ISSN (Online): 2350-0530
ISSN (Print): 2394-3629

International Journal of Research -GRANTHAALAYAH
October 2020, Vol 8(10), 285 – 291
DOI: https://doi.org/10.29121/granthaalayah.v8.i10.2020.1970

PRODUCTION AND FUNGICIDAL ACTIVITY ASSESMENT OF WOOD-WASTE LIQUID SMOKE

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DOI: https://doi.org/10.29121/granthaalayah.v8.i10.2020.1970

Article Type: Research Article

Article Citation: Budy Rahmat, Apip Hermawan, Dedi Natawijaya, and Endang Surahman. (2020). PRODUCTION AND FUNGICIDAL ACTIVITY ASSESMENT OF WOOD-WASTE LIQUID SMOKE. International Journal of Research - GRANTHAALAYAH, 8(10), 285-291. https://doi.org/10.29121/granthaalayah.v8.i10.2020.1970

Received Date: 11 October 2020

Accepted Date: 31 October 2020

Keywords:
Concentration
Fungicide
Liquid Smoke
Soybean
Wood Waste

ABSTRACT

Liquid smoke is known to contain compounds that can control plant disease pathogens. This study aims to produce wood-waste liquid smoke and determine its effectiveness as a fungicide on plant pathogens. This research was conducted in two experimental stages, namely: (i) in vitro test as a preliminary test of the effectiveness of teak waste liquid smoke at concentrations of 0, 0.5, 1, 1.5, 2, and 2.5%; and (ii) in vivo test was arranged in randomized block design consisting of seven levels of liquid smoke concentration, namely 0, 1, 2, 3, 4, 5, and 6%, each of which was repeated four times. The results showed that the pyrolysis of 1 kg of wood waste was produced with the proportions of liquid smoke, charcoal and tar, respectively: 312 mL, 31 g, 367 g and the uncondensed gases. Treatment of liquid smoke in the in vivo test showed that a concentration of 1 to 2.5% liquid smoke was able to suppress the growth of the pathogenic fungus *Sclerotium rolfsii* 100%. The treatment of liquid smoke in the in vivo test showed an effect on inhibition of the growth diameter of fungal colonies, suppressing the disease occurance, and suppressing the lesion diameter.

1. INTRODUCTION

Sustainable agricultural development requires environmentally friendly pest control. In practice, vegetable pesticides are used, one of which is liquid smoke. Internal studies regarding the use of liquid smoke have been carried out and have the potential as a plant regulator for papaya [1], then as a larvacide and repellent for *Spodoptera litura* [2], and able to control the main pests of rice namely the golden snail [3]. As an antifungal, it affects the strawberry fruit rot pathogens [4].

Liquid smoke is known to contain compounds that can control disease-causing pathogens in plants. The compounds in liquid smoke that affect the anti-fungal properties of liquid smoke are phenols and acids.

Phenols and acids are produced by pyrolysis of lignin and hemicellulose. The by-product of processed teak wood has the same properties both in the structure and chemical properties of teak. Therefore, teak wood shavings have the potential to be turned into liquid smoke [5].

Liquid smoke from charcoal furnace processing was able to inhibit the growth of soil-borne fungi *Sclerotium oryzae*, *Pythium spp*., and *Rhizoctonia solani* with the results showing that liquid smoke was able to inhibit pathogenic

activity from a concentration of 2% [6]. Volatile compounds and non-volatile compounds in liquid smoke can inhibit the growth of the fungal mycelium *Rhizoctonia solani* and *Sclerotinia sclerotiorum* [7].

This study aims to produce liquid smoke and its effectiveness as a fungicide.

2. MATERIALS AND METHODS

2.1. MATERIAL

The tools used in this experiment were a set of liquid smoke pyrolysis and distillation tools, container bottles, measuring cups, test tubes, beaker glasses, erlenmeyers, ose needles, cork borer, gauze, aluminum foil, petri dishes, spatulas, tweezers, micropipettes, electric scale, bunsen, gas stove, oven, laminar air flow, thermohigrometer, autoclave, plastic tray, plastic box, hand sprayer, calipers, funnel, filter paper, pH meter, glass object, microscope.

