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Effectiveness of Palm Empty Fruit Bunch Liquid Smoke Against Stem Base Rot Pathogen Oil Palm Plants in *In-Vitro*

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ABSTRACT

Ganoderma species significantly threaten oil palm cultivation, leading to substantial plant mortality. To mitigate the impact of Ganoderma, the application of fungicides has been a common practice. However, the prolonged use of these chemical agents poses risks to environmental sustainability. Consequently, there is a pressing need for alternative, eco-friendly solutions, one of which is using liquid smoke derived from empty oil palm bunches (TTKS). This study aims to determine the effective concentration of liquid smoke from empty oil palm bunches in inhibiting the growth of Ganoderma species. The research was conducted at the Integrated Laboratory of the Faculty of Agriculture, Pasir Pengaraian University, from November 2023 to January 2024. An experimental methodology was employed, utilizing a completely randomized design (CRD) that included six treatment groups with three replications each. The total phenol content was analyzed using the Folin-Ciocalteau method using the Smart Spectro tool. The growth rate of Ganoderma colonies was measured using a ruler, applying a formula based on the Crueger formula (1984) as referenced in Pulungan (2022). The effectiveness of the inhibitory action was calculated using the formula provided by Rakesh (2013). Data analysis was conducted descriptively, followed by statistical tests of variance (ANOVA). The findings of this study indicate that TTKS liquid smoke contains active compounds capable of inhibiting the growth of Ganoderma species, specifically phenol, at a concentration of 2.12 mg/L. The application of TTKS liquid smoke demonstrated 100% effectiveness in inhibiting the growth of Ganoderma in Treatment G3 at a concentration of 3%, followed by G4 at 4% and G5 at 5%.

Keywords: *Ganoderma sp*., Liquid Smoke, Palm Oil

1. INTRODUCTION

Indonesia has the largest oil palm plantations in the world, with a total area of 15,380,981 hectares and an annual production of 48,235,405 tons (Ministry of Agriculture of the Republic of Indonesia, 2022). The Central Statistics Agency of Rokan Hulu (2022) observed that the area of oil palm plantations in Rokan Hulu Regency in 2021 reached 267,791 hectares with a production of 695,965 tons yearly. This indicates that the potential for further expansion in the oil palm industry remains considerable, and there is a need to enhance oil palm productivity in Rokan Hulu Regency.

One of the key challenges to oil palm cultivation is the prevalence of pests and diseases. Fungi are pathogens that cause basal stem rot disease in oil palms (Widiastuti et al., 2016). *Ganoderma sp*. is one of Southeast Asia's most destructive major diseases affecting oil palm plants. In Indonesia, this disease significantly contributes to a decline in oil palm production per unit area in numerous oil palm plantations.

Disease prevention in oil palm plants can be achieved through using fungicides. Synthetic chemical fungicides are typically employed by farmers due to their ease of use and relatively short-term efficacy (Anggraini, 2017). Nevertheless, the prolonged utilization of fungicides may develop resistance, resurgence and the deposition of harmful residues that could compromise environmental sustainability (Susanto et al., 2013). In light of the detrimental effects associated with using synthetic fungicides, it is imperative to explore alternative, more environmentally benign options. One such alternative is using liquid smoke as a fungicide to control pathogens that cause basal stem rot disease in oil palm plantations (Sari et al., 2018).

Liquid smoke contains active compounds that can be employed as antimicrobials (antifungal) (Oramahi et al., 2010). The phenol compounds and organic acids present in liquid smoke can inhibit the growth of microorganisms. Consequently, liquid smoke can potentially be employed in controlling pests (Mugiastuti and Manan, 2009).

Thamrin (2007) posits that liquid smoke can inhibit the growth of *Ganoderma sp* due to the presence of phenol, carbonyl, and acetic acid in the liquid smoke. The efficacy of the inhibitory effect of the liquid substance against the *Ganoderma sp* pathogen is believed to be associated with the concentration of active ingredients present. Furthermore, liquid smoke comprises acid and carbonyl compounds synergistic with phenol, conferring antimicrobial properties (Annisa, 2021). In light of the background, as mentioned earlier, it is imperative to undertake a study examining the efficacy of liquid smoke derived from empty oil palm bunches in combating stem rot pathogens in oil palm plants in vitro.

