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A STUDY ON THE EFFECTIVENESS OF LIQUID SMOKE PRODUCED FROM PALM KERNEL SHELLS IN INHIBITING BLACK POD DISEASE IN CACAO FRUIT IN VITRO

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ABSTRACT: The effectiveness of liquid smoke made from palm kernel shells in inhibiting black pod disease (*Phytopthora palmivora*) in cacao fruit was studied. Palm kernel shells underwent pyrolysis in a slow-pyrolysis reactor at 280°C–400°C. The resulting liquid smoke was then distilled at a temperature of 200°C. The observed parameters were incubation period and spotting diameter. The experimental design was completely randomized, with a 4 × 6 factorial pattern having four repetitions and consisting of two factors. Both temperature and concentration significantly affected the incubation period of the fungi causing black pod disease. Additionally, the liquid smoke concentration had a strong influence on the spotting diameter. Phenolic compounds and acetic acid contained in the liquid smoke serve as antimicrobials and are bacteriostatic. Although the maximum phenol level was found at 280°C, the longest incubation period occurred in liquid smoke produced at a pyrolysis temperature of 360°C. Thus, 360°C was the optimum temperature for producing liquid smoke to inhibit black pod disease caused by *P. palmivora* in cacao.

Keywords: Liquid smoke, Pyrolysis temperature, Biopesticide, Cacao black pod disease, Phytopthora palmivora

1. INTRODUCTION

A major developing industry in Indonesia is oil palm, which results in abundant biomass waste, including kernel shells, fronds and empty bunches. The potential of these waste products has not been utilized properly, even though Indonesia is an agricultural country with a large agriculture biomass. One method to utilize such potential is through pyrolysis to produce liquid smoke [1],[2]. Technology enables the processing of palm kernel shells into liquid smoke, which contains oxidized organic compounds, such as ketone, aldehyde, phenol, and carboxylic acid, resulting from the condensation of the pyrolysis vapor [3]. These compounds give liquid smoke its antioxidant and antibacterial properties, as well as its specific flavor [4]. Phenol and phenolic compounds possess bactericidal and bacteriostatic properties depending on the concentration used. Phenol and its derivatives function to denature proteins in bacterial cells and destroy cell membranes.

Because of the nature of some of its components, liquid smoke can also function as a plant-based insecticide and fungicide to inhibit disease-causing pathogens in agriculture. Previous studies on the effectiveness of liquid smoke in inhibiting anthracnose disease in chili plants have been conducted [1]. Liquid smoke made from palm kernel shells significantly influenced the incubation period of anthracnose, halting the symptoms created by the disease. Thus, the use of liquid smoke in inhibiting plant disease is a step toward supporting the 'go green' organic program.

Presently, farmers still use chemical/synthetic pesticides that can harm the environment and are not suitable for human consumption. The damage caused by synthetic pesticides to soil microbiology has been intensively studied in the past [5],[6]. Plant-based pesticides are potential alternatives [7]. Cacao black pod disease is caused by the Straminipile genus of *Phytophthora* [8]. This fungus can rot the pods at any age and causes a total production loss of ~30% [9],[10]. The infected pods display brownish black spots, usually starting at the stem, middle or end of the pods. Under humid conditions, the spots spread rapidly on the surfaces of the pods, rendering them black and rotten. This pathogen's spores are carried by wind and rain water, infecting other pods and spreading to other plant stems and branches.

This disease is difficult to control in a curative manner; therefore, preventive measures, such as fungicides, are used to stop its spread. Because of the phytopathogenic nature of this fungi, several of

the biological agents used to combat its associated diseases are Trichoderma species, such as *T. asperellum*, *T. harzianum*, *T. polysporum*, *T. viride* and *T. virens* [11]–[14]. A few effective chemical compounds used to control/reduce cacao black pod disease are metalaxyl- and copper-based fungicides [11],[15]. Additionally, chemical components containing essential oils, especially terpene, phenol and alcohol, are being considered for use as biopesticides because they have various biological activities [16],[17], such as insecticidal [18]–[20], antifeedant [21], repellent [22],[23], lure [24], insect growth inhibition [25], and antimicrobial [26],[27]. However, essential oils are mostly volatile, easily decompose, and are unstable when exposed to light and heat [28],[29]. Liquid smoke may serve as a better biopesticide because it is more stable, non- volatile and inexpensive.

