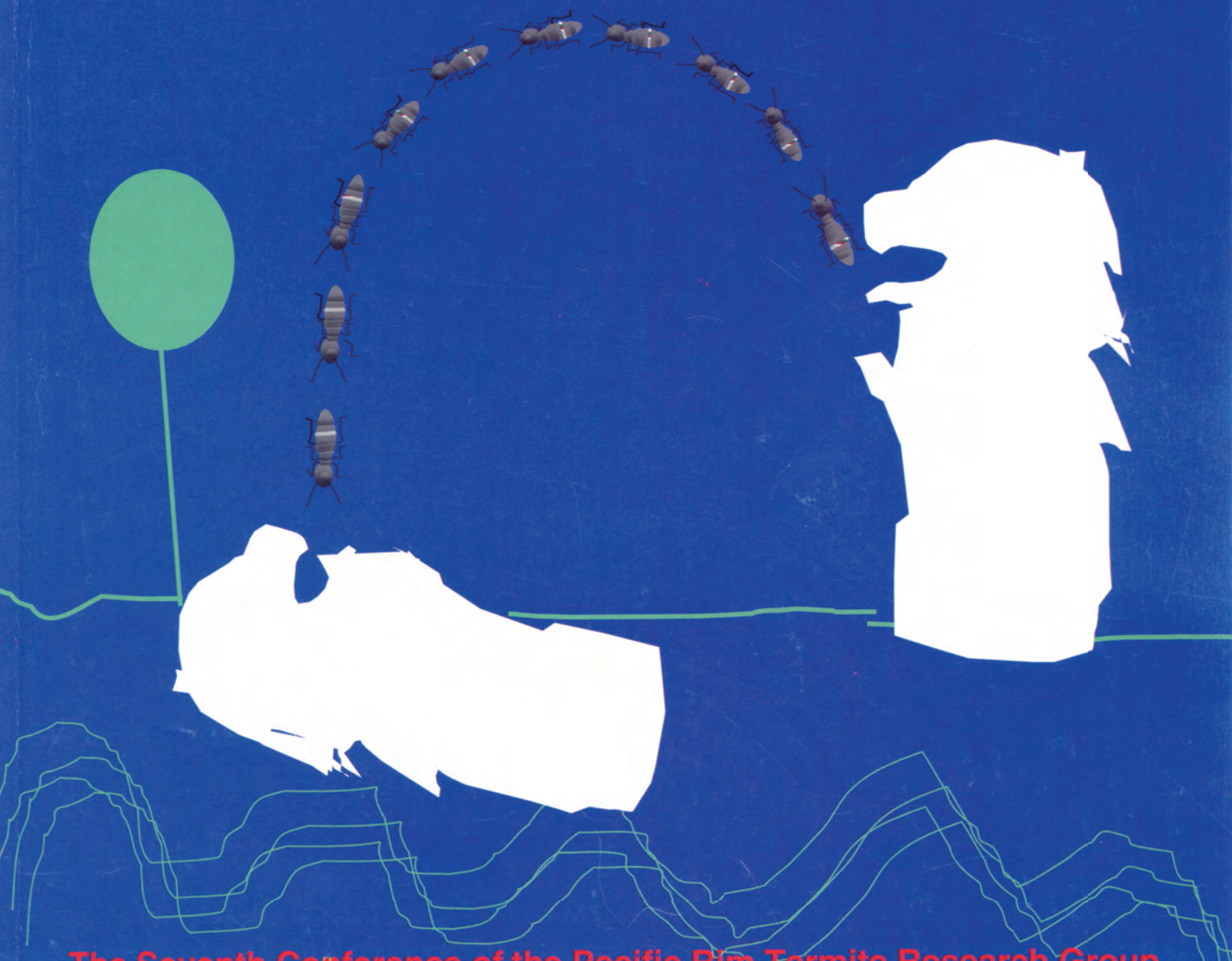
Proceedings – TRG 7



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Pathogenicity of Some Entomopathogenic Fungi Isolates against Dry Wood Termites *Cryptotermes* sp. (Isoptera: Kalotermitidae)

by

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Abstract

Through exploration at soil and sand in some regions of West Sumatera (Padang Pariaman, Padang, Sawah Lunto and Tanah Datar), were found 28 fungi isolates. Most of the isolates (64.28%) pathogenic against drywood termites, *Cryptotermes* sp., that belong to several divisions of fungi such as Ascomycota (*Aspergillus niger, Aspergillus* sp., *A. flavus*, and *Penicillium* sp.), and Deuteromycota (*Metarhizium harzianum, Fusarium* sp., *Beauveria* sp., and *B. bassiana*), and Zygomycota (*Rhizophus* sp.). *A. niger* is the most effective fungus to control *Cryptotermes* sp., that indicated by the ability to kill termites (in 10^7 conidial/ml could kill 96.97%), short lethal time $_{25,50}$, and $_{95}$, high viability and spore formation, but not differed with *Rhizopus* sp., *Aspergilus* sp. and *B. bassiana*.

Key words: Exploration, entomopathogenic fungi, pathogenicity, *Cryptotermes* sp.

Introduction

Dry wood termites, *Cryptotermes* spp. (Isoptera: Kalotermitidae), economically known as very dangerous pest. In Indonesia, Prasetyo (2004) found amount of lost cause of termites damage achieves 224-238 billion Rupiahs annually, while Iswanto (2005) states that drywood termites on the average building damage caused by drywood termites in big cities like Jakarta, Surabaya, Bandung, and Batam more than 70%. Because of that Drywood termites become serious problem in the world.

Drywood termites are an almost ubiquitous part of older wooden structures in regions where they commonly occur. Because drywood termites seek protection from external predation, the cryptic nature of those insects, allowed them to establish colonies without detected and made it difficult to determine the most effective treatment location and it may be difficult to immediately verifying the success of a given treatment.

Termite control mainly use chemical pesticides that may bring environmental problem. The use of chemical pesticides is the cause of many secondary environmental problems aside from the death of the targeted pest. After application of chemical substance, termite may recur. Furthermore, after that, some problems may still occur, chemical application process also can kill the native predator of termite. Long-term use of chemical pesticide in high dose render the termites generate resistance to the applied chemical insecticide. Communities are increasingly in need of natural solutions to pest problems.

The biological control presents an alternative means that can play a role in integrated pest management and reduce dependence on chemical pesticides. Generally, biological agents have little adverse ecological impact due to their specificity for the target host. The long terms environmental hazards and health concerns also are not a factor with biological control agents because chemical residues are not present.

One alternative to confine chemical substance in termites controlling, entails use of biological control agents, such as fungi that have potential as pathogenic agents (Culliney et al., 2000). Using microbial insecticide for termite control has several advantages such as relative have low cost, have many strains and can be germinate strain *invitro* (Oka 1995).

Fungi exhibit qualities that can make them ideal for termites controlling, including a slow-acting nature similar to that of successful chemicals, the ability to self-replicate and the ability of fungal spores to spread by termite social behavior (Grace et al. 1992). Conditions in a termite nest, moderate temperature and high humidity, are also conducive to the growth of fungal species and are important factors in fungal survivability and propagation (Kramm et al. 1982).

Currently, the microbial insecticide used for termites control in the world primarily is *Beauveria bassiana* and *Metarhizium anisopliae.* How ever, there are numerous entomogenous and entomopathogenic fungal species known. Milner et al. (1996) review a wide variety of fungal

pathogens that been reported as potential pathogens to termites. Stamet (2003) found suitable entomopathogenic fungi include Metarhizium, Beauveria, Paecilomyces, Hirsutella, Verticillium and other fungi imperfecti, the Entomophthoracae and other Phycomycetes, and sexually reproducing fungi such as Cordyceps and other Ascomycetes. According to wide variety and potency of fungus that can be function as biopesticide to control drywood termites, it is believe that there are still many fungi that can be found and in various habitat, including in the soil and sand. Because of that it is had been explore and examined some soil and sand fungus against drywood termites.

Materials and methods

Entomopathogenic fungi obtained from various regions in West Sumatera (Padang Pariaman, Padang, Sawah Lunto and Tanah Datar), through dig the soil 5–10cm in depth. In each region taken 4.0 x 500gr soils and kept in plastic bag as an isolates resource. As much as 1gr isolate sources added by 5 ml sterile aquadest and stirred up around 5 minutes with vortex. After that, 1 ml suspension added 9 ml sterile aquadest and stirred up entirely and then 0.1 ml suspension inoculated to PDA medium and incubated in room temperature. Fungus from PDA medium isolated until forming pure isolates. Pure isolates of fungi then cultured in PDA medium and incubated for 3 weeks. Conidial suspension of fungi got out from PDA medium through shake down the petridish use sterile aquadest that contain 0.05% Tween 80. After that those suspension poured into test tube. Serial dilution method used for making suspension to the needs of (10^7 conidia/ml). Conidial density of fungi counted using haemocytometer.

Pathogenicity test of fungi use drywood termites that collected from infested building in Padang. Pathogenicity testing of fungi isolates conducted before identifying the isolates. Fungi application (10^7 conidial/ml) were done through topical method to 1 soldier and 10 worker termites. Infected termites transferred in to plastic bowl that covered with tissue paper which used as food of termites. Infested termites kept in dark room. Mortality of termites counted everyday for a week. Isolate be said to be pathogenic if can cause mortality > 60% (Desyanti, 2007). Isolates that showed pathogenic to drywood termites, then identified by virtue of fungi macroscopic and microscopic characteristics correspond to characteristic that stated by Barneet and Hunter (1972).

Observation on physiology characteristic of fungi carried out on selected entomopathogenic fungi (could kill termites >80%), including viability and spore formation. To determine fungi viability of selected fungi, as much as 0.1 ml of 10^5 fungi conidia/ml put in PDA medium and then incubated on 24^0C. Observation on conidial germinate one day after incubation. Comparison output between germinate conidial amount and conidial total that cast out in medium constitute as fungal viability. Observation on spore formation carried out by incubated 0.1 ml of 10^5 fungi conidia/ml in PDA medium on 24^0C during 15 days. After that, in petridish 50 ml sterile aquadest poured and shook around five minutes, filtered and diluted three times. Conidial concentration counted by haemocytometer and the average of conidial as compared to another isolates. Fungal colony growth (in diameter) observed from each Petri dish during 15 days with 3 days time gap.

Result and discussion

Fungi exploration result from soil and sand in four regions in West Sumatera found 28 isolates (Table 1). Cause of soil fungi found in each region. It indicated that soil is suitable habitat for fungus. Soil contains high organic material that needed for fungi growth. Soil humidity and its nutrition supposed support fungus growth (Storey and Gardner, 1987, 1988 cit. Trizelia, 2005). Soil texture could influence spore formation, persistence and conidia density (Trizelia, 2005). Entomopathogenic fungi were isolated from significantly more samples of soil from the woodland and hedgerow habitats than from the arable field (Chandler, 1997).

Pathogenicity testing to drywood termites showed that 64.28% of isolates pathogenic to drywood termites. It means that generally fungus isolate from West Sumatera pathogenic to drywood termites and West Sumatera fungi potential to be drywood termite biopesticide. Milner et al (1996), state that fungi are the most promising entomopathogens for the development of a microbiological termiticide.

Identifying of pathogenic fungi result showed that entomopatogenic fungi that found in soil and sand from various region of West Sumatera belong to several divisions of fungi such as Ascomycota (*Aspergillus niger, Aspergillus* sp., *A. flavus*, and *Penicillium* sp.), and Deuteromycota (*Metarhizium harzianum, Fusarium* sp., *Beauveria* sp., and *B. bassiana*), and Zygomycota (*Rhizophus* sp.) (Table 2) Milner et al. (1996) review a wide variety of fungal pathogens that reported as potential pathogens

to termites. Gitonga (1996) reports, fungal species of at least 11 genera from four fungi families, i.e. Deuteromycetes, Zygomyceytes, Ascomyceytes and Mitosporic, success tested against termites.

Table 1: Fungi isolates found in soil and sand in various regions of West Sumatera and its pathogenicity against drywood termites

No.	Isolates	Isolates resource	Regions of isolates resources	Mortality of termites (%)	Pathogenicity
1.	A3 10^{-3}	Soil	Padang	72,72	Pathogenic
2.	B1 10^{-2}	Soil	Padang	81,81	Pathogenic
3.	G1 10^{-3}	Soil	Padang Pariaman	36,36	Unpathogenic
4.	A1 10^{-1}	Soil	Padang	45,48	Unpathogenic
5.	A3 10^{-3}	Soil	Padang	87,88	Pathogenic
6.	D2 10^{-3}	Soil	Sawahlunto	54,54	Unpathogenic
7.	F1 10^{-1}	Soil	Tanah Datar	84,85	Pathogenic
8.	C1 10^{-1}	Sand	Tanah Datar	69,69	Pathogenic
9.	F3 10^{-3}	Soil	Tanah Datar	75,76	Pathogenic
10.	E2 10^{-3}	Sand	Padang	63,63	Pathogenic
11.	D2 10^{-3}	Soil	Sawahlunto	18,18	Unpathogenic
12.	C1 10^{-2}	Sand	Tanah datar	96,97	Pathogenic
13.	H2 10^{-2}	Soil	Padang Pariaman	90,91	Pathogenic
14.	D2 10-3	Soil	Sawahlunto	90,91	Pathogenic
15.	C1 10^{-1}	Sand	Tanah Datar	54,54	Unpathogenic
16.	E2 10^{-3}	Sand	Padang	72,72	Pathogenic
17.	D2 10^{-2}	Soil	Sawahlunto	81,82	Pathogenic
18.	B2 10^{-1}	Soil	Padang	36,36	Unpathogenic
19.	G2 10^{-1}	Soil	Padang Pariaman	87,88	Pathogenic
20.	E1 10^{-3}	Sand	Padang	75,76	Pathogenic
21.	D3 10^{-2}	Soil	Sawahlunto	27,27	Unpathogenic
22.	E1 10^{-1}	Sand	Padang	48,28	Unpathogenic
23.	H3 10^{-1}	Soil	Padang Pariaman	63,63	Pathogenic
24.	G3 10^{-3}	Soil	Padang Pariaman	27,27	Unpathogenic
25.	D1 10^{-1}	Soil	Sawahlunto	69,69	Pathogenic
26.	E1 10^{-1}	Sand	Padang	87,88	Pathogenic
27.	C1 10^{-2}	Sand	Tanah Datar	36,36	Unpathogenic
28	D2 10^{-1}	Soil	Sawahlunto	84,85	Pathogenic

Table 2: Mortality of drywood termites after applying various entomopathogenic fungi (%)

Kind of fungi	Termites Mortality			Total	Average	Notation
	1	2	3			
Control	18.18	36.36	27.27	81.81	27.27	A
Metarhizium sp.	54.54	63.63	63.63	181.8	60.60	B
Penicillium sp.	63.64	90.9	54.54	209.08	69.69	B
Beauveria sp.	72.72	72.72	81.82	227.26	75.75	B
Fusarium sp.	72.73	100	54.54	227.27	75.76	B
A. flavus	72.73	100	72.73	245.46	81.82	B
B. bassiana	81.82	81.82	90.9	254.54	84.85	Bc
Aspergillus sp.	81.82	81.82	100	263.64	87.88	C
Rhizopus sp.	81.82	90.9	100	272.72	90.91	C
A. niger	90.9	100	100	290.9	96,97	C

Note: Numbers that followed by the same lowercase indicate that there is no significant different on 5% significant level.

Entomopathogenic fungi seem have differed pathogenicity against drywood termites. Among all of pathogenic isolates, *A. niger* showed highest pathogenicity to drywood termites even though not so differed with *Rhizopus* sp., *Aspergilus* sp. and *B. bassiana*. The difference of fungi pathogenicity indicated that each of fungi has specific potency in controlling drywood termites. Stamet (2003) states that biological control agents had tried with varying results. Fungal control agents are promising group of insect pathogens suitable for use as biopesticides for the control of insects. However, limited availability, cost and reliability have hampered the development of such fungal control agents. Host range and specificity has been a problem as well as an advantage; a fungal pathogen that is virulent and pathogenic to one insect species may be ineffective against other species, even those of the same genus. However, some success had demonstrated. Nasr and Moein (1997) state among the Deuteromycetes tested against termites, *Verticillium indicum* (Petch) Gams and *V. lecanii* (Zimmermann) Viegas were more virulent to *Cryptotermes brevis* Walke (Isoptera: Rhinotermitidae) and *Odontotermes brunneus* Hagen (Isoptera: Termitidae) than *Metarhizium anisopliae* (Metsch) Sorok (Hyphomycetes).

The ability of *A. niger* cause highest termites mortality to be related to it's lethal time (Table 3), viability (Table 4), and spore formation ability (Table 5). It means that *A. niger* could be an alternative biotermicides. Neves et al (2004) state pathogenicity of entomopathogenic fungi determined by several factors including host defense and fungi physiology such as viability, growth rate, spore formation ability and ability to produce enzyme and toxin, and environment.

Table 3: Lethal Time $_{25}$, $_{50}$ and $_{95}$ of various fungi against drywood termites

Jenis Cendawan	Probability	Lethal time (days)
A. niger	0,25	1,99906
	0,50	1,28107
	0,95	2,67018
B. bassiana	0,25	2,10403
	0,50	3,10048
	0,95	7,98076
Aspergilus sp.	0,25	2,01976
	0,50	3,23622
	0,95	10,21716
Fusarium sp.	0,25	5,70270
	0,50	6,50686
	0,95	8,97606
M. anisopliae	0,25	5,14489
	0,50	6,57432
	0,95	11,95386
Beauveria sp.	0,25	3,339203
	0,50	4,68502
	0,95	10,29768
Rhizopus sp.	0,25	2,60232
	0,50	3,73886
	0,95	5,85608
A. plavus	0,25	2,20620
	0,50	3,37139
	0,95	9,48254

Rhizophus sp. that is generally known as fungi saprophyte can cause high termites mortality. It is indicates that this fungus also can used as entomopathogenic fungus. Chamilos et al (2008), state that Rhizopus species cause the majority of Zygomycetes infections, whereas *Mucor, Rhizomucor*, and *Cunninghamella bertholletiae* are less frequently encountered pathogens. Chamilos et al (2008) also found Zygomycetes rapidly infect and kill *Drosophila melanogaster*. The pathogenicity of this Zygomycetes linked with impaired phagocytic cell activity and suppression of induction of genes involved in host defense, stress responses, and tissue repair. Other isolates that found cause mortality to termites known as entomopathogenic fungi with various substances that kill drywood termites.

Aspergillus spp., produce aflatoxins B1, B2, G1, and G2 (Drummon et al 1990), *B. bassiana, M. anisopliae*, and *V. Iecanii*, when grown in liquid cultures containing locust cuticle as sole carbon source, produce a variety of hydrolytic enzymes with activity against the major components of insect cuticle, namely protein, chitin and lipid (St Leger et al., 1986). *B. bassiana* and *V. lecanii* produce a toxin named bassianolide that accounted for the lethality and toxicity accompanying the atonic symptom detected in the dead pupae silk worm infected with each fungus (Murakosih, 1978). *M. anisopliae* has larvicidal activity because produce cyclopeptida, destruxin A, B, C, D, E and desmethyldestruxin. Destruxin has considered as new generation of insecticide (Widiyanti et al, 2004).

Table 4: Viability of selected fungi (%)

Kind of fungi	Viability (%)			Total	Average	Notation
	1	2	3			
B. bassiana	26	27	29	82	27,33	A
Rhizopus sp.	29	32	27	88	28,33	A
Aspergillus sp.	38	40	41	119	39,67	Ab
A. niger	44	47	38	129	43	B
A. flavus	51	49	54	154	51,33	B

Note: Numbers that followed by the same lowercase indicate that there is no significant different on 5% significant level.

Table 5: Spore formation ability of selected fungi (conidial density 10^6 conidia/ml)

Kind of Fungi	Amount of spore			Total	Average	Notation
	1	2	3			
B. bassiana	11	12	9	32	10.67	A
Rhizopus sp.	13	14	16	43	14.33	B
Aspergillus sp.	16	15	19	50	16.67	B
A. flavus	21	23	19	63	21	B
A. niger	23	24	26	73	24.33	B

Note: Numbers that followed by the same lowercase indicate that there is no significant different on 5% significant level.

Conclusion

Several fungi isolates found in soil and sand in fourth regions in West Sumatera. Most of them showed pathogenic to termites that are *Aspergillus* sp., *A. niger, A. flavus, Penicillium* sp., *M. anisopliae*., *Beauveria* sp., *B. bassiana*, *Fusarium* sp., *Rhizopus* sp. that can cause termites mortality 60,6%-96,97% in 2-7 days after inoculation. Among all of the entomopathogenic fungi, *A. niger* seem to be the most pathogenic that determine by it's ability to cause termites mortality, mortality lethal time, viability and spore formation ability.

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Pathogenicity of *Metarhizium anisopliae* to Subterranean Termites *Coptotermes* sp.

by

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Abstract

Bioassay of entomopathogenic fungus *Metarhizium anisopliae* as biocontrol for subterranean termites Coptotermes sp. have been conducted. The purpose of this study is to observe pathogenicity of *M. anisopliae* to termites and to describe of fungal invasion through scanning electron microscopy (SEM). Mortality of termite with application crude filtrate *M. anisopliae* that were cultured in Cxapek-Dox (CD) liquid medium with different carbon and nitrogen ratios took 6-7 days to cause 100% termite mortality. The crude filtrate was fermented in CD liquid medium with yeast extract as single nutrient source (CN7) had highest pathogenicity. Based on electron microscopy (SEM) observation, mycelium fungal invasion to the body of termite through cuticle degradation.

Key words: pathogenicity, *Metarhizium anisopliae*, scanning electron microscopy (SEM).

Introduction

Wood destroying organism such as termite are very serious problem in many tropical country including Indonesia. They attact and damage any building material that contain cellulose, such as wood used in house and building constuction, drywall covered with paper, or siding materials containing cellulose. One of the most prevalent and dangerous wood- destroying organisms is the subterranean termite *Coptotermes* sp., which can cause significant wood damage to a house and other stuctures. The termite's activity generally goes unnoticed until extensive damage becomes visible, and the economic impact is substantial.

The use of persistent insectisides to control soil insects, like termates, is known to cause groundwater contamination and destruction of soil fauna. These problem warrant the search of alternatives to chemical control which are effective and save to to the environtment. In recent years, many trials using entomopathogens to control insect pests have been carried out (Sajab and Kaur, 1990).

Recently, studying on biological insecticides to control termites are becoming of great interest to reduce chemical insecticides uses (Sukartana et al., 2000). Biological control with pathogenic fungi is a promising alternative to chemical control against the subterranean termite. Biological control with pathogenic fungi might provide long lasting insect control without damage to the environment or non-taget organisms. *Metarhizium anisopliae* is one of several natural agents for controlling a broad range of insects by direct penetration of the host cuticle, using a combination of enzymic and physical mechanisms, without any requirement for injection or specialized mode of entry (Ferron, 1985). The purpose of this study is to observe pathogenicity of *M. anisopliae* to termites and to describe of fungal invasion through scanning electron microscopy (SEM).

Materials and methods

Fungal culture and submerged fermentation

Metarhizium anisopliae was obtained from Microbial Culture Collection, Indonesian Institute of Science. Conical flasks (300 ml) were charged with 50 ml autoclave Czapek-Dox (CD) liquid medium (KH_2PO_4, 0.5g/L; $MgSO_4.7H_2O$, 0.5 g/L; KCl, 0.5 g/L; $CaCl_2.2H_2O$, 0.1 g/L; and $NaNO_3$, 3 g/L) containing different composition of glucose and yeast extract as carbon and nitrogen sources (CN1= 30 g/l glucose + 0 g/l yeast extract; CN2= 25 g/l glucose + 5 g/l yeast extract; CN3= 20 g/l glucose + 10 g/l yeast extract; CN4= 15 g/l glucose + 15 g/l yeast extract; CN5= 10 g/l glucose + 20 g/l yeast extract; CN6= 5 g/l glucose + 25 g/l yeast extract; CN7= 0 g/l glucose + 30 g/l yeast extract). Each flasks was inoculated with Entomopatogen fungi that have already cultured on PDA media for 7 days were taken using cock borer (Ø 0.5 cm) as fungal inoculum. The flasks were incubated at 25 °C and 120 rpm on rotary shaker for eight days. Culture filtrates were harverted after

inoculation for eight days and filtered through Whatman filter paper and centrifuged (3000 *g*, 20 min). The crude filtrate used for antitermite test using contact method.

Bioassay of entomopathogenic fungi

Bioassay was carried out using contact method. Fifty workers of *Coptotermes* sp. were sprayed by 2 ml fungal culture filtrate until moisten theirs surface skin. Termites were also sprayed by detilled water as control. Each treatment carried out in three replicates. Each test specimen of *Coptotermes* sp. was placed on filter paper in petridish (Ø 5 cm) and kept at 25 °C and humidity ± 95 % in the dark. The mortality rate of termites was observed during 14 days exposure.

Fungal invasion

Three cadavers of termite were selected randomly after 14 days inoculation. The termites were exposed to 1% osmium tetraxide vapour for 1 to 4 hours. Subsequently, they were freeze dried for another 24 hours. The termites were then attached on standard aluminium stubs at varying positions and coated with gold using a Palaron sputter-coater. The termites were observed under a scanning electron microscope (JEOL JSM-5310 LV SEM® Japan).

Results and discussion

Effect of different media component on termite pathogenicity

Mortality of termite with application crude filtrate *M. anisopliae* that were cultured in Cxapek-Dox (CD) liquid medium with different carbon and nitrogen combinations, could be seen at figure 1. Treatment with fungal filtrate produced 100% termite mortality within 6-7 days.

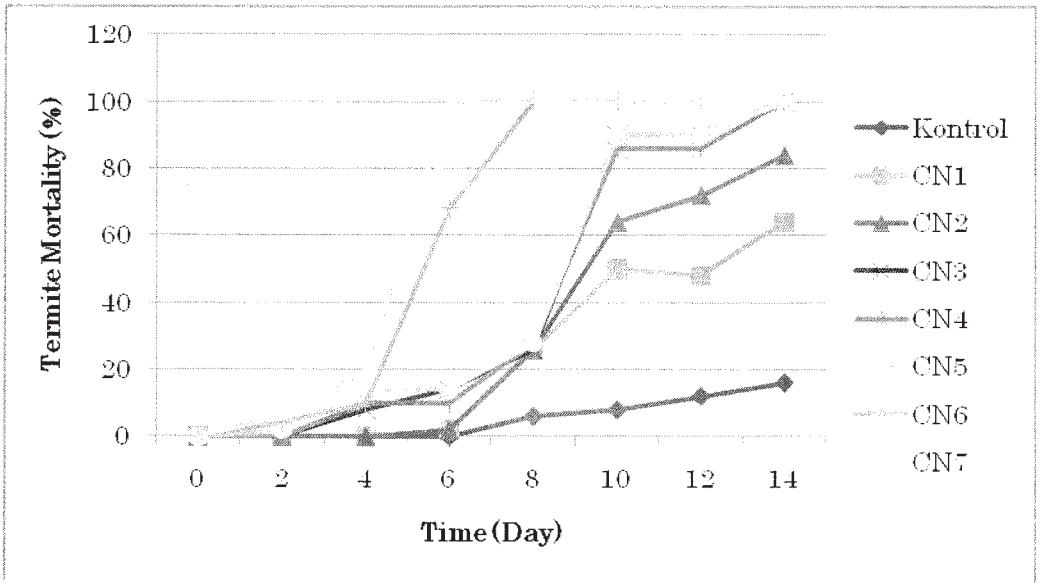


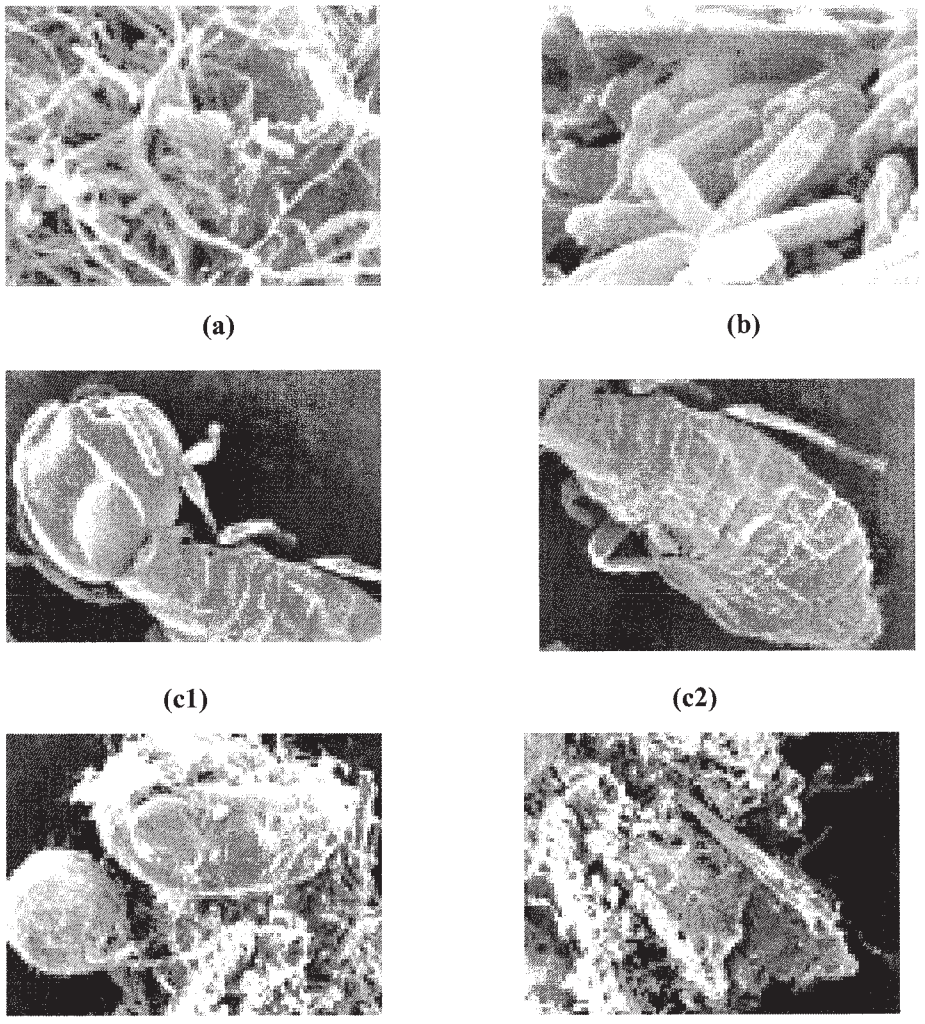
Figure 1. Percentage of termite mortality caused by entomopathogenic fungus *M. anisopliae* with different carbon and nitrogen combinations.

The crude filtrate was fermented in CD liquid medium with yeast extract as single nutrient source (CN7) had highest pathogenicity. The second highest amount in medium CN6 (glucose:yeast extract = 1:5), and then in medium CN5 (glucose : yeast extract = 2:1) resulted in 90-100% death of termites about 10-14 days after treatment respectively, similar to medium CN4 (glucose:yeast extract = 1:1). Medium with glucose as single nutrient source (CN1) was less effective to termite infection.

Entomopatogenic fungi produces specific toxin, such as *M. anisopliae* produced destuxin. Destruxin have been often implicated as one of the cause of insect death infected with *M. anispliae* (Butt, et al., 1994). In vitro toxin secretion of *M. anisopliae* could be significantly influenced by culture condition. Media studies revealed that toxin secretion in liquid medium was highly influenced by different carbon/nitrogen ratios. The amount of destruxin produced by *M. anisopliae* increased with increasing nitrogen content in the medium. Wang et al. (2004) reported the different carbon/nitrogen combinations revealed that higher concentrations of peptone (>60%) in liquid media favours toxin production. Comparatively, highest amount of toxins was produced toxins with peptone as single nutrient source.

Fungal invasion to the body of termite

Based on the electron microscopy (SEM) observation, mycelium fungal have been seen invasion to the body of termite (3b1 and 3b2). The majority of the observed myselium and conidia were on the insect head, but some conidia were also observed on the thoracic or abdominal segments. The whole body was covered by *M. anisopliae* conidia 4 days after inoculation (Figure b3). Similar to Moino Jr. et al. (2002) reported, *M. anisopliae* killed the insects 2 to 3 days after inoculation, and the mycelial extrusion from decadavers happened between 2 to 6 days after inoculation mainly intersegmental areas and, later, in areas with stonger cuticle, inducing complete cuticle degradation.



(a) **(b)**

(c1) **(c2)**

(d1) **(d2)**

Figure 3. Scanning electron microscope of (a) mycelium and (b) conidia of *M. anisopliae* (amplification of 2000X and 7500X, 7 days incubation); (c1, c2) termite body without treatment with crude extract metabolites (50X, and 75X); (d1, d2) mycelium adhesion to the body of termite (50X, and 75X).

The formation of holes around fungal conidia and germination tube on the insect cuticle was observed with conidia adhesion and germination, but mainly during the penetration process (Figure 4b1, 4b2 and 4b3). In the some areas such as the insect thoracic, extensive germ-tubes was observed, probably due to resistence to fungal penetration in these areas with more heavily sclerotized cuticle figure 4b3).

The occurrence of these holes seems to be related to production and excretion of exoenzymes by entomophatogen during the infective process. The enzymatic action of the entomopathogenic fungi *Beauveria bassiana* and *M. anisopliae* on *Heterotermes tenuis* has been observed before with SEM

(Moino Jr. et al., 2002). The combination of proteolitic enzymes and chitinase produces by the fungal mycelium digest the insect cuticle, facilitating the penetration of the insect integument.
The prevailing pathogenesis model of *M. ansopliae* involves (a) attachment of fungal spores (conidia) to insect cuticle, (b) penetration of cuticle via formation of specialized infectious structures known as appresoria and penetrant tubes followed by growth across the surface of the cuticle and within integumental tissues, (c) entry into the haemolymph, (d) reproduction within the haemolymph, producing cells (*in vivo* blastospores or hyphal bodies) that are able to evade the insect immune system and proliferate within the haemolymph, (e) hyphal growth within tissues and out from the insect host leading to development of new conidiogenous (spore-forming) cells on the surface of the cadaver, and (f) conidia formation and dispersal from the host. During infection *M. anisopliae* express and secretes a wide variety of compounds, including proteases, glycosidases, lipases, peptide mycotoxins like destruxin and other secondary metabolites, all of which have been implicated as virulance factors (Diaz *et al.*, 2006; St Leger *et al.*, 1997; Wang and St Leger, 2005).

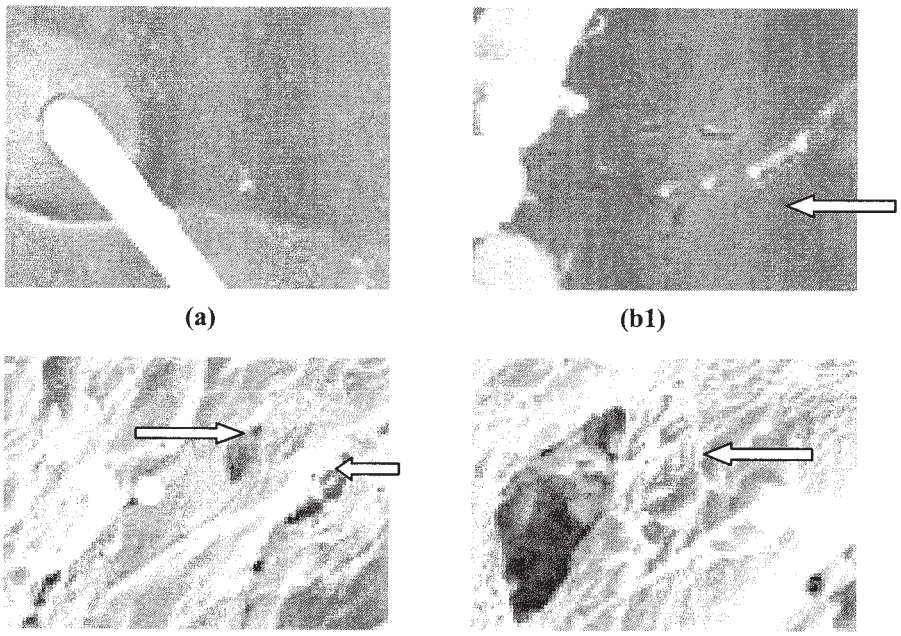


Figure 4. Scanning electron microscope of cuticle of termite: (a) without treatment with crude extract metabolites (7500X); (b1, b2, b3) Extrusion of the mycelium with degradation of the cuticle, in the dorsal (2000X, 2000X and 7500X).

Conclusions

The entomopathogenic fungus *M. anisopliae* was produced in media fermentation with nitrogen as single nutrient source shows the highest termites pathogenicity. The amount of termite mortality increased with increasing nitrogen in the medium. The *M. anisopliae* mycelial extrusion was very intens between 2 to 6 days after inoculation, resulting in a process of cuticle degradation along the whole body of the termite.

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Revirulence of Entomopathogen Fungi Using Chitosan to Eliminate Subterranean termites (*Coptotermes* sp)

by

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Abstract

Subterranean termites are the most destructive and economically important in many tropical country including Indonesia. Nowdays, biological pest control against termites has long been a widely developed approach, especially by using the entomopatogen fungi. Pathogenicity evaluation using entomopatogen fungi has revealed instability termite mortality. It is related with factors that affect entomopatogen fungi to eradicate pest insect. Addition chitosan in cultured media to maintain entomopatogen fungi was supposed to increase its virulence against termites. The research methods are (i) to maintain entomopatogen fungi with addition of chitosan (ii) to formulate fungal material for termite infection (iii) bioassay of fungal entomopathogen against Subterranean termite using contact method. The result revealed that chitosan could induce the increasing virulence of *Metarhizium anisopliae*, *Humicola* sp, *B. bassiana*, *Paecilomyces* sp A and B against Subteranean termites (*Coptotermes* sp).

Introduction

The termites infestation is a serious major problem in many tropical countries including Indonesia. Subterranean termites are considered as one of the most economically pests in the world. In addition, they are the most destructive and economically important pest insect of wood and other cellulose products. Utilization of antitermites chemical such as Dieldrin, pentaclorophenol, sodium pentacholorophenol and sodium arsenite, were effective to control termite population but it has led to environmental problem (Prasetiyo and Yusuf, 2004). Nowdays, biological pest control against termites has long been a widely developed approach, especially by using the entomopatogen fungi such as *Metarhizium anisopliae*, *Aspergillus sp*, *Beauveria bassiana*, *Verticillium lecanii*, and *Fusarium sp* (Kartika *et al, 2006)*. In Indonesia,research to develop entomopatogenic fungi as biocontrol against termites has not been improved yet. Pathogenicity evaluation using entomopatogen fungi has revealed instability termite mortality. It is related with factors that affect entomopatogen fungi to eradicate pest insect. The virulence of entomopatogen fungi is depended several factor such as growth media type, type of fungi species, and the stability production of specific toxic enzim against pest insect.

Many organism such as plants, animal, bacteria, and fungi produce chitinase enzim to reduct chitin into monomer and oligomer's. Insect and fungi activate this chitinase enzim to construct exoskeleton and morphogenesis mechanism of cell wall . Those organisms has chitinase genes which the expression were induced by extracellular chitin (Domsch *et al*, 1980).

Chitosan is the deacetylation's product of chitin which are found on the outer skin of Crustaceae spesies such as shrimps and crabs (Hargono *et al*, 2008). Chitosan is a polysaccharide derived from a low acetyl form of chitin, mainly composed of glucosamine and N-acetyl-glucosamine. Its structure and composition is similar to both cellulose and chitin (Freepons, 1991; Hadwiger and Mc Bride, 2006). Tarmidi *et. al* (2005) revealed that biopolymer chitosan eradicated *Coptotermes* sp in 16 days exposure time with baiting method. Addition chitosan in cultured media to regenerate entomopatogen fungi was supposed to increase its virulence against termites. Furthermore, virulence of entomopatogen fungi that were cultured in media with addition of chitosan would be tested against Subterranean termites (*Coptotermes* sp).

