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Short Articles

Pyroligneous Acid (Wood Vinegar) as a Sustainable Biostimulant for Early Growth of Rice (*Oryza sativa* L.) Under Controlled Conditions

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ABSTRACT

Rice is a vital component in the diet of many countries, but in Cuba, its production is limited by the scarcity of agricultural inputs. This study evaluated the biostimulant effect of pyroligneous acid on the early growth stages of *Oryza sativa* L. under controlled conditions, using certified seeds (viability ≥ 85%) of the INCA LP-5, INCA LP-7, and Selección 1 varieties. Plant height, radicle length, and leaf number were measured in a completely randomized design with factorial arrangement (Factor A: varieties; Factor B: morphological variables) and 10 replications. Data were analyzed using the Kruskal-Wallis test, Spearman correlation, and cluster analysis (InfoStat 2020), revealing significant differences among treatments, with the INCA LP-5 variety exhibiting the greatest stimulation in plant height and radicle length. Positive correlations between variables confirmed the efficacy of pyroligneous acid as a biostimulant, suggesting its potential to improve initial rice growth under resource-limited conditions.

Keywords: Pyroligneous acid, Wood vinegar, Biostimulants, Rice cultivation, Oryza sativa L., Sustainable agriculture, Seedling growth, Root development, Plant growth promoters, Organic fertilizers, Marabou wood, Early growth stage

INTRODUCTION

The agricultural sector faces significant challenges in producing food sustainably to meet the growing needs of a global population. The Food and Agriculture Organization of the United Nations (FAO) forecasts a 13% population growth by 2030, accompanied by a 70% increase in production to meet food and nutritional needs. These forecasts will enable the achievement of the United Nations' Sustainable Development Goals outlined in the 2030 Agenda. [1]

In various countries, the cultivation of rice (*Oryza sativa L.*) is of great importance for promoting food security due to its nutritional, socioeconomic, and cultural values, its high consumption as a staple food, and the production of diverse by-products. Currently, the world's production of this cereal has reached a harvested area of approximately 10% of the planet's cultivated area. The average world production over the last five years is estimated at 764 million tons, with a productivity of 4.68 tons per hectare. Currently, the production volume does not cover the demand for the cereal, where the acquisition and application of synthetic products is considered one of the main constraints. [3]

The production of bioproducts in small and medium-scale agriculture, particularly in countries with limited financial resources, enhances resilience by utilizing endogenous resources of agroecosystems to produce healthier crops, thereby reducing dependence on high-cost inputs from the international market. Among the most widely used alternatives for crop biostimulation and pest control is pyroleanic acid, obtained from wood resin, using different species such as marabú (*Dichrostachys cinerea*). [4]

Different studies have shown scientific results demonstrating its efficacy in biostimulating morphological variables, agricultural yield, and its components in crops of economic interest. On the other hand, its effectiveness in managing various pests by controlling harmful populations that cause financial losses of up to 30% of crops is also of great interest.[5–7] Therefore, the objective of this research is to evaluate pyroleanic acid as an alternative for biostimulating rice (*Oryza sativa* L.) under controlled conditions.

MATERIAL AND METHODS

Location of the research

The research was carried out at the Laboratory of Plant Physiology of the University of Granma, Bayamo, Cuba. Certified seeds from the Empresa Agroindustrial de Granos "Fernando Echenique" with a viability ≥ 85% were used. Ten seeds were placed in each 8.6 cm diameter Petri dish with moistened filter paper, to which 10 mL of distilled water and different concentrations of pyroleanic acid were applied for 15 days. The temperature during the experiment was 30°C. The determination of the main components of the bioproduct is shown in Table 1.

Concentration (%)						**Ppm**				
N	P	K	Ca	Mg	S	B	Zn	Fe	Co	Mn
0,75	0,09	0,10	0,21	0,04	0,08	9	5	2	106	11

Table 1. Chemical composition of pyroligneous acid derived from marabou wood (Dichrostachys cinerea)**. The table presents the macronutrient and micronutrient content expressed in parts per million (ppm), except for the total solution concentration (0.75%). The elements measured include:** N **(nitrogen),** P **(phosphorus),** K **(potassium),** Ca **(calcium),** Mg **(magnesium),** S **(sulfur),** B **(boron),** Zn **(zinc),** Fe **(iron),** Co **(cobalt), and** Mn **(manganese). The absence of a value for manganese (Mn) indicates it was not detected or not reported in the analysis.**

Variables evaluated

The experiment was harvested 15 days after seed germination, and the response to the application of pyroleanic acid was determined by the variables seedling height (PA), radicle length (RL), and the number of leaves (No. H). The evaluation of the different morphological variables was carried out using a 100 cm measuring tape with a measurement error of 0.00001 m. Seedling height was measured from the base of the stem to the leaf apex, while root length was determined from the neck of the seedling to the end of the apparent root system. The number of leaves produced by seedlings in each treatment was recorded.