Nonetheless, there has been nearly no in-depth research on the effectiveness of liquid smoke as a biopesticide. Research related to liquid smoke and its applications in agriculture are required to develop a sustainable agricultural industry. The utilization of waste and organic side products to produce liquid smoke will create an economic added value and an added social cultural value. This research studied the effectiveness of liquid smoke made from palm kernel shells as a plant-based fungicide against black pod disease caused by *Phytophthora palmivora* in cacao fruit (*Theobroma cacao*).

2. RESEARCH METHODS

2.1 Liquid Smoke Production

The slow-pyrolysis reactor made of stainless steel was 32 cm in diameter, 50 cm in height, and had a 5-kg capacity. This reactor was equipped with a tar container and temperature control. The upper temperature was 500°C. Samples of palm kernel shells were put in the reactor for a certain period of time and were pyrolyzed at temperatures ranging from 280°C to 400°C. The smoke was then condensed into liquid smoke using a stainless steel condensation unit (diameter of 50 cm and height of 60 cm), resulting in liquid smoke, Grade 3. This liquid smoke was then distilled at 190°C to become Grade 2. The complete process of liquid smoke production was previously published [1],[2]. The chemical compounds within the liquid smoke were identified using GC–MS based on a method developed by Guillen and Ibargoitia [30].

2.2 Sampling of Cacao Pods

Cacao pod samples were obtained from the People's Plantation at Pidie Jaya, Aceh, Indonesia. The media used was 21 × 21-cm mica boxes, each containing one cacao pod and labeled based on the treatment received.

2.3 Isolation and Cultivation of *P. palmivora*

The *P. palmivora* inoculum was isolated from infected cacao pods before it was cultivated on healthy pods. *P. palmivora* was then inoculated into healthy pods by puncturing the surface of the pod once using a straight pin and then placing these pods into the mica boxes. The punctured sites were then inoculated with *P. palmivora* using a small 3-mm cork borer. These inoculated cacao pods were then incubated for 24 h before liquid smoke was applied in 2–10% concentrations.

2.4 Liquid Smoke Application as Plant-based Fungicide in Cacao Pods

Liquid smoke was applied one day after *P. palmivora* inoculation by spraying the whole surface of the pods. The observed variables were (1) Incubation time: observations began one day after the inoculation of cacao pods with *P. palmivora* until the first symptoms appeared. (2) Spot diameter: Observations began two days after the inoculation of cacao pods with *P. palmivora* using a ruler and continued for the next five days. The data was statistically analyzed using the SPSS program for data in a completely randomized design, with a 4 × 6 factorial pattern having four repetitions and consisting of two factors. The first factor was temperature (T), T1 = 280°C, T2 = 320°C, T3 = 360°C and T4 = 400°C. The second factor was concentration (C), C0 = control, C2 = 2%, C4 = 4%, C6 = 6%, C8 = 8% and C10 = 10%.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Liquid Smoke

Liquid smoke is a compound formed during the burning of cellulose, hemicelluloses and lignin. The pyrolysis of these component results in phenol and acetic acid, which can have antioxidant and antimicrobial characteristics. Phenolic compounds have antibacterial and antifungal effects [31] that can kill rot-causing bacteria that degrade proteins into amino acids, thereby preventing the foul smell. Liquid smoke also possesses strong bacteriostatic properties that inhibit bacterial growth, as well as fungicidal properties that inhibit fungal growth. Phenol itself functions as a bactericide because of its ability to increase the permeability of cell membranes, inactivating essential enzymes and destroying or inactivating functional genetic

materials.