Materials and methods

Microorganisms.

Entomopatogen fungi namely *Metarhizium* sp, *Beauveria* sp, *Humicola* sp, and two *Paecilomyces* sp (*Paecilomyces* A and B) were involved. *Metarhizium* sp was obtained from Microbial Culture collection, Indonesian Institut of science and *Beauveria* sp from Gadjah Mada University. *Humicola* sp was belong to Research and Development Unit for Biomaterial collection. While other isolates

(*Paecilomyces* sp A and B) were entomopatogen fungi from Indonesian Culture Collection (INA-CC), Indonesian Institut of Science.

Chitosan

Granules of chitosan was obtained from Tarmidi's *et al* (2005) previous research. Granules of chitosan was added into media culture PDB to maintain entomopatogen fungi. Function of chitosan as chitin's source, its used without diluted in its solvent.

Production of fungal material for termite infection

Erlenmeyer contained Potato Dextrose Broth (50 ml) without and with addition chitosan (0.5 gram/ml) were sterilized in autoclave at 121°C for 15 minutes. Entomopatogen fungi that have already cultured on PDA media for 7 days were taken using cock borer 0.5 cm (in diameter) as fungal inoculum. The PDB media in which inoculated by each fungi inoculums then incubated and sheaked (120 rpm) at 25°C for 8 days. After 8 days, the fungi that were cultured in PDB was filtered using Whatman filter paper, to isolate fungal filtrat from residue. The fungal filtrat used for antitermite test using contact method, while the fungal residue was dried in oven at 60°C for 3 days, then weighted as fungal biomass.

Bioassay of entomopathogenic fungi

Bioassay was carried out using contact method. Fifty workers of *Coptotermes* sp were sprayed about 250 µl each entomopatogen fungal fitrat until moisten theirs surface skin. Termites were also sprayed by PDB media without chitosan and aquadest as control. Each treatment carried out in three replicates. Each test specimen of *Coptotermes* sp was placed on filter paper in petridish (5 cm in diameter) and kept at 25°C and humidity ± 95 % in the dark for 12 days. The mortality rate of termites was observed during 12 days exposure.

Results and discussion

Mortality of termite with application entomopatogen fungi that were cultured in Potato Dextrose Broth (PDB) without chitosan, could be seen at figure 1. The number of termites mortality, in which tested using entomopathogen fungi, couldn't achieve 50 % from insect population. *Paecilomyces* sp A that were cultured in PDB had highest pathogenicity, this fungi eradicated 43.33% termite population in laboratory. The other fungi show pathogenicity as follow *Beauveria* sp (37.33%), *Paecilomyces* sp B (21.33 %), *Humicola* sp (16.67%) and *Metarhizium* (9.33%).

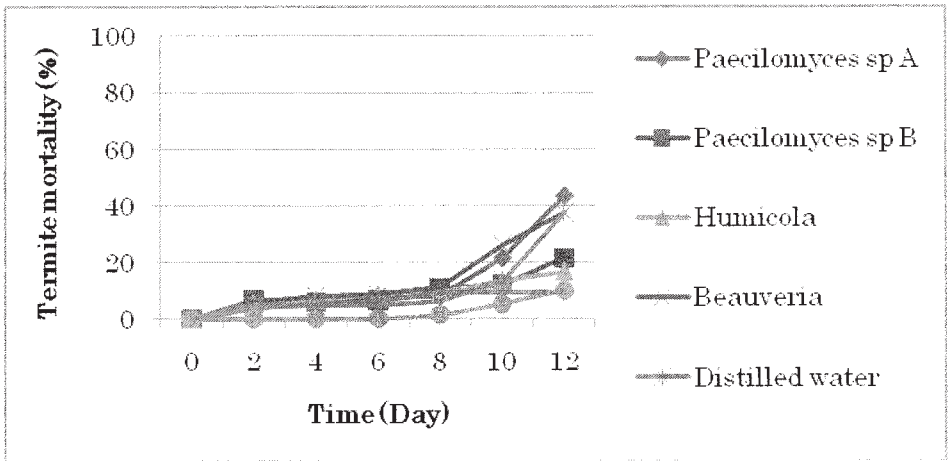

Figure 1. Percentage of termite mortality due to several species of entomopatogen fungi with PDB media without chitosan

The number of termites mortality that were observed for 12 days showing improvement with application of all entomopatogen fungi (Figure 2 below). *Metarhizium* sp had highest pathogenicity, in which caused mortality 93.33% from termites population. The other fungi caused mortality as follow *Humicola* sp (90.67%), *Beauveria bassiana* (86%), *Paecilomyces* sp B (76%) and *Paecilomyces* sp A (75.33%). The percentage of termites mortality in the controlled group (distilled water and PDB) could be negligible because the termite mortality didn't achieve 20%.

When entomopatogen fungi were cultured on media such as PDA or PDB (artificial media that commonly used in fungi regeneration at laboratory), its character could be change especially fungi's

virulence (Figure 1). This research revealed that the using media PDB with addition chitosan to culture *Metarhizium* sp, *Humicola* sp, *Beauveria bassiana*, *Paecilomyces* sp A and B increase the virulence of those fungal filtrat against *Coptotermes* sp. Entomopatogen fungi may produce chitinase or protease enzim (in which caused chitinolysis to termite) whenever their substrat (media) contain micromolecule or material that could induce specific enzim production. Chitosan may provide organic material that could be degraded become micromolecule and used by fungi to produce those enzim. Domsch *et al* (1980) mentioned that *Metarhizium anisopliae*, *B. bassiana*, *Humicola* sp, and *Paecilomyces* sp degraded chitin that was obtained from chitosan (its structure and composition similar with chitin). Each entomopatogen fungi produces specific enzim, such as *Metahizium anisopliae* produced destuxin that had virulence activity against insect.

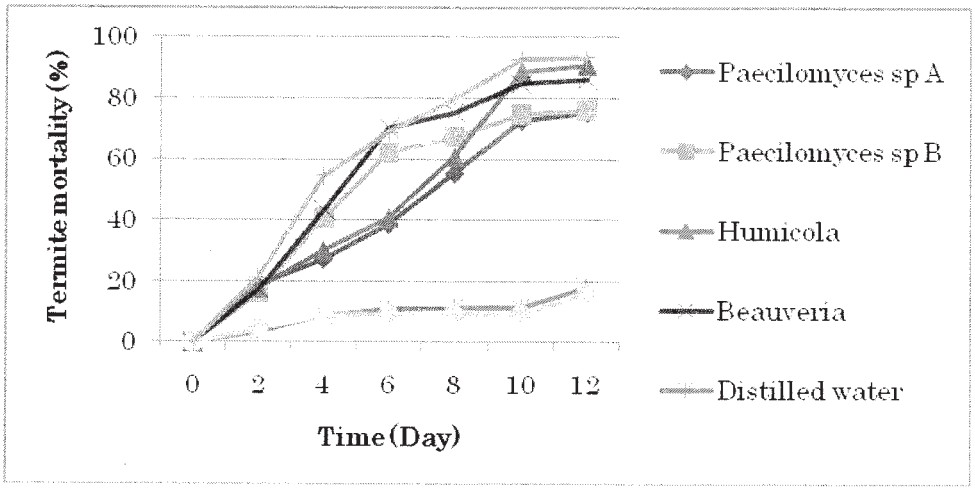

Figure 2. Percentage of termite mortality due to several spesies of entomopatogen fungi with PDB media with chitosan

At figure 2 could be seen that after termites were sprayed with fungal filtrat, the mortality was lowest on the second day and increase rapidly on the fourth day (especially spesies *Metarhizium anisopliae*). According to Brogden, K *et al* (2005) mortality increased on the next two days because specific toxin, destruxin, from the extracts penetrated swiftly in to termites' hemocoel. Saksamprit *et al* (2008) found the band of protein which had 30kDa molecular weight in the supernatant from *Metarhizium* that were culture in PDB media with chitin and N-acetylglucosamin. His research revealed that destruxin which found in the supernatant from *Metarhizium* affects nervous system and immune system of insect.

Fungi species *Humicola* sp also had high activity against *Coptotermes* sp when its cultured in PDB with chitosan addition. Guswenrivo *et al* (2008) maintained *Humicola* sp with PDB and termites's colloidal chitin, in which caused 98% of termite mortality after 12 days. Apparently, *Humicola* sp had better virulence activity when its maintained using colloidal chitin from termites rather than chitosan
(that made from crustacean exoskeleton). There is no much information about the mechanism insect infection caused by *Humicola* sp but Domsch *et al* (1980) mentioned that hyphae or mycelium of *Humicola gricea* also decompose chitin and ceratin, besides mycelia extract showed toxic effect against brine shrimps.

Beauveria bassiana effectively eradicated agricultural pest insect and started to be produced commercially as microbial pesticide in Indonesia. Otherwise, *B. bassiana*'s virulence could decrease if the maintenance of this fungi only on artificial media for long time period's. This research showed that *B. bassiana* had higher virulence against termites when it was cultured in PDB with addition chitosan. *Paecilomyces* sp A and B caused termite mortality more than 70% and could be consider as new candidate entomopatogen fungi against pest insect. Futher research about *Paecilomyces* sp A and B is necessitated to optimize their activity against pest insect especially termites.

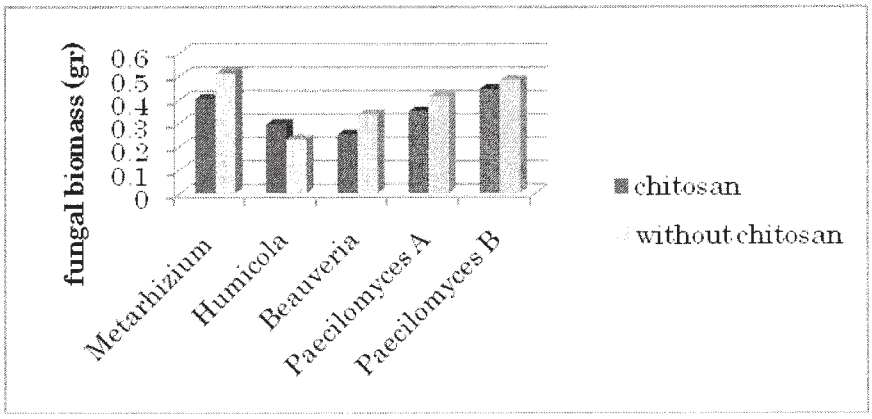

Figure 3. Entomopatogen fungi biomass with cultured period for 8 days

Fungal biomass represented the mycelium's entomopagen fungi growth when it was cultured on the particular media. Fungal biomass of *Metarhizium anisopliae*, *B. bassiana*, *Paecilomyces* sp A and B in which cultured with addition of chitosan was lower than fungal biomass without chitosan, except *Humicola* sp. According to Ghaouth *et al*, 1992 chitosan can induce gross morphological changes, it demonstrated by *R. stolonifer* but not happened by *Botrytis cinerea*. Gross morphological changes increase weight mycelium and this effect could vary with fungi. Gross morphological changes was assumed only exbihited by *Humicola* sp while other species didn't. Gross morphological changes are possibly related to the effect by chitosan indirectly on the formation of hyphal wall.

Conclusion

Chitosan could induce increasing virulence of *Metarhizium anisopliae*, *Humicola* sp, *B. bassiana*, *Paecilomyces* sp A and B against Subteranean termites (*Coptotermes* sp), but its weren't increase entomopatogen fungal biomass.

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Contagious Test of the Entomopathogenic Fungus Originated from West Sumatera Indonesia between Individual in Colony of Subterranean Termites *Coptotermes* gestroi Wasman (Blattodea: Rhinotermitidae)

by

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Abstract

Contagious of entomopathogenic fungus *Myrothecium roridum* Tode ex Steudel and *Metarhizium* sp originated from West Sumatera Indonesia between individual in colony subterranean termite *Coptotermes gestroi* Wasman using some proportion of vector (5%, 10% and 15%) as inoculum's source 7 days after application was carried out. The results showed that there was correlation between proportion of vector and application period with mortality. Statistical analysis shown that the proportion of vector 15% infected by *M. roridum* not significantly different with infected by *Metarhizium* sp. and using vector 10%, but significantly different with vector 5% infected by both species at 0.05 level of confidence according DNMRT test. LT_{95} with vector 5% infected by both species were 59.34 days until 68.31 days. Proportion vector 15% infected by *M. roridum* can caused mortality more highest (77.5%) and generally M. *roridum* was also presented as in vivo sporulation in cadaver of termite body.

Key words: Entomopathogenic fungus, *C gestroi*, contagious, in vivo sporulation, LT_{50}

Introduction

West Sumatera as a province that has been passed by equator is part of territory of Indonesia, its regency or town each other have been varied weather condition, their climate is warm until cool every years. This territory was estimated are harmonizing habitat by some organism of wood degradation and pathogen such as termite, fungi and another insects. Approximately, in the world about 15% from biodiversity of termite species life in surrounding area with manage by human, and about 150 species were known to attack wood structure. Some of those termite species, 10% or more than 200 species have been meet in Indonesia, and Approximately, 20 species role as wood degradation and plant pest (Tarumingkeng 2001; Nandika *et al.* 2003; Sulaeman 2004; Lewis 2006).

Estimated some species of wood degradation termites will be continuous be came part of integral of our ecosystem. It is mean hazard of termite attack to building more higher. The Subterranean termite *Coptotermes gestroi* Wasman. is one of insect can cause the height deterioration of wood construction, presented this insect in housing and another wood structure can make problem, because some time it's coming not known.

Many ways to prevent of termite from wood structure in building: 1) *Chemical barrier* such as using termitiside through soil treatment or impregnation in wood, 2) physical barrier to prevent termite penetration in to building and 3) Technology of bait system. But bio-control using the entomopatogenic fungi not popular yet such as especially in Indonesia, but in developed countries was applied.

Based of termite behavior life hidden in dark condition, apply technique also important to known, because controlling the termites need specials technique, as social insects the termite always to communication with *sensory* (touch and test), The all social interaction of termite's life namely *grooming, trophallaxis and cannibalistic* (Pearce 1997). So that, their behaviors could use full to get success to control termite activity with contagious technique using vector is agent's inoculums source.

Materials and methods

Termites

The used termites in this research are subterranean termite *C. gestroi* (Fig. 1) from society housing in Padang West Sumatera Indonesia. For using as vector, termites were stained with 0.05% (wt/wt) Nile-blue A by forced feeding of stained paper (Whatman no. 42, Θ 125 mm) for 5 days.

Figure 1. The worker and soldier castes of *C. gestroi*

Entomopathogenic fungi

Species of fungus used are: *Metarhizium sp.* and *Myrothecium roridum* (My-Pd). These species of fungi were selected from the early research of pathogenicity test. The selected species were store in room temperature before used. More detail those species show in Table 1.

Table 1. Species of entomopathogenic fungus originated from West Sumatera inoculum's source

Isolates	Isolates Source	Species of fungi	Town origin (year)
1. My-Pd	Sand	*Myrothecium roridum*	Padang (2006)
2. Metar	Soil	*Metarhizium* sp.	Bukittinggi (2009)

Culture procedure

For bio-assay, the Species of fungi were cultured in medium Saboraud Dextrose Agar with Yeast Extract (SDAY) in Petri dish, and than it was incubation for 3 weeks in room temperature

Preparation of conidia suspension

After the fungi were 3 weeks old, suspensions of fungi were prepared by additional of 2 ml sterilized aquadest contained 0,05% Tween 80. The Petri dish was sought to get the conidia and dilute in the sterilized aquadestilata to get the dilution. The haemocytometer was used to count the total of conidia. Conidia suspension from each species of fungi was prepared with concentration 10^7 conidia/ml.

Vector as inoculum's source

Contagious test using vector as inoculum's source, vector is the infected termites by entomopathogenic fungi suspension. Proportion vector were used in this research are 5%, 10% and 15%.

Bio-assay

After prepared the suspension mentioned above, and than the vectors (5%, 10% and 15%) from total colony in unit of treatment infected by suspension of conidia. The filter paper was placed in a Petri dish (Θ 8 cm) together with total individual in colony (20 workers and 2 soldiers) of termite *C gestroi.* The vectors according with the treatments take place between the healthy populations (untreated individual) of each colony in laboratory. The Petri dish was placed in a plastic container and keep in dark condition for 7 days. The dead termites were evaluated every day and termite's mortality was calculated. Beside mortality, LT also evaluated.

Lethal Time (LT)

Lethal Time is the time was needed to kill organism population an amount of definite and is obvious in percentage (%). For known connection regression between the applications time with mortality (LT) was used probite analysis (Finney 1971).

Results and discussion

Mortality (%)

Mortality of termites' *C. gestroi* by treatment of varied proportion vector infected by entomopatogenic fungus (*M. roridum* and *Metarhizium* sp.) until 7 days can see in Table 1 and Figure 2. In all treatments, the results showed that there was correlation between proportion of vector and application period with mortality. Statistical analysis shown that the proportion of vector 15% infected by M. *roridum* not significantly different with vectors 15% infected by *Metarhizium* sp. and vectors 10% infected by *M. roridum* and *Metarhizium* sp. but significantly different with used

vector 5% infected by both species. *M. roridum* can cause mortality highest (77.5%) with vector 15% and generally that fungi presented as in vivo sporulation in cadaver of termite body.

Table 4. Mortality of *C. gestroi* (%) by contagious from various proportion of vectors infected by entomopatogenic Fungus M. *roridum* and *Metarhizium* sp 7 days after grooming.

Fungus Species	Isolates	Vectors		
		5%	10%	15%
M. roridum	My-Pd	28.75 b	47.50 ab	77.50 a
Metarhizium sp.	Metar	33.75 b	62.50 ab	53.75 ab

Mean followed by the same letter are not significantly different at 0.05 level of confidence according DNMRT test. (Control was 22.50%). This treatments Mean with 4 replications

In this research, mortality were counted from total individual in colony or unit of treatment, they are infected individual (vector) and non infected individual (the healthy termites). The all vector die more early than the healthy termites. Used proportion of vector 15% infected by *M. roridum* can caused mortality of *C. gestroi* 77.50% (Fig. 2); that is meaning the vector can contagious the pathogenic fungi to another individual in colony.

There were indicated that contagious of conidia from infected termite (vector) to the healthy individual enough and trans-contamination been helped by behavior of termites are *grooming, trophallaxis, cannibalism* and *Necrophagy* (Fig. 3); they are only can before the all vector die. The continue Contagious my be applied if the fungus resulted the conidia in cadaver of termites by contact or contagious from vectors. Yoshimura *et al.* (1992) mentioned that estimated new conidia can result by entomopatogenic fungus after 5 days the insect die.

The research about transmission the entomopathogenic fungi in colony Dry wood termites *Cryptotermes* sp. by Desyanti *et al* (2009) also resulted trans-contamination from vector inoculated with *Metarhizium brunneum* and *M. roridum* to the another individual (non treated termites). But if we compare with this research, the trans-contamination in colony of subterranean termite *C. gestroi* higher, this was caused their activity in colony more energetic than dry wood termites *Cryptotermes* sp. 50% vector infected by *M. roridum* could kill 82.5% *Cryptotermes* sp., while 15% vector infected by *M. roridum* could kill 77.50% *C. gestroi*

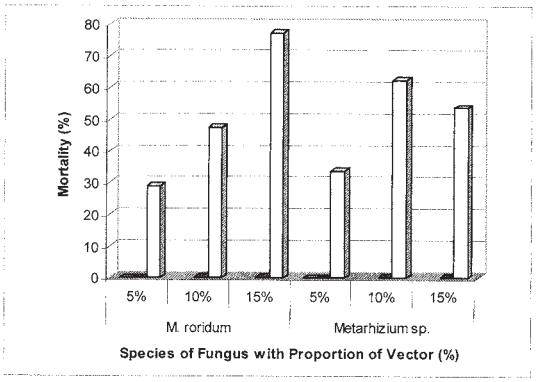

Figure 2. Mortality of *C. gestroi* contagious by several of proportion vectors inoculated by entomopathogenic fungus *M. roridum* and *Metarhizium* sp. 7 days after grooming

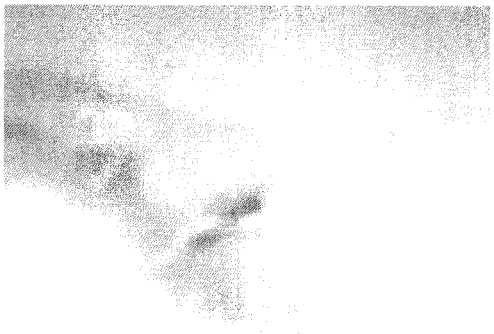

Figure 3. The worker caste of *C. gestroi* is eating the die vector that reated with entomopathogenic Fungi (Necrophagy behavior)

Lethal Time

The Letal Time ($LT_{25, 50, dan\ 95}$) of *C. gestroi* by treatment of varied proportion of vector (5%, 10% and 15%) inoculated with *Metarhizium* sp. and *M. roridum,* their results were confused (my be influenced by another factor with not detected yet), but it were shown that using vector 5% inovulated by *Metarhizium* sp., it's LC_{95} more lower (59.34 days) than infected by *M. roridum* (68.31 days) (Table 2).

Table 2. Lethal Time of *C. gestroi* with varied proportion of vector inoculated by *Metarhizium* sp and *M. roridum*

No	Species	Isolate	Isolate origin	Vector (%)	LT (days)		
					25%	50%	95%
1	*M. roridum*	My-Pd	Padang	5	50.01	55.33	68.31
				10	0.85	0.86	0.89
				15	0.78	0.82	0.89
2	*Metarhizium* sp.	Metar	Bukittinggi	5	43.46	48.08	59.34
				10	0.84	0.84	0.86
				15	0.84	0.88	0.98

Tanada and Kaya (1993) mentioned that period from infection until the insect die need the times 3 days until 12 days, the period varied depend on insect size.

Conclusions

The results showed that there was correlation between proportion of vector and application period with mortality. Statistical analysis shown that the proportion of vector 15% infected by *M. roridum* not significantly different with infected by *Metarhizium* sp. and using vector 10% but significantly different with vector 5% infected by both species at 0.05 level of confidence according DNMRT test, LT_{95} with vector 5% were 59.34 days until 68.31 days. Proportion vector 15% infected by *M. roridum* can caused mortality more highest (77.5%) and generally M. *roridum* was also presented as in vivo sporulation in cadaver of termite body.

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Isolation of Entomopathogenic Fungus *Metarhizium anisopliae* from Soil by Bait Method with Termite

by

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Abstract

Soil-inhabiting entomopathogenic fungi have been isolated mainly using bait method with *Galleria mellonella*. However, various and more susceptible insects should be used to understand the diversity of entomopathogenic fungi in soil. We thought termite as effective insect bait for fungal pathogen in soil because it was reported to be susceptible to a wide range of entomopathogenic fungi. Therefore, we evaluated the potential of termite as insect bait for isolating entomopathogenic fungi, especially *Metarhizium anisopliae*, from soil. From 14 soil samples collected in Japan, we isolated entomopathogenic fungi by using *Reticulitermes speratus* Kolbe as bait for fungal pathogen. *Galleria* bait method and selective media for *M. anisopliae* were also used for comparison. Three well-known entomopathogenic fungi, *Lecanicillium* spp., *M. anisopliae* and *Paecilomyces fumosoroseus* were obtained with termite bait method. As for the isolation of *M. anisopliae*, more various morphology types were isolated by termite bait method than by *Galleria* bait method. In addition, termite bait method was more sensitive in isolating the most common morphology type of *M. anisopliae* than *Galleria* bait method.

Key words: *Metarhizium*, soil fungi, bait method, *Reticulitermes speratus* Kolbe, *Galleria mellonella*

Introduction

Soil-inhabiting entomopathogenic fungi are widely distributed and play a key role in regulating insect populations, particularly soil-dwelling insect pest (Keller and Zimmermann, 1989; Jackson et al., 2000). Many species of these fungi belong to Hypocreales (Ascomycota) and most of them inhabit the soil for a significant part of their life cycle when they are outside of their insect host. Among them, *Beauveria* spp., *Metarhizium anisopliae* and *Paecilomyces* spp. are especially common (Keller and Zimmerman, 1989). Isolation of indigenous entomopathogenic fungi is essential to understand naturally occurring fungal diversity and to exploring potential biological control agent.

Soil-inhabiting entomopathogenic fungi have been isolated using insect as bait for fungal pathogen. For the isolation of *Beauveria bassiana* and *M. anisopliae*, selective media were developed and have been used in some studies (Yaginuma and Takagi, 1986; Shimazu 2002). In general, more strains of fungi are obtained when various isolation techniques are applied to soil. Accordingly, application of various isolation methods is necessary for revealing the diversity of entomopathogenic fungi in soil.

Galleria mellonella has majorly been used as insect bait for isolation of soil-inhabiting insect pathogen (Zimmermann 1986). However, fungal strain with low pathogenicity to lepidopteran insects may not be detected with this species. Kawakami and Naka (1979) reported that three morphology types of *M. anisopliae* were isolated from soils by selective medium but the two of them were not detected by bait method with a pupa of silkworm *Bombyx mori*. In addition, they found that the two types had obviously lower pathogenicity to silkworm in laboratory assay than the other one that was detected from soils by silkworm bait method. Thus, it is necessary to use various orders of insect and an insect that is susceptible to a wide range of fungal pathogens as insect bait.

The two species of subterranean termite, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* Kolbe, were susceptible to a wide range of fungal pathogen in laboratory assay (Shimizu and Yamaji, 2003; Yanagawa and Shimizu, 2005). Additionally, these species were more susceptible when reared individually than when reared as groups. One of the reason for this phenomenon is mutual grooming behavior that plays a major role in the removal of fungal conidia from the cuticle, which is effective in protecting termites from fungal infection (Shimizu and Yamaji, 2003; Yanagawa and Shimizu, 2005). Considering these characteristics of termite, we consider a termite reared individually as appropriate insect bait for isolating entomopathogenic fungi from soils.

Application of termites for bait method to isolating entomopathogenic fungi has already been done by Myles (2002) and Sun (2002), but their experiment was carried out with termites reared as groups. Furthermore, the effectiveness of termite bait for the evaluation of the diversity of entomopathogenic fungi in soil was not researched in their studies.

The objective of this study is to research the effectiveness of termites as insect bait for isolating entomopathogenic fungi from soil by comparing this method to *Galleria* bait and selective medium method. This study is especially concentrated on the isolation of *M. anisopliae* because the species is known to widely distributed in soil and selective medium for this species has already been developed.

Materials and methods

Collection of soil samples: Fourteen soil samples were taken from wood and field in Fukuoka, Japan in 2009 (Table 1). Each soil was collected to a depth of 5-10 cm after removal of surface litter. All soil samples were placed in plastic bags, sieved through a 1.5 mm mesh and stored at 4°C for a maximum of seven days before use.

Isolation of *Metarhizium* by a selective medium: Each soil sample (1.0 g) was suspended in 10 ml of an aqueous solution of 0.05% Tween 80 and thoroughly shaken. Each was then serially diluted 10^1 and 10^2 times. Two to five replicates of each dilution were then spread-plated (100 µl) onto selective agar medium (60 g l^{-1} oatmeal, 12.5 g l^{-1} agar, 0.3 g l^{-1} chloramphenicol, 1 g l^{-1} cycloheximide), a modified medium of Yaginuma and Takagi (1986). Cultures were maintained at 27°C in the dark and examined every three days for 14 days. Conidia, which had characteristic morphologies of *Metarhizium,* were transferred to PDA plates (4 g l^{-1} potato extract; 20 g l^{-1} glucose, 15 g l^{-1} agar) and subcultured. If there was more than one colony type in a soil sample, the conidia of each type were reisolated. Fourteen days after soil dilution plating, the number of colonies on each selective agar was recorded and colony-forming units per 1 g of dried soil (C.F.U./g dried soil) were calculated.

Bait method with termite: The termites, *R. speratus*, were collected in Fukuoka, Japan and maintained in plastic boxes (41 x 31 x 23 cm^3) on half-dried wood in a dark chamber at 25°C. Only worker termites were collected and placed into plastic boxes in a dark chamber at 25°C for one to three weeks. From each of collected soil sample, 20 sub-samples were taken and placed into 20 Petri dishes (30 mm diameter, 10 mm high) up to about 3 mm in depth. The 20 termites were individually released into the Petri dishes and all dishes were maintained at 25°C in the dark. The soil sub-sample in each Petri dish was moistened with sterile distilled water every other day to keep the moisture. Mortality was checked daily for 14 days. This experiment was conducted on sterilized (121°C, 20 min) soil sample of etj8. The cadavers of termite were inspected daily for the presence of mycelium and all potential fungal pathogens were identified microscopically based on morphological characteristics using taxonomic keys of Aoki (1989). Conidia or mycelium of these isolates were transferred to PDA or L-broth (10 g l^{-1} polypeptone; 3 g l^{-1} yeast extract, 20 g l^{-1} sucrose; 5 g l^{-1} NaCl, 20 g l^{-1} agar) agar medium and the morphology of colony and conidiogenesis were reexamined.

Bait method with *G. mellonella*: *Galleria* bait method was conducted based on the protocols described in Zimmermann (1986) and Quesada-Moraga et al. (2007). From each soil sample, two sub-samples were taken and placed into two Petri dishes (90 mm diameter). Five *G. mellonella* larvae (fourth or fifth instar) from SPHERO AQUA (online pet solution store) were placed on the surface of soil of each dish. The dishes were placed in a plastic bag together with moistened paper and the top of the bag was sealed with a loose twist tie to prevent moisture loss. The dishes were incubated at 25°C for five days and inverted daily. After the incubation period, the soil was examined for dead larvae, which were removed immediately and surface-sterilized in 1% sodium hypochlorite for 3 min followed by wash in sterile, distilled water. The larvae were placed in a sterile Petri dish (90 mm diameter) with wet filter paper and incubated at 25°C. After mycelia were formed on the cadavers, morphology of conidiogenesis was observed with optical microscope. Conidia or mycelia of these isolates were transferred to PDA or L-broth agar medium and the morphology of colony and conidiogenesis was reexamined.

Results and discussion

We isolated *M. anisopliae* from 14 soil samples by three methods: termite bait, *Galleria* bait and selective medium. The results of the isolation are shown in Table 1. Three morphology types of *M. anisopliae* (M1, M2 and M3) were isolated by the three methods. When the same morphology type from different soil sample are considered as different strains, a total of 17 strains were obtained. The three types were different in the color of mass of conidia. M1 had black or greenish black conidia and was the most common in the isolates of *M. anisopliae* in this study. M2 and M3 had pale green and green conidia, respectively. M1 and M2 probably correspond to the two of the three morphology types of *M. anisopliae* isolated from soils in mulberry field in Japan by Kawakami and Naka (1979). Other than *M. anisopliae*, three species of entomopathogenic fungi identified as *B. bassiana*, *Paecilomyces fumosoroseus* and *Lecanicillium* spp. were isolated. Some opportunistic pathogens were also isolated, such as *Aspergillus*, *Fusarium*, and *Penicillium*. Soil sample mnm4 contained small predatory mites (they were not be removed by sieving) and unfortunately they ate all of the termite baits and three *Galleria* baits used for the soil sample.

Table 1. Isolation of *Metarhizium anisopliae* from soils with three methods

Soil	Habitat	Termite bait				Galleria bait	Selective medium		
		Isolate and frequency of occurrence (%)				Occurred isolate	C.F.U./g dried soil of isolate		
		M1	M2	M3	Others		M1	M2	M3
etj7	wood	10	0	0			0	0	0
etj8	wood	0	0	15			0	0	$2.7x10^3$
etj11	rice field	20	0	0		M1	$1.8x10^4$	0	$2.5x10^2$
mnm1	wood	20	0	0	L	B, M1	$1.3x10^3$	0	$3.0x10^1$
mnm2	wood	0	60	0		B, P	$6.0x10^2$	$3.5x10^3$	$3.0x10^1$
mnm3	wood	65	0	0		M1	$3.2x10^4$	0	0
mnm4	wood	0*	0*	0*		B, M1	$2.0x10^5$	0	$8.0x10^2$
mre1	riverbank	25	0	0	L, P	M1, P	$2.7x10^3$	0	0
qhc5	field	60	0	0		M1	$1.4x10^4$	0	0
qhc6	bush	15	0	0	L		0	0	0
qhc7	bush	0	0	0			$2.4x10^3$	0	0
qhf1	rice field	0	0	0			0	0	0
qhf2	field	0	0	0	L		$2.4x10^3$	0	0
qhf3	pasture	0	0	0			0	0	0

Abbreviation of occurred entomopathogenic fungi; M1: *M. anisopliae* with black or greenish black conidia, M2: *M. anisopliae* with pale green conidia, M3: *M. anisopliae* with green conidia, B: *Beauveria bassiana*, L: *Lecanicillium* spp., P: *Paecilomyces fumosoroseus*.
* Soil sample mnm4 contains small predatory mites and they ate all of the termite baits.

In the experiment of termite bait method, the termites infected with M1 or M2 died from three to 10 days (largely from three days to seven days) after being released to soil while those infected with M3 died from eight to 13 days. These deaths were followed by mycelium formation within from one to four days. A lot of termite died for other causes, which probably were other pathogens or starvation, and the mortality of seventh day was 30-95% and that of 14th day was 80-100%. The mortality of the termites incubated on sterilize soil sample of etj8 was 55% at seventh day and 90% at 14th day, but no fungi were observed on the cadavers.

Termite bait and selective medium detected all of the three types of *M. anisopliae* while *Galleria* bait method detected only M1. The frequency of isolation of M2 from soil sample mnm2 was 60% while that by *Galleria* bait was 0%, which indicates the higher susceptibility of termite to M2 than *G. mellonella*. *P. fumosoroseus* was detected by both methods while *B. bassiana* was detected only by *Galleria* bait and *Lecanicillium* spp. was detected only by termite bait. These results indicate that the effectiveness of a bait insect to isolate entomopathogenic fungi is different at both inter- and intraspecies level and termite bait has potential to detect a wider range of *M. anisopliae* strains than *Galleria* bait.

In terms of detection sensitivity to M1 of the three isolation methods, none of the M1 occurred from four soil samples (etj7, qhc6, qhc7 and qhf2) by *Galleria* bait but by either termite bait or

selective medium. This result may indicate the higher sensitivity of termite bait and selective media than *Galleria* bait method. Comparing termite bait to selective medium, M1 was not detected from etj7 and qhc6 by selective medium but by termite bait while M1 were not detected from qhc7 and qhf2 by termite bait but by selective medium. The detection sensitivity of the two methods to M1 seems to be about the same, but combined use of the two methods must be better detection sensitivity.

On the relation between the frequencies of occurrence by termite bait (FOT) and C.F.U./g soil, consistent pattern was not observed throughout all of the soil samples. Higher density seems to result in higher FOT in mnm3 and qhc5, but this is not true to etj11 and mnm2. The C.F.U./g soil of M1 from mnm3, qhc5 and etj11 was higher than 1.0×10^4 and FOT was 60, 65 and 20%, respectively, while C.F.U./g soil of M2 from mnm2 was only 3.5×10^3 but its FOT was 60%. This may indicate that the pathogenicity toward termite of M1 from etj11 is relatively low and that of M2 from mnm2 is high. Pathogenicity assay should be done for these strains.

Conclusions

R. speratus and *G. mellonella* were different in the performance for the isolation of entomopathogenic fungi from soil, which probably was resulted from the difference of susceptibility to pathogens. As for the isolation of *M. anisopliae*, termite bait method isolated more various strains than *G. mellonella* and more sensitively isolated the most common morphology type of *M. anisopliae*.

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The Effectiveness of Antiaris and Ki Pahit Bark Extracts against Subterranean Termite *Coptotermes curvignatus* through Maceration and Soxhlet Methods

by

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Abstract

This study was conducted to compare the effectiveness of bark extract of Antiaris toxicaria (Antiaris) and Picrasma javanica (Ki Pahit) with a maceration and soxhlet methods toward subterranean termite *Coptotermes curvignatus*. This research was conducted in some stages. They were sample extraction, the extract application on termites and data analysis. Extraction has been done by two ways. They were maceration and soxhlet. Four different polarity solvents were used. They are n-hexan, ethil acetate, aceton and methanol. The extracts were applied to termites by paper disk baiting. Termites mortality rate was then analyzed. The result showed that bark extract of *Antiaris toxicaria* (Antiaris) and *Picrasma javanica* (Ki Pahit) obtained by maceration was more effective *to Coptotermes curvignatus* rather than the ones obtained by soxhlet.

Key words : extract, maceration, soxhlet.

Introduction

The resistance of wood on termite attack is depend on its extractives. It is well known that wood extractives were responsible for natural durability of wood, but it has different level of toxicity. So, there were many research to isolate the toxic compound from wood. Effectiveness of each extract has been reported by many researcher, such as Indrayati (1987), Syafii (2001), Falah *et al* (2005), Shibutani and Katayama (2005).

Plant basically has the mecanism to protect themselves from insect attack. Falah (2004) reported that bark has some substancees that were distasteful, reppelent, or toxic to termite. Generally, study on evaluation of antitermite activities of extract used maceration method. Due to many kinds of compound in plant, study on extraction using soxhlet method is expected to result more compound that will extracted. The aims of the research are to compare the effectively of bark extract of Antiaris toxicaria (Antiaris) and Picrasma javanica (Ki Pahit) with a maceration and soxhlet methods toward subterranean termite *Coptotermes curvignatus*.

Material and methods

Extraction by maceration

250 grams of powder samples were extracted using n-heksan with ratio 1: 6 (w / w) and then were soaked for 24 hours. Extract and residue were separated by filtering. The residue was extracted with a second solvent ethyl acetate for 24 hours. After that the residue was extracted again with solvent acetone and methanol. Each solution was extracted and then was concentrated with a rotary vacuum evaporator at a temperature of 45 °C, then dried and weighed.

Extraction by Soxhlet

The extraction procedure was refered to ASTM D1107-56. Two grams air dried sample was extracted using soxhlet methods, extraction temperature ranges from 80 – 85 °C, and the extraction was carried out until the time it took. In the last cycle of the heater was turned off, and then samples were removed from soxhlet and the solvent was evaporated. Each performed five replications.

Bioassay Test

Anti-termite test referred to Prianto *et al* (2005). Paper discs with oven-dried at a temperature of 60 °C for three days. Paper disc was treated by 5 % and 10 % extracts and were vacuumed in dessicator to evaporate the solvent. Treated paper disc and 50 termites worker and 5 termites soldier of *Coptotermes sp*, were entered in petri disc coated by plaster paris 3 mm. Each treatment (concentration) and controls were performed three replications. Termites mortality was observed per two days and in the end of observation the weight loss of paper disc also was measured.

Result and discussions

Rendemen

Rendemen was obtained by extraction using two methods and four kinds of solvent. Rendemen of Antiaris bark and Ki pahit bark could be seen in Table 1.

Tabel 1. Extract rendemen of Antiaris and Ki pahit bark

Solvent	Rendemen of Antiaris (%)		Rendemen of Ki pahit (%)	
	Maceration	Soxhlet	Maceration	Soxhlet
n Hexane	0,47	3,17	0,89	5,82
Ethyl acetate	0,45	2,95	1,55	5,38
Aceton	0,14	4,03	1,04	6,55
Methanol	0,99	4,78	7,50	12,78

In Table 1 could be seen that the extractive contents of the Antiaris and Ki pahit bark were varied. The using soxhlet in extraction gave rendemen was greater than maceration, both of the Antiaris and Ki pahit. This was due to temperature extraction of soxhlet was higher than maceration It was indicated that temperature during extraction affected to the solubility level of compounds, if the temperature increased the solubility of the compounds also would increase. From the result of the experiments was known that kind of solvent was also determine the total of rendemen. Methanol produced extract greater than ethyl acetate, acetone, and n-heksan. This was indicated that the compound contained in the bark of Antiaris and Ki pahit polar largely.

