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Antifungal and phytotoxicity of wood vinegar from biomass waste against *Fusarium oxysporum* f. sp. *cubense* TR4 infecting banana plants

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Abstract
Fusarium oxysporum f. sp. *cubense* Tropical Race 4 (*Foc* TR4) infection poses a serious threat to banana cultivation, leading to significant economic losses due to the lack of effective and sustainable disease management strategies. Wood vinegar, derived from biomass waste, offers a potential eco-friendly alternative to chemical pesticides for disease control. This study evaluated the antifungal activity of wood vinegar from coconut shells and Acacia wood residues at different concentrations (1, 2, 4, 6, 8, and 10% v/v) against *Foc* TR4 in vitro using the poisoned food technique and assessed its phytotoxicity on banana plants. Our results showed that wood vinegar from both sources completely inhibited the growth of *Foc* TR4 in vitro at all concentrations tested. The phytotoxicity effects of the different types of wood vinegar on the banana plants were also evaluated using the same concentrations via foliar spray and soil-drenching techniques. Growth parameters, including plant height, stem diameter and leaf number, were recorded at 24, 31, and 39 days after transplanting (DAT). Treatments with lower concentrations (2% v/v) promoted the growth of the banana plants, while at higher concentrations, severe phytotoxicity symptoms such as leaf necrosis and yellowing were observed. These findings provide valuable insights into the potential use of biomass waste-based wood vinegar for controlling Fusarium wilt in bananas.

Keywords Biopesticides · Phytotoxicity assessment · Plant disease management · Fusarium wilt · Pyroligneous acid

1 Introduction

Infection by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4) in banana plants represents one of the most severe threats to banana cultivation, leading to significant economic losses [1]. *Foc* TR4 can reside in the soil for extended periods and attack the root system of banana plants [2], which drastically reduces productivity and even causes plant death in a short time. According to the FAO report in 2019, *Foc* TR4 has spread to major banana-producing regions

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worldwide, including Southeast Asia, Africa, and Latin America, causing enormous economic losses and posing a significant threat to global food security [3].

Given the devastating impact of this disease, it is crucial to seek effective and environmentally friendly control alternatives. The use of chemical pesticides is not only costly, but also potentially harmful to the environment and can lead to pathogen resistance [4–6]. Therefore, the development and implementation of sustainable alternative control methods are urgently needed. One promising approach is the use of natural substances, such as wood vinegar, which has been widely used in agriculture as a botanical pesticide due to its reputation as being more environmentally friendly compared to synthetic chemical pesticides [7, 8].

Wood vinegar, also known as pyroligneous acid or wood acid, is a product obtained from the pyrolysis or torrefaction of organic materials, such as wood and coconut shells, which generates vapor that is then condensed into liquid [7, 9]. The production process of wood vinegar involves the incomplete combustion of organic materials at high temperatures with restricted oxygen, resulting in bioactive compounds with antibacterial and antifungal properties, such as phenols, carboxylic acids, and alcohols [10–12].

Various studies have shown that wood vinegar can inhibit the growth of several plant pathogens and enhance plant resistance to diseases [13]. Wood vinegar, derived from coconut shells, pine wood, and oil palm fronds, effectively inhibited the growth of the pathogenic bacterium *Ralstonia syzygii* subsp. *celebesensis* in banana plants [14, 15]. A study by El-Fawy et al. [16] revealed that wood vinegar derived from guava wood can inhibit the growth of pathogenic fungi in potatoes. Meanwhile, wood vinegar from cocoa pod shells has also shown potential against fungal pathogens like *Candida albicans* and *Aspergillus niger*, where higher concentrations yielded stronger antifungal effects [17]. Recently, a study by Kara et al. [18] showed that wood vinegar from apricot seeds, hazelnut shells, and kermes oak wood could reduce spore production and pathogenic activity of *F. proliferatum* in onion plants. However, there remains a gap in research regarding the effectiveness of wood vinegar against the banana pathogen, *Foc* TR4.

While wood vinegar has demonstrated antifungal potential against various plant pathogens, its application in plant disease management requires careful evaluation of its possible phytotoxic effects. Depending on the types of wood used and the conditions applied during its production, the wood vinegar may differ in its chemical composition and toxicity. Oramahi and Yoshimura [19] showed that wood vinegar derived from *Vitex pubescens* inhibited fungal growth, but required careful dosage management to avoid plant toxicity. Meanwhile, previous studies have indicated that wood vinegar can exhibit toxic effects on banana plants, such as reduced growth when applied at high concentrations [14, 15]. Therefore, it is crucial to evaluate the phytotoxicity of wood vinegar before applying it as a disease control measure in banana plants.

