UDC 634

THE CONCENTRATION AND TYPE OF LIQUID SMOKE TO SUPPRESS THE DEVELOPMENT OF ELSINOE FAWCETTII CAUSING SCAB ON CITRUS PLANT OF JAPANSCHE CITROEN

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ABSTRACT
Citrus is the main fruit commodity in Indonesia. Scab disease is a major disease in citrus plants. Scab disease control usually uses chemical fungicides that cause environmental pollution. Liquid smoke is a natural substance as a safer fungicide. The objective of this study was to analyze the ability of liquid smoke with the most effective concentration of three types of liquid smoke ie coconut shell, teak and falcata in suppressing the development of fungus *Elsinoe fawcettii* in citrus Japansche Citroen (JC). The identification and treatment carried out were analysis of phenol compounds contained in three types of liquid smoke (coconut shell, teak and falcata wood), testing of in vitro antifungal properties on growth of fungus *E. fawcettii* isolate in petri and in vivo sprouts against disease rate scab on JC citrus plant. The results showed that phenol content of coconut shell liquid smoke was 62.747 ml / L, 227.873 ml / L of teak wood and falcata wood was 115.587 ml / L. On observation of *E. fawcettii* fungal colony 14 days after inocculation (dai) highest percentage inhibition was smoke falcata smoke 5% concentration, able to inhibit growth of *E. fawcettii* equal to 77,22% whereas the lowest concentration was coconut shell smoke concentration 2% with 10.14% inhibition rate. Observation of wet weight and dry weight of *E. fawcetti* result of falcata smoke smoke treatment of 5% and 1% concentration have the lowest wet weight and dry weight of 0.867 g and 0.030 g, while on observation of intensity and extent of disease attack in vivo treatment of liquid smoke shell coconut wood and falcata wood have almost the same level of effectiveness. The conclusions of this study indicate that three types of liquid smoke ie coconut shell, teak and falcata wood have the ability to suppress growth and development of *E. fawcetti* fungus both in vitro and in vivo, while the most effective type is falcata wood. The most effective concentration in suppressing growth and development of *E. fawcetti* fungus in vitro and in vivo is the concentration of 5% of the three types of liquid smoke.

KEY WORDS
Liquid smoke, Elsinoe fawcettii, scab diseases, citrus.

The area of national citrus production in 2012 reached 51.793 ha with a production of 1.609.482 tons (Director General of Horticulture, 2014. One breakdown resulting in high loss of yield is the attack of pests. More than 50 types of diseases and 10 types of pests known to cause damage to citrus crops including scab, Citrus Vein Phloem Degeneration (CVPD), diplodia and rotten stem, while the major pests on citrus include fruit flies, aphids and caterpillars (Nirwanto, 2007). Special attention is requiredfor scabwhich are widespread throughout Indonesia (Ferguson, 2002). The symptoms of the disease resembleyellow small warts on leaves, fruit, rootstock and young twigs. Over time,the warts turn to greyish brown and hardened (Semangun, 2000). The scab is caused by *Elsinoe fawcettii* fungus (Semangun, 2000).

Elsinoe fawcettii is one of pathogens usually affects citrus rootstock before grafting. This pathogen commonly attack *Japansche Citroen* since this rootstock type is very sensitive to *E. fawcettii* (Triwiratno, 2005). Effort made in controlling scab is by chemical fungicides, but rather as a cause of environmental pollution. The use of fungicides leave residues that are not degraded by organisms (Rahayu and Akbar, 2003). The use of safer natural ingredients derived from plants as a fungicide is by liquid smoke. The use of liquid smoke as a natural biopesticide is more environmentally friendly due to biodegradable and renewable. Liquid smoke can be derived from many types of waste, but to date, the type of liquid smoke that has been commercialized is derived from coconut shell, teak and falcata.

