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Activity of Bamboo Wulung's Smoke *Gigantochloa atroviolace* againts Subterranean Termites and Fungi Attack

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ABSTRACT

Termites are plants, housing, furniture, and building pests. Termite control has been carried out using synthetic pesticides that are harmful to the environment and human health. Innovation in controlling termites and fungi is required for the preservation of wood and furniture. The aim of this study was to analyze the use of bamboo Wulung's (*Gigochocha atroviolace*) smoke for controlling termites and fungi attacks. The methods used were the manufacture of bamboo's smoke, a test of preference for termites, and mortality of termites. The results showed that liquid smoke at 450°C was effective for controlling termites and fungi. The smoke of bamboo Wulung with a concentration of 10% can control *C. formosanus* termites. It showed 100% mortality within 14 days, while the smoke of bamboo Wulung with a concentration of 5% showed 100% mortality of *R. speratus* within 14 days. Bamboo smoke at pyrolysis temperature 450°C and a concentration of 2.0% can prevent the growth of *T. vesicolor* fungus while effective at the same concentration for *F. palustris*. Bamboo smoke can prevent the growth of *T. vesicolor* mushrooms at a liquid smoke temperature of 450°C with a concentration of 2% and the fungus *F. palustris* at a concentration of 2.0%.

INTRODUCTION

Termites are known as the public's pests whose attacks are increasing from year to year. Losses caused by termites are estimated at IDR 8.7 billion/year reported by Nandika, Rismayadi, & Diba (2015). This is caused by the impact of construction. Naturally, termites live in the forest, but now they become urban pests in plantations, housing, and furniture. Research conducted by Widyastuti, Riastiw, & Suryanto (2019) says that termites can make nests and tunnels in teak logs that are still alive. Termite's attacks are estimated to be around 19% of the total amount of teak in the region.

Termite's control in Indonesia uses synthetic pesticides but the compounds in them endanger humans and the price is expensive. Another disadvantage is not environmentally friendly, not right on the targetted insects, and can cause cancer if exposed to human skin (Subekti, 2019).

The innovation of termite's control in Indonesia with environmentally friendly materials needs to be developed using materials that are easily available, affordable prices, and safe for humans. The use of liquid smoke from bamboo is an alternative system to control termites. Several studies have been carried out using natural materials such as *Aquilaria malaccensis* leaves (Subekti, Widiyaningrum, Nurvaizah, & Mar'Ah, 2019) and *Rhizophora* sp. leave extract (Syahidah & Subekti, 2019). The limited raw material is the weakness of these findings.

Wood and wood products in Indonesia are very resistant to biodeterioration primarily by termites and certain fungi. Research conducted by Hadi, Nurhayati, Jasni, Yamamoto, & Kamiya (2012) says that the results of wood products for buildings such as Alba, pine, and rubber are found to have many termite pests, brown rot fungi, and white-rot fungi.

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Wood as lignocellulose material is the main ingredient in termite food to maintain its life. Cellulose is eaten by termites, and then digested, broken down by cellulase enzymes to produce energy. These enzymes are produced by microorganisms in the termite intestine reported by Susilowati, Subekti, & Bintari (2018). Wood resistance is directly related to the content of extractive substances that are toxic to termites and wood rot fungi reported by Oramahi & Yoshimura (2013).

The mechanism of phenol compounds activity as anti-microbial is the occurrence of reactions with cell membranes that cause disruption of cell membrane permeability processes and the inactivation of essential enzymes, or a combination of both. In addition, compared to phenol itself, phenol oxidation compounds have higher anti-microbial activity, through disruption of cell wall formation and regulation of cell metabolism. In research reported by de Souza Araújo et al. (2018) revealed that the content of liquid smoke compounds of *Eucalyptus urograndis* and *Mimosa tenuiflora* was able to constrain the growth of bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (ATCC 313131 and 102A 10231) and *Cryptococcus neoformans*. In both types of liquid smoke, the dominant components are furfural and phenol. The phenol content of liquid smoke *Eucalyptus urograndis* and *Mimosa tenuiflora*, respectively 44.04 and 56.36%.