Given the increasing need for environmentally friendly alternatives to control *Foc* TR4, wood vinegar is a potential candidate due to its reported antifungal properties. However, research on its effectiveness against *Foc* TR4 is currently lacking, and its impact on banana plant growth, especially with different types of wood vinegar, remains uncertain. Therefore, this study aims to evaluate the effectiveness of wood vinegar derived from coconut shells and acacia wood in inhibiting the growth of *Foc* TR4 in vitro at different concentrations and to assess their phytotoxicity effects on banana plants in a greenhouse experiment. The findings are anticipated to provide new insights into the potential of wood vinegar as an alternative for controlling *Foc* TR4, which supports environmental conservation efforts and sustainability in banana cultivation.

2 Materials and methods

2.1 Plant and fungal materials

Fusarium oxysporum f. sp. *cubense* Tropical Race 4 culture was obtained from the Plant Molecular Biology Laboratory, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia. The tissue culture plantlets of the Cavendish MW 1 variety with 4–5 leaves and a height of 27–31 cm was supplied by PT. Hijau Surya Biotechnology, Asahan, Indonesia.

2.2 Preparation of wood vinegar

Coconut shells were obtained from a local coconut milk producer in Pekanbaru, Indonesia, while acacia wood was sourced from wood sorting residues at a pulp and paper mill in Pelalawan, Indonesia. The coconut shells and acacia

wood were first chopped into pieces (~ 1 cm in length) using a mechanical shredder (Mahkota MWC 500, Indonesia) and dried naturally for 48 h. Then, the chopped coconut shells and acacia wood underwent a torrefaction process where they were heated at 210 °C for 2 h, following previous work [20]. The resulting wood vinegar (coconut shells vinegar—CV and acacia wood vinegar—AV) was filtered and stored in a refrigerator to prevent evaporation. The wood vinegar was then diluted using distilled water in various concentrations (1, 2, 4, 6, 8, and 10% v/v) for future experiments.

2.3 Gas chromatography–mass spectrometry analysis of wood vinegar

To determine the chemical composition of the wood vinegar, an analysis was conducted using Gas Chromatography–Mass Spectrometry (GC–MS, Shimadzu QP2010S). The GC was equipped with a capillary column (30 m × 0.25 mm × 0.25 µm film thickness) with helium as the carrier gas at a flow rate of 1.0 mL/min. The injector temperature was set at 250 °C, and the oven temperature was programmed to start at 40 °C and then increased at a rate of 5 °C per min to a final temperature of 280 °C, where it was held for 5 min. Mass spectra were obtained in electron ionization (EI) mode with an ionization energy of 70 eV. The scan range was set from m/z 28 to 600, and the total run time was 65 min. Identification of compounds was carried out by comparing the mass spectra with those stored in the National Institute of Standards and Technology (NIST) library.

2.4 In vitro antifungal assay

The in vitro antifungal assay was performed using the poisoned food technique, according to Paramita et al. [21]. *Foc* TR4 was first cultured on potato dextrose agar (PDA) at 27 °C for 5 days. Then, the undiluted liquid vinegar was filter-sterilized using a filter membrane of 0.22 µm and mixed with sterile PDA medium in a 90 mm petri dish to reach the following final concentrations: 1, 2, 4, 6, 8, and 10% (v/v). Benomyl was used as the positive control at 0.15% (w/v), and unamended PDA medium served as the negative control. Once the agar solidified, 5 mm of actively growing mycelial plug of *Foc* TR4 was centrally placed on each plate medium (three replicates per concentration). Final observation was made after 7 days of inoculation or after Foc fully colonize the negative control plate.

2.5 Phytotoxicity test

The phytotoxicity test was conducted to assess the impact of wood vinegar on the growth of banana seedlings. The banana seedlings were planted in a cocopeat and soil mixture (1:1 v/v) with a total media weight of 600 g in polybags (size: 15 × 21 cm) and allowed to acclimatize for 10 days in a greenhouse at the Faculty of Agriculture, Universitas Lancang Kuning, Indonesia (0° 34′ 36.5″ N 101° 25′ 30.8″ E). Observations taken 10 days after transplanting (10 DAT) served as the baseline for subsequent evaluations.

Environmental conditions (sunlight intensity, humidity, and temperature) were recorded daily throughout the study. Sunlight intensity was recorded using Smart Sensor AS803 Digital Lux Meter (China) and converted into µmol m^{-2} s^{-1} using a conversion factor of × 1/54, as followed Lestari et al. [22], with an average of 68 ± 18.3 µmol m^{-2} s^{-1}. The average relative humidity (RH) and temperature recorded using HTC-2 Thermohygrometer (China) were 70.7 ± 7.8% and 31.5 ± 1.5 °C, respectively. These data were obtained by taking measurements three times a day at five points in the study area, specifically in the morning (07:00–08:00 GMT + 7), noon (12:00–13:00 GMT + 7), and afternoon (16:00–17:00 GMT + 7), as described in a previous study [22]. Watering was conducted twice a day, in the morning and afternoon, with 150 mL of water for each session. However, during the days when wood vinegar was applied in the afternoon, the scheduled afternoon watering was omitted to avoid excess moisture during the treatment application.

