



Full Length Research Article

Antifungal Activity of Pyroligneous Acid Derived from Mahogany Sawdust Against *Colletotrichum* sp. on Papaya Fruit

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ABSTRACT

Mahogany (*Swietenia* sp.) processing generates a large volume of sawdust residues that remain underutilized despite their potential to be converted into value-added products within a circular forestry system. This study aimed to evaluate the antifungal activity of mahogany sawdust-derived pyroligneous acid (PA) against *Colletotrichum* sp., a major postharvest pathogen of papaya (*Carica papaya* L.). PA was produced through controlled pyrolysis at 400 °C for 90 min under limited-oxygen conditions. Antifungal activity was assessed using both *in vitro* and *in vivo* assays with PA concentrations of 0% (negative control), 1%, 3%, 5%, and 7%, alongside mancozeb at 1.5 g/L (positive control), arranged in a completely randomized design with five replicates per treatment. Data were analyzed using one-way analysis of variance followed by Duncan's Multiple Range Test at a 5% significance level ($p < 0.05$). The results demonstrated that the 7% PA treatment delayed *in vitro* fungal growth until day 10, whereas growth in the negative control began on day 2, and reduced mycelial growth by 70.56%. In *in vivo* assays, the 7% PA treatment effectively suppressed anthracnose development, resulting in a disease inhibition rate of 47.77% and an area under the disease progress curve (AUDPC) of 297.98, which was significantly lower than that of the negative control (570.53). Although the positive control achieved complete *in vitro* inhibition and greater *in vivo* suppression (67.01%; AUDPC = 188.21), the 7% PA treatment exhibited strong dose-dependent antifungal activity, which may be associated with its high acetic acid content and low pH. Lower PA concentrations showed only partial and inconsistent inhibition. These findings indicate that mahogany sawdust-derived PA has considerable potential as a bio-based antifungal agent for postharvest disease management. Further studies on formulation optimization, product stability, and field-scale validation are recommended to support its practical application.

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1. Introduction

Mahogany (*Swietenia* sp.) is an economically valuable tropical timber species widely cultivated in Indonesia. Recent reports indicate that national mahogany roundwood production declined from 58,531 m³ in 2023 to 40,749 m³ in 2024 ([Directorate General of Sustainable Forest Management Indonesia 2025](#)). This decline highlights the importance of using wood resources, including processing residues. Large quantities of sawdust are generated from the mahogany wood

industry, yet these residues remain underutilized (Chanda et al. 2021; Drovou et al. 2022). Transforming such wood-processing waste into value-added products represents an important strategy to improve resource efficiency and support sustainable forest management within a circular framework.

Transforming wood-processing waste into value-added products is an effective strategy to improve the efficiency of forest resource use and support the forestry sector within the framework of sustainable forest management and the circular economy. Pyrolysis technology can turn mahogany sawdust waste from furniture manufacturing into raw material for pyroligneous acid (PA) production (Haryanto et al. 2021). Mahogany wood contains 35–50% cellulose, 20–30% hemicellulose, and 25–30% lignin (Wiranti et al. 2023). This chemical composition makes it an excellent source of PA, as these organic compounds thermally decompose to produce bioactive compounds at high temperatures. Utilizing mahogany sawdust waste to produce PA not only reduces underutilized wood waste but also transforms forest industry residues into valuable products (Cândido et al. 2023; Lee et al. 2022). Forest biomass extractives also show potential as environmentally friendly biological agents (Andika et al. 2025). Furthermore, the environmental impact of naturally sourced PA is lower than that of synthetic alternatives (Leifeld and Walz 2025; Sivaram et al. 2024). This eco-friendly approach aligns seamlessly with sustainable forest resource management and the circular economy concept within the forest industry. Consequently, one practical application of this bio-based product is its use as a natural fungicide to address agricultural disease challenges.

Pyroligneous acid (PA), also known as wood vinegar, is produced through the condensation of vapors generated during the pyrolysis of organic materials. The resulting liquid contains a high concentration of bioactive compounds that can be used as fungicides (Zhou et al. 2024). Organic materials suitable for PA production are usually rich in cellulose, hemicellulose, and lignin (Liao et al. 2025). During thermal decomposition, these polymeric components degrade to form phenolic compounds, organic acids, and carbonyl compounds with antimicrobial properties. Mahogany sawdust is an excellent raw material for the production of PA owing to its high cellulose, hemicellulose, and lignin content (Shobib et al. 2023). The acid derived from this source shows strong activity against phytopathogenic fungi.

Although field testing of mahogany-derived PA is essential for future application, it is first necessary to consider the economic impact of specific crop-pathogen systems. Papaya (*Carica papaya* L.), a major agricultural commodity in Indonesia, represents one such critical system, as its productivity has declined in recent years. For instance, papaya fruit yield per tree in West Java fell from 89.04 kg in 2022 to 86.19 kg in 2023 (Directorate General of Horticulture Indonesia 2024).

