Antitermite and Antifungal Properties of the Soldier Defense Secretions of *Coptotermes curvignathus* Holmgren (Blattodea: Rhinotermitidae)

by

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Abstract

This research aimed to evaluation the antitermites and antifungal activities of soldier defensive secretions from *Coptotermes curvignathus* Holmgren. The secretions were extracted with ethyl acetate solution. *Acacia mangium* wood was preserved with soldier defensive secretions extract. The preservation method was dipping in concentration of 1%, 2% and 3%. After dipping for 48 hour, the wood then exposure to termites and fungi to evaluated the antitermites and antifungal of extract as biopreservatives. The termites used was *Coptotermes curvignathus* Holmgren and the fungal used was *Schizophyllum commune* Fries. The result showed that durability of *Acacia mangium* wood was increased. The average weight loss of wood by termite attack was range between 6.67-15.56% and termite mortality was range between 44.45-90.44% and the average weight loss by fungi attack was range between 4.01-8.78%. The compound then purified by GCMS and the result showed that mainly bioactive compound is Tetradecanal $C_{14}H_{28}O$.

***Keywords**:* soldier defensive secretions, *Coptotermes curvignathus, Schizophyllum commune,* biopreservatives, Tetradecanal

Introduction

Biodegradation is recognized as one of the most significant problems for wood utilization. Among several factors leading to biodegradation, termites are one of the most damaging to wooden structures worldwide. There are several properties that determine wood's natural resistance to termite attack. For example, durability of wood affects the termite's ability to fragment the wood with its mandibles. Insecticide treatment has been widely used to control termites. However, insecticide residues have adverse effects on humans. Hence there is a need for an alternative method for termites control by using biopreservatives. On the other hand, a major cost of social insect's life is the increased exposure to pathogens. This cost of sociality is expected to be particularly high for social insects, which live in crowded, persistent, warm and resource-rich nests providing ideal conditions for the development of microorganisms. Hence, social insects have evolved a variety of behavior and physiological defense mechanisms, including antibiotic-producing symbionts and allogrooming (Roseangus *et al*, 1998), antibiotic secretions (Roseangus *et al*, 2000), removal of wastes and corpses (Roseangus *et al*, 2001) and immune defenses (Traniello *et al*, 2002). Another potential mechanism of defense may be to add plant compounds with antimicrobial properties to the nest. Soldier termites of the subfamilies Coptotermitinae employ chemical secretions in colony defense, applying a lipophilic mixture of electrophilic compounds which act as contact insecticides

(Lamberty *et al*, 2001). So far, the antitermites and antifungal of chemical defense of termites soldier hasn't been investigated. This research aimed to investigation the antitermites and antifungal of soldier defensives secretions from *Coptotermes curvignathus* Holmgren.

Materials and methods

Isolation and extraction soldier defensive secretions

Colonies of subterranean termites *C. curvignathus* Holmgren were obtained from dead *Hevea brasiliensis* tree in Tanjungpura University Campus, West Borneo Indonesia and rearing on Laboratory of Forest Product, Faculty of Forestry Tanjungpura University (*Laboratory reared termites colony)*. Soldiers were removed from the colony and the defensive secretions were isolated and extracted according to Quintana *et al* (2003) methods. Number of soldier used was 4000 termites soldier. The secretions then extracted with ethyl acetate solution.

***Schizophyllum commune* culture**

Schizophyllum commune culture was from Indonesian Sciences Institutes Bogor Botanical Garden West Java Indonesia. The fungus was culture in potatoes dextrose agar and incubation in culture room at 26,5^{o}C.

Wood preservation process

Sapwood blocks of *Acacia mangium* wood measuring 1 cm (T) × 1 (R) × 2 cm (L) were used as the food source for termites and fungi. Concentrations of extract were 1%, 2% and 3% (v/v). The wood was dipping for 48 hour and then air dry for 24 hour before tested to termites and fungi.

Antitermites test

Antitermites test was conducted according to Sornnuwat, *et al* (1995). The glass bottle with volume of 180 cm^3, 4.5 cm in diameter and 11.5 cm in height was used as a container. Thirty gram of sand sieved through 20 meshes was filled in the bottle and moistened with 6 ml of distilled water. One of each block was buried in the sand of glass bottle. 220 termites which contain of 200 workers and 20 soldiers were introduced into the bottle. Each bottle was plugged with cotton wool and kept in the dark under ambient room condition for 21 days. After 21 days, wood blocks were taken out from the containers, cleaned and oven-dried and reweigh to determine percentage weight loss from the equation:

