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Synergistic Effects of Rhizobium Inoculation and Rice Husk Biochar on Growth Performance and Yield of Edamame [*Glycine max* (L.) Merrill] in Alluvial Soil

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ABSTRACT

Edamame production in Indonesia remained sub-optimal, particularly in alluvial soils characterized by extreme acidity (pH 4.35), aluminium toxicity and limited microbial activity. This study evaluated the individual and interactive effects of *Rhizobium* sp. inoculation and rice husk biochar application on soil chemical properties, growth performance and yield of edamame in alluvial soil. A factorial randomized complete block design was employed with two factors: *Rhizobium* sp. inoculation (control, commercial Rhizoka and local isolate) and rice husk biochar rates (25, 50 and 75 g/polybag). No significant interaction was observed between factors. However, *Rhizobium* sp. significantly influenced compound leaf number, and highly significantly affected branch number, fresh pod number and pod weight. The local *Rhizobium* isolate demonstrated superior performance compared to commercial inoculant and control. Rice husk biochar significantly increased root nodule formation, with the highest rate (75 g/polybag) producing 6.0 nodules per plant compared to 4.7 in the lowest rate. Biochar application achieved substantial soil amelioration: pH increased by 1.33 units (4.35’!5.67 average), exchangeable aluminium decreased by 74% (1.05’!0.29 cmol(+)/kg) and organic carbon increased by 22% (1.41%’!1.72%). The optimal treatment combination R, Aƒ yielded 111.59 g/plant (equivalent to 11.2 t/ha), representing a 53% increase over control. Local *Rhizobium* isolate and rice husk biochar independently enhanced edamame productivity through complementary mechanisms, offering sustainable pathways for improving production in challenging tropical soil environments.

Key words: Biological nitrogen fixation, biochar amendment, edamame production, local rhizobia, root nodulation, sustainable agriculture

INTRODUCTION

Edamame [*Glycine max* (L.) Merrill] has gained considerable attention globally due to its exceptional nutritional profile and economic value. The global demand for edamame continues to rise, with Japan alone requiring 100,000 tonnes annually. Despite this growing market opportunity, Indonesia's domestic production remained critically low, meeting only 3% of Japan's annual demand (Directorate General of Horticulture, 2020). The predominant cultivation of edamame in Indonesia occurred on marginal lands, including alluvial soils, which presented several agronomic challenges including extremely low pH (4.35), deficient organic matter content (1.41%) and aluminium toxicity.

Biological nitrogen fixation (BNF) through *Rhizobium*-legume symbiosis represented a cornerstone of sustainable agriculture, potentially reducing chemical fertilizer dependency (Thilakarathna and Raizada, 2017; Asirifi *et al.*, 2025). Native or locally-adapted rhizobial strains often demonstrate superior competitive ability and nitrogen fixation efficiency compared to commercial inoculants (Kawaka *et al.*, 2018; Kolapo *et al.*, 2025; Wu *et al.*, 2025). Biochar, the carbon-rich product of biomass pyrolysis, emerged as a multifunctional soil amendment capable of

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improving soil properties (Kizito *et al.*, 2019; Liu *et al.*, 2025). Rice husk biochar offered particular promise due to its high silica content and favourable pore structure (Shetty and Prakash, 2020). Recent studies demonstrated biochar's capacity to stimulate *Rhizobium* population dynamics and enhance nodulation efficiency (Egamberdieva *et al.*, 2017; Elebiyo and Bachmann, 2024; Liu *et al.*, 2024).

Despite extensive research on *Rhizobium* inoculation and biochar application as independent interventions, their combined effects on edamame production in tropical alluvial soils remained inadequately understood. This study addressed these gaps by investigating: (1) the individual effects of *Rhizobium* sp. inoculation and rice husk biochar application rates on edamame growth and yield; (2) potential interactive effects between these two factors and (3) biochar-mediated changes in soil chemical properties and their relationship to nodulation responses. It was hypothesized that locally-adapted *Rhizobium* isolates would outperform commercial inoculants, that biochar amendment would enhance rhizobial effectiveness through improved soil chemical conditions, and that these improvements would translate to measurable yield benefits.