Colletotrichum sp., a fungus responsible for anthracnose, poses a major threat to papaya production, particularly during postharvest processing. Anthracnose remains the main cause of postharvest losses in papaya-producing countries worldwide (Prasad and Paul 2021). During the growth and development of papaya fruits in the field, disease symptoms are often difficult to detect. However, they become evident after harvest when the fruits start to ripen (Tan et al. 2023). Anthracnose can reduce fruit quality by 30–50% (Liu et al. 2019). Although synthetic fungicides are commonly used to control anthracnose in papaya, their intensive use promotes pathogen resistance and leaves hazardous chemical residues on harvested produce (Islam et al. 2024). Developing eco-friendly alternative fungicides from forest industry waste is a sustainable solution

that addresses both farmers' needs for disease control and the need to maximize the use of forestry by-products.

Recent studies have shown that PA derived from various biomass sources can serve as a natural fungicide against plant pathogens. Wood vinegar made from medang wood (*Cinnamomum* sp.) inhibited the growth of the wood-rot fungus *Schizophyllum commune* by 98.57% at a concentration of 2.5% (Permana et al. 2021). Additionally, wood vinegar obtained from sengon and jabon wood waste completely inhibited the fungus at a concentration of 2% (Oramahi et al. 2023). These antifungal properties are mainly due to the high levels of total phenols and total acids in wood vinegar, as bioactive wood-derived compounds have been widely reported to possess important biological activities (Prayogo et al. 2023). Naufal et al. (2023) discovered that PA from coconut shells at a concentration of 1 mL/L completely inhibited the growth of *C. gloeosporioides* *in vitro*. Despite these promising results from a variety of biomass feedstocks, there remains a notable lack of research on mahogany sawdust-generated PA for controlling *Colletotrichum* sp., highlighting a critical knowledge gap in the use of forest waste for botanical fungicides. Thus, this study aimed to evaluate the antifungal activity of mahogany sawdust-derived PA against *Colletotrichum* sp., thereby contributing to both sustainable forest resource utilization and eco-friendly disease management strategies in papaya production.

2. Materials and Methods

2.1. Study Site and Duration

This study was conducted between April and August 2025 at two distinct facilities. Pyroligneous acid (PA) was produced from mahogany (*Swietenia* sp.) sawdust at the Palm Oil Research Center (PPKS) Unit in Bogor, Indonesia. The *in vitro* and *in vivo* antifungal assays were carried out at the Plant Protection Biotechnology Laboratory (Department of Plant Pests and Diseases, Universitas Padjadjaran, Indonesia).

2.2. Preparation of Mahogany Sawdust-Derived Pyroligneous Acid

Mahogany sawdust was collected from a furniture manufacturing facility in Sumedang, West Java. Approximately 110 kg of air-dried sawdust was subjected to controlled pyrolysis in a stainless-steel reactor at 400 °C for 90 min under limited-oxygen conditions (**Fig. 1**). This thermal conversion process yielded 6 liters of PA, corresponding to a volumetric yield of approximately 5.45% (v/w) relative to the dry sawdust input. The pyrolysis reactor measured 120 cm × 40 cm × 60 cm and was constructed from 2 mm-thick, food-grade 304 stainless steel. It possessed a capacity of 60 liters per batch (5–7 kg) and featured a double-jacket design heated by liquefied petroleum gas (LPG). Furthermore, the system was equipped with a temperature controller, glasswool insulation, and a stainless steel cover. These specific operating parameters ensured thermal decomposition of hemicellulose (200–250 °C), cellulose (280–350 °C), and lignin (300–400 °C). The obtained condensate was allowed to settle for 24 hours, after which the upper clarified layer was separated from the tar sediment. The clarified condensate was then purified by distillation at 126–150 °C for 3 hours to reduce the tar content further and yield the purified PA used in this study. The purified PA was stored in dark glass bottles at room temperature until further use.

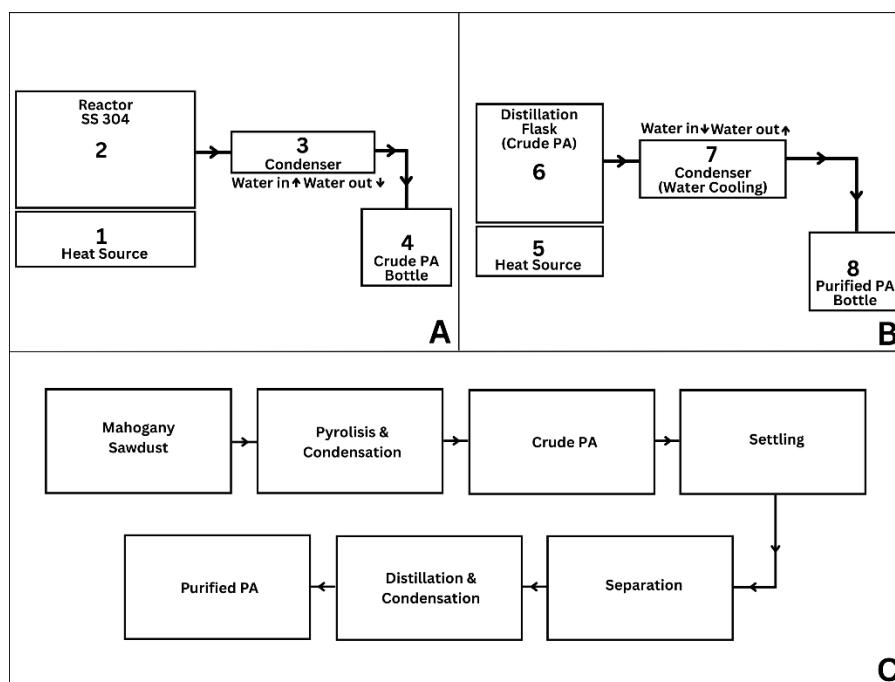


Fig. 1. Schematic diagram of pyroligneous acid (PA) production setup and process flow. Pyrolysis setup (A); Distillation setup (B); Purification process flow from mahogany sawdust to purified PA (C).

