



Research Article

In Vitro Liquid Smoke Potential as Biopesticide on Major Oil Palm Diseases

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ABSTRACT

Liquid smoke is reported as an environmentally friendly pesticide that can effectively manage various crop pathogens. The antimicrobial effect of liquid smoke is believed to be due to its phenol, carbonyl, and organic acid content. The aim of this study was to examine the effect of five commercial liquid smoke at two concentration levels (1% and 2%) on the development of *Ganoderma boninense* and *Curvularia* sp. colonies in vitro. This experiment was carried out using a Completely Randomized Design (CRD) with 11 treatments (Control, A1%, B1%, C1%, D1%, E1%, A2%, B2%, C2%, D2%, and E2%) and three repetitions. Results showed effects of 11 treatments on test pathogen colonies growth. Liquid smoke types B and C showed the best inhibition against *Curvularia* sp. and *G. boninense*. GC-MS analysis revealed that phenol compounds were the dominant compounds, ranging from 24.45% to 85.28%.

Keywords: biopesticides; efficacy; oil palm; pathogens

INTRODUCTION

Basal stem rot and leaf lesion are major disease in palm oil production. Basal stem rot is caused by *Ganoderma* sp.; thus, this disease is commonly mention as *Ganoderma* disease. *Ganoderma* invest basal stem can cause mortality that can reach 68.73% economic loss (Kamu *et al.*, 2021). *Ganoderma* is a major disease due to its increased distribution across Indonesia and its incidence respective to planting period (Soetopo *et al.*, 2022). Meanwhile, leaf lesion is caused by *Curvularia* sp. is commonly found in nurseries. Leaf lesion can affect plant growth and later cause mortality (Priwiratama *et al.*, 2024).

Management of these two disease, especially leaf lesion, heavily rely on synthetic chemicals (Susanto & Prasetyo, 2013). This continuous use of synthetic pesticides will lead to negative effects that later will intrude to palm oil industry (Yigit & Velioglu, 2020; Rani *et al.*, 2021). This conditions encourages multiple stakeholders to discover new pesticides that are environmentally safer and bio-

pesticides are promising options (Samada & Tambunan, 2020). Biopesticides are commonly obtained from processing natural products with high antimicrobial properties (Magierowicz *et al.*, 2020). An example of biopesticide is liquid smoke.

Liquid smoke is obtained from the condensation of wood burning. Burning can be done indirectly in a pyrolysis reactor from material that contain cellulose, hemicellulose, and lignin (Lee *et al.*, 2010). Materials used to make liquid smoke include coconut shell, wood processing waste, and midribs or branches from other plant species (Budijanto *et al.*, 2008; Ginayati *et al.*, 2015; Sari *et al.*, 2018). Liquid smoke components include phenol, alcohol, organic acids, and carbonyl that are known to have antimicrobial properties resulting in researchers attraction to liquid smoke as a preservative, pesticide, and disinfectant (Kim *et al.*, 2008; Lee *et al.*, 2010; Yang *et al.*, 2016).

Liquid smoke effectiveness to manage insect pests and plant disease have also been studied. Kim *et al.* (2008) reported that liquid smoke have insecti-

cidal effects on *Nilaparvata lugens*, *Plutella xylostella* (Sari *et al.*, 2018), and *Spodoptera litura* (Soedijo *et al.*, 2021) while works has also been done to demonstrate liquid smoke's effectiveness against *Pycnoporous sanguinus* (Lee *et al.*, 2010), *Xanthomonas oryzae* (Rusli *et al.*, 2016), and *Phytophthora citrophthora* (Oramahi *et al.*, 2018).

Liquid smoke has been currently commercialize with various formulations and brands. However, standardize testing of liquid smoke does not exist causing variation in management levels. In addition, less work has been done in palm oil causing importances to study its effectiveness against two major diseases, *Ganoderma* sp. and *Curvularia* sp.. This study aims to test the effectiveness of different commercially available liquid smoke products in inhibiting test pathogens.