MATERIALS AND METHODS

The study was conducted from February to May 2025 at Panca Bhakti University, Pontianak, West Kalimantan, Indonesia (0°02′S, 109°20′E, 1 m above sea level). The location experienced a tropical climate with mean temperature ranging from 24.5 to 31.5°C during the experimental period.

Alluvial soil was collected from Sungai Rengas, West Kalimantan. Initial soil analysis was conducted at the Soil Chemistry and Fertility Laboratory, Tanjungpura University. The soil was classified as silt loam with particle size distribution of 2.31% sand, 75.64% silt and 22.05% clay. The soil exhibited extremely acidic conditions (pH H, O 4.35), low organic matter content (C-organic 1.41%), and moderate aluminium saturation (1.05 cmol(+)/kg exchangeable Al).

A factorial randomized complete block design with three replications was employed. The experiment consisted of two factors: Factor 1–*Rhizobium* sp. inoculation (R): R€ –Control (no inoculation), R_1–Commercial inoculant (Rhizoka™), R_2–Local *Rhizobium* isolate. Factor 2–Rice husk biochar rate (A): A_1–25 g/polybag (equivalent to 5 t/ha), A_2–50 g/polybag (equivalent to 10 t/ha), A_3–75 g/polybag (equivalent to 15 t/ha). This yielded nine treatment combinations (R_0A_1, R_0 A_2, R_0A_3, R_1A_1, R_1A_2, R_1A_3, R_2A_1, R_2A_2, R_2A_3), each replicated three times with three plants per replication, totalling 81 experimental units.

Native *Rhizobium* isolates were obtained from edamame root nodules collected from Rasau Jaya, West Kalimantan. Fresh nodules were surface-sterilized, crushed aseptically and streaked onto nutrient agar medium. Pure colonies were selected and sub-cultured for inoculum preparation.

Dolomitic lime was applied at 10.5 g/polybag two weeks before planting. Rice husk biochar was incorporated one week before planting. Edamame seeds (cv. Ryoko 75) were inoculated by soaking in treatment solutions for 15-30 min. Two pre-inoculated seeds were sown at 3-5 cm depth per polybag. Thinning to one plant per polybag was performed seven days after sowing. NPK compound fertilizer (16-16-16) was applied at 1 g/polybag in split doses at 20 and 40 days after sowing. Weed management was conducted manually and pest control utilized botanical pesticides.

Growth parameters measured at 35 days after sowing included plant height, shoot and root dry weight and shoot-to-root ratio. Yield components assessed at 65-70 days after sowing included compound leaf number, branch number, fresh pod number, pod fresh weight, root nodule number and root volume. Soil pH was measured at experiment initiation and conclusion.

Data were subjected to two-way analysis of variance using a factorial model. When significant effects ($P < 0.05$) were detected, means were compared using Tukey's Honestly Significant Difference test at 5% probability level.

RESULTS AND DISCUSSION

Analysis of variance revealed no significant interaction between *Rhizobium* inoculation and biochar application on plant height (Table 1). Individual factor analysis showed that neither *Rhizobium* treatment ($F = 2.97$, $P > 0.05$)

nor biochar rate (F = 1.15, P > 0.05) significantly influenced plant height. Mean plant height across treatments ranged from 31.28 to 35.39 cm, with treatment R, Aƒ producing the tallest plants (35.39 cm). Shoot dry weight did not differ significantly among treatments, with values ranging from 8.21 to 10.96 g. Similarly, root dry weight showed no significant response to either factor, varying between 1.70 and 2.71 g. The shoot-to-root ratio remained statistically uniform across all treatment combinations (3.84-5.09).

Rhizobium inoculation significantly affected compound leaf production (F = 4.60, P < 0.05), while biochar application showed no significant effect (Table 1). Local isolate treatment (R, , 27.28 leaves) produced significantly more leaves than the control (R€ , 23.94 leaves), while commercial inoculant (R , 24.78 leaves) did not differ significantly from either. Among treatment combinations, R, A, yielded the highest leaf count (27.83 leaves). Branch production responded highly significantly to *Rhizobium* inoculation (F = 6.76, P < 0.01) but not to biochar amendment. Local isolate (R,) generated 6.1 branches per plant, significantly exceeding the control (R€ , 5.0 branches), while commercial inoculant (R , 5.7 branches) showed intermediate performance. The treatment R, A, achieved the maximum branch number (6.33).

