***In-vitro* ANTIFUNGAL EFFECT OF THE CAYENNE LEAF EXTRACT MODIFIED WITH LIQUID SMOKE FROM COCOA POD HUSK TO CONTROL *F. OXYSPORUM* ON CHILI (*CAPSICUM ANNUUM L.*)**

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ABSTRACT

This study aims to evaluate the in vitro ability of biopesticides from a mixture of liquid smoke and cayenne pepper leaf extract to control the fusarium wilting on the chili pepper. Liquid smoke was prepared through the pyrolysis of cocoa pod husk at 380 (T1), 420 (T2), and 460°C (T3). The cayenne leaf extract containing flavonoid compounds was obtained by maceration process using n-hexane solvent. Biopesticides were prepared by mixing 2% liquid smoke (v/v) and cayenne leaf extract powder at the concentrations of 0.3, 0.6 and 0.9% (w/v). The test results showed that the cocoa pod husk pyrolyzed at 420°C produced liquid smoke with the highest phenol and acetic acid content of 0.343% and 0.619% respectively. The study showed that liquid smoke (at various pyrolysis temperatures) and the cayenne leaf extract concentrations influenced the inhibition of *Fusarium oxysporum* on the chili pepper. The biopesticide inhibition test in this study showed that the highest inhibition of 81.1% was achieved in T2 liquid smoke and 0.9% cayenne leaf extract (w/v).

Keywords: Liquid Smoke, Cayenne Leaf Extract, Flavonoid, *Fusarium Oxysporum*, Inhibition.

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INTRODUCTION

Indonesia is one of the top producers of cocoa beans. The Ministry of Agriculture of the Republic of Indonesia reported that cocoa production in 2019 amounted to 596,477 tons harvested from a total area of 1,683,868 hectares.¹ With such a volume, the cocoa production leaves a huge amount of agricultural waste in the form of cocoa pod husk, which is potential to be made liquid smoke as it consists of lignin (51.02%), cellulose (17.27%), hemicellulose (18.90%) and other compounds.² Other materials from agricultural by-products that have the potential to be liquid smoke include durian shell,³ oil palm empty fruit bunches (OPEFB),⁴ and palm kernel.⁵

Liquid smoke has a function as a biopesticide because it contains phenol, ketone and acetic acid components⁶ with phenol component itself functions as a bactericide due to its ability to increase the permeability of cell membranes, inactivating essential enzymes and destroying the functional genes of bacteria. Liquid smoke is effective in inhibiting the fungus *Phytophthora palmivora*.⁷ Another material from plants containing phenol compounds is cayenne leaf extract, as phenol is one of the secondary metabolites commonly found in plants.⁸ In addition to phenol compounds, cayenne leaf extract also

contains long-chain carboxylic acid compounds and their derivatives that function as antimicrobials and antioxidants. Cayenne leaf extract is effective in preventing pathogen growth by forming an inhibition zone > 13 mm against *Staphylococcus aureus*.⁹

The agricultural industry in tropical countries including Indonesia is faced with many challenges. Some economically important food crops, for example, chili pepper, are susceptible to various types of plant diseases. One of the diseases that has caused losses and crop failure of nearly 50% in chili pepper production is fusarium wilt caused by *Fusarium oxysporum* (*F. oxysporum*) fungus.¹⁰ *F. oxysporum* is one of the pathogenic fungi that can cause health effects on plants, animals, and humans.¹¹ The pathogenic fungus is soil-transmitted and usually destroys the roots in various horticultural crops such as tomatoes,¹² causing plants to wither and then rot.¹³ In controlling this plant disease, farmers usually rely on synthetic chemicals¹⁴, with trifluoromethylpyrazole acyl urea¹⁵ being the most-frequently-used synthetic pesticide.¹⁴ However, the excessive use of synthetic chemicals can cause harmful effects on humans and the environment and negatively impacts biodiversity. Also, the use of synthetic pesticides causes various chronic diseases in humans.¹⁶

Today the use of natural pesticides has also been widely carried out, including the use of pyraclostrobin to control the pathogenic *F. oxysporum*. However, this method is not effective because it can interfere with the growth of the roots of the adjacent young plants.¹⁷ One other alternative that can be used to inhibit the growth of this pathogenic fungus is liquid smoke from the cocoa pods husk. The phenolic compounds in cocoa pod husk can cause protein coagulation to the microbial cells, thus causing microbial inhibition.¹⁸ According to a study by Lecomte et al.¹⁹, to control the pathogenic *F. oxysporum* fungus, antagonistic microorganisms or extracts from plants can also be used.