Liquid smoke produced from the pyrolysis of coconut shell contains more than 400 components and has a function as a barrier to the development of bacteria and fungi. As reported by Darmadji (1996), pyrolysis ofcoconut shell produces liquid smoke with a phenol content of 4.13%, carbonyl of 11.3% and acid of 10.2%. In addition, the composition of the smoke resulted from burning timber is nearly 1,000 chemical compounds and contain phenols which can act as an antibacterial and antifungal (Swastawati, 2012). Thus,liquid smoke has the potential as an antifungal to deal with pathogens interference in plants. Therefore, it is necessary to do an analysis on the capability of the use of liquid smoke derived fromcoconut shell, teak and sengon with various concentrations to cure scab (*Elsinoe fawcettii*) on *Japansche Citroen* (JC).

This study aims to 1). Analyze the ability of liquid smoke derived from coconut shell, teak and Falcata to suppress *E. fawcettii* fungal growth on citrus plants of JC in vitro and in vivo. 2). Analyze the concentration of liquid smoke that is effective in suppressing the development of fungus of *E. fawcettii*on JC.

MATERIALS AND METHODS OF RESEARCH

The research was conducted in January to May 2016 at the Integrated Laboratory of Indonesian Citrus and Subtropical Fruits Research Institute in Batu and Entomology Laboratory of Brawijaya University. The materials used were citrus plant of type of JC at age of 2 months after transplating, three types of liquid smoke (coconut shell from CV Actindo Malang, teak from CV Madaniah and falcata from CV Agro Good Malang). The method used in this study was identification and experiment with the following stages:

- Analysis of total phenols contained in three types of liquid smoke, namely coconut shell, teak and falcata. Quantitative analysis of total phenolic compounds was made by the method of Folin-ciocalteau developed by Rungruang and Suwanne (2010).
- Testing of the antifungal properties derived from three types of liquid smoke, namely coconut shell, teak and falcata in vitro against fungal isolates of *E. fawcettii* on a petri dish.
- Testing of the antifungal properties derived from three types of liquid smoke, namely coconut shell, teak and falcata in vivo against the attack of scab in JC.

Table 1 – Research treatments *In Vitro*and *In Vitro*

No	Treatments	Description
1	P0K1	Without liquid smoke at concentration of 1%
2	P0K2	Without liquid smoke at concentration of 2%
3	P0K3	Without liquid smoke at concentration of 3%
4	P0K4	Without liquid smoke at concentration of 4%
5	P0K5	Without liquid smoke at concentration of 5%
6	P1K1	Liquid smoke derived from coconut shell at concentration of 1%
7	P1K2	Liquid smoke derived from coconut shell at concentration of 2%
8	P1K3	Liquid smoke derived from coconut shell at concentration of 3%
9	P1K4	Liquid smoke derived from coconut shell at concentration of 4%
10	P1K5	Liquid smoke derived from coconut shell at concentration of 5%
11	P2K1	Liquid smoke derived from teak at concentration of 1%
12	P2P2	Liquid smoke derived from teak at concentration of 2%
13	P2K3	Liquid smoke derived from teak at concentration of 3%
14	P2K4	Liquid smoke derived from teak at concentration of 4%
15	P2K5	Liquid smoke derived from teak at concentration of 5%
16	P3K1	Liquid smoke derived from falcata at concentration of 1%
17	P3K2	Liquid smoke derived from falcata at concentration of 2%
18	P3K3	Liquid smoke derived from falcata at concentration of 3%
19	P3K4	Liquid smoke derived from falcata at concentration of 4%
20	P3K5	Liquid smoke derived from falcata at concentration of 5%
21	P4K1	Chemical fungicide

This study used a completely randomized design (CRD) with 2 factorials, where factor A = 4 (four) types of treatment, namely coconut shell, teak, falcata and distilled water, and factor B = 5 (five) treatment concentrations of 1%, 2%, 3%, 4% and 5%. Positive control using chemical fungicides with three (3) replicates was employed as the comparison.