This experiment used materials including: teak wood shavings liquid-smoke, Potato Dextrose Agar (PDA) media, aquades, *Sclerotium rolfsii* isolate, 3% NaOCl, 70% alcohol, 1% $FeCl_3$, soil, soybean seeds, manure.

2.2. EXPERIMAENTAL SETUP

This experiment was arranged in a randomized block design, which consisted of 7 levels of treatment and was repeated 4 times. The treatment tested was the concentration of teak wood shavings, namely: K_o = control (aquades), K_1 = 1%, K_2 = 2%, K_3 = 3%, K_4 = 4%, K_5 = 5%, and K_6 = 6%.

The data were analyzed by using analysis of variance (Anova) and continued with the Duncan's multiple different test [8].

2.3. PROCEDUR OF EXPERIMENT

Making liquid smoke uses the following tools and procedures used by Rahmat et al. [3] and Rahmat et al. [5]. Raw liquid smoke is redistilled once to produce liquid smoke in grade 2.

In vitro testing was carried out by mixing liquid smoke with PDA media according to the desired concentration. The concentrations of liquid smoke to be used are 0, 0.5, 1, 1.5, 2, and 2.5%. The next step, the *Sclerotium rolfsii* culture was cut using a cork borrer with a diameter of 5 mm, then placed right in the center of the media, done in duplicate, and observed for 4 days.

In vivo testing was carried out on 5 day old soybean plants. The plants were then sterilized using 3% NaOCl by soaking them for 5 minutes then rinsing with distilled water and drying them. The plastic box was filled with seven plants which had been surface sterilized and maintained their physiological activity. The plant roots were wrapped in cotton soaked in water. Pathogen inoculation by attaching pieces of hyphae with a diameter of 5 mm to each plant stem and then incubated for 6 hours. After that, the application of liquid smoke was carried out by soaking the inoculated plants in a liquid smoke solution according to the treatment for 5 minutes. Then the soybean plants are returned to the box in their original position.

2.4. DATA COLLECTION

The parameters observed included: characteristics of teak wood shavings liquid smoke, identification of pathogenic isolates, in-vitro anti-fungal index, disease incidence rate and length of lesions. The characteristics of liquid smoke observed included color, aroma, pH, specific gravity, acid content and phenol test results. Fungi identification was done by observing the characteristics and morphology of hyphae and sclerotia on PDA media both macroscopically and microscopically.

In the *invitro* test, the fungisidal action (FA) value was obtained using the following formula:

$$FA = 1 - \frac{CT}{CC} \text{ x } 100\%$$

Note: CT = colony diameter in treatment; CC = colony diameter in control

The disease occurrence rate (DO) was calculated by the following equation:

$$DO = \frac{SI}{ST} \times 100\%$$

Note: SI = number of infected seedling; ST = number of total observed seedling

From the disease occurrence rate (DO), the suppression of disease occurrence (SDO) can be determined with the following equation:

$$SDO = \left(\frac{DOC - DOT}{DOC}\right) \times 100\%$$

Note: DOC = disease occurrence rate in control; DOT = disease occurrence rate in treatment

3. RESULTS AND DISCUSSIONS

3.1. CHARACTERISTICS OF LIQUID SMOKE

The characteristics of teak wood shavings liquid smoke produced are: reddish-brown in color, pH 3, and a very strong smoke aroma. The yield resulting from the pyrolysis process of 1 kg of teak wood shavings was produced with the proportions of liquid smoke, charcoal and tar, respectively: 312 mL, 31 g, 367 g and the uncondensed gases. The lower yield of liquid smoke than that produced by Rahmat et al. [4] and Wagini and Sukaryono [7] possibly caused by differences in the moisture content of the materials, pyrolysis kiln design, temperature and pyrolysis time.