MATERIALS AND METHODS

Preparation and Characteristic Testing of Liquid Smoke

Liquid smoke solutions used in this study were commercially available liquid smoke products, consisting of five different liquid smoke types from three different brands encoded as A: grade 1 (brand I [Akago]), B: grade 2 (brand I [Akago]), C: grade 3 (brand I [Akago]), D: grade 2 (brand II [Assyifa]), and E: grade 3 (brand III [D'dak]). All products tested were produced from a pyrolysis process using coconut shell as the main ingredient source. Pyrolysis stages include preparation of main material of dried coconut shell, material is then burned in anaerobe pyrolysis reactor, produced smoke will move in condensers and condensate periodically into liquid smoke. Liquid smoke produce from pyrolysis tools is categorized into grade 3, redistillation of grade 3 liquid smoke will produce grade 2, and redistillation of grade 2 will produce grade 1. Each product was assess based on its color, thickness, tar contaminants, and odor. Character test of liquid smoke included quantitative observation of density using a pycnometer, water content, and pH using a pH detector (Tooy *et al.*, 2024).

Pathogen Isolate Preparation

Ganoderma boninense and *Curvularia* sp. isolates used in this study were a collection of the Research

and Development PT Astra Agro Lestari Tbk. Laboratory of Biology. Pathogens isolates were rejuvenated on PDA (Merck, 1101300500) at five days before testing. Inoculum source were sampled using a 0.5 cm cork borer.

In Vitro Fungal Activity

Five liquid smoke types were tested with liquid smoke type A–D being produced from coconut shells while type E did not enlist its material sources on its label. Liquid smoke type A to C was produced by the same company while liquid smoke type D and E was produced by respectively different companies.

Testing was done using PDA media that was infused with 1 or 2% (v/v) of each liquid smoke type and inoculum sources sampled using cork borer were placed in the middle of the media. Antifungal activity was tested using a Completely Randomized Design (CRD). No liquid smoke was infused in the untreated control. All treatments were replicated three times. The list of treatments was provided below:

Treatment	Type of liquid smoke	Brand	Concentration (%)
Untreated control	-	-	-
A1	grade 1	Akago	1
B1	grade 2	Akago	1
C1	grade 3	Akago	1
D1	grade 2	Assyifa	1
E1	grade 3	D'dak	1
A2	grade 1	Akago	2
B2	grade 2	Akago	2
C2	grade 3	Akago	2
D2	grade 2	Assyifa	2
E2	grade 3	D'dak	2

Diameter of fungal colonies were done every two days until the 6th day by measuring the diameter length of the colony and calculating using the following formula (Yuliawati *et al.*, 2020):

$$D = \frac{D1 + D2}{2}$$

Description: D = colony diameter; $D1$ = 1st colony diameter; $D2$ = 2nd colony diameter.

Analysis of Chemical Content using Gas Chromatography–Mass Spectrometry (GC-MS)

GC-MS analysis used Agilent 7890B (GC) and Agilent-5977A (MS). Column used was HP-5ms (30m × 0.25 mm × 0.25 μm) with Helium as its mobile gas. As much as 1 mL was injected into the system using the following GC and MS settings: GC was set for injector temperature of 250 °C, initial temperature of 60 °C, second temperature of 80 °C, third temperature of 110 °C. Temperature was held for five minutes at each temperature increment until it reached the maximum temperature of 180 °C while the MS was set at the scanning mode range of 50–550 amu and scan rate of 2 scan/s. After electron mode was set at 70 eV with temperature of 250 °C and used NIST MS 14.0 for mass data collect (Budaraga *et al.*, 2016; Wijaya *et al.*, 2017).

Data Analysis

Diameter *Curvularia* sp. and *Ganoderma* sp. were analyzed using ANOVA and Duncan post-hoc test with confidence interval of 95%.

RESULTS AND DISCUSSION

Liquid Smoke Characteristics

Liquid smoke used in this study have different characteristics. In general, liquid smoke formulations colors varied between bright yellow to blackish brown and have strong smoky odors with pH between 2–4, density 1.01–1.02, and water content between 91.3–95.07 (Table 1).

Based on standardized liquid smoke quality from Japan, liquid smoke is categorized as good quality if pH is between 1.5–3.7, density > 1.005, color of bright yellow to brownish yellow, and transparent (Yatagai, 2002 as cited in Alpian *et al.*, 2012). Liquid smoke A, B, and C was categorized as good quality based on standards from Japan (Table 1). Liquid smoke D

color was dark and not transparent while liquid smoke E had high pH compared to ranges for good quality liquid smoke. Thus, liquid smoke D and E do not meet standards of good quality liquid smoke.