The local *Rhizobium* isolate (R,) consistently outperformed both the commercial inoculant and non-inoculated control across multiple yield-related parameters. This superior performance corroborated findings demonstrating that locally-adapted strains often exhibited enhanced competitive ability, environmental stress tolerance and host compatibility compared to exotic inoculants (Kawaka *et al.*, 2018; Elebiyo and Bachmann, 2024; Kolapo *et al.*, 2025). The enhanced performance of local isolates likely reflected evolutionary adaptation to specific soil conditions, including pH extremes and aluminium toxicity characteristic of alluvial soils (Lindström and Mousavi, 2020; Wu *et al.*, 2025). The significant increases in branch number (22% over control) demonstrated substantial agronomic benefits, aligning with the critical role of nitrogen in vegetative development and reproductive allocation in soybeans (Santachiara *et al.*, 2017; Ciampitti and Salvagiotti, 2018).

Fresh pod number exhibited highly significant response to *Rhizobium* inoculation (F = 9.58, P < 0.01; Table 2). Both inoculated treatments (R : 41.9 pods and R, : 46.8 pods) significantly surpassed the non-inoculated control (R€ : 34.9 pods). Treatment R, A produced the highest pod count (47.67 pods). Pod weight mirrored pod number trends, showing highly significant response to *Rhizobium* inoculation (F = 16.36, P < 0.01). Local isolate (R, , 109.1 g) and commercial inoculant (R , 98.8 g) both significantly exceeded control (R€ , 81.2 g). The treatment combination R, Aƒ yielded the heaviest pods (111.59 g).

Root nodule number demonstrated significant response to biochar application (F = 4.34, P <

Table 1. Vegetative growth parameters of edamame as affected by *Rhizobium* inoculation and rice husk biochar application in alluvial soil

Treatment	Plant height (cm)	Shoot DW (g)	Root DW (g)	S/R ratio	Leaf number	Branch number
R_0A_1	32.39	8.53	2.18	3.84	24.83	5.33
R_0A_2	35.33	8.21	2.04	3.95	24.33	4.83
R_0A_3	34.11	9.58	2.25	4.25	22.67	4.83
R_1A_1	31.28	9.19	1.94	4.75	24.33	5.50
R_1A_2	31.83	9.57	2.36	4.06	24.67	5.50
R_1A_3	32.44	8.91	2.17	4.20	25.33	6.00
R_2A_1	33.67	8.51	1.70	5.09	26.67	6.17
R_2A_2	33.33	10.96	2.71	4.11	27.83	6.33
$R2A_3$	35.39	10.65	2.39	4.46	27.33	5.83
C. V. (%)	6.61	27.30	25.43	14.63	9.58	11.54
R effect	NS	NS	NS	NS	*	**
A effect	NS	NS	NS	NS	NS	NS
R × A	NS	NS	NS	NS	NS	NS

DW – Dry weight, S/R – Shoot-to-root, NS – Not Significant, *,**Significant at P<0.05 and Highly Significant at P<0.01, respectively.

Table 2. Yield components and root parameters of edamame as affected by *Rhizobium* inoculation and rice husk biochar application in alluvial soil

Treatment	Pod number	Pod weight (g)	Nodule number	Root volume (cm³)
R_0A_1	33.33	73.00	4.74	18.00
R_0A_2	34.67	82.53	5.52	15.00
R_0A_3	36.83	88.08	4.93	17.00
R_1A_1	44.00	97.95	4.21	12.33
R_1A_2	41.33	93.94	4.83	18.00
R_1A_3	40.33	104.39	6.55	16.67
R_2A_1	47.67	106.84	5.11	13.00
R_2A_2	46.17	108.82	5.97	23.00
R_2A_3	46.50	111.59	6.61	20.67
CV (%)	13.98	10.85	17.95	30.85
R effect	**	**	NS	NS
A effect	NS	NS	*	NS
R × A	NS	NS	NS	NS

NS – Not Significant; *,**Significant at P=0.05 and *Highly significant at P<0.01, respectively.