The results of redistillation obtained that the average proportion of liquid smoke was 82.25%, and 17.75% tar and uncondensable compounds. Redistillated liquid smoke changes color, pH and aroma. Grade 2 liquid smoke has characteristics: pale yellow color, pH 2, and reduced smoke aroma. This teak wood shavings liquid smoke distillate has a density of 1.0107, the acid content is 7.026%. The results of the characterization of liquid smoke can be seen in Table 1, with a comparison of the standard Japanese liquid smoke distillate [9].

The phenol test results show positive, there was a dark black color, which indicates the presence of phenol content in the liquid smoke.

Table 1: Comparison of sample liquis smoke and Japenese quality standard

Parameter	Liquid smoke distillate	Liquid smoke by Japanese standards
pH	2	1,3 to 3,7
Density	1.0107	> 1,001
Color	Pale yellow	Colorless to reddish brown
Transparency	Transparent	Not cloudy (transparent)
Acid content	7.026%	1 to18%

3.2. FUNGICIDAL ACTION OF LIQUID SMOKE *IN VITRO*

In the control treatment, the pathogenic fungal mycelium *S. rolfsii* began to grow on agar media at 9 hours after incubation. At 24 hours after incubation, the diameter of the mycelium colonies reached 1.1 cm. Then at 4 days after incubation the fungal mycelium growth has filled the entire area of the petri dish (Fig.1.). The maximum growth of *S. rolfsii* on PDA media is in the range of 4 and 7 days after incubation. Furthermore, the measurement of the fungisidal action rate the liquid smoke treatment was only carried out for four days [10]. The results of measuring the fungisidal action can be seen in Table 2.

Table 2: Effect of liquid smoke concentration on fungisidal activity

Liquid Smoke Concentration	Fungicidal activity rate (%)			
	1 DAI	2 DAI	3 DAI	4 DAI
0,5%	100	73,469	66,667	46,389
1%	100	100	100	100

1,5%	100	100	100	100
2%	100	100	100	100
2,5%	100	100	100	100

Note: DAI = days after incubation

In media with a liquid smoke concentration of 0.5% the fungi began to grow at 2 days after incubation. The decrease in antifungal activity began at 2, 3 and 4 days after incubation. However, at a concentration of 1 to 2.5% there was no decrease in antifungal activity, it remained 100%, until the measurement time was complete.

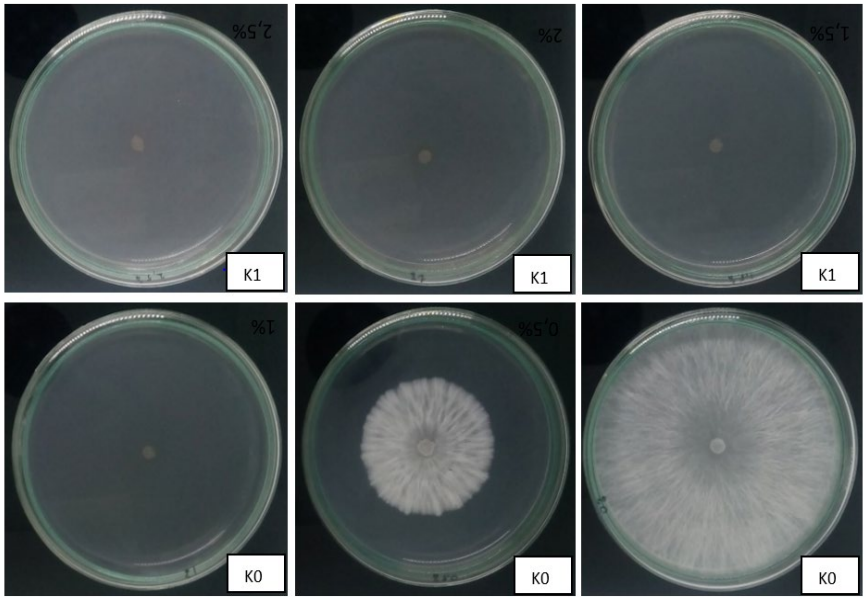

Figure 1: The top row is a medium with a concentration of 1% liquid smoke (K_1) at 1 to 3 DAI; and the bottom row is a medium with a concentration of 0 % liquid smoke (K_0/control) at 1 to 3 DAI.