Termites Mortality

Termite mortality rate by extract with maceration method could be seen in Figures 3 and 4, whereas the mortality rate by extract with soxhlet method could be seen in Figures 5 and 6.

Antiaris and Ki pahit were extracted by maceration caused termite mortality rates from 84.24% to 100% at the end of the observation. Figure 3 and 4 showed that there were five extracts with concentrations of 10% which gave 100% termites mortality, such as antiaris extract with ethyl acetate and methanol solvent and Ki pahit extract with ethyl acetate, acetone and methanol solvent. Whereas at 5% concentration extract, there were only acetone and methanol solvent in Antiaris extract that gave 100% of termite mortality, on the other hand Ki pahit at 5% concentration extract gave similar condition with 10% extract concentration. The result of bioassay showed that in the method maceration, Ki pahit extract caused higher termite mortality than Antiaris bark extract. It was indicated that the active compounds contained in the bark of Ki pahit more toxic to termites than Antiaris.

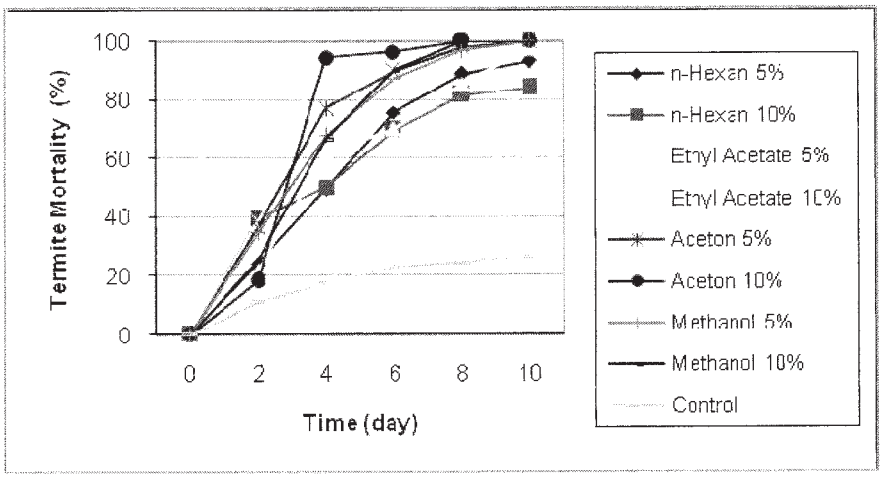

Figure 3. Termite mortality by the extract of Antiaris bark with maceration methods.

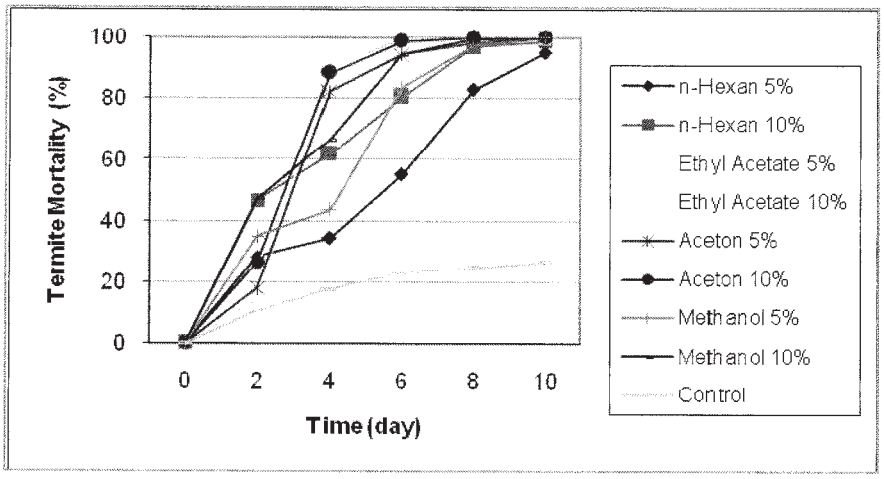

Figure 4. Termite mortality by the extract of Ki pahit bark with maceration methods.

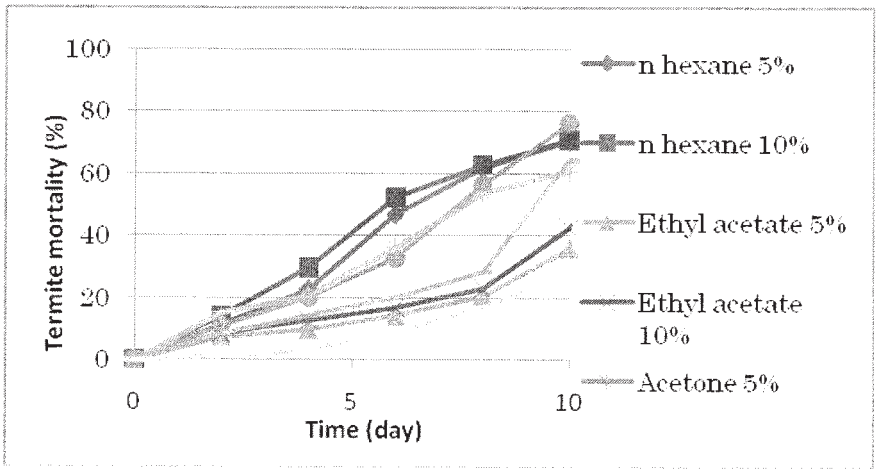

Figure 5. Termites mortality by the extract of Antiaris bark with Soxhlet methods.

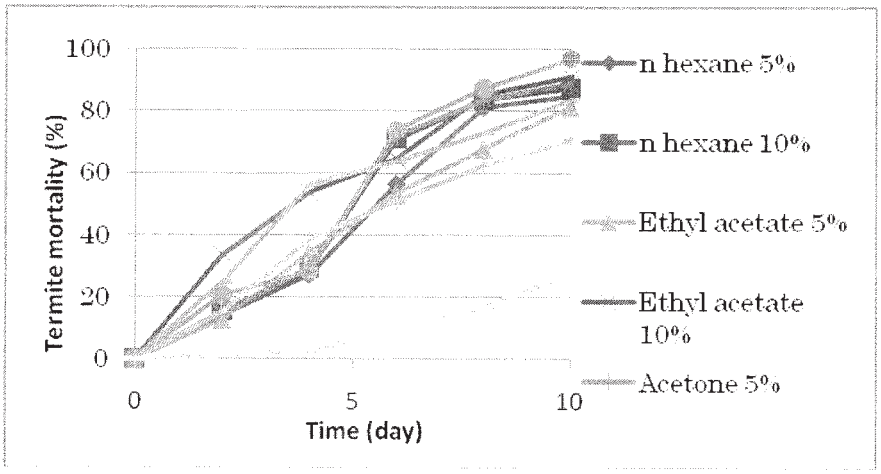

Figure 6. Termites mortality by the extract of Ki pahit bark with Soxhlet methods.

According to Figure 5 and 6, Antiaris was similar in termite mortality with Ki pahit by acetone solvent at the 10 % concentration of extract. The result of bioassay showed that in the soxhlet method, Ki pahit extract caused higher termite mortality than Antiaris bark extract.

According to Figure 3, 4, 5, and 6, termites mortality were varies depending on the kind of material extracts, solvent, and method of extraction. Termite mortality on the maceration method was higher than soxhlet method. It was possible that the active compounds contained in the materials were not stable to heat, so at the time of extraction the active compounds were changing its basic form.

Weight Loss

Table 2 showed the percentage of weight loss from paper disc after baited to termite. Extract treatment affected paper disc resistance to termite attack significantly. On the same concentration treated paper disc of Ki pahit gave less weight loss than treated paper disc of Antiaris. Each extract with high termite mortality has resulted low weight loss (Prianto, 2005). Based on percentage of weight loss, termite's ability to consume paper disc was affected by concentration, if the concentration was greater, the consumption of paper disc would be decrease.

Tabel 2. *Weigth Loss* of *Paper Disc* after feeding to termite

Solvent	*Weight loss(%)*				
	Concentration (%)	Maceration		Soxhlet	
		Antiaris	Ki Pahit	Antiaris	Ki Pahit
n-hexane	5	17,19	15,35	43,05	25,72
	10	8,82	9,01	32,76	21,43
Ethyl acetate	5	15,48	9,89	36,08	4,76
	10	11,42	6,87	18,93	0,00
Aceton	5	15,22	7,87	38,77	21,23
	10	10,83	2,95	13,98	8,12
Methanol	5	20,03	13,65	22,84	14,24
	10	15,74	7,85	14,85	0,00
Control		43,96		68,75	

Conclusions

From discussion above, it could be concluded that:

1. Extracts that were obtained from maceration method was more effective against termite *Coptotermes curvignatus* than soxhlet method, both of the Antiaris and Ki pahit.
2. Ki pahit bark extract more effective againts termite *Coptotermes curvignatus* than Antiaris bark extracts.

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Antitermite Activity of *Carbera manghas* L. Seed Extract

by

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Abstract

Carbera manghas L. is well-known as a poisonous plant. This research was conducted to study anti-termite activity of *Carbera manghas* L. seed extract and its chemical properties. Dry-powdered *Carbera manghas* L. seed was extracted by methanol then evaporated by rotary vacuum evaporator at 40 °C followed by waterbathing process to obtain dry extract. Fractionation process was initiated by liquid vacuum chromatography, with silica gel G60 as stationary phase. Gradual elution was carried out with MTC followed by various eluen composition, MTC: EtoAC (8:2, 5:5, 2:8) and EtoAC: Acetone (10:0, 8:2, 5:5, 2:8) then Aceton: H_2O (10:0, 8:2, 5:5). Each fractions was identified by thin layer chromatography. The bioassay test against subterranean termite, *Coptotermes gestroi*, conducted by forced-feeding method. The result confirmed that *Carbera manghas* L. seed extract showed high-toxicity against subterranean termite, *Coptotermes gestroi*. The highest anti-termite activity given by fraction A and phytochemical analysis confirmed that active compounds of *Carbera manghas* L. seed extract consist of alkaloid, saponin, tanin, tryterponoid and steroid.

Keywords: *Carbera manghas* L. seed extract, antitermite activity, *Coptotermes gestroi*.

Introduction

The utilization of plant extractives as natural preservatives material is the appropriate solution along with the rise of corcern toward sustainable green-environment. The effort on developing some suitable natural preservatives aims to reduce the use of chemicals-toxic pesticide. In Indonesia, wood preservation treatment and building protection against termite are very important, consider that most building construction consist of timber materials. In other hand, scarce supply of high durable wood caused lesser-known wood is mass-processed as building material so that timber protection with natural-based preservatives is the main priority consedered on its safe for enviromental and human-health. More over, this termite attack has risen high economic loss untill billions Rupiah per year (Prasetiyo dan Yusuf, 2004).

Carbera manghas L. is well-known as a poisonous plant. Previous study (Tarmadi et. al., 2007) reported that bark and leaf extract of *Carbera odollam* was proven high-toxic against subterranean termite, *Coptotermes sp.* The research objective is to study anti-termite activity of *Carbera manghas* L. seed extract and its chemical properties.

Material and methods

Extraction and Fractionation Procedure

Carbera manghas L. seed kernel was dried and powdered into 40 mesh. 150 g *Carbera manghas* L. seed powder extracted with methanol at room temperature for 24 h and repeated four time. The extract evaporated by rotary vacuum evaporator at 40 °C followed by waterbathing process to obtain dry extract. Fractionation process was initiated by liquid vacuum chromatography, with silica gel G60 as stationary phase. Gradual elution was carried out with MTC followed by various eluen composition, MTC: EtoAC (8:2, 5:5, 2:8) and EtoAC: Acetone (10:0, 8:2, 5:5, 2:8) then Aceton: H_2O (10:0, 8:2, 5:5). Each fractions was identified by thin layer chromatography and grouped based on its time retention.

Bio-assay test

The bioassay test method against *Coptotermes gestroi* referred to Guswenrivo *et. al.* (2005) and Prianto *et. al.* (2005). This force feeding test method use paper disc as termite bait. Paper disc was treated by extract with various concentration and entered into desicator for six hours to evaporate methanol solvent. Then, fifty workers and five soldiers of *Coptotermes gestroi*, and extract-treated paper disc was entered into petri disc coated by plaster paris 3 mm. The Bioassay test periode will

run for 14 days. Termite mortality was observed per two days periods and in the final period observation, the mass loss of paper disc also determined.

$$\text{Persentase mortalitas} = \frac{(R1 - R2)}{R1} \times 100\%$$

Where,
R1= Total termite quantity before test
R2= Total termite quantity after test
Weight loss percentage = ((ODS1-ODS2)/ODS1) x 100%
Where, ODS 1 = oven-dried sample before test
ODS 2 = oven-dried sample after test

Phytochemical analysis

Alkaloid test

Condensed methanol extract of *Carbera manghas* L. seed solved on chloroform and filtrated. 2 N Sulfuric acid added to the filtrat and the acid layer separated to further test. Alkaloid contain analysis was conducted by reacting Dragendorf, Mayer and Wagner reactants in the acid layer solution, and sediment formation observed (Harborne, 1987). Positif test confirmed by various colour of sediment formed, Dragendorf showed by orange sediment; Mayer by lemon-colored sediment, and Wagner by brown sediment.

Steroid, Tryterpenoid and Saponin Test

Condensed methanol extract of *Carbera manghas* L. seed filtrated and evaporated followed by adding diethylether and reacting with Liebermann-Buachard reactant, then color change observed. Residual extract added by hot aquadest, shaked until form foam layer and left for 30 m. If the foam layer is still persistence, acid chloride solution added followed by extraction with diethylether and reacting with Liebermann-Buachard reactant, then color change observed (Harborne, 1987). Steroid noticed by green or blue color, tryterpenoid on red, violet or brown.

Phenolic test

Condensed methanol extract of *Carbera manghas* L. seed placed on plate and dropped by 1% $FeCl_3$ solution, the color change observed. Phenolic compounds noticed by green or blue color.

Results and discussion

The rendemen of *Carbera manghas* L. seed extract is 15,75 g (10,5% w.t). Figure 1 shows termite mortality rate of *Carbera manghas* L. seed extract in various concentration. The result shows the higher concentration, the higher termite mortality rate. The highest termite mortality (100%) given by 10% (w/v) extract concentration on the eighth day of test period, while the lowest termite mortality (72,67%) given by 2% (w/v) extract concentration on the end of test period. It corfirms that concentration has correlation with termite mortality rate. Based on this result can be concluded, *Carbera manghas* L. seed extract has high toxicity against subterranean termite, *Coptotermes gestroi*.

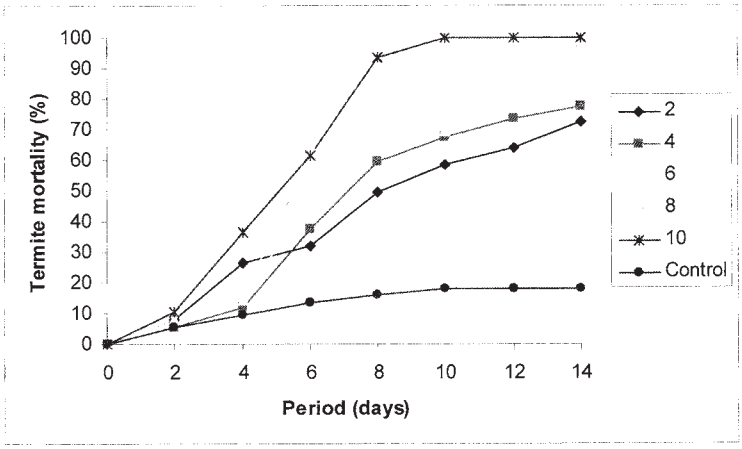

Figure 1. Termite mortality rate after 14 days test

Fractionation process by liquid vacuum chromatography gives 37 eluates, and grouped by thin layer chromatography into 3 group fraction. From 5 g dry *Carbera manghas* L. seed extract fractionated, collected 0,83 g fraction A; 1,408 g fraction B; and 1,827 g fraction C. Termite

mortality rate of each fractions displayed on Figure 2, indicates that the amount of fraction rendemen is not correlated to its antitermite activities. Fraction A, the least yield rendemen, gives 100% termite mortality, fraction B 62% and fraction C 65,33%. The result indicates chemical compounds in fraction A has higher toxicity toward subterranean termite, *Coptotermes gestroi.*

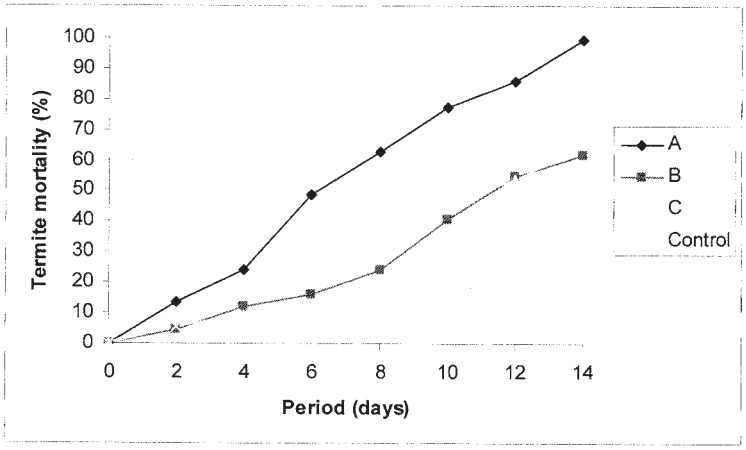

Figure 2. Termite mortality rate fractions after

Sample consumption rate of subterranean termite *Coptotermes gestroi* toward treated paper discs were generally depend on extract concentration. Figure 3 shows the higher extract concentration, the less sample weight loss. The highest weight loss, 31,17%, given by 2% extract concentration, while the least weight loss, 12,77%, showed by 10% extract concentration. It indicates that extractives presence is able to resist termite consumption toward paper disc

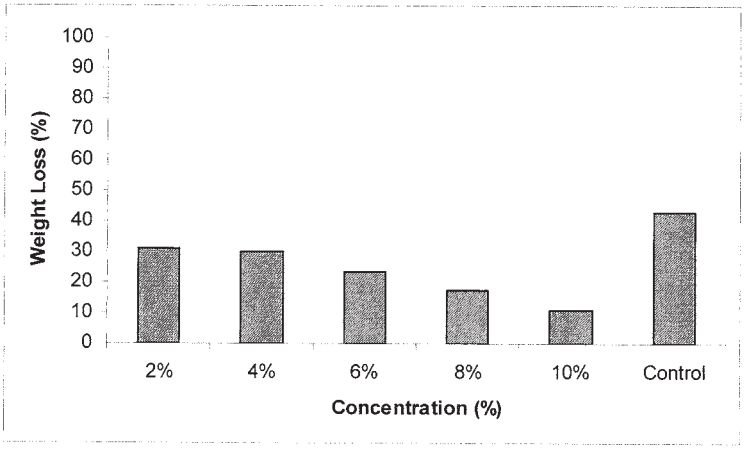

Figure 3. Weight loss percentage of paper disc after test

Figure 4 displays the average of sample weight loss percentage from each fraction group of *Carbera manghas* L. Fraction A gives 24,11% weight loss, fraction B 32 %, while fraction C 43,30%. Further attention need to analyze the result of fraction B and C. As reported above, mortality of fraction C is higher than fraction B. But the same condition happened on weight loss percentage, where fraction C also gives higher result than fraction B. It can be assumed that both fraction contain less toxic bio-active compounds, and the weight loss anomali result is influenced by termite condition itself. The clear result given by fraction A, where the highest mortality is confirmed by the least weight loss percentage. It indicated that anti termite bio-active compound is highly possible contained in the fraction A.

The result displayed on Figs. 3 and 4 confirm that all treated paper disc are consumed by termite. This phenomena gives indication, termite mortality caused by poisoning mechanism in termite disgestion, where bio-active agent of *Carbera manghas* L. seed kernel extract disrupt protozoa in the termite gut so that termite is unable to digest cellulose.

Phytochemical analysis of *Carbera manghas* L. seed kernel extract affirm the chemical compounds group contains such as alkaloid, saponin, tanin, tryterpenoid, and steroid (Table 1). The main intensity possessed by saponin, steroid, and alkaloid groups. It well-known that insect repeller

and plant-growth control. Saponin has poisonous effect toward insect, while steroid influence insect hormon insect exfoliation cycle (Harbore, 1987).

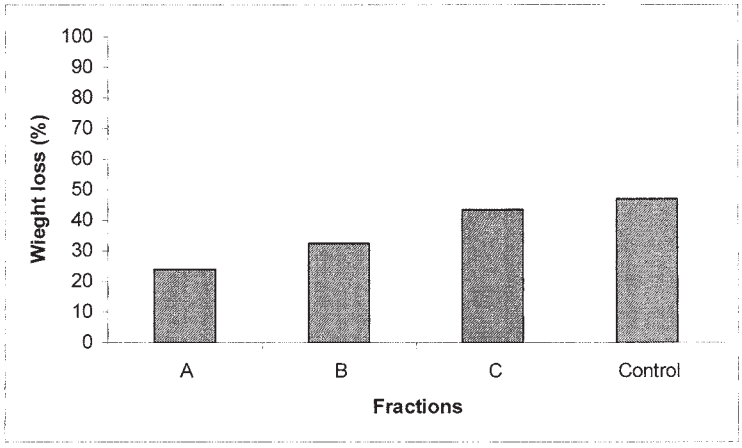

Figure 4. Weight loss percentage of paper disc after test

Table 1. Phytochemical analysis of *Carbera manghas* L. seed kernel extract

No	Group Name	Intensity
1.	Alkaloid	+++
2.	Saponin	++++
3.	Tanin	+
4.	Tryterpenoid	++
5.	Steroid	++++

Key: (-) = negative; (+) = low concentration; (++) = medium; (+++) = high; (++++) = very high

Conclusion

Carbera manghas L. seed kernel extract has high toxicity against subterranean termite, *Coptotermes gestroi*. Anti termite bio-active compounds is highly possible contained in fraction A, and phytochemical analysis confirm that *Carbera manghas* L. seed kernel extract has some chemical group name compounds alkaloid, saponin, tanin, tryterpenoid, and steroid.

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Laboratory Evaluation of Indonesia's Essential Oil against The Subterranean Termite *Coptotermes gestroi*

by

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Abstract

Recently, intense study has been conducted by researchers to utilize essential oils from tropical origin to be active ingredients in various insecticide formulations against some pests. The objective of this research was to evaluate the termite activity of Indonesian essential oil Clove *(Eugenia caryophyllata* Tumberg), Cubeb pepper *(Piper cubeba* L*),* Lemon grass *(Cymbopogon winterianus* Jowitt) against subterranean termite (*Coptotermes gestroi* WASMANN). Mortality and weight loss test was conducted by subjecting various concentration of Indonesian essential oil toward *C. gestroi* in forced-feeding test method. Subterranean termite, *C. gestroi* mortality rate was observed per two days periods until two weeks test period. The result showed paper disc treated by clove oil gave 100% termite mortality at 10% (v/v) in day tenth, while the smallest concentration 2% just gave 75% mortality in the end test period. According to the same test, lemongrass showed higher termite mortality than cubeb oil. Lemongrass caused 100% mortality at 10% concentration (v/v), whereas cubeb oil just caused 76% mortality at same concentration in the end of test period. Clove oil has the highest termiticidal potency compared to other essential oils against *C. gestroi.*

Key words: Clove, Cubeb pepper, Lemon grass, *Captotermes gestroi*

Introduction

The negative effect on environment by synthetic pesticide had raised so many researching to find a environmental-safe biopesticide. Indonesia as major producer of several essential oils are also being examined as green alternatives to subtitute the dangerous synthetic chemical-based pesticides. Essential oils are a mixture of many chemical compounds which are extracted from odoriferous plants. Essential oils are commonly used in perfume fragrance, food enhancers, pharmaceuticals and insecticides (Zhu et al. 2001). Some of the Indonesian essential oil is leaf of clove Clove (*Eugenia caryophyllata*), Cubeb pepper (*Piper cubeba* L) and Lemon grass (*Cymbopogon winterianus* Jowitt). Clove oil has antifungal activity on *Candida*, *Aspergillus* and dermatophyte species (Pinto et al., 2009). Some of them have been evaluated for their antimicrobial and insecticidal activity (Isman 1999). Cubeb piper used as traditional medicine and insecticide. Essential oils are preferable as termiticides due to their low mammalian toxicity and minimal environmental hazard (Isman 2000).
C. gestroi is the Asian subterranean termite which thought to have originated from Assam through Bruma and Thailand to Malaysia and the Indonesian archipelago (Kirton and Brown 2003). The most destructive species in South East Asia are from the genus *Coptotermes* (Kirton and Wong 2001, Lee and Chung 2003). It attacks living plants and finished products or almost any material that contains cellulose (xylophagous). This destructive termite species is frequently found infesting wooden structures in houses, cargo on-board ships and sailing vessels (Kirton and Brown 2003, Jenkins et al. 2007).

The objective of this research was to evaluate the usefulness of Indonesian essential oil Clove *(Eugenia caryophyllata),* Cubeb pepper *(Piper cubeba* L*),* Lemon grass *(Cymbopogon winterianus* Jowitt*),*) as a candidate of termiticide. The effect of Indonesian essential oil on tunneling behavior, termite mortality and weight loss were also investigated.

Materials and methods

Indonesian essential oils in this experiment (cubeb oil, leaf clove oil and lemon grass oil) was purchased from Aroma Kimia Industry, Yogyakarta, Indonesia. These essential oil re-distillated to obtain more pure oil.

Anti-termite activities of Indonesia's essential oil from clove, Cubeb pepper, Lemon grass was done by force feeding test method, which is drops in paper disc and was fed to *C. gestroi.* Dry paper disc was treated by various concentrations (2% until 10%) of essential oil and evaporated in

temperature room to evaporate solvent. The bioassay test method against *C. gestroi* referred to Setiawan et al., (2008), where fifty worker and fifth soldier of *C. gestroi*, and treated paper disc was entered in petri dish coated by Plaster of Paris 3 mm in thickness. Termite mortality was observed per two days until 14 days observation, the mass loss of paper disc also determined in the end of observation.

Tunneling activity was done using modification of petri dish method by Yeoh & Lee 2007. In this experiment, we used petri dish (16 cm diameter x 2 cm height) which separated into 2 sections by a piece of aluminum paper. One section contained 30 g of treated by hexane as solvent and another contained an equal amount of sand which have treated with Indonesian essential oil. This experiment used 1.5 ml of 10% concentration of essential oil at the ratio 1:20 (v/w). A piece of rubber wood (*Hevea brasiliensis*) measuring 2x2x1 cm was placed in the treated by essential oil section. Two hundred worker and 10 soldier were introduction into solvent treated section and allowed to acclimatize for 48 hour. After that period, the aluminum paper was removed and termites were allowed for 2 weeks. Experiment was replicated 3 time and held in a dark room. Tunnel structure were scanned using Hp Deskjet F2200 series. Tunneling activities in solvent and essential oils treated section were qualitative scored with 0 for no tunneling activity, 1 for tunneling activities covering ≤25% of the total arena, 2 for tunneling activities covering 26-50%, 3 for tunneling activities covering 51-75% of the total arena, and 4 for tunneling activities ≥75% of total arena (Yeoh, B.H & C.Y. Lee, 2007)

Differential in weight loss by each or concentration and period and tunneling activity were determined using one-way analysis of variance (ANOVA) and means were separated by using Tukey's HSD.

Results and discussion

The results showed there are distinctive differences in mortality, weight loss and tunneling pattern of the type of essential oil. Leaf of clove oil demonstrated a higher termite mortality than lemongrass and cubeb oil against *C. gestroi*. Termiticide bioassays were done in the various concentration from 2% (v/v) to 10% (v/v). Paper disc with clove oil treated gave 100% termite mortality at 10% (v/v) in day ten, while the smallest concentration 2% just gave 75% mortality in the end test period. According to the same test lemongrass showed higher termite mortality than cubeb oil. Whereas lemongrass caused 100% mortality at 10% concentration (v/v) and cubeb oil just caused 76% mortality at same concentration in the end of test period. Based on Table 1, the higher extract concentration caused the higher termite mortality rate, while in control specimens

Table.1. Termite mortality rate of essential oil evaluated

Name of Essential oil	Concentration (% v/v)	Percentage of termite mortality after test periode (%)						
		2	4	6	8	10	12	14
Clove *(Eugenia caryophyllata)*	10	50	73	85	91	**100**	**100**	**100**
	8	43	67	76	83	89	94	**100**
	6	36	43	51	62	75	88	**100**
	4	14	26	31	43	64	76	**92**
	2	8.	18	21	33	54	60	**75**
Cubeb pepper *(Piper cubeba* L*)*	10	16	22	24	59	71	72	**100**
	8	13	18	22	33	59	66	**91**
	6	8	9	20	36	64	64	**84**
	4	8	9	12	21	40	64	**72**
	2	0	2	4	6	12	28	**52**
Lemon grass *(Cymbopogon winterianus* Jowitt*)*	10	2	16	27	34	44	58	**76**
	8	4	12	28	32	43	45	**69**
	6	4	13	24	34	41	46	**52**
	4	7	17	18	22	26	46	**48**
	2	3	4	9	17	22	26	**33**

there were no termite mortality during test period. It can be concluded that solvent doesn't influence termite mortality rate and termite mortality caused both of concentration and type of chemical

compound on essential oil. So, from this results Indonesian leaf clove oil have a higher termicide effect against *C. gestroi.*

This study was observing that repellent or non-repellent properties were depended on concentration used by weight loss and tunneling test. Figure 1. shows there are correlation between concentration and sample weight loss, where as higher concentration caused moreless percentage weight loss. This is demonstrated repellent character, which clove oil has a higher repellent character more than lemongrass and cubeb oil. Decreasing of feeding activity occurred at increasing concentration when weight loss value be decreased. It indicated that essential oils have repellent characteristic, where as the essential oil was volatile compound to natural enemies in this test (subterranean termite). The interaction between plants and herbivores involves volatile and other chemical compounds. Plants respond to herbivore damage by releasing chemicals (Tallamy and Raupp 1991; Baldwin 1994). Previous studies showed that corn (*Zea mays*) and cotton (*Gossypium hirsutum*) when stimulated by insect feeding damage can release certain types of chemical compounds including caryophyllene (Loughrin *et al.* 1994).

Table.2. Termite weight loss rate of Essential oil evaluated

Concentration (% v/v)	Clove *(Eugenia caryophyllata)*	Cubeb pepper *(Piper cubeba L)*	Lemon grass *(Cymbopogon winterianus Jowitt)*
2	3.88±1.46	54.99±36.49	32.86±11.33
4	10.76±1.46	26.28±9.94	31.39±6.89
6	9.61±2.95	26.93±5.29	33.09±11.88
8	11.70±2.46	29.11±14.53	27.83±11.88
10	10.56±1.36	15.09±8.17	16.86±11.22

Mean followed by the different within the same column and insecticide are significantly different (Tukey HSD; $P<0.05$)

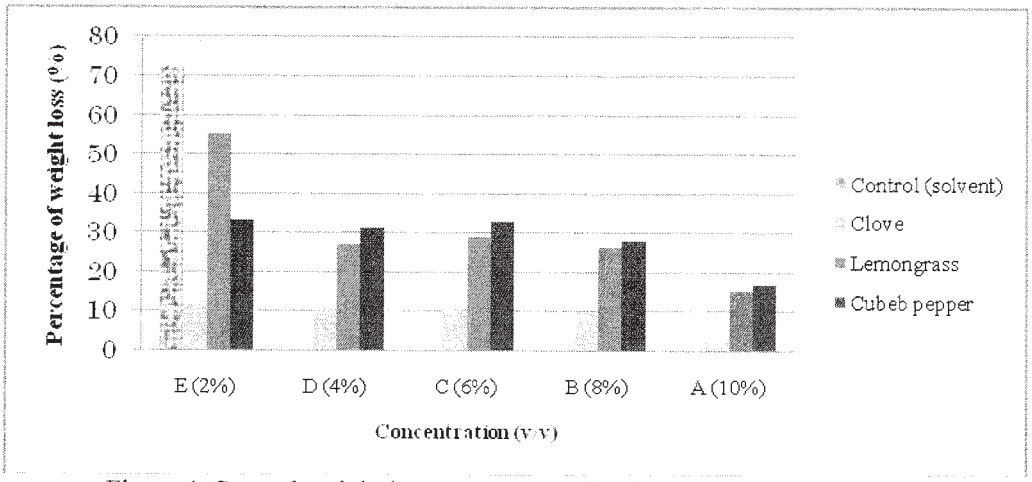

Figure 1. Sampel weight loss percentage of Indonesian essential oil

Tunneling results showed that all essential oil evaluated indicated repellent characteristic. This is demonstrated by a small value of tunneling activity (Table 3). Tunneling readily done in cubeb oil sand treated, lemongrass oil and clove oil. Clove oil have a higher repellent characteristic, it has showed by a small value of tunnel activity and weight loss value which in smallest concentration than other essential oil. Clove oil contains acetyl eugenol, beta caryophyllene in a higher concentration. It's suggested caused a repellent character in against *C. gestroi* which have showed moderate acute toxicity to Formosan subterranean termites (Qiaoling, 2009).

Meanwhile the sand treated by lemongrass and cubeb oil showed a higher attacked by termite. Even as repellent, lemongrass and cubeb oil treated sand section still have been attacked by termite. Javanese lemongrass from Indonesia showed percentage constituent such as (Sastrohamidjojo, 2004): Sitronelal 32-45%, Geraniol 12-18%, Sitronelol 11-15%, Geranil asetat 3-8%, Sitronelil

acetate 2-4%, Sitral, Khavikol, Eugenol, Kadinol, Kadinen, Vanilin, Limonen, and kamfen. Cubeb oil has a higher tunneling activity which have unless termite mortality. Even as essential oil, cubeb oil just have a few of volatile oil and contain seskuiterpene, kubebate acid, kubebin, piperina, piperidine as a toxic compounds. Foraging activity in cubeb oil treated sand section caused a low of repellent compound. So, termite mortality suggested caused by toxicity of chemical compound which have slow action like reported by Kintzios and Barberaki, 2003 where as piperidine compound contain on cubeb oil have toxic activity as anticancer. Clove and lemongrass oil have a repellent compound. Termicide effect on clove and lemongrass oil depended type and concentration of a chemical compound, this has needed further experiment.

Table 3. Tunneling activity rate of Essential oil evaluated

Name of Essential oils	Mean (±St Dev) ranking for tunneling activity
Control (methanol)	3.83±0.21
Clove *(Eugenia caryophyllata)*	0.20±0.20
Cubeb pepper *(Piper cubeba L)*	1.93±0.50
Lemon grass *(Cymbopogon winterianus Jowitt)*	0.93±0.25

Mean followed by the different within the same column and insecticide are significantly different (Tukey HSD; $P<0.05$)

A control specimen in tunneling was using sand which treated by water and solvent (hexane). This has showed the high foraging of tunnel in all the control area. It indicated that solvent doesn't have any influence in the tunneling activity. Further research has been needed to identify what kind of the chemical compound contained on essential oil which has termicidal activity against *C.gestroi.*

Conclusion

The present study has confirmed that Indonesian essential oil of the Clove *(Eugenia caryophyllata),* Cubeb pepper *(Piper cubeba* L*),*and Lemon grass *(Cymbopogon winterianus* Jowitt*)* oils have a potential termicidal activities against *Coptotermes gestroi* . These essential oil were showing haracteristic base on termite consumption behaviour and mortality data.

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Termiticidal Activity of Eugenol Derived from Indonesian Clove Leaf Oil (*Eugenia caryophyllata* Tumberg) against Subterranean Termite, *Coptotermes gestroi* WASMANN

by

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Abstract

Research on termite control using bio-based preservatives agent had been conducted in recent years. But, some problem arise, such as difficult process and low availability of bio-active compounds; and unsustainable supply of raw material, had possessed high production cost so that bio-based preservatives are commercially unproper. This study aims to propose termite control using eugenol as bio-active agent, derived from indonesia clove leaf oil, *Eugenia caryophyllata* Tumberg, to overcome these problems mentioned. Destillation process to purify crude clove leaf oil gave 91,68%, with eugenol concentration is 75,25%. Bio-assay test of distilled clove leaf oil showed high toxicity against subterranean termite, *Coptotermes gestroi*. To study termicidal effect, eugenol was isolated from distilled clove leaf oil using reduced-pressure dillation method. The result gave three fraction, Fraction 1 (F-1) is no eugenol contain; Fraction II (F-2) with 45,5% eugenol contain and Fraction III (F-3), 84% eugenol contain. The redistillation of F-3 was success to optimize eugenol concentration, 96,7% (F-4). The bioassay test of F-3 and F-4 against *C. gestroi* showed that eugenol has very good termiticidal activity.

Keywords: Eugenol, *Eugenia caryophyllata* Tumberg, *Coptotermes gestroi* WASMANN

Introduction

Wood-base material continues to be very popular and economical raw material for building construction considered on its availability, renewable, easy on working, and very esthetic. Unfortunately, wood-base material are susceptible for termite infestation. Proper protection is a solution to avoid economical loss caused by termite. Chemical-based treatment such as imidacloprid, pipronil and pyrethroids, are the most common method for termite control. But, even these chemical-base material are very effective against termite, these chemicals posses many negative effect toward environment and humanosphere.

Many study were conducted to propose new alternatif preservatives which are more safe for human and environment, based on natural extractives (Peterson & Ems-Wilson, 2003; owusu et. al., 2008) and entomopatogenic fungi (Tarmadi et. al, 2005; Guswenrivo et. al., 2008). Even though all these study reported the high termicidal activity, the main problems arise about the commercial prospect of bio-active compounds. The difficult process and low availability of bio-active compounds isolation, and sustainable supply of raw material were some problems faced by many researcher. These problems has risen production so that bio-based termite agent is unproper from economic point of view. This research aims to propose termite control by bio-active compounds derived from indonesia clove leaf oil, *Eugenia caryophyllata* Tumberg. Easy bio-active compound isolation and sustaibility supply of clove leaf oil became main reason to study termicidal activity.

Materiasl and methods

Commercial Indonesian clove leaf oil (*Eugenia caryophyllata* Tumberg) was purchased from Aroma Kimia Industry, Yogyakarta, Indonesia. Reduced-pressure distillation of commercial clove leaf oil at 65-120 ^{0}C/1 mmHg was conducted to purify clove leaf oil. Gas Chromatography analysis was performed to identify eugenol concentration in the distilled clove leaf oil. GC Instrument used was Hewlett Packard 5890 Series II, with column HP-5 (30 m, Film thickness is 0.25μm), the carrier gas was helium at 60 Kpa. Injection port was kept at 280 ^{0}C. The column temperature was initiated at 160 ^{0}C, continually at 5 ^{0}C per minute 280 ^{0}C.

Bioassay Test

The bioassay test method against *Coptotermes gestroi* was referred to forced-feeding test according

to Tarmadi et. al. (2007). Paper disc was treated by extract with various concentration of distilled clove leaf oil 2%, 4%, 6%, 8% and 10 % (v/v). Treated paper disc was left for 12 h at room temperature to evaporate solvent hexane. Then, fifty workers and five soldiers of *Coptotermes gestroi*, and treated paper disc was entered into petri disc coated by moistened plaster paris 3 mm. The Bioassay test periode will run for 14 days. Termite mortality was observed per two days periods and in the final period observation, the mass loss of paper disc also determined.

