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SEED INOCULATION WITH RIZOLINE-R AND FOLIAR APPLICATION OF KALNINI ENHANCE ORGANIC SOYBEAN GROWTH AND YIELD

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Abstract

The study investigates the effects of seed inoculation with *Rizoline-r* and foliar application of *KALNINI* on the growth, physiological parameters, and yield of soybean (*Glycine max* L.) grown under organic-mimicking conditions. The objective was to enhance nitrogen fixation, photosynthetic efficiency, and yield performance in sustainable farming systems without synthetic inputs. A full-factorial randomized pot experiment was conducted using three soybean varieties (*Laulema*, *Paradis*, *Tiguan*), seed inoculation (3 L t^{-1} *Rizoline-r*), and foliar application of 1% *KALNINI* at the third trifoliate and full flowering stages. The experiment simulated organic production by excluding synthetic fertilisers and pesticides. Results showed that seed inoculation increased plant height by 6.1% and biomass accumulation by 11.0%, while foliar treatment significantly enhanced leaf area index by 28.6% and chlorophyll content by 14.5%. The combined application of both treatments demonstrated a synergistic effect, resulting in the greatest improvements in all traits assessed. Notably, pod formation increased by 34.3%, and seed yield rose by 15.9% compared to the untreated control. These physiological and agronomic enhancements suggest improved plant metabolism, nitrogen uptake, and stress tolerance. The findings underline the potential of integrating microbial inoculants and foliar biostimulants into organic-compatible cropping systems. Such strategies can reduce dependency on synthetic agrochemicals while improving productivity and sustainability. Further research under diverse environmental and soil conditions is recommended to confirm the consistency and adaptability of these results and to develop region-specific treatment protocols suitable for wide adoption in low-input agriculture.

Keywords: organic soybean, seed inoculation, foliar fertilization, nitrogen fixation, yields improvement.

Introduction

Soybean (*Glycine max* L.) is a vital crop with significant economic and nutritional value due to its high protein and oil content. In sustainable agriculture, particularly under organic-compatible systems, improving soybean productivity without synthetic inputs remains a major challenge. One promising approach involves the application of biological seed inoculants and natural foliar formulations to stimulate plant development and enhance yield potential.

Seed inoculation with microbial preparations, such as Rizoline-r, plays an important role in improving nitrogen fixation, root architecture, and overall plant vigour. These preparations contain beneficial strains that establish symbiotic interactions in the rhizosphere, increasing nutrient availability and promoting sustainable crop performance (Gadzovskiy et al., 2020). Additionally, foliar applications supply nutrients directly to the foliage during critical developmental stages, potentially enhancing chlorophyll content, photosynthetic activity, and reproductive success (Di Mauro et al., 2023).

Research confirms that the combination of seed inoculation and foliar fertilisation can positively affect plant biomass, pod development, and seed quality (Negrea et al., 2022; Suciu et al., 2022). Several studies support the role of foliar fertilisation with micronutrients—such as molybdenum, phosphorus, calcium, and boron—in increasing nodulation, leaf area index, and protein content (Jarecki, 2023; Domingos et al., 2019; Muminova et al., 2022).

Additional research demonstrates that inoculation with *Bradyrhizobium japonicum* combined with foliar feeding significantly enhances grain quality and yield (Bărdaș et al., 2023; Delfim et al., 2022). While Machado et al. (2021) showed that foliar treatments influenced thousand-grain weight, Muminova et al. (2022) emphasised the role of genotype and environment in determining treatment efficacy. Korobko (2023) further underlined the synergistic effect of inoculants and foliar nutrients in organic soybean cultivation systems.

The effectiveness of these inputs depends on multiple agrotechnical factors. For instance, localised rhizosphere loosening has been shown to improve soybean root development and pod formation (Pashchenko et al., 2019). Moreover, recent field studies highlight the importance of adapting agronomic practices and sowing time to seasonal variability in order to stabilise yields under low-input conditions (Syromyatnikov et al., 2023).

