IN VITRO EFFICACY OF VARIOUS CONCENTRATIONS OF COCONUT SHELL LIQUID SMOKE AGAINST *FUSARIUM OXYSPORUM* F. Sp. LYCOPERSICI

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Abstract

Fusarium oxysporum F. sp. lycopersici is one of a fungi that causes wilt disease in tomato plants. To overcome this problem is by developing bioactive agents, namely coconut shell liquid smoke, which contains phenolic compounds, organic acids and alcohol that act as antibacterial and antifungal agents. The purpose of this study was to determine the efficacy of coconut shell liquid smoke in inhibiting the growth of F. oxysporum F. Sp. lycopersici in vitro. The design used was a non-factorial completely randomized design (CRD) consisting of 6 treatments and 3 replications with coconut shell liquid smoke factor (A), consisting of 6 levels, namely A0: 0% (Control), A1: 2%, A2: 4%, A3: 6%, A4: 8% and A5: 10%. Parameters observed were colony diameter, percentage of colony relative inhibition, sporulation and growth compatibility of Fusarium oxysporum F. sp. lycopersici with coconut shell liquid smoke. The results showed that coconut shell liquid smoke had a very significant effect on colony diameter and colony relative resistance percentage. Coconut shell liquid smoke had a very significant effect on colony diameter at concentrations of A4 (8%) and A5 (10%), while the percentage of relative inhibition of colonies in A4 and A5 treatments with 90% inhibition. Liquid smoke also affects the sporulation and growth compatibility of Fusarium oxysporum F. sp. lycopersici in treatment A4 (8%) and A5 (10%) each had a percentage of 0 with 100% inhibition.

Keywords: *Fusarium oxysporum, Liquid smoke, Concentration, efficacy, in vitro.*

1. INTRODUCTION

Fusarium oxysporum F. is a soil-borne fungus that has been reported as the pathogen of horticultural crops including chili (Semangun, 2007; Sastrahidayat, 2017). This fungus develops in the plant body, especially in transport tissue, xylem (Fabiola, 2004) and has a toxin compound in the form of a polypeptide named lycomarasmin and fusaric acid. These two compounds cause wilting of plants due to loss of osmotic efficiency in vascular tissue (Wibowo, 2005).

According to Putri *et al,* (2005) the symptoms of this disease begin with the occurrence of necrosis on the lower leaves of the plant. Furthermore, the symptoms will increase to the upper leaves, and in advanced attacks cause the plant to fall and die. In mature plants, although

infected by this fungus, they tend to survive and even form fruit despite their small size and very little production (Semangun, 2007). Summerell (2011) reported that *F. oxysporum* attack on tomato plants resulted in losses and crop failure of up to 50%. Likewise, the attack on chili plants can reduce production by up to 50% and even crop failure can occur (Rostini, 2011)

The treatment and control of this wilt disease has been carried out in various ways, including the use of inorganic pesticides. Although it has been proven that the use of inorganic pesticides is able to reduce the loss or damage to agricultural products due to pest, the negative impacts they cause must also be considered. According to Aisyiah et al (2012) this is because inorganic pesticides are usually synthesized from non-renewable materials (such as coal and oil) so they are generally toxic and have a negative impact on the environment. Considering the possible side effects caused by inorganic pesticides, it is necessary to develop pesticides that are environmentally friendly and non-toxic to non-target organisms.

The utilization of bioactive agents derived from plants has the potential to be an alternative for controlling plant pest and pathogen in addition to inorganic pesticides. One of them is the bioactive compounds contained in coconut shells. According to Karseno *et al*. (2002) coconut shell contains phenolic compounds, organic acids and alcohol which can act as antioxidants and antimicrobials (antibacterial and antifungal). Based on research conducted by Luditama (2006), the quality of coconut shell liquid smoke, namely phenol 19.23% and acetic acid 128.13%.