Eugenol isolation from clove leaf oil

150 g distilled clove leaf oil stirred with 25 g NaOH. The solution was entered into separating funnel and left for 15 minutes until formed two layer, bottom layer contain of Na-eugnolat; and upper layer contain other clove leaf compound. Bottom layer was collected and extracted with 3 ml diethyl eter and left until form two layer again. The bottom layer separated and added with 25% (v/v) HCl until pH 3. The solution extracted once more with diethyl eter in the separating funnel and in the end of reaction will be formed two layer; upper layer contain eugenol while bottom layer isolate NaCl. The upper layer collected followed by reduced pressure distillation at 103 – 106 ^{0}C/3 mmHg. The redistilled oil characterized by gas chromatography-mass spectrometry and infra red spectrophotometry to determined eugenol concentration. The bioassay test was conducted to study termicidal activity of eugenol derived from clove leaf oil.

Results and discussion

The research was inisiated by reduce-pressure distillation to obtain purified clove leaf oil. The result presented on table 1, where distillation yield is 91,68 %. Gas chromatography analysis (figure 1) showed there are eight peak represented of chemical compounds contained.

Table 1. The result of clove leaf oil reduced-pressure distillation

Crude clove leaf oil (g)	*distillate (g)*	*Vol. (ml)*	*Temp. (^{0}C)*	*Pressure (mmHg)*
300	274,8	276	65 – 120	1

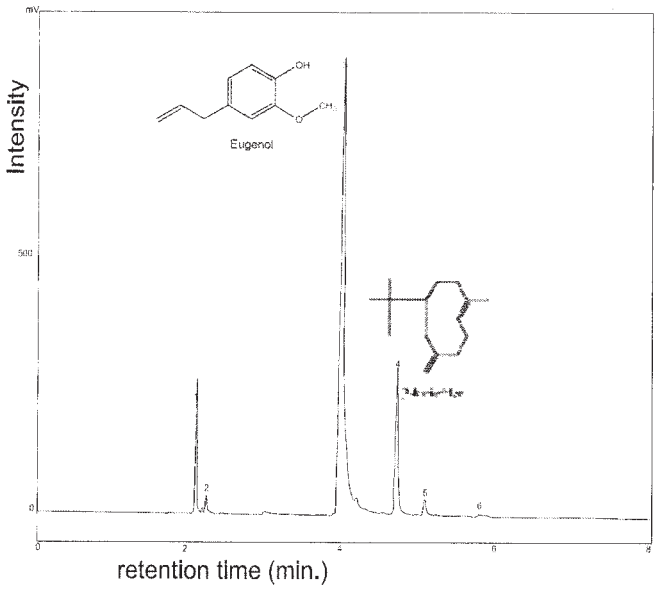


Figure 1. GC Spectrum of distilled clove leaf oil

Eugenol is represented by peak with retention time (T_R) 3,96 minute with concentration 75,25 %. The other peak (T_R 4,7 min.) is β-caryophyllen with 14,63% concentration. As reported by Hardjono (2004), clove leaf oil has two main component, eugenol and β-caryophyllen. The other component are non-phenolic compunds such as α-cubene, α-copaen, humulen, and β-cadien.

Anti termite activity of clove leave oil distillate was conducted by bioassay test against subterranean termite, *Coptotermes gestroi*. The result showed the higher clove leaf oil concentration, the higher termite mortality (fig. 2.a). The highest response given by 10% (v/v) concentration, where 100% termite mortality achieved on day ten. Even the least concentration (2%, v/v) is still able to give high termite mortality rate, 75%, in the end of test period. It indicated that clove leaf oil has termical activities. Weight loss data (fig. 2.b) shows the higher concentration, the lower termite consumption rate. The data support the presumption that clove leaf oil is quite effective against subterranean termite

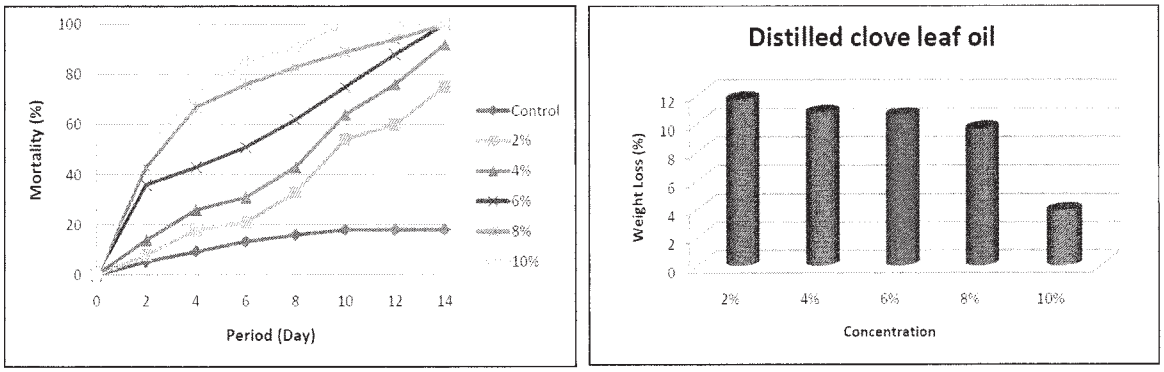


Figure 2. Termite mortality rate of distilled clove leaf oil against subteranean termite, *C. gestroi* (2.a); Percent weight loss in the end of test period (2.b.)

Hardjono (2004) reported that eugenol is the dominant compound of clove leaf oil, so that based on bioassay result (fig. 2.a & 2.b) can be assumed that eugenol plays the most important role on termite killing. Eugenol isolation was conducted to prove this hypothesis. 150 g clove leaf oil was redistilled in reduced-pressure condition and gave three fraction, fraction I (F-1); fraction 2 (F-2) and fraction III (F-3).

Table 2. Eugenol isolation from distilled clove leaf oil

Distillate	*Temp. (^{O}C)*	*Pressure (mmHg)*	*Yield (g)*	*Eugenol conc. (%)*
Fraction I (F-1)	90-99	3	29,1	-
Fraction II (F-2)	100-103	3	41,4	45,5
Fraction III (F-3)	104-120	3	64,8	84,0
Redistilled Fraction III (F-4)	109 -120	3	35,25	96,7

Eugenol analysis by gas chromatography was resulting that the highest eugenol concentration was found in F-3 by 84,0%, then F-2 by 45,5% (Table 2). Fraction 1 (F-1) is not contain eugenol because the fraction temperature is far below eugenol boiling point (142 ^{o}C). The similiar condition happened in F-2, where eugenol concentration is not significant. The fraction temperature is heat enough to vaporize eugenol but the fractionation condition is not in eugenol's optimum temperature, so that the other compounds is still presence (fig 3.a). F-3 temperature is the optimum condition, and able to isolate eugenol in higher level (Fig. 3.b).

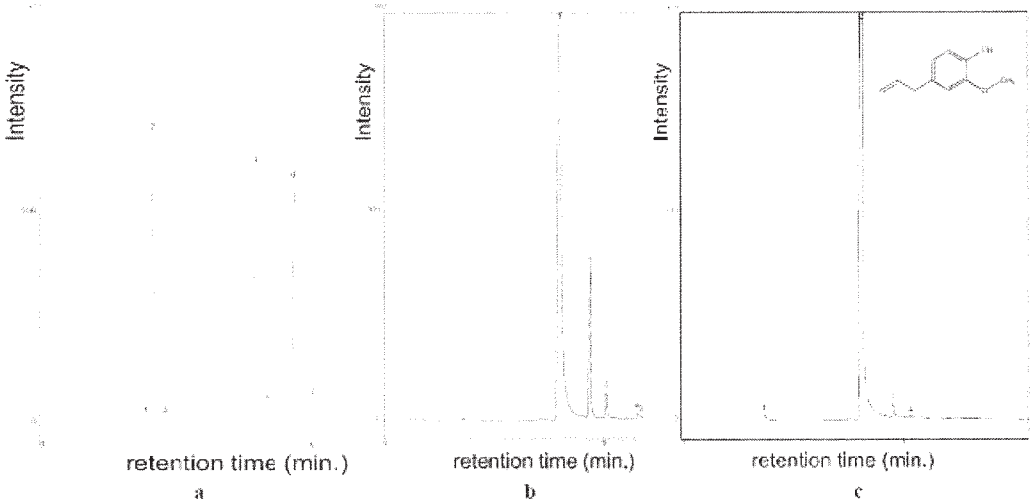


Figure 3. Gas chromatography spectrum of: a. Fraction II (F-2); b. Fraction III (F-3); and c. Redistilled of Fraction III (F-4)

Redistillation process of F-3 in was conducted to optimize eugenol concentration. Gas chromatography spectrum above (fig. 3.b and 3.c) describe that in the redistilled of fraction III (F-4), eugenol is the dominant compounds (96,7%) rather than F-3 where other compounds are still present, even though are not in significant level (fig. 3.b).

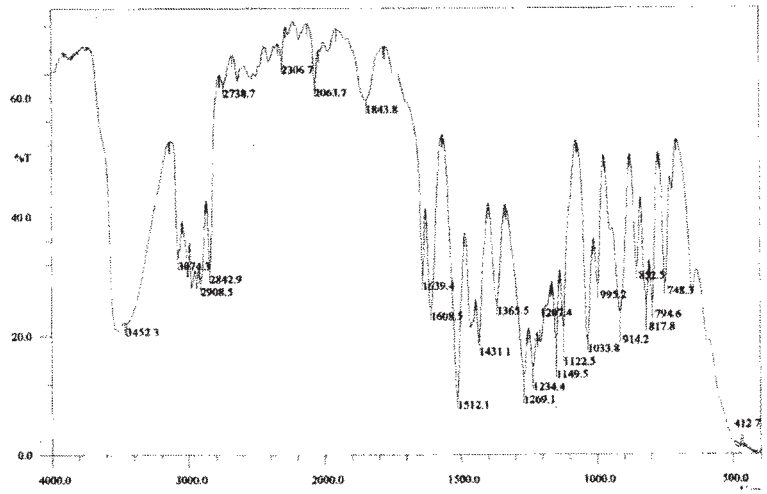
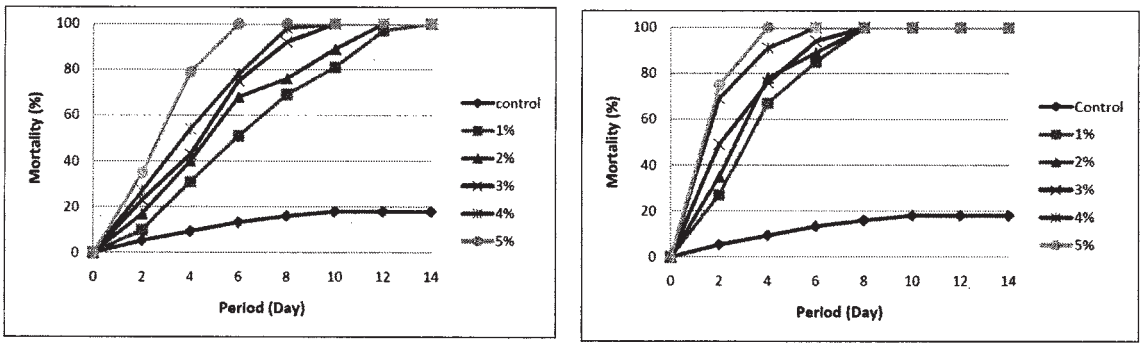


Figure 4. Infra Red spectrum of Redistilled of Fraction III (F-4)

To prove the eugenol presence, further analysis by infra red spectrophotometry toward F-4 was conducted. The result (figure 4) showed wide band between 3600 – 3200 cm^{-1} is specific area for hydroxyl (–OH); acute band between 1660-1670 cm^{-1} is specific area for alkil bonding (C–H, sp^3); specific area for alkene (C=C) from allil bonding is found at around 1515 cm^{-1}; while C=C bonding for aromatic compounds was showed at 1200 – 1100 cm^{-1}. Based on gas chromatography and infra-red spectrophotometry analysis can be concluded that the chemical compound in F-4 is eugenol.

Termicidal activities of eugenol was conducted by subjecting F-3 and F-4 against *C. gestroi* in forced-feeding test method. F-2 is not considered for bioassay because eugenol presence in this fraction is still bound with other compounds, so the result will not reflect eugenol termicidal activity itself. More over, the chemical properties of F-2 is quite similiar with distilled clove leaf oil (fig. 2) above.

In these kind of test (fig. 5), the fraction concentrations used were 1%, 2%, 3%, 4% and 5% (v/v) reduced by half-point of distilled clove leaf oil termite test. The reason of concentration reduction is based on the result that distilled clove leaf oil has high termicidal activity (fig.2). Bioassay test result showed that eugenol has high termicidal activity. 100 % termite mortality achieved in just 4 days in 5% F-4 concentration (fig. 5.b), and 6 days in the same concentration of F-3 fraction (fig. 5.a). The very high termicidal activity was affirmed even in 1% fraction concentration, where 100% mortality is given in eighth day by F-4 and the end of test period by F-3.



a b

Figure 5. *C. gestroi* mortality rate of: a. Fraction III (F-3); and b. Redistilled of Fraction III (F-4)

Comparing the F-3 and F-4 result, it can be concluded that F-4 gives more reliable and consistent result. This is influenced by eugenol purity and concentration. Based on, its fast killing, eugenol has toxic effect against termite, as reported by Cornelius (1997).

Sample weight loss is represent of termite consumption behaviour. Figure 5 shows that samples weight loss average are below three percent, generally insignificant amount. Beside its fast killing

effect, eugenol also indicates has repellent effect, showed by low termite consumption. However, it need further study such as termite tunneling behaviour and soil treatment, to affirm this hypothesis. But, based on the data, can be conclude that eugenol is proved has high termicidal activity against subterranean termite, *Coptotermes gestroi.*

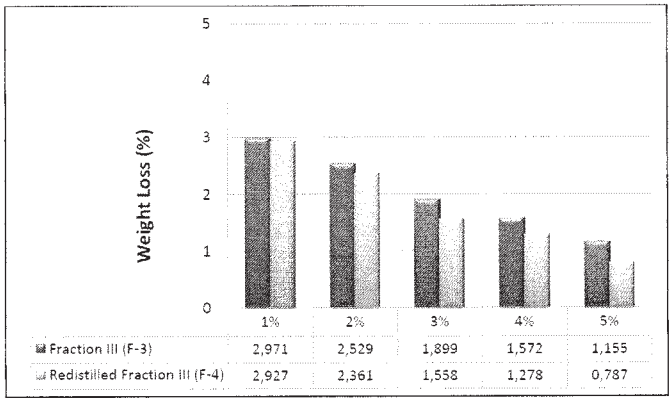


Figure 5. Weigt loss percentage of Fraction III (F-3) and Redistilled of Fraction III (F-4)

Conclusion

Eugenol derived from indonesia clove leaf oil has been proved high termicidal activity against subterranean termite, *Coptotermes gestroi.* Based on the data, eugenol is not only showed toxicity against termite considered on its fast killing mecanism, but also repellent activity conducted to low sample consumption by termite.

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Effect of Oil Palm Vinegar on Hydrogen and Methane Emission of *Coptotermes formosanus* Shiraki (Blattodea: hinotermitidae)

by

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2) RISH Kyoto University Japan

Abstract

Hydrogen and methane emission by the termites *Coptotermes formosanus* Shiraki was investigated in relation to the effect of oil palm vinegar on the wood bait. Concentrations of oil palm vinegar were 1%, 2% and 3%. Hydrogen and methane emission rates were observed at 1 day, 3 days, 6 days, 9 days, 16 days, and 21 days after setting-up. The concentration 2% showed maximum emission rates of 1.307 nmol/termite/hour for hydrogen and 0.355 nmol/termite/hour for methane. Meanwhile hydrogen and methane emission rates of 1% and 3% concentration were quite similar. The wood consumption was ranged 14.6%-19.3% and termite mortality was ranged 11.3%-16.3%. Ethanoic acid as the major compound of oil palm vinegar might play some role in preventing termites attack on wood.

Keywords: hydrogen emission, methane emission, *Coptotermes formosanus*, oil palm vinegar

Introduction

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is one of the most destructive wood pests in Japan. Presently, termites are managed through chemical control. However, some synthetic chemicals cause serious environmental problems due to their persistence and toxicity. There is growing interest in using natural compounds with low toxicity to mammals to control termites. Oil palm vinegar is a liquid generated from the gas and combustion of fresh oil palm empty fruit bunch burning in airless condition. When the gas from the combustion is cooled, it condenses into liquid. Raw vinegar has more than 200 chemicals, such as acetic acid, formaldehyde, ethyl-valerate, tar, methanol, etc. Wood vinegars have been use in several purposes such as industrial product, livestock, household and agriculture (International Bio-Energy, 2003; Yatagai *et al,* 2002, Apai and Tongdeethare, 2001). The production and consumption of wood vinegars have been increasing recently. Today, around 6.000-7.000kl of wood vinegar is produced and consumed annually; mainly for agricultural use (Pangnakon, 2008).Although several ways to use wood vinegar have been known, there have been few detailed studies of its properties as wood preservatives. The objective of this research was to evaluate the possibilities of oil palm vinegar with different concentrations as wood bait to *C. formosanus.* Hydrogen and methane emission was used as indicator of bait consumed by termite.

Materials and methods

Preparation of oil palm vinegar

Oil palm vinegar was made from burning wood meal from oil palm empty fruit bunch. The material was collected from Pontianak West Borneo Indonesia and converted into wood meals in a willey mill with 40-60 mesh screens, and air dried to about 12% of moisture content. About 1000 grams of air-dried wood meals were then put in a kiln and burn at temperatures 400°C. The smoke from carbonization was cooled by the outside air when passing through the chimney. The hot steams condensed into liquid were collected.

GCMS analysis

The oil palm vinegar was 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 x 0.25 mm diameter). The GC settings were as follows: initial column temperature set at 40°C for 5 min; temperature program from 40°C to 330°C with a rate 4 °C/min. Mass spectra resolution was 1000, with interval 0.5 sec; ionization 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

Feeding bioassay

No-choice feeding bioassay was conduct to know the effect of oil palm vinegar on wood consumption and hydrogen and methane emission rates. Sapwood block of Japanese Cedar (*Cryptomeria japonica)* measured 20 mm (R) x 20 mm (T) x 10 mm (L) were dipped into the oil palm vinegar for 5 hours and were air-dried before used as bait. Concentrations of the oil palm vinegar were 1%, 2% and 3% (v/v). Distilled water was used as a solvent for the vinegar.

The procedure of no-choice feeding bioassay was as follows: matured 100 workers and 10 soldiers of *C. formosanus* taken from a laboratory colony maintained in termite culturing room at the Laboratory Innovative Humano-habitability Research Institute for Sustainable Humanosphere, Kyoto University in the dark at 28±2°C and >85% RH were put into an acrylic cylinder (60 mm in diameter and 50 mm in height) with a plaster bottom. They were kept in acrylic for two days (starvation treatment) at 28±2°C and >85% RH by serving only distilled water through the bottom of acrylic. After that, a plastic sheet was set on the center of the acrylic and wood block was put on the plastic sheet. Three replicates were employed. Survival rates and weight changes of workers were observed at 1 day, 3 days, 6 days, 9 days, 16 days, and 21 days after setting-up. All test units were kept in the termite culturing room for 21 days and the wood blocks were recovered. Mass loss and termite mortality were calculated.

Measurements of hydrogen and methane emission rates

Hydrogen and methane emission rates were measured as follows: at the 1 day, 3 days, 6 days, 9 days, 16 days, and 21 days all the workers were harvested and transferred into a clean glass vial (50 ml) with a silicon rubber rid, and the vial was kept in the termite culturing room for one hour. One ml of gas sample from vial was taken by a syringe and injected to a gas analyzer equipped with a semiconductor sensor (New Cosmos Electric) to measure hydrogen and methane concentrations.

Results

Oil palm vinegar

After 2 hour process, oil palm vinegar was achieved and yielded 200 ml with light yellow in color. Its specific gravity was 1.008, temperature was 29°C, and pH value was 4.0. Figure 1 show the chromatogram of oil palm vinegar. The chemical compound were consist of ethanoic acid ($C_2H_4O_2$), phenol (C_6H_6O), furanone ($C_4H_6O_2$), dimethyl ketone (C_3H_6O) and butanoic acid,2-propenyl ester ($C_7H_{12}O_2$).

Hydrogen and methane emission rates

Table 2 shown hydrogen and methane emission rates of workers of *C. formosanus* fed on wood bait treated with various concentration of oil palm vinegar as expressed nmol per termite per hour. Beforestarvation (normal condition for the test insects), workers of *C. formosanus* emitted hydrogen and methane at the rates of 0.732 nmol/termite/h and 0.257 nmol/termite/h, respectively. By the two days starvation, the emission rates drastically decreased to 0.099 nmol/termite/h and 0.147 nmol/termite/h for hydrogen and methane emission, respectively. Hydrogen emission rates gradually increased in workers fed on wood, wood dipped in 1% oil palm vinegar and wood dipped in 2% oil palm vinegar

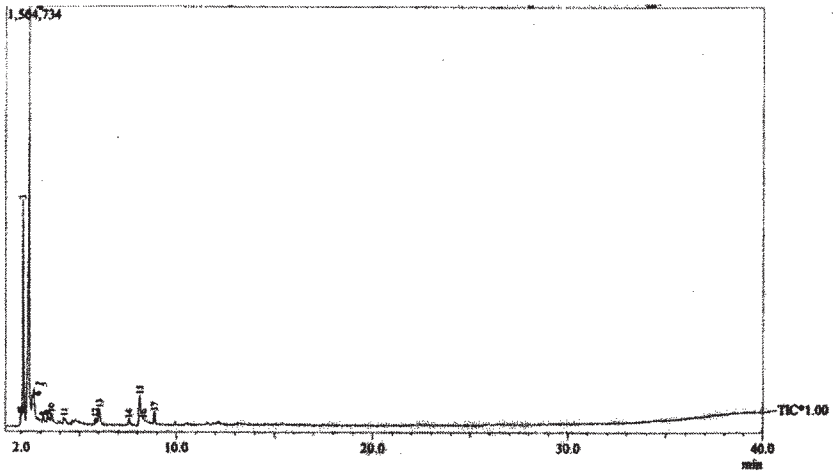

Figure 1. Chromatogram oil palm vinegar

during 6 days, and the highest rate of 1.307 nmol/termite/h was obtained in the case of wood dipped in 2% oil palm vinegar. Meanwhile, workers fed on wood dipped in 3% oil palm vinegar gradually increased the hydrogen emission rate for 16 days with the highest rate of 0.591 nmol/termite/h.

Table 1. Chemical Compound and Retention Time of Oil palm Vinegar

No.	Peak No.	Retention Time (min)	Area (%)	Height	Chemical Compound
1	4	2.381	46.39	1540383	Ethanoic acid ($C_2H_4O_2$)
2	3	2.067	21.04	832117	Dimethyl ketone (C_3H_6O)
3	5	2.626	9.68	135403	Butanoic acid,2-propenyl ester ($C_7H_{12}O_2$)
4	15	8.094	7.04	113090	Phenol (C_6H_6O)
5	13	6.032	3.09	58151	Furanone ($C_4H_6O_2$)

Table 2. Hydrogen and methane emission rates of workers of *C. formosanus* fed on wood with various oil palm vinegar concentration

Substrate	Gas	Emission Rates (nmol/termite/h)								Termite Mortality (%)	Wood Weight Loss (%)
		Before Starvation	After Starvation	1 day	3 days	6 days	9 days	16 days	21 days		
Wood	H_2	0.732	0.099	0.127	0.258	0.347	0.262	0.304	0.255	5.3	23.86
	CH_4	0.257	0.147	0.185	0.278	0.244	0.233	0.209	0.155		
1% oilpalm Vinegar	H_2	0.732	0.099	0.099	0.569	0.848	0.844	0.632	0.361	13.0	16.56
	CH_4	0.257	0.147	0.137	0.311	0.311	0.328	0.233	0.106		
2% oilpalm Vinegar	H_2	0.732	0.099	0.150	0.890	1.307	1.246	0.972	0.958	11.3	19.31
	CH_4	0.257	0.147	0.141	0.353	0.355	0.318	0.222	0.074		
3% oilpalm Vinegar	H_2	0.732	0.099	0.083	0.174	0.440	0.570	0.591	0.377	16.3	14.57
	CH_4	0.257	0.147	0.111	0.203	0.236	0.244	0.210	0.164		

Note: values are means of three replicates

Methane emission rates in workers fed on wood was recovered after one day starvation meanwhile on workers fed on wood with oil palm vinegar need three day to recover. Methane emission rates in workers fed on wood was ranged 0.155 nmol/termite/h -0.278 nmol/termite/h. Workers fed on wood which dipped in 2% oil palm vinegar has the highest methane emission rates at 6 day (0.355 nmol/termite/h) meanwhile workers fed on wood which dipped in 1% and 3% oil palm vinegar reach the highest for 9 day (0.328 nmol/termite/h and 0.244 nmol/termite/h, respectively).

Wood consumption was range between 14.57%-23.86% and termite mortality was range between 5.30%-16.33%. Wood dipped in 2% oil palm vinegar was the highest consumption (19.31%) than wood dipped in 1% and 3% oil palm vinegar (16.56% and 14.57%, respectively). Termites mortality on wood dipped in 3% oil palm vinegar was the highest value (16.3%) followed by 1% and 2% oil palm vinegar (13.0% and 11.3% respectively).

Discussion

Ethanoic acid was the major compound of oil palm vinegar. Lin *et al* (2006) found that bamboo vinegar contains of organic acids, phenolic compounds, alkanone compounds, alcohol compounds, and aldehyde compounds. Higashino *et al* (2005) stated that some wood vinegar contain of acetic acid, hydroxyacetone, propionic acid, butyric acid, phenol, guaicol, *n*-butanoic acid, and furfural. Those finding showed that oil palm vinegar compound quite similar to other vinegar compound even made from different source.

Wood consumption and survival of termites on wood dipped in oil palm vinegar was lower than untreated wood. It can be assumed that oil palm vinegar has repellent effect activity to termites.

Yatagai *et al* (2002) reported wood vinegar made from *Cryptomeria japonica* and *Pseudotsga menziesii* had high termiticidal activity and acetic acid which is the largest content of wood vinegar are considered to plays some role in preventing termites attack on wood. As ethanoic acid was the major compound of oil palm vinegar, it can be presumed that ethanoic acid contributes on repellent effect.

Table 2 showed the methane emission was stable meanwhile hydrogen emission generally higher after three days, but did not recover after the starvation treatment. This indicated the symbiotic protist could not utilize the substrates directly and made the bacteria depression. Boga and Brune (2003) stated that hydrogen is produced by the anaerobic fermentation of glucose by protist and bacteria, followed by acetogenesis and methanogenesis by the bacteria. Hydrogen is well known as a fundamental resource for material and energy. The hydrogen emission by termites depends on the digestion of cellulosic matter by termites. The digestion depends on the presence of a dense and diverse population of microorganisms in their guts, particularly cellulolytic flagellated protists and bacteria. Inoeu (2007) studies postulated that the gut symbiotic protists phagocytose wood or cellulose particles and then depolymerize and ferment them to produce amounts of acetate, carbon dioxide, and hydrogen. The symbiotic protists, accounting for up to one-third of the total insect volume, are densely packed mainly in the voluminously dilated anterior part of the hindgut, where the hydrogen partial pressure is the highest. Thus, cellulolytic protists are considered to be the major producers of hydrogen in the termite gut.

Doolittle *et al* (2007) stated that all members of lower termites are provided with a complex gut fauna consisting of bacteria and protozoa. Gut bacteria seem to have a special role in nutritional metabolism in lower termites, although they are not considered as the major contributors to cellulose decomposition. The low wood consumption also indicated that protists in termite gut could not digest the wood. Baht *et al* (2009) reported that oil palm liquor has antioxidant activity. This report indicated that some chemical in oil palm vinegar may have effect on termite gut activity. Pester and Brune (2007) said that acetic acid is one of the main short chain fatty acids, which can affect intestinal functions and metabolism. Acetic acids were reported to control the balance of intestinal micro flora and pathogen. These reports suggest that the present of ethanoic acid in oil palm vinegar give effects to termite gut activity.

Kawaguchi *et al* (2005) tested the hydrogen and methane emission from termites *C. formosanus* with fed on cellulose diets. The result showed that hydrogen emission before starvation was 1.72 nmol/termite/h and methane emission was 0.69 nmol/termites/h before starvation, meanwhile on this research the hydrogen and methane emission was lower, 0.732 nmol/termite/h and 0.257 nmol/termite/h, respectively. Yoshimura et al (1994) surveyed the seasonal fluctuation of wood attacking activities of the termites *C. formosanus* colony and found that the activities tended to be lowest in winter and to increase linearly to the maximum values in the autumn. Despite of the constant temperature and humidity in termite culturing room all through the year, it is well recognized that the foraging activities of workers fluctuate seasonally to some extent. Therefore, significantly lower hydrogen and methane emission in this test might be caused by seasonal changes of physiological conditions of termites.

After the 16 days, hydrogen and methane emission by workers appeared to slightly decrease possibly because of the effect of oil palm vinegar and on the health conditions of workers tested. Further research is needed to construct the effect of oil palm vinegar on activity of termites gut microbes. Limiting termite growth through the destruction of the gut microbes is a control method that has shown some success.

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Attractant and Arrestant Chemicals from *Cryptomeria* for Japanese Subterranean Termite *Reticulitermes speratus*

by

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Abstract

Commercialized termite monitoring-and-baiting program is currently applied to control subterranean termites in Japan, and effective to *Coptotermes formosanus*. It is, however, less effective to *Reticulitermes speratus*, so we search for additional attractant and/or arrestant chemicals from its habitat to improve the effects on *R. speratus* of this system. Chips of pine (*Pinus densiflora*), cedar (*Cryptomeria japonica*) and North American spruce (*Picea*) were separately immersed in hexane or diethyl ether. Only the hexane extract of the cedar had potent activity as attractant and/or arrestant to *R. speratus*. It contained several characteristic compounds of which Kovats Indices were between 1400 and- 1700 and between 2000 and- 2400. Most of those compounds were eluted in 30% ether-in-hexane when chromatographed on silica gel, and the fraction presented the activity. Preparative HPLC further separated the fraction into three fractions. One of those fractions was effective to arrest the termites, but not to attract them. When mixing all those fractions, however, it effectively attracted and/or arrested the termites. GC-MS analyses suggested that the major compounds in the active fraction were diterpenoid alcohols.

Key words: *Reticulitermes speratus*, *Cryptomeria japonica*, attractant, arrestant, wood extract

Introduction

Termites consist of over 2800 species (Constantino, 1998), and about 180 species are known to damage buildings and about 80 species cause significant damage (Edwards and Mill, 1986). Most of them are subterranean species, including mound building and arboreal ones. In Japan, two subterranean termites, *Coptotermes formosanus* and *Reticulitermes speratus*, often cause serious damage to wooden buildings including historical ones, and the damage costs reach to 100-300 billion yen every year (Yoshimura, 2003), being much higher than the fire damage costs. They are expanding their habitat to north year by year because of high adiabaticity of the recent houses (Mori, 2003).

Various types of soil termiticides, including organophosphate and carbamates, have been used to control these termites in the last half century. Such termiticides were effective to eliminate the termite colonies partially, but not completely. Furthermore, there is danger of causing health hazard in human. Alternative termite monitoring-and-baiting program is recently getting popular (Su and Scheffrahn, 1998; Forschler and Jenkins, 2000). In this program, several monitoring bait stations are placed in the soil surrounding a building, and the baits are replaced to ones contained slow-acting toxin, such as the chitin synthesis inhibitor when the termites are in. This program is not harmful for mammals, and enables to decrease other negative environmental impacts because the use of the toxic baits is limited. It has been already applied to control subterranean termites in many countries including Japan, and succeeds in *C. formosanus*. However, it is not so effective to *R. speratus*, which also causes damages in buildings. It is important to keep the termites staying at the station even after the replacement of the baits to apply the monitoring-and-baiting program to *R. speratus*. We focused on the food-and-nest orientation signals of this species, and tested several wood extracts to search for attractant and/or arrestant chemicals. We succeeded in finding such chemicals in the Japanese cedar trees. In this study, we further separated the chemicals by means of several chromatographies for identification of the active compound.

Materials and methods

(a) Collection and rearing of study insects

Two mature *R. speratus* colonies were collected in Yoshida-yama, Kyoto Prefecture in September 2009. They were individually maintained in plastic containers (250×350×40mm) at 28°C, supplied with water and wood pieces every day.

(b) Preparation and separation of wood extracts

Powdered *C. japonica* (50g) was immersed in 500ml of hexane for 24 h at room temperature, and this was separately repeated 5 times. The extracts were then stored at -30°C in a deep freezer.

Preparative high performance liquid chromatography (HPLC) was conducted on a Shimadzu LC-6A equipped with a UV detector (UV: 230nm) and a reversed-phase column LiChrosorb RP18-5 (150mm length, 2.1mm i.d., GL Science). Successive 3 fractions were collected every 5 min as Fr.1, Fr.2 and Fr.3, respectively.

(c) Bioassays

A cylinder (6cm diam., 4cm height) made of a transparent sheet was placed in the center of a plastic container (250×350×40mm), and 4 small filter papers (2cm diam., 180μm thickness, Whatman Int., England) were displayed on the circumference of the cylinder at equal intervals. The filter papers were treated with 50μl of test samples of *C. japonica* extracts, or one of the HPLC- fractionized sample, and solvent (hexane). All they were impregnated with 50μl distilled water to retain moisture. Only one filter paper was not impregnated with distilled water. 100 termites were taken inside of the cylinder. The cylinder was gently removed after evaporation of the solvents, and the number of termites staying at each filter paper was counted at the following intervals; 1, 3, 5, 10, 15, 20 min. This was repeated 3-6 times with rotating the position of the test samples. Post hoc multiple comparisons were made by using Newman-Keuls test.

(d) Chemical analyses

Gas chromatographic (GC) analyses were conducted on a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector and an apolar capillary column InertCap 1 (15m length, 0.25mm i.d., and 0.25μm film thickness, GL Science). Helium was the carrier gas and the column head pressure was at 98kPa. Injection was made in the split mode at 300°C. The detector was also set at 300°C. The column oven temperature was kept at 60°C for 1 min, programmed to 320°C at 10°C/min, and held at the final temperature for 5 min. Gas chromatograph-mass spectrometer (GC-MS) analyses were performed on a Shimadzu QP-5000 equipped with GC-17A and an apolar capillary column, DB-1HT (15m length, 0.25mm i.d., 0.1μm film thickness, J&W Scientific). Helium was the carrier gas with the column head pressure at 100kPa. Injection was made in the split mode at 250°C. The detector was set at 280°C. The column oven temperature was programmed from 60°C to 300°C at 10°C/min. EI mass spectra was measured at 70eV.

Results and discussion

The number of termites staying at filter papers treated with test samples of hexane extract for 20 min after releasing the termites was shown in Fig.1A. Significantly larger number of termites were kept staying at the filter paper that was treated with *C. japonica* extract. Among three fractions obtained by HPLC, termites significantly preferred Fr.2 (eluted 5-10min from HPLC) to the others, and to the mixture of the three fractions. The gas chromatogram of Fr.2 work as the active fraction was shown in Fig.2C. Several characteristic peaks of which equivalent chain length (ECL) values were equivalent to *n*-alkanes of C20-C24 were observed. Some of these chemicals should have potent attractant and/or arrestant activity to the *R. speratus*. Mixture of three fractions showed weaker attractant and/or arrestant activity. This fact suggests that other two fractions (Fr.1 and Fr.3) contain some other chemicals that suppress attractant and/or arrestant activity of Fr.2 to *R. speratus*. Of Fr.2, the compounds corresponding to C20-C24 provided molecular ions at *m/z* 286. This suggests the compounds would be diterpenoid alcohols, $C_{20}H_{30}O$. So it is suggested this diterpenoid is the attractant and/or arrestant chemical of *R. speratus*.

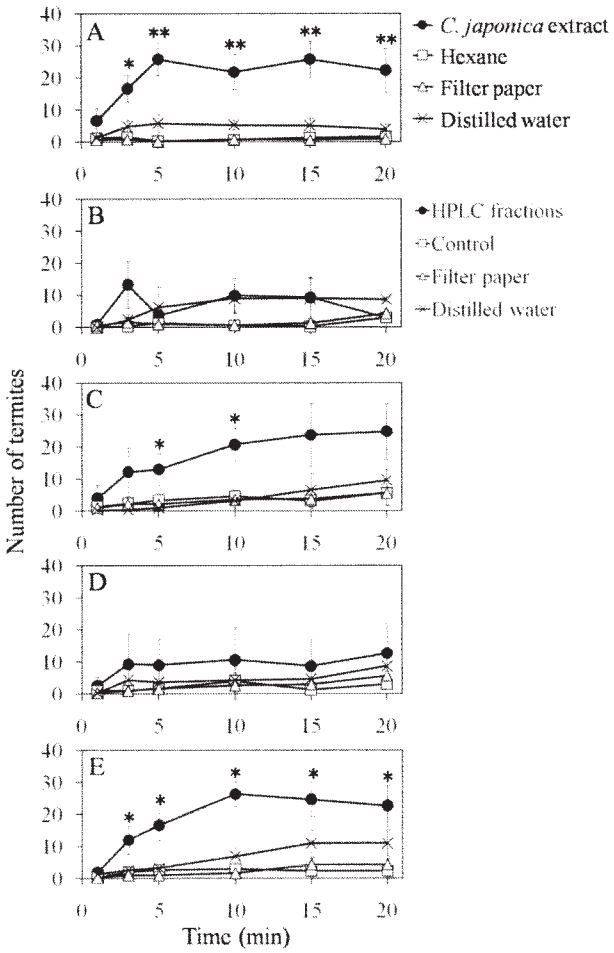

Figure 1. Transition of the number of the termites staying at each test sample for 20 min (A; *C. japonica* extract (n=6), B; Fr.1 (n=3), C; Fr.2 (n=5), D; Fr.3 (n=3) and E; mixture of the three HPLC fractions (n=3)). Asterisks show the numbers of attracted and/or arrested termites are significantly different between the test samples and the others at $P<0.05$ (*) and $P<0.001$ (**) by Newman-Keuls test.

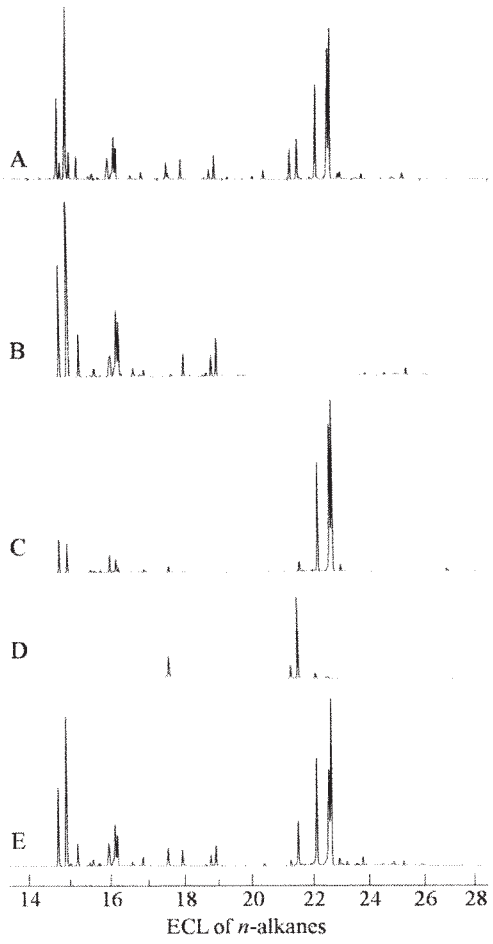

Figure 2. Gas chromatograms of the hexane extract of *C. japonica* (A), three HPLC fractions of *C. japonica* extract (B: Fr.1, C: Fr.2 and D: Fr.3) and mixture of three HPLC fractions (E).