NPK fertilizer (16:16:16) was applied at a rate of 1 g per plant at 10 and 24 days to support optimal plant growth. Wood vinegar was sprayed at 17 and 31 days, with different concentrations as follows: 0% (0), 1% (1), 2% (2), 4% (3), 6% (4), 8% (5), and 10% (6). Each plant received 50 mL of wood vinegar using two application methods: foliar spraying (S1) and soil drenching (S2). Growth parameters, including plant height, stem diameter, and number of leaves were measured weekly starting from 10 DAT over 4 weeks (17 DAT, 24 DAT, 31 DAT, and 39 DAT). Root length (cm) and plant length (cm) were measured on the final observation day, plant length represents the combined measurement of the root length and the plant height. Additionally, observations were made for phytotoxicity symptoms such as chlorosis (leaf yellowing) and necrosis (tissue death marked by leaves or stems turning brown and dry) [15].

2.6 Experimental design and data analysis

The experimental design employed a factorial Completely Randomized Design (CRD) with three factors for the phytotoxicity test: the first factor being the type of wood vinegar (CV and AV), the second factor consisting of five concentration levels (0%, 1%, 2%, 4%, 6%, 8%, and 10%), and the third factor being the application method (foliar spray and soil drenching). Each combination was replicated four times. For the in vitro antifungal assay, five concentrations were tested with three replicates each. Data from both the phytotoxicity and antifungal activity tests were analyzed using analysis of variance (ANOVA) using IBM SPSS Statistics v.26 (IBM Corp., Armonk, NY, USA).

3 Results and discussion

3.1 Chemical composition of wood vinegar

The chemical composition of wood vinegar is significantly influenced by the type of feedstock, pyrolysis temperature, and processing conditions [23]. Based on the GC–MS analysis, the wood vinegar produced from coconut shells and acacia wood in this study exhibited notable differences in its chemical composition (Table 1). The major compounds identified in the wood vinegar included acetic acid, phenol, guaiacol, and cresol, among others. These compounds are known to have antimicrobial properties and contribute to the bioactivity of the wood vinegar.

Acetic acid was identified as the predominant component in the wood vinegar from coconut shells and acacia wood, with a concentration of 42.3% and 44.3%, respectively. Acetic acid is known for its strong antimicrobial properties, which can lower the pH of the microbial environment, making it unfavorable for microbial growth. For example, while acetic acid has been shown to inhibit spoilage bacteria such as *Rahnella aquatilis* [24], its efficacy also extends to plant pathogens like *Foc* TR4, highlighting its broad-spectrum antimicrobial potential.

In addition to acetic acid, other compounds such as 2-propanone (also known as acetone) and phenol were found in substantial amounts in coconut shell vinegar, with concentrations of 8.4% and 8.3%, respectively. 2-Propanone has been shown to exhibit significant antimicrobial properties against *Listeria monocytogenes* and *Escherichia coli*, effectively inhibiting their growth under various conditions [25]. Phenol, on the other hand, displays strong antimicrobial activity through mechanisms such as protein denaturation and microbial cell membrane disruption, as described by Nassarawa et al. [26]. It also affects various physiological and biochemical processes, including nutrient absorption, enzyme activity, and interactions with soil microorganisms [27]. The presence of phenol in wood vinegar can impact the physical and chemical properties of soils, as well as the physiological characteristics of plants.

Other significant components in the wood vinegar from coconut shells and acacia wood include methanol, furfural, and cresol. Methanol has been shown to inhibit the growth of bacteria and fungi, while furfural is noted for its broad antimicrobial activity, especially against gram-positive bacteria. Cresol, although present in lower concentrations, also exhibits considerable potential in pathogen control due to its proven antibacterial and antifungal activities in various studies [28]. Compounds such as guaiacol (2.33%) and phenol (2.19%) are also present in the wood vinegar from acacia wood. While their concentrations are lower compared to those found in CV, these compounds play a significant role in influencing plant growth and soil health.

Table 1 Major chemical compounds detected in CV and AV

Wood vinegar	No. of identified compounds	Major compounds* identified
Coconut shell (CV)	33	Acetic acid 42.3%, 2-propanone 8.4%, phenol 8.3%, methanol 8%, furfural 5.2%, propanoic acid 3.1%, cresol 2.7%, guaiacol 2.6%
Acacia wood (AV)	29	Acetic acid 44.3%, methanol 13.3%, 2-propanone 6.6%, furfural 6.1%, acetaldehyde 3.8%, propanoic acid 2.6%, guaiacol 2.3%, phenol 2.2%

*Compounds showing peak areas greater than 2.0% relative to the total peak area in gas chromatography (GC). The percentage values of compounds are relative to the total wood vinegar

The chromatograms of wood vinegar from coconut shells and acacia wood, presented in Fig. 1, provide a visual representation of their differing chemical compositions. This variation is crucial for the study, as it suggests that the wood vinegars from these sources may have different potentials for antimicrobial activities and plant growth. The presence of acetic acid, phenol, and other compounds in high concentrations suggests that wood vinegar has antimicrobial properties, which aligns with previous studies on its pesticidal potential. However, certain compounds, such as 2-propanone, have been linked to phytotoxic effects in some plants, including *Arabidopsis* [29]. Thus, further investigation is required to determine the specific phytotoxic thresholds for banana plants.