The utilization of coconut shells as biopesticides for pest and plant pathogen control has been carried out in the form of liquid smoke (Aisyiah *et al*, 2018; Isa *et al*, 2019). The results of the study Isa *et al,* (2019) showed that coconut shell liquid smoke with a concentration of 7% caused the mortality of armyworm (*Spodoptera litura*) of 88.89%. Meanwhile, the test for controlling wilt disease with the application of liquid smoke with a concentration of 7%, was able to inhibit the growth of Fusarium oxysporum up to 100% (Aisyiah *et al*, 2019).

The purpose of this study was to determine the efficacy of coconut shell liquid smoke in inhibiting the growth of the *F. oxysporum* F. sp. Lycopersici in vitro.

2. LITERATURE REVIEW

1.1 Liquid smoke

Liquid smoke is a result of distillation or condensation of steam resulting from indirect or direct combustion of materials that contain a lot of carbon and other compounds. Liquid smoke varies according to process conditions and raw materials. Most studies have focused on liquid smoke from rapid pyrolysis processes which generally consist of hydroxyaldehydes, hydroxyketones, carboxylic acids, compounds containing furan/pyran rings, anhydro sugars, phenolic compounds and oligomeric fragments of lignocellulosic polymers. This product is derived from the original biomass composition consisting of cellulose, hemicellulose, lignin, extractives, lipids, proteins, simple sugars, starch, water, hydrocarbons, ash, and other compounds (Dickerson, 2013). The content of liquid smoke is influenced by the chemical content of the raw materials used and the temperature reached in the pyrolysis process. From the results of the analysis of the types of components of liquid smoke using the GCMS technique, at least 61 compounds were identified consisting of ketones (17 compounds), phenolics (14 compounds), carboxylic acids (8 compounds), alcohol (7 compounds), esters (4 compounds), aldehyde (3 compounds), and others 1 compound.

2.2 Fusarium oxysporum

One of the diseases that commonly attack tomato plants is Fusarium wilt (Fusarium oxysporum) – Fusarium wilt caused by the fungus Fusarium oxysporum, is one of the most feared diseases, especially by horticultural farmers because it has the potential to cause huge losses. In fact, it is not uncommon for this disease to be the cause of cultivation failure. At high attack rates, fusarium wilt disease can kill the entire plant, especially during the rainy season

and the planting area is easily flooded. Early symptoms of tomato Fusarium wilt disease are pale bone leaves, especially the upper leaves, followed by drooping of the stalks, and finally the plant wilts as a whole. Often wilting is preceded by yellowing of the leaves, especially the lower leaves. Withering can occur unilaterally. On the stems sometimes form adventitious roots. In young plants, it can cause sudden death of the plant because the base of the stem is damaged.

3. RESEARCH METHODS

This research was carried out at the Plant Protection Laboratory, Faculty of Agriculture, Teuku Umar University, in August-September 2019. The materials used included aquades, PDA (Potato Dextrose Agar), coconut shell liquid smoke, label paper, and isolate of *Fusarium. oxysporum* F. sp. Lycopersici, plastic wrap, and aluminum foil. While the tools used are petri dishes, oven, spatula, Bunsen lamp, tweezers, scales, measuring cup, autoclave, and erlenmeyer.

This study was arranged in a non-factorial Completely Randomized Design (CRD) consisting of six treatments and three replications. The observed factor was the concentration of coconut shell liquid smoke (A), namely: A0 (0%), A1 (2%), A2 (4%), A3 (6%), A4 (8%), and A5 (10%). If the F test shows a significant effect, then it is continued with a further test, namely the Tukey HSD (Honestly Significant Different) test at the 5% level.

3.1. Production of coconut shell liquid smoke

Coconut shell liquid smoke was made using a simple distillation system following the modified Sutin (2008) method. The first step is by preparing the coconut shells that has been split with a size of ± 6-8 cm and then put it into the combustion tool. The material is heated for 5 hours, until a liquid is formed which is flowed by an iron pipe from the combustion apparatus into a glass bottle prepared at the end of the pipe. The next process is the filtering of liquid smoke using filter paper and collected back in a clean glass bottle and then tightly closed so that outside air does not enter. After that, the liquid smoke can be applied

3.2. Preparation of PDA (Potato Dextrose Agar)

PDA medium was made by using boiled potato water, sugar, and agar. All ingredients are cooked and stirred until boiling then poured into a 1000 ml elenmeyer and then covered with cotton. The PDA (Potato Dextrose Agar) media was cooled and then put into an autoclave for 15 minutes at a temperature of 120^{o}C. then the media was stored at room temperature for 24 hours.

