

The Liquid Smoke from Empty Oil Palm Bunches (*Elaeis Guineensis* Jacq.) as a Natural Fungicide and Its Ability to Inhibit the Growth of *Colletotrichum* sp. Fungus

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Abstract

The primary pest of red chili plants is anthracnose disease. Anthracnose is caused by the fungus *Colletotrichum* sp. Anthracnose is controlled with synthetic fungicides, but their long-term usage can harm the environment by leaving residues in the soil that are difficult to degrade, causing environmental degradation and a severe impact on the health of human. The goal of this study was to see how much potential liquid smoke had as a natural fungicide in preventing *Colletotrichum* sp. growth. It is recognized that liquid smoke emitted by empty oil palm bunches includes antioxidant compounds that function as antifeedants, contact poisons, and stomach poisons against plant pests. These compounds have the ability to act as natural fungicides, preventing the rise of *Colletotrichum* sp. The pyrolysis process produces liquid smoke at temperatures ranging from 350°C to 450°C. Total phenol and total acid content are determined from the obtained liquid smoke. The total phenol a material was identified using the well diffusion method, and the findings were 12.57%. Meanwhile, the maximum total acid content was found in liquid smoke from pyrolysis at 450°C (38.4 mg/L) with a volume of liquid smoke produced of 790mL. The ideal liquid smoke concentrations for limiting the increasing number of *Colletotrichum* sp. fungus are 20% and 30%, with average values of 4.28 and 7.73 mm, respectively. With a very strong inhibitory response category.

Keywords: Liquid smoke, total phenol, total acid, fungicide, *Colletotrichum* sp.

Introduction

Indonesia is recognized for its tropical climate and plentiful natural resources. These weather conditions are the ideal cause for Indonesians to enter the agriculture sector, particularly horticultural crops. Horticultural crops are plants with a high market value because they can provide farmers with revenue. Red chili is a popular horticultural crop among the general people. However, its productivity has not been maximized [1]. The availability of soil nutrients, attacks by plant-disturbing organisms, and meteorological variables can all have an impact on the production of red chili plants [2]. Red chili

plants are frequently attacked by anthracnose bugs, also known as patek on plants. This pest attack is produced by pathogenic fungus such as *Colletotrichum capsici* [3], which infects all sections of the plant, particularly the fruit, with symptoms ranging from blackish brown blotches on the surface of the chili fruit to the top dying [4]. This pathogenic fungus can diminish red chili plant output because it can live in the surviving plants and spread chili plant seeds or seedlings, as well as infected fruit, resulting in crop failure [5].

The use of synthetic fungicides is one of the methods commonly employed by farmers to

control fungi detected in red chili plants. Long-term use of synthetic fungicides can leave difficult-to-degrade residues in the soil, negatively impacting the environment and the health of human. The synthetic fungicides is not only harmful to the damage of environment and health, but it is also relatively expensive. Furthermore, long-term usage of synthetic fungicides might result in disease resistance and the mortality of non-target organisms. Therefore, its use needs to be combined with organic fungicides with a low level of toxicity but remains toxic to pathogenic organisms, does not pollute the environment because it is easily degraded naturally, and is relatively safer for plants and the health of human [6].

The use of liquid smoke as a natural fungicide is a less harmful way to reduce plant pests. Liquid smoke's high amount of phenol, alcohol, and organic acid components makes it efficient for inhibiting fungal development [7]. Liquid smoke is the product of incomplete combustion of organic molecules at a pyrolysis temperature of 400°C, which then condenses. Organic sources for liquid smoke are often derived from wood including lignin, cellulose, hemicellulose, and other carbon compounds [8]. In most cases, bioactive materials derived from plants have allelopathic abilities, which means they have the ability to produce biomolecular compounds into the environment, which can have an impact on the increasing and development of other organisms around them [9].

