Application of Coconut Shell Liquid Smoke to Control Fusarium Wilt Disease on *Hevea brasiliensis* Muell. Arg.

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**ABSTRACT**

The study aims to observe coconut shell liquid smoke as a bio-fungicide in controlling *F. oxysporum* growth. The treatment was applied on sample i.e., coconut shield liquid smoke as a bio-fungicide, compared with a chemical fungicide as a positive control, and without treatment as a negative control. The direct observation was conducted for four weeks, with treatment application every seven days. The analysis of variance test shows the difference of three treatments in controlling the *F. oxysporum* growth for each observation period. Still, it has no difference in average in fungal growth after treatment. According to the results of weekly observations, coconut shell liquid smoke bio-fungicide reduces the disease severity on the tapping fields infected by *F. oxysporum* in the range of 13-21%, whereas chemical fungicide reduces the disease severity in the range of 1-10%. In conclusion, the ability of coconut shell liquid smoke bio-fungicide to control is relatively shorter compared to chemical fungicide. Based on the inhibition ability of coconut shield liquid smoke, it is recommended that utilize various grades of coconut shell liquid smoke as a comparison.

**INTRODUCTION**

*Hevea brasiliensis* Muell. Arg is one of Indonesia's top seven export commodities. Based on the data from the Association of Natural Rubber Producing Countries (ANRPC) in 2021, Indonesia is the second-largest rubber-producing country. Indonesia exports about 85% of its rubber to the international market. Rubber plantations distribution in Indonesia is large enough. Around 80% of them are accounted for by small farmers, including many independently personal-owned plantations. One of the rubber plantations owned by the local community in Alur Mentawak Village, Aceh Province, was attacked by *Fusarium oxysporum* on the tapping fields area. The character of *F. oxysporum* as a fungal pathogen is supported by its ability to grow as symptomless endophytes under many conditions and cause root or stem rots, cankers, wilts, fruit, or seed rots, and leaf diseases with varying severity (Leslie & Summerell, 2006).

*Fusarium* sp. also causes skin necrosis disease/bark necrosis (Ramadiani, Ramadhani, Jundillah, & Azainil, 2019).

*Fusarium* spp. is one of the plant pathogens with a diversity of affected hosts commonly found in terrestrial ecosystems and has several pathogen taxa and types of habitat (Ploetz, 2006). The fungus spreads very quickly and infects other plants. The tapping process potentially infects the other plant from tapping knives (Semangun, 2000). The fungus spore also spread through the wind (Fourie, 2008), groundwater, and soils infected and carried by agricultural and human tools (Agrios, 1996). *F. oxysporum* attaches to the tapping area, which is still wet or newly tapped, causes decaying, damages the stem bark, and causes an interrupted rubber tapping. A persistent condition of this lowers the rubber tree productivity (Li, Shi, Liu, Lin, & Huang, 2014).

Fungicides are used to control the fungus that infects the rubber trees. Chemical fungicides and bio-fungicides is used to manage the fungus *F*.
oxysporum. According to Yang, Hamel, Vujanovic, & Gan (2011), chemical fungicides negatively affect the fertility of soil microorganisms. In this research, bio-fungicides are used to inhibit the F. oxysporum and reduce the negative effects of chemical fungicide usage.

Liquid smoke is a natural material derived from plants with the anti-fungal potential to deal with pathogen interference in plants so that people have long used it as preservatives and insecticides (Darmadji, 2002). Liquid smoke is more environmentally friendly because it is biodegradable and renewable. Liquid smoke is made from various types of waste, but the kind of liquid smoke commercialized comes from coconut shells, teak, and falcata wood. Liquid smoke from the pyrolysis process contains lignin, cellulose, hemicellulose, and other carbon compounds. Liquid smoke that is for sale, usually made by going through various techniques, which are fractionation, purification, and concentration. The quality of liquid smoke depends on its chemical composition and is affected by the making process. Further, the refining process will remove undesirable polycyclic aromatic hydrocarbons (PAH) and adjust the intensity of flavor and color of the liquid smoke (Cadwallader, 2007). Compared to full-strength liquid smoke products, the application of refined liquid smokes generally will be more flexible to particular food systems.