3.3. Propagation of *Fusarium oxysporum* F. sp. lycopersici

Fusarium oxysporum was propagated by inoculating on new PDA (Potato Dextrose Agar) media carried out in LAF (Laminar air flow) so that colonies of *F. oxysporum* F. sp. Lycopersici with the desired amount to be used as a source of inoculum.

3.4. Application of coconut shell liquid smoke on PDA medium

The coconut shell liquid smoke is purified by using filter paper. The pure coconut shell liquid smoke is diluted to obtain a concentration of 2% (2 ml liquid smoke + 98 ml aquadest), 4% (4 ml liquid smoke + 96 ml aquadest), 6% (6 ml liquid smoke + 94 ml aquadest) , 8% (8 ml of liquid smoke + 92 ml of distilled water), and 10% (10 ml of liquid smoke + 90 ml of aquadest) then the concentration results are mixed with PDA media (Potato Dextrose Agar) that become a growth medium for *Fusarium oxysporum* F. .sp. lycopersici.

3.5. Inoculation of *Fusarium oxysporum* F. sp. lycopersici on PDA medium

Fusarium oxysporum F. sp. lycopersici was inoculated on PDA (Potato Dextrose Agar) medium by using a 5 mm diameter corebore in the middle of the medium circle, then incubated at 25 °C in LAF (laminar air flow) until the mycelium filled the petri dish (Noveriza and Tombe, 2003).

3.6. Observations

The observations were carried out after 24 hours incubation of *Fusarium oxysporum* F. sp. lycopersici. The parameters observed are:

1. Colony Diameter

Colony diameter was measured every day for 12 days or until the control filled the petri dish. Measurement of diameter using a ruler whose calculation method is to make vertical and horizontal lines where the point of intersection of the two lines is right in the middle of the fungal colony. The method of measurement in a petri dish is based on the following formula:

$$D = \frac{d1 + d2}{2}$$

Note:

D = Colony diameter of *Fusarium oxysporum* F. sp. *lycopersici*
d1 = Vertical colony diameter of *Fusarium oxysporum* F. sp. *lycopersici*
d2 = Horizontal colony diameter of *Fusarium oxysporum* F. sp. *lycopersici*

2. Percentage of Colony Relative Inhibitory

The relative inhibitory ability of fungicides to the growth of *Fusarium oxysporum* F. sp. lycopersici were counted until the fungus had grown. The percentage of inhibitory calculated according to the formula Noveriza and Tombe (2003) is as follows:

$$HR = \frac{dk - dp}{dk} \times 100\%$$

Note:

HR = Relative Inhibitory
dk = Control diameter of *Fusarium oxysporum* F. sp. *lycopersici*
dp = Treatment diameter of *Fusarium oxysporum* F. sp. *lycopersici*

The effect of a fungicide is assessed from the categories according to Irasakti and Sukatsa (1987) as follows:

0 = no effect
>0-20% = very less influential
>20-40% = less influential
>40 – 60% = quite influential
>60 – 80 % = influential
>80% = very influential

3. Sporulation

The observation of spore density was carried out by harvesting spores from pure cultures of *F.oxysporum* F. sp. lycopersici 7 days incubation. The spore harvest was carried out on the 12th day by dredging the colony so that the media would not be lifted using a spatula to obtain a concentrated suspension of spores, then the concentrated suspension was put into a test tube containing 9 ml of sterile water and homogenized using a vortex mixer for 1 minute. A total of 1 ml of concentrated solution which was put into a test tube was taken and added to 5 ml of distilled water. After completion, 1 ml of *F. oxysporum* F. sp. was taken. lycopersici was placed on a glass slide covered with a cover glass, then observed for microscopic form using a microscope with a magnification of 40x100.