Statistical processing and treatments used

A completely randomized design with a factorial arrangement was used, consisting of Factor A (3 varieties) and Factor B (3 morphological variables), with 10 replications. Before conducting statistical analysis, the data were checked for compliance with ANOVA assumptions using a modified Shapiro-Wilk normality test. [1.] Then, they were transformed using different equations when they did not comply with these assumptions. They were then analyzed using nonparametric variance, as determined by the Kruskal-Wallis test, with varieties and treatments serving as classification criteria. Comparisons were made pairwise using an ascending list presentation at a significance level of 0.05.

In the multivariate analyses, Spearman correlation coefficients were used to examine the relationships between the variables' seedling height, radicle length, and number of leaves, with matrix presentation. In the clusters, varieties and treatments were used as classification criteria. The method used was Average linkage by Euclidean distance. The cut-off line was set at 50%. Data were processed with the statistical package InfoStat ver. 2020. [5]

Treatments

T1: 1 mL^{-1} of pyroleanic acid.
T2: 2 $mL.l^{-1}$ of pyroleanic acid.
T3: 3 $mL.l^{-1}$ of pyroleanic acid.
T4: 4 $mL.l^{-1}$ of pyroleanic acid.
TC: Control (without pyroleanic acid).

RESULTS

The plant height variable showed significant differences between treatments in the cultivars used. The highest results were obtained with the application of treatments T1 and T2 on cultivar LP5; however, the interactions with cultivar Selection 1 showed the lowest biostimulation. These results could reveal the efficacy of the bioproduct on cultivar development.

Varieties	Treatments				
	T1	T2	T3	T4	TC
LP5	3,38 a	2,93 a	2,76 abc	2,35 cd	2,23 de
LP7	2,66 abc	2,62 abc	2,54 bcd	2,98 ab	1,98 ef
S1	1,86 ef	1,56 fg	1,74 fg	1,75 fg	1,37 g

T: treatment, CT: control treatment. Different letters indicate significant differences.

Table 2. Biostimulation of the variable plant height in rice with the application of pyroleanic acid obtained from marabú (*Dichrostachys cinerea*) under controlled conditions.

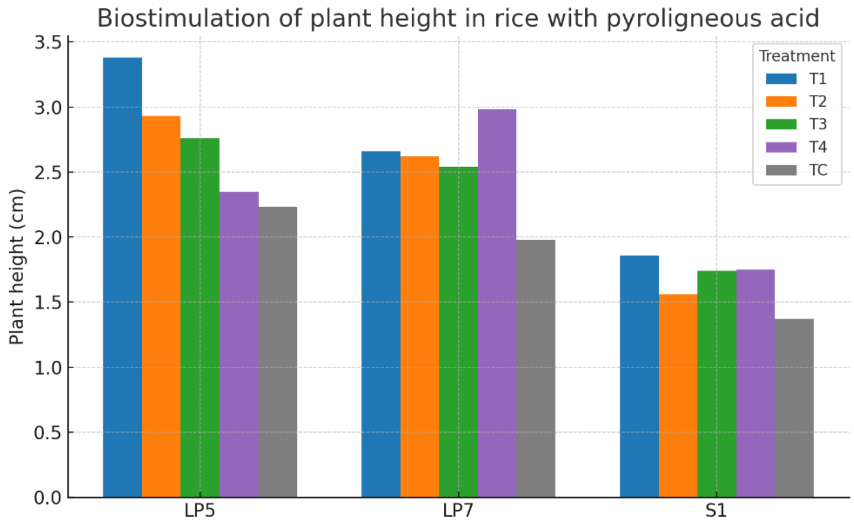

Figure 1. Effect of pyroligneous acid obtained from marabou wood (*Dichrostachys cinerea*) on plant height of three rice (*Oryza sativa L.*) varieties (LP5, LP7, and S1) under controlled conditions. Values represent means of 10 replicates per treatment. Treatments: T1 = 1 mL·L⁻¹, T2 = 2 mL·L⁻¹, T3 = 3 mL·L⁻¹, T4 = 4 mL·L⁻¹, TC = control (no pyroligneous acid). Error bars indicate standard deviation. Different letters above the bars denote statistically significant differences according to the Kruskal–Wallis test ($p < 0.05$).