$$\text{Weight loss (\%)} = (W_1 - W_2) / W_1 \times 100$$

W_1 : weight of wood block before exposure to termite

W_2 : weight of wood block after exposure to termite

Number of dead termites was recorded at the end of the test. Mortality test was determined from the equation :

$$\text{Mortality (\%)} = (N_1 - N_2) / N_1 \times 100$$

N_1 : number of initial workers

N_2 : number of dead workers

Antifungal test

Antifungal test was conducted according to Loman (1970) in Syafii and Yoshimoto (1993), using agar block test methods. The petri dish with Ø 9 cm and 1 cm in height was used as a container. Ten ml potatoes dextrose agar (PDA) was filled to petri dish as a substrate for fungi growth. Then one isolate of *S. commune* fungi (Ø6 mm, 5 days hyfa) was introduced in the center of petri dish. One of each block then was introduced on the top of fungi. Each petri dish then kept in the dark under ambient room condition for 12 weeks. After 12 weeks, wood blocks were taken out from the containers, cleaned and oven-dried and reweigh to determine percentage weight loss from the equation:

$$\text{Weight loss (\%)} = (W_1 - W_2) / W_1 \times 100$$

W_1 : weight of wood block before exposure to fungi

W_2 : weight of wood block after exposure to fungi

Analysis the bioactive compound

The soldier defensive secretions extract were identified by gas chromatography mass spectra (GCMS) analysis. The GC was Shimadzu QP 5050 model equipped with GC 17a , column DB5 MS (30 m length and 0.25 mm diameter). The GC settings were as follows : initial column temperature set at 40^{o}C for 5 min; temperature programmed from 40^{o}C to 330^{o}C with a rate 4 ^{o}C/min. Mass spectra resolution was 1000, with interval 0.5 sec; ion energy 0.90 kV and retention time 1.6-56.0. Identified the compound were obtained according to Clement *et al* (2001) and compared with data on NIST 62 (*National Institute Standard and Technology)*, WILEY 229 and PESTICD.LIB.

Result and discussion

Antitermite properties

Result of the research showed that secretions from *C. curvignathus* was increased the durability of *Acacia mangium* wood against *C. curvignathus* termites. The average weight loss of *Acacia mangium* wood was 6.67-15.56%, meanwhile on control wood was 63.33%. The lowest weight loss was on concentration 3%. Termites produced defensive secretions to prevent the microorganisms attack in the soil (Christe *et al.* 2003). The physiology and mechanism of soldier defensive secretions was variety, including antibiotic on *Pseudacanthotermes spiniger* (Lamberty *et al.* 2001) and *Nasutitermes costalis* and *N. nigriceps* (Roseangus *et al.* 2000), *allogrooming* on *Zootermopsis angusticolis* (Roseangus *et al.* 1998), and *immune defense)* (Traniello *et al.* 2002). The average weight loss value of *A. mangium* wood which has been preservative with termites secretions in different concentration after exposure to termites *C. curvignathus* was shown on Table 1.

Table 1. The average values of termites mortality, *Acacia mangium* wood weight loss by termites and fungi *S. commune* attack (mean ± SE)

Values	**Concentration**			**Control**
	1%	**2%**	**3%**	
Termites mortality (%)	44.45 ± 2.66	74.12 ± 4.32	90.44 ± 4.73	10.22 ± 1.29
Wood weight loss by termites attack (%)	15.56 ± 5.27	8.44 ± 1.24	6.67 ± 0.78	63.33 ± 2.55
Wood weight loss by fungi attack (%)	8.78 ± 0.53	6.89 ± 1.58	4.01 ± 0.87	14.78 ± 3.56

Note: each value is means of three replicates

Antitermite properties of soldier defensive secretions from *C. curvignathus* have the mechanism as toxic/poison to termites. The average termite mortality value was 44.45-90.44%. Meanwhile the average termites mortality value on control treatment was only 10.22%. The highest termites mortality was on concentration 3%. Table 1 explained the termites mortality value on Acacia wood after 21 days exposure.

Antifungal properties

Result of the research showed that extract of soldier defense secretions has increased the durability of Acacia wood. The average wood weight loss value by fungus attack was range between 4.01-8.78%, meanwhile on control wood was 14.78%. The lowest wood weight loss was on concentration 3%. Table 1 shown the average wood weight loss by fungus *S. commune* attack. Diba (2008) have shown that soldier defensive secretions from *C. curvignathus* have secondary metabolite and have a function as antibiotic. This is the possibilities as antifungal also. The mechanisms inhibition the fungus growth by antibiosis system. Antibiosis was the growth inhibition mechanisms using secondary metabolite in the organisms (Billen *et al*. 2000). Roseangus *et al.* (2000) explained that Nasutitermes genus has secondary metabolites which function as antibiotic and inhibited the growth of *Metarhizium anisopliae* fungus. Lamberty *et al.* (2001) said that insect secretions have secondary metabolite which functions as antifungal and antibacterial such as *Heliothis virescens* which produce antifungal and namely heliomycyn. The fly *Drosophila melanogaster* produce secondary metabolite which functions as antifungal and namely drosomicin (Landon *et al.* 1997), meanwhile the spider also produce secondary metabolite, namely gomesin which can inhibited the growth of fungus and bacteria (Mandard *et al.* 2002).