Smoke from plants is a product of the combustion reaction that produces water, its evaporate increases if the material burned has high water content. Ingredients from smoke produce many acids and phenols. The results of this fogging produce functional properties as wood preservatives and anti-microbial materials reported by Hadi, Efendi, Massijaya, Arinana, & Pari (2016). Making smoke from bamboo can overcome the previous problem because bamboo raw materials are abundant and easy to find.

Liquid smoke resulting from brownish condensation needs to be purified by distillation to remove harmful compounds and clearer colors. The pyrolysis component is a phenol, carbonyl group with antioxidant and antimicrobial properties reported by Adfa et al. (2017). These compounds include phenols and phenol ethers such as guaiacol (2-methoxy phenol), siringol (2,6-dimethoxy phenol),

and their derivatives. In hardwood, pyrolysis will produce siringol as the main component, while in softwood, pyrolysis will produce guaiacol and its derivatives reported by Hadi, Nurhayati, Jasni, Yamamoto, & Kamiya (2010). The group of compounds is able to prevent the growth of bacteria and fungi and viruses reported by Faisal, Utari, Hayvia, & Maulana (2019). The advantages of this liquid smoke can be produced commercially, while the advantages of distillation are reducing the content of unneeded PAH (Polycyclic Aromatic Hydrocarbon) compounds such as benzo(a)pyrene, removing fat and salt reported by Montazeri, Oliveira, Himmelbloom, Leigh, & Crapo (2013).

Wood as lignocellulose material is the main ingredient in termite food. A termite attack on wood is not determined by its specific gravity but is related to extractive substances in wood that are toxic to termites and microorganisms in their intestines reported by Ni & Tokuda (2013). This can be understood because there is a mutualism relationship between termites and microorganisms, namely bacteria, fungi, and protozoa in their intestines reported by Subekti, Fibriana, Widyaningrum, & Adfa (2017). Therefore, to control termites and wood rot, the fungus can use toxic compounds from the smoke. The innovation of this research is the use of liquid smoke from bamboo Wulung (*Gigantochloa atrovioleacea*) as an environmentally friendly biopesticide for the control of soil termites and wood rot.

MATERIALS AND METHODS

Preparation and Smoke Liquid of Bamboo

Smoke from bamboo Wulung was collected from Sekaran village in Semarang, Central Java, Indonesia and transformed to bamboo meals in Wood Workshop Laboratory, LITBANG Bogor, West Java, Indonesia by 40–60 mesh screens Willey mill, air-dried to about 15% of moisture content. The dried material (1.000 g) was put into a sealed reactor (2.000 g capacity) and was heated up to 350 and 450°C with a 5°C/minutes heating rate. Smoke was funneled to a cooling column by a channel. The cold smoke was then preceded to the other column with a pump to restore the evaporated vinegar. All smoke of bamboo produced at a temperature of 350 and 450°C is for an antifungal test, whereas the smoke from 450°C is for the test of anti-termites.

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Anti-termites

Soldiers and workers of *Reticulitermes speratus* (Kolbe) were from three field colonies at the Uji Campus of Kyoto University, Japan. Subterranean termites *Cryptotermes formosanus* Shiraki was from the Research Institute for Sustainable Humanosphere (RISH) laboratory colony, Kyoto University. This colony has been maintained at more than 85% RH in the dark with $28 \pm 2^\circ\text{C}$. To get liquid smoke, condensation is needed using fresh water, where the pipeline from the output of the gasification results is passed to the condensation basin filled with water. Then the tar will be condensed and collected down at the tar shelter reported by Abnisa, Arami-Niya, Wan Daud, Sahu, & Noor (2013).

Antifungal Assay

Liquid smoke was mixture with agar media. Incubation 1 x 24 hour. Fungal cultures, i.e., brown-rot fungus strain, fungal inoculation was carried out by means of the media being poured into Petri plates and then inoculated in the center with a 5 mm single plug of each fungus. Controls were assembled by Petri plates with PDA (Potatoes Dextrose Agar). All concentrations and controls were prepared in four replicates. After inoculation, the prepared dishes were incubated at 27°C . The measurement of fungal inhibition activities was performed by daily determining the colony diameter and calculating the inhibition rate of mycelia by the following equation (Oramahi, Yoshimura, Diba, Setyawati, & Nurhaida, 2018).

$$I = \frac{(C-T)}{C} \times 100 \dots\dots\dots 1)$$

Where:

I is the inhibition rate,
C is the mycelium diameter of control (mm), and
T is the mycelium diameter of smoke bamboo treatment (mm).