The application of pyroleanic acid showed significant differences between treatments on the varieties used in the variable radicle length. The greatest development of the root system was obtained in varieties LP5, LP7, and Selection 1, respectively. The interactions with the highest results in terms of variety and biostimulation were T3 and T2, and similarity was observed in the rest of the treatments. These results suggest that the application of high concentrations may inhibit root growth in the crop, while medium dilutions could improve nutrient uptake under similar soil conditions.

Varieties	**Treatments**				
	T1	T2	T3	T4	TC
LP5	8,38 ab	9, 40 a	9,18 a	7,87 abc	5,10 ef
LP7	6,09 bcde	6,83 abcd	6,00 cde	6,69 bcde	1,93 h
S1	5,43 def	4,07 fg	6,39 de	5,09 ef	2,50 hg

T: treatment, CT: control treatment. Different letters indicate significant differences.

Table 3. Biostimulation of the variable radicle length in rice with the application of pyroligneous acid obtained from marabú (*Dichrostachys cinerea*) under controlled conditions.

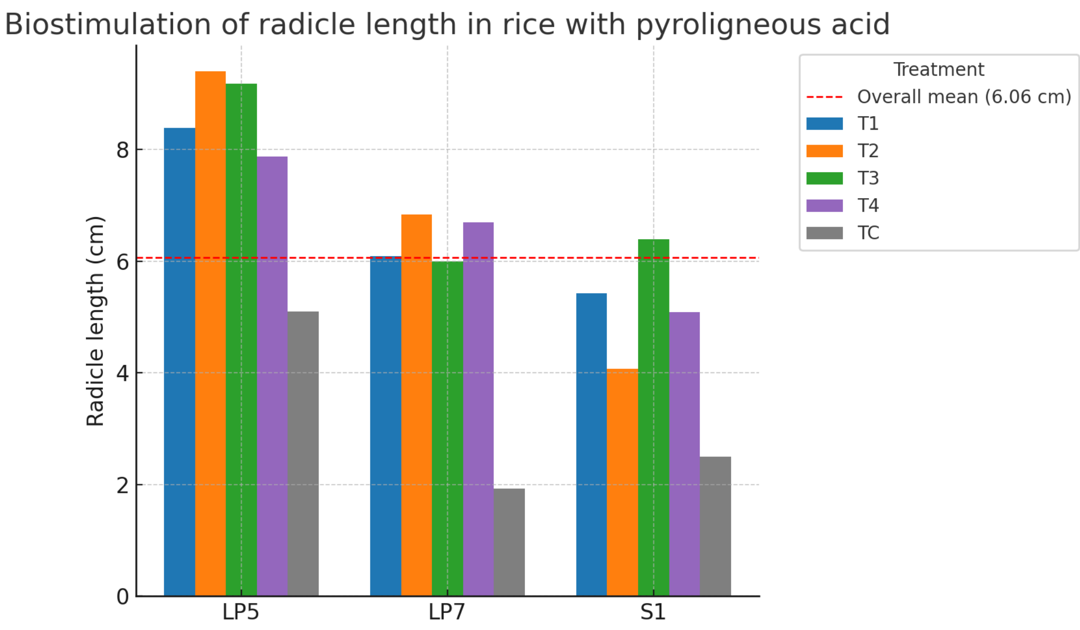

Figure 2. Effect of pyroligneous acid obtained from marabou wood (*Dichrostachys cinerea*) on radicle length of three rice (*Oryza sativa L.*) varieties (LP5, LP7, and S1) under controlled conditions. Values represent means of 10 replicates per treatment. Treatments: T1 = 1 mL·L⁻¹, T2 = 2 mL·L⁻¹, T3 = 3 mL·L⁻¹, T4 = 4 mL·L⁻¹, TC = control (no pyroligneous acid). The dashed red line indicates the overall mean radicle length across all treatments and varieties. Error bars indicate standard deviation. Different letters above the bars denote statistically significant differences according to the Kruskal–Wallis test ($p < 0.05$).

