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Research Article

Potential of Coconut Fiber-Based Liquid Smoke as Biofungicide to Suppress *Phytophthora palmivora* Growth In Vitro[(*)]

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ABSTRACT

Pod rot caused by *Phytophthora palmivora* (Butl.) is a major constraint in cocoa production. This disease management needs organic approachs that are environmentally friendly such as by using biopesticides from plant materials. Coconut fiber liquid smoke is one of the materials that can be used as an environmental friendly botanical pesticide. This study aims to determine the effective concentration of liquid smoke made from coconut fiber to inhibit pathogenic fungus *P. palmivora* growth in vitro. The experiment was set as a non-factorial Randomized Complete Block Design (CRD) consisting of six treatments and five replicates, for a total of 30 experimental units. The experiment was conducted using the food poisoned technique by mixing 10 mL PDA with liquid smoke (2 mL of each concentration). Treatments consisted of 0% (control), 2%, 4%, 6%, 8% and 10% concentrations. Results of the in vitro test showed that the application of coconut fiber liquid smoke at 10% suppressed growth and inhibit *P. palmivora* (Butl.) fungal colony development to 0 cm.

Keywords: coconut fiber; concentration; liquid smoke; *Phytophthora palmivora*

INTRODUCTION

An obstacle of cocoa production, a leading commodity of Central Sulawesi, is the presence of cocoa pod rot disease caused by the pathogenic fungus *Phytophthora palmivora* (Butl.). Infected cocoa pods rot to the seed, causing economic losses by reducing production. Madani (2019) reported that *P. palmivora* can cause yield loss up to 90% especially during the rainy season or dry season in fields with a large ant populations. The pathogen is difficult to control as it survives inside the cocoa pods especially when buried in the soil for months, even years. This means that the inoculum of the pathogen is always available in the soil and field. *P. palmivora* can maintain its life in the soil for years in the form of chlamydospores or cysts that have thick walls (Efendi, 2014).

Current cocoa pod rot management rely on the use of synthetic fungicides due to their easiness for application, effective eradication, and quickly show results. However, farmers tend to use fungicides excessively causing negative impacts on the environment. The use of biopesticides from plant-based materials such as liquid smoke from plants is expected to control pathogenic fungi without polluting the environment. Plant-based pesticides in the form of liquid smoke after pyrolysis process have been shown to effectively suppress plant pathogens. Results from Fatimah (2011) showed that coconut fiber liquid smoke was able to inhibit the growth of *Salmonella choleraeaeus, Bacillus subtilis* and *Staphylococcus aureus*. Coconut shell liquid smoke can inhibit growth *Corynespora cassiicola* on rubber (Mahmud *et al*., 2020), *Colletotrichum capsici* which causes anthracnose disease (Zuanif & Despita, 2019) and *Fusarium oxysporum* f.sp *capsici* which causes *Fusarium* wilt in chilies (Wildan *et al*., 2021). Furthermore, liquid smoke from rubber tree stumps can control white root mould disease (*Rigidoporus microporus*) by more than 75% (Dalimunthe & Tistama, 2018).

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Liquid smoke derived from natural materials such as wood, cashew seeds, coconut shells, coconut fibres, and so on, is a liquid resulting from the condensation of smoke vapours from wood pyrolysis that contain acetic acid, phenol and carbonyl as a result of thermal degradation of cellulose, hemicellulose and lignin components (Pamori *et al.*, 2015; Siswanto, 2020). Phenol, acetic acid, and carbonyl compounds are antimicrobial, antioxidant, and antifungal compounds so that liquid smoke can be used as a natural preservative and pesticide (Siswanto, 2020; Akkus *et al.*, 2022). Other compounds contained include furans, alcohols, and esters (Siswanto, 2020). The natural compounds phenol (C_6H_6O) and acetic acid ($C_2H_4O_2$) have bioactive properties as antimicrobials, so they can be utilised for plant protection against plant pest organisms (Mugiastuti & Manan, 2009). Phenol and acetic acid are compounds that play the most antimicrobial activity. The more the content of these compounds, the higher the ability of liquid smoke to suppress the growth of microorganisms (Wildan *et al.*, 2021).