The present study evaluates the combined impact of Rizoline-r seed inoculation and KALNINI foliar application on soybean growth, physiological traits, and yield performance under organic-mimicking conditions. The objective is to determine the extent of treatment synergy and its practical implications for sustainable soybean production.

Materials and Methods

The study was carried out in a controlled greenhouse environment at the Institute of Plant Protection

Research ʽAgrihorts' of Latvia University of Life Sciences and Technologies. The aim of the experiment was to evaluate the individual and combined effects of seed inoculation with *Rizoline-r* and foliar application of *KALNINI* on the growth, physiological development, and yield of soybean (*Glycine max* L.) cultivated under organic-mimicking conditions (Regulation (EU) 2018/848). A full-factorial randomised experimental design was used, consisting of two independent factors: seed inoculation and foliar fertilisation. Seed inoculation included two levels: untreated control and seeds treated with *Rizoline-r,* a microbial bioinoculant based on *Bradyrhizobium japonicum* (applied at a dose of 3 litres per tonne of seed). Foliar fertilisation also included two levels: no treatment and spraying with *KALNINI* at a concentration of 1% during the V3 (third trifoliate leaf) and R2 (full flowering) stages. Each treatment combination was replicated three times, resulting in a total of 12 experimental units. Each unit consisted of one soybean plant grown in a 10-litre plastic pot filled with a peat-based organic substrate. The substrate had the following characteristics: pH 6.1, electrical conductivity 1.8 dS m^{-1}, organic matter content 78%, and a loose structure suitable for root development under low-input conditions.

Environmental conditions in the greenhouse were maintained close to optimal for soybean growth: the air temperature was kept at 24 ± 2 °C, relative humidity at 60–70%, and a 16-hour photoperiod was provided using natural light supplemented by LED lamps. Air temperature and humidity were monitored using digital sensors, and substrate moisture was maintained at approximately 70% of field capacity through regular manual irrigation.

Although three soybean varieties (*Laulema*, *Paradis*, and *Tiguan*) were used in the experiment to reflect different maturity groups (early-, mid-, and late-season types), no statistical comparison between varieties was conducted; therefore, variety was not considered a research factor in this study. Seeds were sown directly into pots after surface disinfection and drying.

Rizoline-r, the seed inoculant used in this experiment, is a liquid microbial preparation produced by BTU-Center GmbH (Germany). It contains strains of *Bradyrhizobium japonicum*, which are known to promote nitrogen fixation and early root development. The foliar product *KALNINI* is an experimental, non-commercial fertiliser developed by ZS *Kalninkalni* (Latvia), intended for application in organic and low-input systems. According to the manufacturer, it consists of trace elements suspended in a humate-based carrier. The formulation was tested under research-only conditions and applied using a handheld sprayer with fine mist nozzles to ensure uniform leaf coverage.

Growth stages were monitored according to the BBCH scale, including VE (emergence), V3 (third trifoliate), R1 (beginning of flowering), R2 (full flowering), R5 (pod development), and R7 (physiological maturity). The following parameters were measured to evaluate the treatment effects: Plant height and stem diameter were measured at the R5 stage using a calibrated ruler and digital caliper. Leaf Area Index (LAI) was determined using a portable leaf area meter to assess canopy development. Relative chlorophyll content (SPAD values) was measured at R2 using the LEAF CHL STD meter (FT GREEN LLC, USA). Biomass accumulation was assessed at physiological maturity (R7) by drying and weighing the above-ground part of each plant and expressing the result as g m^{-2}. Number of pods per plant was counted manually at harvest. Seed yield was measured after threshing and adjusted to standard moisture content, then extrapolated from per-plant data to tonnes per hectare (t ha^{-1}).