Palm oil waste, which is used as a raw material for liquid smoke, has antifungal qualities and can prevent the *Colletotrichum* sp. fungus, which produces patak in chili plants. The efficacy of liquid smoke to prevent fungal development is affected by the raw material utilized in the manufacturing process, the pyrolysis temperature, and the chemical compounds contained in the liquid smoke [10]. Several related investigations have demonstrated that liquid smoke from empty oil palm bunches (EFB) can prevent fungal development [11]. Melani's (2020) study found that a 5% concentration of liquid smoke had a substantial influence on *Curvularia* sp.'s 86% inhibition, decreased growth rate, and

antifungal index. A 7% concentration of liquid smoke can completely suppress the increasing number of the *Fusarium oxysporum* fungus [12]. Liquid smoke from empty oil palm bunches has the capacity to inhibit several species of fungi, therefore it could be used as a natural fungicide to manage *Colletotrichum* spp. The goal of this investigation is to see if liquid smoke from empty oil palm bunches (*Elaeis guineensis* Jacq.) can inhibit the *Colletotrichum* sp. fungus.

Experimental

Equipment

The tools used in this study were UV-vis spectrophotometry 1800 (Shimadzu, Japan), shaker-vortex (Test Tube Shaker LJ-039, Panchkula), pyrolysis equipment, autoclave (Merck, Germany), drum, incubator, hotplate stirrer (Thermo Scientific Cimarec SP88857105, America), micropipette, disc paper (484000 MN 827 ATD, Germany) diameter 6 mm, digital caliper, analytical balance USS-DBS15-3, dropper pipette, 0.2 µm filter membrane (Millipore, Germany) and other glassware.

Material

The materials used in this experiment were empty oil palm bunch waste taken from Ujung Gading village (1° 43' 44.4" N, 99° 51' 14.4" E), South Labuhanbatu Regency, North Sumatra, 1M NaCO₃ (Sigma Aldrich, Germany), Folin-ciocalteu reagent (Sigma Aldrich, Germany), 1N NaOH (Merck, Germany), acetic acid p.a (Millipore, Germany), PDA powder (Millipore, Germany), 10% chloramphenicol (Sigma Aldrich, Germany), and DM aquadest (Brataco).

Methods

Making liquid smoke from empty oil palm bunches

The liquid smoke is produced utilizing the previously employed approach, namely the pyrolysis method. This method is a thermal breakdown procedure that employs high temperatures and little or no oxygen. The combustion process is performed by utilizing a reactor coupled to a succession of condensers, as

well as a measuring cup to contain liquid smoke. The trash from empty oil palm bunches is first treated to remove contaminants. Empty oil palm bunches are weighed to determine their original weight, then sliced into 10-15 cm pieces, air-dried till the water content is 15%, and reweighed. 300 g of dry empty oil palm bunches are placed in the combustion reactor (height 60 cm, diameter 40 cm, and capacity 5 kg). Pyrolysis is carried out for two hours at 300°C, with a thermometer reading every fifteen minutes. The combustion temperature varies between 350, 400, and 450°C. The smoke generated during the pyrolysis process is directed into a tube that serves as a condenser, and the distillate is collected in a 1 L measuring cup and allowed to cool. The liquid smoke is separated from the tar in the distillate using a 0.2 µm membrane filter [13].

Total Phenol Analysis

Total phenol assessment was performed according to the technique performed by Yusmar, et al (2023) using the Follin-Ciocalteu method with the aim of determining the amount of phenol contained in the sample. The total phenol content was done by adding 1 mL of empty oil palm bunch liquid smoke into an Erlenmeyer flask and then diluting it using distilled water to a volume of 100 mL. The dilution solution was taken as much as 1 mL then added 5 mL of 1 M NaCO₃ solution and left at room temperature for 10 minutes. The solution was then added with 0.5 mL of Folin-Ciocalteu reagent, stirred using a vortex shaker until the solution was homogeneous, then left for 30 minutes. The absorbance was read at a wavelength of 750 nm using a Shimadzu UV-Vis 1800 spectrophotometer, and the phenolic concentration of the sample solution was calculated based on the standard curve obtained from the pure phenol solution [14].

Total Acid Content Analysis

The determination of acid sample solution has been calculated with the typical curve produced from the pure phenol solution content was performed by using the Akbar, et al (2013)

method. A total of 2 mL of liquid smoke from various pyrolysis temperatures was diluted to a volume of 100 mL using distilled water and then titrated using 0.1N NaOH solution with pp indicator added until the liquid smoke solution was pink.

Making Potato Dextrose Agar Media

A total of 39 g of PDA powder was dissolved in 1 L of distilled water and then boiled using a hot plate until all the mixture was completely dissolved. Chloramphenicol (10%) was added to the media and sterilized using an autoclave at a temperature of 121 °C and a pressure of 2 atm. Sterilization was carried out for 15 minutes [14].