The number of leaves showed significant differences among treatments in the cultivars used, with superior results in cultivars LP7, Selection 1, and LP5, respectively. The greatest effectiveness in the formation of these plant organs was shown in treatments T3 and T4 in the variety with the greatest biostimulation, with low association in the rest of the interactions. The results show the effectiveness of the biostimulation of this variable with the application of pyroleanic acid, which could be included in the technological package of alternative products in the crop.

Varieties	**Treatments**				
	T1	T2	T3	T4	TC
LP5	1,85 cde	1,08 e	1,55 abc	1,42 bcd	1,08 e
LP7	1,39 bcd	1,72 ab	1,69 a	1,68 a	1,20 de
S1	1,54 ab	1,54 ab	1,50 ab	1,32 cde	1,36 bcd

T: treatment, CT: control treatment. Different letters indicate significant differences.

Table 4. Biostimulation of the variable leaf number in rice with the application of pyroleanic acid obtained from marabú (*Dichrostachys cinerea*) under controlled conditions.

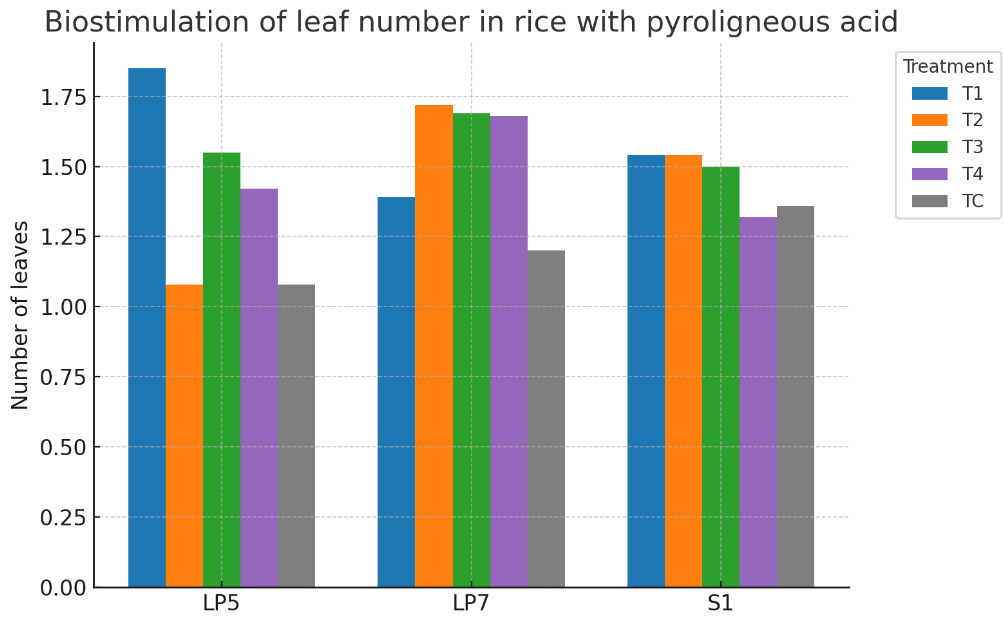

Figure 3. Effect of pyroligneous acid obtained from marabou wood (*Dichrostachys cinerea*) on the number of leaves in three rice (*Oryza sativa L.*) varieties (LP5, LP7, and S1) under controlled conditions. Values represent means of 10 replicates per treatment. Treatments: T1 = 1 mL·L⁻¹, T2 = 2 mL·L⁻¹, T3 = 3 mL·L⁻¹, T4 = 4 mL·L⁻¹, TC = control (no pyroligneous acid). Error bars indicate standard deviation. Different letters above the bars denote statistically significant differences according to the Kruskal–Wallis test ($p < 0.05$).

Spearman correlation coefficients between the variables analyzed showed highly significant differences. All associations were positive, where the variables plant height and radicle length showed a high correlation between them (0.61). At the same time, low correlations were found between the number of leaves and the variables plant height (0.18) and radicle length (0.19). These correlations show the agricultural relationship of the application of bioproducts in the biostimulation of these crop morphological variables.

	AP	**LR**	**No. H**
AP	1.00		
LR	0,61***	1.00	
No. H	0,18***	0,19***	1.00

AP: plant height, LR: radicle length, No. H: number of leaves. Different points indicate significant differences.