No Choice Test

This method was to evaluate the activity of soil termite and check the toxic effects of bamboo Wulung's liquid smoke. As much as 0.3 ml Wulung bamboo smoke was then pipetted after the concentration test prepared into Whatman No. 1 filter papers. The filter paper was settled on a 60 mm Petri plate. Then, fifty-five active termites (5 soldiers and 50 workers) were placed on each filter paper. Control paper filters only used deionized water, with concentrations of smoke Wulung bamboo 1.0,

3.0, 5.0% (v / v); and 2.5, 5.0, 7.5, 10.0% (v / v) (respectively for *R. speratus* and *C. formosanus*). Later, it was covered and incubated at 26.5°C incubator and 80% RH. All treatments were in four replication when observing the termites for 21 days. It is only liquid smoke with pyrolysis temperature 450°C was tested.

Choice Test

The bioassay method is done to observe the behavior of soil termites against bamboo Wulung's smoke. This observation was done by using 60 mm-diameter Petri dishes and a pair of 8 mm-diameter paper discs (Advantec Co. Ltd. Tokyo, Japan): smoke winding bamboo treatment disc (treated) and a DI-treatment disc (untreated). Both paper discs were arranged to a Petri dish by about 25 mm apart. The observed smoke bamboo concentrations were 100, 50, and 10% (v/v), and the paper discs were saturated by 0.1 ml of bamboo smoke solutions or DI. Thirty workers were arranged in the center of the paired disc on the Petri. Ten replications were done for each concentration. The number of workers in contact with each disc was then determined every 5 minutes for 60 minutes (n = 12 counts). It is only the smoke at 450°C was used in the testing. A completely randomized design observation of wood rot fungus activity was done by the factorial of 2×5 . The first factor was temperature of pyrolysis (300°C and 450°C), and the second factor was liquid smoke concentration (0%, 0.5%, 1.0%, 1.5%; 1.0%; and 2%). All data were analyzed by SPSS 23.0.

RESULTS AND DISCUSSION

Bamboo Wulung's Smoke (*Gigantochloa atroviolacea*) Properties

The smoke of Bamboo Wulung used was liquid smoke, i.e., 300°C and 450°C . Table 1 shows that the acidity and phenol content was from 1.97 to 4.47% and 5.53 to 8.03%, respectively. The color of the smoke was observed from bright yellow to yellowish-brown, whereas the shade of the smoke was in accordance with the temperature rise. The difference in color from bamboo Wulung's smoke is caused by a large amount of acid contained therein. The type of bamboo and liquid smoke technique greatly affect the quality of the acid and phenol content in it. It also greatly influences the compound characteristics of the extraction and chemical content of bamboo Wulung including cellulose, hemicellulose, lignin, carbon, and ash content.

Table 1. The total concentrations of smoke bamboo acid and phenol

Temperature (°C)	Total acid (%)	Total phenol (%)
300	4.47±0.02 a	5.33±0.02 a
450	1.97±0.01 b	8.03±0.03 b

Remarks: Numbers by different letters in the similar column are significantly different at the level of $p < 0.05$ in Tukey's test.

In this study, the comparison of bamboo Wulung smoke obtained with liquid smoke temperatures of 300°C and 450°C is observed. There is a difference in the content of Acid and Phenol in both temperatures. Significant differences are indicated by the letter next to the value, differentiated by total acid and phenol. Table 1 shows that the highest phenol content in pyrolysis results is 450°C. Total acid has a significant contribution to the growth of fungi. In fact, acid content, phenol in the smoke of bamboo Wulung also affects the antimicrobial activity.