This paper reports a preliminary study on the combination of liquid smoke from cocoa pod husk (LS-CPH) and cayenne leaf extract as an alternative biopesticide for controlling in vitro the fusarium wilt in pepper plants.

EXPERIMENTAL

Preparation of Liquid Smoke

Three kg of raw materials of cocoa pod husk was put into the pyrolysis reactor. It was pyrolyzed at the temperatures of 380 (T1), 420 (T2), and 460°C (T3) and the resulting smoke was condensed to produce liquid smoke. The preparation procedure was reproduced verbatim from the previous research methodology.²⁰ The resulting liquid smoke was tested for phenol and acetic acid content using UV-Vis Spectrophotometry (Agilent Cary 60 UV-Vis) and High-Performance Liquid Chromatography (HPLC) (Hitachi L-4200H).

Extraction and Evaporation of Cayenne Leaf

One kg of cayenne leaf was dried (desired moisture content: less than 5%) and mashed. The dried powder of cayenne leaf was macerated using n-hexane solvent (1:3 w/v) for 24 hours to remove the sample fat, then filtered using filter paper. The fat-free powder was dried and macerated in 95% ethanol (1:3 w/v) for 3 days, then filtered and evaporated at 80°C using a rotary evaporator (Eyela N-1100SWD).²¹

F. oxysporum Preparation and Inhibition Test

The equipment used was first sterilized using an autoclave at 121°C for 15 minutes. The media was prepared as follows: PDA powder (potato dextrose agar) was weighed, put into an Erlenmeyer, then dissolved with distilled water and stirred until it was homogeneous. The agar media was then sterilized using an autoclave. After sterilization, the media was cooled to $\pm$ 45-50°C. A 1 mL mixture of liquid smoke and cayenne leaf extract was put into a petri dish using a micropipette, and then 9 mL of PDA gelatin was added. The agar media was then left to harden. *F. oxysporum* fungus was obtained from the isolation at the Laboratory of Plant Diseases. The fungus was inoculated into agar media that had been pierced with a 9 mm cork borer. The media was incubated at room temperature and observations of the diameter of the fungus were carried out once every 24 hours for 7 days.

The chili pepper (*Capsicum annum* L.) media was prepared as follows. Red chili pepper was sterilized and a 4 mm hole on the chili surface was drilled, into which *F. oxysporum* fungus was inoculated. Then the entire surface of the red chili was sprayed with a mixture of liquid smoke and cayenne leaf extract.

Each of the fungus diameters on the chili was observed and measured every 24 hours for 3 days. For comparison, a controlled media was provided, to which no mixture of liquid smoke and cayenne leaf extract was added. The inhibition of the fungus growth was calculated as follows.²²

$$P = \frac{(a-b)}{a} \times 100 \% \quad (1)$$

P : Inhibition

a : Diameter of fungi colony (control)

b : Diameter of fungi colony (treatment)

RESULTS AND DISCUSSION

Analysis Results of Liquid Smoke Content

The test results of liquid smoke on HPLC and UV-Vis Spectrophotometry for the percentage of its phenol and acetic acid content at various pyrolysis temperatures are presented in Table-1.