2.3. Pyroligneous Acid Characterization

Prior to the bioassays, the physicochemical properties of the mahogany sawdust-derived PA were characterized. The acidity of the solution was determined using an AMT20 pH/mV Meter (AMTAST, Jakarta, Indonesia), yielding a pH of 2.8. Volatile compound identification was performed via Gas Chromatography-Mass Spectrometry (GC-MS) utilizing a GCMS-QP2010 Ultra instrument (Shimadzu Corporation, Kyoto, Japan). The chromatographic conditions included an initial oven temperature of 70 °C (held for 1 min), ramped at 9 °C/min to 300 °C (held for 7 min), an ion source temperature of 230 °C, and an interface temperature of 280 °C. Finally, the detected compounds were identified by comparing their resulting mass spectra against the WILEY7 reference library.

2.4. Fungal Isolation, Identification, and Inoculum Preparation

Colletotrichum sp. was isolated from papaya (*Carica papaya* L.) fruits exhibiting typical anthracnose symptoms, which were obtained from the area of Cikancung, Bandung, West Java, Indonesia (7°01'48.0" S–107°49'12.0" E). Symptomatic tissue fragments (approximately 3 mm²) were surface-sterilized sequentially with 1% sodium hypochlorite and 70% ethanol for 1 min each. The tissue was then rinsed with sterile water and plated onto potato dextrose agar (PDA) media. The plates were incubated at room temperature for seven days until visible growth appeared.

Macroscopic characteristics of the pure colonies, including colony pigmentation and mycelial morphology, were recorded. Additionally, microscopic features, including conidial shape and dimensions, were observed and measured using an Olympus CX23 binocular microscope (Olympus Corporation, Tokyo, Japan) at 400x magnification. For inoculum preparation, a seven-day-old culture of *Colletotrichum* sp. grown on PDA was flooded with 10 mL of sterile distilled water, and the conidia were gently dislodged from the colony surface using a sterile L-shaped cell

spreader. The resulting suspension was transferred into a sterile test tube, sealed with sterile aluminum foil, and homogenized via vortex mixing. The suspension was then filtered through sterile cotton to remove mycelial fragments, and the conidial density was adjusted to 1×10^6 conidia mL^{-1} using a hemocytometer. This standardized suspension was utilized for the *in vivo* assays.

2.5. In Vitro Antifungal Assay

The antifungal activity of mahogany sawdust-derived PA was evaluated using the poisoned food technique. PA was incorporated into molten potato dextrose agar (PDA) at final concentrations of 0% (negative control), 1%, 3%, 5%, and 7% (v/v), while mancozeb at 1.5 g/L served as the positive control. 10 mL of the amended medium was poured into each Petri dish, and a 5-mm mycelial plug excised from an actively growing culture of *Colletotrichum* sp. was placed at the center of each plate. The experiment was arranged in a completely randomized design (CRD) with five replicates per treatment, and the plates were incubated at 27 ± 2 °C. Colony growth was recorded daily by measuring two perpendicular diameters of the colony using a standard ruler, with the mean value representing the colony diameter. Observations were conducted daily until the mycelial colony in the negative control treatment entirely covered the Petri dish (14 days). The percentage of mycelial growth inhibition was calculated according to Marroquín et al. (2022) using Equation 1.

$$\text{Inhibition (\%)} = \frac{D_c - D_t}{D_c} \quad (1)$$

where D_c is the colony diameter of the negative control, and D_t is the colony diameter of the treatment. The calculated inhibition percentages were further classified into five categories to evaluate the efficacy of the treatments, based on the criteria established by Mori et al. (1997), as shown in Table 1.

Table 1. Classification of antifungal activity level (Mori et al. 1997)

Antifungal activity (AFA)	Activity level
$\text{AFA} \geq 75\%$	Very Strong
$50\% \leq \text{AFA} < 75\%$	Strong
$25\% \leq \text{AFA} < 50\%$	Moderate
$0\% < \text{AFA} < 25\%$	Weak
$\text{AFA} = 0\%$	Not Active

2.6. In Vivo Assay on Papaya Fruits

Uniform, healthy 'California' papaya fruits weighing approximately 1200 g each were selected for the assay. These fruits were characterized by an MS1 green skin color with a trace of yellow (approximately 15% ripe), according to the classification by Garillos-Manliguez and Chiang (2021). The selected fruits were washed, surface-sterilized sequentially with 1% sodium hypochlorite for 1 min and 70% ethanol for 1 min, rinsed three times with sterile distilled water, and air-dried. Subsequently, the fruits were immersed in treatment solutions containing 1%, 3%, 5%, and 7% (v/v) PA, along with a 0% negative control and a 1.5 g/L mancozeb positive control, and allowed to air-dry. Each fruit was then wounded at three separate points using a sterile needle (creating wounds of approximately 2 mm $\times$ 2 mm) and inoculated with 20 μL of a *Colletotrichum*

sp. conidial suspension (10^6 conidia mL^{-1}). The *in vivo* assay was arranged in a completely randomized design (CRD) with five replicates per treatment, each consisting of a single papaya fruit. Treated fruits were placed in high-humidity containers (about 90% relative humidity) and incubated at 27 ± 2 °C. Observations were conducted daily until the lesion in the negative control treatment covered the entire fruit surface (7 days).

