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RESEARCH ARTICLE
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Effect of Liquid Smoke from the Temperature Stratification Technique as an Antipathogenic Agent

Santiyo Wibowo [1,*], Wasrin Syafii [2], Gustan Pari [1], Elis Nina Herliyana [3], Lisna Efiyanti [1], Andianto [1], Gusmailina [1], Dian Anggraini Indrawan [1], Saptadi Darmawan [1], Rozza Tri Kwatrina [1], Aswandi [1] and Cut Rizlani Kholibrina [1]

[1]National Research and Innovation Agency, Jl. Raya Bogor KM 46, Bogor 16911, Indonesia
[2]Department of Forest Products, IPB University, Kampus Dramaga, Bogor 16680, Indonesia
[3]Department of Silviculture, IPB University, Kampus Dramaga, Bogor 16680, Indonesia
*Corresponding author: santiyowibowo1973@yahoo.co.id

ABSTRACT

Agriculture and animal husbandry cannot be separated from pest and disease attacks, which can cause losses to farmers. Pathogenic microbes generally cause disease in plants and livestock. Synthetic chemicals can cause poisoning in other living organisms, leading to the development of microbial resistance. Liquid smoke is a renewable natural material derived from biomass pyrolysis that can be used as an antipathogenic agent. The various pyrolysis processes and lignocellulose types, including hardwood, softwood, and nonwood, produce liquid smoke with differing chemical compositions, particularly phenol, due to their distinct lignin compositions. This study aimed to evaluate the effectiveness of liquid smoke phenols derived from the temperature stratification technique (200 and 400°C) and three raw materials of forest-industrial waste against pathogenic microbes: *Xanthomonas oryzae*, *Staphylococcus aureus*, *Fusarium oxysporum,* and Bean Common Mosaic Virus (BCMV). The liquid smoke used for antimicrobial analysis was obtained from *Tectona grandis*, *Pinus merkusii*, and Andong bamboo (*Gigantochloa pseudoarundinaceae* (Steudel) Widjaja). The liquid smoke concentrations used for the antimicrobial analysis were 0.5, 1, 2, 5, 10, 15, 20, and 30%, and for the plant antiviral, it was 1%. The results showed that pine liquid smoke at 400°C and 30% concentration had a higher inhibitory effect on *X. oryzae*. The teak liquid smoke (400°C, 30%) had a higher inhibitory diameter for *S. aureus.* Bamboo liquid smoke (400°C, 30%) had a higher inhibitory diameter for *F. oxysporum* than the other treatments. Teak liquid smoke (400°C, 1%) had a more substantial inhibitory effect on BCMV in *Chenopodium amaranticolor*.

Keywords: Antimicrobial, Liquid smoke, Lignocellulose, Pathogenic microbes, Pyrolysis

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INTRODUCTION

Bactericides, antibiotics, disinfectants, and antiseptics made from synthetic chemicals are not recommended because their long-term effects can harm the environment and humans (carcinogenic, dermatological, and reproductive effects). It can also result in resistance or even give rise to new, more resistant pathogen strains (Mancuso et al., 2021). This has encouraged the continued search for alternative materials that come from nature but are safe for humans, animals, and the environment. One natural ingredient that can potentially control pathogenic agents is liquid smoke. Liquid smoke is extracted using the smoke condensation technique, which results from the pyrolysis of lignocellulosic biomass. Several studies have reported the fairly good effectiveness of liquid smoke in inhibiting microbes (Chukeatirote & Jenjai, 2018; Suresh et al., 2019).

According to Ooi et al. (2022), leaf blight caused by *Xanthomonas oryzae* attacks causes significant yield losses, that is, approximately 70% of crop yield losses. *Fusarium oxysporum* causes significant losses in agricultural products, where plants wilt and die. The damage caused by *F. oxysporum* to chili plants can reach 79% (Ferniah et al., 2018). *Staphylococcus aureus* causes mastitis in the udders of dairy cows in up to 40% of cases, potentially reducing milk production in dairy cows by 2.6-43.1% (Olives et al., 2020).

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Liquid smoke generally originates from a one-stage pyrolysis process from room temperature to 400-500°C, and has been used for various purposes, including antimicrobial, natural pesticide or herbicide, and odor removal. In addition, it is used for plant growth purposes by diluting it to a certain concentration. However, improper dilution of liquid smoke from one-stage pyrolysis causes plant damage. Pyrolysis temperature stratification allows the production of two or more liquid smoke products, for example liquid smoke at 200°C and 400°C temperatures, for different uses. Wibowo et al. (2024) showed that liquid smoke produced by stratifying at the pyrolysis temperature of 200°C resulted in a better growth response in cat's whiskers plants than liquid smoke at 400°C at the same concentration of 0.25%. In addition, each type of lignocellulose has a different chemical content, which affects its liquid smoke content. The redistillation of liquid smoke also influences the levels of phenol, acetic acid, and other chemicals (Theapparat et al., 2018; Cheng et al., 2021).

Reports on the effectiveness of liquid smoke from three types of lignocellulosic raw materials, the pyrolysis temperature stratification technique, and liquid smoke redistillation against the bacteria *X. oryzae, S. aureus, F. oxysporum* (fungi), and *Bean common mosaic virus* (BCMV) have not been widely reported, particularly the concentration level of the liquid smoke used. This study aimed to evaluate liquid smoke from three types of raw material waste, namely teak wood, pine wood, and bamboo, which were obtained by temperature stratification on the inhibitory power of the microbes *X. oryzae, S. aureus, F. oxysporum* and BCMV. This study can help manage lignocellulose waste, especially from Indonesian plants. Not allowing lignocellulosic waste to decompose but utilizing it as liquid smoke will help reduce greenhouse gas emissions and support SDG 13 climate action, which also has economic value.