Besides the various processes, raw materials, such as the wood type and moisture content, will affect the chemical compound of liquid smokes, the pyrolysis temperature, and the duration of smoke generation (Cadwallader, 2007). The coconut shell is a raw material composed of cellulose, hemicelluloses, and lignin in this research. According to Tegang et al., (2020), some factors that affect the composition of cellulose, hemicellulose, lignin, and extractive content, such as the age of the material, harvest period, and the climatic conditions when harvesting it. Further, the pyrolysis of lignin mainly will produce phenols and their derivatives. The condensation of material affect the compound and the condensate collected at 160°C can affect the biological activity of bacteria and fungi (Chu et al., 2019).

Through the pyrolysis process, a thermochemical process, the coconut shell will convert into a liquid (bio-oil/liquid smoke), gas, and solid (Zuraida, Sukarno, & Budijanto, 2011). Decomposition of thermal cellulose produces anhydroglucose, carbonyl-containing compounds, and furans. In comparison, acetic acid and carbon dioxide are produced by hemicellulose decomposition. The results of thermal degradation have a complex mixture of compounds. The lignin of the coconut shell generates various types of phenolic compounds (Miler & Sikorski, 1990). The whole pyrolysis process would produce the chemical compounds with organoleptic, antioxidative, and antibacterial characteristics (Wei et al., 2010). Liquid smoke contains acidic compounds, phenols, carbonyls, and tar (Darmadji, 2002; Indrayani, Oramahi, & Nurhaidai, 2011). The contents of acid and phenol compounds could kill bacteria and insects (Haji, 2013; Pangestu, Suswanto, & Supriyanto, 2014). According to Pangestu, Suswanto, & Supriyanto (2014), the major compounds in liquid smoke used as anti-microbial agents are phenol and acetic acid.

The coconut shell is agricultural waste, which usually is burned and used for cooking, then emission from it causes air pollution (Lombok, Setiaji, Trisunaryanti, & Wijaya, 2014). Because of lignin and cellulose, coconut shells are used as a raw material of liquid smoke, which is useful as a preservative through the pyrolysis process. The liquid smoke of coconut shells has the potential to kill bacterial (Hattula, Elfving, Mroueh, & Luoma, 2001). Compared to the other materials, coconut shells have several advantages. Coconut shell is categorized as hardwood with high lignin and low cellulose content (Grimwood & Ashman, 1975). The high lignin content in coconut shells will produce liquid smoke with high phenol content, which is acts as an anti-microbial (Kailaku, Syakir, Mulyawanti, & Syah, 2017; Lingbeck et al., 2014). From the pyrolysis process, there are three grades of coconut shell liquid smoke i.e., grades 1, 2, 3, which the extent of filtration length will affect each grade difference. Grade 1 is more refined which visible slightly very yellowish than other grades. Grade 1 is also commonly used as food preservatives. The Second Grade with yellowish color can be used as antioxidants and anti-microbial agents. In contrast, Grade 3 is darker than the other two grades and has a sharp odor, which can be used as rubber coagulant, wood preservatives, also odor absorbers (Kailaku, Syakir, Mulyawanti, & Syah, 2017).

Control of fungi by administering fungicides. The fungicide used can be in the form of chemical fungicides or bio-fungicides. Fungicides generally inhibit and act on cells or pathogenic parts and inhibit many functions of fungal metabolism (Misato & Kakiki 1977). The use of chemical fungicides harms humans and the environment. To reduce the negative impact on the environment, it is now widely used plant-based fungicides. One of the biofungicides used to control the growth of F. oxysporum is coconut shell liquid smoke. The purpose of
this study was to observe the ability of coconut shell liquid smoke bio-fungicide in controlling *F. oxysporum* growth on the rubber-tapping area of a rubber tree compared to chemical fungicide.

**MATERIALS AND METHODS**

**Research Location and Experimental Design**

The experiment was conducted from October to November 2017 in Alur Mentawak Village, Aceh. The rubber plantation owner produced liquid smoke with a pyrolysis reactor. This reactor was made of stainless steel, equipped with stainless steel pipe, tar catcher, cooling containers, and fluid smoke reservoir. The reactor served to burn coconut shells, and the pyrolysis process produces three substances, namely solid, liquid, and gas. The coconut shell liquid smoke produced in Alur Mentawak Village used in this study was created directly from pyrolysis without distillation and filtering. It is Grade 3 liquid smoke, according to Ariyani, Mujiyanti, & Harlianto (2015).