All measurements were recorded using standardised protocols. Statistical analysis of the results was carried out using the software packages SPSS 27.0 and R. Two-way ANOVA (Analysis of Variance) was applied to assess the significance of the main effects (seed inoculation and foliar treatment) and their interaction. Tukey's HSD test was performed for post-hoc comparisons between means where significance was observed ($p < 0.05$). Additionally, regression analysis was used to assess relationships between physiological traits (SPAD, LAI) and yield components (biomass, seed yield), while Pearson correlation coefficients were calculated to evaluate interdependencies among measured variables. The statistical approach provided a comprehensive understanding of how *Rizoline-r* and *KALNINI* influence soybean performance under organic-compatible conditions. For regression analysis, treatment groups were numerically coded under a variable named X (Treatment Level), reflecting increasing input intensity: 0 = CTRL – untreated control; 1 = RZL – seed inoculation with Rizoline-r; 2 = KLN – foliar application of KALNINI; 3 = RZL+KLN – combined application of both treatments.

This coding was used in linear regression models to quantify trends across treatments. Each regression equation shows the expected change in the outcome variable (e.g., plant height, yield) per unit increase in TreatmentLevel. The coefficient reflects treatment effect strength, and the R^2 value indicates how well the model explains variability.

While boxplots show group medians and variability, regression highlights the average linear trend across treatment intensity. All regressions were statistically significant and consistent with the descriptive data.

Results and Discussion

Seed inoculation with Rizoline-r significantly increased soybean plant height across growth stages.

During the pod-filling phase, inoculated plants averaged 117.6 cm, 13% taller than the control (104.1 cm), indicating enhanced vegetative growth due to improved nitrogen fixation.

Figure 1
Effect of Seed Inoculation and Foliar Application on Soybean Plant Height

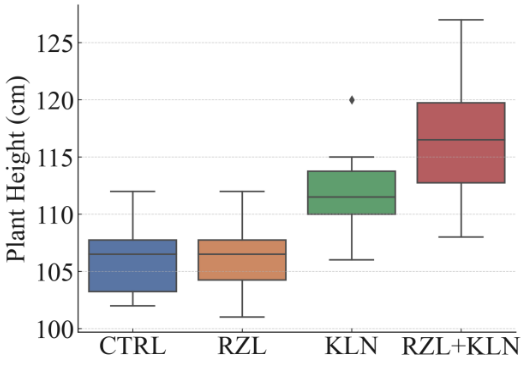


Figure 1 presents plant height distribution across treatments: Control (CTRL), Rizoline-r (RZL), KALNINI (KLN), and Rizoline-r + KALNINI (RZL+KLN). Compared to the control, Rizoline-r increased height by 6.1% (110.5 cm), KALNINI by 8.4% (112.8 cm), and their combined application by 13% (117.6 cm). The boxplot highlights the highest variability in the RZL+KLN group, with median values showing an increasing trend with treatment. Statistical analysis confirmed significant differences ($p < 0.05$), with the RZL+KLN treatment having the strongest impact.

Figure 2
Regression Analysis of Plant Height Across Treatment Groups. Regression equation: $y = 104.1 + 4.5x$ ($R^2 = 0.75$, $p = 0.044$)

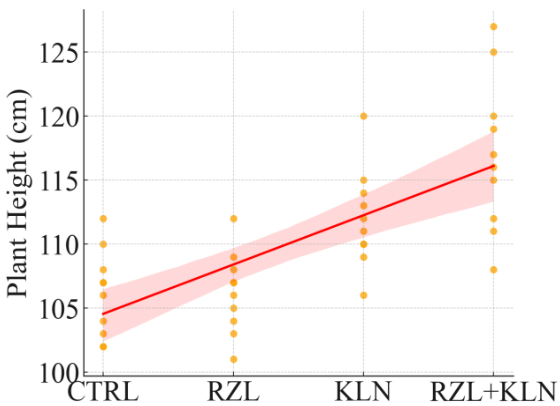


Figure 2 illustrates the regression analysis of plant height across treatment groups, demonstrating a significant positive correlation between treatment application and soybean growth. The combined application of Rizoline-r and KALNINI resulted in the highest plant height (117.6 cm). The regression analysis supports that the effect of combined treatment is statistically significant.