2. MATERIAL AND METHOD

This study was carried out between November 2023 and January 2024 at the Integrated Laboratory of the Agrotechnology Study Program within the Faculty of Agriculture at Pasir Pengaraian University, located at coordinates 0.930653°N, 100.328758°E. An experimental approach employing a completely randomized design (CRD) was utilized, featuring six distinct treatments, each replicated three times. The total phenol content was analyzed at the Fishery Product Chemistry Laboratory of the Faculty of Fisheries and Marine Sciences at Riau University, utilizing the Folin-Ciocalteau method with the Smart Spectro instrument. The observational data were subjected to descriptive analysis and statistical variance analysis (ANOVA). In instances where treatment differences were identified, the analysis proceeded with the Least Significant Difference (LSD) test.

The study utilized several materials, including liquid smoke derived from empty oil palm bunches sourced from the Faculty of Engineering at Pasir

Pengaraian University, isolates of *Ganoderma sp* obtained from the Integrated Laboratory of the Faculty of Agriculture at the same university, as well as potato media, Dextrose Agar (PDA), distilled water, and 70% alcohol.

The equipment employed in this research comprised a laminar air flow cabinet, autoclave, test tubes, petri dishes, microscopes, glass slides, cover slips, spatulas, aluminum foil, Erlenmeyer flasks, cork borers, measuring cups, markers, plastic wrap, stationery, analytical balances, and gloves.

The parameters observed in this study encompassed both macroscopic and microscopic characteristics of *Ganoderma sp* colonies, the growth rate of these colonies, and the efficacy of liquid smoke in inhibiting *Ganoderma sp*. Data analysis was performed using SPSS software version 23, focusing on the parameters mentioned earlier, including the macroscopic and microscopic traits of *Ganoderma sp* colonies, the pathogen colonies' growth rate, and the inhibitory effects assessment.

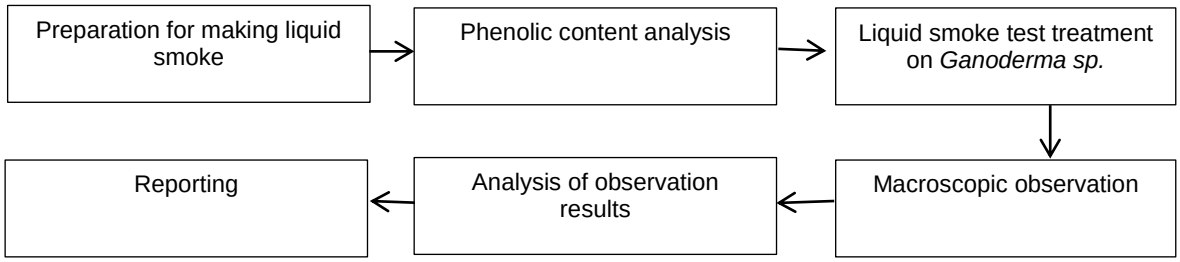

Figure 1. Research flow diagram

3. RESULT AND DISCUSSION

3.1. Analysis of Total Phenol Content of Liquid Smoke from Empty Oil Palm Bunches

The results of the research on the total phenol content in liquid smoke in this study can be seen in table 1 below:

Table 1. Total Phenolic Liquid Smoke of Oil Palm Empty Bunches

Sample Test	Total Phenol Content mg/L
Smoke Liquid	2,12

Source: Laboratory of Marine and Fisheries Products Chemistry, University of Riau

Table 1 illustrates that the analysis of phenol content yielded a result of 2.12 mg/L. This indicates that the phenol content of the present study is relatively low. Nevertheless, it can still impede the growth of *Ganoderma sp*. This is because, as Thamrin (2007) asserts, liquid smoke can inhibit the growth of *Ganoderma sp*. due to the presence of phenol and acetic acid in the liquid smoke. The efficacy of liquid substances in inhibiting the growth of *Ganoderma sp*. pathogens is believed to be associated with the concentration of active ingredients they contain. Furthermore, liquid smoke comprises acid and carbonyl compounds synergistic with phenol, conferring antimicrobial properties to the substance (Annisa, 2021).

3.2. Macroscopic and Microscopic Characteristics of Pathogenic Fungi *Ganoderma sp*

The results of macroscopic and microscopic observations of *Ganoderma sp* can be seen in Table 2. The results of the macroscopic and microscopic observations, as presented in Table 2 and Figure 2, indicate that the *Ganoderma sp* exhibits a white upper mycelial surface, a yellowish-white lower mycelial surface, a texture that is shaped like fibrous threads, and a wavy appearance. Subsequently, the morphology of *Ganoderma sp*. assumes a rounded shape aligned with the Petri dish's surface. The growth pattern is lateral.