There are three types of treatment given on the tapping fields of rubber plants. Coconut shell liquid smoke used as bio-fungicide, Derosal 60 WP as chemical fungicide contains Carbendazim active compound was applied as a positive control, and plants without treatment served as a negative control. The liquid smoke bio-fungicide and chemical fungicide were applied by brushing them on the predetermined samples of a rubber plant in the observation area. The dosage is used depending on the size of the fungus attack on the tapping field areas.

The experiment was conducted in a 0.20 ha (80 m x 25 m) of plantation area, which includes 133 trees consisting of 32 unproductive (untapped) trees and 101 productive trees (tapped). The real abundant rubber trees were identified, whereas 36 trees were attacked by *F. oxysporum* and used as samples.

Data were collected from the weekly field observation after the treatment application. The data were recorded on the ability of fungicides to control fungal growth on tapping areas and records of the decrease of disease intensity. After seven days, the treatment was reapplied and then further observed.

**Disease Observation**

The observation of the rubber plant disease caused by *F. oxysporum* was conducted using the direct observation method for symptoms appearing on the plants. The severity of the disease (%) was measured concerning the formula proposed by Towsend & Heuberger (1943) is sum of scorings of different rating divided by (number of sample x highest rating) x 100%. The damage scale was measured using direct measurement, i.e., severity assessment and severity monitoring, by assigning a value from 0 to 100% depending on the affected parts (Kranz, 1988). The scale of disease severity shows in the Table 1 and Fig. 1.

**Data Analysis**

All experiments were observed and recorded once a week for one month. Statistical testing was carried out to see the difference in the average control of *F. oxysporum* growth after getting the three treatments i.e., coconut shell liquid smoke as a bio-fungicide, chemical fungicide as a positive control, and without treatment a negative control. The data obtained were analyzed using variance (ANOVA) with Microsoft Office Excel 2011 and SPSS Statistics version 17.0 software. Significantly different treatments were further tested using the Tukey Test.

**Table 1. The scale of disease severity**

| Scale   | Description                                                                 |
|---------|------------------------------------------------------------------------------|
| 0%      | No symptom of damage                                                          |
| 1-20%   | White fungus attached to the tapping area                                     |
| 21-40%  | Greyish white fungus                                                         |
| 41-60%  | Blackish gray fungus, smelly, and attacking cambium                          |
| 61-80%  | Blackish gray fungus, smelly, attacking cambium until turn black             |
| 81-100% | The fungus attacks the cambium until the tapping field is blackened and cannot be tapped again |
RESULTS AND DISCUSSION

Effect of the Coconut Shell Liquid Smoke on the Growth of *F. oxysporum*

The analysis of the variances test shows the difference between the three treatments. Each observation period shows the different variations of inhibition on the *F. oxysporum* growth (Table 2). The average of *F. oxysporum* growth without treatment has shown a significant difference from the early observation to week 4, compared to biofungicide (coconut shell liquid smoke) and chemical fungicides treatment. From the field observation, the growth of *F. oxysporum* is relatively fast within 4 weeks without treatment. The development is also affected by high humidity caused by rain when the observation is conducted. Rubber tapping fields are susceptible to fungal attacks during the rainy season and are influenced by environmental factors such as weather, wind, and humidity (Jayasinghe, 1999; Muklasin & Matondang, 2013; Ramadiani, Ramadhani, Jundillah, & Azainil, 2019; Semangun, 2000).

The difference shows on the rubber tapping fields treated with coconut shell liquid smoke biofungicide. At first week observation, after being treated by coconut shell liquid smoke, the *F. oxysporum* on the surface of the tapping field looks reduced (Fig. 2). Coconut shell liquid smoke bio-manages to inhibit the growth of a colony of *F. oxysporum* and prove that liquid smoke is toxic to this fungus (Mugiastuti & Manan, 2009).

The active compound content influences the coconut shell liquid smoke’s ability to control *F. oxysporum* growth is influenced by the dynamic compound content. This experiment uses grade 3 liquid smoke containing high phenol, which is also used as an anti-microbial, and it has the best inhibitory effect on microbes (Kailaku, Syakir, Mulyawanti, & Syah, 2017).