MATERIALS & METHODS

Materials

Liquid smoke from teak wood (TLS), pine wood (PLS), and bamboo andong (BLS) uses liquid smoke, was prepared as described by Wibowo et al. (2023). Microbial cultures were obtained from the Indonesian Culture Collection (InaCC) BRIN Indonesia; *Xanthomonas oryzae* (InaCC B16), *Fusarium oxysporum* (InaCC F642) and *Staphylococcus aureus* (ATCC 25923) were obtained from the Biotechnology Laboratory Collection, BRIN. *Chenopodium amaranticolor* plant, nutrient broth (Himedia), nutrient agar (Himedia), Mueller-Hinton agar (Himedia), pure sucrose, amoxicillin, nystatin, distilled water, and other chemicals.

Preparation of Liquid Smoke Dilution

Liquid smoke dilution is done by reducing the concentration of liquid smoke from its original concentration (100%) to a more dilute one by adding water. The liquid smoke was diluted from crude liquid smoke to 0.5, 1, 2, 5, 10, 15, 20, and 30% for antipathogenic activity. The determination of acetic acid and phenol value was performed using a modified method from Wibowo et al. (2023).

Evaluation of the Antimicrobial Activity against Bacteria

A total of 1mL of *X. oryzae* and *S. aureus* suspension at a concentration of 10^6 CFU/mL was put in a sterile petri dish, and 20mL of MHA (Mueller–Hinton Agar) medium at a temperature of ±45°C was added. The agar medium was perforated with a diameter of 6mm with a sterile cork borer and filled with 20µL of liquid smoke at concentrations of 0.5, 1, 2, 5, 10, 15, 20, and 30%, negative control using sterile distilled water, and positive control using amoxicillin.

Evaluation of The Antimicrobial Activity against *Fusarium oxysporum*

The petri dish contained 10mL of PDA (Potato Dextrose Agar) medium and 20µL of *F. oxysporum* suspension at a concentration of 10^6 CFU/mL and was allowed to solidify. After the PDA solidified, a diffusion well with a diameter of 6mm was created using a sterile cork borer. Each diffusion well was filled with 20µL of liquid smoke at a concentration according to the treatment; the negative control was sterile distilled water, and the positive control was nystatin. The medium in the petri dishes was incubated at 37°C for 24h.

Antiviral Activity against Bean Common Mosaic Virus (BCMV)

The study of BCMV inoculum propagation followed the procedure described by Damayanti & Panjaitan (2014). The concentration of liquid smoke was 1%, produced at 200 and 400°C. *C. amaranticolor* was used as a test plant, which is an indicator plant used to test the physical properties of viruses. Preparation of inoculant: An amount of 0.1g of long bean leaves infected with BCMV was added to 1mL of pH 7 phosphate buffer solution and crushed.

Procedure of Antiviral Activity

(a) Treatment of spraying liquid smoke after 1 day of the test plants being inoculated with the BCMV virus. Six leaves were prepared per test plant as a replication, sprinkled with carborundum, and then gently rubbed to injure them. Furthermore, the entire leaf surface was rubbed with an inoculant preparation. One day after inoculation, the plants were sprayed with a liquid smoke solution according to the treatment (repeated three days later). (b) Liquid smoke spraying treatment before BCMV inoculation. Six leaves per plant were prepared and sprayed with liquid smoke according to the treatment. After 1 day of spraying, the leaves were sprinkled with carborundum at treatment point a, and then inoculated with the BCMV virus preparation. (c) Sick control plants were not treated with liquid smoke but only inoculated with BCMV. (d) Healthy control plants that is test plants that were not administered any treatment.

Observation of Liquid Smoke Inhibition against BCMV

The observation variables for liquid smoke selection on *C. amaranticolor* are as follows:

(a) Number of Local Necrotic Lesions (LNL) that appear after treatment

(b) Relative Inhibition Levels (RIL) is calculated using the following equation:

$$RIL = \frac{K-P}{K} \times 100\% \quad (1)$$

Where: RIL = Relative Inhibition Level.
K = Number of LNL in the control

Statistical Analysis

Data were stated as mean ± standard deviation and analyzed with ANOVA followed by DMRT using SPSS 22 software.

RESULTS & DISCUSSION

Phenol Compound

The results of the Pyr-GCMS analysis (Table 1) show that TLS liquid smoke at 200°C detected three phenols, and eight phenols were detected at 400°C. At pine liquid smoke (PLS) 200℃, four types of phenol were detected, and at PLS 400℃, seven types of phenols were detected. Meanwhile, at BLS, four types of phenol were detected at 200°C, and at bamboo liquid smoke (BLS) 400℃, ten types of phenol were detected. All types of phenol have molecular weight between 94.11 and 374.4g/mol.

Phenol and Acetic Acid Value

The analysis results of phenol and acetic acid value showed a decrease in phenol and acetic acid levels in liquid smoke dilution on to 0.5%-30% concentration Fig. 1. In general, the concentration of 0.5% had the lowest and the highest levels at 30%. The lowest phenol content was found in TLS 200 ℃ at a concentration of 0.5% (0.001%) (Fig. 1. A1), and the highest content was found in PLS 400 ℃ at a concentration of 30% (0.45%) (Fig. 1. B1). Meanwhile, the lowest acetic acid content was also found in TLS 200 ℃ at a concentration of 0.5% (0.009%) (Fig. 1. A2) and the highest was found in BLS 400 ℃ at a concentration of 30% (4.18%) (Fig. 1. B2). The high and low content of phenol and acetic acid in liquid smoke can determine the type of liquid smoke application. Liquid smoke with low phenol and acetic acid contents is suitable for plant growth, whereas high phenol and acetate contents are suitable for pesticide, herbicide, rubber coagulant, and odour remover applications (Li et al., 2018a; Wibowo et al., 2023).