Measurement of the Inhibitory Power of Liquid Smoke from Empty Oil Palm Bunches against *Colletotrichum sp. Fungus*.

The measurement of inhibitory power was performed based on the method carried out by Purwantisari, et al (2023), namely using the well diffusion method which aims to determine the optimal concentration of empty oil palm bunches liquid smoke in inhibiting the increasing number of *Colletotrichum sp.* This method involves making wells (small holes) in the agar medium that has been inoculated with the test microorganism. This measurement was carried out using grade 2 liquid smoke from distillation as a sample. *Colletotrichum sp.* fungi were inoculated on PDA media using sterile cotton swabs. Wells were made on the PDA media with 5 holes measuring 0.5 cm using a cork borer. Each hole was given liquid smoke with a concentration variation of 0% (without liquid smoke) as a negative control, 10%, 20%, 30% and 2% mancozeb was employed as a positive control [15]. The experiment was done twice and incubated for two days at 35 °C. After incubation, the inhibition zone (zone free from microorganism growth) around the well was measured to determine the antimicrobial activity of the material being tested. The clear zone formed is measured using a vernier caliper and determined using the formula:

$$X = (Z1 + Z2 + Z3 + \dots Zn)/n$$

Where :

X : Raverage resistance (cm)

Z : Barrier Diameter (cm)

n : Number of repetitions

Antifungal activity is expressed by the antifungal index (Oramahi, 2023) with the following equation:

$$(1 - \frac{D_t}{D_c}) \times 100\%$$

Where :

IAJ = Antifungal Index

Dt = Diameter of the treated fungal colony (mm)

Dc = Diameter of control fungal colony (mm)

Results and Discussion

Volume of Liquid Smoke produced

Figure 1 shows the measurement results of the volume of liquid smoke obtained during the pyrolysis process. Based on the figure, the optimal volume of liquid smoke was obtained at a temperature of 450°C with a volume of 188 mL for 35 minutes. The resulting volume decreased during the pyrolysis process until it stopped for 60 minutes (0 ml) because all cellulose, hemicellulose and lignin had been completely decomposed.

Pyrolysis temperature is one of the factors that affects the volume of liquid smoke produced. This can be seen from Figure 1 where the higher the temperature, the greater the volume of liquid

smoke. The volume produced from temperatures of 350, 400 and 450°C is 798 mL, 795 mL, and 790 mL, respectively. Pyrolysis lasts 30-35 minutes and creates the most liquid smoke before decreasing. This is related to the breakdown of chemical substances in empty oil palm bunches. The decomposition of organic molecules in raw materials is caused by increasing the pyrolysis temperature. An increase in temperature causes cellulose, hemicellulose, and lignin to breakdown and condense into liquid smoke. The volume of liquid smoke produced is determined by the cooling system utilized, such as water for heat transfer. High temperature pyrolysis can reduce the volume of liquid smoke produced by increasing the water temperature, causing the smoke to fail to condense perfectly [16].

Total Phenol Analysis of Liquid Smoke from Empty Oil Palm Bunches

The total phenol test was performed to quantify the amount of phenol in the liquid smoke sample. Phenolic compounds are compounds that act as antimicrobials. Liquid smoke whose total phenol is determined is grade 2 liquid smoke. As stated by Saputra, et al. (2020), that grade 2 empty oil palm bunches liquid smoke produces a brownish blue to blackish color due to the reaction of acetic acid compounds which are the dominant compounds in liquid smoke. Compounds that have a carboxylate functional group (R-COOH) when reacting with the Folin-Ciocalteu reagent will make the solution turn blackish blue.