Fig. 1. Various damage caused by *F. oxysporum* (a) Scale 0%; (b) Scale 1-20%; (c) Scale 20-40%; (d) Scale 40-60%; (e) Scale 60-80%; (f) Scale 80-100%
Table 2. ANOVA result

| Observation Period | Without Treatment | Coconut Shell Liquid Smoke | Chemical Fungicide |
|--------------------|-------------------|----------------------------|--------------------|
|                    | n  | Mean | F_{stat} | p-value | n  | Mean | F_{stat} | p-value | n  | Mean | F_{stat} | p-value |
| Initial Condition  | 10 | 1.900 | 3.887    | 0.009    | 12 | 2.166 | 9.749    | 0.000    | 14 | 2.285 | 26.766 | 0.000   |
| Week 1             | 11 | 2.273 |          |          | 12 | .667  |          |          | 14 | .071  |          |         |
| Week 2             | 9  | 3.000 |          |          | 12 | .667  |          |          | 14 | .500  |          |         |
| Week 3             | 10 | 3.300 |          |          | 12 | .667  |          |          | 14 | .214  |          |         |
| Week 4             | 10 | 3.300 |          |          | 12 | 1.083 |          |          | 14 | .286  |          |         |
| Total              | 50 | 2.740 |          |          | 60 | 1.050 |          |          | 70 | .671  |          |         |

Remarks: Source: Processed data

Fig. 2. Daily visual observation of sample with coconut shell liquid smoke bio-fungicide; (a) before treatment; (b) coconut shell liquid smoke bio-fungicide application; (c) day 1 after treatment; (d) day 2 after treatment; (e) day 3 after treatment; (f) day 4 after treatment the fungus begin to grow

F. oxysporum grows in this area
The concentration of liquid smoke significantly affects the inhibition of colony diameter and dry weight of the fungus *F. oxysporum* (Mugiastuti & Manan, 2009). Based on the statistical test, the average growth of *F. oxysporum* with bio-fungicide treatment has no difference (Table 3), compared to chemical fungicide treatment which show the difference (Table 4). Based on direct observation, the disease severity average at 43% in the first week shows a decrease of up to 13% for three consecutive weeks but then rise to 21% in the 4th week. The inhibitory ability of bio-fungicides on the growth of *F. oxysporum* is relatively short.

**Table 3.** The different test of *F. oxysporum* growth average by observation period with coconut shell liquid smoke treatment

| Test Different Treat | Mean Difference | p-value |
|----------------------|----------------|---------|
| Tukey HSD            |                |         |
| Initial Condition    | Week 1         | 1.500'  | 0.000** |
|                      | Week 2         | 1.500'  | 0.000** |
|                      | Week 3         | 1.500'  | 0.000** |
|                      | Week 4         | 1.083'  | 0.005** |
| Week 1               | Week 2         | 1.000   | 1.000   |
|                      | Week 3         | 1.000   | 1.000   |
|                      | Week 4         | 0.620   | 0.620   |
| Week 2               | Week 3         | .00000  | 1.000   |
|                      | Week 4         | -0.416  | 0.620   |
| Week 3               | Week 4         | -0.416  | 0.620   |

Remarks: * = alpha 5%, ** = alpha 1%; Source: Processed data

**Table 4.** Different test of *F. oxysporum* growth average by observation period with chemical fungicide

| Test Different Treat | Mean Difference | p-value |
|----------------------|----------------|---------|
| Tukey HSD            |                |         |
| Initial Condition    | Week 1         | 2.21429 | 0.000** |
|                      | Week 2         | 1.78571 | 0.000** |
|                      | Week 3         | 2.07143 | 0.000** |
|                      | Week 4         | 2.00000 | 0.005** |
| Week 1               | Week 2         | -0.42857| 0.434   |
|                      | Week 3         | -0.14286| 0.979   |
|                      | Week 4         | -0.21429| 0.912   |
| Week 2               | Week 3         | 0.28571 | 0.784   |
|                      | Week 4         | 0.21429 | 0.912   |
| Week 3               | Week 4         | -0.07143| 0.999   |

Remarks: * = alpha 5%, ** = alpha 1%; Source: Processed data
The Potential of Coconut Shell Liquid Smoke as an Agent for Inhibiting *F. oxysporum*

At week 4, *F. oxysporum* begins to grow again, but on a minimum scale of damage. The disease severity average of 13% stayed for three consecutive weeks, then rose to 21% in week 4. This means liquid coconut shell smoke inhibits the growth only in a relatively short time. Grade 3 of liquid smoke is not undergoing a filtration process. Thus it contains phenol compounds higher than other compounds (Table 5). According to Fardiaz (1992), phenol could damage the cell membranes, inhibit cell growth, or cause the death of fungal cells. Phenol compounds diffuse in the fungus cell membrane and interfere with the metabolic pathway (Omidpanah, Sadeghi, Sarcheshmeh, & Manayi, 2015). Phenol compounds also denature the protein cells, and their anti-fungal mechanisms are involved in cell lysis (Cowan, 1999). Liquid smoke also contains levels of acid content that can kill bacteria and fungi (Darmadji, 2002; Pangestu, Suswanto, & Supriyanto, 2014).