The microscopic observations of *Ganoderma sp* revealed distinctive characteristics, particularly those about the basidiospores of the fungus. The microscopic observations of basidiospores revealed that they exhibited an oval shape, akin to an egg, with yellowish-brown outer walls and green inner walls, which resembled a core with a slightly concave surface.

The results of these observations are consistent with those reported by Luanghan (2021), who posited that the defining characteristic of the genus *Ganoderma sp* is the presence of double-walled and truncated basidiospores. This indicates that the pathogen isolates employed in the present study is highly likely to be *Ganoderma sp*..

Table 2. Macroscopic and Microscopic Observations of Pathogenic Fungi *Ganoderma sp*.

No	Observation	Result
1	Microscopic	
	a) Color of the upper surface of mycelium	a) White
	b) Color of the lower surface of mycelium	b) Yellowish white
	c) Texture of the surface of mycelium	c) Surface-like fibrous threads, and wavy
	d) Colony shape	d) Round following the petri dish
	e) Growth direction	e) Sideways
No	Observation	Result
2	Microscopic Basidiospores	Yes
	a) Basidiospores Shape	a) Ellipse
	b) Basidiospores Color	b) Dark green

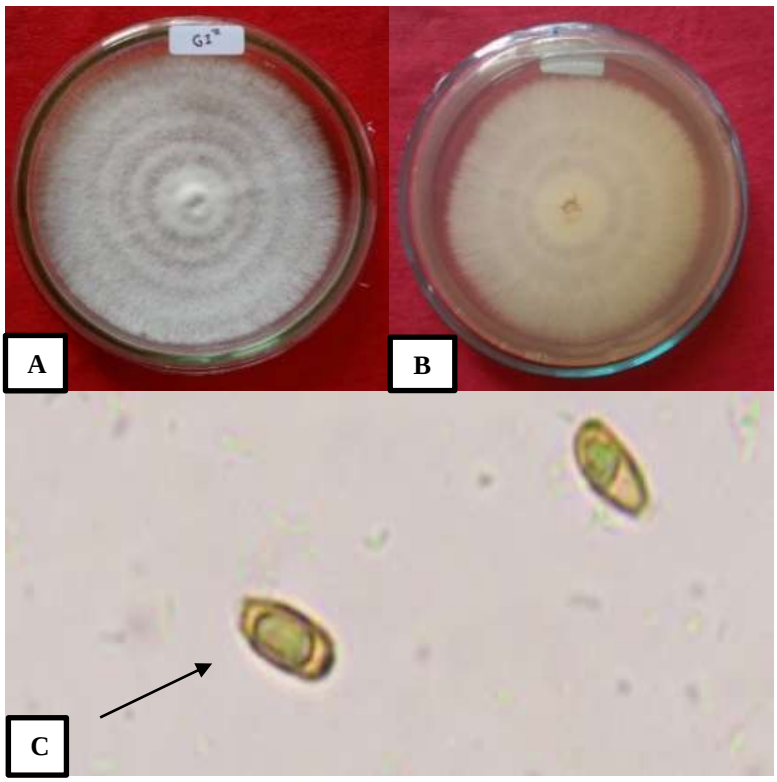

Figure 2. Macroscopic and Microscopic Pathogenic Fungus *Ganoderma sp*
(A) Macroscopic *Ganoderma sp* top view
(B) Macroscopic *Ganoderma sp* bottom view
(C) Microscopic *Ganoderma sp* basidiospores

3.3. Growth Rate of Pathogenic Fungi *Ganoderma sp*

The results of observations of the average growth rate of the pathogenic fungus *Ganoderma sp* can be seen in Figure 3. The observations presented in Figure 3 indicate that the growth rate of *Ganoderma sp* exhibits daily variations.

The peak growth rate of *Ganoderma sp* pathogens was recorded on the sixth-day post-incubation (HSI), achieving a growth measurement of 1.37 cm. Conversely, the lowest growth rate was observed on the first day of HSI, attributed to the lag phase experienced by the pathogen.