Effect of Liquid Smoke on *Curvularia* sp. and *Ganoderma boninense* Colony Growth

This study showed that liquid smoke affected *Curvularia* sp. colony growth. Liquid smoke A1%, B1%, C1%, and E1% showed significantly lower *Curvularia* sp. colony diameter compared to untreated control with B1% providing the best inhibition. All liquid smoke at the concentration of 2% showed complete inhibition, except D2 that showed only some colony growth at day 6 (Table 2). Liquid smoke showed different effects on *G. boninense* where treatment C1 was most effective in inhibiting colony with diameter of 1.32 cm while A2% and D2% still showed *G. boninense* colony growth since the second day (Table 3). These results demonstrated the different effects of liquid smoke based on the target species.

Liquid smoke effectiveness in this study were consistent with previous findings. Gani *et al.* (2014) reported that liquid smoke from palm oil waste at concentration of 5% can inhibit anthracnose on chili. Rahmat *et al.* (2020) also reported that liquid smoke from teak wood at concentrations of 1% could inhibit *Rhizoctonia solani* root rot in soybean. Liquid smoke from coconut shell has also been reported to effectively inhibit *Fusarium oxysporum* in rubber (Ristiani, *et al.* 2022). Liquid smoke from yellow balau at concentrations of 2% was also reported to effectively inhibit *Phytophthora citrophthora* *in vitro* (Oramahi *et al.*, 2018).

The effective concentration in this study was 2% and was consistent with the the studies previously cited that showed effective concentrations between 1–5%. Effective concentration from liquid smoke

Table 1. Characteristic of five liquid smoke used against *Curvularia* sp. and *Ganoderma boninense*

Liquid smoke	Color	pH	Density	Water content (%)
A	Bright yellow	2	1.01	92.61
B	Yellow	3	1.01	91.69
C	Yellow	3	1.02	91.30
D	Blakish brown	2	1.01	94.60
E	Dark yellow	4	1.01	95.07

is based on the material and temperature used during pyrolysis as reported by Komarayati *et al.* (2020) that was able to demonstrate that different materials affect yield and concentration of aktif compound. According to Budaraga *et al.* (2016) pyrolysis temperature affected compound composition in liquid smoke with optimum temperature between 300–400 °C. In this study, the material source of commercial liquid smoke used in this study and pyrolysis temperature could not be confirmed.

Table 2. *Curvularia* sp. colony diameter after *in vitro* testing

Treatment	Colony diameter (cm)		
	2 DAI	4 DAI	6 DAI
Untreated control	2.30 b	5.80 a	8.27 a
A1%	1.95 c	4.10 c	6.77 b
B1%	1.10 e	3.43 e	6.17 c
C1%	1.20 de	3.80 d	6.60 b
D1%	2.47 a	5.13 b	7.97 a
E1%	1.33 d	3.70 d	6.40 bc
A2%	0.00 f	0.00 f	0.00 e
B2%	0.00 f	0.00 f	0.00 e
C2%	0.00 f	0.00 f	0.00 e
D2%	0.00 f	0.00 f	1.83 d
E2%	0.00 f	0.00 f	0.00 e

Notes: Numbers followed by the same letter in the same column were not significantly different based on Duncan post-hoc test at alpha of 5%. DAI: days after inoculation. A–E: liquid smoke type, 1% and 2%: concentration of liquid smoke.

Liquid smoke effectiveness depend on the target pathogen as shown in this study, *G. boninense* was more resistant to liquid smoke compared to *Curvularia* sp. (Table 2 and Table 3). This may be caused by the different composition of cell walls between both pathogens. *Curvularia* sp. belongs to Ascomycota with chitin (39%), glucan (29%–60%), protein (7%–13%), and lipid (6%–8%) (Wabster & Waber, 2007). Meanwhile *G. boninense* cell wall was different to *Curvularia* sp. Liu *et al.* (2022) reported that *Ganoderma* sp. cell wall contained glucan ($76.7 \pm 2.9\%$) and chitin ($3.85 \pm 0.55\%$). More chitin was contained in *Curvularia* sp. while glucan concentration was higher in *G. boninense*. Besides that, it is possible that *G. boninense* have higher adaptation to antimicrobial compounds.