This research was conducted directly on the tapping field in the rubber plantation so that environmental conditions affected the growth of fungi. The complexities of *F. oxysporum* species include a variety of strains, and most of these are saprotrophs. They present in soils ubiquitously (Bao et al., 2004) and rapidly recolonizes treated soils since they are facultative saprophytes (Ploetz, 2007). The distance between the tapping field and the ground is quite close, thus allowing the surface of the tapping field to be contaminated with *F. oxysporum*. The damage due to the fungus is difficult to inhibit. Besides, many factors also support fungal growth, a fungus spore that can spread through the wind (Fourie, 2008). Besides phenol, other types of compounds still need to be investigated to inhibit *F. oxysporum*.

Inhibition of Coconut Shell Liquid Smoke on *F. oxysporum* Compared to Chemical Fungicides

The application of bio-fungicides and biological fertilizers can increase the absorption of nutrients from the soil or atmosphere, as well as produce bioactive compounds, enzymes, and hormones that stimulate plant growth, even useful to reduce the potential of plant diseases by inhibiting pathogens growth (Kaewchai, Soytong, & Hyde, 2009). Coconut shell liquid smoke controls the fungus and is used as an alternative substitution to chemical fungicides. Bio-fungicide is more eco-friendly use.

**Table 5.** Analysis result of coconut shell liquid smoke by GC-MS

| Retention Time (Minutes) | Chemical Compounds                  | % Area  |
|--------------------------|------------------------------------|---------|
| 3.165                    | Tetrahydro-1,3-tgiazine-2-thione    | 0.242   |
| 3.550                    | 2(3H)-Furanone, dihydro-(CAS) Butyrolactone | 2.53   |
| 3.580                    | 2(3H)-Fusarone, dihydro-4,5-dimethyl | 1.55   |
| 3.650                    | Oxirane, trimethyl- (CAS) 2-METHYL-2,3-EPOXY-BUTANE | 0.91   |
| 3.680                    | Tybamate                           | 2.05    |
| 3.845                    | 2-Hexenoic acid, 5-hydroxy-        | 0.64    |
| 3.935                    | LAURINDEAURE, BUT-3-ENYLESTER       | 0.30    |
| 4.556                    | Phenol (Cas) Izal                  | 91.16   |
| 42.730                   | 1-(Pent-4-ynil)pyranol[3,4]indo-3-one | 0.30   |
| 44.102                   | Silicone grease, silisonfett        | 0.33    |
The coconut shell liquid smoke bio-fungicide controls the fungus that grows only relatively quickly. *F. oxysporum* colonies can still grow in the area that is contaminated with coconut shell liquid smoke. The statistical test result (Table 4) proves that there is a difference in the average inhibition of *F. oxysporum* from the chemical fungicides treatment. The chemical fungicides treatment has inhibited *F. oxysporum* growth better than the bio-fungicides treatment. The disease severity average of samples with chemical fungicide treatment decreased from 45% to 10%. This result is in line with the research result of Channon & Thomson (1973), that Carbendazim active compound can prevent infection caused by *F. oxysporum* by protecting plant cell walls. It makes coconut shell liquid smoke inefficient compared to the use of chemical fungicides that can inhibit the growth of fungi for a longer time.

CONCLUSION

The statistical test shows the difference in the treatment for each observation period. Coconut shell liquid smoke bio-fungicide application could control the growth of *F. oxysporum*. Based on direct observation, three days after treatment, the fungus begins to grow. The disease severity is reduced to the range of 13-21% with coconut shell liquid smoke bio-fungicide treatment, whereas chemical fungicide can reduce to the range of 1-10%. The difference test result shows that there has no difference in average in fungal growth after treatment. The control ability of coconut shell liquid smoke bio-fungicide is relatively shorter compared to chemical fungicide. Based on the knowledge of coconut shell liquid smoke bio-fungicide, to control the fungus growth, it is recommended to try to use various grades of coconut shell liquid smoke as a comparison and add the length of observation time. Environmental factors such as humidity, wind speed, weeds, the season also need to be considered in the experiment.

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