Test of Wooden Mold Fungus Activities

The effect of the rising concentration of the liquid smoke of bamboo Wulung on the *T. versicolor* growth is compiled in Table 2. All bamboo has significant restraint to the growth of fungi, and the effectiveness of the liquid smoke toward fungi raised as liquid smoke temperature elevated. Bamboo that smoked at 450°C showed a significantly higher restraint in *T. versicolor* than at 300°C at concentrations at 0.5%. As in Table 1, the phenol of liquid smoke produced at 450°C was significantly higher than liquid smoke of 300°C.

The effect of increasing concentration and temperature in *T. versicolor* and *F. palustris* samples is shown in Table 2. Overall, the treatment of both samples had a significant effect and the level of inhibition increased with the addition of concentrate. Treatment at 450°C showed a significantly higher level of resistance for *T. versicolor* and *F. palustris* samples compared to treatments at 300°C at a concentration level of 2.0%.

Bamboo smoke at 450°C could prevent the growth of *T. versicolor* fungi at the highest concentration of 1.5%, while *F. palustris* was effective at the same concentration. Bamboo smoke could prevent the growth of *T. versicolor* fungi at a pyrolysis temperature of 300°C with a concentration of 2% and *F. palustris* at a concentration of 1.5%.

Table 3 shows untreated (7-21 days), the percentage of termite mortality was significantly different from the treatment concentration of 1.0% for 14 days. At a concentration of 1.0% for 7 days, termite mortality was significantly different from the treatment concentration of 3.0% for 14 days. Treatment concentration of 1.0 for 21 days, termite mortality was significantly different with a treatment concentration of 3.0% for 7 and 21 days and a concentration of 5.0% with a duration of 7-21 days.

Table 2. The smoke bamboo effect on *Trametes versicolor* and *Fomitopsis palustris* growth

Treatment	Concentration (%)	Inhibition (%)	
		<i>T. versicolor</i>	<i>F. palustris</i>
Control	0	2.89±1.33 d	2.14±0.92 d
W1 (450°C)	0.5	1.93±1.18 c	1.43±1.11 c
	1.0	0.25±0.05 a	0.10±0.10 a
	1.5	0.02±0.06 a	0.00±0.00 a
	2.0	0.25±0.05 a	0.00±0.00 a
W2 (300°C)	0.5	1.51±0.96 c	0.86±0.55 c
	1.0	1.77±1.15 c	1.34±0.89 c
	1.5	1.34±0.91 b	0.00±0.00 a
	2.0	0.87±0.63 a	0.00±0.00 b

Remarks: Numbers followed by diverging letters in a similar column are significantly different at $p < 0.05$ according to Tukeys test.

Table 3. Toxic effect of the smoke of bamboo Wulung at 450°C in the no-choice test toward *Reticulitermes speratus*

Treatment	Mortality of termite (%)		
	7 days	14 days	21 days
Untreated	0.67±1.15 a	3.06±8.33 a	2.08±1.33 a
1.0%	9.66±1.15 a	8.67±0.58 a	34±1.73 c
3.0%	14.33±2.08 b	15±2.65 b	35±0.00 c
5.0%	19±1.73 b	35±0.00 c	35±0.00 c

Remarks: Numbers followed by diverging letters in a similar column are significantly different at $p < 0.05$ according to Tukeys test.

Table 4 shows that for untreated termites (7-21 days), termite mortality was significantly different with a concentration of 2.5% for 7 and 14 days and a concentration of 5.0% for 7 days. Termite mortality at a concentration of 5.0% for 14 days was

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significantly different from a concentration of 7.5 with duration of 7 and 14 days. Termite mortality at a concentration of 5.0% for 21 days was significantly different from a concentration of 7.5 for 21 days and a concentration of 10.0% for 21 days. In summing up, each treatment of the sample gives a significant effect/difference. Each treatment and the duration of observation gave different mortality rates. Judging from the mortality rate, 10.0% of treatment had the most significant effect on termite mortality because it gave a very sharp increase from an average of 7.33 on day 7 to 35 on day 14.

Table 4. Bamboo Wulung smoke toxic effect at 450°C in the no-choice test on *Coptotermes formosanus*

Treatment	Termite mortality (%)		
	7 days	14 days	21 days
Untreated	0.00±0.00 a	3.33±0.58 a	15.33±2.52 a
2.5	2.00±1.00 a	11.00±1.00 a	23.67±9.87 b
5.0	1.67±0.58 a	19.67±0.58 b	35±0.00 c
7.5	3.33±1.15 b	30.67±2.08 b	33.33±2.89 c
10.0	7.33±1.15 c	35±0.00 c	35±0.00 c

Remarks: Numbers followed by diverging letters in the related column are significantly different at the level of $p < 0.05$ according to Tukeys test.