The mechanism of phenol activity as antimicrobials is to inhibit biosynthesis of ergosterol which disrupts cell membrane permeability, causing irregularities in the cytoplasmic membrane in fungi. As a result, the fungus will lose its cell contents. The phenol reaction on cell membranes will also inactivate essential enzymes resulting in metabolic imbalances that inhibit growth and cause fungal cell death (Fitriani *et al.*, 2022). Zuanif and Despita (2019) stated that phenol is a compound that has a synergy function as a protein denaturant and lipid hydrolyser that can damage cell membranes of fungal tissue and inactivate fungal secreted enzymes. Phenolic compounds also affect mitochondrial function thereby disrupting cellular respiration that leads to inhibition of fungal growth. Meanwhile, the general mechanism of microbial growth inhibition by acetic acid activity is through acidification of the cell cytoplasm caused by the release of excess protons after acid dissociation (Suryani *et al.*, 2020). This causes enzyme denaturation and instability of microbial cell membrane permeability, thus inhibiting microbial cell growth and survival (Ahadiyat *et al.*, 2020). Dalimunthe & Tistama (2018) stated that this acidification can penetrate microbial cell wall and cause cell lysis and disrupt permeability, thus inhibiting microbial metabolism and growth. The combination of phenol functional components and acetic acid content works synergistically to prevent and control microbial growth.

Palu City is area area in Central Sulawesi that has abundant agricultural waste resources, particularly coconut fibre, which has not been fully utilized for making planting media and organic fertilizer. Currently coconut fibre in Palu is solely used as a burning medium or even thrown away and left unprocessed. Therefore, to maximize the function of coconut fibre waste, it needs to be developed towards other processing in the form of liquid media that can be used as a botanical pesticide. This is because liquid smoke made from coconut fibre has the potential to control plant pathogens because it is antimicrobial (Santoso, 2015). Research on cocoa pod rot disease using liquid smoke made from coconut fiber has never been conducted. This study aims to determine the concentration of liquid smoke made from coconut fiber that is effective in inhibiting the growth of fungal pathogen *P. palmivora* in vitro.

MATERIALS AND METHODS

This research was conducted at the Plant Disease Laboratory, Faculty of Agriculture, Tadulako University from June to December 2022. The equipment used were a set of pyrolysis tools, scales, petri dishes, goblets, measuring cups, test tubes, micropipettes, tweezers, oz needles, Erlenmeyer glass, aluminium foil, haemocytometer, Bunsen burner, Laminar Air Flow (LAF) refrigerator, and autoclave. Materials used include inoculum of the fungus *P. palmivora* that causes fruit rot disease in cocoa, Potato Dextrose Agar (PDA) (EMD Millipore), tissues, 96% alcohol, coconut fibre, methylated spirits, distilled water, and alcohol.

The experiment was arranged as a non-factorial Randomized Complete Block Design (CRD) consisting of six treatments and five replicates totaling of 30 experimental units. This test was conducted in vitro using the media poisoning method (food poisoned technique) (Paramita *et al.*, 2014) by mixing 10 ml PDA media with 2 mL of liquid smoke at each concentration , inoculating test pathogenic microbes on the growth media and measuring their

growth. Liquid smoke treatments consisted the following concentrations: A = 0% (no liquid smoke + 10 mL distilled water), B = 2% (0.2 mL liquid smoke + 9.8 mL distilled water), C = 4% (0.4 mL liquid smoke + 9.6 mL distilled water), D = 6% (0.6 mL liquid smoke + 9.4 mL distilled water), E = 8% (0.8 mL liquid smoke + 9.2 mL distilled water), and F = 10% (1 mL liquid smoke + 9 mL distilled water).