3.2 The inhibitory effects of wood vinegar on the mycelial growth of Foc TR4

The antifungal properties of wood vinegar were evaluated by assessing its impact on the mycelial growth of *Foc* TR4 in vitro. Wood vinegar, derived from acacia wood chips and coconut shells, was tested at various concentrations (1%, 2%, 4%, 6%, 8%, and 10% v/v). The experimental setup included treatment plates with these wood vinegar concentrations, a positive control plate treated with benomyl (a known antifungal agent), and a negative control plate treated with distilled water. The results demonstrated that wood vinegar effectively inhibited the growth of *Foc TR4* across all tested concentrations. Specifically, no mycelial growth of *Foc TR4* was observed in the treatment plates for any concentration of wood vinegar (Fig. 2). This indicates that the wood vinegar, regardless of its concentration, was highly effective in preventing fungal proliferation.

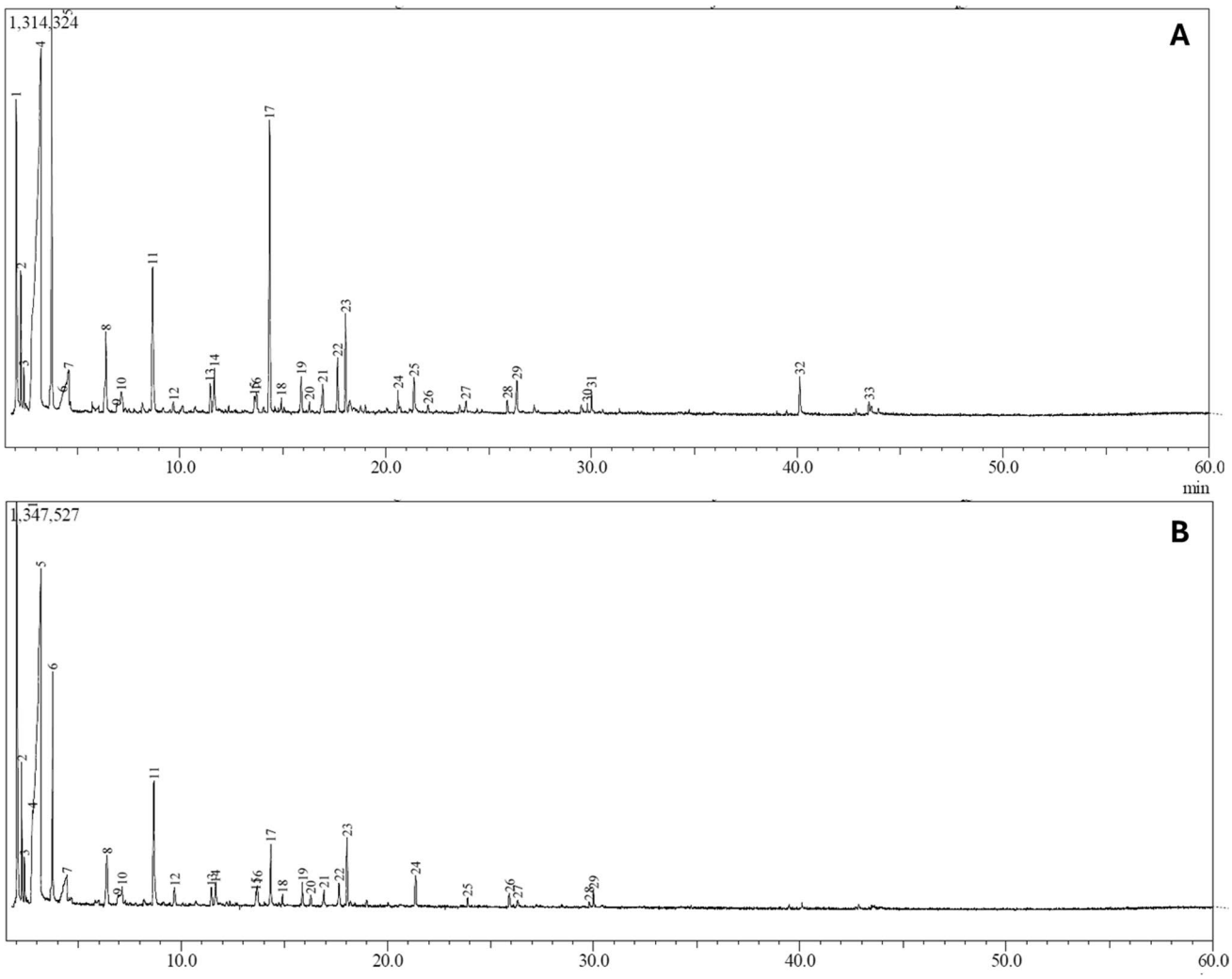

Fig. 1 Chromatograms of wood vinegar from **A** coconut shell and **B** Acacia wood

In contrast, the negative control plate, which lacked any antifungal treatment, showed significant colonization by *Foc* TR4 after 7 days of incubation, as evidenced by the extensive spread of mycelia across the entire plate. This confirms that the fungus was able to grow and spread in the absence of inhibitory substances. The positive control, treated with benomyl, showed no fungal growth, corroborating the effectiveness of the antifungal treatment and validating the experimental setup. The observed complete inhibition of mycelial growth in the treatment plates can be attributed to the bioactive compounds present in the wood vinegar. However, the concentrations tested in this study might be relatively high, and further investigation using a lower range of concentration is warranted to determine the minimum inhibitory concentration (MIC) required to suppress the growth of *Foc* TR4 effectively.