Tabel-1: Phenol and Acetic Acid Content in Liquid Smoke at Various Pyrolysis Temperatures

Liquid Smoke	Phenol (%)	Acetic Acid (%)
T1	0.307	0.202
T2	0.343	0.619
T3	0.334	0.150

From Table-1, it can be seen that T2 liquid smoke has the highest content of phenol and acetic acid, which is 0.343% and 0.619% respectively. The phenol content found in liquid smoke is influenced by the lignin content in it. The higher the lignin content is, the higher the phenolic content will be.²³ Liquid smoke has two main compounds with a bactericidal/bacteriostatic effect, namely, phenol and acetic acid in the form of a combination of compounds that work effectively to influence microbial growth.²⁴ Detail composition of liquid smoke from cocoa pod husk has been discussed by Desvita *et al.*²⁵ Based on this, liquid smoke can act as a natural pesticide to control various pest organisms in various crops. Several previous studies have also reported the content of phenols and acetic acid in liquid smoke with various concentrations. It was reported that cashew nut husk contained 36.6% phenol and 18.8% total acid.²⁶ Liquid smoke from corn cobs had a phenol content of 1.22% and a total acid of 9.60%.²⁷ Liquid smoke from coconut shells had phenol content of 2.71% and acid 0.07%, while liquid smoke from palm shells contained phenol compounds of 3.06% and acid compounds of 5.13%.²⁸

F. oxysporum Inhibition by LS-CPH

Test Result of PDA Media

The inhibition test of LS-CPH against *F. oxysporum* fungus on PDA media was carried out by inoculating pure fungus by diffusion method on PDA media. The amount of LS-CPH inhibition in PDA media treated with various types of liquid smoke (T1-T3) and concentration of cayenne leaf extract can be seen in Figs.- 1 and 2.

Fig.-1: LS-CPH Inhibition to the Growth of *F. oxysporum* Fungus on PDA Media for Various Liquid Smoke (T1-T3) with an Incubation Period of 24 hours.

Fig.-2: LS-CPH Inhibition to the Growth of *F. oxysporum* Fungus on PDA Media for Various Liquid Smoke (T1-T3) with an Incubation Period of 72 hours

Figures-1 and 2 show LS-CPH inhibition to the growth of *F. oxysporum* fungus on PDA media with an incubation period of 24 hours and 72 hours. LS-CPH inhibition against *F. oxysporum* fungus on T2 liquid smoke and cayenne leaf extract at 0.9% concentration produced the highest inhibitory antifungal activity, which was 40.1% (72 hours of incubation period). The results of the inhibition analysis indicated the influence of the mixture concentration of liquid smoke and cayenne leaf extract on PDA media on the growth of *F. oxysporum* fungus. The inhibition results were in line with Table-1, where T2 liquid smoke has high phenol and acetic acid content. A higher concentration of liquid smoke will have a better inhibitory effect on fungus growth. The antimicrobial effect of liquid smoke is due to its phenol content. The higher the phenol content is, the higher the antimicrobial activity will be. The antimicrobial mechanism of the phenol compound in liquid smoke takes place by damaging the cell wall and increasing the permeability of the cell membrane.²⁹ The cayenne leaf extract can function as an antimicrobial because the extract in the form of ethanol, ethyl acetate, and methanol compounds can inhibit bacterial activity by interfering with cell membrane synthesis.²¹ Cayenne leaf extract contains phenols, which is 26.15 ± 0.76 (mg/g of dry extract weight) and flavonoids 12.84 ± 0.22 (mg/g of dry extract weight).^{9,30} The application of liquid smoke from cocoa pod husk pyrolyzed at 500°C to inhibit the growth of *F. oxysporum* fungus was carried out by Pallawagau et al.¹⁸ The study found that the fungal inhibition in the application of 8% liquid smoke was 83.30%, while at 9% concentration of liquid smoke, *F. oxysporum* fungus did not grow anymore. Also, another studies³¹ found that cocoa pod husk had the potential to inhibit *F. oxysporum* in tomato plants at the liquid smoke extract concentrations of 8% (v/v), with an inhibition result of around 23.8%.

Test Results on Chili Media

Fungal inhibition test on chili pepper was carried out by inoculating *F. oxysporum* fungus on the surface of the chili that had been pierced. The fungus was allowed to grow for a day, and then LS-CPH was applied to the entire surface of the chili.