The fungisidal action shows the ability of liquid smoke to inhibit the development of fungi, namely the presence of phenol compounds in liquid smoke. The mechanism of activity of phenolic compounds as antimicrobial substances includes reactions in cell membranes that cause increased permeability and result in loss of cell contents, inactivation of essential enzymes, and destruction or functional inactivation of genetic material. The higher the phenol concentration will precipitate all cell proteins, while the lower the concentration will effectively inhibit essential enzymes [11].

Organic acids, such as acetic acid, propionic acid, lactic acid, benzoic acid, and salicylic acid can act as antimicrobials mainly due to the formation of free H^+ ions. At concentrations of acetic acid above 0.5% it is able to penetrate into cells and denaturate cell plasma proteins. Propionic acid can inhibit microbes by blocking the cellular metabolic system through inhibition of enzyme activity. In addition, propionic acid is able to reduce the intracellular pH value which can result in inhibition or cell killing [12].

3.3. DISEASE OCCURRENCE RATE *IN VIVO*

Table 3 showed that, since 1 day after application there was a difference in the occurrence of disease between the control and all treatments from the concentration of liquid smoke. The incidence in the control was 70.36%, while in all treatments the concentration of liquid smoke was able to withstand 0%. This shows that in the liquid smoke there ws anti-fungal activity of liquid smoke that suppressed *S. rolfsii* growth.

There was no disease occurrence since 1 day after application in all liquid smoke treatments, because the active compounds in liquid smoke were at an intolerance level for fungal life. At 2 days after application, it began to show differences in the disease occurrence, namely the decrease in the occurrence with the increasing concentration of phenol and organic acids in the liquid smoke solution.

Table 3: Effect of liquid smoke concentration on desease occurance rate

Liquid smoke concentration	Disease occurrence rate (%)							
	1 DAI		2 DAI		3 DAI		4 DAI	
K_0 (0%)	70,36	a	100,00	a	100,00	a	100	a
K_1 (1%)	0,00	b	32,50	b	42,50	b	47,50	b
K_2 (2%)	0,00	b	9,17	c	21,67	c	32,50	b
K_3 (3%)	0,00	b	4,17	c	16,07	c	25,24	bc
K_4 (4%)	0,00	b	3,57	c	10,71	c	10,71	cd
K_5 (5%)	0,00	b	0,00	c	0,00	d	4,17	d
K_6 (6%)	0,00	b	0,00	c	0,00	d	3,57	d

Note: Numbers followed by the same letter indicataes not significantly different according to Duncan's Multiple range test level of 5%. The data was transformed by $\sqrt{(x + 0.5)}$. DAI = days after inoculation.

The facts mentioned above show that liquid smoke is most effective as an antifungal in affecting the incidence rate of disease due to *Sclerotium rolfsii* at a concentration of 4 to 6%. At these concentrations, the quantity of phenolic compounds is thought to be able to inhibit the development of fungi. The difference in the concentration of the liquid smoke given causes a difference in the quantity of the compounds present in the liquid smoke. The higher the concentration of liquid smoke, the higher the anti-fungal activity. Accordingly the study that reported the higher concentration of liquid smoke that was applied was able to reduce the percentage of strawberries infected with the *Rhizopus stolonifer* fungus [4]. Furthermore, other study reported that at a concentration of 5% coconut shell liquid smoke was able to inhibit the growth of *Fusarium oxysporum* and *Colletotrichum gloeosporioides* [13].

The role of phenol compounds and their derivatives is associated with impaired integrity and permeability of fungal cell membranes which can cause permanent damage to cell walls and membranes. Observations using a Scanning Electron Microscope (SEM) regarding the effect of eugenol on *Zygosaccharomyces rouxii* with the results showing that eugenol at the minimum inhibitory concentration (0.4 µl / ml) is able to cause the cell surface to shrink, while increasing the concentration to the minimum fungicide concentration (0.8 µl / ml) causes cell destruction and the presence of a torn cell surface [14].