Preparation of Pathogen Isolates

The *P. palmivora* isolates used were collected from the Plant Disease Laboratory, Department of Plant Pests and Diseases, Tadulako University. The isolate was in the form of a fungal culture on PDA media which was stored at 5°C. The isolates were obtained from the isolation of cocoa pods with pod rot symptoms from Donggala Regency (Central Sulawesi).

Fungi Rejuvenation

Rejuvenation of *P. palmivora* pathogenic fungal isolates was done by re-growing isolates on new PDA medium. Isolates were taken in the form of plates using a sterile cork borer, then planted in the center of the PDA medium in a petri dish. Microbial cultures were incubated at room temperature for 7 days.

Liquid Smoke Manufacturing

The process of making coconut fiber liquid smoke referred to Reta (2013). Ten kg of coconut fiber was cleaned and chopped, then dried in the sun for three days or until dry to reduce the water content in the raw liquid smoke. Dried coconut fiber was placed into the used pyrolysis reactor and tightly closed. Furthermore, the combustion process was carried out at temperatures of 400°C for 8 hours. The material in the reactor tube was warmed and underwent pyrolysis. The smoke generated from the combustion was flowed into the condenser to condense and result in liquid smoke that was collected into the smoke reservoir. Liquid smoke was allowed to stand for 48 hours to precipitate the tar content in the liquid smoke. After settling, liquid smoke was filtered using Whatman filter paper size 40, and filtered again to reduce tar content contained in the liquid smoke using a 0.2 μm filter membrane and the liquid smoke obtained was used as a biopesticide. Coconut fiber liquid smoke from the first combustion is considered to have a concentration of 100%.

Liquid Smoke Preparation

The coconut fiber liquid smoke used in the experiment was grade 3, which means the liquid smoke produced from the first combustion process in the pyrolysis reactor. The classification of this liquid smoke was based on the amount of harmful compounds in the liquid smoke. The preparation of coconut fiber liquid smoke was done by diluting grade 3 liquid smoke according to the treatment concentration. Determination of liquid smoke concentration was done with the following formula (Trisnawati *et al.*, 2019):

$$z = \frac{x}{x + y} \times 100\%$$

z = concentration of coconut fiber liquid smoke; x = volume of liquid smoke obtained from combustion (mL); y = volume of distilled water added (mL); x + y = total volume of liquid smoke and distilled water (10 mL)

Observational Variables

Colony Growth of *P. palmivora*

P. palmivora growth was observed by measuring fungal colonies diameter daily for 7 days. Measurements done on vertical and horizontal lines that intersected exactly at the midpoint of the fungal colony on the Petri dish (9 cm) using a ruler. Measurement of colony diameter was carried out when the mycelium grew for the first time and began to spread every day. Measurement of the diameter of *P. palmivora* colonies with the following formula (Fatihah *et al.*, 2022):

$$d = \frac{d_1 + d_2}{2}$$

d = diameter of the fungal colony; d_1= vertical diameter of *P. palmivora* colony (mm); d_2= horizontal diameter of *P. palmivora* colony (mm)

The results of measuring the diameter of each fungi colony will be used to calculate the percentage of inhibition of coconut fiber liquid smoke.

Inhibition Test

Two milliliters of liquid smoke at each concentration was added to 10 mL of sterile PDA media, homogenized until evenly distributed and allowed

to stand until it solidifies. After cooling and solidifying, the center of the PDA media in the petri dish was perforated with a cork borer with a diameter of 0.5 cm. *P. palmivora* fungi mycelium from pure culture was inoculated right into the middle of the PDA medium using a looped needle, then incubated at room temperature for 7 days. Each experiment was repeated 3 times. Observations were made by measuring the growth of colony diameter every day until the fungi mycelium covered the entire surface of the petri dish. Percentage inhibition of each concentration is done by the formula (Suganda *et al*., 2020):

$$dh\% = \frac{x_2 - x_1}{x_2} \times 100\%$$

dh = inhibition percentage; x_2 = *P. palmivora* colony diameter in control (mm); x_1 = *P. palmivora* colony diameter on treatment media (mm)