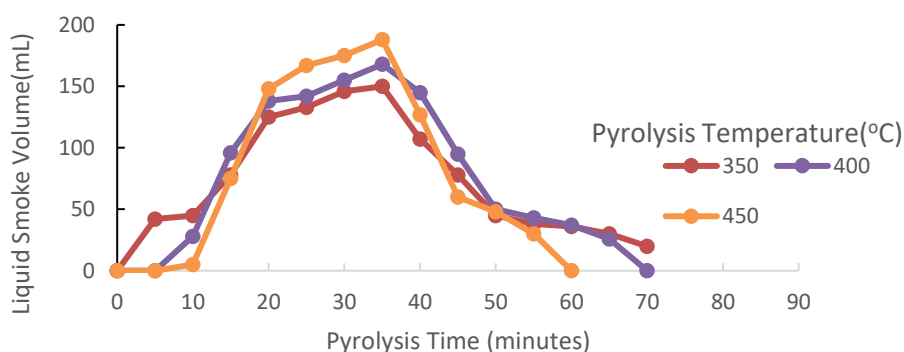


Figure 1. Relationship between pyrolysis time and the volume of liquid smoke obtained at several temperatures.

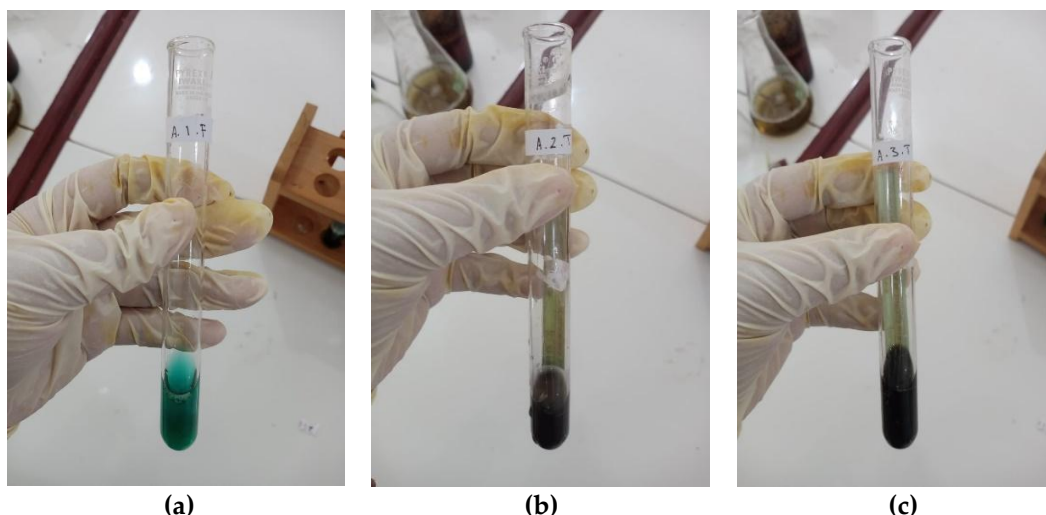


Figure 2. Analysis of Total Phenol Content with variations in pyrolysis temperature (a) 350°C, (b) 400°C and (c) 450°C

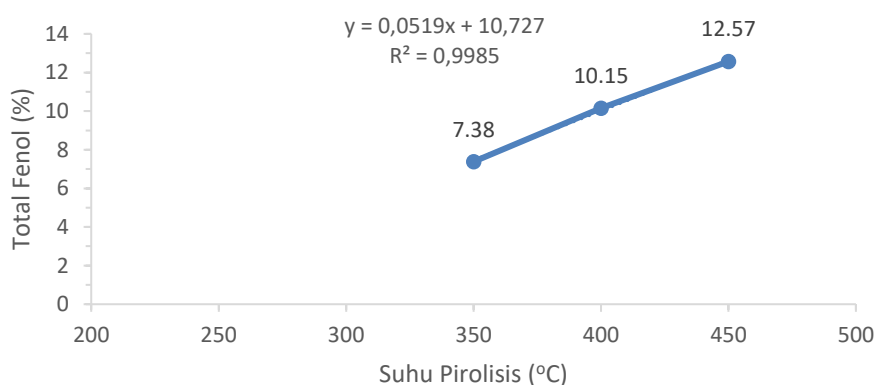


Figure 3. Effect of Pyrolysis Temperature on Total Phenol Content

In line with this, Figure 2 shows that the liquid smoke given the Folin-Ciocalteu reagent is blackish blue. It can be concluded that empty oil palm bunches liquid smoke contains phenol compounds. The Folin-Ciocalteu reagent has several advantages including short analysis time, easy processing, inexpensive reagents and uncomplicated instruments [17].