The pyrolysis of cellulose produces acetic acid and carbonyls, such as acetaldehyde, glucose and acrolein. The pyrolysis of lignin results in phenol, guaiacol and syringol, along with their homologs and derivatives. The numbers and types of compounds contained in the resulting liquid smoke depend on the pyrolysis temperature and the raw materials used [1]. An analysis of the liquid smoke composition at T3 is shown in Table 1. The phenol and acetic acid contents of liquid smoke are shown in Table 2. The maximum phenol content occurred at the T1 pyrolysis temperature, while that of acetic acid occurred at T2.

Tabel 1 Liquid smoke composition at 360°C

Peak	R. Time	Area	Conc.(%)	Name
1	5,700	1828077467	28.02	Acetic acid (CAS) Ethylic acid
2	6,110	451840165	6.93	Acetic acid (CAS) Ethylic acid
3	7,058	792450881	12.15	Propanoic acid (CAS) Propionic acid
4	7,322	632271906	9.69	Acetic acid (CAS) Ethylic acid
5	11,877	1477952354	22.65	Benzenamine (CAS) Aniline
6	12,658	164876203	2.53	2-Cyclopenten-1-one, 2-hydroxy-3-methyl- (CAS) Corylon
7	13,095	280722335	4.30	Phenol, 4-methoxy
8	13,640	103445521	1.59	cis-1,3-Dideuterio-1,3-cyclohexandiamine
9	14,258	74541045	1.14	2-Methoxy-4-methylphenol
10	14,863	153070215	2.35	1,3-Benzenediol (CAS) Resorcin
11	15,159	44016691	0.67	Phenol, 4-ethyl-2-methoxy- (CAS) p-Ethylguaiacol
12	15,292	67054314	1.03	3-Methoxy-pyrocatechol
13	15,925	195663312	3.00	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol
14	16,758	83136367	1.27	1,2,4-Trimethoxybenzene
15	17,887	175252398	2.69	1,6-Anhydro-beta-D-Glocopyranose

Tabel 2 The content of phenol and acetic acid in liquid smoke

No.	Pyrolysis temperature	Acetic acid (%)	Phenol (%)
1	280°C	1.29	0.73
2	320°C	2.06	0.62
3	360°C	1.20	0.31
4	400°C	1.86	0.36

Note : % = gr/100 ml

3.2 Incubation Time

Incubation time was observed to establish the effectiveness of liquid smoke concentrations applied on the cacao pods to halt the fungal growth that causes black pod disease. Incubation periods were marked by the presence of brownish black spots on the surface of the pods, which will look clear under a magnifying glass. The application of liquid smoke produced at various temperatures and concentrations affected the incubation time differently. However, the temperatures and concentrations did not have visible interactions. The average incubation times of *P. palmivora* after the application of liquid smoke produced at different temperatures are shown in Figure 1.

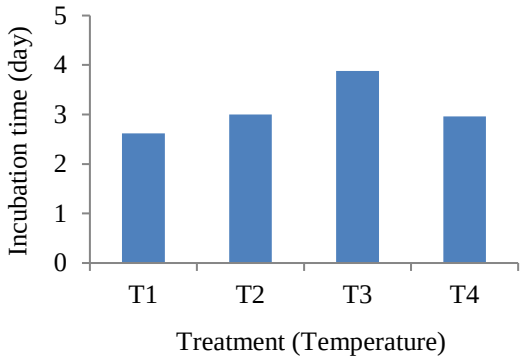

Fig.1 Average incubation time of *P. palmivora* after the application of liquid smoke (6%) produced at different temperatures.

The temperature with the longest incubation time of 3.88 days was T3. Meanwhile T1, T2 and T4 had similar incubation times, namely 2.62 days, 3.00 days and 2.96 days, respectively. Thus, T3 was effective in inhibiting the growth of *P. palmivora* as proven by the resulting longer incubation time, which is a criterion of resistance against pathogen infection. Longer incubation times mean that the symptoms are slow to appear because the pathogen's growth is inhibited.