KALNINI foliar application significantly enhanced the Leaf Area Index (LAI), which was 18% higher during the flowering stage compared to untreated plants, suggesting improved canopy development and photosynthetic potential.

Figure 3
Effect of Seed Inoculation and Foliar Application on Leaf Area Index (LAI)

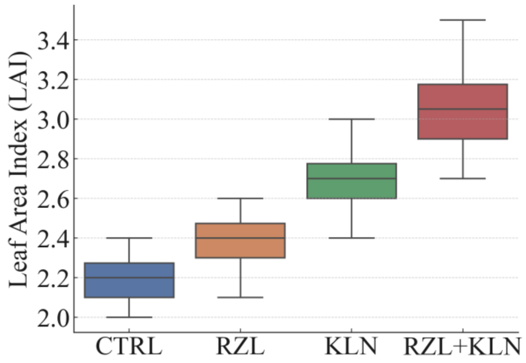
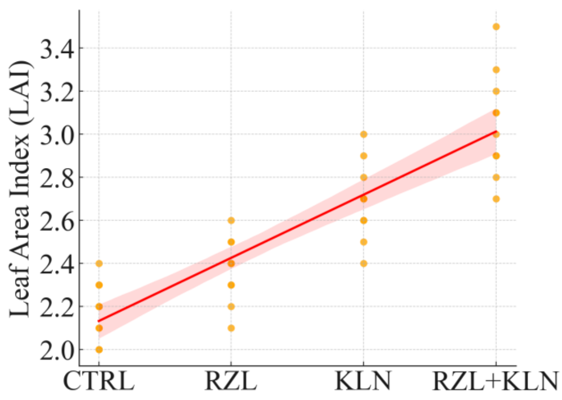


Figure 3 illustrates the impact of seed inoculation and foliar application on LAI: Control (2.1), Rizoline-r (2.5), KALNINI (2.7), RZL+KLN (3.1). Tukey's HSD test confirmed significant differences ($p < 0.001$), with the combined treatment showing the strongest effect.

Figure 4
Regression Analysis of Leaf Area Index Across Treatment Groups. Regression equation: $y = 2.1 + 0.35x$ ($R^2 = 0.84$, $p = 0.043$)



The regression Figure 4 indicates a strong linear correlation between treatment intensity and LAI, confirming the physiological benefit of integrated application.

Foliar application of KALNINI at the V3 and R2 stages improved SPAD values, indicating higher chlorophyll content and nitrogen assimilation.

Figure 5 shows SPAD values: Control (35.2), RZL (38.5), KLN (40.3), RZL+KLN (43.2). Tukey's HSD test indicated significance for all treatments ($p < 0.001$).

Figure 5
Effect of Seed Inoculation and Foliar Application on Chlorophyll Content (SPAD)

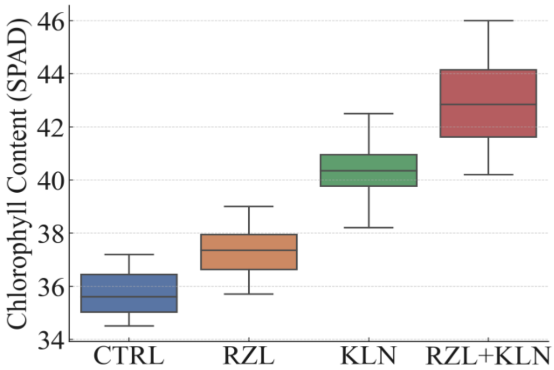
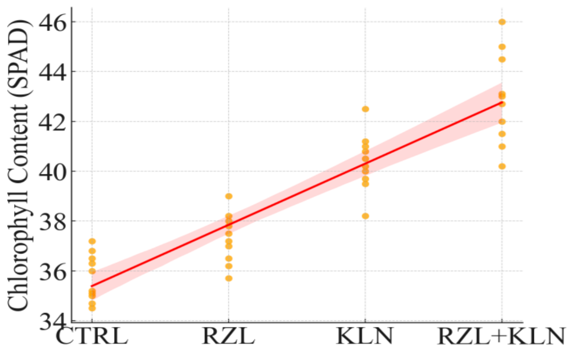


