biological nitrogen sources to achieve optimal soybean growth and yield.

CONCLUSION

The study demonstrated that nitrogen fertilization, particularly the application of 100% recommended nitrogen (T₄), significantly enhanced grain and hay yield in soybean, making it the most effective treatment under the tested conditions. Treatments involving Rhizobium inoculation alone (T₂) and 50% nitrogen (T₃) also performed well, highlighting the potential of biological nitrogen fixation and reduced nitrogen inputs as sustainable alternatives.

In contrast, FYM-based treatments (T₅, T₈, T₉, T₁₀) were less effective in enhancing yield, likely due to delayed nutrient release and potential microbial competition. These findings emphasize the importance of nutrient timing, source, and integration strategy in optimizing soybean productivity.

Further research on soil nutrient dynamics, microbial interactions, and the long-term impact of combining organic and inorganic inputs is recommended to develop resilient and sustainable nutrient management systems for soybean cultivation. Integrated Pest Management (IPM) in soybean should combine cultural, biological, and need-based chemical control practices to minimize pest pressure and enhance crop health. Use of rhizobium, resistant varieties, crop rotation, timely sowing, and

balanced fertilization can reduce the risk of pest outbreaks. Botanical extracts and Rhizobium inoculation should be integrated as eco-friendly options, while chemical pesticides are applied only when pest populations exceed economic thresholds. This holistic approach can ensure sustainable soybean production with reduced environmental impact. Plant-based extracts from neem, garlic, or chili can be used for sustainable pest management in soybean cultivation. Plant materials should be thoroughly washed, crushed, soaked in clean water, and filtered before application. Extracts should be applied evenly on leaves during early morning or late afternoon, while protective clothing is worn to ensure user safety. Any leftover extracts should be stored in clearly labeled containers away from children and livestock, and pest populations should be regularly monitored to maintain effective and safe crop management.

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AUTHORS CONTRIBUTIONS:

The research study was conceptualized and methodologically designed by Durani, who also provided primary academic supervision, critical review, and comprehensive guidance throughout the research process.

Gulab served as the co-supervisor and contributed substantially to the technical review and refinement of the manuscript. Safi and Noor technically supported me throughout the study.

Hilali carried out fieldwork and data collection at various phenological stages of soybean growth and yield development. He was responsible for statistical analysis and has used SPSS software, interpretation of results, preparation of visual representations, and drafting the original manuscript. He also independently managed project logistics, mobilized necessary resources, and secured funding required for the successful execution of the current study. All authors have read and approved the final version of the manuscript for publication.

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