The longer T3 incubation time was probably because of the chemical contents of the liquid smoke produced at T3. According to Lin *et al.* [32], liquid smoke's antifungal properties were influenced by its pyrolysis temperature. Similar results were shown on the effectiveness of liquid smoke in inhibiting *anthracnose* disease in chilies [1], and temperatures significantly affected the *anthracnose* incubation time.

Figure 2 shows the average incubation time of P. palmivora after the application of liquid smoke at several concentration levels. The highest concentration at C4 produced markedly different results from C0 and C2, but not significantly different from C6, C8 and C10, although the

incubation times at C2, C4, C6, C8 and C10 were markedly different from that of C0 (control). The lower incubation time at C0 occurred because no liquid smoke had been applied, leaving the pathogen free to infect the surface and the fruit of the cacao pods, resulting in the rapid display of symptoms. Thus, C4 should be used to control the disease because it inhibited black pod disease longer in cacao.

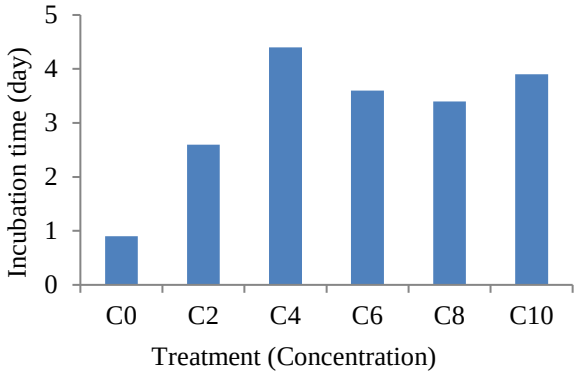

Fig. 2 Average incubation time of P. palmivora after the application of liquid smoke (from T3) at several concentration levels.

The high content of the functional components, phenol and organic acid, in C4 was able to destroy the fungal cell membranes, slowing its growth. The phenol compound increases the permeability of the fungal cell membranes, leading to the loss of nuclei, and the functional inactivation of genetic materials and essential enzymes [33].

3.3 Spot Diameter

Diameter observation by consistently measuring the spots on the long side was carried out every day from the pathogen inoculation until infection symptoms began to show. The growth of *P. palmivora* was marked by mycelia that form brownish black spots or concave dry black spots on the surface of the infected cacao.

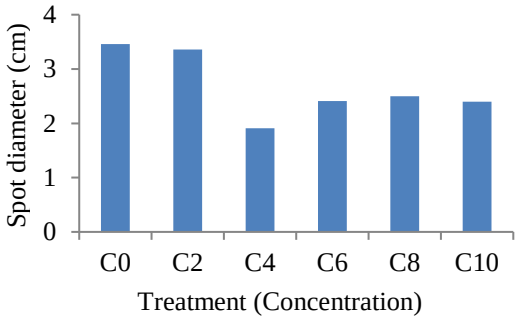

Fig. 3 Average spot diameters of the fungus after the application of liquid smoke at several concentration levels.

This pathogen can infect internal tissues of the fruit, causing the cacao seeds to wrinkle and change colors during infection [34]. An analysis of variance showed that several liquid smoke concentration levels produced significant effects on the spot diameter of the fungus that causes cacao black pod disease.

4. CONCLUSION

Different temperatures and concentrations had significantly different effects on the incubation time of *P. palmivora*, the causal agent of black pod disease, and the concentration had markedly significant effects on the diameter of the spots. The phenol and acetic acid compounds contained in the liquid smoke have antimicrobial and bacteriostatic properties. The optimum conditions for inhibiting the spread of black pod disease in cacao were 360°C during liquid smoke preparation and the use of 4% liquid smoke.

5. ACKNOWLEDGEMENTS

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