Conclusions

We succeed to separate the mixture of chemicals that have potent attractant and/or arrestant activity from the hexane extract of *C. japonica*. The characteristic mass spectrum suggested the attractant and/or arrestant chemicals should be the diterpenoids. We have to conduct bioassays to confirm those activity is also observed in the field condition. If the attractant and/or arrestant chemicals are also effective in the field bioassays, the chemical can apply to the novel monitoring-and-baiting system also effective to *R. speratus*.

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Learned from "Raisa House of Excellence" on how to Control Termite Attack on Furniture Products

by

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Abstract

Raisa House of Excellence (RHE) produces furniture for export especially to Europe and USA with mainly using mahogany wood. One of quality requirement of wooden furniture products is clean from insect or termite attack, whereas in Jepara *Lyctus brunneus* Stephenns and *Dinoderus minutus* Fabricius are found. To ensure products are free from termite attack RHE did brushing and dipping with dragnet. This paper describes the research conducted by the company to control termite attack for their furniture products.

The research results shown that (1) The effectiveness level of preservative treatment depends on the application method (2) Dipping method is more reliably effective to be applied rather than brushing method (3) Brushed dragnet can be executed for temporary treatment and dipping can be performed to obtain maximum result (4) Lentrek is more effective against termites but it is prohibited because of its poison level.

Key words: Furniture, mahogany, *Lyctus brunneus* Stephenns, and *Dinoderus minutus*

Introduction

Raisa House of Excellence is an Indonesian Furniture Manufacturer based in Jepara, Central Java, Indonesia which is world widely known as the centre of furniture industry. The company has established for 15 years and has been exporting worldwide. Realizing that the market demand of furniture is vary, the company has divided into two (2) divisions with its own specialty to have more market segmented and get to the point to whom requires what kind. The first division RAISA HOUSE OF EXCELLENCE is specializing in French furniture style. The company makes furniture restoration in the style of French, Louis XV, Louis XVI, Louis Philippe, and Chateau. The second division is INTACT ANTIQUE specializing in American furniture style which mainly produces wooden furniture adopted from American style that has been created and developed by the artist and designer since the 14th century. The company brings the beauty of Chippendale style furniture, Victorian style furniture, Edwardian style furniture, Queen Anne style furniture, Adam style furniture, and has been reproducing furniture come from excessively period like Renaissance, Tudor, George I, II and III and many inspiring classic styles of furniture. They are all having their own characteristics on the carvings, ornaments and curvatures. All is made for export quality. The company mainly use mahogany *(Swietenia mahagoni* Jacq.*)*, mindi (*Melia azedarach*), durian (*Durio zebathinus*), and teak (*Tectona grandis*) depend on customer request. The company welcome for any custom made designs, hotel project or restaurant project with the best material. The company commitment is high quality furniture and customer satisfaction.

The Importance of preservatives for the company

The company main concern of business is totally related to the wood and its characteristics. The company is committed to produce high quality furniture and guarantee their customer satisfaction. It means the company is expecting zero complaint up to products they exported worldwide. Products complaints could come from lack of manufacturing process and lack on the material quality. Customer do not tolerate imperfection include termite infection.

And, one of the main issues for most of wooden furniture industry is termite attack. Most countries prevailing policy against termite and conducted law acts for everybody who deliberately or not disseminate products contaminated by termites. One of law firm in Florida provides Representative Lawsuit Verdicts and Settlements for case of Termite Damage Claim against Major Retail Chain at $235,000. (www.floridainjuryaccident.com). In June 1999, the French National Assembly voted for a law on termites after the Adoption by the National Assembly of the modified law "aimed at

protecting buyers and building owners from termites and other xylophagous insects" t May 1999. (www.dowagro.com/sentritech/en/law).

That's why termite issue becoming one of nightmares for wooden furniture industry. Termite control management must be well-performed by wooden furniture industry actors. Otherwise, they will lose their customer and business and facing the law against termite claims.

Inventory policy and its implications

Raisa House of Excellence is one of leading wooden furniture manufacturer and exporter in Jepara supplying customer worldwide for furniture need. The company strategic manufacturing and inventory policy prevailed were driven by market demand which has regularly and certain graphic time path. Market demand on wooden furniture attaining peak season during summer. The production lead time must meet this market demand. By prevailing inventory policy, the company does stock some regularly and mostly ordered items. So that, once the orders coming onto the desk, they will be ready for finishing process.

Shortage in time of processing the orders to container loading initiate company's ability to increase the capacity in a time unit of peak season. Customer will be satisfied by shorten lead time, they do not have to wait for such time to be able to obtain their orders. Reliability and time assurance are fulfilled as instruments to measure service quality which will affect on customer satisfaction. It is explained by Chang-His Yu, Hsiu-Chen Chang & Gow-Liang Huang (2006), describing customer satisfaction fulfilled by service quality has significant relationships to customer loyalty. And added by Heskett et.al (1997) that the relationship is direct and strong between Service Quality, Customer Satisfaction and Profitability.

Above descriptions reflect the positive side view. The operational cost to manage the inventory is the contrary. But, balancing and even pressure of profitability increasing against cost rising could be reach by applying efficiency and effectiveness in whole company's activities.

Problems facing by the company

The inventory merchandises were firstly treated by preservatives before coming into inventory list. Treatment prevailed mainly to prevent bugs and termites. The company used to perform the treatment using lentrek, since it was used by almost all of furniture company in Jepara and empirically proven to prevent and to protect furnitures from the attacks (see www.scorecard.org/chemical-profiles/product.tcl?reg_nr=06271900076 &prodname=LENTREK%206 for product profile). The inventory strategy running well with lentrek treatment until the Environmental Protection Agency prohibit lentrek usage in Industry (www.epa.gov/EPA-PEST/2000/September/Day-20/p24211.htm)

The company is starting an in-house examination by applying some of preservatives trade mark on samples of furniture. Several trademark preservatives were applied and the most suitable and effective is being undertaken for deeper and advance examination. Dragnet is one of the preservative which has suitability for furniture products protection from termite attack.

Materials and methods

The company study regularly the treatability and durability of furniture samples treated using Dragnet against subterranean termite and powder post beetle. The treated and untreated samples were tested against subterranean termite and powder posts beetle for 4 months. The preservative is applied in two different methods on the two different samples to be treated, namely Brushing Method and Dipping Method.

The sample specimens used for the in-house examination is furniture made of Mahogany (*Swietenia Mahogany*) in average EMC 13%. A preservative used is Dragnet 380 EC with active substance permethrin 38, 6% using ratio 1 : 1 to solvent kerosene.

In the end of study, level of attack defined based on criteria belong to Martawijaya and Sumarni (1978), namely:

- 100 = No damage (no termite bite)
- 90 = slightly attack (attack on surface only)
- 70 = moderately attack (attack inside the wood but not extensive)
- 40 = heavy attack (attack inside the wood and extensive)
- 0 = very heavy attack (the wood is destroyed)

Exclusive storage of samples was not specially performed. They were located in the warehouse with well-airflow.

Principles of performing this preservatives examination were adopted from noviantoblog.blogspot.com. In this blog articles are also written the advantages and disadvantages of each method of preservative applications.

Data Analysis

Data analysis was taken in a very simple method and practical way which could be performed in the warehouse at minimum cost. The main objective was finding out the effectiveness of preservative products to be used in company and wooden furniture industry in common. Sampling method was not referring to sampling theory but its availability in the warehouse. Stool was taken as sample for dipping since it does not need big chamber to perform the dipping.

Results and discussion

The results of the experiment using preservative Dragnet can be seen in Table 1. According to the experiment results, it was found that the wood destroyer for furniture made from mahagony wood is Lictus and Dinoderus. This research results shown the same results with research before which stated that wood destroyer organism mostly found in Jepara is Lyctus brunneus Stephenns and Dinoderus minutus Fabricius or traditionally known as *"cocoh"* in Javanese (www.indonetwork.co.id/bintang_agroart /1220761/dragnet-380-ec-obat-pelindung-kayu.htm.

Lyctus brunneus is a species of beetles with a worldwide distribution, being present in tropical Africa, Oceania, the Pale arctic (including Europe), the Nearctic, the Neotropics, North Africa and East Asia. It is absent from the Near East. In Europe, it is found in Austria, the Azores Islands, Belgium, the British Isles, the Canary Islands, Cyprus, the Czech Republic, mainland Danemark, Finland (doubtful), mainland France, Germany, mainland Greece, the Republic of Ireland, mainland Italy, Malta, mainland Norway (doubtful), Poland, mainland Portugal, northern Russia (doubtful), Sardinia, Slovakia, mainland Spain, Sweden (doubtful), Switzerland and the Netherlands (doubtful) (Wikipedia, the free encyclopedia).

An observation on Dinoderus Minutus was performed in Bangladesh by K N Ahmed and C M Zulfiqr (2006) found that *Dinoderus minutus* (Fab.) is an important insect pest of dried bamboo and wooden materials. The pest seriously damages these commodities and makes them unsuitable for use.

Treatment methods executed in this study appear in different result. The untreated sample was attacked by powder beetles within a month only and getting worst infected at the end of study. Sample preserved by Dragnet with brush applicator using ratio 1 : 1, at the fourth month appeared a little infection on the surface. And the dipping method using dragnet showed that the sample was clean from attacks. This is because the Dragnet penetration using brushing method only 0.5 mm in average, while using dipping method for 24 hours the Dragnet penetration is 7.00 mm in average. Research results concerning Lentrek shown that brushing method is very effective to control termite attack. Up to four months, the effectiveness to Dragnet dipping method is the same as Lentrek brushing method. However, Lentrek is not allowed to be used as furniture preservative, the only way to protect the furniture is using Dragnet with dipping method. This research results recommended The Raisa House of Excellence to build new facilities for preservation using dipping method.

Conclusions and suggestions

1. Preservative Dragnet is effective for wooden furniture treatment. However, the effectiveness level depends on the application method.
2. Dipping method is more reliably effective to be applied rather than brushing method. So that, brushing method for dragnet can be executed for temporary treatment.
3. Dipping method for Dragnet can protect the furniture 100% until the end of the test period (4 months), it can be performed to obtain maximum result.
4. Lentrek is more effective against termites but it is prohibited because of its poison level.
5. The company is recommended to build a chamber for dipping facility with big enough capacity. It will be layout next to the kiln-dry for effective process-flow.
6. What to dip should be studied carefully, whether to dip sawn-timber, components or half-finished product (raw).

Table 1. Research Results on Furniture Preservation Using Dragnet

SAMPLING	Nett Weight (KGS)	EMC (%)	Treat-ment.	Preser-vative.	Applica-tionn.	Ratio	Level of Attack (Time unit)			
							28/3/2007	28/4/2007	28/5/2007	28/6/2007
							09.21	09.02	09.08	09.02
SAMPLE 1 (Cheveret)	22	13	No	-	-		-	70 (one area)	40 (three areas)	0 (many areas)
SAMPLE 2 (Writing desk)	48	14	Yes	Dragnet	Brushing	1 : 1	-	-	-	70 (one area)
SAMPLE 3 (Stool)	11	11	Yes	Dragnet	Dipping	1 : 1	-	-	-	-
SAMPLES X (Chair) (*)	18	10	Yes	Lentrek	Brushing	1 : 1	-	-	-	-

* Sample was not intentionally taken for this study.

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Survivorship and Tunneling Activity of *Heterotermes indicola* (Wasmann) in Response to the Soil Treated with Different Insecticides in the Laboratory

by
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Abstract

Heterotermes indicola is economically important urban and agricultural pest of Pakistan. Laboratory behavior of field collected colony of *H.indicola* was examined to determine a) cumulative tunnel distance b) maximum tunnel height and c) number of tunnels after 7 days of treatment. Similarly, survivorship and tunneling activity of *H.indicola* was also studied using four commercially available insecticide formulations: imidacloprid, chlorfenapyr, bifenthrin, and fipronil, each at concentrations of 1.56, 3.125, 6.25, 12.50, 25, 50, 100 ppm. *H. indicola* showed tunneling with chlorfenapyr and imidacloprid at all tested concentrations while in fipronil tunneling was observed at 1.56, 3.125, 6.25, 12.50, 25 ppm and with bifenthrin tunneling occurred in only two tested concentrations i.e. 1.56 and 3.125 ppm. It was also revealed from present study by soil contact toxicity test that at higher concentrations, there was quick termite mortality and limited tunneling.

Key words: Tunneling, Survivorship, *H. indicola,* soil toxicity

Introduction

H. indicola is one of the important termite species widely distributed in different parts of Pakistan. It accounts for significant proportion of damage to agricultural crops, forest plantations; wood works in buildings, paper, cloth, cotton, leather and even plastic goods (Akhtar, 1983). The present study aims to investigate tunneling behavior and survivorship of the worker caste of *Heterotermes indicola* through soil contact toxicity test and using tunneling apparatus.

Materials and methods

Termite Collection

Workers, 3rd instars larvae and soldiers of *H. indicola* were collected by bucket traps placed at different locations of university. Before exposure to insecticide, termites were maintained inside clear plastic containers in the laboratory at 26±2 °C and 80% Relative Humidity (R.H) until used in this experiment. Moistened filter paper and sawdust were provided as food.

Soil Contact Toxicity Test

In a forced-exposure (no-choice) assay, soil was treated with different concentrations of different insecticides. Ten grams of soil was treated with 2 ml of seven different concentrations of each insecticide, mixed thoroughly and left for evaporation. Treated soil was placed in plastic Petri plates (90mm×15mm).1 ml distilled water was added evenly to the soil in order to provide moisture and hundred termite workers were placed in the dish. A strip of paraffin film was stretched around the petri plates to reduce moisture loss. Termite tunneling activity and mortality was recorded after one day and 7th day of treatment. Three replicates were performed with each tested concentration of insecticides.

Tunneling bioassay of subterranean termites

Tunneling bioassays were performed in the laboratory with modifications as adapted by Grace (1991). It

consisted of 2 plastic microscope slides (2.5×7.5 cm) spaced 3-4 mm apart and secured in a horizontal upright position on one long edge by chloroform to a third flat glass slide as a base. The ends of chamber were sealed with plastic spacers and silicon caulking, with a1.5 cm long tygon tube at base of each end of the sandwich leading into the base of one of the two 55 ml polystyrene vials (60×30mm diameter).Each of these vials contain 10 gm untreated soil, 2 ml distilled water and filter paper as food. The treated soil was poured into tunneling arena, and 2 ml distilled water was added by the pipette along the open top edge. This water moistened the soil of the arena. The top edge of the each tunneling arena was sealed with transparent plastic tape in order to reduce evaporation.100 termites were released in one of the two vials. The vials were capped and the caps were pierced air holes by heated insect pin (Fig 1). Each concentration

of insecticide and control were replicated three times. All experimental units were kept in incubator in 26±2°C. Test setups were examined at 24 hour intervals for 7 days.

At each examination, the number of tunnels, the length of tunnels, and the number of surviving and affected individuals were measured and counted.

The data were analyzed using Mean, Standard error, one –way ANOVA Test using Graph pad Prism Version 4.00 for Windows,(www.graphpad.com). Results with $P<0.05$ were considered statistically significant.

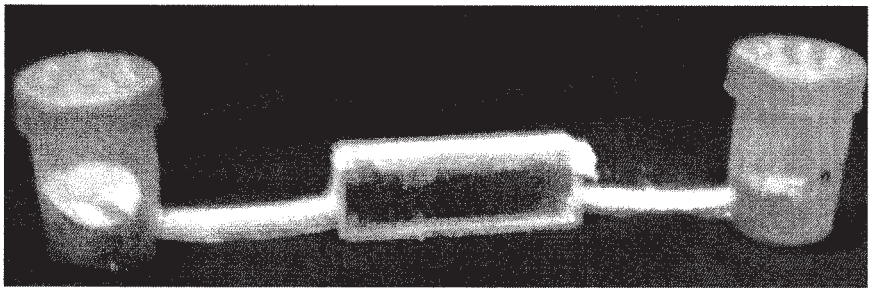

Fig 1: Tunneling test apparatus

Results and discussion

As discussed in materials and methods, tunneling behavior of termites was tested for cumulative tunnel distance, maximum tunnel height and number of tunnels for day 1 and day 7th of treatment (table 1).The tunneling behavior of subterranean termite *H. indicola* observed during the experiment and termites were found to travel along the sides of test chamber and they could begin tunneling at any point along the circumference of the entrance hole of the test apparatus.

Considering the control termites constructed more tunnels directed upward along the both walls of the chamber. In view of the fact that termites showed an overall tendency to spread out horizontal and upward direction more than they did in down ward direction. Becker, (1981, 1989) also concluded that in *H. indicola* the direction of tunnel building is influenced by magnetic and electric field even though in present study we did not account for such factors but further investigation is needed for studying the influence of geomagnetic and electrical fields on termite tunneling behavior.

When *H. indicola* was tested for soil contact toxicity against imidacloprid, chlorfenapyr at seven different concentrations 1.562, 3.125, 6.25, 12.5, 25, 50, 100 ppm and control, it was observed that there was minor tunneling activity at higher concentrations and active tunneling at lower concentrations after day 1 of treatment. Mean percentage mortality and tunneling activity also increased after 7th day of treatment. When treated with fipronil, there was significant mortality and no tunneling at 100 and 50 ppm even after 7th day of treatment. Percent response of *H. indicola* to different insecticides by contact toxicity is shown in table 3. In these results highest %age mortality (84.7±2.603) was observed for bifenthrin at 100ppm and at the same ppm the lowest %age mortality (6.0±0.577) was for imidacloprid after one day of treatment. Bifenthrin is non-repellent insecticide so there was no tunneling activity at 100, 50, 25, 12.5 and 6.25 ppm against *H. indicola*. Reduced tunneling and significant mortality was observed at 3.125 and 1.562 ppm after day 1 and 7 of treatment. There was extensive tunneling observed in control set (Table 2).

For the last many years, researchers have been testing the repellent and non-repellent termiticides (Hu, *et .al,* 2005; Su, 2005). Various studies have been carried out to test the efficacy of different insecticides on different termite species in different parts of the world. Mauldin (1986) in his ground board method evaluated six insecticides to study termite control. Regarding chlorpyrifos, Su, *et. al.,* (1982) reported that both species *Coptotermes heimi* and *Micromeres obesi* do not penetrate Permethrin treated soil. Rummen and Su (2005) also proved the efficacy of fipronil and found that 2 ppm of fipronil can fully stop the penetration of *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* Kollar in treated barrier layer. In present study, for all the tested insecticides extensive tunneling and maximum foraging was observed in chlorfenapyr and imidacloprid and tunneling was seen all around the container, at edges and in bottom also.

Our results also indicate that *H. indicola* can tunnel slightly into soil treated with high concentration and these results agree with the previous research carried by Tamashiro *et.al.,* (1987) and Johns(1989) showed that in soil treated with chlorfenapyr at 500ppm, no tunneling occurred.

When fipronil was tested for tunneling behavior, it was revealed that termites showed extensive tunneling at concentrations 25, 12.5, 3.125 and 1.562 ppm but no tunneling was observed at higher concentrations i.e. 50 and 100 ppm. So it was observed that fipronil has non-repellent properties and is slow acting insecticide and showed limited tunneling at higher concentrations i.e. 50 and 100 ppm. So we conclude from present study that at higher concentrations, there was quick termite mortality and limited tunneling. biflex showed tunneling at .562 ppm and 3.125 ppm. Biflex inhibited tunneling at other concentrations 100, 50, 25, 12.5 and 6.25 ppm. Biflex is also repellent at all these concentrations 100, 50, 25, 12.5 and 6.25 ppm and non-repellent to lowest concentrations 3.125 and 1.562 ppm and limited tunneling activity was observed. Although our findings are limited as study was carried out in a small chambered apparatus and may be changed under different experimental conditions such as temperature, moisture, concentration and experimental duration.

Despite, the economic importance of *H.indicola* there is little information available on its life history and sociobiology. This lack of understanding may be due to their cryptic life style and social organization behavior of termite that make them difficult to study. Therefore, most studies are required for examining the social structure and foraging activity of subterranean termite colonies in Pakistan.

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Table 1: Tunneling behavior of *H. indicola* (Mean ± SE) after exposure to imidacloprid, chlorfenapyr, fipronil and bifenthrin treated soil (after day 1 and day 7 treatments).

Conc. (ppm)	Imidacloprid						chlorfenapyr					
	Cumulative tunnel distance (mm)		Maximum tunnel height (mm)		No. of tunnels		Cumulative tunnel distance (mm)		Maximum tunnel height		No. of tunnels	
	1 day after	7 days after	1 day after	7 days after	1 day after	7 days after	1 day after	7 days after	1 day after	7 days after	1 day after	7 days after
Control	232.6±0.406	282.5±0.491	61.53±0.376	82.6±0.491	11.0±0.577	17.7±0.333	234.4±0.612	381.2±0.608	62.5±0.635	81.8±0.240	12.3±0.333	18.0±0.577
1.562	225.6±0.524	374.6±0.406	60.27±0.617	80.3±0.208	10.0±0.577	17.0±0.577	222.9±0.929	370.2±0.639	63.2±0.491	80.3±0.393	13.0±0.577	16.7±0.333
3.125	209.4±0.433	369.3±0.569	58.83±0.581	76.7±0.491	9.0±0.577	15.7±0.333	206.3±0.617	364.4±0.578	57.5±0.694	76.2±0.643	8.0±0.577	16.0±0.577
6.25	180.7±0.436	338.7±0.463	55.60±0.462	75.5±0.433	7.0±0.577	15.0±0.577	177.0±1.012	334.3±0.593	54.5±0.784	74.4±0.674	6.0±0.577	16.0±0.577
12.50	154.8±0.520	302.3±0.754	47.47 ±0.491	70.8±0.467	6.0±0.577	13.0±0.577	151.9±0.874	299.4±0.406	46.8±0.318	70.3±0.520	5.7±0.333	15.0±0.577
25	140.7±0.410	257.6±0.463	44.57±0.491	65.5±0.406	4.3±0.333	12.0±0.577	136.4±0.639	253.4±0.722	42.5±0.578	64.4±0.578	4.7±0.333	13.0±0.577
50	120.8±0.657	239.4±0.404	36.80±0.551	61.6±0.436	3.3±0.333	8.0±0.577	116.5±0.593	232.5±0.561	36.4±0.513	61.4±0.657	3.7±0.333	12.0±0.577
100	103.3±0.433	208.3±0.462	33.63±0.491	53.6±0.462	2.3±0.333	5.0±0.577	99.3±0.382	202.2±0.635	33.6±0.448	51.5±0.351	2.7±0.333	8.0±0.577
Conc. (ppm)	Fipronil						Bifenthrin					
	Cumulative tunnel distance (mm)		Maximum tunnel height		No. of tunnels		Cumulative tunnel distance (mm)		Maximum tunnel height		No. of tunnels	
	1 day after	7 days after	1 day after	7 days after	1 day after	7 days after	1 day after	7 days after	1 day after	7 days after	1 day after	7 days after
Control	224.4±0.612	385.3±0.491	53.7±0.754	76.4±0.289	6.7±0.333	18.0±0.577	254.4±0.612	381.2±0.608	62.5±0.635	81.8±0.240	13.0±0.577	18.0±0.577
1.562	247.8±0.551	279.2±0.555	45.3±0.339	76.1±0.176	8.0±0.577	14.0±0.577	246.0±0.252	260.9±0.939	45.3±0.953	49.8±0.581	8.0±0.577	14.0±0.577
3.125	200.4±0.582	260.3±0.550	42.2±0.177	76.1±0.376	6.3±0.333	10.0±0.577	200.9±0.953	214.6±0.581	39.7±0.186	41.5±0.296	4.7±0.333	8.0±0.577
6.25	169.4±0.625	243.4±0.549	40.4±0.122	63.4±0.639	5.7±0.333	8.3±0.882	-	-	-	-	-	-
12.50	167.6±0.521	238.6±0.561	39.2±0.385	61.5±0.635	4.3±0.333	7.0±0.577	-	-	-	-	-	-
25	165.6±0.578	214.5±0.498	36.0±0.291	57.6±0.669	3.3±0.667	4.7±0.333	-	-	-	-	-	-

Table 2: Contact toxicity (Mean±S.E.) of soil treated with imidacloprid, chlorfenapyr, fipronil and bifenthrin to *H. indicola* workers in a forced-exposure assay

	Conc. (ppm)	Day 1		Day 7	
		Mortality (%)	Tunneling activity	Mortality (%)	Tunneling activity
Imidacloprid	100	6.0±0.577	Minor - throughout dish	54.0±2.082	Active-throughout dish
	50	4.3±0.333	Minor - throughout dish	44.7±2.028	Active-throughout dish
	25	3.0±0.577	Minor - throughout dish	29.3±3.283	Active-throughout dish
	12.5	1.7±0.333	Minor - throughout dish	26.7±2.404	Active-throughout dish
	6.25	1.3±0.333	Active - throughout dish	13.7±3.180	Very active-throughout dish
	3.125	0.7±0.333	Active - throughout dish	5.3±0.333	Very active-throughout dish
	1.562	0.3±.333	Active - throughout dish	2.3±0.333	Very active-throughout dish
	Control	0.0±0.000	Active - throughout dish	1.0±0.000	Very active-throughout dish
Chlorfenapyr	100	9.0±0.577	Minor - throughout dish	65.0±2.887	Active-throughout dish
	50	6.0±0.577	Minor - throughout dish	45.0±2.887	Active-throughout dish
	25	3.3±0.333	Minor - throughout dish	31.7±4.055	Active-throughout dish
	12.5	1.7±0.333	Minor - throughout dish	26.0±2.309	Active-throughout dish
	6.25	1.3±0.333	Active - throughout dish	14.7±2.906	Very active-throughout dish
	3.125	0.7±0.333	Active - throughout dish	6.7±0.882	Very active-throughout dish
	1.562	0.3±0.333	Active - throughout dish	3.0±0.577	Very active-throughout dish
	Control	0.0±0.000	Active - throughout dish	1.3±0.333	Very active-throughout dish
Fipronil	100	67.4±3.180	None	100.0±0.0	-
	50	55.7±1.201	None	100.0±0.0	-
	25	40.7±3.180	Minor-at edge of apparatus	62.7±1.453	Active-throughout dish
	12.5	26.7±3.283	Minor - at edge of apparatus	42.0±1.155	Active-throughout dish
	6.25	16.3±1.764	Minor-at edge of apparatus	32.0±1.528	Active-throughout dish
	3.125	7.3±0.882	Active - throughout dish	22.7±1.76	Very active-throughout dish
	1.562	2.3±0.882	Active - throughout dish	14.3±2.333	Very active-throughout dish
	Control	0.0±0.000	Active - throughout dish	2.0±0.577	Very active-throughout dish
Bifenthrin	100	84.7±2.603	None	100.0±0.0	-
	50	75.0±3.464	None	100.0±0.0	-
	25	55.0±2.887	None	100.0±0.0	-
	12.5	38.3±2.848	None	100.0±0.0	-
	6.25	30.0±3.464	None	100.0±0.0	-
	3.125	19.3±2.333	Minor- At edge of apparatus	63.0±4.041	Active-throughout dish
	1.562	6.7±1.764	Minor - At edge of apparatus	31.3±3.757	Active-throughout dish
	Control	0.0±0.00	Active - throughout dish	4.7±1.202	Very active-throughout dish

- No tunneling activity

Xterm™ (Bistrifluron) Solid Bait Pellets Eliminate *Coptotermes* Colonies Rapidly

by

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Abstract

The time to eliminate *Coptotermes acinaciformis* colonies was measured using the chitin synthesis inhibitor bistrifluron in cellulose bait pellets (tradename Xterm™ Defence Against Termites). Two concentrations of bistrifluron were used: 1.0%, 0.5%, plus 0% (untreated control). Both were effective; 83% of colonies (10 of 12) were either eliminated (termites completely absent from nests) or moribund (reproductives absent and decreased workforce) after eight weeks, compared with none of the control colonies. The remaining treated colonies were in decline. The earliest signs that bistrifluron was affecting the colonies included: three weeks after baiting mound temperatures showed a loss of metabolic heat, and four weeks after baiting foraging activity in feeding stations was reduced or absent, with dissection of two mounds at four weeks showed they were moribund. Colony elimination was achieved in half or less the time, and with less bait toxicant, than other bait products tested under similar conditions in the field, due either to the active ingredient, the high surface area of the pellets, or a combination of both. Thus the sometimes long times reported for control using baits may be reduced significantly.

Key words: Baiting, chitin synthesis inhibitor, nest temperature, peritrophic membrane

Introduction

Baiting offers two benefits compared with other termite management methods: low environmental impact and colony elimination. The low environmental impact comes from the small amounts of toxicant that are relatively toxic to insects but not to mammals; toxicants are contained in a cellulose food of interest only to termites within an impervious bait station (Su 1994; Su et al. 1995; Forschler & Ryder 1996). Baiting aims to eliminate colonies; i.e. 100% colony mortality, by spreading slow acting toxicants by the foragers to the nest. These are desirable benefits; yet baiting has one drawback compared with other termite management options: it is slow. "The biggest complaint, common to all the current baiting systems, is that it is slow, time-consuming and tedious" (Potter 1999); an unchanged attitude a decade later (Anon. 2008). Published field studies of termite baiting using five toxicants report control times of up to half a year or more: 12-23 weeks in Australia (Peters & Fitzgerald 1999, 2003), 15-39 weeks in Asia (Tsunoda et al. 1998; Su & Hsu 2003; Wang et al. 2007), 10-43 weeks in the USA (Su 1994; Forschler & Ryder 1996; Su et al 2000, 2002; Rojas & Morales-Ramos 2003), and 17-64 weeks in Latin America (Su et al 2000; Ripa et al. 2007). Of course variation in local species and conditions will influence control times; regardless baiting control times are long compared with those for soil insecticides. Perhaps faster acting bait products will reduce the biggest criticism of bait systems.

This study aimed to test the efficacy of bistrifluron in cellulose bait pellets (tradename Xterm™ Defence Against Termites developed by Sumitomo Chemical) using the mound-building form of the subterranean termite *Coptotermes acinaciformis* (Froggatt) in tropical northern Australia. Bait pellets have a much greater surface area compared with other matrices, which increases bait consumption (Evans & Gleeson 2006). The study aimed to use standard monitoring methods (present and condition of termites in bait and monitoring stations) plus effects on the colony and mound (presence of reproductives, eggs and larvae, building activity, and mound temperature) to identify more precisely the level and timing of the effect of the bait toxicant on the termite colony.

Materials and methods

Installation. The field site was in wet-dry tropical northern Australia (12°23.S 131°10.E) ca. 50 kilometres north east of Darwin. *Coptotermes acinaciformis* is the most important economic pest species

in Australia; the mound it builds has an outer clay wall, a middle layer of partially digested wood called 'carton material', and an inner 'nest' of royal chambers (occupied by the queen and king) and nursery (occupied by eggs, larvae and any moulting individual).

On the 26th June 2007, 16 healthy *C. acinaciformis* colonies (no structural damage) were selected. Four feeding stations (11 L drums with ~3 kg of wood) were installed 1 m from each mound; n.b. large stations attract more termites and are less affected by inspection disturbance (Evans & Gleeson 2006). Feeding stations were linked to mounds for quick discovery via a wood filled trench (following Evans et al. 1999; Evans 2001). The temperature in the termite mounds was monitored to track colony health; populous colonies maintain warm temperatures in their mounds (Holdaway & Gay 1948; Korb & Linsenmair 1998), unlike low population colonies (Greaves 1964). Probes from HOBO® data loggers (Onset Computer Corporation, Bourne, MA) were placed 300 mm into the mound. The openings in the clay wall of the mounds (200 x 200 mm) made for the probes were repaired the within 24 hours, showing colony health (Evans 2006). Twelve weeks later (21st August) the bait pellets were placed. Colonies were allocated to treatments randomly (four controls, six 0.5% Bistrifluron, and six 1.0% Bistrifluron). Bait pellets (total of 800g wet weight, 766.4 g dry weight) were placed into two feeding stations.

Inspections. The first inspection was four weeks later (18th September). All feeding stations were inspected, and termite activity and health (walking speed, colour) were noted. Colony health was tested with a mound-damage-and-repair manipulation: the clay walls of the mounds were damaged by creating an opening similar to that made for the data logger installation. The broken pieces of clay wall were replaced into the opening to allow for easier repair and examined one day later. There were three categories of repair. A full repair had complete re-building of the clay wall so that no broken clay wall pieces were visible. A clay wall seal was less complete; new clay was placed between the broken clay pieces, which were still visible. A carton material seal had minimal sealing of the carton material, there as no building with clay; the broken clay wall pieces were visible and movable. Two mounds fed 0.5% Bistrifluron bait were dissected.

The second inspection was another weeks later (16th October). Inspections followed the four week inspection. All remaining bait was collected and dry weighed to determine bait removal. All mounds were dissected and colony status assessed in four categories. (1) Healthy = normal colony with reproductives, larvae, many workers, no fungus or other termite species. (2) Declining = colony with reproductive capacity in some form (actual reproductives or the capacity to generate replacement reproductives), fewer and less healthy termites, fungus and other termite species could be present. (3) Moribund (near death) = colony without reproductive capacity and few, sick termites, fungus and other termite species present. (4) Eliminated = no live termites in colony. Note that workers and soldiers are sterile in *Coptotermes* spp., and only nymphs and alates can form secondary reproductives (Lenz & Barrett 1982; Costas-Leonardo et al. 2004). The HOBO® data loggers were retrieved and temperature data were analysed.

Data recorded from feeding stations (feeding stations with *Coptotermes*, with protective mudding built by *Coptotermes*, and with other termites species) were analysed using Kruskal-Wallis tests (due to the low maxima, 4 per colony). The dry weight of bait removed was analysed by ANOVA and t-tests. Mound repair data were analysed with chi-squared tests.

Results and discussion

Observations on feeding stations. Observations on feedings stations during the entire experiment are shown in Table 1. The feeding stations around control colonies almost always contained *C. acinaciformis*, with protective mudding, and never contained other species. This was observed for both bistrifluron baits at the time of bait placement, but this changed over time. At the first inspection, *Coptotermes* were observed often in stations, but fewer and sickly (walked slowly and white with uric acid build up), protective mudding was found in less than half the stations, and other species were found, especially in 0.5% Bistrifluron treated mounds. At the second inspection, *Coptotermes* were not observed in stations for 0.5% Bistrifluron treated mounds and less often in 1.0% mounds, but very few and sickly. The protective mudding was not found in stations with 0.5% Bistrifluron bait, and less than one

station for 1.0% bait. Other species were found more frequently, especially for 0.5% Bistrifluron stations.

Table 1. Observations on *Coptotermes acinaciformis* mound-colonies baited with Bistrifluron. Numbers are the average ± standard error of feeding stations per colony for each treatment (maximum of four). nb two colonies from the 0.5% treatment were dissected after the first inspection and were not available for the second inspection Superscript letters indicate significant differences along rows.

Activity Date	Feeding stations with	Control	0.5% Bistrifluron	1.0% Bistrifluron
Bait placement	*Coptotermes*	3.8 ± 0.2^{a}	4.0 ± 0.0^{a}	3.8 ± 0.2^{a}
21st August 2008	Protective mud	3.5 ± 0.3^{a}	3.5 ± 0.2^{a}	3.7 ± 0.2^{a}
	Other sp.	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
First inspection	*Coptotermes*	4.0 ± 0.0^{a}	3.8 ± 0.2^{a}	3.8 ± 0.2^{a}
18th September 2008	Protective mud	4.0 ± 0.0^{a}	1.7 ± 0.6^{b}	1.8 ± 0.6^{b}
	Other sp.	0.0 ± 0.0^{a}	0.8 ± 0.5^{a}	0.2 ± 0.2^{a}
Second inspection	*Coptotermes*	4.0 ± 0.0^{a}	0.0 ± 0.0^{b}	1.5 ± 0.8^{b}
16th October 2008	Protective mud	4.0 ± 0.0^{a}	0.0 ± 0.0^{b}	0.7 ± 0.4^{b}
	Other sp.	0.0 ± 0.0^{a}	1.8 ± 0.8^{b}	$1.3 \pm 0.7^{a,b}$

First inspection. At the first inspection one colony fed 0.5% bistrifluron bait appeared to be very unhealthy. The four feeding stations had no *Coptotermes* termites, no protective mudding, and three were occupied by other termite species. A strong smell of decay was obvious through small hole dug in the clay wall of the mound. This mound was dissected and was categorised as moribund; no queen, nymphs, eggs or larvae, few unhealthy workers, many rotting cadavers, neither royal chamber nor nursery found. Two other species were feeding on the carton material, along with patches of fungus. These other species in the mound demonstrated the inability the *Coptotermes* colony to defend itself, indicating it was almost eliminated.

The mound damage and repair manipulation showed stark differences between colonies in the different treatments. Of the colonies fed control bait, all (4 of 4) built full repairs, of those fed 0.5% bistrifluron bait, 2 build clay wall seals and 3 built carton material seals, and of those fed 1.0% bistrifluron bait, 4 build clay wall seals and 2 built carton material seals; a significant difference ($\chi^2_4 = 16.067$, $p = 0.003$). A second mound-colony (also fed 0.5% bistrifluron) with the poorest repair and with the fewest and least healthy *Coptotermes* in the feeding stations was dissected. It was categorised as moribund, with observations similar for the first dissected mound.

Second inspection. At the second inspection all mounds were dissected. The mounds of all four colonies fed control bait were assessed as healthy. They had full reproductive capacity (queens, many eggs and larvae), many healthy live and no dead termites. The carton material inside the mounds was dry to touch, contained royal chambers and nurseries, and no fungus.

The 4 remaining colonies treated with 0.5% bistrifluron baits and 3 of the 6 colonies treated with 1.0% bistrifluron baits were assessed as eliminated. No live *Coptotermes* were found, only decaying cadavers. The carton material inside the mounds was moist and had wet patches. No royal chambers were found and only one mound had an identifiable nursery, but this was filled with decaying cadavers. All mounds had other species of termites (up to 3) and fungus in the carton material. One colony fed 1.0% bistrifluron bait was assessed as moribund, with observations as for the two mounds dissected after four weeks. Two colonies fed 1.0% bistrifluron bait were assessed as declining. The bases of the mounds with the royal chambers and nurseries were wet and filled with decaying cadavers and fungus (as found for eliminated and moribund colonies). The queens and few sickly workers had relocated from the now uninhabitable base of the mounds to the upper parts of the mound; small numbers of eggs had been laid.

Termites had removed an average of 727.4 ± 16.0g (dry weight) of untreated control bait, 43.8 ± 10.6 g of the 0.5% bistrifluron bait and 23.3 ± 1.8 g of the 1.0% bistrifluron bait. These differences were significant ($F_{2, 13} = 718.02$, $p < 0.001$); due entirely to control versus both treated (Bonferonni corrected $p < 0.001$) as there was no significant difference between 0.5% and 1.0% bistrifluron baits (Bonferonni corrected $p > 0.05$). The amount of the active ingredient bistrifluron in the removed bait

was 218.8 ± 52.9 mg in the 0.5% treatment, and 233.2 ± 18.2 mg in the 1.0% treatment, which was not significantly different ($t_{10} = 0.183$, $p = 0.859$). The amount of the active ingredient in the removed bait from eliminated colonies was 179.9 ± 28.3 mg, and from moribund colonies was 281.3 ± 139.9 mg, which was not significantly different ($t_7 = 0.634$, $p = 0.546$).