Data Analysis

The research data obtained, tested by Analysis of Variety (ANOVA) and if it has a significant effect, it will be continued with the LSD post-hoc test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Colony Growth

Phytophthora palmivora fungi colony diameter after treatment with different concentrations of coconut fiber liquid smoke is presented in Figure 1. Results showed that the coconut fiber liquid smoke significantly affected the diameter size of *P. palmivora* fungi colony. Treatment of 10% concentration was significantly different from all treatments. At concentrations of 2% (B), 4% (C),6% (D), and 8% (E), the size of the colony diameter were 6.87 cm, 4.43 cm, 3.27 cm, and 1.78 cm respectively, while at a concentration of 10% (F) no fungal growth occurred during the observation period. This shows that each concentration treatment of coconut fiber liquid smoke can suppress *P. palmivora* growth respective to the used concentrations. Increasing the coconut fiber liquid smoke will increase the toxic bioactive compound against these pathogenic fungi due to their antimicrobial activity. According to Fatimah (2011), coconut fiber liquid smoke contains ethylene glycol, acetone, acetic acid, oxalic acid, pyrazine, 1-hydroxyl-2-propanone, furfural, dihydro furanone, phenol, cycloethane, 2-methyl phenol, 2-methoxy phenol, 2-methoxy-4-methyl phenol, 2,5-dimethoxitoluene, and 2,6-dimethoxy phenol. The two main compounds in fiber liquid smoke that are known to have antimicrobial activity are phenol and acetic acid (Aisyah *et al*., 2013).

The control treatment showed the largest colony diameter (8.64 cm) compared to other ones treated with coconut fiber liquid smoke. Fungal colonies appeared to continue to grow and develop without inhibition so that the size of the colony diameter is getting bigger, wider, and forming aerial mycelium with a colony shape that is not patterned, distributed, white, and smooth like thick cotton. This is in line with the statement of Wibowo *et al.* (2017), *P. palmivora* fungi can grow well on PDA agar media with irregular colony shapes, flat colony surfaces, and cotton-like mycelium. Figure 2 shows *P. palmivora* growth on PDA media at concentrations of 0%, 2%, 4%, 6%, and 8%, while at 10% concentration there was no growth due to the high concentration of liquid smoke. Based on the size of the colony diameter, the low concentration of coconut fiber liquid smoke showed that the colony size of *P. palmivora* fungus was larger than the colony size at a high concentration of liquid smoke in PDA media.

The enlarged colony diameter is an indicator of microbial colony growth. One of the parameters of microbial growth is the increase in cell volume due to the increase in protoplasm and nucleic acid compounds (Naim *et al*., 2020). Rahmawati *et al.* (2020) reported that the growth of microbial colonies is irreversible. Microbial colonies are used in growth measurements because the cell mass is generally derived from one cell. In general, fungal colonies start from one invisible cell to become visible, namely from fungal spores or conidia to mycelium or colonies (Amir *et al*., 2018). The growth of fungal colonies has an important role in the process of the fungal life cycle because the spores or conidia produced are used as a means of asexual reproduction, spread and defence of fungal life against the environment (Amaria *et al*., 2015). In this study, the growth of *P. palmivora* fungi occurred optimally during the 7th incubation period where the entire surface of the PDA media was covered by mycelial mass.