The fungal colony growth data obtained was an average of four times the diameter measurements in different areas. These observations were made on the 12th day after

inoculation. Calculation of the number of spores was carried out three times in each treatment. The rate of spore count was calculated in five medium box samples (on the 7th day after inoculation) by the formula Syahnen *et al* (2014).

$$S = R \times K \times F$$

Note:

S = Number of spores
R = Average number of spores in 5 haemocytometer field of view
K = Coefficient constant of equipment ($0{,}25 \times 10^6$)
F = Dillution factor

4. Compatibility of *Fusarium oxysporum* F. sp. lycopersici and Coconut Shell Liquid Smoke

To determine the effect of coconut shell liquid smoke on *F. oxysporum* F. sp. lycopersici, the data from the compatibility observations are entered into the T formula according to Alves *et al*. (1998), in Trizelia and Rusli (2012) as follows:

$$T = \frac{20(PK) + 80(SP)}{100}$$

Note:

T = Compatibility value
PK = Relative value of treatment colony growth compared to control (0%)
SP = Relative sporulation value of the treatment compared to the control (0%) The T value was divided into the following categories: 0-30 very toxic, 31-45 toxic, 46-60 less toxic, and >60 not toxic or compatible.

4. RESULTS AND DISCUSSION

1. Colony Diameter

The growth of *Fusarium oxysporum* F. sp. Lycopersici colony diameter after application of coconut shell liquid smoke at 1-12 day after incubation showed variations in growth on various days of observation and treatment. The lowest colony diameter growth at 1-12 DAI was found in treatments A4 and A5 which were significantly different from other treatments (Table 1).

Table 1. Average growth of *Fusarium oxysporum* F. sp. Lycopersici colony diameter at 1-12 DAI

Days	The Concentration of liquid smoke						HSD 5%
	A0 (0%)	A1 (2%)	A2 (4%)	A3 (6%)	A4 (8%)	A5 (10%)	
1	6,68 b	0,57 a	0,57 a	0,57 a	0,57 a	0,57 a	0,28
2	8,73 b	0,57 a	0,57 a	0,57 a	0,57 a	0,57 a	0,16
3	10,93 b	2,35 a	0,57 a	0,57 a	0,57 a	0,57 a	2,51
4	12,67 c	5,57 b	3,39 a	0,57 a	0,57 a	0,57 a	2,83
5	13,81 c	6,96 b	6,26 b	0,57 a	0,57 a	0,57 a	1,94
6	14,67 c	8,75 b	8,58 b	3,66 a	0,57 a	0,57 a	2,74
7	14,94 d	10,23c	10,41c	4,46 b	0,57 a	0,57 a	3,23
8	15,35 c	11,34c	11,80c	5,88 b	0,57 a	0,57 a	3,93
9	15,86 c	12,31c	12,17c	6,66 b	0,57 a	0,57 a	4,66
10	16,34 c	13,33c	13,25c	8,33 b	0,57 a	0,57 a	5,57
11	17,05 c	14,49c	14,13b	8,72 b	0,57 a	0,57 a	5,75
12	17,32 c	14,93c	14,33b	8,85 b	0,57 a	0,57 a	5,84

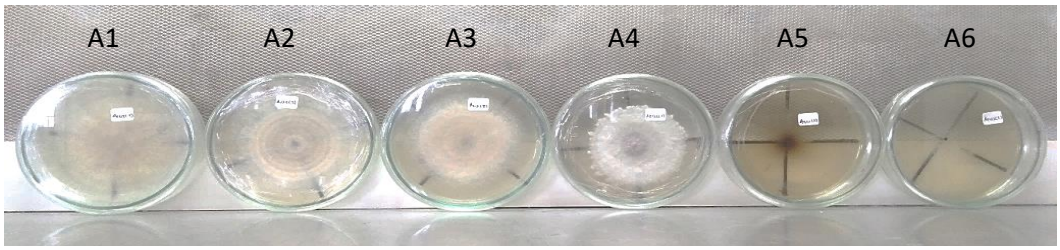
Note: Numbers followed by the same letter in the same column are not significantly different at the 0.05% test level.