Phenolate ions cause the formation of a blue color in the sample which is caused by the decomposition process of H^+ ions in phenolic compounds. The addition of sodium carbonate (Na_2CO_3) provides a basic atmosphere to the solution so that oxotungstate and oxomolybdate compounds are formed. The more the concentration of phenolic compounds in an extract, the more phenolate ions are formed,

which causes the sample to become more concentrated [18].

Picture 3 explains the effect of pyrolysis temperature used on phenol content, where the higher the pyrolysis temperature used, the higher the total phenol content will be, which is 12.57%. The higher the heating temperature of liquid smoke, the more lignin components in the wood will break down. Thus, the phenol content produced will increase, which is indicated by the increasing absorption measured [19].

The coefficient of determination value obtained is close to 1 indicating that the regression equation obtained is linear with the equation $y = 0.0519x + 10.277$, and the R^2 value = 0.9985. The results show that there is a significant

relationship between pyrolysis temperature and total phenolic content.

Burning of bunches Empty palm oil using limited oxygen causes the decomposition of lignin compounds into phenolic compounds and their derivatives. The resulting phenolic compounds will later be used as natural fungicides.

Total Acid Content Analysis

The acid content there is on liquid smoke determine the quality of liquid smoke produced. The acid content in liquid smoke is used as an antimicrobial [20]. Total acid content values of liquid smoke at various temperatures pyrolysis can be seen in Table 1.

Based on the data in table 1, it shows that the highest total acid content was obtained from the pyrolysis of empty oil palm bunches using a temperature of 450°C, namely 38.4%, followed by temperatures of 400°C and 350°C of 32.5 mg/L and 20.6 mg/L respectively. The higher the pyrolysis temperature used, the higher the total acid content obtained. This is in line with the research of Akbar et al (2013) which states that the total acid produced is based on the high pyrolysis temperature. The higher the pyrolysis temperature, the more cellulose and lignin compounds are decomposed into acidic chemical compounds, such as acetic acid. This is reinforced by the research of Juwita, et al. (2016) stating that liquid smoke produced at a pyrolysis temperature of 350°C has a low total acid content, because organic compounds such as phenol, guaicol and syringol only decompose at temperatures above 350°C. The total acid content is also influenced by the phenol content contained in liquid smoke, the higher the phenol content, the higher the total acid content [21].

Inhibitory Power of Empty Oil Palm Bunches Liquid Smoke Against *Colletotrichum* sp.

Fungus

To see the effectiveness of empty oil palm bunches liquid smoke, the antifungal index was determined against various variations of liquid smoke concentrations of 10%, 20% and 30% at pyrolysis temperatures of 350°C, 400°C and 450°C respectively. Determination of the antifungal index was performed by measuring the diameter of the inhibition zone of the *Colletotrichum* sp. fungus tested using a caliper, so that measurement data was obtained from the results of observations during the 2-day incubation period, then calculated using the antifungal index formula, and the data was processed statistically to obtain the data in Table 2.

Based on table 2, it is known that liquid smoke from empty oil palm bunches has antifungal properties. The administration of liquid smoke from empty oil palm bunches with a pyrolysis temperature of 450°C at all concentrations of 10%, 20%, 30% showed a very significant difference against mancozeb 2%, but the administration of liquid smoke from empty oil palm bunches at temperatures of 350°C and 450°C did not have a significant effect on the control treatment of mancozeb 2%, then the administration of liquid smoke at levels of 2% to 5% each showed a significantly different effect on each treatment. The results of the study showed that liquid smoke from empty oil palm bunches has antifungal properties as indicated by the results that are directly proportional between the increase in the concentration of liquid smoke from empty oil palm bunches and the resulting inhibition power.

Table 1. Results of analysis of total acid content from pyrolysis at various temperatures

Pyrolysis Temperature (oC)	Total Acid (mg/L)
350	20.6
400	32.5
450	38.4

Table 2. Effect of pyrolysis temperature and liquid smoke concentration on antifungal index

Treatment		Antifungal Index (%)
Pyrolysis Temperature (oC)	Liquid Smoke Concentration (%)	
350	Control (0%)	0.00a
	10	10.75b
	20	23.21b,c
	30	41.10c,d
	Mancozeb 2%	56.61d
400	Control (0%)	0.00a
	10	11.47b
	20	31.85 b,c
	30	56.12d
	Mancozeb (2%)	53.13d
450	Control (0%)	0.00a
	10	18.21b
	20	84, 28e
	30	97.19f
	Mancozeb (2%)	57.79d

Information: Means followed by the same letter in the same column are not significantly differ ($P>0.05\%$).