Antimicrobial Activity

There was a wide variation in antibacterial activity across the liquid smoke types and concentrations tested. A diameter of the inhibition zone (DIZ) of 6.00mm has no

Table 1: Types of phenol in liquid smoke sample

Temp. ℃	No.	TLS	MF, MW, LT*	PLS	MF, MW, LT*	BLS	MF, MW, LT*
200℃	1	2 methoxy-4 methylphenol	$C_8H_{10}O_2$ 138.16 G	Vanilin (4-hydroxy-3-methoxy-Benzaldehyde)	$C_8H_8O_3$ 155.17 G	3-ethyl-phenol (m-Ethylphenol)	$C_8H_{10}O$ 122.16 H
	2	2 methoxyphenol (Guaiacol)	$C_7H_8O_2$ 124.14 G	2-methoxy-4-propylphenol atau 5-Propyl-Guaiacol	$C_{10}H_{14}O_2$ 166.22 G	Phenol, 4-ethyl-2-methoxy-(CAS) p-Ethylguaiacol	$C_9H_{12}O_2$ 152.19 G
	3	4-Ethyl-2-methoxyphenol	$C_9H_{12}0_2$ 152.19 G	Acetovanillone (Ethanone, 1-(4-hydroxy-3-methoxyphenyl)	$C_9H_{10}O_3$ 166.17 G	2,6-dimethoxyphenol	$C_8H_{10}O_3$ 154.16 S
	4	-	-	2-methoxy-4-(2-propenyl)-phenol (CAS) Eugenol	$C_{10}H_{12}O2$ 164.20 G	1,2,3-Trimethoxybenzene (CAS) Methylsyringol	$C_9H_{12}O_3$ 168.19 S
400℃	1	Guaiacol atau 2-methoxypenol	$C_7H_8O_2$ 124.14 G	Phenol (CAS) Izal	$C_9H_{12}O_2$ 152.19 H	Phenol (CAS) Izal	C_6H_6O 94.11 H
	2	2-Methoxy-4-Methylphenol	$C_8H_{10}O_2$ 138.62 G	4-methoxyphenol (Hydroquinone monomethyl ether)	$C_7H_8O_2$ 124.14 G	2-methoxy-phenol (CAS) Guaiacol	$C_7H_80_2$ 124.14 G
	3	4-Ethyl-2-methoxyphenol	$C_9H_{12}0_2$ 152.19 G	2-methylphenol (CAS) o-Cresol	C_7H_8O 108.14 H	3-ethylpehenol (CAS) m-Ethylphenol	$C_8H_{10}O$ 122.18 H
	4	3-Methoxy-pyrocatechol (catechol)	$C_7H_8O_3$ 140,14 G	2-Methoxy-4-methylphenol	$C_8H_{10}O_2$ 138.62 G	Phenol, 4-ethyl-2-methoxy-(CAS) p-Ethylguaiacol	$C_9H_{12}O_2$ 1 52.19 G
	5	2,6-Dimethoxyphenol (syringol)	$C_8H_{10}O_3$ 154.16 S	4-ethyl-2-methoxyphenol	$C_9H_{12}O_2$ 152.19 G	1,4-Benzenediol, 2-methoxy-	$C_7H_8O_3$ 140.14 G
	6	1,4 benzenediol (Hydroquinone)	$C_6H_6O_2$ 110.11 G	1,4-Benzenediol (Hydroquinone)	$C_6H_6O_2$ 110.11 G	2,6-Dimethoxyphenol	$C_8H_{10}O_3$ 154.16 S
	7	4-Methoxy-3-(methoxymethyl) phenol	$C_9H_{12}O_3$ 168.19 S	(-)-Nortrachelogenin	$C_{20}H_{22}O_7$ 374.4 G	1,3-Benzenediol, 4,4'-thiobis-	$C_{12}H_{10}O_4$ 250.27 S
	8	Benzene, 1,2,3-trimethoxy-5-methyl-	$C_{10}H1_4O_3$ 182.22 S	-		1,2,4-Trimethoxy benzene	$C_9H_{12}O_3$ 168.19 S
	9	-	-	-	-	1,2,3-trimethoxy-5-methyl-benzene	$C_{10}H_{14}O_3$ 182.22 S
	10	-	-	-	-	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-Acetosyringone)	$C_{10}H_{12}O_4$ 196.2 S

*TLS: Teak liquid smoke, PLS: Pine liquid smoke, BLS: Bamboo liquid smoke, MF: Molecular Formula, MW: Molekul Weight, LT: Lignin Type; syringil (S), guaicyl (G), p-hidroxyfenil (H)