0.05; Table 2), representing the only parameter showing significant biochar effect. The highest biochar rate (Aƒ , 75 g/polybag{ ¹) produced significantly more nodules (6.0 nodules/plant) compared to the lowest rate (A , 25 g/polybag; 4.7 nodules). Treatment R, Aƒ achieved the highest nodule count (6.61). Root volume showed no significant response to either factor, with values ranging from 12.33 to 23.00 cm³.

The significant positive effect of rice husk biochar on root nodule formation, particularly at the highest application rate, represented a key finding supported by substantial soil chemical improvements. The biochar-induced enhancement of nodulation could be explained through multiple complementary mechanisms. Biochar's porous structure provided expanded habitat space for rhizobial populations, potentially increasing their survival and proximity to infection sites (Elebiyo and Bachmann, 2024; Liu *et al.*, 2024). More critically, biochar's liming effect contributed to substantial pH amelioration, bringing soil conditions within the acceptable range for both *Rhizobium* survival and nodule formation (Ma *et al.*, 2019; Zhao *et al.*, 2021).

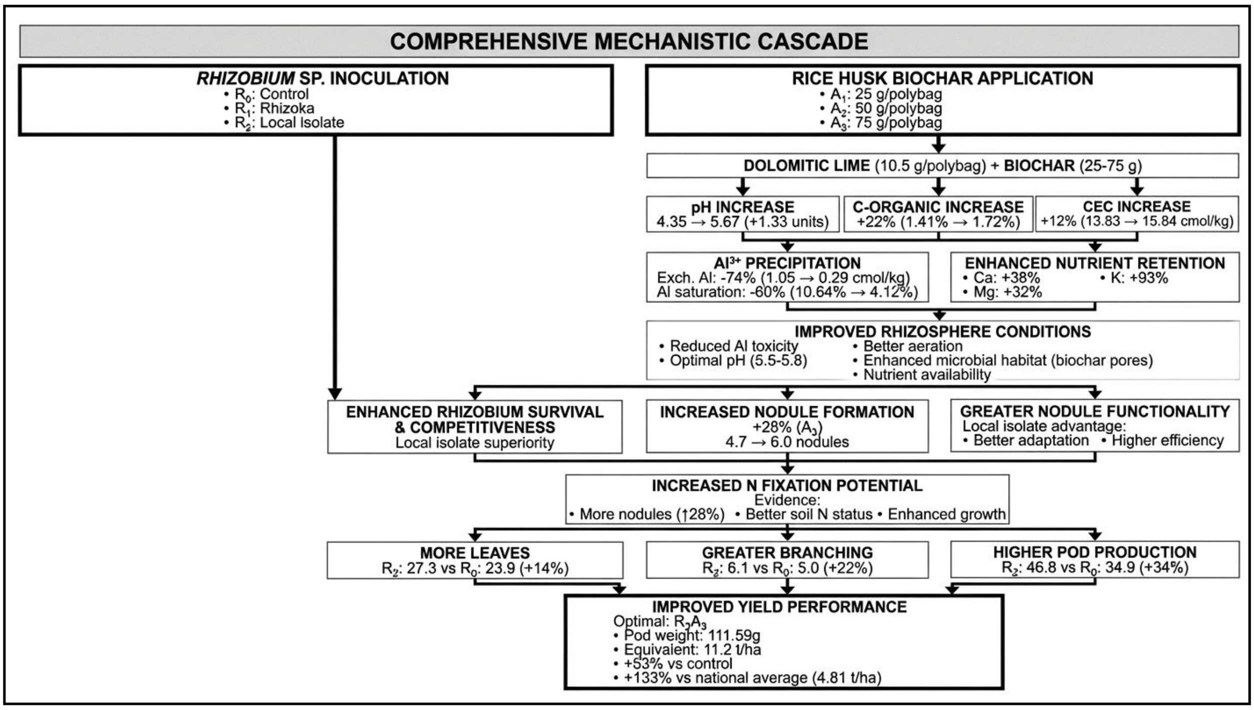

Fig. 1. Mechanistic cascade showing the integrated effects of *Rhizobium* inoculation and rice husk biochar amendment on edamame productivity in alluvial soil.