Parameter of observations included area of fungi colonies, wet weight of *E. fawcettii* mycelium, dry weight of *E. fawcettii* mycelium, intensity of disease attack, and area of disease attack. Data analysis included antifungal test was analyzed using ANOVA (F test) on a 5% level of significance. Further test using Duncan test was performed if the ANOVA result indicated a significant difference. Software used for these analyses was Dsaastat (Onofri, 2007).

RESULTS AND DISCUSSION

Analysis result indicated that phenol content in the liquid smoke of coconut shell was 62.747 ml / L, that of teak was 227.873 ml / L, and that of falcata was 115.587 ml / L. Each type of liquid smoke had different phenol content, where teak had a total phenol content higher than coconut shell and falcata. According to Hardianto (2015), the analysis result of liquid smoke derived from different materials will produce different content of phenol, carbonyl and total acid. However, the results of phenol content measurement in these samples were not in accordance with the literature, where the literature states that the highest phenol content found in coconut shells. Total phenol contained in liquid smoke of coconut shell was 6.70% (Hardianto, 2015), of teak was 2.70% (Wijaya, 2008) and of falcata was 0.50% (Adalina, 2008). High phenol content in liquid smoke of coconut shell was due to more degradation of lignin, because lignin content in coconut shell was higher (Himawati 2010).

Table 1 – Measurement of phenol concentration in samples

Sample	Absorbance	Concentration (ml/L)	Dilution (Kali)	Concentration (ml/L)
Coconut shell	0,27	6,2747	10	62,747
Teak	0,52	22,7873	10	227,873
Falcata	0,35	11,5587	10	115,587

The difference between total phenol in samples and in literature might occurred because of the differences in pyrolysis temperature in the manufacturing process. This was supported by Manshuri (2008) that the role of temperature in the manufacturing of liquid smoke mostly determined the quality of the smoke produced. Furthermore, liquid smoke containedvarious compounds due to the pyrolysis of three wood components, namely cellulose, hemicellulose and lignin which had various proportion, depending on the type of materials pyrolyzed (Fatima, 2005). The difference in total phenols can also be affected by the raw material. Further, Luditama (2006) stated that the liquid smoke composition dependent on the raw materials features including type, moisture content, particle size, combustion temperature, oxygen adequacy and stages of the process.

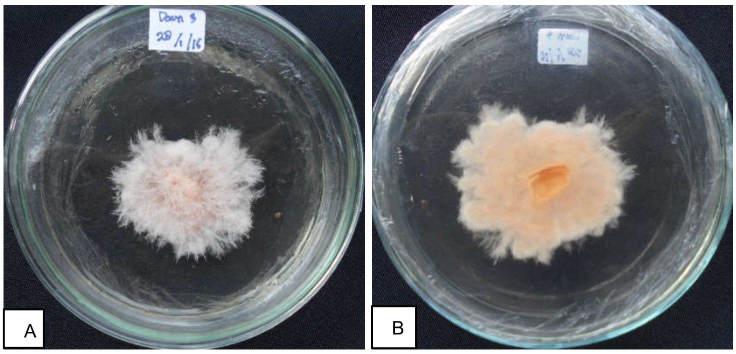

Figure 1 – Pure culture of *E. Fawcettii*on PDA medium at the seventh day, isolated from *Japansche Citroen* (a: front view, b: back view)

The color of fungal colonies of *E. Fawcettii*was reddish white with irregular form of *E. fawcettii* colonies. The growth of *E. fawcettii* colony was very slow at only 3 cm in 7 days, the texture was thick, fibrous and had a small lump in the middle (Figure 1a). A white orange colony clearly appeared (Figure 1b). This was consistent with the statement of Timmer (2001) that the characteristics of *E. fawcettii* colony on PDA were highly variable, even within the same isolates.

Microscopicidentification (Figure 2) showed that the conidiaof *E. fawcettii*was oval-shaped with a tapered end and had a size of 6 x 3 µm. This was consistent with the literature indicating that the fungus of *E. fawcettii* hadaservulus that were separable or united, slightly rounded and generally less than 1 mm in size. Hyaline conidia were 4-8 x 3-4 µm, conidiophores were very tight, tubular, tapered, composed of 1-3 cells, size of 12-22 x 3-4 µm, and hyalinecolor (Semangun, 1989).