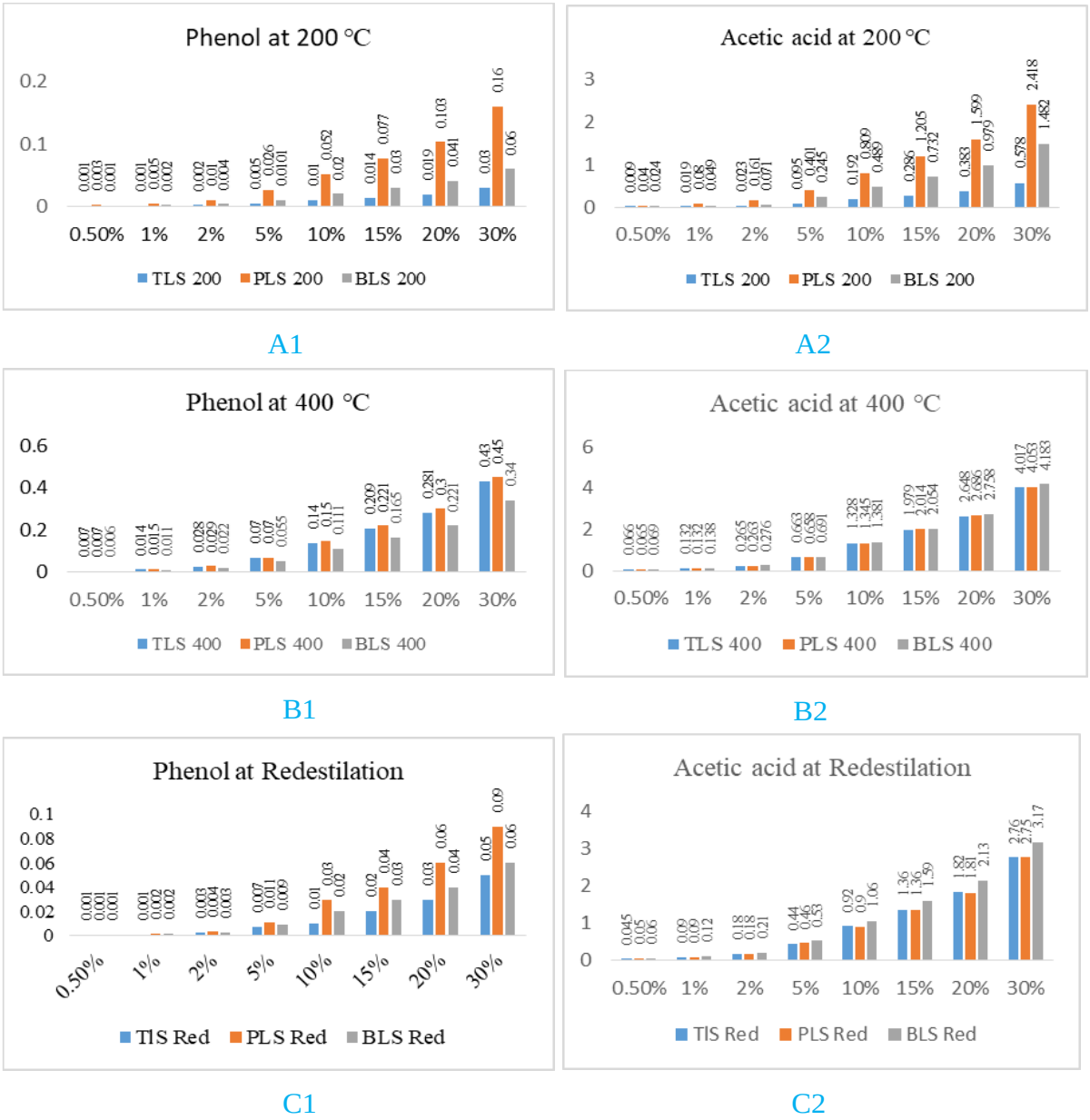

Fig. 1: Phenol and acetic acid value after liquid smoke dilution. A1. Phenol at 200℃, A2. Acetic acid at 200℃, B1. Phenol at 400℃, B2. Acetic acid at 400℃, C1. Phenol at liquid smoke redistillation, C2. Acetic acid at liquid smoke redistillation.

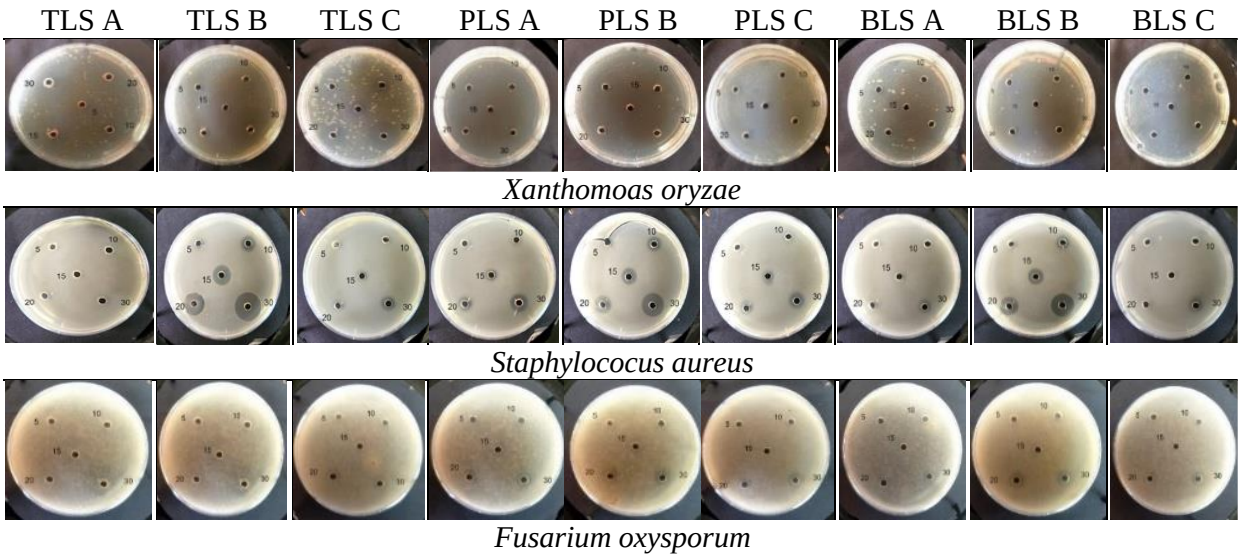

Fig. 2: Antimicrobial activity; TLS: Teak liquid smoke, PLS: Pine liquid smoke, and BLS: Bamboo liquid smoke, A = 200 ℃, B = 400 ℃, C = Redistillation liquid smoke from concentrations of 5, 10, 15, 20, and 30%.

Table 2: The *diameter* of *inhibition zone* of liquid smoke against *X. oryzae, S. aureus*, and *F. oxysporum*