Lesion diameters were measured daily by recording two perpendicular axes of each lesion. The average value was used to quantify lesion size, consistent with the procedure applied in the *in vitro* assay. Disease suppression was calculated relative to the negative control. Disease development over time was quantified using the Area Under the Disease Progress Curve (AUDPC) following the methodology of [Campbell and Madden \(1990\)](#).

2.7. Data Analysis

Both *in vitro* and *in vivo* experiments employed a completely randomized design (CRD) with five replicates per treatment. The collected data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at 5% significance level. Percentage data were arcsine-transformed before analysis. Statistical processing was performed using IBM SPSS Statistics 26.0 (IBM Corporation, Armonk, New York, United States of America).

3. Results and Discussion

3.1. Isolation and Morphological Characterization of *Colletotrichum* sp.

The fungal isolate used in this study was obtained from naturally infected papaya (*Carica papaya* L.) fruits exhibiting typical anthracnose lesions collected from Cikancung, West Java, Indonesia. Symptomatic tissues were surface-sterilized and plated onto potato dextrose agar (PDA), where fungal growth emerged within seven days. The resulting colonies displayed white-grey aerial mycelia with a pale to brownish coloration on the reverse side (**Fig. 2**), which is consistent with the morphological description of the *Colletotrichum* species complex reported in previous studies ([Arneti et al. 2020](#); [Gautam 2014](#)).

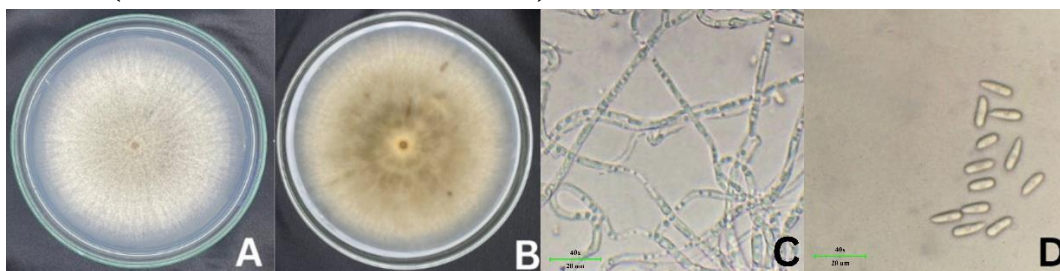


Fig. 2. Macroscopic and microscopic characteristics of the *Colletotrichum* sp. isolate. Colony surface (A); Colony reverse (B); Hyphal morphology (C); Conidial morphology (D).

3.2. In Vitro Inhibition of Mycelial Growth

The application of mahogany (*Swietenia* sp.) sawdust-derived pyroligneous acid (PA) inhibited the mycelial growth of *Colletotrichum* sp. on PDA (**Fig. 3**). Slower colony expansion was observed at 1%, 3%, and 5% concentrations relative to the negative control (PA 0%). Conversely, the 7% concentration showed the most significant suppression, reflected by a notably

delayed onset of growth and the smallest final colony size (2.65 cm), which was statistically significant based on Duncan's Multiple Range Test (DMRT). Specifically, mycelial growth commenced on the second day after inoculation in the negative control, 1%, and 3% treatments, on the fourth day in the 5% treatment, and not until the tenth day in the 7% treatment.

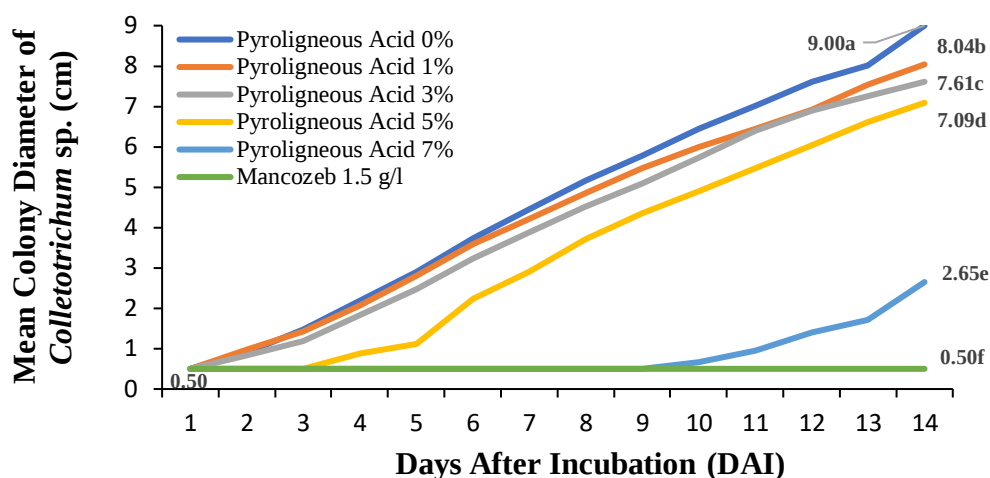


Fig. 3. Mycelial growth of *Colletotrichum* sp. on potato dextrose agar (PDA) under different concentrations of mahogany sawdust-derived pyroligneous acid (PA) over 14 days of observation *in vitro*. Note: Different letters next to the data points indicate significant differences according to Duncan's Multiple Range Test ($p < 0.05$).