Chemical constituents of soldier defensive secretions

The chemical composition of soldier defensive secretions and the relative amount of each component were determined by GCMS analysis. Chromatogram of extract soldier defensive secretions in ethyl acetate solution was shown in Figure 1 and the chemical compound was shown in Table 2. The chemical compound were consisted aldehyde group, namely Tetradecanal, Pentadecanal, and Undecanal, Ethyl propionate and n-butyl acetate from Ester group and 1-butanol from alcohol group.

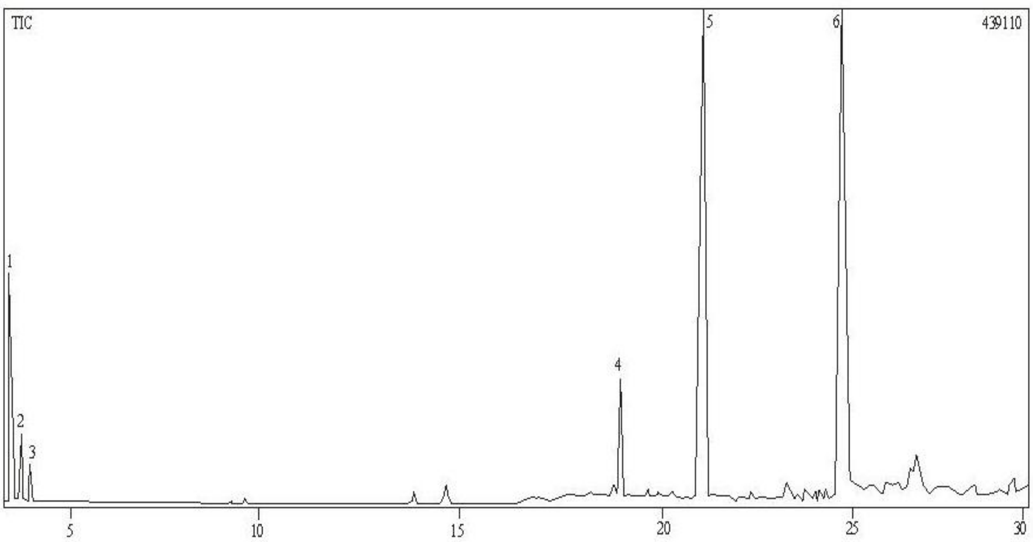

Figure 1. Chromatogram of *C. curvignathus* secretions in Ethyl acetate Extract
Peak Number is based on Number on Table 2.

Table 2. Chemical Compound and Retention Time from Ethyl acetate Extract *C. curvignathus* secretions

Peak No.	Retention Time	LRIexp	LRIref	Chemical Compound	Group
1.	1.954	0	696 [(a)]	Ethyl propionate	Ester
2.	2.067	0	-	1-butanol	Alcohol
3.	2.925	0	795 [(a)]	n-butyl acetate	Ester
4.	19.422	1025	-	Undecanal	Aldehyde
5.	21.669	1096	1107 [(b)]	Tetradecanal	Aldehyde
6.	23.717	1137	-	Pentadecanal	Aldehyde

Remarks : LRI experiment from GC-MS, DB-5 Column
LRI reference (a) Boscaini *et al*. (2003), DB-5 Column
LRI reference (b) Mahattanatawee *et al*. (2004), DB-5 Column

Conclusion

Soldier defensive secretions from *C. curvignathus* have increased the durability of *Acacia mangium* wood against *Schizophyllum commune* fungi and *C. curvignathus* termites. The average weight loss of Acacia wood was 6.67-15.56%, meanwhile on control wood was 63.33%. The average termites mortality value was 44.45-90.44%, meanwhile on control wood was 10.22%. The average wood weight loss value by fungus attack was range between 4.01-8.78%, meanwhile on control wood was 14.78%. The optimum concentration was 3%. Gas chromatography mass spectra (GCMS) analysis resulting 6 volatile compounds, mainly aldehyde compounds, including tetradecanal, pentadecanal, and undecanal. The bioactive compound which functions as antitermites and antifungal is Tetra decanal.

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