The occurrence of variations in the growth of *Fusarium oxysporum* colonies at 7-12 DAI, presumably because the concentration of coconut shell liquid smoke in treatments A4 and A5 had a higher content of phenolic compounds compared to other treatments. This is in line with what was reported by [18] that the toxicity of fungicides which play an important role in inhibiting the growth of fungi in coconut shell liquid smoke is derived from the components of phenolic compounds and organic acids. Another thing was also reported by Ray and Sandine, 1993; Ray, 1996 in Aisyiah *et al* (2012) which states, liquid smoke from coconut shell raw materials contains compounds such as alcohol, phenol and organic acids that have functional abilities as antibacterial and antifungal. Karseno *et al* (2002) also stated that coconut shell liquid smoke contained 5.13% phenolic compounds; carbonyl 13.28%; and 11.39% acid which acts to inactivate the fungi Rizhopus stolonifer and *Fusarium oxysporum*, because they have fungicidal, fungistatic and germicidal properties.

Fungicidal is one of characters of chemical compounds that have the ability to control fungi, while fungistatic is the characters of chemical compounds that are able to inhibit the growth of fungi without killing them. Meanwhile, germicidal is the nature of chemical compounds that have the ability to kill fungi (fungi) and bacteria (Tranggono *et al.,* 1996).

Observations also showed that the growth of the Fungi Fusarium oxysporum F. sp. colony was inhibited. lycopersici by coconut shell liquid smoke. This was indicated by a change in the color of the media and the fungal colonies of Fusarium oxysporum F. sp. lycopersici at various concentrations, as presented in Figure 1.

Figure 1. Media color and colonies of *Fusarium oxysporum* F. sp. lycopersici at various concentrations of coconut shell liquid smoke (A0 (control), A1(2%), A2 (4%), A3(6%), A4 (8%) and A5 (10%))



Treatments A0, A1, A2 and A3 did not show any difference in the color of the media and the colonies of *Fusarium oxysporum* F. sp. lycopersici, but the color changes to brown in the A4 and A5 treatments, namely a change in the color of the media while the fungal colonies did not grow at all. This condition is suspected because the higher the concentration of liquid smoke, the more visible the color and the chemical content of the concentrated liquid smoke on PDA (Potato Dextrose Agar) media. Noor *et al.* (2016) stated that basically the characteristics of liquid smoke are brownish yellow to blackish with a pungent odor and consist of 80-90% water and 10-20% organic matter including more than 200 chemical substances. Therefore, when liquid smoke is applied to the PDA media, the color changes along with the increase in the concentration of liquid smoke applied to the PDA media (A5).

2. Percentage of Colony Relative Inhibitory

Percentage of relative inhibition of *Fusarium oxysporum* F. sp. lycopersici increased after application of coconut shell liquid smoke at the growth of 1-12 DAI. A4 and A5 treatments were effective in inhibiting the growth of Fusarium oxysporum F. sp. colonies. lycopersici up to 90% (Table 2)..

Table 4.2. The average percentage of relative inhibition of *Fusarium oxysporum* F. sp. Fungal colonies. lycopersici at 1-12 HSI

Days	The Concentration of liquid smoke	HSD 5%

	A0 (0%)	A1 (2%)	A2 (4%)	A3 (6%)	A4 (8%)	A5 (10%)	
3	0,57a	78,91b	90,00b	90,00b	90,00b	90,00b	15,63
4	0,57a	63,18b	75,14b	90,00c	90,00c	90,00c	14,77
5	0,57a	58,84b	62,72b	90,00c	90,00c	90,00c	9,62
6	0,57a	52,51b	53,59b	75,86c	90,00d	90,00d	13,25
7	0,57a	46,12b	46,38b	72,18c	90,00d	90,00d	14,75
8	0,57a	41,58b	42,22b	67,24c	90,00d	90,00d	17,39
9	0,57a	38,74b	38,53b	61,76c	90,00d	90,00d	21,25
10	0,57a	34,88b	35,57b	57,65b	90,00c	90,00c	23,88
11	0,57a	31,32b	31,61b	57,22c	90,00d	90,00d	23,22
12	0,57a	30,05b	31,23b	56,92c	90,00d	90,00d	23,49