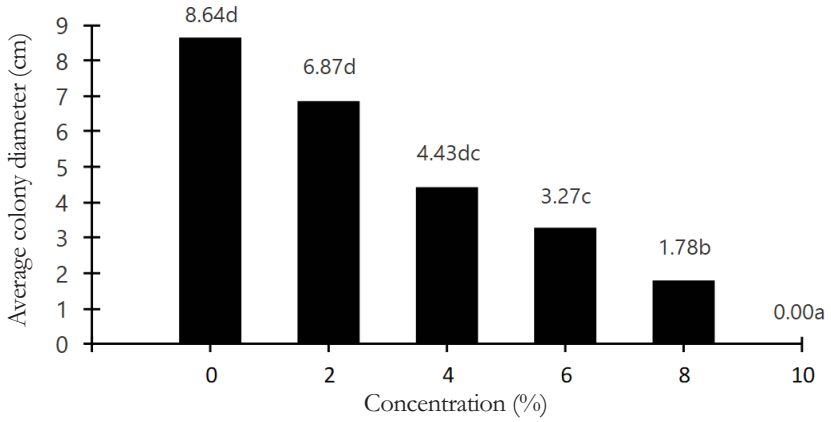

Figure 1. Average colony diameter (cm) of *Phytophthora palmivora* after treatment using various concentrations of coconut fiber liquid smoke during the 7-day incubation period

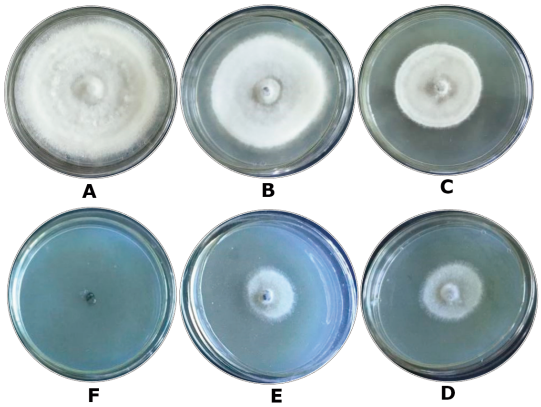

Figure 2. Growth of *Phytophthora palmivora* on PDA medium: concentration 0% (A), concentration 2% (B), concentration 4% (C), concentration 6% (D), concentration of 8% (E), and concentration of 10% (F)

Inhibition

Results showed that the application of fiber liquid smoke significantly inhibited *P. palmivora* colony growth in vitro. The highest coconut fiber liquid smoke concentration (10%) significantly inhibit growth compared to all treatments (Figure 3). The inhibition of colony growth increased as the concentration of liquid smoke increased. The high inhibition may be related to the bioactive content in the liquid smoke. Coconut fiber liquid smoke contains bioactives that are antimicrobial (Pamori *et al.*, 2015).

The use of coconut fiber liquid smoke at a concentration of 2% inhibited colony growth. Likewise, at concentrations of 4%, 6%, 8%, and 10%, coconut fiber liquid smoke was able to inhibit colony growth compared to controls. Thus, the concentration of liquid smoke indicated that the initial inhibition of colony occurred at a concentration of 2% and so on. In this study, there were differences in the inhibition at each liquid smoke concentration. This difference was influenced by the amount of antimicrobial compounds contained in each concentration. This is due to high concentrations, the content of bioactive compounds in liquid smoke was higher and had higher ability to suppress and inhibit the growth of fungal colonies. *P. palmivora* was no longer able to grow in medium containing liquid smoke at a concentration of 10%. The higher the concentration of liquid smoke used, the higher the content of bioactive compounds that can inhibit the growth of fungi (Melani, 2020; Oramahi *et al.*, 2021).

If the medium contains large amounts of antimicrobial compounds, they will be absorbed in larger amounts by the pathogenic fungi. This ab-