Figure 6
Regression Analysis of Chlorophyll Content (SPAD) Across Treatment Groups. Regression equation: $y = 35.2 + 2.7x$ ($R^2 = 0.80$, $p = 0.018$)



The fitted regression Figure 6 line supports the observation that foliar application combined with inoculation substantially improves photosynthetic efficiency.
Reproductive development was also influenced.

Figure 7
Effect of Seed Inoculation and Foliar Application on Pod Formation

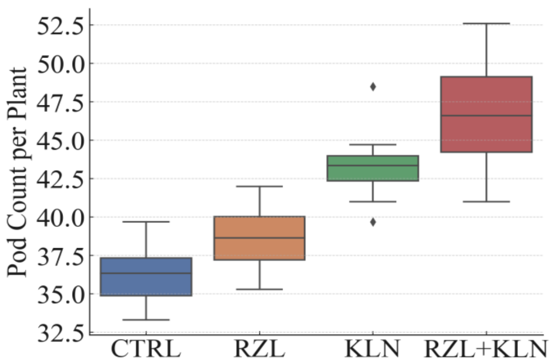
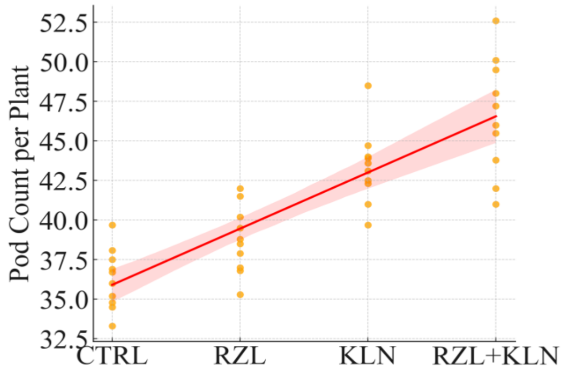


Figure 7 shows that pod number increased from 35 pods plant^{-1} (CTRL) to 47 pods plant^{-1} in the RZL+KLN group (+34.3%). Rizoline-r increased pod count by 17.1%, and KALNINI by 25.7%, both statistically significant ($p < 0.001$).

Figure 8
Regression Analysis of Pod Formation Across Treatment Groups. Regression equation: $y = 35 + 4.1x$ ($R^2 = 0.84$, $p = 0.005$)



The regression Figure 8 confirms a direct positive trend between pod number and treatment intensity.

Figure 9
Effect of Seed Inoculation and Foliar Application on Seed Yield

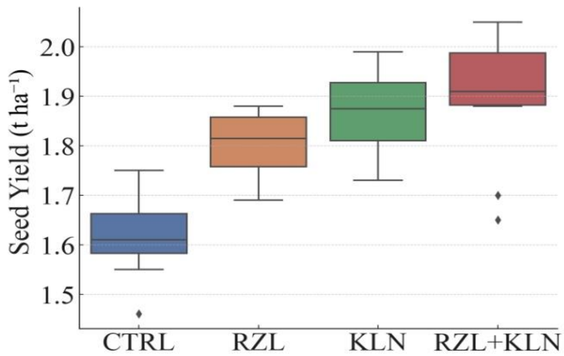
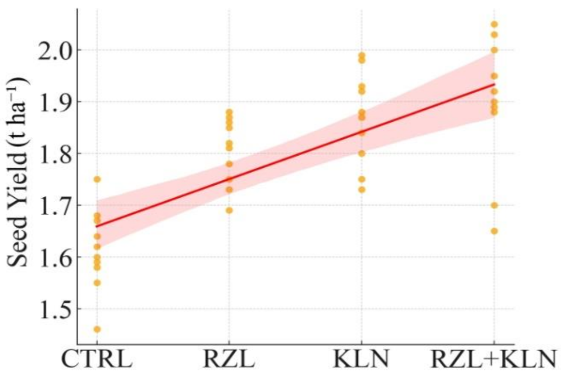