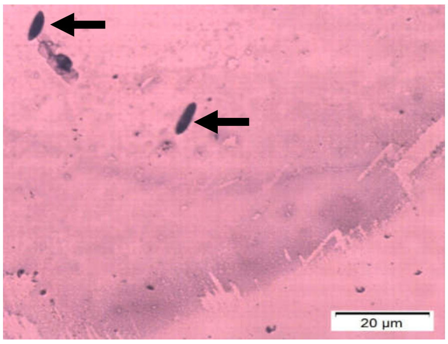

Figure 1 – Conidia of *E. fawcettii*

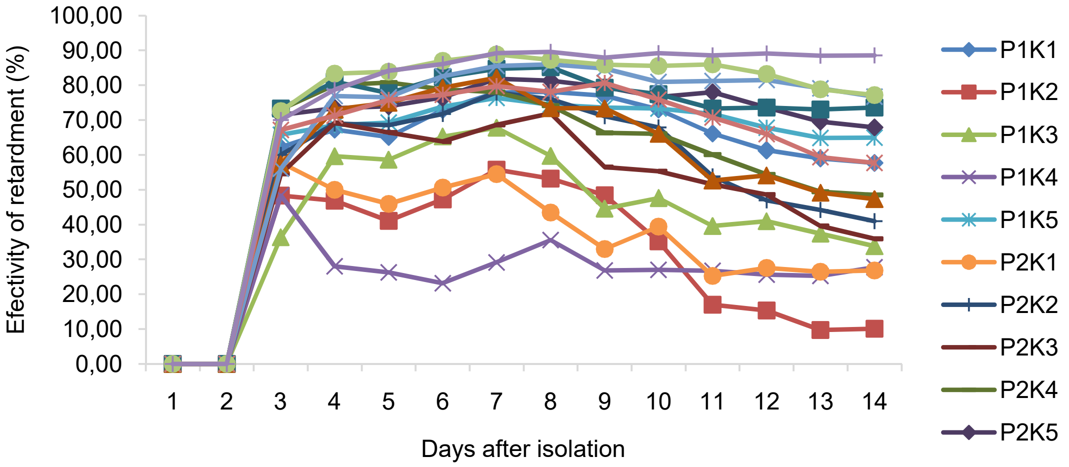

Figure 3 – The effectivity of liquid smoke against*E. fawcettii*growth in vitro method of food poisoning test. Description: P1K1: Coconut shell 1%, P1K2: Coconut shell 2%, P1K3: Coconut shell 3%, P1K4: Coconut shell 4%, P1K5: Coconut shell 5%, P2K1: Teak 1%, P2K2: Teak 2%, P2K3: Teak 3%, P2K4: Teak 4%, P2K5: Teak 5%, P3K1: Falcata 1%, P3K2: Falcata2%, P3K3: Falcata3%, P3K4: Falcata4%, P3K5: Falcata5 %, P4K1: Chemical Fungicides.

In addition to macroscopic and microscopic identifications, pathogenicity test was also conducted to determine whetherscab was caused by *E. fawcettii* or not.Pathogenicity test result indicated that this fungus was a pathogen causing scab disease in citrus. This can be seen from the presence of brownish warts on citrus leaves that have been injected with suspension of *E. fawcettii*conidia. This was supported by the literature stating that scab was caused by a fungus of *E. fawcettii*, where the symptoms of the disease such asyellow little warts on the leaves, fruits and twigs of young branch (Semangun, 2000).