Temperature data. Four data loggers failed during the experiment: one in a control baited mound and three in 1.0% bistrifluron baited mounds. The daily 3 am temperatures were chosen for comparison as these were typically the coolest temperatures recorded, when metabolic heat from termites was most essential to maintain mound temperature. Data from four weeks prior to bait placement (24th July – 20th August) was included for comparison with the eight weeks of post baiting temperature data (21st August – 16th October). There were four broad patterns (Figure 1):

(1) Air temperatures increased over the experiment due to seasonal change.

(2) During the pre-baiting period, mound temperatures in all mounds were the same. Metabolic heating from the termites raised mound temperatures about 10°C.

(3) Three to five weeks after bait placement the temperatures in the bistrifluron baited mounds began to drop whereas those of control mounds do not, likely due to the loss of metabolic heat.

(4) Seven weeks plus after bait placement the temperatures in bistrifluron treated mounds is roughly the same as for air temperature, with control mounds maintaining a temperature around 4°C higher.

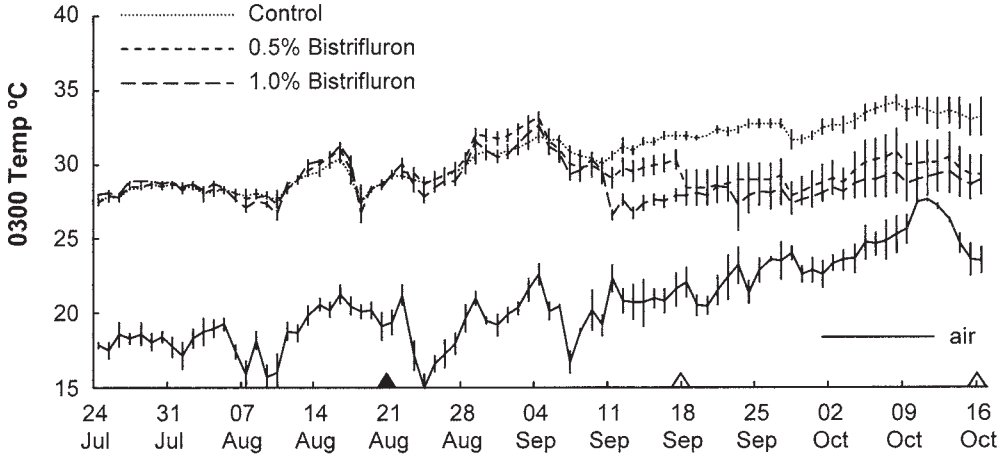

Figure 1. The mean (± standard error) 3 am temperatures recorded in the bistrifluron baiting trial against *Coptotermes acinaciformis* near Darwin, Australia. The solid black triangle (21st August) indicates bait placement, the open triangles (18th September and 16th October) indicate first and second inspections.

The results from this study show that the bistrifluron bait was effective at eliminating *Coptotermes acinaciformis* colonies rapidly. In the eight weeks of the baiting period of this study, 7 of the 12 colonies were eliminated, and 3 were moribund (without reproductive capacity and so a short time from elimination), an 83% success rate. The remaining two colonies were declining, the colony population had been reduced and the nest in the mound had been abandoned, yet the colonies did retain reproductive capacity. It is not possible to predict whether these two colonies would have survived or succumbed (a real possibility as the lower mounds were filled with rotting cadavers and entomopathogenic fungi) had the experiment continued for a longer period.

All 6 colonies fed 0.5% bistrifluron bait, and 4 of the 6 colonies fed 1.0% bait succumbed. This may be due to reduced palatability: the average amount of 0.5% bait removed was double the amount of the 1.0% bait; as found by Kubota et al. (2006) for *C. formosanus*. Alternatively, colonies may have varied in size of vigour that was not apparent during installation. E.g. two colonies fed 0.5% bistrifluron bait: moribund colony #10 removed the most bait (~112 g containing 560 mg AI), whereas eliminated colony #2 removed the least bait (~25 g containing 127 mg AI).

The mound dissections demonstrated that the bistrifluron bait can eliminate colonies in eight weeks from bait placement. The temperature data suggested that morbidity or elimination occurred earlier; three to five weeks from bait placement. The exact time of elimination is difficult to determine, due to the variation in the temperature data and variation in the effect of the bistrifluron. It is clear moribund status was achieved three to four weeks after placement, using both temperature data and the four week inspections.

This is rapid compared with other bait toxicants. It is difficult to compare all previous baiting studies because these aimed to demonstrate that elimination was possible, whereas the present study aimed to determine the time to elimination. The highly variable time to control reported in previous studies (from 10 to 64 weeks inferred from absent of termites in bait stations, see references in introduction) would depend on toxicants, concentrations, termite species, colony sizes, environment, geography, season, and so forth. Yet comparison is possible as mound-building *C. acinaciformis* have been baited in tropical Australia using similar methods with two other active ingredients (Table 2). The rate of colony control (eliminated and moribund) was similar for chlorfluazuron and bistrifluron, which were both higher than hexaflumuron. Time to control was two to three times longer for chlorfluazuron and three to four times longer for hexaflumuron relative to bistrifluron. The amount of active ingredient was about 25% more for chlorfluazuron and three times more for hexaflumuron.

Table 2. Observations on *Coptotermes acinaciformis* mound-colonies baited with three toxicants in tropical Australia. Controlled = eliminate + moribund.

	Hexaflumuron	Chlorfluazuron	Bistrifluron
Source	Peters & Fitzgerald 1999	Peters & Fitzgerald 2003	current study
# colonies controlled /baited	10 / 16	11 / 13	10 / 12
% controlled	62.5	84.6	83.3
Time to control (weeks)	23	12-17	3-8
Bait AI removed (mg)	670	220	180

There are two possible reasons why the bistrifluron bait acted more rapidly than chlorfluazuron and hexaflumuron: (1) greater surface area of the pellets and (2) greater toxicity of bistrifluron. Termites explored all over the bait pellets as shown by chewing marks and faecal spotting, plus almost all control pellets (~700 g) were eaten in 4 weeks confirming the relationship between surface area and consumption (Evans & Gleeson 2006). Unfortunately the effect of surface area and toxicity cannot be separated using these studies as the hexaflumuron and chlorfluazuron baits had low surface area.

The toxicity of these chitin synthesis inhibitors may differ because of two modes of action. All CSIs work by disrupting α-chitin synthesis during moulting, yet this mode of action is probably too slow to explain rapid elimination as it is unlikely all ca. one million individuals in a colony (Evans et al. 1999) moulted during the same month. Disruption of γ-chitin synthesis for the peritrophic membrane in the midgut is likely to cause death faster than disruption of moulting because this will interrupt digestion and lead to starvation.

The current study using the mound building *C. acinaciformis* allowed the measurement of colony level effects, such as loss of reproductive capacity, loss of breeding sites, loss of temperature control in the nest, and the invasion of other termites and fungi. Such colony level effects are difficult to demonstrate using termites without obvious nests. The results from the current study showed that metrics from feeding stations were correlated to colony level effects; however final elimination of the colony lagged the disappearance of termites in the feeding stations. Therefore the time to actual elimination of termite colonies in previous studies is probably longer than those times reported.

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Bio-Efficacy of a Termite Powder against Philippine Subterranean Termites

by

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Abstract

The efficacy of a termite powder was evaluated against four economically important Philippine species of subterranean termites namely: milk termites (*Coptotermes vastator* Light)), Los Baños termites (*Microcerotermes losbañosensis* Oshima), Luzon point headed termites (*Nasutitermes luzonicus* Oshima) and mound building termites (*Macrotermes gilvus* Hagen). Incised tunnels and infested wood with active termite population served as loading sites for the application of the termite powder using a micropipettor.

Termite powder with 0.5% fipronil was effective against Philippine subterranean termites. Elimination period was very fast from 1 (*N. luzonicus*) to 7 or 8 weeks (*M. gilvus*) after application with minimal volume of termite powder. The amount required to eliminate termite infestation depends on termite species, size of colony and degree of infestation in houses and buildings. Complete elimination of termite populations was manifested by inactive population, dry and collapsed tunnels and nests, no reconstruction of injection or termite powder loadings sites and presence of ants.

Thorough inspection, identification of termite species and regular follow-up monitoring are the major factors for the success of termite powder application to eliminate termite infestations. As long as it is properly applied, this will provide an alternative control measure to eliminate termite infestations.

Key words: termite powder, fipronil, Philippine subterranean termites, non-repellent termiticide, *Coptotermes vastator, Microcerotermes losbañosensis, Macrotermes gilvus, Nasutitermes luzonicus*

Introduction

For several decades, termite control is dependent on the application of synthetic pesticides as soil treatment (Garcia 1972; Ibrahim 2003; Garcia and Giron 2004; Tsunoda and Yoshimura 2008) and is still currently used by our local pest control applicators. Organo-chlorine compounds which include chlordane, aldrex, aldrin, dieldrin heptachlor, DDT, mirex, dioxins, furans, hexachlorobenzene, PCB's (Garcia 1972; Reyes and Garcia 2003; Garcia and Giron 2004) were the first group of termiticides used in the 1950's to early 1990's. These compounds were replaced by organophosphates like chlorpyriphos and pyrethroids like deltamethrin, cypermethrin, fenvalerate, bifenthrin in different brand names which are now commercially available in the market. The efficacy of these chemicals applied as soil barrier is from 3 to 5 years and cannot compare with the more persistent organo-chlorine type of termiticide.

In the early 2000, a new concept in termite control, the termite baiting system using hexaflumuron or chlorfluazuron as insect growth regulator (IGR) was introduced in the country to eliminate subterranean termites in houses and buildings (Garcia and Giron 2004, 2007). However, the major drawbacks of this method include variability of response of termite species to bait, preparation or handling of bait during application efficiency, long period of termite elimination and its high cost compared to conventional chemical treatment.

Research and development continue to evolve and one of the potential approaches is focused on the non-repellency action of some newly introduced termiticides like fipronil which has been proven very effective against *Coptotermes formosanus* (Tsunoda and Yokoyama 2005; Yeoh and Lee 2006; Tsunoda 2007; Tsunoda and Yamaoka 2007).

In this study, the bio-efficacy of a new termite powder containing 0.5% fipronil was evaluated against destructive species of Philippine subterranean termites of wooden components of houses and buildings.

Materials and methods

Test Insects

The efficacy of a termite powder was evaluated against four economically important species of Philippine subterranean termites: 1) Los Baños termites, *M. losbañosensis* Oshima; 2) mound building termites, *M. gilvus* Hagen; 3) Luzon-point headed termites, *N. luzonicus* Oshima; and 4) milk termites, *C. vastator* Light. Nests of *M. losbañosensis* and *N. luzonicus* containing active termite population were collected and individually set-up in termite chamber. Each nest was set-up at the center of a half-sawn 200 li capacity plastic drum provided with soil. Wood interceptors of 2.5 cm x 5.0 cm x 30 cm Moluccan sau, *Paraserianthes falcataria* (L.) Fosb. and coco-wood chamber support served as food source for the termite population to sustain the establishment and construction of earthen tunnels. On the other hand, mounds of *M. gilvus* were selected in the field while houses infested with active population of *C. vastator* were used as experimental units. The degree of wood damage, level of termite population and conditions of termite tunnels and nests were inspected, observed and marked prior to application of the termite powder.

Test Formulation and Application

The test compound is a powder formulation containing 5g fipronil per kg of powder as a carrier. The termite powder was dispensed into active termite population in incised tunnels or infested wood or mound using an Oxford® BenchMate™ pipettor. The powder was gently sucked by a 5-ml plastic pipette tip up to the marked level with pre-determined volume of powder. The pipette tip was inserted to the incised tunnels or pointed directly to termite population in infested wood and the plunger was slowly depressed to dispense the powder. The volume and frequency of application in each experimental unit were recorded.

Evaluation of the Efficacy of a Termite Powder

The loading sites of the termite powder (termite tunnels and infested wood with active termite population) were monitored at regular weekly interval for 4 weeks and monthly thereafter for 3 months. The reconstruction of loading sites, abundance of termite population, signs of termite elimination, volume of powder applied and elimination period were used as the basis in evaluating the efficacy of the termite powder against the four species of subterranean termites. After signs of elimination of termite population were observed, destructive sampling of termite nests and tunnels in both termite powder-treated and untreated groups were done. An untreated termite nests for each species were included for comparison.

Results and discussion

1. Efficacy of the Termite Powder against Los Baños Termites *M. losbañosensis*

There were no active population of *M. losbañosensis* in treated tunnels and infested wood 1 to 2 weeks after treatment (Table 1). The previously intact and attached tunnels on wood surface became dry, brittle and started to collapse suggesting that there were no more workers to maintain the termite tunnels. Dead termites were observed inside the tunnel. The reconstruction of powder loading sites ranged from 14 to 100% during the 1st week and no loading sites or openings were restored after 2 weeks. The toxicant was acquired by workers during the reconstruction of incised termite tunnels through patching treated openings with mixed matrix of wood, soil and saliva and by directly hitting their bodies with the powder during the dispensing. The powder was presumably spread then to other members of the population by the termites' grooming behavior and trophyllaxis activity.

The nests of *M. losbañosensis* in the treated group containing active and abundant population became few to moderate after 1 to 3 weeks and abundant population was still noted in the 3rd nest during the 4th week. There were no active populations in the first two nests after 3 weeks while it took 2 months in the case of the 3rd nest. The differences in the period of elimination among the three nests were mainly due to the size of population used in the study. However, the application of 0.16 g to 0.40 g termite

powder was enough to arrest the foraging activity of *M. losbañosensis* in 3 weeks to 2 months. All treated nests turned dry and brittle and contained dead termites as well as head capsules. Ants invaded the treated nests due to the absence of soldiers that will defend these nests against intruders.

In untreated nests, termite population remained moderate to abundant and all termite tunnels remained intact and hard. Earthen tunnels continued to spread extensively and caused heavily damage to termite chamber support after the observation period.

Table 1. Efficacy of the Termite Powder against *M. losbañosensis*

Nest No.	Treatment	Sites of Powder Appl'n	Population Level*							Vol. of Powder Applied (g)
			Before Treatment	After Treatment						
				1wk	2wks	3wks	4wks	2mos	3mos	
1	Powder	T & I	A	A	M	0	-	-	-	0.40
2	Powder	T & I	A	M	F	0	-	-	-	0.24
3	Powder	T & I	A	M	F	F	A	0	-	0.16
	Sampling Sites									
1	Control	T, I & N	A	A	M	A	M	M	M	0
2	Control	T, I & N	M	M	M	A	M	M	M	0
3	Control	T, I & N	M	M	M	M	A	M	A	0

Legends: T-Termite tunnels with active population, I-Infested wood with active population, N-Nest

Termite Population in Nest or Infested Wood	Termite Tunnel	Classification of Termite Population
0	0	0 - None
1 to 20 termites	1 to 3 termites	F - Few
21 to 50 termites	4 to 6 termites	M - Moderate
over 50 termites	over 7 termites	A – Abundant

*Population level was estimated from base the of each termite nest.

Table 2. Efficacy of the Termite Powder against *N. luzonicus*

Nest No.	Treatment	Sites of Powder Appl'n	Population Level*						Vol. of Powder Applied (g)
			Before Treatment	After Treatment					
				1wk	2wks	3wks	4wks	2mos	
1	Powder	T & I	M	F	0	-	-	-	0.20
2	Powder	T & I	M	F	0	-	-	-	0.54
3	Powder	T & I	A	A	0	-	-	-	1.4
	Sampling Sites								
1	Control	& N	A	A	A	A	M	M	0
2	Control	& N	A	M	A	M	M	M	0

Legends: T - Termite tunnels with active population, I - Infested wood with active population, N – Nest

Termite Population in Nest or Infested Wood	Termite Tunnel	Classification of Termite Population
0	0	0 - None
1 to 20 termites	1 to 3 termites	F - Few
21 to 50 termites	4 to 6 termites	M - Moderate
over 50 termites	over 7 termites	A – Abundant

*Population level was estimated from base the of each termite nest.

2. Efficacy of the Termite Powder against Luzon Point Headed Termites, *N. luzonicus*

An average of 67% to 100% of termite tunnels made as powder loading sites were reconstructed by *N. luzonicus* after 1 week while all termite powder loaded tunnels were not rebuilt after 2 weeks. The termite tunnels became brittle and started to detach from the surface of the wood or concrete. This indicates the absence of workers or maintenance caste.

The population abundance of termites inside the nest became few to abundant 1 week after treatment and no active termites were noted on the 2nd week (Table 2). Feeding activity was arrested in wood interceptors and termite chamber support thus no further damage was observed. The nest appeared dry, brittle and contained plenty of dead termites during sampling. The population of *N. luzonicus* was eliminated in 2 weeks by applying 0.20 to 1.4 g of termite powder.

Abundance and feeding activity of termite population in untreated nests showed no difference over the study period. However, more tunnels were constructed by the population and damage of wood interceptors and termite chamber support became moderate to heavily attack.

3. Efficacy of the Termite Powder against Mound Building Termites, *M. gilvus*

The termite powder was dispensed directly to active population of *M. gilvus* observed in the dug holes drilled along the perimeter of the mound. Approximately 40 to 100% of treated dug holes were restored by workers by patching the openings with mixed mud and saliva after each treatment application.

The abundant population in 3 experimental mounds became few to abundant on the 1st to the 7th week of test (Table 3). The population in 2 mounds started to decline in week 5 and abundant immature

Table 3. Efficacy of the Termite Powder against *M. gilvus*

Nest No.	Treatment	Sites of Powder Appl'n	Population Level *						Vol. of Powder Applied (g)
			Before Treatment	After Treatment					
				1wk	3wks	5wks	7wks	9 wks	
1	Powder	P	A	F	F	A	F	0	
2	Powder	P	A	M	A	F	A nymphs	0	1.80
3	Powder	P	A	A	A	M nymph	M nymphs	0	1.8
	Sampling Sites								
1	Control	P & Mo	A			A	M	A	0
2	Control	P & Mo	A	A	M	M	A	A	0
3	Control	P & Mo	A	M	M	M	A	A	0

Legends: P - Perimeter with active population, Mo – Mound

Termite Population in Nest or Infested Wood	Termite Population in Termite Tunnel	Classification of Termite Population
0	0	0 - None
1 to 20 termites	1 to 3 termites	F - Few
21 to 50 termites	4 to 6 termites	M - Moderate
over 50 termites	over 7 termites	A – Abundant

*Population level was estimated from base the of each termite nest.

nymphs were observed in most termite powder treated areas in week 7. Destructive sampling of mounds was conducted on week since no active population was observed along the perimeter. The mounds of termites appeared dry and a hairline fracture was developed indicating the disappearance of members responsible for he maintenance of the mound. Abundant dead termites, presence of head capsules and occurrence of ants were observed inside the destroyed mounds. There were no active workers, soldiers and nymphs. There were no eggs deposited in the fungus garden inside the mound. Fungus garden which serve as food for the immature were blemished and appeared unpalatable for termites. Likewise, the king

and queen in royal chamber were not found in all treated mounds which suggest that the colony of *M. gilvus* was most likely eliminated in 7 to 8 weeks after application of 1.45 to 1.8 g of termite powder.

In untreated group, 100% of the drilled openings made during sampling of population were plugged by termites. This is the instantaneous response of workers to repair destroyed mound and tunnels precisely to prevent the entry and attack of intruders like ants. Untreated mounds were intact and contained active and abundant termite population throughout the period of test. Eggs and nymphs were deposited in fungus garden and also noted inside the royal chamber. Soldiers and workers were found inside the destroyed mounds. The king and queen were observed inside the dissected royal chambers.

4. Efficacy of the Termite Powder against Milk Termites, *C. vastator*

a. Elimination of *C. vastator* in Heavily Infested Experimental Unit

The experimental unit number 1 was a 2-storey residential house heavily infested by *C. vastator*. A total of 20 loading sites were applied with powder in the lower floor (2 secondary nests, 7 termite tunnels, 1 infested door jamb of *C. vastator* and 1 secondary nest of *M. losbañosensis*) and upper floor (7 infested wooden beams, 1 plywood partition and 1 wooden post) (Table 4).

Table 4. Efficacy of the Termite Powder in Houses Infested by *C. vastator*

House Unit No.	Degree of Damage	Sites of Powder Appl,n	No. of Loading Sites	Vol. of Powder Applied (g)	Population Level/Additional Powder					Vol of Powder Applied (g)
					Before Treatment	After Treatment				
						1wk	2 wks	3 wks	4 wks	
1	Heavily	T (7) I (10) N (3)	20	0.07 to 1.81 0.09 to 1.51 0.22 to 0.44	A - -	M - -	M (0.11g) (0.11g)	F - -	F (0.08g) (0.11g)	9.44
2	Moderate	T & I	13	0.02 to 0.32 0.05to1.03	A -	F -	0 -	- -	- -	2.58
3	Slight	T & I	6	0.03 to 0.44 0.01 to 0.44	A -	0 -	- -	- -	- -	1.1

Legends: T = termite tunnels with active population, I = Infested wood with active population, N = Nest

Termite Population in Nest or Infested Wood	Termite Tunnel	Classification of Termite Population
0	0	0 - None
1 to 20 termites	1 to 3 termites	F - Few
21 to 50 termites	4 to 6 termites	M - Moderate
over 50 termites	over 7 termites	A – Abundant

Generally, all incised termite tunnels treated with termite powder were not restored and abundant termite population became inactive after 1 week without any additional powder. The tunnels remained intact in 2 weeks but became brittle and partly detached on the 3rd week. Dead termites were noted inside the termite tunnels. Likewise, loading openings in infested wooden beams were not restored after treatment. The previously active population became inactive in 1 week except for the 2 wooden beams and 1 column where few to moderate population of termites remained active in 2 to 3 weeks. Additional 0.11g of termite powder was applied twice every other week and no further feeding activity was noted on the beams and column after 4 weeks.

There were no active termite populations of *C. vastator* in the two secondary nests applied with the powder after 1 week. The abundant population in the secondary nest of *M. losbañosensis* became few after 1 week of treatment. The population of *M. losbañosensis* invaded the vacated two nests of *C. vastator* but injection it with 0.11g of powder every other week resulted to the former species' complete

elimination. All powder injected holes were not restored, the nest became brittle and detached from the concrete wall after 4 weeks. Dead termites and ants were observed during destructive sampling of termite nests. Termite infestation of the heavily infested house by *C. vastator* was arrested in 4 weeks using 9.44 g of termite powder. No further feeding activity of termites was observed in 4 month post-inspection period.

b. Elimination of *C. vastator* in Moderately Infested House

Infestation of *C. vastator* in the experimental unit was noted mainly in the cabinets in the kitchen area and in plywood double wall partition which extended towards a portion of the ceiling. Termite population was abundant in infested cabinets. The abundant termite population became inactive and absent after 1 week of treatment (Table 4). Out of the 13 powder loading sites, only 1 injection site was reconstructed by *C. vastator*. Sampling of reconstructed tunnel showed no active termites. All powder treated tunnels remained intact but turned dry, brittle and partly detached after 2 weeks of treatment. It is speculated that the colony was eliminated in 2 weeks by treatment application of 2.58 g of termite powder. There was no occurrence of termite population and no further destruction on wood components during the 4 month monitoring period.

c. Elimination of *C. vastator* in Slightly Infested House

The 3rd experimental house unit was a bungalow type and termite infestation was observed in doorjamb, 2 kitchen cabinets and a small portion of the eaves. The formerly abundant populations in 6 termite powder loading sites became inactive and no active population was noted in 1 week (Table 4). All treated tunnels were not restored by termites. Termite treated tunnels remained intact but became brittle and collapsed. Likewise, the openings in the powder treated infested wood was not restored. Dead termites were noted inside the tunnels and infested wood during sampling period. It is most likely that the population was eliminated in 1 week after application of 1.1 g of termite powder. There was no occurrence of termites and such result arrested the further destruction of wooden components of the house within the 4 month monitoring period.

Conclusions and recommendations

1. The 0.5% fipronil in termite powder is effective to exterminate 4 species of Philippine subterranean termites.
2. The volume of termite powder required to eliminate termite infestation depends on termite species, size of colony and degree of infestation in houses and buildings.
3. Elimination period is very fast using minimal volume of the termite powder. Test termite colonies of *M. losbañosensis, M. gilvus, N. luzonicus* and *C. vastator* can be eliminated using 0.16 to 0.42 g; 1.4 to 1.8; 0.3 to 1.4g and 1.09 to 9.42g of termite powder in 3 to 12 weeks; 7 to 8 weeks; 2 weeks and 4 weeks, respectively.
4. Complete elimination of termite populations was manifested by inactive population, dry and collapsed tunnels and nests, no reconstruction of injection sites and presence of ants.
5. The termite powder was most likely acquired by workers of termites through the reconstruction of injection sites. Contaminated termites can transmit the toxicant to other members of the colony by body contact during foraging, grooming behavior and mouth to mouth feeding.
6. Thorough inspection, identification of termite species and regular follow-up monitoring are the major factors for the success of termite powder application to eliminate termite infestations.
7. Difficulty in dispensing the powder into the incised tunnel is a major drawback but patience in application and frequent practice will precisely resolve the problem.

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Diversity of Termite Species in Vietnam

by

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Abstract

Total of 141 termite species belonging to 4 families, 8 subfamilies and 38 genera in Vietnam was described in the research, among them the scientific name of 120 species were determined and there were 21 species whose name were not determined.

Five new genera were first recorded for the Vietnam termite region: Archotermopsis, Indotermes, Xiaitermes, Pseudocapritermes and Sinocapritermes.

Introduction

Termite (Isoptera) is small social insects. In a colony, they are clearly castes and function division. There are more than 2600 termite species in the world. Termites are widely distributed in tropical and subtropical regions. Vietnam, located on the Southeast Asia, has a tropical monsoon climate with natural resources of high diversity and characteristics, as well as places suitable for the growth and development of termite. Besides researchs on some biological of some high economic value termite (examples: *Coptotermes formosanus, Odontotermes hainanensis*), researchers also concern of research on diversity of termite species in Vietnam.

Researchs on termites in Vietnam focus on three main items: Investigation into termite species in some National Park and natural reserve in the country; research on biological of some value species and proposed means test solutions for prevention. Researchs often associated with each object that need protect such as construction projects, dams and dikes and crops.

The first publication of the termite species of Vietnam by Bathellier (1927) reported only 18 species (among total 19 species in Indochina).

At the first half of 20 century, some documentations of some authors were publised as Bathellier (1933, 1937), L. Caresch (1937) and Allouard (1947). But those were about the harmful effects and some new methods for termite control and prevention on constructions and crops without helpful for classification and taxonomy.

The first book caught attention's everybody was Termite of North Vietnam published in 1976 by Duc Kham Nguyen. The auther described the bio-ecological characteristics and distribution characteristics of 61 species, 21 genera in northern Vietnam. Before this, Harris (1968) had been published a work on termites in Vietnam, Cambodia and Thailand.

Tan Vuong Nguyen (1997) reported 14 termite species belonging to the research on Macrotermes in South Vietnam. Van Quang Nguyen (2003) also researched on Macrotermes but in North Vietnam. He reported 17 species of Macrotermes in North Vietnam.

Termite composition in national parks and nature conserve are also concerned in recent years. But there were no guidline given for the termite classification in these researchs.

Duc Kham Nguyen et al. (2007) published a book "fauna of Vietnam" with bio-ecological and distribution characteristics of 101 termite species in Vietnam.

Termite specimens were collected by the Institute for Termite control and Works protection in the past 20 years. Over 7,000 specimens were collected and preserved in the Institute. The result is a scientific basis for further studies on diversity of termite species in Vietnam.

Materials and methods

Termite specimens were collected from 64 provinces all over Vietnam both on the natural forests, plantations, fruit orchards, hills, buildings, dikes and dams. Over 7,000 specimens were collected and preserved in 75% ethyl-alcohol in the past 20 years. Voucher specimens are deposited at the Institute for Termite control and Works protection.

Method of taxonomic study

Termites were identified through external morphology of unbroken soldier caste. The major characteristics employed to distinguish termite genera were the shape and size of head, fontanelle, labrium clypeus, mandible characteristics, the place of teeth, and pronotum and number of antennae articles.

First, with the help of microscope, termites specimens were surveyed and described in terms of their external morphology, then their body parts were measured and relerant indexs were calculated according to Roonwal's instructions (1969). All measurements were recorded in millimeters. Based on Systematic Key to termites and decriptions in identification documents the scientific names for specimens were determined.

Documents used: Measurement of termites (Isoptera) for taxonomic purpose (Roonwal, M. L.); Key to Indo - Malayam termites (Ahmad, 1958); Termites of Thailand (Ahmad, 1965); Fauna sinica (insecta, Vol.17, isoptera) (Huang Fusheng và cs., 2000); Termites of Sabah (East Malaysia) (Thapa, R.S., 1981); Fauna Vietnamese – Termite (Isoptera), Vol. 15. Nguyen Đuc Kham et al 2007); Study on the genus Coptotermes from China, Isoptera: Rhinotermitinae (Xiakailing and He Xin Xong, 1986); Phylogeny and generric reclassification of the Capritermes complex (Isoptera, Termitidae, Termitinae) (Kumar Kishna, 1968).

Results and discussion

Composition of termite and their distribution on Vietnam

According to animal researchers, Vietnam's territory is divided into 6 Geo-zoological distributions namely: Northeast, northwest, North of Central part, South of Central part, Middle of Central part and Southern part Vietnam. Composition of termites in each region is presented in Table 1.

Results showed that there were 38 genera with total 141 termite species belonging to 4 familie, 8 subfamilies and in Vietnam, the scientific name of 123 species were determined and there were 21 species whose name were not determined. The genera has the lagrest amount of species is Odontotermes, accounting for 12.1, while the number of most genera are small (less than 5 species).

In each of research regions, the result showed not only difference in species diversity but also special characters of termite species, not or rarely seen in other regions.

The Northeast of Vietnam's territory is made up of midlands plain and mountains with many blocks of limestone mountains and soil mountains, so species composition not only has characteristic of the high mountain, jungle species (such as *Hodotermopsis sjostedti*, *H.japonicus*...) but also has characteristics of the delta and midland species (termite species having fungus garden such as Macrotermes or Odontotermes...) so level of species found only at an altitude of 1,000 metres and over above sea level as Termopsidae (*Hodotermopsis sjostedti*, *H.japonicus*). Besides, this area is also very suitable for the development of cold termite species such as Reticulitermes spp.

The Northwest of Vietnam's territory is made up of mainly limestone mountain blocks and rock plateaus, so the composition species here are mostly Reticulitermes spp., Archotomopsis spp. Preferring cold and altitude. This is also the only region where Archotermopsis sp1. have been found up to now. It is one of the largest in size of living termites. This species live at 2800 to 9000 feet, mostly above 4000 feet, in the subHymalaya in Indian, West Pakistan and Afghanistan, northeast of Kabul (Harris, 1967) and in Vietnam, we have found it at the altitude of from 1700 to 2000 metres in Hoang Lien Son mountain.

North central part's terrain is a long, narrow corridor, s bordered in the west by Truong Son and Lao, and in the East by the Eastern Sea. Territory includes narrow plain, midland and also mountainous and coastal area. This region concentrates some national parks and nature reserves such as Benen nation park, Pumat national park, Phongnha-Kebang national park... So the termite composition here is very diverse not only in species but also in genera. There are 30 termite genera found here, (this area has the largest number of gerena in research areas (ratio 30/38 genera discovered in Vietnam). Some termites like Na. dimopphus and Sub. major are only distributed in this region.

South of Central part have such termites as *Neotermes* sp1, *O.thachkhensis* and *H.dabanensis*. Their distribution is known here only. This area also is a place marking the emergence of *M.carbonarius* very popular in the southern provinces of Vietnam. It is the place where the distribution of *M.barneyi* that is fairly common in northern and noeth-central part ends.

Table 1. List of termite genera and their geographical distribution

No.	Termite genera (No. of species)	Geographic area					
		Northwest	Northeast	North of Central part	South of Centra part	Middle of Centre part	South Vietnam
1	*Hodotermopsis* (2 sp.)	+	+				
2	*Archotermopsis (1 sp.)*	+					
3	*Cryptotermes (2. sp.)*	+	+	+	+		
4	*Neotermes (3 sp.)*		+	+	+		
5	*Glyptotermes (3 sp.)*		+	+	+		
6	*Coptotermes (7 sp.)*	+	+	+	+	+	+
7	*Coptermopsis (2 sp.)*		+				
8	*Schedorhinotermes (6 sp.)*	+	+	+	+	+	+
9	*Prorhinotermes (2 sp.)*					+	
10	*Reticulitermes (9sp.)*	+	+	+	+	+	
11	*Micerotermes (3 sp.)*		+	+	+	+	+
12	*Indotermes (2 sp.)*	+		+		+	+
13	*Speculitermes (1 sp.)*		+	+			
14	*Amitermes (1 sp.)*						
15	*Euhamitermes (1 sp.)*			+			
16	*Globitermes (1 sp.)*			+	+	+	+
17	*Odontotermes (17 sp.)*	+	+	+	+	+	+
18	*Hypotermes (4 sp.)*	+	+	+	+	+	+
19	*Macrotermes (14 sp.)*	+	+	+	+	+	+
20	*Microtermes (3 sp.)*	+	+	+	+	+	+
21	*Pericapritermes (5.sp)*	+	+	+	+	+	+
22	*Procapritermes (3 sp.)*	+	+	+		+	+
23	*Pseudocapritermes (4 sp.)*	+	+	+	+		+
24	*Sinocapritermes (1sp.)*						+
25	*Dicupiditermes (5 sp.)*	+	+		+	+	
26	*Termes (4 sp.)*			+	+	+	+
27	*Nasutitermes (14 sp.)*	+	+	+	+	+	+
28	*Subuliotermes (1 sp.)*			+			
29	*Hospitalitermes (3sp.)*		+	+	+		+
30	*Aciculitermes (3 sp.)*		+	+		+	
31	*Bulbitermes (2 sp.)*			+	+	+	+
32	*Peribulbitermes (2 sp.)*	+	+	+			
33	*Pilotermes (1 sp.)*		+	+	+		
34	*Xiaitermes (1 sp.)*	+					
35	*Ahmaditermes (3 sp.)*		+	+	+		
36	*Haviladitermes (2 sp.)*		+		+		
37	*Lacestitermes (2 sp.)*				+		
38	*Proaciculitermes (1sp.)*			+	+		

The Middle of central part of Vietnam characterized by bazan red soil, the termite composition in diverse with various species belonging to the rhinotermitinae. Among them species of Schedorhinotermes, Procapritermes are big in size.

And finally the South of Vietnam with representatives of the termite Macrotermes chaiglomi, this soil termite species distribution is quite common in KienGiang province in particular and the South in general, but has never been detected in other parts of Vietnam.

However, in order to confirm the presence or absence of a species in a certain region further studies on the distribution of local fauna, historical origins geographic distribution ect are needed.

Table 2. Termite species of Vietnam reported from 1927 - 2009

No.	Termite genera (No. of species)	Reference			
		Bathellier (1927)	Nguyễn (1976)	Nguyễn (2007)	Present Study (2009)
1	*Hodotermopsis* (2 sp.)	0	2	2	2
2	*Archotermopsis (1 sp.)*				1
3	*Cryptotermes (2. sp.)*	1	2	2	2
4	*Neotermes (3 sp.)*		2	2	3
5	*Glyptotermes (3 sp.)*		2	2	3
6	*Coptotermes (7 sp.)*	2	5	7	7
7	*Coptermopsis (2 sp.)*		1	1	2
8	*Schedorhinotermes (6 sp.)*	1	1	6	6
9	*Prorhinotermes (2 sp.)*			1	2
10	*Reticulitermes (9sp.)*	1	6	6	9
11	*Micerotermes (3 sp.)*	1	2	3	3
12	*Indotermes (2 sp.)*				2
13	*Speculitermes (1 sp.)*		1	1	1
14	*Amitermes (1 sp.)*			1	1
15	*Euhamitermes (1 sp.)*		1	1	1
16	*Globitermes (1 sp.)*	1	1	1	1
17	*Odontotermes (19 sp.)*	2	9	11	17
18	*Hypotermes (4 sp.)*	1	1	3	4
19	*Macrotermes (15 sp.)*	1	4	13	14
20	*Microtermes (3 sp.)*		1	3	3
21	*Pericapritermes (5.sp)*		3	4	5
22	*Procapritermes (3 sp.)*		2	2	3
23	*Pseudocapritermes (4 sp.)*				4
24	*Sinocapritermes (1sp.)*				1
25	*Dicupiditermes (5 sp.)*			3	5
26	*Termes (4 sp.)*	2	1	3	4
27	*Nasutitermes (24 sp.)*	3	10	7	14
28	*Subuliotermes (1 sp.)*			1	1
29	*Hospitalitermes (4sp.)*		1	3	3
30	*Aciculitermes (3 sp.)*			2	3
31	*Bulbitermes (2 sp.)*			2	2
32	*Peribulbitermes (2 sp.)*			1	2
33	*Pilotermes (1 sp.)*			1	1
34	*Xiaitermes (1 sp.)*				1
35	*Ahmaditermes (3 sp.)*			2	3
36	*Haviladitermes (2 sp.)*			1	2
37	*Lacestitermes (2 sp.)*			2	2
38	*Proaciculitermes (1sp.)*			1	1
Tổng		**17**	**61**	**101**	**141**

In comparison with research by Nguyen Duc Kham et al, 2007, our research added 40 species and 5 genera. The five new termite genera are Archotermopsis, Indotermes, Sinocapritermes, Xiaitermes and Pseudocapritermes. This is the most complete list of termite composition in Vietnam up to now.