At 14 days post-inoculation, the negative control exhibited the largest colony diameter (9.00 cm), whereas all PA treatments significantly reduced colony expansion, with the 7% concentration yielding the smallest diameter (2.65 cm). This indicates a dose-dependent response, where higher concentrations of mahogany PA are associated with more potent antifungal activity, likely due to the cumulative effects of its acidic constituents. Based on the inhibition percentages calculated on the final day of observation (day 14), the 7% treatment achieved the highest inhibition at 70.56%. In comparison, the 1%, 3%, and 5% treatments showed 10.67%, 15.44%, and 21.22% inhibition, respectively (**Fig. 4**). In contrast, the commercial fungicide mancozeb (1.5 g/L), serving as the positive control, completely inhibited the mycelial growth of *Colletotrichum* sp. (0.50 cm) maintaining a 100% inhibition rate throughout the 14 days of observation. According to the fungicide efficacy classification by Mori et al. (1997), the 7% concentration falls into the “strong” inhibition category, whereas the lower concentrations (1–5%) fall into the “weak” category.

The 7% concentration demonstrated the strongest antifungal activity (70.56% inhibition), which can be attributed to the cumulative effect of higher concentrations of bioactive compounds, particularly acetic acid, the dominant volatile compound (77.72% of total peak area), and the low pH (2.8) of the PA. At elevated concentrations, organic acids more effectively disrupt cellular pH homeostasis and the proton motive force, thereby impairing ATP synthesis and cellular metabolism (Antunes and Sa-Correia 2024).

This concentration-dependent efficacy aligns with previous studies evaluating PA from various biomass sources. Permana et al. (2021) reported 98.57% inhibition of *Schizophyllum commune* using medang wood (*Cinnamomum* sp.) vinegar at 2.5%, while Oramahi et al. (2023) demonstrated complete inhibition of the same fungus at 2% using wood vinegar from sengon and jabon. Furthermore, Naufal et al. (2023) achieved complete inhibition of *C. gloeosporioides* *in vitro* at 1 mL/L with coconut shell PA. The higher concentration required in the present study (7%)

likely reflects differences in biomass feedstock composition, pyrolysis conditions, and the inherent tolerance of the specific pathogen tested. Although the commercial fungicide mancozeb (1.5 g/L) achieved 100% inhibition throughout the observation period, the 7% PA treatment still exhibited competitive antifungal performance and falls within the 'strong' antifungal activity category according to the classification by Mori et al. (1997). These findings collectively confirm that mahogany sawdust-derived PA exhibits robust antifungal activity *in vitro*, with its efficacy closely correlated to concentration.

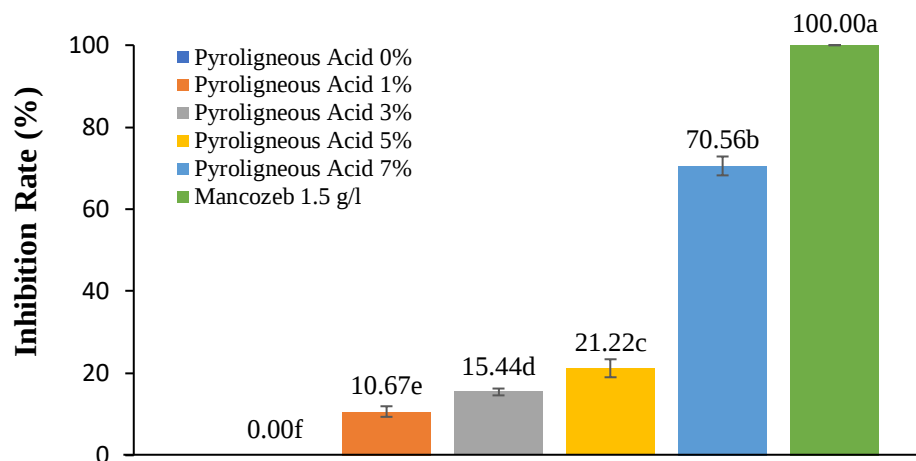


Fig. 4. Inhibition rate of mahogany sawdust-derived pyroligneous acid (PA) against *Colletotrichum* sp. after 14 days of incubation *in vitro*. Note: Different letters next to the data points indicate significant differences according to Duncan's Multiple Range Test ($p < 0.05$).