Studies have shown that wood vinegar contains various phenolic compounds, acetic acid, and other organic acids, which possess antifungal properties [25, 28]. These compounds can disrupt fungal cell membranes, inhibit enzyme activity, and interfere with metabolic processes crucial for fungal growth. For instance, acetic acid, a major component of wood vinegar, has been reported to exhibit potent antifungal activity by causing structural damage to fungal cells [30]. Similarly, phenolic compounds have been shown to possess broad-spectrum antifungal activity, which can effectively inhibit the growth of various fungal pathogens [26, 27]. The in vitro findings highlight the potential of wood vinegar as an effective natural antifungal agent against *Foc TR4*.

3.3 Phytotoxicity of wood vinegar on banana plants

Phytotoxicity refers to a condition where a substance or chemical compound causes toxic effects on plants, leading to disrupted growth and development [31]. In this experiment, the phytotoxicity of wood vinegar pertains to the potential negative impact that exposure to wood vinegar may have on banana plants in the greenhouse. At the initial observation, 17 DAT (within a few hours after the first wood vinegar application), no changes were observed in plant growth parameters, including plant height, stem diameter, or leaf count, for either source of wood vinegar. This indicates that wood vinegar does not immediately impact the growth of banana plants.

However, by 24 DAT, and continuing through 31 and 39 DAT, significant changes in growth parameters began to emerge. For instance, plants treated with 2% wood vinegar from coconut shells showed an increase in height by 10.5 ± 1.5 cm at 24 DAT and 20.5 ± 2.5 cm by 39 DAT. In contrast, higher concentrations, such as 10% from coconut shells, led to significantly slower growth, with plant height reaching only 1.5 ± 0.2 cm at 24 DAT and 12.5 ± 1.0 cm at 39 DAT, indicating a concentration-dependent inhibition of growth. This aligns with previous studies suggesting that the effects of wood vinegar on plants tend to be gradual and require time to manifest [32].

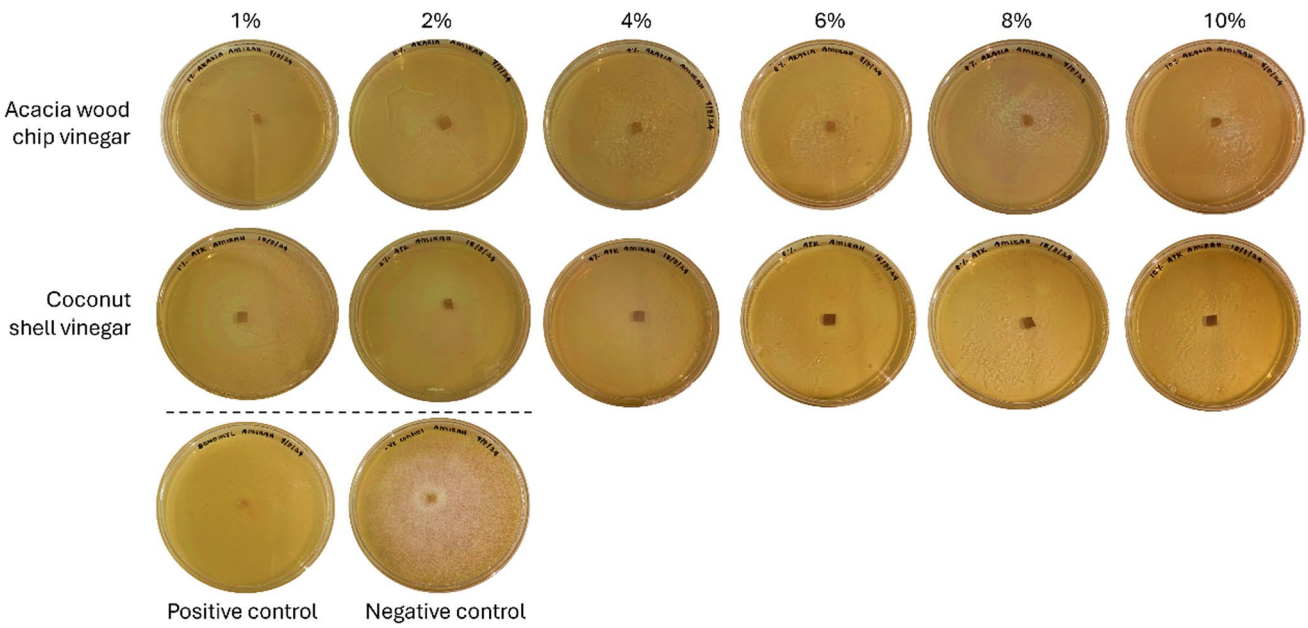

Fig. 2 *Foc* TR4 inhibition by acacia vinegar and coconut vinegar in different concentrations (1–10% v/v) after seven days of incubation