Table 3. List of termite species in Vietnam

No	Species	No.	Species	No.	Species
1	*Hodotermes sjotedti**	54	*O. horni*	106	*D. sp2 **
2	*Ho. Japonicus**	55	*O. jampeensis*	107	*Termes comis**
3	*Archotermopsis sp1**	56	*O. graveli**	108	*Termes laticornis**
4	*Cryptotermes domesticus**	57	*O. pahamensis**	109	*Termes propinquus**
5	*Cr. declivis**	58	*O. measodensis**	110	*Termes sp1 **
6	*Neotermes koshunensis**	59	*O. yunanensis**	111	*Nasutitermes curtinasus**
7	*Ne. termillesemus**	60	*O. longignathus**	112	*Na. medoensis**
8	*Ne. sp1**	61	*O. khechensis**	113	*Na. regularis**
9	*Glyptotermes fucus**	62	*O. thachkheensis**	114	*Na. matangensiformis**
10	*Glypt. satsumensis**	63	*O. oblongatus**	115	*Na. matangensis**
11	*Glypt. sp1**	64	*O. bruneus**	116	*Na. tiantongensis**
12	*Coptotermes emersoni**	65	*O. sp1**	117	*Na. sinensis**
13	*C. curvignathus**	66	*O. sp2**	118	*Na. ovatus**
14	*C. travians**	67	*Hypotermes sumatrensis**	119	*Na. ninhthuanensis**
15	*C. ceylonicus**	68	*H. makhamensis**	120	*Na. dimorphus**
16	*C. formosanus**	69	*H. obscuricep**	121	*Na. bulbicep **
17	*C. gestroi**	70	*H. dabanensis**	122	*Na. moratus**
18	*C. havilandi**	71	*Macrotermes barneyi**	123	*Na. sp1*
19	*Coptotermopsis dimorphus**	72	*M. annadalei**	124	*Na. sp2*
20	*Co. sp1**	73	*M. maesodensis**	125	*Na. Disparatus*
21	*Schdotermopsis malaccensis**	74	*M. menglongensis**	126	*Na. Cuphus*
22	*Sch. tarawakensis**	75	*M. beaufortensis**	127	*Na. Pavonasutus*
23	*Sch. medioobscurus**	76	*M. latignathus**	128	*Na. Deltocephalus*
24	*Sch. magnus**	77	*M. malaccensis**	129	*Na. Communis*
25	*Sch. Javanicus*	78	*M. serulatus**	130	*Na. Gardneri*
26	*Sch. sarawakensis**	79	*M. tuyeni**	131	*Na. Orthonasus*
27	*Prorhinotermes sp1s **	80	*M. gilvus **	132	*Na. Senae*
28	*Prorhino. tibiaoensiformi**	81	*M. carbonarius**	133	*Na. sp3**
29	*Reticulitermes chinensis**	82	*M. catbanensis**	134	*Na. sp4**
30	*Re. magdalenae **	83	*M. sp1**	135	*Subulioditermes major**
31	*Re. dangi**	84	*M. chaiglomi**	136	*Hospitalitermes jepsoni **
32	*Re. microcephalus**	85	*M. estherae*	137	*Hospi. damenglongensis**
33	*Re. flaviceps**	86	*Microtermes pakistanicus**	138	*Hospi. medioflavus**
34	*Re. speratus**	87	*Mi. incertoides**	139	*Hospi. Luzonensis*
36	*Re. assamensis**	88	*Mi. obesi**	140	*Aciculitermes sarawakensis*
37	*Re. wuganensis**	89	*Pericapritermes tetraphilus**	141	*Aciculi. holmgreni**
38	*Re. sp1**	90	*Peri. sermarangi**	142	*Aciculi. oditermes**
39	*Microcerotermes burmanicus**	91	*Peri. nitobei**	143	*Bulbitermes prabhae**
40	*Micero. bugnioni**	92	*Peri. latignathus**	144	*Bulbi. laticephalus**
41	*Micero. crassus**	93	*Peri. sp1**	145	*Peribulbitermes dinghuensis**
42	*Indotermes vietnamensis**	94	*Procapritermes albipennis**	146	*Peribul. sp1**
43	*Indotermes sp1**	95	*Proca. sowerbyi**	147	*Pilotermes jiangxiensis**
44	*Speculitermes donhanensis**	96	*Proca. suoivangensis **	148	*Xiaitermes sp1**
45	*Amitermes longignathus**	97	*Pseudocapritermes pseudolaetus**	149	*Ahmaditermes perisiniosus**
46	*Euhamitermes hamatus**	98	*Pseudo. parasilvaticus **	150	*Ahmad. tianmuensis**
47	*Globitermes sulphureus**	99	*Pseudo. jiangchengensis**	151	*Ahmad. sp1**
48	*Odontotermes feae**	100	*Pseudo sp1 **	152	*Havilanditermes atripennis**
49	*O. hainanensis**	101	*Sinocapritermes sp1**	153	*Havila. sp1 **
50	*O. formosanus**	102	*Dicuspiditermes orientalis**	154	*Lacesititermes batavus**
51	*O. proformosanus**	103	*Di. nemorosus**	155	*Lacesti. albipes**
52	*O. angustignathus**	104	*Di. grathawaitei**	156	*Lacesti. homgreni**
53	*O. ceylonicus**	105	*Di. sp1 **	157	*Proaciculitermes orientalis**

Note. '*': termite species found in the report.

Conclusion

1. There were 141 termite species belonging to 4 families, 8 subfamilies and 38 genera in Vietnam, the scientific name of 120 species were determined and there were 21 species whose name were not determined.

2. Five new genera were first recorded for the Vietnam termite region: Archotermopsis, Indotermes, Xiaitermes, Pseudocapritermes and Sinocapritermes.

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The Taxonomy of Some Chinese *Heterotermes* Revealed by COII Gene

by

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Abstract

The taxonomy of Chinese *Heterotermes* is always controversial in China. Although *Heterotermes* has been thought to be confined to tropical areas, some Chinese taxonomists suggest that this genus is also distributed in subtropical to temperate areas and treat some Chinese species as *Heterotermes*. We sequenced 642 bp of the mitochondrial COII gene of several *Reticulitermes* and *Heterotermes* species from different city in China. The inferred molecular phylogeny showed that *Heterotermes* and *Reticulitermes* species from China formed a monophyletic group suggesting that the Chinese *Heterotermes* should be belong to *Reticulitermes*.

Key words: mtDNA, COII, *Reticulitermes*, *Heterotermes*

Introduction

Reticulitermes has a Holartic distribution and is the principal termite genus present in China. In Fauna Sinica (Insecta, Isoptera), the genus *Reticulitermes* are classify to 3 subgenus, (*Planifrontotermes* Tsai et Huang 1977), *Frontotermes* Tsai et Huang 1977) and (*Reticulitermes* S. str. Holmgren 1912). There are more than 100 species described in china, among which about 21 species were treated as *Heterotermes* by some Chinese termitists (Tsai & Huang, 1983; Huang et al., 1989).

Traditionally, the diagnosis of *Reticulitermes* species is based on the morphological characters of the soldiers and alates. But more often than not, the alates of termites are not easy to collected , and we have but only the soldiers (not numerous) and some workers in the majority of specimen sets, since the morphological characters of soldiers are variety as the ages change of a nest. It is difficult to get accurate species identification. The taxonomy of termites (particularly in *Reticulitermes*) could not come to an agreement. This genus is currently under review, as the use of molecular markers provides new phylogenetic insights (Jenkins et al, 2001; Austin et al, 2002). In genus of *Reticulitermes*, The main divergence is that whether there is *Heterotermes* in China.

It is controversial whether *Heterotermes.sp* in China is actually a member of *Heterotermes*. The main morphological characters of *Heteromermes* are that the mandible of the soldier is long and fragile, and the apex of the transparent part of the labrum is sharp, with a long tip. Whereas in *Reticulitermes*, the mandible is robust and the accordant part of the labrum is tongue-like, without a long tip (Huang *et al.*, 1989). In addition, most taxonomists believed that *Heterotermes* is distributed only in tropical areas (Pearce& Waite, 1994).

These few years, many research works about *Heteromermes* and *Reticulitermes* have been development. Zhang Fangyao (1994) using Scanning Electron Microscope(SEC) to study the wing microsculpturing between *Heterotermes* and *Reticulitermes*, From the shape and arrangement of the papillae, they found no difference between *Heterotermes* and *Reticulitermes*. And then, Xing Lianxi (2001) used COII gene to compare *Heterotermes aculabialis* in China with *Heterotermes* various localities of the world, suggesting that the Chinese *Heterotermes aculabialis* should be transferred to *Reticulitermes aculabialis*.

More recently, Cuticular hydrocarbon (CHC) profiles have been used for taxonomic purposes (Zhang Hongbing, 2005). According to their experiments. there is *Heterotermes* in China. The main difference between *Heterotermes* and *Reticulitermes* is that *Heterotermes* lacks such hydrocarbons as heptadecane, eicosane, heneicosane, docosane, tricosane, tetracosane, hexacosane and so on, but has the isoquinoline. So they believe that there is *Heterotermes* in China.

In this study, we attempted to collecte *Heterotermes* samples from different city in China, but, to us disappointed, among 220 *Retilulitermes* samples we got, we just have only 19 *Heterotermes* samples. So we used COII mitochondrial gene of 19 *Heterotermes* samples from different city in China, to compare with Genbank related sequences, to illuminate the relationships among the *Heterotermes* and *Retilulitermes* in China.

Materials and methods

Termites used in this study were collected from Guangdong, Hubei, Jiangsu, Hunan and Zhejiang Province (See Table 1), and preserved in 100% ethanol (others in 75% ethanol for morphological identification). Only the head and thorax parts of a worker were used for DNA extraction in order to prevent DNA pollution from protistan symbionts in the hindgut of the termite. Total DNA was extracted following CTAB DNA isolation method. Polymerase chain reaction (PCR) was conducted using the primers TL2-J-3037 (5-ATGGCAGATTAGTGCAATGG-3) designed by Liu and Beckenbach (1992) and described by Simon et al. (1994) and Miura et al. (1998) and primer TK-N-3785 (5-GTTTAAGAGACCAGTACTTG-3) from Simon et al. (1994). These primers amplify a 3- portion of the mtDNA COI gene, tRNA-Leu, and a 5-section of the COII gene. PCR reactions were conducted using 2ul of the extracted DNA in 50ul volumes, amplification profile consisted of an initial three-minute denaturation at 94 °C, followed by 35 cycles of 94°C for 45 s, 55 °C for 45 s, and 72 °C for 60 s, and finally a 10-minute cycle at 72 °C, The PCR products were analyzed by electrophoresis on 1.5% agarose gel in 0.5TBE buffer and visualized under UV-light after staining with Goldview. The PCR products of interest were purified using the E.Z.N.A.Cyclpure Kit (OMEGA BIO-TEK). Sequencing was carried out by Invitrogen Biotechnology Co.,Ltd.

The sequences were corrected with Chromas, and aligned by Seqman (DNASTAR. Lasergene.v7.1.0) and clustalx1.83, and then calculate the haplotype with arlequin311, finally imported the sequences of haplotype and Genbank into Mega4 to construct phylogenetic trees based on neighbour joining analysis with Kimuura-2-parameter model (transition/trasversion rates set to 2:1). Bootstrap confidence intervals on each branching were calculated from 1000 replications of samples.

Table1. Summary of the collected termites used in this study

Samples code	Species Name	Collecting sites
01	Heterotermes .sp01	Hangzhou West Lake,Zhejiang province
02	*H.sp02*	Hangzhou West Lake, Zhejiang province
03	*H.sp03*	Hangzhou West Lake, Zhejiang province
04	*H.sp04*	Hangzhou West Lake, Zhejiang province
05	*H.sp05*	Hangzhou West Lake, Zhejiang province
06	*H.sp06*	Hangzhou West Lake, Zhejiang province
07	*H.sp07*	Hangzhou West Lake, Zhejiang province
08	*H.sp08*	Hangzhou botanical garden, Zhejiang province
09	*H.sp09*	Hangzhou botanical garden, Zhejiang province
10	*H.sp10*	Yangzhou, Jiangsu province.
11	*H.sp11*	Yangzhou, Jiangsu province.
12	*H.sp12*	Yangzhou, Jiangsu province.
13	*H.sp13*	Nanjing, Jiangsu province.
14	*H.sp14*	Nanjing, Jiangsu province.
15	*H.sp15*	Nanjing, Jiangsu province.
16	*H.sp16*	Nanjing, Jiangsu province.
17	*H.sp17*	Huazhong Agricultural University, Wuhan province.
18	*H.sp18*	Jishou district, Hunan province.
19	*R.guangzhouensis*	Heyuan, Guangdong province.
20	*R.flaviceps*	Guangzhou Longdong, Guangdong province.
21	*R.affinis*	Shaoguan,,North of Guangdong province.
22	*R.ampliceps*	Genebank AB050704
23	*H.tenuior*	Genebank AB050714
24	*H.vagus*	Genebank EF442711
25	*H.cardini*	Genebank AY453590
26	*H.ferox*	Genebank AY536411
27	*H.darwin*	Genebank AB050715
28	*H.longiceps*	Genebank AY553138
29	*H.platycephalus*	Genebank DQ442137
30	*P.queenslandicus*	Genebank AB005585

Table2. Kimura2-parameter distances between COII sequences used in this study.

Table2. Kimura2-parameter distances between COII sequences used in this study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.*H.sp*11																	
2.*H.sp*10	0.002																
3. *H.sp*01	0.011	0.009															
4.*H.sp*12	0.048	0.046	0.048														
5.*H.sp*02	0.011	0.009	0.006	0.050													
6.*H.sp*17	0.050	0.048	0.050	0.005	0.052												
7.*H.sp*18	0.013	0.011	0.014	0.055	0.014	0.057											
8. *R.ampliceps*	0.053	0.052	0.057	0.034	0.059	0.039	0.061										
9. *R.flaviceps*	0.078	0.076	0.076	0.072	0.072	0.070	0.078	0.077									
10. *R.affinis*	0.076	0.074	0.076	0.072	0.074	0.070	0.080	0.078	0.031								
11. *H.cardin*	0.186	0.183	0.181	0.196	0.178	0.199	0.191	0.204	0.211	0.220							
12. *H.ferox*	0.171	0.173	0.171	0.173	0.164	0.176	0.178	0.193	0.170	0.178	0.129						
13. *H.darwin*	0.173	0.175	0.173	0.181	0.170	0.188	0.180	0.196	0.182	0.187	0.114	0.050					
14. *H.tenuior*	0.178	0.181	0.188	0.199	0.186	0.201	0.183	0.204	0.203	0.212	0.172	0.164	0.157				
15. *H.vagus*	0.164	0.166	0.169	0.167	0.166	0.169	0.174	0.179	0.180	0.194	0.114	0.120	0.108	0.134			
16. *H.longiceps*	0.167	0.169	0.167	0.170	0.165	0.172	0.172	0.177	0.181	0.184	0.134	0.086	0.097	0.158	0.121		
17. *H.platycephalus*	0.160	0.162	0.160	0.163	0.153	0.165	0.169	0.182	0.174	0.182	0.129	0.061	0.078	0.140	0.108	0.086	
18. *P.queenslandicus*	0.254	0.257	0.262	0.275	0.251	0.275	0.271	0.299	0.282	0.282	0.257	0.254	0.241	0.283	0.247	0.266	0.242

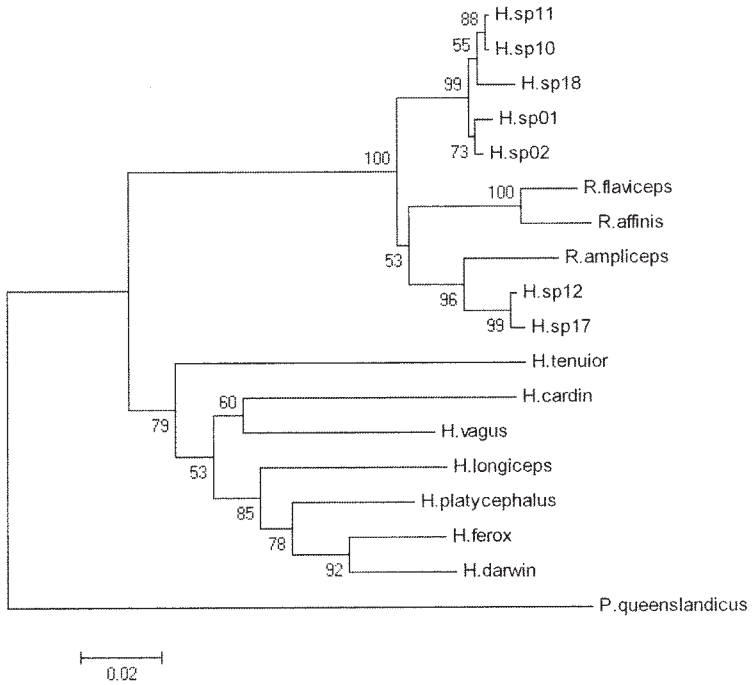

Fig.1.N-J tree created with Mega4. *Parrhinotermes queenslandicus* was used as an outgroup

Results and discussion

Average amplicon size resulting from DNA sequencing was about 760 base pairs(bp). To make sure of the true results and to be compared with the sequences from the Genbank, 118 bp from the two ends of the amplicon was excluded, and the remaining 642-bp COII portion was used. Among all the currently available COII sequences, no indel was observed. The average base frequencies were A-0.39, C-0.24, G-0.14, and T-0.23 by Arlequin311. The pairwise genetic distances of DNA sequences corrected by Kimura's method (1980) are shown in Table2. The genetic distances among

Reticulitermes (all species from China used in this study) were below 0.080. While the distances range from 0.153 (between *H.sp*02 and *H.platycephalus*) to 0.201 (between *H.sp*17 and *H.tenuior*).

In the Neighbor-joining tree, three distinct clades were obtained (see Fig 1.), All the *Heterotermes* species from foreign country were included in a single clade, whereas the rest species from China formed a monophyletic group, supported with bootstrap values of 100%. And the outgroup *Parrhinotermes queenslandicus* was in a single clade. *H.sp* was shown to be closely related to *R.ampliceps* (*Planifrontotermes* Tsai et Huang 1977).

The current results suggest the phylogenetic relationships: (i) *Heterotermes* species from foreign country are obviously different from those species in China; (ii) *Heterotermes* species from China are close related to *R.ampliceps* (*Planifrontotermes* Tsai et Huang 1977), and should be belong to *Reticulitermes*.

For other similar *Heterotermes* species in China, further taxonomic work should be carried out to confirm whether they belong to *Reticulitermes*. In addition, the present findings strongly that not only external morphological characteristics, but also molecular information are useful in examining the precise taxonomic position of lower termites. Phylogenetic analysis could be a good way to verify the morphological describes. This finding suggests a combination of molecular and morphological approaches are necessary for accurate species identification.

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RNA Interference in the Termite *Reticulitermes speratus*: Silencing of the Hexamerin Gene Using a Single 21 Nucleotide Small Interfering RNA

by

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Abstract

Termites express polyphenism, in that nymphs can differentiate into either the alate or the nymphoid form, which is one of the reproductive caste phenotypes (neotenics). Using RNA interference, we identified hexamerin in the termite *Reticulitermes speratus* Kolbe by cDNA sequencing. We used a single 21 nucleotide siRNA fragment to silence the hexamerin gene in order to avoid off-target effects. The siRNA injection treatment used to silence hexamerin caused moderate suppression of hexamerin gene expression in workers for 8 days after the injection, while hexamerin gene expressions rose significantly for 2 – 9 days in the body section of nymphs. Promotion of nymph differentiation to nymphoid occurred with the siRNA injection treatment. These findings suggest a unique differentiation mechanism for the development of nymphs to the reproductive caste phenotype, nymphoid, caused by the elevated expression of the hexamerin.

Key words: double-stranded RNA, RNAi, siRNA, social insects, caste regulation

Introduction

Termite caste differentiation is a post-embryonic developmental process. Caste differentiation and development in the genus *Reticulitermes* can follow either an apterous or an imaginal pathway (Zhou et al. 2006a). The apterous pathway for workers includes five instars, and the imaginal pathway for nymphs includes six instars, and is distinguished from other castes by the possession of wing buds (Takematsu 1992). The initial separation of the two developmental lines occurs after two larval instars. Nymphs have two developmental potentials: differentiation into adult alates that establish a new colony as primary reproductives, or differentiation into nymphoids that supplement the reproductive capacity within colonies. Workers have three developmental potentials: status quo molts remaining as workers, differentiation into pre-soldiers (followed immediately by molting into soldiers), or differentiation into ergatoids that assume identical functions as nymphoids. Caste differentiation in termites is influenced strongly by changes in the juvenile hormone (JH) titer (Lüscher 1960). High titers of JH lead to differentiation of workers to pre-soldiers (Yin and Gillot 1975), whereas low titers result in status quo worker-to-worker molts (Zhou et al. 2006a). JH has a key role in egg development as well as in caste differentiation. In *Reticulitermes flavipes* Kollar, egg development is related to the rate of JH synthesis (Elliott and Stay 2007).

Hexamerins, lipophorins, and vitellogenins have been documented as JH-binding proteins (JHBPs) in insects (Engelmann and Mala 2000; Gilbert et al. 2000). JHBPs have two major roles in insect developmental physiology. JHBPs protect JH from degradation by JH esterase and JH epoxide hydrolase, and serve as hemolymph carrier proteins that deliver JH to the target tissue (Gilbert et al, 2000). In *R. flavipes*, significant increases in expression of the hexamerin and vitellogenin genes after JH exposure demonstrated changes of protein and gene expression of such JHBPs during termite caste differentiation (Scharf et al. 2005). More recent studies using RNA interference (RNAi) suggest that hexamerins function in caste differentiation of *R. flavipes* via regulation of the JH levels (Zhou et al. 2006a, 2007). In *R. flavipes*, two hexamerin proteins suppress JH-dependent worker differentiation to the soldier caste phenotype.

Hexamerins are insect storage proteins that have been found at high concentrations in the hemolymph of many species (Hagner-Holler et al. 2007). Hexamerins serve as a source of energy and amino acids during non-feeding periods (Telfer and Kunkel 1991). Termite hexamerins are hypothesized to act as a signaling mechanism for nutritional status and to suppress pre-soldier differentiation when certain nutritional requirements are met (Zhou et al. 2006a). An alternative hypothesis is that the hexamerins are part of the mechanism that sequesters JH, preventing it from

eliciting downstream effects on developmental gene expression (Zhou et al. 2006a). The JH-sequestration hypothesis is supported by the increase in JH-dependent caste differentiation of workers after silencing of the *R. flavipes* hexamerin genes by RNAi (Zhou et al. 2006a). Although the role of hexamerins in the caste regulation of workers seems to be unveiled, the question of how hexamerins function in the termite caste regulation of nymphs has not been answered. The studies reported here were undertaken in an effort to identify the hexamerin gene in *Reticulitermes speratus* Kolbe, and to examine the role of hexamerin in caste regulation of nymphs, e.g. differentiation of nymphs to nymphoids, alates or status quo molts by hexamerin gene silencing using a single 21 nucleotide siRNA to avoid off-target effects. Additionally, hexamerin gene silencing using a single siRNA (21 bp) was applied to workers to examine the effect of a single siRNA (21 bp) on the caste differentiation of workers.

Materials and methods

Insects. *R. speratus* individuals were collected from a colony located in an infested wood in the Wakayama Prefecture, Japan, and maintained in the laboratory at 26°C with their nest materials and with blocks of *Pinus densiflora* as food source. Non-reproductives, nymphs with wing buds on the thorax, and apterous workers were collected from the colony maintained in the laboratory.

Partial sequencing of hexamerin cDNA. mRNA was extracted from the head and decapitated body of 20 worker caste *R. speratus* termites using a QuickPrep micro mRNA purification kit (GE Healthcare, UK). First-strand cDNA was constructed using an oligo(dT) primer and Superscript II reverse transcriptase (Invitrogen, USA) according to the manufacturer's instructions. PCR amplification of the first-strand cDNA was done with a My Cycler™ thermal cycler (Bio-Rad Laboratories, USA) with the primers as follows: forward, 5'-GATCCATTCCACAAGCACG-3'; reverse, 5'-ACATTCTCCACCGTCACTCC-3', and the Takara Ex Taq polymerase hot start version (Takara Bio, Japan). Each reaction (100 µl) contained 2.5 units of *Taq* DNA polymerase, 10 µl of 10 × Ex Taq buffer, 8 µl of dNTP mix (2.5 mM each dNTP), 1 µl of template DNA, and 10 pmol of each primer. The thermal cycler profile consisted of 35 cycles of 30 sec at 94°C, 30 sec at 58.8°C, and 1 min at 72°C. These primers were designed from *R. flavipes* hexamerin cDNA sequences (Zhou et al. 2006b). The PCR products were subjected to electrophoresis in a 1% agarose gel. A fragment of ~100 bp amplified from the mRNA of *R. speratus* was purified using a MinElute gel extraction kit (Qiagen, USA). The purified products were cloned into the pGEM-T vector (Promega, USA) with the JM109 bacterial host according to the manufacturer's instructions. The plasmids were extracted from the host bacterial cells using the Wizard® Plus Minipreps DNA purification system (Promega, USA) and were used as sequencing templates. The sequences were determined in both orientations with SP6 and T7 oligonucleotides as sequencing primers using a DYEnamic™ ET terminator cycle-sequencing kit (GE Healthcare, UK) with an ABI3100 automated DNA sequencer.

Rapid amplification of cDNA end (RACE) of hexamerin. Total RNA was extracted from 10 *R. speratus* worker caste individuals using the PureLink™ Micro-to-Midi total RNA purification system (Invitrogen, USA). For 3'- RACE, the first-strand cDNA was constructed using the 3'-RACE system for rapid amplification of cDNA ends (Invitrogen, USA). Hexamerin-specific primers for 3'-RACE were: first PCR primer (1221 – 1241 bp), nested-PCR primer (1285 – 1307 bp), third PCR primer (1770 – 1791 bp). For 5' RACE, first strand cDNA synthesis was primed using a hexamerin-specific antisense oligonucleotide (5'-GCTCATTCAAGTCCCATTCC-3') and the 5'-RACE system (version 2.0 Invitrogen, USA) according to the manufacturer's instructions. Hexamerin-specific primers for 5'-RACE are shown as follows: first PCR primer (1477 – 1496, compl.), nested PCR primer (1396 – 1414, compl.), third to sixth PCR primers (1058 – 1079, 765 – 784, 743 – 762, 85 – 104 bp, compl.). Sequences of primers and oligonucleotide for 3'- and 5'-RACE were designed from partial sequences analyzed in the present study from *R. speratus* hexamerin cDNA.

siRNA synthesis. Table 1 gives the sequences of oligonucleotides used for synthesis of siRNA to silence the hexamerin gene. The target sequence (5' -GAAGTAAGCACCATGTTTC-3') was designed using the siRNA Target Designer program (http://www.promega.com/siRNADesigner/program/). The target sequence (Hex-1, Table 1) and the complement sequence (Hex-4, Table 1) had T7 RNA polymerase recognition sequences (5'-TAATACGACTCACTATAGGG-3') plus random sequences (5'-GGATCC-3') appended to their 5'-ends, and TT onto their 3'-ends, respectively. Complement sequences (Hex-2 and Hex-3, Table 1) of these templates were prepared. siRNA was

synthesized using a commercially available kit (*in vitro* Transcription T7 Kit for siRNA synthesis, Takara Bio, Japan) and stored at –80°C in the RNase-free water provided with the kit. We minimized the possibility of non-target effects by choosing the target sequence with an e-value >0.7 by BLASTN search using the GenBank, EMBL, RefSeq and KEGG nucleotide databases; i.e. the target sequence of 19 nucleotides (Hex-1) had virtually no sequence similarity to any sequence in the nucleotide databases (Itakura et al. 2009).

RNAi. The synthesized siRNA of hexamerin (Table 1) was diluted in RNase-free water to 217.4 pg/nl for injection into worker and nymph termites. The siRNA (500 pg) was injected into the side of the thorax with an auto-nanoliter injector Nanoject II (Drummond Scientific Company, USA) with a custom-pulled glass needle and with a custom-built holder composed of multi-layered plastic tape with a hole in the shape of a rectangular solid (2 mm × 7 mm × 1 mm depth) covered with a pair of coverglasses to facilitate termite immobilization (Fig. 1). Controls were injected with an equivalent volume (2.3 nl) of RNase-free water.

Bioassays. After siRNA injection, replicated groups of 15 workers or nymphs were held within 9 cm diameter plastic Petri dishes on moistened filter paper for 30 days. Mortality and neotenic formation (ergatoid from worker or nymphoid from nymph) were recorded every day. Termites were observed with an SMZ1000 stereoscopic zoom microscope (Nikon, Japan) and the images were captured using a Handycam HDR-CX7 high-definition camcorder (Sony, Japan) equipped with an NY-VS adapter (Microscope Network , Japan). At assay days 1, 2, 4, 8 and 9, workers and nymphs were sacrificed and dissected into head, gut and decapitated, degutted body (referred to here simply as body) for RNA isolation and cDNA synthesis.

RNA isolation, cDNA synthesis and quantitative real-time PCR (qRT-PCR). All qRT-PCR primer sequences and Genbank accession numbers are given in Table 2. qRT-PCR was done with the Smart Cycler® II System (Cepheid, USA) with SuperScript™ III Platinum® two-step qRT-PCR kit with SYBR® Green (Invitrogen, USA). The cDNA used as the template for qRT-PCR was synthesized from total RNA of the head, gut, and body of a single worker or nymph. Total RNA was obtained with the SV total RNA isolation system (Promega, USA) followed by treatment with DNase using a TURBO DNA-free™ kit (Ambion, USA). The suitability of two reference genes, *β-actin* and *nicotinamide adenine dinucleotide-dehydrogenase* (*NADH-dh*), were evaluated, and *NADH-dh*, which had less variance, was chosen as the reference gene. Relative gene expression was determined as described by Livak and Schmittgen (2001). Means and 95% confidence intervals were determined by averaging the relative expression levels across three independent replicates. Mean expression levels were compared statistically by Student's *t*-test.

Results

Characterization of the hexamerin gene. Before RNAi, we had cloned and characterized full-length cDNA for hexamerin from *R. speratus* (*RsHex*) and submitted the sequence to the DDBJ/GenBank (accession number AB371986). Initially, partial cDNA fragments were obtained by PCR amplification using cDNA of *R. speratus* worker caste termites as template and primers designed from the nucleotide sequences of *R. flavipes* hexamerin (Zhou et al. 2006b). The complete sequence of cDNA was assembled by 3'- and 5'-RACE, as described in Materials and Methods. Sequence similarities were examined using the BLASTN programs (http://blast.genome.jp/). The nucleotide sequence of the cDNA is 98% identical with *R. flavipes* hexamerin I cDNA (accession number AY572858). The translated amino acid sequence for *RsHex* shares 97% amino acid identity with *R. flavipes* hexamerin I. RsHex has a predicted open reading frame of 688 amino acids, a signal peptide for transmembrane transport of 20 amino acids (MNTALLFATVVAVLVCGAFS), two conserved hexamerin signature motifs, ^{211}YFTEDVGL^{218} and ^{401}TALRDPAYYQ^{411} (Zhou et al. 2006b). The cDNA of *RsHex* had a polyadenylation site (^{2565}AATAAA^{2570}) and a poly(A) tail at the 3'-end, as well as multiple stop codons (2101–2103, 2173–2175, 2179–2181, 2320–2322, 2359–2361, 2386–2388, 2319–2321, 2467–2469, 2497–2499, 2554–2556 bp) in the 3'-downstream region of the first stop codon at 2065–2067 bp.

Effect of RNAi on hexamerin gene expression. The effect of RNAi on expression of the target and control genes was investigated by qRT-PCR in head, gut, and body sections of individuals sampled at 1, 2, 4, 8, and 9 days after siRNA injection. The results are reported as the proportion of gene expression in water-injected controls. The expression of *RsHex* in worker caste termites tended downward but the suppression of *RsHex* was not significant in head, gut, or body up to day 9 (Fig. 2).

In the body of nymph caste termites, expression of *RsHex* was increased significantly from 2 to 9 days after suppression at 1 day. An increase in *RsHex* expression was observed but it was not significant in the head or gut of nymphs (Fig. 3).

Phenotypic effects of hexamerin RNAi on caste differentiation. Fig. 4 shows the effect of RNAi on nymphoid differentiation for nymphs, which occurred earlier in nymphs injected with RsHex siRNA compared with the controls. The total number of newly emerged nymphoids was greater from nymphs injected RsHex siRNA compared with the controls. No ergatoid differentiation from workers injected with RsHex siRNA occurred during the 30 days of the assay (data not shown). The difference in mortality was not great between nymphs (Fig. 5), or between workers (Fig. 6) injected with RsHex siRNA compared with the controls.

Discussion

Hexamerin structure. Hexamerin-1 from *R. flavipes* is the closest homolog to RsHex (97% amino acid identity). Sequencing the full-length *RsHex* cDNA open reading frames revealed similarities and differences between *R. speratus* and *R. flavipes*. While most of translated protein sequence of RsHex (residues 1–665) and hexamerin-1 of *R. flavipes* were highly conserved, RsHex lacked a unique C-terminal hydrophobic region that has been found only in hexamerin-1 of *R. flavipes* among hexamerins sequenced (Zhou et al. 2006b). On the basis of the existence of a stop codon followed by multiple stop codons and a polyadenylation site plus poly(A) tail, cDNA of *RsHex* appeared to be adequately analyzed to the end of C-terminus. The hexamerin subunit precursor from *Blaberus discoidalis* (49% amino acid identity, accession number U31328), hexamerin-2 from *R. flavipes* (49% amino acid identity, accession number AY572859), and hexamerin-1 from *Perla marginata* (45% amino acid identity, accession number AM690365) are close homologs of RsHex. The RsHex protein is a moderately aromatic hexamerin with 6.5% phenylalanine and 9.0% tyrosine; the King and Jukes average is 4.0% and 3.3%, respectively (King and Jukes 1969). The methionine content of RsHex is 4.4%, while the King and Jukes average for methionine is 1.8%. Most other hexamerins have similar high contents of aromatic amino acids and methionine (Hagner-Holler et al. 2007).

Phenotypic effects of RNAi on caste differentiation. In the phenotypic experiments, we evaluated RsHex siRNA injected treatments and water injected controls. With our injection approach, the difference in mortality between siRNA-injected and water injected controls was small in both nymph and worker caste termites (Figs. 5, 6). The mortality of noninjected nymph caste termites was greater than that of the siRNA- and the water-injected nymphs (Fig. 5), while the mortality of noninjected worker caste termites was less than that of siRNA- and water-injected workers (Fig. 6). This difference in the mortality of noninjected nymphs and workers could be caused by their tasks in a natural colony, i.e. workers forage and feed themselves but nymphs are fed by workers. No pre-soldier differentiation from workers occurred in either RsHex siRNA-injected treatments or water-injected controls (data not shown). In *R. flavipes*, silencing hexamerin by injection of a mixture of siRNAs of various lengths prepared by digestion of ~500bp dsRNAs was reported to increase pre-soldier differentiation significantly relative to the water-injected controls (Zhou et al. 2006a). The difference in pre-sodier differentiation from workers by silencing hexamerin using siRNA between *R. speratus* and *R. flavipes* could come from the difference in siRNAs injected into the workers; i.e. a single siRNA for *R. speratus* but a mixture of siRNAs for *R. flavipes*, which might lead to off-target effects. Nymphoid differentiation from nymph occurred at day 3 after RsHex siRNA injection, while nymphoid differentiation occurred at day 17 in the water-injected controls (Fig. 4). The number of nymphoids differentiated from RsHex siRNA-injected nymphs was greater than that from water-injected nymphs throughout the assay period of 30 days. In *R. speratus* RsHex siRNA-injected nymphs, *RsHex* gene expression in body increased following the depression at day 1, as mentioned above. This finding suggests that increased hexamerin expression promotes nymphoid differentiation from nymphs in *R. speratus*, and supports the JH-sequestration hypothesis, in that the hexamerins are part of the mechanism that sequesters JH, thus preventing it from eliciting downstream effects on developmental gene expression (Zhou et al. 2006a).

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Table 1. Sequences of templates for siRNA synthesis and synthesized.

Template	Sequence (5' -3')
Hex-1	GGATCC-TAATACGACTCACTATAGGG-GAAGTAAGCACCATGTTTC-TT
Hex-2	AA-GAAACATGGTGCTTACTTC-CCCTATAGTGAGTCGTATTA-GGATCC
Hex-3	AA-GAAGTAAGCACCATGTTTC-CCCTATAGTGAGTCGTATTA-GGATCC
Hex-4	GGATCC-TAATACGACTCACTATAGGG-GAAACATGGTGCTTACTTC-TT
siRNA	5'-GAAGUAAGCACCAUGUUUC-UU-3' 3'-UU-CUUCAUUCGUGGUACAAAG-5'

Shaded box: target sequence, open box: complement of target sequence. Underline: T7 promoter sequence. Hex-2: a complement of Hex-1, Hex-3: a complement of Hex-4.

Table 2. Gene identities, accession numbers and qRT-PCR primer sequences.

Gene identity	Accession no.	Forward primer (5' - 3')	Reverse Primer (5' - 3')
Hexamerin	AB371986	CCCGCTCATGTTCTGGTATT	GGTGTGATGTTCAGGTGTGC
β-actin	DQ206832	AGAGGGAAATCGTGCGTGAC	CAATAGTGATGACCTGGCCGT
NADH-dh	BQ788175	GCTGGGGGTGTTATTCATTCCTA	GGCATACCACAAAGAGCAAAA

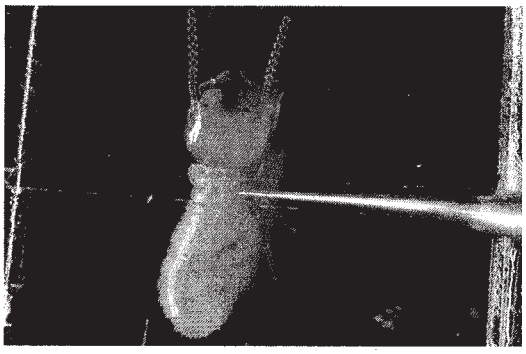

Fig. 1. siRNA injection into an *R. speratus* worker caste termite via the lateral thorax. The siRNA (500 pg, 2.3 nl) was injected with a micro-injector fitted with a custom-pulled glass needle and a custom-built holder composed of multi-layers of plastic tape with a hole in the shape of a rectangular solid (2 mm x 7 mm x 1 mm depth) covered with a pair of coverglasses.

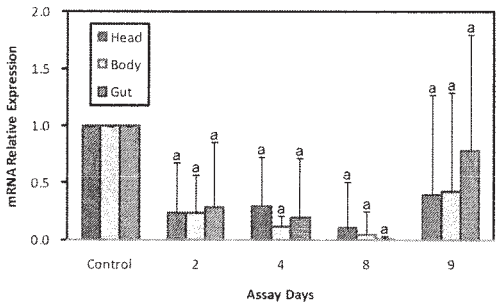

Fig. 2. Effects of RNAi in *R. speratus* workers after injection of siRNA for hexamerin silencing. The results are expressed as the ratio of hexamerin-gene expression in siRNA-injected individuals to that of the water-injected controls. Means labeled with the same letter within head, body, and gut are not significantly different (n = 3, P <0.05). Error bars represent the 95% confidence intervals.

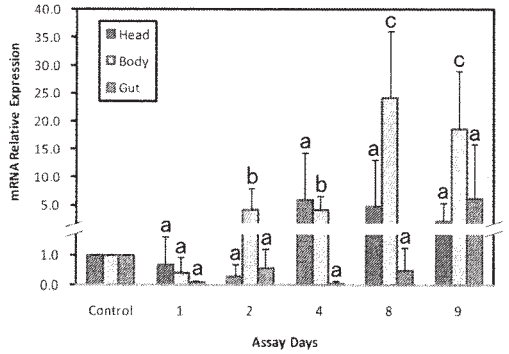

Fig. 3. Effects of RNAi in *R. speratus* nymphs after the injection of siRNA for hexamerin silencing. The results are expressed as the ratio of examerin-gene expression in siRNA-injected individuals to that of water-injected controls. Means labeled with the same letter within head, body, and gut are not significantly different (n = 3, P <0.05). Error bars represent 95% confidence intervals.

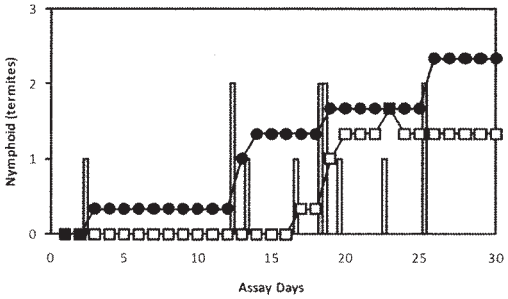

Fig. 4. Phenotypic effects of RNAi on nymphoid differentiation from nymphs. The average cumulative nymphoid induction after siRNA injection for hexamerin silencing (●, n = 3) and water injection as a control (□, n = 3). The shaded bar indicates the daily total of newly emerged nymphoids from nymphs that had been injected with siRNA for hexamerin silencing (n = 3), and the open bar indicates the daily total of nymphoids from the water-injected nymphs (n = 3).

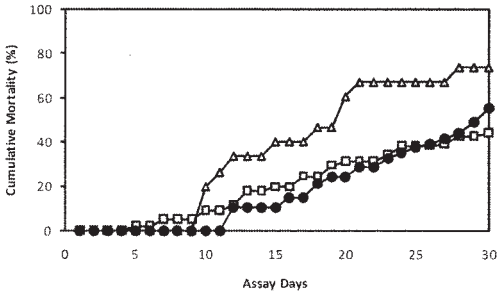

Fig. 5. The cumulative mortality of *R. speratus* nymphs injected with siRNA for hexamerin silencing (●, n = 3), water-injected (□, n = 3) and non-injected (Δ, n = 1) controls.