Table 3. *Ganoderma boninense* colony diameter after *in vitro* testing

Treatment	Colony diameter (cm)		
	2 DAI	4 DAI	6 DAI
Untreated control	2.30 a	4.73 a	9.00 a
A1%	2.18 a	4.72 a	9.00 a
B1%	1.08 a	4.53 ab	9.00 a
C1%	1.32 bc	3.37 bc	7.67 ab
D1%	2.07 a	4.83 a	9.00 a
E1%	1.90 ab	4.27 ab	9.00 a
A2%	1.03 c	2.12 c	5.25 c
B2%	0.00 d	0.00 d	0.00 d
C2%	0.00 d	0.00 d	0.00 d
D2%	1.03 c	2.57 c	6.28 bc
E2%	0.00 d	0.00 d	0.00 d

Notes: Numbers followed by the same letter in the same column were not significantly different based on Duncan post-hoc test at alpha of 5%. DAI: days after inoculation, A–E: liquid smoke type, 1% and 2%: concentration of liquid smoke.

The main compounds contained in liquid smoke in this study was phenol. Phenol percentage in every liquid smoke were different depending on the liquid smoke types. Lowest phenol percentage was found in liquid smoke E of 24.45% and the highest in liquid smoke B of 85.28% (Table 4). Previous studies reported that compound composition depends on material source and pyrolysis temperatures. Budaraga *et al.* (2016) showed that pyrolysis temperature of 300 °C using coconut shell resulted in higher phenol (35%) compared to temperatures of 100 °C, 200 °C, and 400 °C that only resulted in 18–23% of phenol.

Phenol that was the major compound found in this study, is suspected as the main compound to inhibit *Curvularia* sp. and *G. boninense*. Phenol is a well known antimicrobial compound that disrupt cell wall or inhibit growth of pathogen. Phenol can penetrate and disrupt cell wall and membrane (Manso *et al.*, 2022). Rao *et al.* (2010) reported that phenol can disrupt fungal metabolism by affecting pH in vacuole and cytoplasm and Ca^{2+} transfer. These mechanisms will disrupt fungal cell and result in cell death.

This study also demonstrate that phenol content in liquid smoke is not the sole indicator of liquid smoke effectiveness to inhibit pathogens.

Table 4. Liquid smoke chemical content analysis using GC-MS

Liquid smoke	Main compound	Retention time	Area (%)
A	17-Octadecynoic acid	4.13	2.66
	Phenol	9.91	83.45
	2-Methyl Phenol	15.22	2.11
	2-Methoxy Phenol	17.27	9.48
B	Phenol	9.93	85.28
	2-Methyl Phenol	15.23	2.32
	2-Methoxy Phenol	17.28	8.87
	2,6-dimethoxy-phenol	40.19	0.92
C	Phenol	9.95	84.62
	2-Methyl Phenol	15.22	1.83
	2-Methoxy Phenol	17.29	10.11
	Creosol	26.29	1.83
D	Phenol	9.87	81.40
	3-Methyl Phenol	15.18	2.11
	p-creosol	17.02	2.68
	2-Methoxy Phenol	17.24	6.08
E	Phenol	9.88	24.45
	p-creosol	17.03	6.94
	2-Methoxy Phenol	17.27	16.14
	3-Ethyl-Phenol	24.56	6.54

Liquid smoke D had phenol percentage area of 81.40%, but lower inhibition compared to liquid smoke E with percentage area of 24.45%. Other compounds are thought to have antimicrobial properties, such as phenol derivatives. Acetate acid was a compound commonly found in liquid smoke (Montazeri *et al.* 2012) that may also have roles in this study. Organic acid compound are known to have antifungal properties by affecting pH around cell membrane, disrupting cell metabolism reactions, and causing anion poisoning (Hassan *et al.*, 2015). Further studies are required to indicate compounds with antimicrobial properties in liquid smoke.

CONCLUSIONS

Treatment using five different liquid smoke types resulted in different *Curvularia* sp. and *Ganoderma boninense* colony growth. Highest *Curvularia* sp. inhibition was shown by liquid smoke type B and liquid smoke type C showed the highest inhibition of *G. boninense*. Main components contained in liquid smoke was phenol consisting of 24.45%–

85.28%. Phenol was one of the main component thought to inhibit *Curvularia* sp. and *G. boninense*.

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