Table 5. Correlations between biostimulated variables with the application of pyroleanic acid obtained from marabou (*Dichrostachys cinerea*).

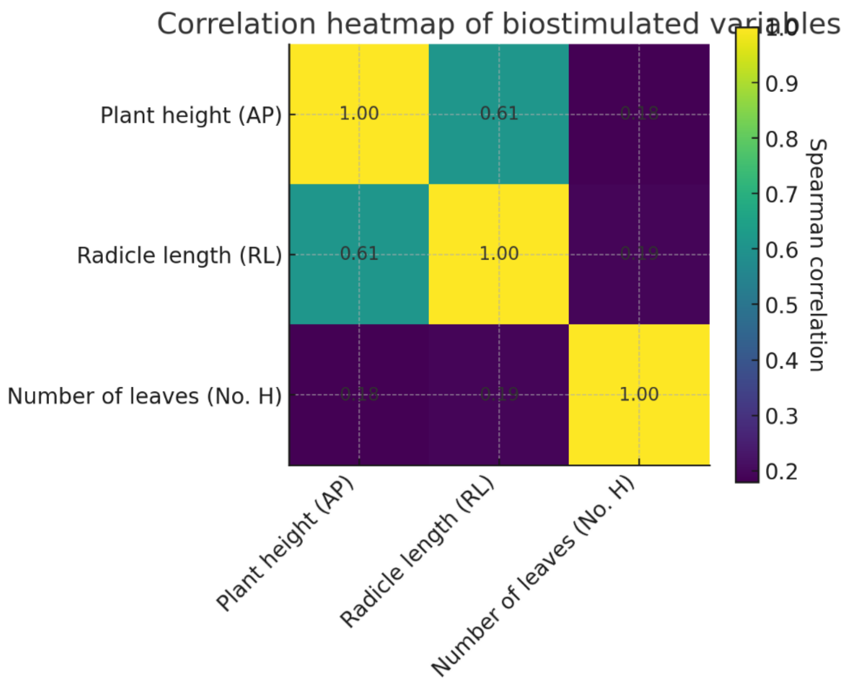

Figure 4. Spearman correlation heatmap among plant height (AP), radicle length (RL), and number of leaves (No. H) in rice seedlings biostimulated with pyroligneous acid under controlled conditions. Coefficients are shown within cells; all reported associations were positive, with a strong correlation between AP and RL (ρ = 0.61) and weaker correlations involving No. H (ρ = 0.18–0.19). All coefficients were significant in the original analysis (*, p < 0.001).**

In the cluster analysis, eight groups were obtained. The interactions of treatments T2, T4, T3, and T1 in the variety Selection 1 showed similarities among them. The control treatments of cultivars LP7 and LP5, while the behavior of the control and T2 in LP5 formed independent groups. In LP7, treatments T3, T4, and T2 showed similarities when grouped; the rest of the interactions did not show grouping. All this could demonstrate the effectiveness of pyroleanic acid in the homogeneous behavior of morphological variables in cultivars Selection 1 and LP7.