Table-2: The Infection Diameter on Chili infected by *F. oxysporum* Fungus and the Inhibition Percentage of LS-CPH

Liquid Smoke	Cayenne Leaf Extract (%)	Infection Diameter (mm)/Inhibition (%)		
		24 hours	48 hours	72 hours
T1	0.3	0/0	13.6/30.6	18.6 /17.7
	0.6	0/0	11/43.9	16.1/28.7
	0.9	0/0	10.8/45.2	14.8/34.8
T2	0.3	0/0	6.1/68.8	7.8/65.7
	0.6	0/0	4.8/75.8	7.5/66.9
	0.9	0/0	2.9/85.4	4.1/81.8
T3	0.3	0/0	12.4/36.9	14.4/36.5
	0.6	0/0	8.8/55.4	10.8/52.5
	0.9	0/0	8.1/58.6	10.1/55.2

Table-2 shows the infection diameter on chili infected by *F. oxysporum* fungus and the inhibition percentage of LS-CPH. Fungus diameter increased from 24 hours to 72 hours. Media with T1 liquid smoke showed a larger diameter of infection than that with T2 and T3 liquid smoke. In the first 24 hours of incubation, chili media did not show any infection, while at 48 hours of the incubation period, the media showed *F. oxysporum* fungal infection, marked by the infection diameter on the chili media. The largest inhibition was obtained in media with T2 liquid smoke combined with 0.9% cayenne leaf extract concentration, that is, 85.4%. Meanwhile, the smallest inhibition was in T1 liquid smoke combined with 0.3% cayenne leaf extract, which was 30.6%.

At 72 hours of incubation, chili media had an increase in infection by *F. oxysporum*. This was indicated by the increasing diameter of infection of the media infected by the fungus. The largest inhibition was obtained in T2 liquid smoke combined with 0.9% leaf extract concentration, that is, 81.8%. The smallest inhibition was achieved in media with T1 liquid smoke combined with 0.3% extract concentration, which was 17.7%. High concentrations of phenol and acetic acid in T2 liquid smoke could increase the inhibitory effects of the infection diameter by *F. oxysporum* fungal infections. Furthermore, the increase in the concentration of cayenne leaf extract affected the effectiveness of the diameter inhibition as well. Cayenne leaves contain antibacterial compounds, namely, capsaicin and dihydrocapsaicin. Capsaicin has an antibacterial mechanism by interfering with cell membrane synthesis, whereas dihydrocapsaicin is selectively active against bacterial cell walls.²¹ A study by Rachmawaty et al.³¹ proved the potential of liquid smoke from cocoa pod husk to inhibit *F. oxysporum* fungal infection in tomato plants, with the highest inhibition obtained at 2% liquid smoke (v/v), that is, 54%. This shows that the inhibition obtained from the current study is higher than that of the previous study.

Fig.-3: The Preliminary Test of LS-CP as Biopesticide for *F. oxysporum* (a. Liquid Smoke T3 and 0.9% Cayenne Leaf Extract; b. Liquid Smoke T1 and 0.3% Cayenne Leaf Extract) (after 72 hours Incubation)

Figure-3 shows the differences in fungal infections on chili. Observation on the morphology of the fruit identified the symptoms of fusarium wilt characterized by the presence of brownish spots on the chili surface and it will become soft and rot. Symptoms of *F. oxysporum* fungal infection on plants are characterized by abnormal growth in leaves, where the leaves' green color fades away. Symptoms of wilting generally start from the leaves in the lower part of the plants then develop upward as the base of the stem begin to rot.³¹

In comparison, a study by Ceylan et al.³² found that the use of liquid smoke combined with thymol could inhibit bacterial growth by up to 60%. Fusarium wilt disease in tomato plants could also be overcome by one of the biological controls, namely the bacterium *Bacillus amyloliquefaciens*, but the inhibition percentage of *F. oxysporum* fungal growth is 48.3%.³³

CONCLUSION

LS-CPH has the potential as a biopesticide to control fusarium wilting in chili plants caused by *F. oxysporum* fungus. The smallest diameter of infection by *F. oxysporum* in chili pepper was obtained in T2 liquid smoke combined with 0.9% concentration of cayenne leaf extract. Under these conditions, the highest *F. oxysporum* fungal inhibition of 81.1% was achieved. The growth inhibition of *F. oxysporum* fungus was influenced by antimicrobial substances found in liquid smoke and cayenne leaf extract. The LS-CPH biopesticide is expected to replace the synthetic pesticides that have been used so far.

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