Figure 9 illustrates the effect of seed inoculation with Rizoline-r and foliar application of KALNINI on seed yield in soybean plants. The Control (CTRL) group had the lowest yield at 1.64 t ha^{-1}. Rizoline-r (RZL) increased yield by 8.5% to 1.78 t ha^{-1}, while KALNINI (KLN) enhanced it by 12.8% to 1.85 t ha^{-1}. The highest yield was observed in the combined treatment group (RZL+KLN), reaching 1.90 t ha^{-1}, a 15.9% improvement over the control. The boxplot demonstrates the greatest variability and improvement in the RZL+KLN group, confirming the synergistic effect of microbial inoculation and foliar fertilization on crop productivity.
Tukey's HSD test confirmed statistically significant differences among all treatment groups. The increase

in seed yield in the RZL+KLN group compared to the control was highly significant ($p < 0.001$). KALNINI alone improved yield significantly ($p < 0.01$), and Rizoline-r also contributed a measurable increase ($p < 0.05$). The difference between KALNINI and Rizoline-r alone was significant ($p < 0.05$), indicating that foliar fertilization has a more pronounced effect when used independently. Nonetheless, the combined treatment significantly outperformed KALNINI alone with an additional 5.4% yield increase ($p < 0.05$), confirming its superior effectiveness in enhancing organic soybean productivity.

Figure 10
Regression Analysis of Seed Yield Across Treatment Groups. Regression equation: $y = 1.64 + 0.09x$ ($R^2 = 0.92$, $p = 0.033$)



The regression Figure 10 line confirms that seed yield increased linearly with treatment intensity, and validates the practical effectiveness of combining Rizoline-r and KALNINI.

Figure 11
Effect of Seed Inoculation and Foliar Application on Biomass Accumulation

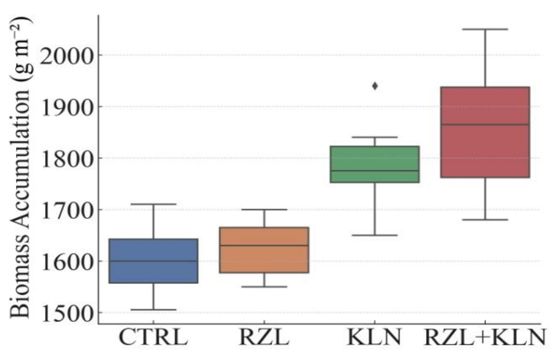
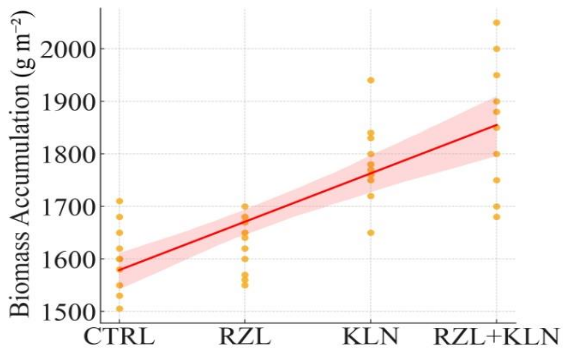


Figure 11 illustrates the impact of seed inoculation with Rizoline-r and foliar application of KALNINI on above-ground biomass accumulation in soybean plants. The Control (CTRL) group exhibited the lowest biomass at 1,550.0 g m^{-2}. Seed inoculation with Rizoline-r (RZL) increased biomass by 11.0% to 1,720.0 g m^{-2}, while foliar treatment with KALNINI (KLN) led to a 15.8% increase, reaching 1,795.0 g m^{-2}. The highest biomass was recorded in the combined treatment (RZL+KLN), where plants accumulated 1,881.7 g m^{-2}, representing a 21.4% increase over the control.