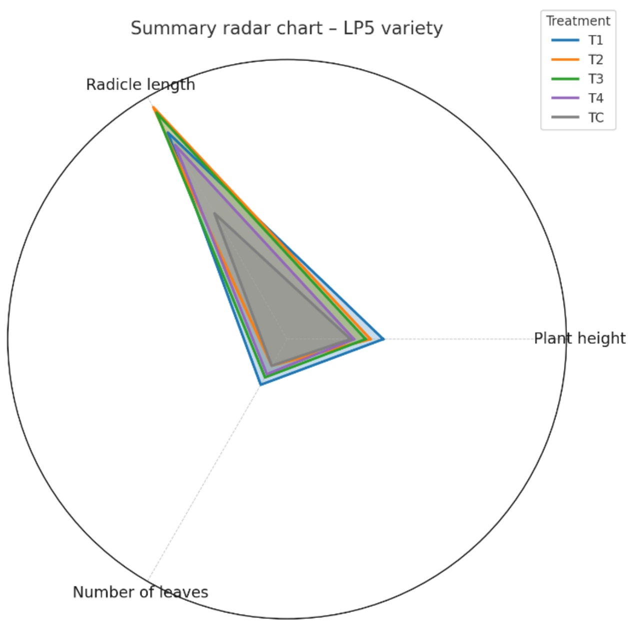

Figure 5. Summary radar chart showing the effect of pyroligneous acid obtained from marabou wood (*Dichrostachys cinerea*) on three morphological variables—plant height, radicle length, and number of leaves—in the LP5 rice (*Oryza sativa L.*) variety under controlled conditions. Treatments: T1 = 1 mL·L^{-1}, T2 = 2 mL·L^{-1}, T3 = 3 mL·L^{-1}, T4 = 4 mL·L^{-1}, TC = control (no pyroligneous acid). Values represent means of 10 replicates per treatment.

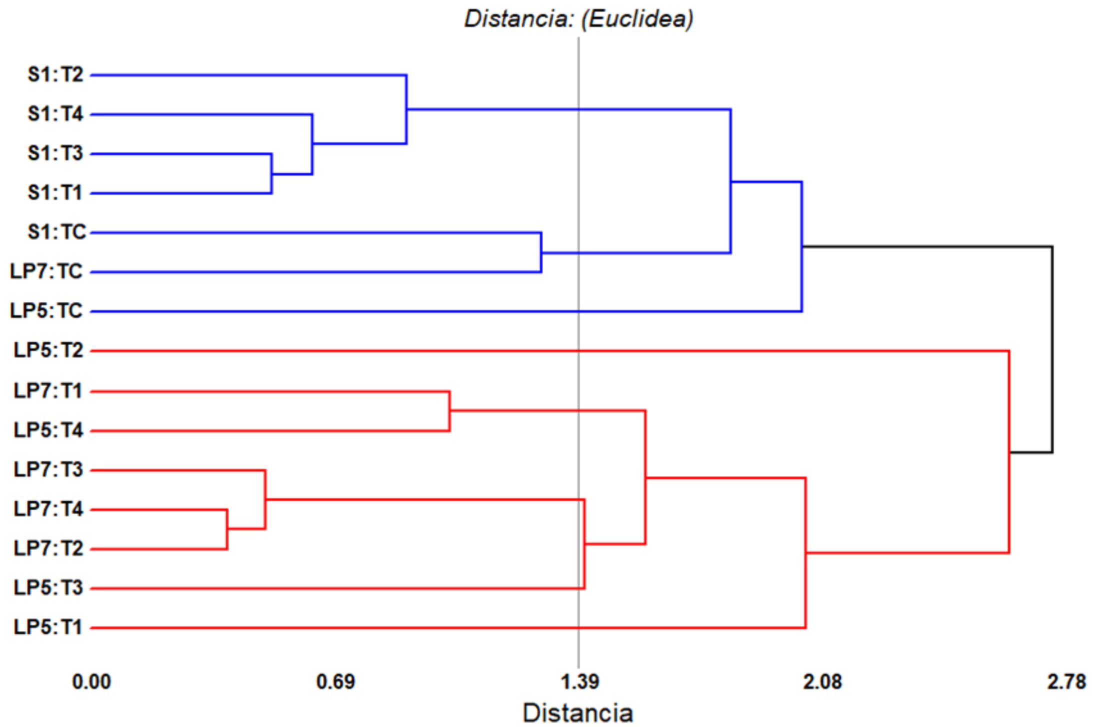

Figure 6. Cluster analysis between the variables biostimulated with the application of pyroleanic acid obtained from marabú (*Dichrostachys cinerea*).

DISCUSSION

The visual representation of the results (Figures 1–5) reveals clear and consistent patterns in the biostimulant effect of pyroligneous acid on early rice development, which were less evident when observing the raw numerical tables alone.

Plant height (Figure 1) showed a pronounced positive response in the LP5 variety, particularly at low to moderate concentrations (T1 and T2). This aligns with Carril et al. [6], who demonstrated positive effects on yield, protein content, and nutritional composition in legumes such as chickpea (*Cicer arietinum L.*), lentil (*Lens culinaris L.*), and bean (*Phaseolus vulgaris L.*). Similarly, Hur et al. [21] reported that moderate doses of pyroligneous acid enhanced shoot elongation in rice by elevating photosynthetic efficiency. The decline at higher doses (T4) for LP5 suggests a possible inhibitory effect at excessive concentrations, also noted by Weber et al. [9] in maize and bean.