Foliar application of CV at a 2% (CV2S1) concentration resulted in the greatest increase in plant height, particularly at 24 DAT (10.50 ± 1.5 cm), 31 DAT (13.50 ± 0.5 cm), and 39 DAT (20.50 ± 2.5 cm) (Fig. 3A), indicating that lower concentrations of CV bioactive components are more effective in promoting vegetative growth. However, beyond 2%, the growth-promoting effect diminished, suggesting potential phytotoxicity at higher concentrations. When applied through soil drenching, CV showed moderate height increases, with the most significant growth at 1% concentration at 24 DAT (8.50 ± 0.5 cm) and 31 DAT (11.00 ± 1.0 cm), and a notable increase at 2% concentration at 39 DAT (18.00 ± 0.0 cm). In contrast, foliar application of AV produced more variable results, with a 6% concentration showing consistent height increases at 24 DAT (2.00 ± 0.0 cm), 31 DAT (2.50 ± 0.5 cm), and 39 DAT (13.00 ± 1.0 cm) (Fig. 3B). Soil drenching with acacia wood vinegar did not demonstrate a consistent growth pattern, with variations observed across different concentrations and time points.

The most significant increase in stem diameter was observed with a 2% concentration of CV, reaching 0.40 ± 0.1 cm at 24 DAT, 0.50 ± 0.1 cm at 31 DAT, and 1.00 ± 0.2 cm at 39 DAT (Fig. 3). This indicates that lower concentrations of CV are more effective in promoting stem growth, while concentrations above 2% reduce efficacy and may cause phytotoxic effects. Similarly, leaf spraying with CV showed optimal growth at a 2% concentration. In contrast, soil drenching with CV was most effective at a 1% concentration, with diminishing returns at higher levels. At 10% concentration, growth effects were minimal, suggesting that excessive CV application may reduce benefits or cause adverse outcomes. The application of AV exhibited variable results. For foliar spraying, a 4% concentration (AV4S1) consistently increased stem diameter to 0.20 ± 0.0 cm at 24 DAT, 0.40 ± 0.1 cm at 31 DAT, and 0.75 ± 0.1 cm at 39 DAT. Other concentrations were less consistent, suggesting responses may depend on environmental factors or plant physiology. Soil drenching with AV also revealed mixed growth patterns, with the highest increase at a 2% concentration (AV2S2) (0.20 ± 0.0 cm at 24 DAT, 0.35 ± 0.1 cm at 31 DAT, and 0.95 ± 0.0 cm at 39 DAT). Higher concentrations produced inconsistent or minimal growth effects.

Figure 3 depicts the average change in the number of leaves of banana plants. At 24 DAT, foliar application of coconut shell wood vinegar at 2% concentration (CV2S1) resulted in the greatest increase in leaf number, while higher concentrations (6%, 8%, and 10%) showed no increase, suggesting potential negative effects. Soil drenching with CV consistently increased leaf number up to 2% (CV2S2), but higher concentrations did not enhance growth further. By 31 DAT, foliar spraying with AV achieved consistent leaf growth at 1% and 2%, while CV was effective only at lower concentrations. Soil drenching with both vinegars at 2% concentration (CV2S2 and AV2S2) promoted the most leaf growth, with higher concentrations having reduced or no effect. At 39 DAT, foliar application of acacia wood vinegar at 1% attained the