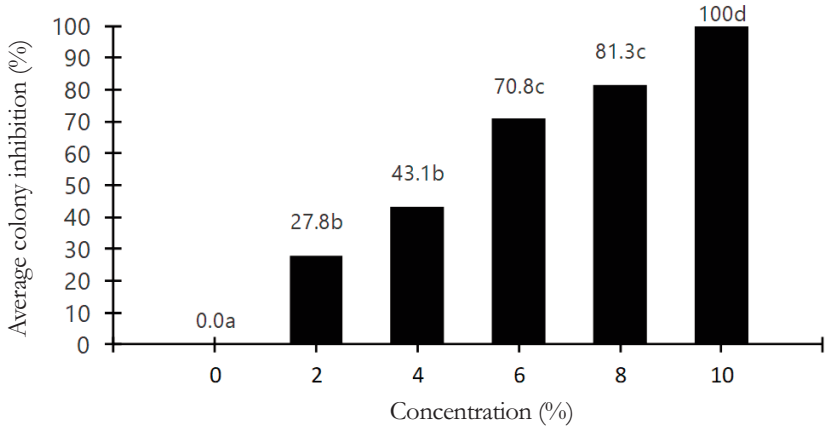

Figure 3. Average inhibition percentage of *Phytophthora palmivora* fungal colonies using various concentrations of coconut fiber liquid smoke after 7-day incubation period

sorption causes a strong reaction from the antimicrobial compounds of fiber liquid smoke against the pathogen *P. palmivora*. The greater the concentration of liquid smoke given, the stronger the reaction will be so that the growth of pathogenic fungal colonies becomes suppressed and slow. The content of phenol compounds and acetic acid as antimicrobial substances in fiber liquid smoke is thought to play a role in suppressing the growth of pathogenic fungal colonies so that growth is inhibited. The results of research by Pamori *et al.* (2015) showed that fiber liquid smoke contained acetic acid and phenol. Phenol and acetic acid compounds have a major role as antimicrobial substances or organic pesticides. Both compounds have functional abilities as antibacterial and antifungal (Siswanto, 2020). This is in line with the report of Jayanudin and Suhendi (2012), that liquid smoke components such as phenol and acetic acid function as antibacterial and antifungal compounds. The phenol and acetic acid compounds in liquid smoke can interfere with microbial metabolism, thus inhibiting the growth of fungi and bacteria. Melani (2020) reported that phenol compounds can inhibit microbial enzyme activity, while acetic acid can inhibit the growth of developing microbes.

The mechanism of activity of phenol compounds is to diffuse in the fungal cell membrane and interfere with metabolic pathways, and change protein cells so as to inhibit cell growth, or cause fungal cell death (Ristiani *et al.,* 2022). Habibi *et al.* (2017) added, phenol compounds will react with cell membranes causing cell membrane permeability to increase so that cell contents come out, inactivation of essential enzymes, destruction or functional inactivation of genetic material, and work as lipid hydrolysers so as to damage cell membranes. This membrane damage will allow organic ions nucleotides coenzymes and amino acids to leave the cell and prevent the entry of essential materials into the cell because the cytoplasmic membrane in charge of controlling essential materials in the cell does not function properly (Novita *et al.*, 2012). This will disrupt microbial growth and cause death. The effect of antimicrobial activity of acetic acid compounds from liquid smoke is thought to directly acidify the cytoplasm and damage the surface tension of the membrane, as well as the loss of active transport of food through the membrane, causing destabilization of various functions and structures of cell components (Mahmud *et al.*, 2020). Phenol and acetic acid can also cause protein denaturation, hydrolysed lipids so that they can damage cell membranes in fungal body tissues, and inactivate enzymes secreted by the fungus (Melani, 2020). Damage to proteins and lipids in the cytoplasmic membrane of pathogenic cells causes the membrane to leak and as a result the permeability of the cell membrane is disrupted. This causes the membrane to become permeable and allow permease enzyme to disrupt cells and absorption of nutrients. The activity of nutrient absorption from the host for metabolism is disrupted resulting in biological and physiological activities of the fungus being disrupted and eventually causing the death of fungal cells (Aisyah *et al.*, 2013).

CONCLUSION

Results from this study indicated that liquid smoke derived from coconut fiber could be used as an effective management strategy for inhibiting *P. palmivora* growth. This suggests that the liquid smoke of coconut fiber holds potential as a control method for *P. palmivora*. Application of coconut fiber liquid smoke at a concentration of 10% was able to inhibit fungal mycelium growth until it could not grow (0 cm) with 100% colony growth inhibition in vitro.

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