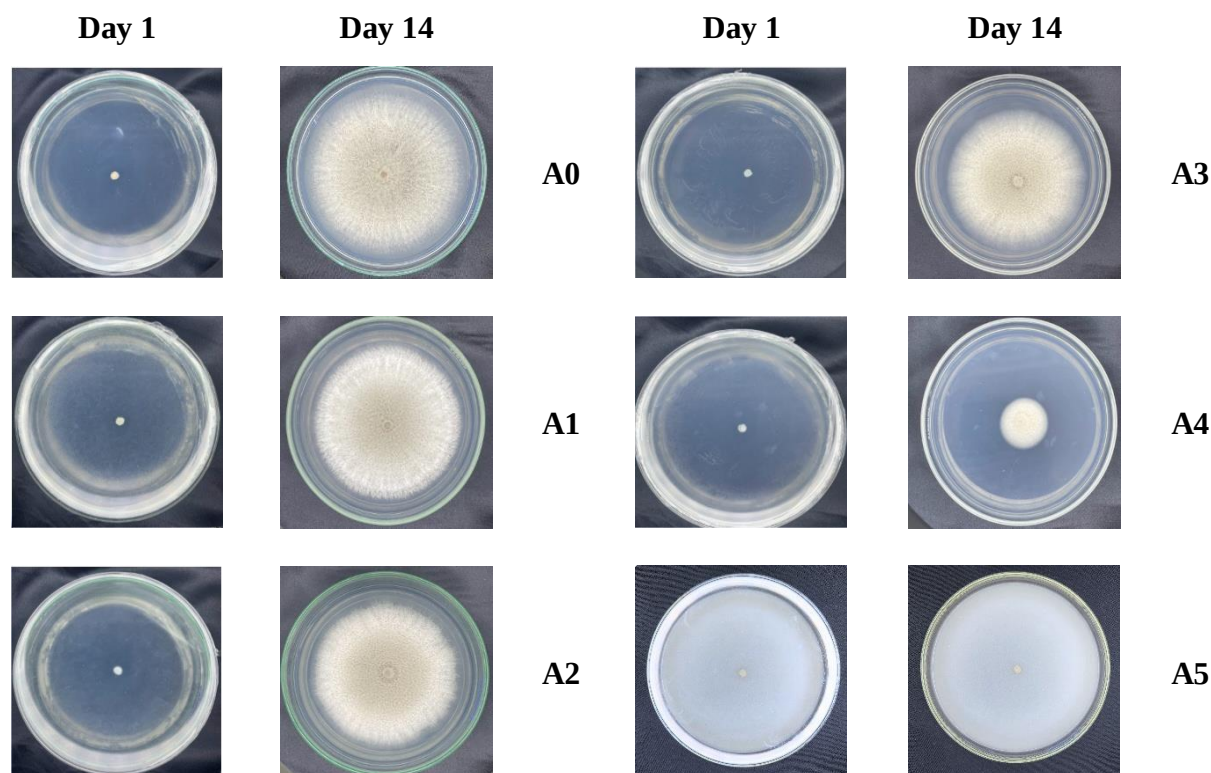


Fig. 5. Visual assessment of the inhibitory effect of mahogany sawdust-derived pyroligneous acid (PA) on the colony diameter of *Colletotrichum* sp. at Day 1 and Day 14 of observation. Treatments consist of A0: 0% PA (negative control); A1: 1% PA; A2: 3% PA; A3: 5% PA; A4: 7% PA; and A5: mancozeb at 1.5 g/L (positive control).

Visual comparisons of colony development across treatments further illustrate the dose-dependent inhibition pattern, with the 7% concentration exhibiting markedly reduced colony expansion between the initial and final observation days (Fig. 5). The inhibition pattern observed in this study parallels findings reported for other wood-derived PA, which have been shown to suppress a broad range of phytopathogenic fungi, including *Colletotrichum* sp. (El-Fawy et al. 2023; Wattana et al. 2025). Consequently, these visual assessments corroborate that higher concentrations of mahogany PA induce pronounced suppression of fungal growth *in vitro*.

3.3. In Vivo Suppression of Anthracnose on Papaya Fruit

The application of mahogany sawdust-derived PA suppressed anthracnose symptom development on papaya fruit (Fig. 6). Lower disease severity was observed at the 3%, 5%, and 7% concentrations compared to the negative control. Among the tested PA concentrations, the 7% concentration produced the smallest lesion area, averaging 135.48 cm² at 7 days after inoculation (DAI). The 5% and 3% treatments recorded lesion areas of 192.93 cm² and 216.21 cm², respectively, whereas the 1% treatment (237.79 cm²) was not significantly different from the negative control (239.14 cm²). The positive control, mancozeb at 1.5 g/L, achieved the smallest overall lesion area (111.93 cm²), which was significantly lower than all PA treatments.