Table 5. Filter paper repellency with the smoke bamboo produced at 450°C on *Reticulitermes speratus* workers in the choice test

Experiment	Number of termites in contact with the filter paper discs (60 min ^a)
Control	0.51±1.11 a
Smoke of bamboo (10%)	7.88±10.03 b
Control	0.64±1.40 a
Smoke of bamboo (50%)	6.93±8.82 b
Control	0.25±0.47 a
Smoke of bamboo (100%)	5.55±4.49 b

Remarks: A paired comparison t-test was employed for each experiment $df = 9$, ^a mean ± SD no of counts in a 60 minutes period (n=12). Each means is occupied on 300 termites (ten replicated x 30 termite worker per replication).

Table 5 shows that the total termites in contact with filter paper in the control treatment for bamboo smoke were 10% significantly different from

the total termites in the control for 50% and 100% bamboo smoke. Meanwhile, the total termites in contact with filter paper in the 50% bamboo smoke did not have a significant difference to the 100% treatment. The greater the concentration of bamboo smoke, the more termites is not interested in filter paper. This is understandable because the higher the concentration of bamboo Wulung's smoke, the more phenol content in the filter paper, and the more termites avoid. The nature of volatile phenols is also the reason termites away from filter paper.

Table 3 and Table 4 explain termite mortality and weight loss of filter papers in the no-choice test with the liquid bamboo smoke from temperatures of 450°C. In *R. speratus* termites, termite mortality increases with increasing concentrations from 1.0 to 5.0% after 7, 14, and 21 days (Table 3). In *R. speratus* termites, smoke bamboo with a liquid smoke temperature of 450°C, at a concentration of 5.0% can termite mortality 100%, while termite *C. formosanus* requires a concentration of 10% can termite mortality 100% (table 4). This is due to differences in body size and feeding activities. In fact, *C. formosanus* is bigger compare to *R. speratus* in size as well as has higher activities of feeding.

The same study was conducted by Yoshimura, Imamura, & Takahashi (2003) that the average consumption requirements of *C. formosanus* and *R. speratus* as soil termites were 0.0961 mg/termite/day and 0.0737 mg/termite/day in the *P. densiflora* smoke wood treatment. The results showed that the concentration of smoke wood 10% can cause mortality of *C. formosanus* termites 100%, meanwhile the concentration of smoke wood 5% mortality of *R. speratus* termite 100%.

research with smoke originating from the wood of *Cryptomeria japonica* (Thunb. Ex L.f.) D. Don and *Pseudotsuga menziesii* (Mirb.) Franco, *Q. serrata*, and *P. densiflora*, has significant effects on controlling soil termites due to the content of was acetic acid. Liquid smoke produced from the 3 types of wood mentioned above is very high in toxic nature to control *R. termite* soil termites. Organic matters from liquid smoke and acetic acid have different abilities to control termites.

Testing using termites of *R. speratus* and *C. formosanus* workers made regular association with both water treatment-filter papers (control) with no significant difference ($P > 0.05$, $df = 9$, paired comparisons t-test, as in Table 5). The same study

1 conducted by Bläske & Hertel (2001) said that the paper discs with the smoke solution at 100, 50, and 10% make the workers cast away from the paper discs.

CONCLUSION AND SUGGESTION

The results showed that liquid smoke of *Gigantochloa atroviolece* can be used to control soil termites and wood rot fungi. The termite mortality of *Coptotermes formosanus* was the highest in bamboo smoke with a concentration of 10% within 14 days. Mortality is the highest in soil termites *Reticulitermes speratus* at a concentration of 5% smoke bamboo within 14 days. Liquid smoke with a temperature of 450°C with a concentration of 2% is the best in inhibiting *T. vesicolor* and *F. palustris* fungi. For the future, further research is needed to test the effectiveness of this compound in the field test.

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