The combined application of dolomitic lime and rice husk biochar substantially improved soil chemical properties compared to initial conditions (Table 3). Soil pH increased from extremely acidic (pH 4.35) to moderately acidic levels (pH 5.56-5.79), representing an average improvement of 1.33 pH units. This pH amelioration successfully brought soil conditions within the acceptable range for edamame cultivation and *Rhizobium* activity. Concomitant with pH improvement, exchangeable aluminium decreased by an average of 74% (from 1.05 to 0.15-0.42 cmol(+)/kg), effectively mitigating aluminium toxicity constraints (Shetty and Prakash, 2020; Shetty *et al.*, 2021; Liu *et al.*, 2025).

Soil organic carbon content increased by an average of 22% (1.41% to 1.58-1.89%). This improvement was accompanied by enhanced cation exchange capacity (+12%, from 13.83 to 14.95-16.72 cmol(+)/kg). A dose-dependent relationship was evident across biochar application rates, with the highest rate (A_3, 75 g/polybag) producing the greatest improvements in pH (5.73-5.79), organic carbon (1.80-1.89%), lowest aluminium levels (0.15-0.21 cmol(+)/kg), and highest CEC (16.20-16.72 cmol/(+)/kg). This dose-response pattern aligned well with the nodulation response.

The most critical mechanism underlying biochar's positive effect was the substantial pH amelioration achieved (+1.33 units average), which brought the initially extremely acidic soil (pH 4.35) into the acceptable range for *Rhizobium* activity (pH 5.56-5.79). This pH improvement resulted from the combined alkalinity of rice husk biochar and dolomitic lime application. At the initial pH of 4.35, aluminium existed predominantly as phytotoxic Al^{3+} species that severely inhibited root growth and reduced *Rhizobium* survival (Shetty *et al.*, 2021). As pH increased above 5.5, aluminium progressively precipitated as $Al(OH)_3$, effectively removing it from soil solution. This was evidenced by the 74% reduction in exchangeable aluminium. The 1.33-unit pH increase achieved in this study aligned well with reported effects of rice husk biochar applications in acidic tropical soils (Hossain *et al.*, 2020; Liu *et al.*, 2025; Yao *et al.*, 2025).

The absence of significant interactions between *Rhizobium* inoculation and rice husk biochar application across all measured parameters contrasted with several studies reporting enhanced nodulation when biochar and rhizobial inoculants were combined (Egamberdieva *et al.*, 2017; Asirifi *et al.*, 2025). However, results aligned with recent work by Liu *et al.* (2024) and Fan *et al.* (2025), who similarly observed independent rather than interactive effects in legume systems. Several factors may have explained this lack of interaction. First, the relatively short experimental duration (70 days) may have been insufficient for biochar-mediated soil conditioning effects to fully manifest (Hossain *et al.*, 2020). Second, the high initial fertilizer input may have masked subtle synergistic effects by satisfying plant nutrient requirements independently of biological nitrogen fixation efficiency (Santachiara *et al.*, 2017).

Table 3. Changes in soil physicochemical properties before and after *Rhizobium* inoculation and rice husk biochar application in alluvial soil

Soil property	Before treatment	After treatment (70 DAS)	Change	Optimal range
pH (H_2O)	4.35	5.56-5.79	+1.21 to +1.44	5.5-7.0
pH (KCl)	3.89	5.12-5.35	+1.23 to +1.46	5.0-6.5
C-organic (%)	1.41	1.58-1.89	+0.17 to +0.48	>2.0
Total N (%)	0.17	0.19-0.23	+0.02 to +0.06	>0.20
C/N ratio	8.29	8.22-8.31	-0.07 to +0.02	8-12
Ca (cmol(+) kg^{-1})	6.10	7.25-8.40	+1.15 to +2.30	>5.0
Mg (cmol(+) kg^{-1})	3.52	4.10-4.65	+0.58 to +1.13	>1.5
K (cmol(+) kg^{-1})	0.14	0.18-0.35	+0.04 to +0.21	>0.20
Exch. Al (cmol(+) kg^{-1})	1.05	0.15-0.42	-0.63 to -0.90	<0.50
Exch. H (cmol(+) kg^{-1})	1.15	0.35-0.58	-0.57 to -0.80	<1.0
Al saturation (%)	10.64	1.56-4.12	-6.52 to -9.08	<20
CEC (cmol(+) kg^{-1})	13.83	14.95-16.72	+1.12 to +2.89	>15
Base saturation (%)	71.51	78.35-84.60	+6.84 to +13.09	>50

Initial analysis at Soil Chemistry Laboratory, Tanjungpura University, 2024 and DAS – Days after sowing.