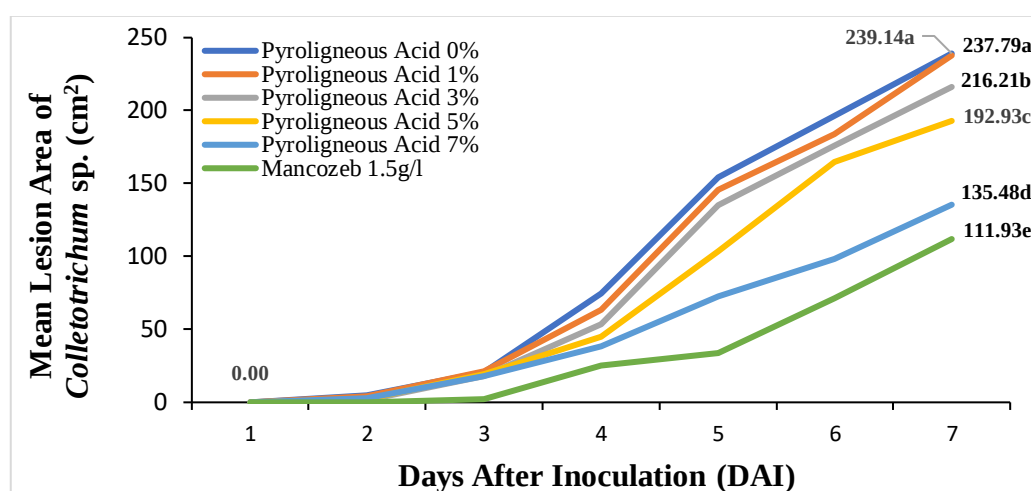


Fig. 6. Development of anthracnose lesion area on papaya fruit treated with mahogany sawdust-derived pyroligneous acid (PA). Note: Different letters next to the data points indicate significant differences according to Duncan's Multiple Range Test ($p < 0.05$).

Anthracnose disease symptoms on the papaya fruit began to appear on the second day after inoculation. Initial symptoms manifested as small, circular lesions at the *Colletotrichum* sp. inoculation sites. These lesions progressively expanded as the fruit ripened (Fig. 7).

As detailed in Table 2, the negative control (0% PA) exhibited the highest average AUDPC value (570.53), serving as the baseline for disease progression. Among the PA treatments, the 7% PA treatment exhibited the lowest average AUDPC value (297.98), which was significantly lower than those of the other PA treatments, and resulted in the highest disease inhibition (47.77%). In contrast, the positive control (mancozeb at 1.5 g/L) recorded the lowest average AUDPC value (188.21) and the highest disease inhibition rate (67.01%). Overall, statistical analysis revealed that PA treatment significantly affected anthracnose disease development *in vivo* ($p < 0.05$).

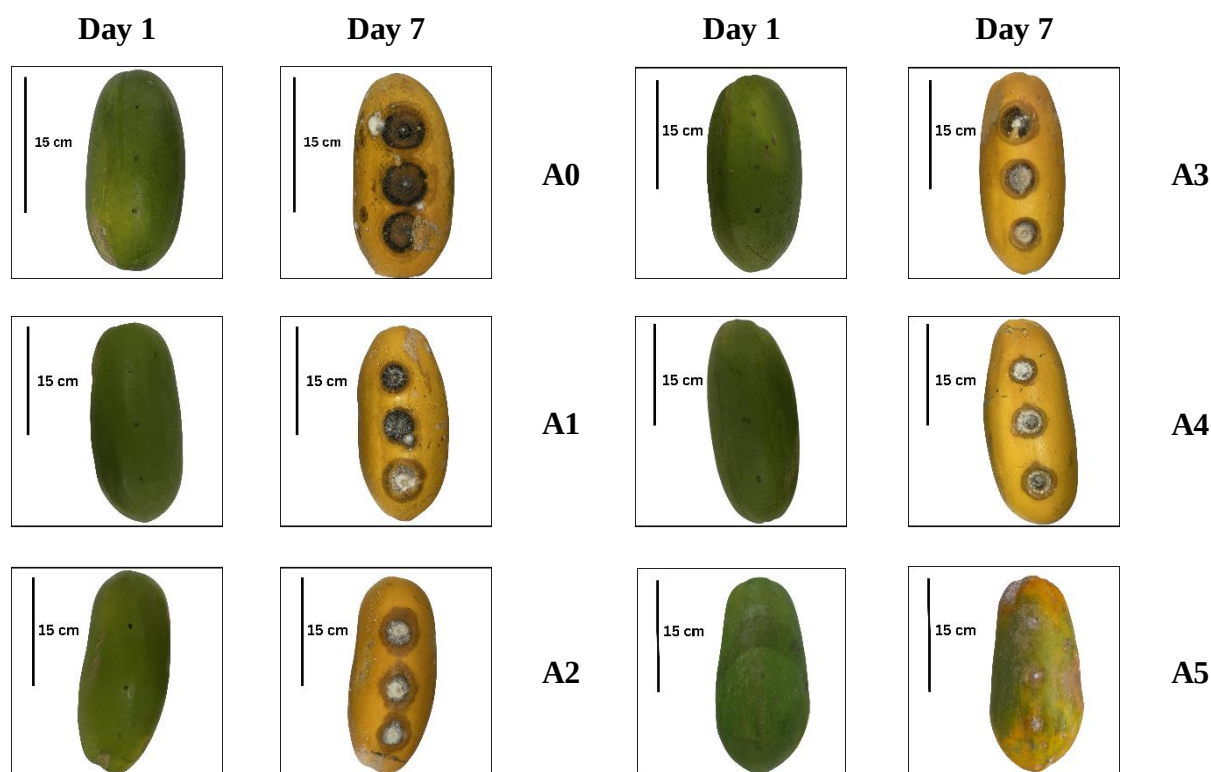


Fig 7. Development of anthracnose lesion area on papaya fruit treated with mahogany sawdust-derived pyroligneous acid (PA) on Day 1 and Day 7 of observation. Treatments consist of A0: 0% PA (negative control); A1: 1% PA; A2: 3% PA; A3: 5% PA; A4: 7% PA; and A5: fungicide mancozeb at 1.5 g/L (positive control).