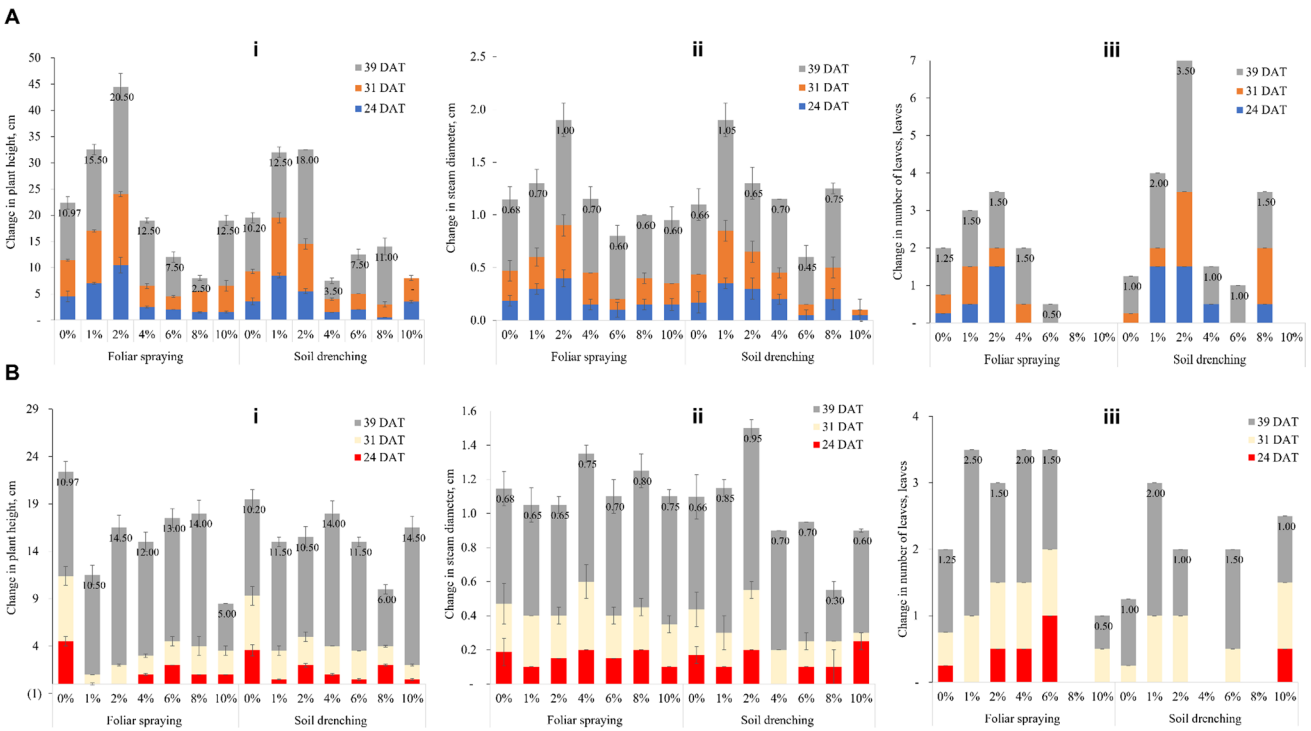

Fig. 3 Changes in (i) plant height, (ii) stem diameter, and (iii) leaf number at 24, 31, and 39 DAT for banana seedlings treated with wood vinegar from **A** coconut shells and **B** acacia wood

highest increase in leaf number, while other concentrations maintained or reduced leaf numbers. Soil drenching with coconut shell vinegar at 2% again resulted in the most significant leaf increase, with higher concentrations showing no improvement or even a decrease in leaf number.

Wood vinegar contains various organic compounds, including phenols, organic acids, and other substances that can affect plant growth. At appropriate concentrations, these compounds can act as growth stimulants, enhancing various physiological aspects of plants, such as photosynthesis, nutrient uptake, and cell division. However, at excessively high concentrations, these compounds can become phytotoxic, inhibiting growth processes, or even causing plant death [33, 34], as observed in treatments CV6S2 and AV5S2, in Table 2. Although AV5S2 exhibited signs of toxicity, including no new leaf growth since the beginning of the treatment and leaf death, there was still an increase in plant height and stem diameter, though not significantly different from the control.

Meanwhile, observations on root length and overall plant length indicated that the CV2S2 treatment yielded the best results, as shown in Figs. 4 and 5, even though the increases in height and stem diameter were less pronounced compared to CV2S1. The significant root length contributes to the stability and nutrient absorption capacity of the plant, which, in turn, enhances overall growth in the long term [35]. This study suggests that a 2% concentration of wood vinegar is optimal for promoting overall growth in banana plants without causing phytotoxicity.

The analysis of changes in plant height, stem diameter, and leaf number indicates that neither the type of wood vinegar (CV and AV), the concentration levels, nor the method of application (foliar spraying or soil drenching) had a statistically significant effect on the growth of banana plants. However, trend analysis reveals that both the type of wood vinegar and its application method can influence banana plant growth over time, as shown in Tables S1–S3 and Figures S1–S3. Lower concentrations (0%-4%) generally favor growth, while higher concentrations may reduce or inhibit growth. Additionally, ANOVA results indicate that neither the type of wood vinegar nor the application method significantly affects root length or total plant length under the tested conditions and concentrations.

The reduction in plant height observed at higher wood vinegar concentrations can be attributed to physiological disruptions caused by phytotoxic compounds, particularly the increased concentration of acetic acid. A study by Iacomino et al. [34] demonstrated that high concentrations of acetic acid in wood vinegar can impair growth processes and fruit production in strawberries. This effect aligns with our findings, where plants exposed to higher concentrations appeared less capable of utilizing resources for optimal growth. The decrease in leaf number suggests interference in leaf formation and vegetative development, likely linked to phytotoxic effects on chlorophyll synthesis and plant hormone activity. This is further supported by Khatoon et al. [36], who reported that high concentrations of wood vinegar can disrupt the production of plant growth hormones, leading to stunted plant growth.