Sample	%	*Xanthomonas orizae*			*Staphilococus aureus*			*Fusarium oxysporum*		
		A	B	C	A	B	C	A	B	C
TLS	0.5	6.00±0.00 a*	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	1	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	2	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	5	6.00±0.00 a	14.64±1.72 def	6.48±0.830 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	10	6.00±0.00 a	19.25±2.01 jkl	11.04±1.15 bc	6.00±0.00 a	9.53±1.7 e	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	15	6.00±0.00 a	23.83±2.28 no	12.42±0.40 cde	6.00±0.00 a	12.35±1.81 g	6.22±0.09 a	6.00±0.00 a	6.73±0.72 bc	6.00±0.00 a
	20	9.44±0.84 b	26.75±2.18 pqr	15.85±2.92 fghi	6.00±0.00 a	15.21±0.66 h	6.39±0.13 ab	6.00±0.00 a	7.38±0.53 de	6.54±0.94 b
	30	17.97±0.69 hijk	29.81±2.25 st	22.21±6.12 mn	6.00±0.00 a	18.19±0.65 i	7.07±0.79 bc	6.00±0.00 a	8.29±0.26 h	7.29±0.45 d
PLS	0.5	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	1	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	2	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	5	12.51±0.93 cde	17.84±2.04 ghijk	12.57±0.09 cde	6.00±0.00 a	6.39±0.28 ab	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	10	17.64±1.10 ghijk	22.59±1.30 mno	15.03±1.10 defg	6.33±0.42 ab	7.51±0.66 cd	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	15	19.85±0.36 klm	25.19±0.26 opq	18.39±0.58 ijk	6.81±0.25 ab	10.41±0.32 f	6.09±0.03 a	6.00±0.00 a	7.03±0.89 cd	6.00±0.00 a
	20	22.56±1.8 mno	28.84±3.18 rs	23.59±2.00 no	7.56±0.24 cd	12.42±0.87 g	6.57±0.01 ab	6.00±0.00 a	8.21±0.49 gh	6.82±0.71 bc
	30	26.65±1.94 pqr	32.05±3.07 t	27.38±1.29 qrs	9.9±0.53 ef	14.74±0.59 h	8.09±0.76 d	7.84±0.2 fg	8.97±0.39 h	7.76±0.11 ef
BLS	0.5	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	1	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	2	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	5	8.86±0.39 ab	12.27±0.90 cd	12.82±0.73 cde	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	10	12.29±1.17 cd	16.66±2.53 fghij	15.87±1.18 fghi	6.00±0.00 a	6.62±0.36 ab	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	15	15.18±1.43 efgh	21.74±3.19 lmn	18.77±0.69 jk	6.00±0.00 a	9.58±0.15 e	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
	20	17.57±1.09 ghijk	24.52±3.76 nop	24.45±1.08 nop	6.00±0.00 a	11.81±0.43 g	6.00±0.00 a	6.00±0.00 a	7.74±0.44 ef	6.00±0.00 a
	30	20.3±0.34 klm	28.22±1.19 rs	27.06±3.64 pqr	6.15±0.19 a	14.95±0.5 h	6.61±0.07 ab	6.00±0.00 a	9.26±0.44 i	8.2±0.73 gh

*The mean diameter of the inhibitory zone (DIZ)±standard deviation (SD) of three replicates (mm), the diameter of the well disk (6mm) is included. A (DIZ) of 6.00mm is considered as no antimicrobial activity. A = 200℃, B = 400℃, C = Redestilation liquid smoke. Negative control (distilled water) = 6.00mm, positive control bacteria Amoxicilin (1000mg/L) on *X. oryzae* = 42.29mm, *S. aureus* = 22.53mm, positive control *F. oxysporum*: Nystatin (10.000mg /L) = 18.99mm

antimicrobial activity, while a DIZ greater than 7mm is considered positive (Aljumaah et al., 2020). The DIZ analysis results are presented in Table 2 and Fig. 2. The results showed that the highest DIZ value for antibacterial activity in *X. oryzae* was found in pine liquid smoke (PLS) at a temperature of 400°C with a concentration of 30%, i.e., 32.05mm (Table 2), while the lowest was found in bamboo liquid smoke (BLS) at 200°C, that is, 8.06mm.

In teak wood liquid smoke (TLS) at a temperature of 200 ℃, bacterial inhibition occurs at concentrations of 20 and 30%, while concentrations of 0.5-15% have no antibacterial activity. DIZ in bacteria occurred at concentrations starting from 5% in PLS and BLS liquid smoke at 200°C (Table 2). In BLS, TLS, and PLS, liquid smoke at 400°C and redistilled liquid smoke (RLS), DIZ in bacteria begins at a concentration of 5%. When the concentration increased, the DIZ value increased.

The antibacterial activity of liquid smoke against *S. aureus* showed the highest DIZ value at a TLS temperature of 400°C and concentration of 30% (18.19mm). At TLS 200°C, no bacterial inhibitory activity was observed, even up to a concentration of 30%. At a PLS temperature of 200°C, bacterial inhibition began at a concentration of 10–30% (Table 2).

Meanwhile, at a BLS of 200°C, DIZ only occurred at a concentration of 30%. When PLS is applied at a temperature of 400°C, it inhibits bacteria starting at a concentration of 5%, whereas PLS redistillation inhibits bacteria starting at a concentration of 15%. At TLS and BLS temperatures of 400°C, inhibition of *S. aureus* started at a concentration of 10%. In contrast, TLS redistillation inhibition began at a concentration of 15%, while BLS redistillation inhibition only started at a concentration of 30%.

The antifungal activity of liquid smoke against *Fusarium oxysporum* showed that BLS at 400°C with a concentration of 30% provided the highest DIZ value, i.e., 9.26mm. In liquid smoke at 200°C, only a PLS of 30% provided inhibition with a DIZ value of 7.84mm, while TLS and BLS did not cause inhibition (Table 2). When liquid smoke was applied at a temperature of 400°C, TLS and PLS provided inhibition starting at a concentration of 15%. In contrast, fungal inhibition begins at a concentration of 20% in liquid smoke from BLS at 400°C. Meanwhile, its re-distillation only provided inhibition at a concentration of 30%. ANOVA analysis showed that the factors of liquid smoke type, concentration, and their interaction were significantly different ($P<0.05$) in the diameter of the inhibition zone for *X. oryzae*, *S. aureus*, and *F. oxysporum*.