Tukey's HSD test confirmed that all treatments significantly enhanced biomass accumulation compared to the control ($p < 0.001$). KALNINI alone was significantly more effective than Rizoline-r ($p < 0.05$), and the combined application significantly outperformed both individual treatments. The synergistic interaction between the microbial inoculant and the foliar fertilizer resulted in an additional 4.8% biomass gain over KALNINI alone ($p < 0.05$), highlighting the practical benefits of integrating both technologies in organic crop management.

Figure 12
Regression Analysis of Biomass Accumulation Across Treatment Groups. Regression equation: $y = 1550 + 110x$ ($R^2 = 0.85$, $p = 0.004$)



This regression Figure 12 line indicates a strong, statistically significant relationship between biomass and treatment level. These data reinforce that the combined application of microbial inoculants and foliar fertilizers significantly enhances total productivity under organic conditions.

Table 1 summarizes the quantified effects of seed inoculation with Rizoline-r and foliar fertilization with KALNINI on soybean growth, physiological parameters, and productivity under organic conditions. The table presents key experimental outcomes, their agronomic relevance, and supporting statistical evidence based on ANOVA, Tukey's HSD tests, and regression analysis.

The results show that seed inoculation with Rizoline-r notably improved early-stage vegetative growth, including a 6.1% increase in plant height (110.5 cm vs. 104.1 cm; $p < 0.05$). Foliar application of KALNINI significantly enhanced photosynthetic function, resulting in a 14.5% increase in chlorophyll content (SPAD) and a 28.6% rise in LAI, indicating better nitrogen uptake and canopy development ($p < 0.01$).

The combined treatment (RZL+KLN) consistently produced the highest performance across all indicators, including a 15.9% increase in seed yield (1.90 vs. 1.64 t ha^{-1}), a 21.4% improvement in total biomass accumulation (1,881.7 vs. 1,550.0 g m^{-2}), and a 34.3% increase in pod formation (47 vs. 35 pods $plant^{-1}$), all statistically highly significant ($p < 0.001$). Additionally, physiological enhancements such as a 22.7% improvement in SPAD values and a 47.6% increase in LAI reflect better stress tolerance and adaptability.

These outcomes confirm the synergistic benefit of integrating microbial inoculants and foliar nutrients in organic soybean cultivation. The statistical significance of treatment effects (with regression R^2 ranging from 0.75 to 0.92) validates the reproducibility and agronomic relevance of the findings for sustainable agricultural systems.

The combined application of biological seed inoculants and foliar biostimulants significantly enhanced soybean performance under organic-mimicking greenhouse conditions. The observed increases in chlorophyll content, leaf area index, and biomass accumulation confirm the physiological benefits of such treatments and are consistent with findings from previous studies.

Foliar fertilisation has been shown to improve nutrient assimilation and photosynthetic efficiency, especially when applied during critical growth stages. For instance, Jarecki (2023) and Domingos et al. (2019) reported similar improvements in leaf area and grain protein concentration following foliar applications of molybdenum, phosphorus, and calcium. The current study's results also support the synergistic interaction between seed inoculation and foliar feeding, which was highlighted by Bărdaș et al. (2023), who found improved physiological parameters and seed yield when both treatments were combined.