The presence of other volatile organic compounds (VOCs), such as 2-propanone and methanol, may also contribute to the observed growth suppression. Ramírez et al. [37] found that exposure to elevated levels of methanol can lead to oxidative stress, affecting cell division and elongation processes in plants. Additionally, phenolic compounds, which are abundant in wood vinegar, have been reported to inhibit enzyme activity essential for plant metabolism, further limiting growth potential [38]. The variation in growth responses across different application methods (soil drenching *vs*. foliar application) suggests that the mode of exposure plays a role in determining phytotoxicity. While low concentrations ($\leq 2\%$) of wood vinegar showed minimal differences between application methods, higher concentrations ($\geq 6\%$) resulted in more severe phytotoxic effects, particularly in foliar application. This suggests that leaf tissues might be more sensitive to direct contact with wood vinegar components, whereas soil application allows for dilution and gradual absorption, reducing toxicity.

To better understand which compound has the most pronounced effect, further tests on the main components of wood vinegar—such as acetic acid, phenol, and methanol—are needed to isolate their individual impacts on plant development. Future studies should also investigate potential detoxification mechanisms in plants that might mitigate the negative effects of high wood vinegar concentrations, including the role of antioxidant enzymes and metabolic adaptations. These insights will be essential in refining wood vinegar formulations to balance antifungal efficacy with minimal phytotoxicity, ensuring its safe and effective use in agricultural applications. While the current study provides valuable insights into the antifungal efficacy and phytotoxic effects of wood vinegar, the findings would benefit from more replications under varying environmental conditions to assess potential seasonal or environmental influences on the results.

Table 2 Effects of different concentrations of coconut shell (CV) and acacia wood (AV) on banana plants at 31 days after treatment (DAT)

Treatment	Growth of Plants at 31 DAT	Treatment	Growth of Plants at 31 DAT
CV0S1		AV0S1	
CV2S1		AV2S1	
CV2S2		AV2S2	
CV5S2		AV5S2	
CV6S2		AV6S2	

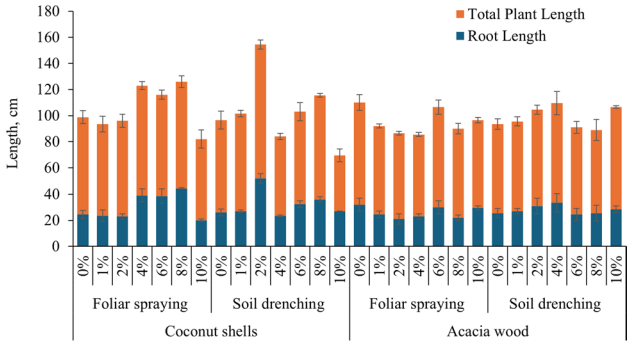

Fig. 4 Effect of different concentrations of coconut shell and acacia wood vinegar on root and total plant length of banana at 39 days after treatment (DAT)

Fig. 5 Comparison of root development in banana plants under coconut vinegar CV2S1 (left) and CV2S2 (right) treatments

4 Conclusion

This study confirms the potential of wood vinegar from coconut shells and *Acacia* wood residues as an effective antifungal agent against *Foc* TR4. In vitro, all tested concentrations (1%–10%) inhibited fungal growth, demonstrating strong antifungal activity. However, phytotoxicity assessments revealed that 2% was beneficial for plant growth, while higher concentrations (≥ 4%) caused chlorosis, leaf necrosis, and stunted growth, with foliar application having stronger negative effects than soil drenching. Despite the lack of repetition in the experimental design, the findings from this study are still valuable to guide future research, which includes in vivo field trials to optimize application rates and evaluate long-term effects. Integrating wood vinegar into banana disease management could provide an eco-friendly alternative to synthetic fungicides.

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Author contributions D.A conducted the experimental work, collected and analyzed the data, and drafted the manuscript. I.P. supervised the study, contributed to the experimental design, provided guidance throughout the research process, and critically revised the manuscript as a corresponding author. N.B.S provided expertise in the in vitro assays, contributed to the analysis of results, and critically revised the manuscript, also serving as a corresponding author. F.N contributed to the research design, particularly in phytopathology, and reviewed the manuscript. N.A.B supervised laboratory work at Universiti Putra Malaysia and contributed to the discussion of the results. A.M. assisted in data collection

and provided laboratory support, while N.A.S.B.R. assisted in the execution of the in vitro assays. R.B. contributed insights on the formulation for wood vinegar and reviewed the final manuscript.

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Data availability The data supporting the findings of this study are available from the corresponding author upon reasonable request. All relevant data are included in the manuscript, and additional datasets generated and analyzed during the current study are available upon request.

Declarations

Ethics approval and consent to participate The authors confirm that all methods were carried out in accordance with relevant guidelines and regulations of Universitas Lancang Kuning, Indonesia. The banana plants used in this study were obtained from local nurseries and cultivated under controlled conditions in compliance with national and international plant research guidelines. No additional permits were required for the cultivation and use of these plants. The experimental protocols were reviewed and approved by the Research Ethics Committee of Universitas Lancang Kuning.

Competing interests The authors declare no competing interests.

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