Table 3. Fungal inhibition response *Colletotrichum* sp. on the provision of liquid smoke from empty oil palm bunches with various concentrations.

Treatment		Fungal Power <i>Colletotrichum</i> sp. (mm)	Inhibitory Response (Growth Inhibition)	Barrier
Pyrolysis Temperature (oC)	Liquid Smoke Concentration (%)			
350	Control (0%)	0.00	Weak	
	10	0.87	Weak	
	20	1.00	Weak	
	30	1.00	Weak	
	Mancozeb 2%	1.26	Currently	
400	Control (0%)	0.00	Weak	
	10	1.47	Currently	
	20	1.65	Strong	
	30	2.00	Strong	
	Mancozeb (2%)	1.36	Currently	
450	Control (0%)	0.00	Weak	
	10	1.85	Strong	
	20	4, 28	Very strong	
	30	7.73	Very strong	
	Mancozeb (2%)	1.87	Strong	

Table 4. Growth inhibition categories based on Purwantisari, et al. (2023).

Clear Zone Diameter (Clear Zone Diameter)	Growth Barrier Response (Growth Inhibition Response)
> 2mm	Very strong
1.6 – 2mm	Strong
1.0 – 1.5mm	Currently
< 1mm	Weak

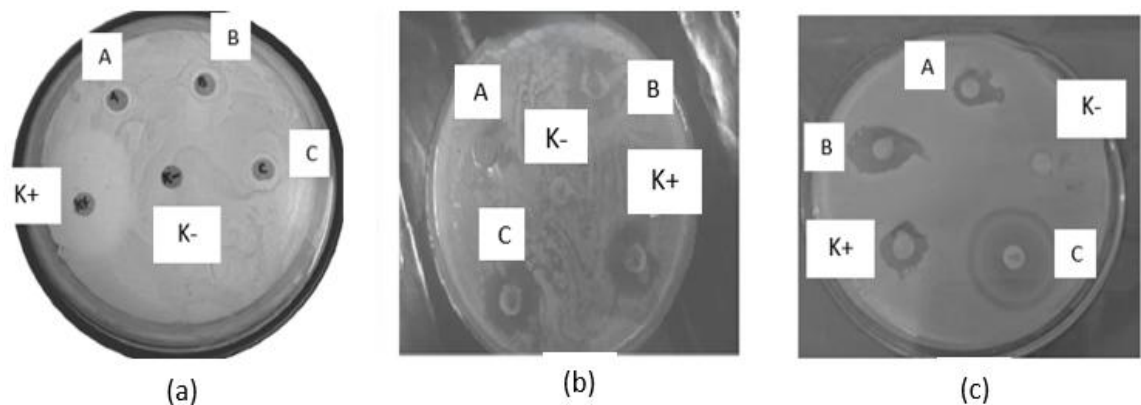


Figure 4. Clear zone formed from the treatment of giving liquid smoke from empty oil palm bunches at pyrolysis temperatures of (a) 350°C, (b) 400°C, and (c) 450°C, with liquid smoke concentrations of (A) 10%, (B) 20%, (C) 30%, K⁻ (control) and K⁺ (Mancozeb 2%)

This is in accordance with the inhibitory response of *Colletotrichum* sp. fungi to the administration of liquid smoke from empty oil palm bunches which can be seen in table 3. Based on the data obtained in table 3, there is an inhibitory response from liquid smoke and mancozeb as a positive control in inhibiting *Colletotrichum* sp. fungi. which is marked by the presence of a clear zone formed, but not in the negative control (0% liquid smoke) there was no clear zone formed. The presence of an inhibitory response is determined by influencing the clear zone obtained. The larger the diameter of the clear zone formed, the stronger the inhibitory response of the empty oil palm bunches liquid smoke.