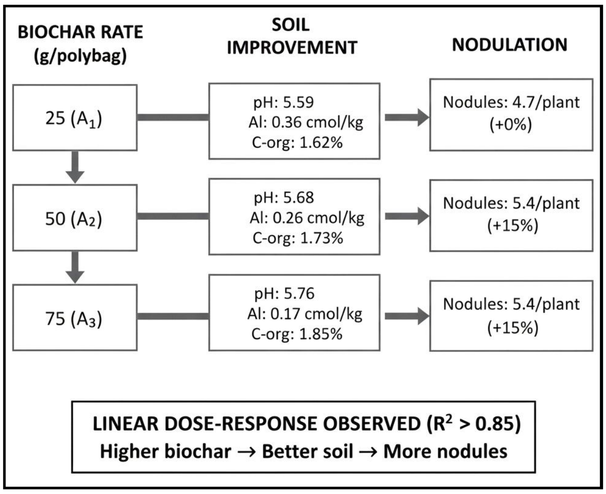

Fig. 2. Dose-response relationship between biochar application rate, soil chemical improvements, and root nodulation in edamame cultivation.

The treatment combination R, Aƒ (local isolate + 75 g biochar / polybag) emerged as optimal for several key yield parameters including plant height (35.39 cm), pod weight (111.59 g), and nodule number (6.61), representing increases of 13.2, 52.9 and 39.4%, respectively, compared to the control (R€ A). This combination effectively leveraged the complementary benefits of enhanced biological nitrogen fixation and maximized soil chemical improvements. For vegetative biomass accumulation, treatment R, A, proved most effective, yielding the highest shoot dry weight (10.96 g), root dry weight (2.71 g), and branch number (6.33).

From a commercial production perspective, the R, Aƒ combination would likely be preferred given its superior total pod yield (111.59 g / plant, equivalent to approximately 11.2 t / ha at standard plant densities). This productivity level substantially exceeded current Indonesian averages (4.81 t / ha) and demonstrated potential for 2.3-fold yield improvements. These findings had important implications for developing sustainable intensification strategies for edamame cultivation in tropical alluvial soils. The superior performance of locally-adapted *Rhizobium* isolates highlighted the value of exploring native microbial resources (Kawaka *et al.*, 2018; Kolapo *et al.*, 2025). The demonstrated benefits of rice husk biochar presented opportunities for circular economy approaches (Kizito *et al.*, 2019; Asirifi *et al.*, 2025; Wu *et al.*, 2025).

CONCLUSION

This study demonstrated that local *Rhizobium* isolates and rice husk biochar independently enhanced edamame productivity in alluvial soil through distinct but complementary mechanisms. Although no synergistic statistical interactions were detected, both interventions contributed meaningfully to improved plant performance. The local *Rhizobium* isolate significantly outperformed commercial inoculant across multiple yield-related parameters including compound leaf number (+14% versus control), branch production (+22%), pod number (+34%) and pod weight (+34%). Rice husk biochar application achieved substantial soil chemical improvements: pH increased by 1.33 units (from pH 4.35 to 5.67), exchangeable aluminium decreased by 74% (from 1.05 to 0.29 cmol(+) / kg), and organic carbon content enhanced by 22% (from 1.41 to 1.72%). These soil ameliorations translated to significantly increased root nodulation (+28% in highest biochar rate). Optimal treatment combinations achieved pod yields of 111.59 g / plant (R, Aƒ , equivalent to 11.2 t / ha), representing a 53% improvement over non-amended controls. The findings supported the integration of locally-adapted rhizobial inoculants and rice husk biochar as complementary components of sustainable intensification strategies for edamame production in tropical alluvial soils.

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