The inhibition mechanism of TLS, PLS, and BLS liquid smoke on the growth of bacteria and fungi is due to the presence of certain levels of chemical compounds, especially phenols and acids, which are the dominant chemical compounds found in liquid smoke (Li et al., 2018a). Higher levels of acetic acid and phenol increased microbial inhibition. Liquid smoke produced at 200°C has lower levels of phenol and acetic acid than that produced at 400°C. The liquid smoke redistillation process can also reduce the phenol and acetic acid levels. Additionally, diluting the solution to a specific concentration can reduce the levels of phenol and acetic acid, potentially affecting the ability to inhibit microbes. A high concentration of liquid smoke was in line with the high acetate and phenol content. Conversely, a low concentration of liquid smoke implies lower levels of phenol and acetic acid. This indicates that concentrations of 0.5% to 15% at 200°C and 0.5-2% at 400°C cannot inhibit microbial growth.

The diameter of the inhibition zone produced at a TLS concentration of 30% (18.9mm) against *S. aureus* was not significantly different from that reported by Zhang et al. (2019), who used a liquid smoke concentration of 100% mulberry branches and obtained a DIZ of 19.8mm against *S. Aureus*. As well, the results of research by Araujo et al.

(2018), which used *Eucalyptus urograndis* liquid smoke with concentrations of 100 and 50%, produced a DIZ of 27.0mm and 9.0mm, respectively, the results of this research provide a better DIZ. Different types of raw material sources of liquid smoke produce different antimicrobial responses.

Antimicrobial inhibition zones were classified as follows: >20mm, very strong; 10-20mm, strong; 5–10mm, medium; and <5mm, weak (Ouchari et al., 2019; Addo-Mensah & Holland, 2022). Based on the inhibition zone classification, this research reveals that liquid smoke produced at a temperature of 400°C (non-redistillation) with a concentration of >15% produces an inhibition zone in the very strong classification.

The type of microbe is thought to influence resistance to antimicrobial agents. Fungi are known to be more resistant to low-pH conditions than bacteria (Demain & Martens, 2017). There are two types of bacteria: gram-negative and gram-positive. The main difference between Gram-positive and Gram-negative peptidoglycans lies in the thickness of the layer surrounding the plasma membrane. Gram-negative peptidoglycan is only a few nanometers thick or thin, with one to several layers (<10nm), but has an additional outer membrane with several pores. Meanwhile, Gram-positive peptidoglycan is 30-100nm thick and contains many layers (Mai-Prochnow et al., 2016).

Antibacterial substances inhibit bacterial growth by damaging proteins, nucleic acids, and bacterial cell walls. In Gram-negative bacteria, antibacterial substances enter through porins, namely the outermost layer of gram-negative bacteria, which function as a channel through which several molecules, including antibacterial compounds, can reach the peptidoglycan layer, bind to proteins, and cause the cell to undergo lysis. Meanwhile, in Gram-positive, antibacterial substances will enter the peptidoglycan layer, then bind to the protein and cause lysis (Zahid, 2015).

The presence of organic acids can cause the cytoplasm of pathogenic bacterial cells to become acidic and inhibit transmembrane potential and substrate transport. Lipid-soluble, undissociated organic acid molecules can freely diffuse into the cell and break down to generate protons (H^+) and acidic ions (ROO−). As a result of cytoplasmic acidification caused by H^+ accumulation in the cytosol, the cell actively transports H^+ out of the cytosol to maintain the intracellular pH. This process requires a large amount of adenosine triphosphate (ATP). As H^+ is released, cells exchange and pump potassium ions, which increases intracellular osmotic pressure and destroys bacterial transmembrane proton motility. This causes the cytoplasmic membrane to rupture and leak its contents (Ji et al., 2023). Meanwhile, the presence of phenol denatures proteins in bacterial cells, resulting in the cessation of cell metabolic activity. At low levels, phenol can inactivate bacterial enzyme systems. At high levels, phenol can penetrate cell walls and precipitate proteins (Wang et al., 2020).

According to Jubeh et al. (2020), the cell walls of gram-positive bacteria do not have an outer membrane. However, it has a thick peptidoglycan layer that covers the plasma membrane to protect it from the harsh environment in which it lives. In contrast, gram-negative bacteria have a thin peptidoglycan layer, but an outer membrane consisting of phospholipids, lipopolysaccharides, lipoproteins, and β-barrel protein porins. The outer membrane functions as an additional barrier to block the transport of toxic compounds, such as antibiotics and chemicals (including antibiotics) with a molecular weight of more than 600 Da, which are generally unable to penetrate the envelope of gram-negative bacteria (Gupta & Datta, 2019). Some antibiotics, such as vancomycin and daptomycin, which have a molecular weight of more than 1400g/mol (1.449,3g/mol and 1.619,71g/mol, respectively), cannot pass through the outer membrane of Gram-negative bacteria, which are therefore considered more resistant to antibiotics (Choi & Lee, 2019).

Several studies reveal that Gram-negative bacteria are more resistant than Gram-positive bacteria regarding the structure of the bacterial cell wall (Epand et al., 2016; Gupta & Datta, 2019). However, the results of this study show that the inhibitory activity of *Xanthomonas oryzae* (Gram-negative) bacteria is more significant than *Staphylococcus aureus* (Gram-positive). This condition can be caused by gram-negative bacteria with abundant porin protein on the outer membrane, which also functions as a channel through which several molecules, including antibacterial compounds with a molecular weight of <600 Da (g/mol), can pass through the membrane. Chemical compounds such as acetic acid and several types of phenol in liquid smoke have molecular weights lower than 600 Da ((Epand et al., 2016).

It is known that the molecular weight of some chemical compounds in liquid smoke is lower than 600 Da (g/mol); for example, acetic acid has a molecular weight of 60.05g/mol, while phenol, 94.11g/mol and its derivatives, for instance, 2-methylphenol 108.14g/mol, 2-methoxyphenol 124.14g/mol and 2,4-dimethylphenol 122.16g/mol (Table 1). These conditions allow the wider penetration of phenol and acetic acid compounds into the cell membrane, primarily through porin proteins in gram-negative bacteria. Thus, liquid smoke can inactivate detoxification enzymes in *X. oryzae* bacteria, thereby damaging the peptidoglycan and plasma membrane, and ultimately resulting in bacterial cell lysis.