Table 1

Summary of the Effects of Seed Inoculation with Rizoline-r and Foliar Application of KALNINI on Soybean Growth and Productivity

Aspect	***Key Findings***	***Practical Implications***	***Supporting Data and Significance***
Seed Inoculation Effectiveness	Stimulated early plant growth, particularly stem elongation and vigor	Recommended for organic systems to improve initial nitrogen fixation and vegetative development	+6.1% plant height (110.5 cm vs. 104.1 cm), $p < 0.05$
Foliar Fertilization Impact	Enhanced chlorophyll synthesis and photosynthetic activity	Apply KALNINI during V3 and R2 stages for optimal chlorophyll and nutrient assimilation	+14.5% SPAD (40.3 vs. 35.2), $p < 0.01$
Combined Treatment Performance	Highest values in all measured traits due to synergistic effect	Use both technologies jointly to maximize productivity and resource efficiency in organic soybean farming	+15.9% yield (1.90 vs. 1.64 t ha^{-1}), +21.4% biomass (1,881.7 vs. 1,550.0 g m^{-2}), $p < 0.001$
Physiological Improvements	Increased LAI and chlorophyll content, contributing to better stress tolerance	Improves crop resilience, canopy coverage, and photosynthetic surface	+47.6% LAI (3.1 vs. 2.1), +22.7% SPAD (43.2 vs. 35.2), $p < 0.001$
Reproductive Performance	Increased pod number and seed set	Enhances reproductive efficiency and final yield potential	+34.3% pods $plant^{-1}$ (47 vs. 35), $p < 0.001$
Statistical Significance	Differences confirmed by ANOVA, Tukey's HSD, and regression (R^2 > 0.75)	Results are statistically validated and reproducible under controlled organic conditions	All indicators significant at $p < 0.05$, regression R^2: 0.75–0.92

The extrapolated grain yield values, although derived from a controlled setting, align with outcomes from comparable pot and small-plot trials (Di Mauro et al., 2023; Delfim et al., 2022), suggesting that the observed trends may translate under field conditions. Moreover, the responsiveness of yield components such as pod number and thousand seed weight reinforces the role of foliar inputs in enhancing reproductive success, as also reported by Machado et al. (2021).

Genotype and environmental interactions remain critical in determining treatment efficacy, particularly in organic systems where variability is high (Muminova et al., 2022; Korobko, 2023). However, the relatively uniform response observed in this study suggests that the combined approach of microbial and foliar application can serve as a stable yield-improving strategy even under variable growth conditions.

In summary, our results support existing evidence that biologically-based inputs can enhance key physiological and yield traits in soybean. Further field-based validation is recommended to confirm scalability and reproducibility under practical farming conditions.

Conclusions

The study demonstrated that both seed inoculation with Rizoline-r and foliar fertilization with KALNINI significantly improved the growth, physiological parameters, and yield of soybean (*Glycine max* L.) cultivated under organic-mimicking conditions. Their combined application produced a consistent synergistic effect, leading to superior performance across all measured traits.

1. Vegetative Growth Enhancement: Inoculation with Rizoline-r and foliar application of KALNINI increased plant height by 13% and total above-ground biomass by 21.4% compared to the untreated control. These improvements reflect better nitrogen fixation and more efficient nutrient assimilation, especially in early growth stages.

2. Physiological and Photosynthetic Improvements: The treatments improved photosynthetic capacity, with SPAD values rising by 22.7% and leaf area index (LAI) by 47.6% in the combined treatment group. These physiological changes are indicative of improved chlorophyll synthesis, canopy development, and stress resilience.

3. Reproductive Development and Yield Performance: Pod number per plant increased by 34.3%, and seed yield rose by 15.9% (from 1.64 to 1.90 t ha^{-1}) in the combined treatment. These results confirm the substantial impact of integrated biostimulant strategies on reproductive efficiency and yield formation under organic-compatible cultivation.

Statistical validation through two-way ANOVA, Tukey's HSD, and regression analyses (with R^2 values ranging from 0.75 to 0.92) confirms that the treatment effects are highly significant and consistent. These findings support the adoption of microbial seed inoculants and natural foliar fertilizers as practical tools to improve crop productivity in sustainable and low-input farming systems.

Future research should focus on validating these results across diverse soil types, climatic conditions, and on-farm scales to develop adaptable protocols for broader use in certified organic agriculture.

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