Note: Numbers followed by the same letter in the same column are not significantly different at the 0.05% test level

The ability of liquid smoke in A4 and A5 treatments to effectively inhibit the growth of *Fusarium oxysporum* F. sp. colonies. lycopersici (90%), due to the high content of phenolic compounds. As reported by Pangestu *et al.* (2014), that the largest group of compounds in liquid smoke is phenol and acid compounds that work synergistically and function as antibacterial and antifungal agents. Davidson *et al.* (2005) also stated that the process of inhibiting fungal growth was influenced by antimicrobial compounds in coconut shell liquid smoke, namely phenol compounds in the form of, 2-ethylphenol, 3-Methylphenol, 2,6-Dimethylphenol, 2,4-Dimethylphenol, and 3-hylphenol and acid compounds such as 2,3-dihydroxy-benzoic acid, 3-methoxybenzoic acid methyl ester, and 4-Hydroxy-benzoic acid methyl ester.

3. Sporulations

The average value of sporulation at various concentrations of coconut shell liquid smoke showed that the higher the concentration of coconut shell liquid smoke, the fewer spores formed. In treatment A4 and A5 the average inhibition of sporulation reached 100% (Table 3).

Table 3. The average of *Fusarium oxysporum* F. sp*. Lycopersici* sporulation

Treatments	Sporulation average
A0 (control)	2.165×10^6
A1 (2%)	1.015×10^6
A2 (4%)	4.75×10^8
A3 (6%)	5.25×10^8
A4 (8%)	0
A5 (10%)	0

Treatment of liquid smoke (A4 and A5) showed no spores growing, in contrast to the other concentrations. This phenomenon indicates that coconut shell liquid smoke effectively inhibits the growth of *Fusarium oxysporum* F. sp. spores. lycopersici by 100%. The content of alcohol, phenol and organic acids in coconut shell liquid smoke can inhibit the germination of *Fusarium oxysporum* and *Colletotricbum gloeosporoides* spores, with each inhibiting power of 100% (Aisyiah *et al*., 2012) This is added by Pelczhar and Chan (1988) that alcohol, phenol and acetic acid contained in liquid smoke also indicate that they are compounds that have a synergistic function as protein denaturation and lipid hydrolyzing, so that they can damage cell membranes in fungal body tissues and inactivate enzymes excreted. the fungus.

4. Compatibility of *Fusarium oxysporum* F. sp. lycopersici and Coconut Shell Liquid Smoke

Compatibility is a value that indicates the level of toxicity possessed by a compound as a biopesticide. Alves et al. (1998), in Trizelia and Rusli (2012) classified the compatibility values of a pesticide as follows: 0-30 very toxic, 31-45 toxic, 46-60 less toxic, and >60 not toxic or compatible.

Based on the results of the study, the compatibility value of A4 and A5 was 0, meaning that coconut shell liquid smoke was very toxic to Fusarium oxysporumF. Sp. lycopersici. The best concentration of coconut shell liquid smoke to inhibit the growth of Fusarium oxysporum F. Sp. lycopersici were found in treatments A4 and A5 (Table 4).

Table 4. Compatibility category of *Fusarium oxysporum* F. Sp. lycopersici and coconut shell liquid smoke

Treatments	Compatibility value	
A1 (2%)	52,47	Less Toxic
A2 (4%)	31,65	Toxic
A3 (6%)	30,79	Toxic
A4 (8%)	0	Very Toxic
A5 (10%)	0	Very Toxic

Based on Table 4, coconut shell liquid smoke can be used as a bioesticide because it has a compatibility value of 0, which means that liquid smoke plays a role in inhibiting the growth of *Fusarium oxysporum* F. Sp. colonies. lycopersici. This is in accordance with the statement Elisa(2006) a biopesticide will be toxic to microbes if it is not compatible with microorganisms applied.

CONCLUSION

To sum up, coconut shell liquid smoke was able to inhibit the growth of *Fusarium oxysporum* F. sp. lycopersici in vitro. The best concentration to inhibit the growth of *Fusarium oxysporum* F. sp. lycopersici were found in A4 (8%) and A5 (10%).

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