Meanwhile, *S. aureus* bacteria do not have an outer membrane, although they have porins in the cell walls of gram-positive bacteria. However, these numbers are lower than those of gram-negative bacteria (Ghai & Ghai, 2018)Thicker walls are thought to cause *S. aureus* bacteria to have less resistance compared to *X. oryzae*.

Liquid smoke also contains antibacterial compounds, such as quinone derivatives, namely *hydroquinone* (found in liquid smoke from teak wood and bamboo at a temperature of 400°C), which are cytotoxic to cell channels in bacteria where the integrity of the cell membrane is disturbed (Carcamo-Noriega et al., 2019). Acetovanilone (apocynin) and *nortrachelogenin*, which can disrupt the fluidity of the bacterial cell cytoplasmic membrane, reducing the activity of membrane-related enzymes and ultimately bacterial cell death, were detected (Sammaiah et al., 2014; Lee et al., 2016).

This research shows that liquid smoke can be used as

an antimicrobial agent against *X. oryzae*, which attacks rice plants. A possible application to plants is the short soaking of rice seeds in liquid smoke before sowing, to prevent the development of plant diseases. Liquid smoke can also be applied as an alternative to directly treat wounds on livestock caused by *S. aureus* or sprayed in animal stalls.

Antiviral Activity of Liquid Smoke against BCMV

Viral diseases that attack plants are a major constraint that causes significant losses of agricultural crops worldwide and account for approximately 30% of plant diseases (Manjunatha et al., 2022). One of the most detrimental plant viruses is the Bean Common Mosaic Virus (BCMV), which attacks long bean plants.

In general, all liquid smoke treatments showed lower LNL (Local Necrotic Lesions) results than the diseased control (without liquid smoke treatment but inoculated with BCMV (Fig. 3). The treatment of spraying liquid smoke from TLS at a temperature of 400°C with a concentration of 1% resulted in the lowest LNL compared to the other treatments (Table 3).

The results of the analysis of variance showed that only the type of liquid smoke factor had a very significant effect on the LNL, whereas the watering condition factor and the interaction of the two factors had no significant effect. There was no significant difference between treatment with liquid smoke before BCMV inoculation and administration of liquid smoke after BCMV inoculation. This shows that liquid smoke can inhibit the development of BCMV virus attacks, both when administered at the beginning of plant growth and when plants are attacked by BCMV. The results showed that teak liquid smoke produced a lower LNL number (8.17 points) than pine and bamboo liquid smoke.

Table 3: Effect of liquid smoke treatment on the number of Local Necrotic Lesions (LNL) and relative inhibition level (RIL) in *C. amaranticolor*

Liquid smoke (LS)	After viral inoculation		Before viral inoculation	
	LNL	RIL (%)	LNL	RIL (%)
TLS 200 1%	27.83±7.3 b*	62.51±9.8 c	30.33±14.9 b	59.15±20.04 c
400 1%	8.17±3.2 a	89.00±4.3 d	9.66±5.92 a	86.98±7.97 d
PLS 200 1%	45.8±11.8 d	38.31±15.86 a	53.16±9.86 d	28.39±13.28 a
400 1%	25.5±10.6 b	65.66±14.29 c	24.33±12.30 b	67.23±16.57 c
BLS 200 1%	59.00±13.7 d	20.54±18.47 a	54.54±20.96 d	27.27±28.23 a
400 1%	43.5±8.4 c	41.41±11.32 b	38.17±13.19 c	48.15±17.77 b
Control	76.5±28.76 d	-	-	-

*Numbers followed by the same letter indicate no significant difference based on Duncan's test (P>0.05). LS = Liquid smoke, LNL = Local Necrotic Lesions, RIL = Relative Inhibition Level. TLS = Teak liquid smoke, PLS = Pine liquid smoke, BLS = Bamboo liquid smoke

The liquid smoke treatment that produced the largest LNL number (less inhibition of the virus) was bamboo liquid smoke treatment at 200°C, with an LNL number of 59.00. Liquid smoke at a temperature of 400°C with a concentration of 1% appears to be better at inhibiting the occurrence of LNL in test plants. This is due to the presence of phenol compounds in the teak liquid smoke.

Based on the results of the Py-GCMS analysis, it showed that the compounds 3-methoxy-pyrocatechol and 2,6-dimethoxyphenol were detected in teak liquid smoke at a temperature of 400℃. These phenol compounds were also detected in liquid smoke in the study by (Li et al., 2018b), namely phenol compounds; 3-methoxy-pyrocatechol or also known as 3-methoxy-1,2 benzenediol which has a very high inhibition of encephalocarditis virus (EMCV) reaching 99.9%, followed by the phenol compound 2,6-dimethoxyphenol which has an inhibition of EMCV of 98-99.2%.

In addition, several other types of phenols were also detected, such as phenol, 2-methylphenol, 3-methylphenol, 2-methoxyphenol, and 4 methoxyphenol, capable of inhibiting the EMCV virus by 78-99%. This indicates that the inhibition of TLS at a temperature of 400°C against BCMV attack symptoms is quite high, with a relative inhibition level (RIL) reaching 89% or the lowest LNL of 8.17 (Table 3).

These results are in line with previous research (Damayanti & Panjaitan, 2014) that used plant extracts to inhibit BCMV virus attacks, where leaf extracts of *Bougainvillea spectabilis*, *Mirabilis jalapa*, and *Celosia cristata* provided the most effective inhibition of BCMV infection. This inhibition is possible because leaf extracts contain compounds such as flavonoids, polyphenols, triterpenoids, saponins, and others. The use of liquid smoke has been shown to inhibit the development of LNL in test plants, due to the presence of phenolic compounds, acetic acid, and others. Similarly, Sun et al. (2020) showed that eugenol can stimulate the immune response of tomato plants against Tomato Yellow Leaf Curl Virus (TYLCV). The results of this research strengthen the idea that liquid smoke also contains polyphenols, including eugenol. According to Mani et al. (2020), several polyphenols, such as guercetin, myricetin, and caffeic acid, have antiviral activity against SARS-CoV-2. However, further work is required to test liquid smoke on long bean host plants and ELISA (Enzyme-Linked Immunosorbent Assay) analysis to detect, identify virus types and monitor plant immune responses to virus infection.