K_0 K_1

Figure 2: *S. rolfsii* attack on K_o (control); and on K_1 (1% concentration of liquid smoke).

3.4. DIAMETER OF THE LESION *IN VIVO*

The results showed that there was an effect of anti-fungal activity on the length of fungal lesions infecting plant stems. At 1 day after application, there was a very significant effect of the antifungal activity of liquid smoke, and at

2 days after application, there was an increase in the diameter of the lesion in the control treatment, followed by the concentration of liquid smoke from 1 to 4%. The highest increase in lesion length occurred in the control and it was different for all the liquid smoke treatments that were tried. In the same treatment, the concentration of liquid smoke there was a tendency that the more the concentration of liquid smoke would decrease the length of the fungal lesion. This is in line with the study results showed that coconut shell liquid smoke in the range of 5 to 10% was able to suppress the development of pod rot disease by pressing the length of spots on the fruit caused by *Phytoptora palmivora* [15].

Table 4: Effect of liquid smoke concentration on diameter of lesion

Liquid smoke concentration	Diameter of the lesion (mm)							
	1 DAI		2 DAI		3 DAI		4 DAI	
K_0 (0%)	4,61	a	11,14	a	16,53	a	22,58	a
K_1 (1%)	0,00	b	2,01	b	4,09	b	4,52	b
K_2 (2%)	0,00	b	0,77	bc	1,69	c	1,83	c
K_3 (3%)	0,00	b	0,40	c	1,09	cd	1,18	cd
K_4 (4%)	0,00	b	0,03	c	0,80	cd	0,86	cd
K_5 (5%)	0,00	b	0,00	c	0,00	d	0,20	d
K_6 (6%)	0,00	b	0,00	c	0,00	d	0,16	d

Note: Numbers followed by the same letter indicataes not significantly different according to Duncan's Multiple range test level of 5%. The data was transformed by $\sqrt{(x + 0.5)}$. DAI = days after inoculation.

The process of formation of lesions through several series of biochemical processes with enzymatic mechanisms. The mechanism of enzyme inactivation was thought to be the most influencing factor in the formation of lesions on plant stems. Phenolic compounds as antimicrobials in several mechanisms that occured include inactivation of essential enzymes, as well as functional destruction or inactivation of genetic material in the fungal body [13]. This was supported by the study, which found that the mechanism of phenolic compounds as anti-fungal was through inhibition of enzyme activity needed when infecting plants [16].

The effect of acid content in low concentrations of liquid smoke was thought to have little effect on fungal development, because fungi are able to adapt to fairly acidic environmental conditions. However, environmental conditions such as low pH are thought to directly acidify the cytoplasm, damage the surface tension of the membrane and lose the active transport of food through the membrane, causing the destabilization of various functions and structures of cell components [13].

4. CONCLUSION

The results of the study provided the following conclusions:

1) The pyrolysis of 1 kg of teak wood shavings was produced with the proportions of liquid smoke, charcoal and tar, respectively: 312 mL, 31 g, 367 g and the uncondensed gases.
2) Liquid smoke from teak wood shavings was effective as fungicide on the damping off pathogen (*Sclerotium rolfsii*). The effectiveness of this liquid smoke includes: inhibition of the diameter of fungal colonies, suppression of disease occurance rates, and suppression of lesion diameter.
3) Concentrations of liquid smoke ranging from 4% were effective as a fungicide to control *Sclerotium rolfsii* as a pathogen for soybean damping off.

SOURCES OF FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The author have declared that no competing interests exist.

ACKNOWLEDGMENT

The authors are grateful for the support of the Directorate General of Research and Development of the Ministry of Research, Technology and the Higher Education Republic of Indonesia under the Grant Agreement of the Basic Research Number 209 /SP2H/LT/DRPM/2019.

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