Table 2. Area under disease progress curve (AUDPC) and disease inhibition of anthracnose on papaya fruit

Treatment	AUDPC	Inhibition (%)
Pyroligneous Acid 0%	570.53a $\pm$ 3.96	0.00a $\pm$ 0.00
Pyroligneous Acid 1%	537.95b $\pm$ 5.84	5.71b $\pm$ 1.02
Pyroligneous Acid 3%	492.24c $\pm$ 6.71	13.72c $\pm$ 1.77
Pyroligneous Acid 5%	431.64d $\pm$ 11.78	24.34d $\pm$ 2.06
Pyroligneous Acid 7%	297.98e $\pm$ 6.62	47.77e $\pm$ 1.17
Mancozeb 1.5 g/L	188.21f $\pm$ 10.10	67.01f $\pm$ 1.77

Notes: Values are presented as Mean $\pm$ Standard Deviation. Values followed by different letters indicate significant differences according to Duncan's Multiple Range Test ($p < 0.05$).

Higher concentrations of mahogany sawdust-derived PA led to a greater reduction in the development of anthracnose disease on papaya fruit. This dose-dependent suppression is consistent with findings from related studies on wood vinegar applied against postharvest pathogens (Akkuş et al. 2022; Yıldızlı et al. 2024). For instance, El-Fawy et al. (2023) reported that guava wood vinegar significantly reduced disease severity caused by *Colletotrichum coccodes* on potato, demonstrating the capacity of wood-derived pyroligneous acids to suppress *Colletotrichum*-related diseases *in vivo*. Similarly, Wattana et al. (2025) reported concentration-dependent antifungal effects of wood vinegar against various phytopathogenic fungi, further supporting the dose-response relationship observed in the present study.

In the present study, the 7% PA treatment achieved an *in vivo* disease suppression of 47.77% (AUDPC = 297.98), significantly reducing disease development compared with the negative control (AUDPC = 570.53). Although this suppression level was lower than that achieved by mancozeb at 1.5 g/L (67.01%; AUDPC = 188.21), the results indicate the potential of PA as a bio-based alternative for postharvest disease management. The lower efficacy observed *in vivo* compared with the *in vitro* inhibition rate (70.56%) is a commonly reported phenomenon. According to Guo et al. (2022), host tissue characteristics, environmental humidity, and wound-inoculation dynamics may limit the direct antimicrobial activity of PA under *in vivo* conditions. Nevertheless, concerns about intensive fungicide use, including pathogen resistance and chemical residues on harvested produce, highlight the potential of PA as an environmentally friendly alternative while promoting the use of forestry residues.

The antifungal activity of mahogany PA *in vivo* can be attributed to its chemical composition. As shown in Table 3, GC-MS analysis identified acetic acid as the dominant volatile compound, accounting for 77.72% of the total peak area, followed by 2,5-pyrrolidinedione (succinimide) at 11.35%. In addition, physicochemical characterization revealed a highly acidic pH of 2.8. These characteristics create an unfavorable environment for *Colletotrichum* sp., which typically grows optimally at pH 6.0–7.0 (Hailmi et al. 2017). Similar observations were reported by Iacomino et al. (2024), who noted that wood vinegar typically has a low pH and a high organic acid content. Mechanistically, acetic acid disrupts cellular pH homeostasis and the proton motive force, leading to impaired ATP generation and growth retardation (Antunes and Sá-Correia 2024). Furthermore, organic acids inhibit microbial enzymatic activity and reduce cell membrane permeability, thereby impairing nutrient uptake and cellular metabolism and ultimately reducing pathogen viability (Anggrayni et al. 2025). Moreover, 2,5-pyrrolidinedione has been reported to exhibit antimicrobial activity and may further contribute to the overall antifungal effect (Albakhit et al. 2023). Collectively, these findings suggest that mahogany sawdust-derived pyroligneous acid suppresses anthracnose development primarily through organic acid-mediated mechanisms and pH suppression, supporting its potential as an environmentally friendly approach for postharvest disease management.

Table 3. Volatile compounds identified in mahogany sawdust-derived pyroligneous acid by GC-MS analysis

Peak	Compound	Retention Time (min)
1	Acetic acid	1.615
2	2,5-Pyrrolidinedione (Succinimide)	7.618

4. Conclusions

Mahogany sawdust can be successfully converted into pyroligneous acid (PA), an effective natural antifungal agent against *Colletotrichum* sp., the causal pathogen of papaya anthracnose. The application of 7% mahogany sawdust-derived PA consistently inhibited fungal growth in both *in vitro* and *in vivo* experiments. Although the commercial fungicide mancozeb (1.5 g/L) remained the most effective treatment, the 7% PA demonstrated substantial antifungal activity and showed promise as an environmentally friendly alternative to synthetic fungicides for postharvest disease control. These findings support the utilization of mahogany sawdust as a value-added product within a circular forestry framework while highlighting the potential of PA for sustainable

agricultural applications. Further research on formulation refinement, product stability, and field-scale validation is required to facilitate its practical implementation.

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Author Contributions

C.N.: Conceptualization, Methodology, Supervision, Validation, Data Curation, Project Administration, Writing – Original Draft Preparation; A.B.P.: Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft Preparation, Software; S.: Supervision, Validation, Data Curation, Writing – Review and Editing; D.A.N.: Investigation, Visualization, Formal Analysis, Writing – Original Draft Preparation.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation

Not applicable.

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