Phenolic compounds can inhibit virus multiplication in the virus life cycle, for example by disrupting the interaction between cellular receptors and viruses (attachment function), inhibiting the fusion of viral pseudoparticles to the host membrane (penetration function), inhibiting replication, inhibiting the hydrolysis activity of microsomal triglyceride transfer protein (MTP), and inhibiting secretion from infected cells. In addition, phenolics affect the life cycle of the virus by affecting biochemical processes in host cells (Kowalczyk et al., 2021).

The complexation of metal ions in phenolic compounds increases their antioxidant properties of phenolic compounds (Parcheta et al., 2021). Several metal ions, such as Cu (II), Zn (II), Mn (II), Mg (II), and Fe (III), have been reported to have antiviral properties (Ishida, 2018; Wibrianto et al., 2020). Zn (II) ions have been shown to inhibit nidovirus polymerase activity and the synthesis of RNA components of foot and mouth disease viruses, as well as being strong inhibitors at the fusion stage of several alphaviruses. In addition, zinc and cuprum (Cu) ions can inhibit the growth of foot and mouth disease viruses in cell culture. Manganese (Mn) ions have also been reported to inhibit the reverse transcriptase activity of the HIV virus (Ishida, 2018; Kowalczyk et al., 2021). Based on the results of the analysis, it is known that liquid smoke from teak, pine and bamboo wood contains a number of metal nutrients

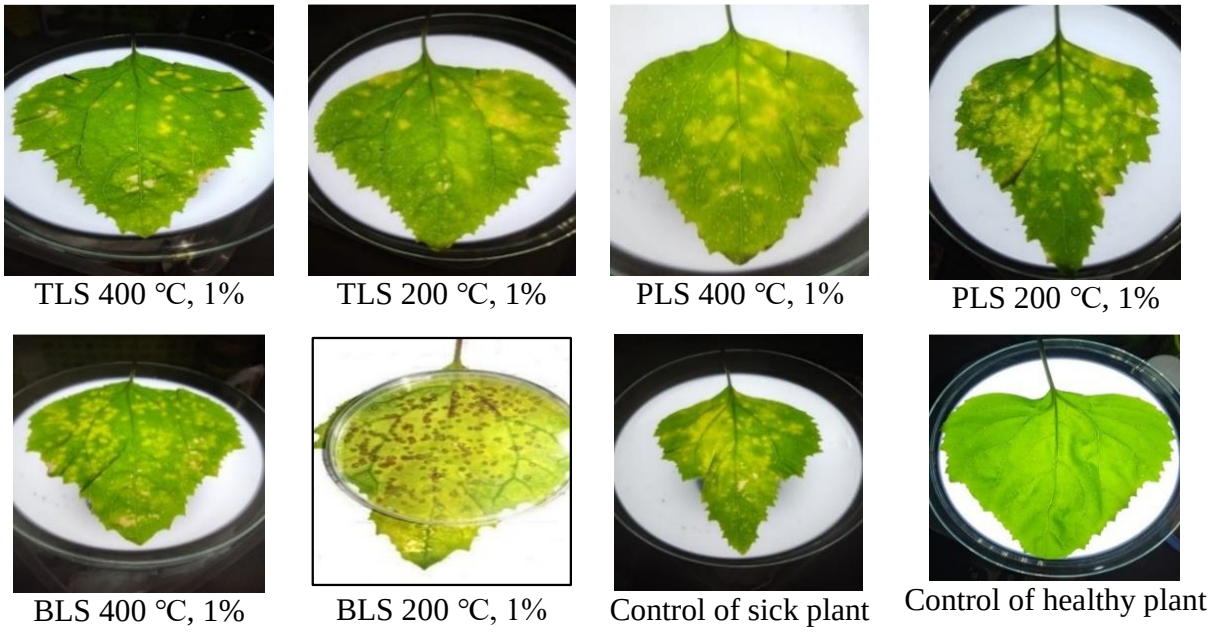

Fig. 3: LNL symptoms of BCMV attack on the leaves of the test plant *C. amaranticolor*.

such as Fe, Mg, Cu, Zn, and Mn. Thus, in addition to providing nutrients for plants, it also serves as an antivirus, containing phenolic compounds, acetic acid, alcohol, and other compounds.

This study demonstrated that lignocellulose can serve as a source of natural chemicals through pyrolysis techniques, particularly for those that have not been previously utilized (waste). This supports the concept of zero waste, where all parts of the plant are utilized without leaving waste/waste from the forestry, plantation, and agricultural industries. From the results of this study, it can be considered that in the future, in the production of liquid smoke, a temperature stratification technique can be used where the liquid smoke is separated at certain temperatures, such as 200°C, 300°C, and 400°C, to obtain several liquid smoke products for different applications.

Conclusion

The liquid smoke produced at 200°C had a lower microbial inhibitory ability than that produced at 400°C and redistillation. The redistillation of liquid smoke reduced its ability to inhibit microbes compared to that of crude liquid smoke. Low concentrations of liquid smoke (0.5-2) could not inhibit microbes. *Fusarium oxysporum* was more resistant to liquid smoke than *X. oryzae* and *S. aureus*. Teak wood liquid smoke produced at 400°C and 1% concentration inhibited BCMV attack by 89%.

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Author's Contribution: SW, WS, and GP conceived and designed the experiments. SW and LE performed the study; AD and RTK confirmed the plant nomenclature and preparation of raw materials; and GS and SD prepared the pyrolysis equipment. ENH supervised and coordinated the experiments. AW and CRK performed statistical analyses of the experimental data. SW and GP drafted the manuscript and prepared the figures. All authors have revised and approved the manuscript.

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