Radicle length (Figure 2) responded optimally to intermediate concentrations (T2, T3) across all varieties, surpassing the overall mean indicated by the dashed reference line. Yuan et al. [7] demonstrated that the combined application of biochar and pyroligneous acid increased the yield of groundnut crops under controlled conditions and significantly improved soil biological indicators, consistent with our finding that improved root architecture enhances nutrient uptake. The strong correlation between radicle length and plant height (ρ

= 0.61; Figure 4) supports the role of root development in driving above-ground biomass, in line with Sandhu et al. [11], who linked root traits to yield potential in rice.

Leaf number (Figure 3) displayed a more variable pattern, with LP7 and S1 outperforming LP5 at higher concentrations (T3, T4). This may reflect different physiological controls over leaf initiation compared to shoot or root elongation, as observed by Praveena et al. [10] in *Vigna radiata*. The weaker correlations between leaf number and the other traits ($\rho = 0.18–0.19$) suggest partial independence in their response to biostimulation.

The radar chart (Figure 5) for LP5 integrates these results, highlighting T1 and T2 as balanced treatments that enhance all three traits simultaneously, while higher doses improved some parameters but not all. This reinforces the concept of dose optimization to maximize multiple growth indicators.

The correlation heatmap (Figure 4) visually confirms statistical associations, with plant height and radicle length forming the strongest functional link. Iqbal et al. [12] noted that such root–shoot synergy is crucial for sustainable rice production under low-input conditions.

Beyond our controlled-environment results, literature indicates that pyroligneous acid is effective under field conditions both as a biostimulant and as a phytosanitary agent. Martoreli et al. [8] reported improvements in peanut seedling development, although less pronounced than in our study. Its phenolic compounds and organic acids have been linked to improved resistance against pathogens and abiotic stresses such as drought and salinity [15], as well as enhanced beneficial microbial activity and nutrient availability in soils [17,18]. These mechanisms likely explain the morphological improvements observed here.

However, as Weber et al. [9] and Muduli et al. [13] point out, responses vary by genotype, environment, and dose, making it essential to conduct field trials with multiple varieties. Our data clearly show genotype-specific differences, particularly the strong performance of LP5 at moderate doses, underscoring the need for tailored recommendations.

A key limitation of this study is that it focuses exclusively on early growth stages. As noted by Iqbal et al. [12] and Garcés et al. 14, full agronomic potential must be evaluated through yield components such as panicle number, grain weight, and quality under field conditions. Furthermore, we did not address interactions with soil type, nutrient dynamics, or pest pressure, which could significantly affect efficacy.

In summary, the integration of detailed statistical results with visual analysis enhances our understanding of pyroligneous acid's action on rice seedlings. The data support its role as a sustainable growth promoter, provided that concentration and varietal sensitivity are carefully managed. These insights contribute to optimizing biofertilization strategies for low-resource systems in Cuba and similar agroecological contexts.

CONCLUSIONS

This study demonstrates that pyroligneous acid derived from marabou wood (*Dichrostachys cinerea*) exerts a measurable and dose-dependent biostimulant effect on the early growth of rice (*Oryza sativa L.*) under controlled conditions.

Visual analysis of the data revealed that:

- Moderate concentrations (1–2 $mL \cdot L^{-1}$) consistently enhanced plant height and radicle length, particularly in the LP5 variety, indicating an optimal dose range for early vigor.
- Radicle length exhibited the strongest positive correlation with plant height ($\rho = 0.61$), highlighting the central role of root system development in promoting above-ground biomass accumulation.
- Leaf number responded differently across varieties, with LP7 and S1 benefiting more from higher concentrations, suggesting trait-specific sensitivity to pyroligneous acid.
- The radar chart confirmed that no single concentration maximized all traits simultaneously, reinforcing the importance of dose optimization.

These findings position pyroligneous acid as a promising **sustainable biostimulant** for rice cultivation in low-input systems. However, its use should be tailored to specific varieties and applied at concentrations that balance shoot and root development.

Future research should:

1. Extend trials to field conditions to validate the controlled-environment results.
2. Integrate yield components and stress tolerance indicators into the evaluation.
3. Explore the physiological and molecular mechanisms underlying the observed morphological responses.

By adopting a genotype-specific and evidence-based approach, pyroligneous acid can contribute to more resilient and productive rice systems while reducing dependence on synthetic inputs.

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Additional Information

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