The use of mancozeb as a positive control showed a clear zone formed of 1.87 mm. Mancozeb is a synthetic fungicide that can

control the development of pathogenic fungi. The active ingredient included in the M3 group, namely Dithio-Carbamate, the Dithio-Carbamate group is a fungicide that reacts (works) and inactivates the sulfhydryl group of amino acids and fungal cell enzymes that cause lipid metabolism and respiration disorders. However, based on Figure 4, the clear zone of *Colletotrichum* sp produced by liquid smoke is relatively larger than that of mancozeb. This is because liquid smoke contains phenol, acid and carbonyl compounds which have antimicrobial properties. Determination of the increasing inhibition response category is determined according to Purwantisari, et al. (2023) in Table 4.

Liquid smoke of empty oil palm bunches at pyrolysis temperatures of 400°C and 450°C has a clear zone with a strong and very strong

inhibitory response category. Liquid smoke concentration of empty oil palm bunches 20% and 30% with a pyrolysis temperature of 450°C provides a very strong inhibitory response to the growth of *Colletotrichum* sp fungi with values of 4.28 and 7.73 mm respectively. While at a pyrolysis temperature of 350°C with various concentrations provides a weak growth inhibition response. The results of the study indicate that there is a relationship between pyrolysis temperature and the concentration of liquid smoke of empty oil palm bunches where the higher the temperature, the higher the ability to inhibit fungi, due to the large number of active ingredients contained in liquid smoke such as phenol groups, organic acids, and carbonyl compounds which are known to have antimicrobial properties that can inhibit the growth of pathogenic fungi.

From the image above, it can be seen that the liquid smoke produced at a pyrolysis temperature of 450°C provides the largest clear zone diameter at various concentrations. This is in line with research conducted by Romauli, et al. (2020) which states that the higher the pyrolysis temperature, the more biomass substances (lignin, cellulose and hemicellulose) are decomposed, resulting in more phenol and organic acid compounds [22]. Factors that affect the content of secondary metabolites in liquid smoke include pyrolysis temperature, cellulose, hemicellulose and lignin content in the raw materials. In addition, the phenol and organic acid content in liquid smoke also greatly affects the response of fungal inhibition by damaging the pathogen membrane which causes the metabolic process to run imperfectly. The mechanism of damage to the pathogen cell membrane occurs because phenol compounds and their derivatives form bonds with one of the components of the pathogen cell membrane called ergosterol. The bond between phenol and ergosterol in the fungal cell membrane can form holes that cause components inside the cell to come out and the loss of cell contents, inactivation of essential enzymes and destruction or functional inactivation of genetic material. This is reinforced by research by Oramahi et al (2010) which states that liquid

smoke from empty oil palm bunches contains phenol (2-ethylphenol, 3-Methylphenol, 2,6-Dimethylphenol, 2,4 Dimethylphenol, and 3-hylphenol) and acid compounds (2,3 dihydroxybenzoic acid, 3-methoxybenzoic acid methyl ester, and 4-Hydroxy-benzoic acid methyl ester) contained in liquid smoke has the ability to denature proteins contained in the tested bacterial membrane because phenol is known as an antifungal compound. The same thing was also stated by Velmurugan et al. (2009) stated that the components of 2,6 dimethoxy phenol, dehydroacetic acid and 2,3,5 trimethoxytoluene in liquid smoke of sawdust and bamboo can act as antifungal [23]. In addition to the phenol content which acts as an antifungal, acid also has the same role as an antifungal agent. Karseno et al (2001) stated that the acids contained in liquid smoke of empty oil palm bunches include formic acid, ethanoic acid, acetic acid, and carbonyl acid. The antimicrobial properties of acetic acid in liquid smoke of empty oil palm bunches are related to pH conditions. The higher the pH, the less the ability of acetic acid to inhibit fungal growth. This is because acetic acid at high pH conditions (6-7) is unable to dissociate so that it penetrates more quickly into the fungal cell membrane. Meanwhile, formic acid and propionic acid are able to inhibit microbes by blocking the cell metabolism system through inhibition of enzyme activity [24].

Conclusions

Liquid smoke produced from various temperature variations used during the pyrolysis process positively contains phenol with the highest total phenol content at a pyrolysis temperature of 450°C, which is 12.57% and the volume of liquid smoke produced is 790 mL. The fungal inhibition zone test showed the most optimal results at a concentration of 20 and 30% empty oil palm bunches liquid smoke with a pyrolysis temperature of 450 oC which gave a very strong inhibitory response to the growth of *Colletotrichum* sp fungi with an average value of 4, 28 and 7.73 respectively.

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