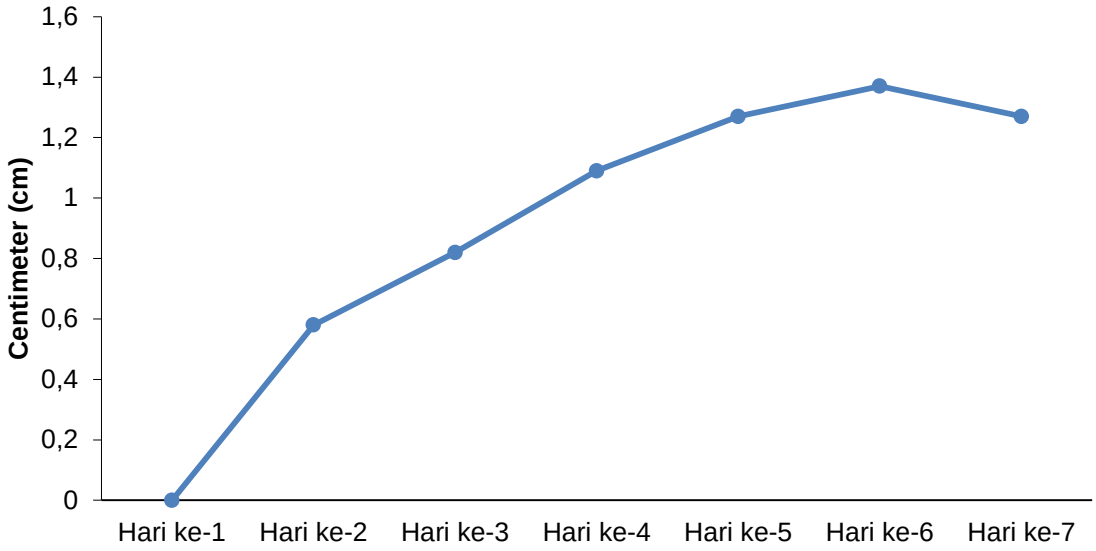

Figure 3. *Ganoderma sp* growth rate

Rolfe MD (2012) noted that the lag phase represents a period during which the pathogen acclimatizes to its new environment. The duration of this lag phase can vary significantly, influenced by factors such as the media composition, pH levels, temperature, aeration, initial inoculum density, and the physiological characteristics of the microorganisms present in the preceding media.

A relative decline in fungal growth was noted on the seventh day, marking the onset of the stationary phase. This phenomenon occurs when the growth rate of the *Ganoderma sp* pathogen stabilizes concerning the number of dead cells. Although the essential nutrient carbon has been depleted during this phase, it does not signify a complete cessation of growth for the *Ganoderma sp* pathogen, as lysis continues to play a role in its development (Hamdi, 2018).

3.4. Effectiveness of Inhibitory Power of Liquid Smoke from Empty Oil Palm Fruit Bunches on Pathogenic Fungi *Ganoderma sp* Growth.

The results of observations of the inhibitory power of liquid smoke from empty oil palm bunches on *Ganoderma sp* with various concentrations showed a significant effect. This can be seen in the following table 3:

Tabel 3. Average Effectiveness of Inhibitory Power of TTKS Liquid Smoke Against *Ganoderma sp* Pathogen

Treatment	Average Effectiveness of Inhibitory Power (%)
G0	0a
G1	5,55b
G2	27,77c
G3	100d
G4	100d
G5	100d

Description: Numbers followed by the same letter in the same column are significantly different based on the BNT test at the 5% level. G0: Control, G1 (Concentration 1%), G2 (Concentration 2%), G3 (Concentration 3%), G4 (Concentration 4%), and G5 (Concentration 5%).

The analysis of variance indicates that there are significant differences among the various treatments applied. This investigation identified the effective concentrations for controlling *Ganoderma sp* as 3%, 4%, and 5%. These concentrations completely inhibited *Ganoderma sp* growth, reaching 100%. This outcome can be attributed to the increased levels of active compounds in liquid smoke at these concentrations, which hindered the growth and development of *Ganoderma sp* on PDA media.

Mahmud (2021) notes that the ability to inhibit the growth of *Ganoderma sp* pathogens suggests the presence of active antimicrobial substances in liquid smoke derived from empty oil palm bunches. It was observed that higher concentrations resulted in a larger area devoid of fungal growth.

Supporting this, Zahrina (2020) emphasizes the correlation between concentration and the quantity of active ingredients in the formulation. As the concentration of a formulation increases, so does the amount of active ingredient, thereby enhancing its efficacy in suppressing the growth of *Ganoderma sp* pathogens.

4. CONCLUSION

Liquid smoke derived from empty oil palm bunches contains a phenol concentration of 2.12 mg/L. This liquid smoke demonstrates the ability to inhibit the growth of *Ganoderma sp*., achieving 100% effectiveness in Treatment G3 at a concentration of 3%. Additionally, Treatments G4 and G5, with concentrations of 4% and 5% respectively, also exhibit inhibitory effects.

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