From the treatment of liquid smoke, the best interactionwas demonstrated by Falcata with a concentration of 5%, where this treatment had the highest percentage of growth inhibition of *E. fawcettii* than other liquid smoke treatments. Nevertheless, in general, liquid smoke of Falcata had a high percentage of growthinhibition of *E. fawcettii*. The percentage of fungal growth inhibition of *E. fawcettii* by liquid smoke of Falcata with a concentration of 1%, 2%, 3%, 4% and 5% was 73.58%, 47.30%, 76.75%, 57.73% and 77.22% respectively. The high percentage of the inhibition occurred due to the total phenols content in the liquid smoke of Falcata. This was also described by Shiah (2006) that the phenol content inliquid smoke of Falcata was relatively high, so that it can be used as antimicrobial, especially antifungal. Fungal growth was inhibited as the concentration of liquid smoke was higher than that on fungal media. This was due to the high absorption of acids and phenols from liquid smoke on fungal media. Acids and phenols disturbed fungal cell membranes causing the increase of permeability, and eventually, losing their cell contents.

The results show that liquid smoke has been generated from several types of plants and is effective for controlling various pathogens, including those reported by Milly *et al*., (2005) that Liquid smoke fractions possess antimicrobial properties against a variety of Gram-positive and Gram negative bacteria, yeast, and molds. Vitt, *et al* (2001) We found a specific type of commercial liquid smoke that was equally inhibitory in vitro to *L. monocytogenes* and *L. innocua*. Cold-smoked salmon prepared using Charsol Supreme resulted in a 3-$\log_{10}$/gr reduction of *L. Innocua* previously inoculated on the raw fish.

Liquid smoke characters have been studied by several researchers whose results are among others. Study report by Montazeri, *et al*., (2012) the refined liquid smoke fractions AM- 3, AM-10 and 1291 had significantly lower acidity, lighter color, and rather distinct chemical make-up when compared with the full-strength Code 10-Poly. The observed differences demonstrate that knowledge of the chemical characteristics of liquid smoke preparations is important to better understand their interactions with food components. Martin, *et al*., (2010) This study indicated that liquid smoke was an effective antimicrobial for frankfurters in that it provided at least a one log initial reduction of surface inoculated Lm and suppressed growth during the extended shelf life of this product.

Liquid smoke from some plants can control the disease. A study was designed to validate the antimicrobial effect of liquid smoke as an ingredient against L. monocytogenes and its effects on the shelf life and quality of frankfurters (Morey *et al*. 2010). Aisyah, *et al*., (2013), reported that liquid smoke from dry distillation of coconut shells in strongly indicative cucumber plants was able to inhibit the growth of *Collelotrichum gloeosporoides* and *Fusarium oxysporum* fungi in vitro and in vivo. Reported also that Coconut shell liquid smoke used in cocoa plants as a organic fungicide with a concentration of 0.11%, can inhibit the growth of *Phytophthora* sp (EC_{50} by 0.11%) (Pangestu, *et al*., 2014).

liquid smoke from coconut wood waste 3% concentration can inhibit *Fusarium oxysporum* growth, while in concentration 4% can kill *nematode* in laboratory equal to 83,799% (Mugiastuti E. and Abdul Manan, 2009). Reported also that Liquid smoke can also be consumed in the food field, ie avoiding harmful *Listeria monocytogenes* bacteria can contaminate beef (Maria C, *et al*., 1988). liquid smoke from acacia wood sawdust 2% concentrations are reported as anti-fungal, which can inhibit the growth of *Aspergillus flavus* fungus, which is often the dominant toxigenic fungus that grows on agricultural products (Oramahi, H.A., *et al*. 2011).

CONCLUSION

Total phenol in each sample showed different results, where coconut shell was 62.747 ml / L, teak was 227.873 ml / L, and falcatawas 115.587 ml / L. Liquid smoke of teak had a total phenol content that was higher than the coconut shell and falcata.

Liquid Smoke derived from coconut shell, teak and falcata had the ability to suppress the growth and development of *E. fawcetti* with differen level of inhibition. The most effective liquid smoke in suppressing the development of *E. fawcetti* both in vitro and in vivo was liquid smoke of falcata.

The most effective concentration in suppressing the growth and development of *E. fawcetti*in vitro and in vivo was 5% at all types of liquid smoke.

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