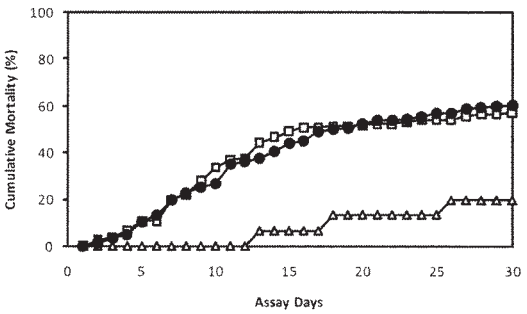

Fig. 6. Cumulative mortality of *R. speratus* workers injected with siRNA for hexamerin silencing (●, n = 16), water-injected (□, n = 6) and noninjected (Δ, n = 1) controls.

Genetic Diversity of the Formosan Subterranean Termite, *Coptotermes formosanus* Shiraki in Relation to the Distribution of Staphylinid Termitophiles

by

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Abstract

Coptotermes formosanus is assumed to be introduced to Japan before the 1600s, but believed to originate in the mainland China and Taiwan. To test the validity of this opinion, we examined the termite nests in Okinawa and Iriomote Islands for staphylinid termitophiles, the presence of which is supposed to be the evidence of the original places, and determined COII gene sequences of the termites. Incorporating sequences deposited in GenBank, we compared the genetic diversities among the mainland China, Taiwan, Japan and the U.S., where *C. formosanus* has been reported to be introduced in the 1900s. The haplotype and nucleotide diversities of the Japanese populations were usually as high as those in the mainland China and Taiwan and significantly higher than those of the U.S, suggesting that Japan as well as the mainland China and Taiwan is the original areas of *C. formosanus*. We did not necessary found staphylinid termitophiles in the termite nests in Iriomote Island, where its occurrence has been reported previously. On this basis, there is no inconsistency between the known distribution areas of staphylinid termitophiles and our suggested original areas (natural distribution areas) of *C. formosanus*.

Key words: COII gene sequence, haplotype, genetic diversity, the Ryukyu Archipelago

Introduction

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki is an invasive pest species and has been recently distributed to a wide range of zoogeographic regions, especially the United States. Historically the distribution of *C. formosanus* was restricted to the mainland China, Taiwan and Japan until the early 1900s when it was reported from Hawaii (Su 2003). In 1957, the first specimen was collected in the continental U.S., where this species has rapidly spread over south provinces (Su 2003).

In order to prevent further dispersion of the termite infestation in the U.S., the origin of introduction(s) and the route(s) have been studied by using molecular techniques. Jenkins et al. (2002) first inferred the origin of *C. formosanus* infestation in the U.S. by analyzing cytochrome oxidase subunit II (COII) gene sequences of the termites collected from the continental U.S., Hawaii and the mainland China. Although the reliability of the obtained COII sequences has been discussed elsewhere (Austin et al. 2006, Fang et al. 2008), the study showed the applicability of COII sequences for tracing the origin of the termite infestations. Austin et al. (2006) conducted an intensive sampling in the continental U.S. and analyzed a more comprehensive data set of COII sequences, showing two distinct lineages (haplotypes) spanning the continental U.S., Hawaii, Japan and the mainland China as well as the evidence of at least two introductions from the mainland China/Japan through Hawaii to the continental U.S.

Identifying the original areas/populations of *C. formosanus* may help us understand biological features of introduced populations (Vargo et al. 2003), and potential natural enemies/control agents (Kistner 1985, Iwata 2000). Fang et al. (2008) examined the termite populations in the mainland China, which has been supposed to be the center of origin (Kistner 1985; see below), and found a higher haplotype diversity of COII sequences than that in the U.S and recognized several unique haplotypes. It was expected from the theory that populations of endemic species have higher genetic diversity in the center of origin than introduced areas. Li et al. (2009) reported a high variation of COII sequences and a distinct sequence from southeast Taiwan, suggesting that Taiwan should be included in the original areas. Although Japanese populations of *C. formosanus* have been reported to be exotic (Su and Tamashiro 1987, Su 2003), the situation still remains unclear due to the lack of such a comprehensive analysis of COII sequences in Japan.

So-called termitophiles are animals that live in termite nests and depend on the termites, and Kistner (1985) has presumed that the presence of termitophiles in termite nests in a given area usually means that the termites have been there naturally for a long time. It was in 1982 that Kistner discovered in the mainland China the first *C. formosanus*-specific termitophile, which was a rove beetle (Staphylinidae) later named *Sinophilus xiae*, and pinpointed the center of origin of *C. formosanus* there (Kistner 1985). After Kistner (1985), Maruyama and Iwata (2002) found staphylinid termitophiles from the termite nests in the Ryukyu Archipelago and proposed the extension of the original areas. These findings, however, should be evaluated in connection with phylogeography of *C. formosanus*, because at least currently, there is no clear evidence that staphylinid termitophiles are not involved in a human-aided transportation of the host termites and that there are not non-secondary/-facultative relationships.

Here we observed nests of *C. formosanus* in Okinawa and Iriomote Islands for staphylinid termitophiles, conducted a comprehensive analysis of COII sequences of *C. formosanus*.

Materials and methods

Nests of *C. formosanus* (and wood infected by the termite) were collected from Okinawa and Iriomote Islands, which are located in the southern part of the Ryukyu Archipelago, in June and December 2009 and checked carefully for staphylinid termitophiles by hand-sorting. According to Maruyama and Iwata (2002), our efforts focused on finding *Sinophilus yukoae*. The staphylinids as well as some termite soldiers were preserved in absolute ethanol. Voucher specimens are maintained at Forest Ecology Laboratory, Graduate School of Agriculture, Kyoto University, Japan.

As described elsewhere (Fang et al. 2008), one individual of soldier termites from each nest was used for sequencing of COII gene. DNA extraction from the soldier heads and PCR were performed by using the Ampdirect kit (SHIMADZU) and the primers A-tLeu and B-tLys (Miura et al. 1998). We followed the manufacturer's suggested protocols with the modifications of 10 min-sonication before the lysis step and the utilization of Ex-Taq HS (TAKARA) instead of Nova Taq. The PCR conditions are as follows: an initial denaturation step of 95 °C for 1 min followed by 35 cycles of 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 1 min. The obtained PCR fragments were purified with Sephacryl S-400 HR (GE Healthcare) and used for sequencing reactions with the same primers used for the PCR with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were purified by using BigDye Xterminator (Applied Biosystems) analyzed with an automated sequence analyzer (ABI PRISM 3130xl Genetic Analyzer, Applied Biosystems).

For comprehensive comparisons with other COII sequences deposited in GenBank, the first 632 bp were used for analyses. From the 125 sequences found in the database, a total of 93 sequences were selected: AB109529 (Ohkuma et al. 2004), AB262474 (Noda et al. 2007), AB262501 (Kitade et al. unpbl.), AF107488 (Jenkins et al. unpbl.), AF525317 (Austin et al. 2002), AY027489 (Jenkins et al 2001), AY168204 (Ye et al. 2004), AY453588 (Austin et al. 2004), AY536403-07 (Ye et al. unpbl.), AY553136 (Lo et al. 2004), AY683212-17 (Jenkins et al. 2002), AY683218-21 (Jenkins et al unpbl.), DQ386170 (Austin et al. 2006), EF056702-39 (Fang et al. 2008), EF379940, 42-43 (Yeap et al. 2007), EU805758-72 (Li et al. 2009), and FJ384636-43, 45-48 (Yeap et al. 2009). The sequences of EF379941 and FJ384644 were excluded because of the presence of deletions, and the sequences of FJ870566-93, DQ493744 and FJ423459 were also excluded because the sampling locations were ambiguous or the lack of the information on hand. Since DQ386170 (Austin et al. 2006) represents 37 populations, a total of 129 populations were analyzed together with the

populations we examined here. We calculated haplotype diversity (h) and nucleotide diversity (π) by using DnaSP v5 (Librado & Rozas 2009).

Results and discussion

Genetic diversity of COII sequences

A total of seven nests of and one wood infected by *C. formosanus* were collected from the two islands (Table 1). COII sequences were retrieved from five populations. All the five sequences were identical with each other, regardless of occurrence of the staphylinids as well as nest locality. Incorporating the five populations, we made a data set consisting of a total of 134 populations, which have been collected from either the mainland China, Taiwan, Japan or the U.S. This data set included 14 haplotypes, and the mean haplotype diversity ($h \pm$ s.d.) was 0.721 (± 0.039), 0.417 (± 0.191), 0.484 (± 0.138), and 0.112 (± 0.059) in the mainland China ($n = 55$), Taiwan ($n = 9$), Japan ($n = 18$), and the U.S. ($n = 52$), respectively. The mean nucleotide diversity ($\pi \pm$ s.d.) was 0.00161 (± 0.00016), 0.00281 (± 0.00183), 0.00189 (± 0.00072), and 0.00042 (± 0.00023), in that order. Comparing by Welch's t-test with Bonferroni correction, the U.S. populations were significantly less diverse than the others ($P < 0.01$ for h, $P < 0.05$ for π), while the Chinese populations had significantly higher haplotype diversity than the others ($P < 0.01$). In addition, there were several unique haplotypes in Japan.

Table 1. List of *C. formosanus* populations examined in the present study.

Population	Locality	Date	Habitat	Termitophile
				Sinophilus yukoae
UR01CF	Okinawa Is., Nishihara	June 2009	Nest	-
IR00CF	Iriomote Is., Funaura	June 2009	Nest	+
IR06CF	Iriomote Is., Kura Riv.	June 2009	Nest	-
IR07CF	Iriomote Is., Kura Riv.	June 2009	Nest	-
IR08CF	Iriomote Is., Ohmija Riv.	December 2009	Nest	+
IR09CF	Iriomote Is., Ohmija Riv.	December 2009	Nest	-
IR10CF	Iriomote Is., Ohmija Riv.	December 2009	Nest	+
SK01CF	Okinawa Is., Naha	December 2009	Wood	-

Coptotermes formosanus is believed to have been introduced to Japan before the 1600s (Su and Tamashiro 1987, Su 2003); however, the present analysis resulted in a higher diversity of COII sequences in Japan compared to the introduced area (i.e. the U.S), and showed that the genetic diversities were well comparable to those in the mainland China and Taiwan (Table 3). In line with the logic employed in Fang et al. (2008) and Li et al. (2009), Japan as well as the mainland China and Taiwan should be the original places of *C. formosanus*. In reviewing the previous account, it is based on that the known oldest reliable record of *C. formosanus* in Japan is the German doctor's work written in 1712 (Mori 1987). The doctor stayed in Nagasaki, which was the solo port that foreign ships were allowed to enter at that time, between 1690 and 1692. On this basis, it was supposed that *C. formosanus* was transported by trading ships sailing from southern China to Nagasaki (Su and Tamashiro 1987, Su 2003), while Kistner (1985) had reported the first discovery of the *C. formosanus*-specific termitophile, *Sinophilus xiai* in the mainland China. Even if any older records of *C. formosanus* could not be found in Japan, the previous account should be tested scientifically.

This hypothesis that Japanese populations of *C. formosanus* originate from human-aided introduction(s) could be supported if the occurrence of a genetic bottleneck were detected, because the size of the immigrant population in exotic places is usually small. Although the termite population of Charleston in the U.S. has shown the evidence of a recent bottleneck as expected (Vargo et al. 2006), there was not detected any clear evidence for Nagasaki populations (Vargo et al. 2003). Vargo et al. (2003) attributed the lack of evidence to a sufficiently large number of generations that have passed after the introduction so as to eliminate detectable traces. Alternatively they suggested serial introductions from the mainland China or other areas to Nagasaki. Such multiple introductions have been reported to be associated with increased genetic diversity (Dlugosch & Parker 2008). The former, however, is clearly not inconsistent with the idea that Japan

is one of the original areas. Although several introductions not only to Nagasaki but also to the Japan main islands are well possible, the unique COII haplotypes in Japan would support a much longer history of *C. formosanus* than several hundred years in Japan.

Distribution of staphylinid termitophiles

Of the seven nests of *C. formosanus* we examined, four nests were inhabited by *Sinophilus* staphylinids. So far, two genera and four species of staphylinid termitophiles have been recorded from nests of *C. formosanus*. Their distribution areas range from southern China to the Ryukyu Islands (Iriomote, Ishigaki Iheiya, Yaku, Nakano and Suwanose Islands), possibly up to Japan main islands (Kistner 1985, Pace 1998, Maruyama & Iwata 2002). Although genetic analysis of the termite populations has resulted in that Taiwan is one of the original places of *C. formosanus* (Li et al. 2009), Kistner (1985) reported that it is unlikely that *C. formosanus* originated there, because he could not find any termitophiles from several nests of the termite. Our observations, however, indicate that termitophiles are not necessarily found in the termite nests even where they occur (Table 1); thus, there is apparently no inconsistency between the logics derived from genetic analyses and staphylinid termitophiles.

Origin and phylogeopraphy inference

Combining our results with the inferred phylogeopraphy for some other termite species currently distributed in Japan (Maekawa et al. 1998, Park et al. 2006), a possible phylogeopraphical of *C. formosanus* can be proposed as follows: *C. formosanus* extended their distributions northwards along the land-bridges that connected the mainland China, Taiwan, the Ryukyu Archipelago and the Japan main Islands in the Pleistocene, and the populations were later separated into the current regions and isolated from each other by straits as a result of elevated sea level. These dispersal processes are in agreement with the termite fauna of Japan being a subset of those of Taiwan and the mainland China (Sugio & Yamada 2010). On this basis, the areas termites are naturally distributed are the original areas, while the higher haplotype diversity in China (see above) than those in Taiwan and Japan may mean that *C. formosanus* evolved in China.

Conclusions

Our results suggest that Japan as well as the mainland China and Taiwan is the original areas of *C. formosanus,* on the basis of the relatively high genetic diversities of the populations compared to those of exotic populations (the U.S. populations) as well as the sympatric distribution of staphylinid termitophiles and the termite populations with high genetic diversities.

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The Resistance of Areca Palm (*Areca catechu* L.) Trunk against Subterranean Termite Attack

by

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Abstract

Areca palm (*Areca catechu* L.) is a kind of palm crops that commonly found in Indonesia and it used for many purposes. This study objective was to evaluate the resistance of Areca palm trunk against subterranean termite attack in graveyard test based on height and depth of the trunk. The test was conducted for 3 months and weight loss of specimens attacked by *Macrotermes gilvus* Hagen was determined. The result showed that weight loss increased from the outer to inner part along the trunk height. In general, the outer part was more resistance than the central and inner part. Almost all of the part of the trunk belonged to level V (very weak resistance), only the outer part in the bottom of the trunk belonged to level II (strong resistance).

Keywords: resistance, areca palm, graveyard test, subterranean termite

Introduction

Areca palm or areca nut palm (*Areca catechu* L.) is one of the palm crops found throughout Indonesia mainly in Sumatera Island. It was found both individually and in the population, commonly planted as a plant of garden fence or barrier. It has many uses e.g. for food, cosmetic, medicine and dye materials (Novarianto and Rompas, 1990; Staples and Bevacqua, 2006 in Maskromo and Miftahorrachman, 2007). And also the trunk has been used as a raw material for building, bridge and water pipe (Sihombing, 2000).

Since areca palm trunk is used as a building material, it needs to study its resistance against subterranean termite attack. Subterranean termite is the most important wood destroyer organism in tropical region including Indonesia. One of them is *Macrotermes gilvus* Hagen.

This paper presents part of a series of studies of areca palm trunk properties. This study objective was to evaluate the resistance of areca palm trunk against subterranean termite attack in graveyard test based on height and depth of the trunk.

Materials and methods

The areca palms for this study were taken from Binjai, North Sumatera, Indonesia. The specimens were cut from three sections of trunk height and trunk depth with 3 replications, as shown in Figure 1.

The dimension of specimen was 2 cm in width and thickness and 25 cm in length. The specimens were tested for resistance to subterranean termite attack in graveyard test. All specimens were planted vertically in the soil at the Campus of University of Sumatera Utara, Medan, Indonesia for 3 months. Before and after graveyard test, the specimens were dried in the oven at 103 ± 2° C for determining of weight loss. The weight loss values of specimens were compared to resistance level (Table 1) according to Indonesian standard of test of wood and wood products against wood destroyer organism (SNI 01-7207-2006).

Table 1. Resistance level of wood against termite attack based on weight loss

Level	Resistance	Weight loss (%)
I	very strong	< 3,52
II	strong	3,52 – 7,50
III	moderately strong	7,50 – 10,96
IV	weak	10,96 – 18,94
V	very weak	18,94 – 31,89

Source: SNI 01-7207-2006

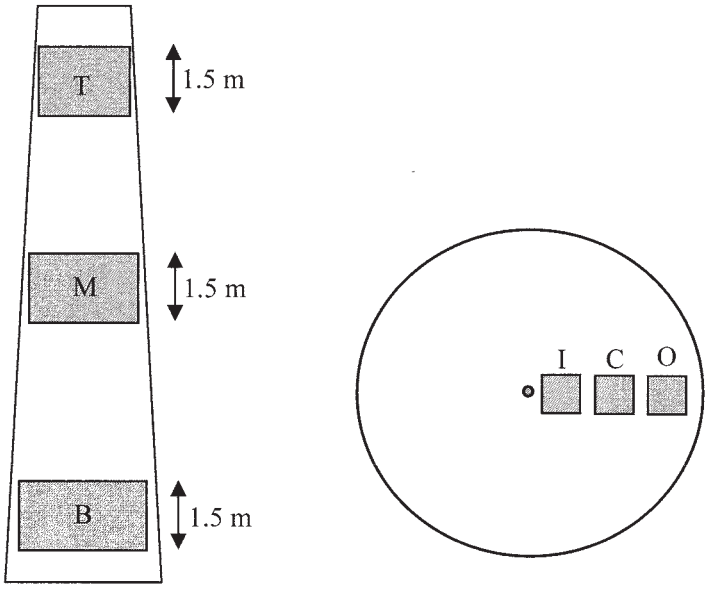

Figure 1. Location of specimens based on (a) height (where B is bottom, M is middle and T is top of the trunk) and (b) depth (where O is outer, C is central and I is inner at transverse sectional view) of the trunk

Results and discussion

The average value of weight loss of specimens after graveyard test for 3 months ranged from 4.82 to 85.83% as shown in Figure 2.

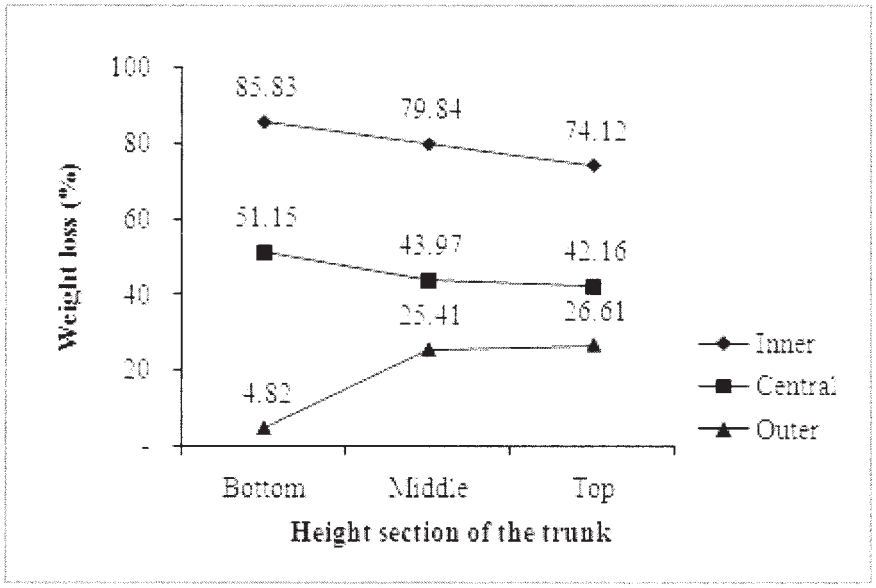

Figure 2. Weight loss of specimens based on height and depth of the trunk

Based on depth of areca palm trunk, the inner part had the highest value of weight loss along the trunk height. Weight loss increased from the outer to inner part along the trunk height. Meanwhile, weight loss of the inner and the central part decreased from the bottom to the top of the trunk but the outer part showed different trend. In general, the outer part was more resistance than the other parts.

Based on height of the trunk, the average values of weight loss of bottom, middle and top section were slightly different i.e. 47.27, 49.74 and 47.63%, respectively. According to the resistance level (Table 1), almost all of the part of the trunk belonged to level V (very weak resistance), only the outer part in the bottom of the trunk belonged to level II (strong resistance).

The previous study showed that the outer part consisted high content of vascular bundles. Vascular bundles is essential for supporting the structural features. Vascular bundles decreased from the outer to the inner part of the trunk. Whilst parenchymatous tissue increased from the outer to the inner part. The inner part consisted high content of parenchyma cells and moisture. Therefore, the outer part was the hardest part of the trunk, and on the contrary to the inner part. Further, the density values decreased from the outer to the inner part. The outer part density ranged from 0.62 to 1.07 gr/cm^3 whilst the inner part density ranged from 0.12 to 0.14 gr/cm^3 (Trisnawati, 2009).

As a monocotyledons species, the areca palm does not have cambium, secondary growth, growth rings, ray cells, sapwood, heartwood, branches and knots. Studies on other monocotyledons species such as coconut wood and oil palm showed that some properties were the same with its properties. A study by Wardhani *et al.* (2004) reported that the end and the inner part of coconut trunk had the highest content of sugars and starches. Soepijanto (2004) stated that the bottom part of coconut trunk had better strength and durability compared to the inner and the end part. A study by Bakar et al. (1999) reported that that oil palm wood belonged to level V, very weak resistance against attacks of wood destroyer.

Therefore, the attack level of subterranean termite (*Macrotermes gilvus* Hagen) for the inner part more severe than the outer part. It relates to anatomical and physical properties and chemical compounds along the trunk of areca palm.

Conclusions

Weight loss increased from the outer to inner part along the trunk height. In general, the outer part was more resistance than the other parts. Based on resistance level, almost all of the part of the trunk belonged to level V (very weak resistance), only the outer part in the bottom of the trunk belonged to level II (strong resistance).

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The Resistance of Albizia Wood from Timber Estate against Termite at Various Level of Tree Ages

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Abstract

Resistance of particular wood species depends on various kinds of factors such as extractive content, wood specific gravity, tree ages at the moment of its cutting/feeling, growth rate of tree, origin of tree from natural forest or plantation forest, tree species variety, and environment factor, like temperature and humidity, which in all can affect wood durability. This research aimed to look into wood resistance against dry-wood termite and subterranean termite, based on levels of tree ages. Wood species as used was albizia (*Paraserianthes falcataria* (L) Nielsen) species with 5 levels of its tree ages, i.e. 5 years, 6 years, 7 years, 9 years and 10 years. The size of albizia wood sample for dry-wood termite and subterranean termites in laboratory experiment measured consecutively 5 cm x 2.5 cm x 2 cm and 2.5 cm x 2.5 cm x 0.5 cm.

Laboratory results revealed that the resistance of albizia wood from all of five ages level at 5 – 10 years, against dry-wood termite belonged to class IV (Indonesia National Standard 01-7207-2006). However, the albizia wood against subterranean termite belong into class V. It is necessary to preserve albizia wood prior to be used.

Key word: albizia wood, dry-wood termite, subterranean termites

Introduction

The success of Indonesia's plantation forest (IPF) is not unrelated with its management uses. There are three kinds of IPF wood uses that comprise merchant wood, fiber wood, and energy wood. Each of those uses requires different specific criteria. For merchant wood, as example, it will be preferred woods with characteristics such as attractive decoration, ease in working, high strength and dimensional stability, and effective resistance against wood-destroying organisms (Anonim, 1992). Establishment of IPF actually has been started since 1985. Then it is assumed that there must have been IPF trees with ages of about 20 years. According to Karnasudirdja and Kadir (1989), tree rotation of IPF's wood merchant ranges about 10-30 years. In the past, IPF which was established in Indonesia, was intended to meet the needs of raw material for pulp and paper industries. However, with appropriate management and handling, production of IPF's wood besides being allocated for pulp/paper industries, can also comply with the raw material needs for merchant-wood industries (Anonim, 1992).

Recently, ini Indonesia, albizia/sengon wood (*Paraserianthes falcataria*) has been being used more intensive by making the species for various products both of solid and composite. The wood does not as secondary material anymore which is used in wood industry, even the species is as main wood material for industry. Nevertheles, due to the species is produced from forest plantation and belong to fast growing species, the species is still hesitated because of the weakness of the properties. Commonly, albizia wood is harvested on young age relatively as well as various ages in the cutting. Therefore, research on basic properties, included the resistance against termite, should be done due to values of particular wood species are determined by, not only physical and mechanical strength, but also by the resistance against wood-destroying organisms. Related with such, various wood species afford different resistances against kinds of organisms that attack. Even, in the same wood species, its resistance is not similar against different organisms, i.e. dry-wood termites and subterranean termites. Besides, resistance of particular wood species is affected by age of tree, when it is felled; growth rate of trees; tree origin whether it is from natural forest or from plantation forest; and physical-chemical properties of wood itself. All those factors can significantly affect wood resistance and hence its durability (Martawijaya, 1996).

Albizia wood presents one of the several species used for IPF establishment, and its trees are planted much by the community as well as by the company. The species wood has been used a lot as raw material for plywood, furniture, and hand-craft items. In general, natural durability of albizia wood belongs to class IV/V, it means not durable (Martawijaya *et al*, 2005; Seng, 1990); however, it is not yet know definitively whether each of the various ages of its trees afford as similar resistance against particular organism as it is against another organism. In relevance, therefore, it is necessary to conduct research regarding resistance of albizia wood against two different wood-destroying organisms, i.e. dry-wood termites and subterranean termites (both in the laboratory test). The result of this research will remind to alert on the uses of the wood species.

Materials and methods

Materials

Main material as used was wood of albizia/sengon (*Paraserianthes falcataria* (L) Nielsen) species with 5 levels of its tree ages, i.e. 5 years, 6 years, 7 years, 9 years and 10 years. This wood was procured from Ciamis, West Java. Meanwhile, the test organisms were dry-wood termites (*Cryptotermes cynocephalus* Light.) and subterranean termites (*Coptotermes curvignathus* Holmgren).

Methods

Preparing test wood specimens

Albizia wood at five age levels was each cut to test specimens measuring 5 cm in length x 2.5 cm in width x 2 cm in thickness (for resistance test against dry-wood termites), and measuring 2.5 cm in length x 2.5 cm in width x 0.5 cm in thickness (for resistance against subterranean termites).

Specimens of albizia wood which had been prepared in their intended sizes (as specified above) were used in the test for the resistance against those two organisms (dry-wood termites and subterranean termites). The testing procedures were referring to those of modified ASTM (Anonim, 1995) and Indonesian National Standard (SNI) No. 01-7207-2006 (Anonim, 2006).

Wood resistance against dry-wood termites

Five albizia-wood specimens for each of the five age levels (5, 6, 7, 9, 10 years) were prepared with sizes specified for the test against dry-wood termites (5 cm x 2.5 cm x 2 cm) and then placed in contact with a glass tube (3 cm in height and 1.8 cm in diameter), one specimen for one glass tube. Wood specimen was placed horizontally under the glass tube vertically installed on it such that the larger surface of specimen was in contact with the lower mouth (hole) of glass tube. In other to make the contact between lower glass-tube and upper specimen-surface airtight for the possible small air-spaces, those spaces was sealed using wax. Afterwards, inside the glass tube were inserted as many as 50 dry-wood termites of the worker type. In this way, therefore, the termites could not move out of the glass tube during the test. Instead, the termites would eat wood specimens thereby leaving a kind of tunnels on the specimen surface. This test arrangement was allowed to proceed for 12 weeks. In this test, those five albizia-wood specimens as described previously were regarded as replicates.

After 12 weeks, the examination or assessment was carried out on the wood specimens covering percentage of wood-weight losses, percentage of survival termites, and degree of attack by termites. The data were used to determine the resistance class of albizia wood with various age levels using the classification of wood resistance as described in Table 1 (Anonim, 2006). In addition, to assess the degree of attack by termites, the related criteria by the AWPA were also consulted (Anonim, 1972).

Table 1. Classification of wood resistance against the attack by dry-wood termites (*Cryptotermes cynocephalus* Light)

Class	Resistance criteria	Weight loss (%)
I	Very resistant	< 2.0
II	Resistant	2.0-4.4
III	Moderate	4.4-8.2
IV	Poor	8.2-28.1
V	Very poor	> 28.1

Source: Anonim (2006)

Wood resistance against subterranean termites

Five albizia-wood specimens for each of the age levels were also prepared with sizes specified for the test against subterranean termites (2.5 cm x 2.5 cm x 0.5 cm) and then placed into the glass bottles, one specimen for one bottle. Inside the bottle, the wood specimen was places lengthwise rather vertically such that one of the widest specimen-surface leaned against the inner wall of the bottle. Further, into the bottle was put 200 grams of wet sand with moisture content of 7% (below its water-holding capacity). Subsequently, into the bottle were put as many as 200 healthy, active subterranean termites (*Coptotermes curvignathus* Holmgreen). Afterwards, the arrangement test (i.e. wood specimen, wet sand, subterranean termites, and bottle) was stored in dark room and then let for 12 weeks. In this test similar to dry-wood termites, those five albizia-wood specimens were also regarded as replicates.

After 12 weeks, the examination or assessment was carried out on the wood specimens covering also percentage of wood-weight losses, percentage of termite survival, and degree of attack by termites. The data were used to determine the resistance class of albizia wood with various age levels using the classification of wood resistance as described in Table 2 (Anonim, 2006). In addition, to assess the degree of attack by termites, the related criteria by the AWPA were consulted as well (Anonim, 1972).

Table 2. Classification of wood resistance against the attack by subterranean termites (*Coptotermes curvignathus* Holmgreen)

Class	Resistance criteria	Weight loss (%)
I	Very resistant	< 3.52
II	Resistant	2.52-7.50
III	Moderate	7.50-10.96
IV	Poor	10.96-18.94
V	Very poor	18.94-31.89

Source: Anonim (2006)

Wood resistance against subterranean termites using the field test

Albizia-wood specimens for each of the age levels (17-28 years) were prepared with sizes specified for the field (graveyard) test against subterranean termites (2.5 cm x 2 cm x 2 cm) and then buried vertically, with the upper portion about 5 cm below the soil-ground surface. The burial was carried in Bogor, and lasted for 3 months. Afterwards, examination that comprised wood-specimen failure was carried out the modified ASTM procedures (Anonim, 1995).

Results and discussion

In detailed scrutiny, resistance of test albizia wood specimens against dry-wood termites appeared to be significantly different from that against subterranean termites, as described in the following.

Resistance Against Dry-Wood Termites in Laboratory Test

In laboratory test, resistance of test albizia wood specimens against dry-wood termites was assessed according to each age level of those specimens. Results of the resistance test (i.e. survival termites, weight loss, and degree of attack) that lasted for 12 weeks are presented in Table 3.

Table 3. Weight loss, survival termites, and degree of attack on test albizia wood specimens due to the attack by dry wood termites

No	Age levels of wood specimens (years)	Weight Loss (%)	Resistance class	Survival of termites (%)	Degree of attack	
					K (%)	T
1	5	10,34	IV	37.8	24,5	C
2	6	10,14	IV	24.4	20,5	C
3	7	9,73	IV	36.0	21,0	C
4	9	10,00	IV	26.8	17,8	C
5	10	9,74	IV	32,0	20	C

Remarks: K = wood defect; T = level

Referring to the test as above (Table 3), there were differences as well as similarities in albizia wood resistance based on age levels. All of ages of albizia woods belong to class IV, based

on weight loss (Anonym, 2006). This is because all weight losses for those ages were in the range of 8.2-28.1%.

Wood resistance, in this regard albizia species, against dry-wood termites can also be specified by the degree of attack and percentage of survival termites (Table 3). For albizia wood specimen at 5-year age, it revealed the highest degree of attack, i.e. 24.5% compared to the others at ages with the lowest degree of attack (17.8%) at 9 years, but have the same level regarded as C. In addition, in order to scrutiny resistance of albizia woods at their different age levels, it can also be assessed from the percentage of mortality termites. In albizia wood with 5 year age, percentage of mortality termites was the lowest (62.4%), while the highest percentage were belonged to 6 and 9 years old revealed 75.6% and 73.2%.

Based on the results, confirmed that the higher of the tree age, the resistant of the wood species, although the resistance class did not increase. The increasing of resistance suspected by the increasing of extractive content in older wood. Martawijaya (1996), stated that extractive content determines wood resistance toward wood destroyer organism.

Resistance Against Subterranean Termites in Laboratory Test

Assessment on resistance test against these termites and parameters as used were similar to those as previously described in the test against dry-wood termites, both in laboratory tests. Results of the resistance test that lasted for 6 weeks are shown in Table 4.

Table 4. Weight loss, survival termites, and degree of attack on test albizia wood specimens due to the attack by subterranean termites

No	Age levels of wood specimens (years)	Weight Loss (%)	Resistance class	Mortality of termites (%)	Degree of attack	
					K (%)	T
1	5	42,59	V	24,45	89	D
2	6	33,31	V	37,3	80	D
3	7	38,16	V	30,85	76,5	D
4	9	40,72	V	27,65	87	D
5	10	35,77	V	28,85	76	D

Remarks: K = condition of test wood specimens; T = level

Viewing results of the test (Table 4), there are not differences in albizia wood resistance class or degree of attack based on age levels, althogh the weight loss or K value for each age level were different. Nevertheles, the weight loss and degree of attack percentages are still in the range with very poor grades. Albizia woods with all of age levels revealed similar resistance, and therefore at both ages, they could be judged belonging to class V (level D), and it also conformed to the SNI (Anonym, 2006).

Similar to the cases for dry-wood termites, wood resistance of albizia species against subterranean termites could also be specified by the degree of attack and percentage of living-termites or the mortality (Table 4). It could be indicated that albizia wood with ages of 5 years and 9 years sustained the highest degree of attack, i.e. 89% and 87% (ludged as level D. Besides, resistance of wood albizias at their different age levels could also be determined by the portion (percentage) of survival termites (Table 4). Albizia wood with ages 6 years revealed the highest survival termites, followed in increasing order by albizia woods with ages consecutively 7 years, 10 years, 9 years, and 5 years (as the lowest). In all, this again confirmed that there are not different resistance group of albizia wood against subterranean termite attack.

Conclusions

Resistance of albizia wood against the attack by dry-wood termites in laboratory test was not affected by the tree ages. All of the age, wood resistance belonged to class IV. Resistance of albizia wood against the attack by subterranean termites in laboratory test also was not affected also by albizia tree ages. For all tree ages level, wood resistance belonged to class V. The result confirms that it is important to preserve albizia wood before used.

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Dry Wood Termite and Subterranean Termite Test for Natural Durability of Six Species of Indonesia Wood in Laboratory and Graveyard Tests

by

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Abstract

This experiment about natural durability of six species of Indonesia wood have been done in Laboratory and field of Forest Product Technologi Faculty of Forestry, Bogor Agricultural University. The experiment was conducted for six species of wood: jati (*Tectona grandis*), sengon (*Parasianthes falcataria*), balsa (*Ochroma* sp.), mindi (*Melia azedarach* L), pinus (*Pinus merkusii*) and kamper (*Dryobalanops* sp.), every species was repeated 3 replication. These experiment contain of two methods: the first in Laboratory test and the second in graveyard test.

The result of this study: 1. In Laboratory test: the highest mortality was goten from sengon (*P. falcataria*) (84 %) and the highest of wight loss in kamper (*Dryobalanops* sp.) (7.087 %). 2. In graveyard test, the highest of wight loss found in balsa (*Ochroma* sp.) (62.24 %).

Keywords: natural durability, termite, six Indonesia wood

Introduction

Wood is a material of natural that many used as construction materials for humans need, this is caused by wood properties with many advantage, such as: strong, easy works, renewable, easy re-decomposition, but wood also have properties un-advantage, one of there, wood very easy degradation by organism and its properties different each other (between species, between tree and in stem).

Natural durability of wood is durability wood species for microorganism like: fungi, insect and sea worm (Hunt dan Garrat, 1986). Toxicity ecstractive to organism wood decay selective, likely one species for fungi not yet resistance to other invasion species .

All of these have been done research for natural durability of six species of fast growing woods at laboratory and field test, with goal for determine resistances to termite (dry wood termite, and subterranean termite).

Materials and methods

Wood species for laboratory test were: pinus (*P. merkusii)*, jati (*T. grandis*), kamper (*Dryobalanops* sp.), balsa (*Ochroma* sp.), mindi (*M. azedarach* L.) and sengon (*P. falcataria*), and for graveyard test is: pinus (*P. merkusii),* balsa (*Ochroma* sp.) and sengon (*P. falcataria*) wood. Material wood for graveyard-test with size: 5 cm x 5 cm x 50 cm, and material wood for laboratory-test with size 5 cm x 2 cm x 2 cm. Samples are not different among heartwood, sapwood, and distance mid or tip. Each wood sample was exposed to 50 drywood termites (45 workers and 5 soldiers) in the laboratory test, and to subterranean termites in the graveyard test.

Natural durability test

Indicator for laboratory test is loss t material test and mortality termite after 1 (one) month trap, and indicator for field test after 2 (two) month with scoring.

Laboratory test

Material wood for laboratory tests, first moisture water 12 – 14 %. Each material test to determine moisture water, then material into in boxes test from glass with measured: 6 cm x 3 cm x 3 cm. Sebanyak 50 species dry wood termite with 45 species workers and 5 species soldier in to boxes test with material test wood, and then close keep with soft paper and places in dark places. One month later, mortality test for termites doing and loss weight material test with formula:

$$\text{Mortality (\%)} = [(\text{summary termite die})/(\text{summary total termite})] \times 100$$

Loss weight (%) = [(W1 – W 2)/ W1] X 100, where: W1 = weight of material test oven drying before application and W2 = weight of material test oven drying after application

Durability test classes indicator based loss weight sample, calculate with classification according to Sornnuwat *at al*, 1995 *in* Febrianto *et al* (2000), like at Table 1.

Graveyard test

Material test buried with distance 30 cm, collecting data after 2 (two) month application with scoring at Table 2.

Tabel 1. Termite resistance classification

Weight loss (%)	*Resistance classes*
0	High durability
1 – 3	Durability
4 – 8	Low
9 – 15	Weak
> 15	Very weak

Table 2. Standard damage material test for graveyard test

Gradea	Condition material test	Value
A	Wood still good (no damage)	0
B	With light feed termite	1 – 20
C	Low damage, like canal small and thin	21 – 40
D	With high damage, like canal small and thin	41 –60
E	Over 50 % wood feed by termite	61 -80

Results and discussion

Laboratory test

Weight loss

Loss weight material data and mortality termite from laboratory test can see at Table 3. Loss weight material test and mortality termite result from laboratory can see at Table 3, respectively. From Table 3 loss weight material tests for six wood not different, and relatively same for pinus, jati, balsa, mindi and sengon is **"durability"** with loss weight between 1.109 % - 1.858 % except for kamper wood into is "**medium durability**" with loss weight 7.087 %.

Table 3. Average loss weight material test six fast growing wood and mortality termite from laboratory test (%)

Species	Loss weight	Classes durability	Mortality
Pinus	1.689	Durable	34
Jati	1.290	Durable	54
Kamper	7.087	Medium durable	34
Balsa	1.204	Durable	12
Mindi	1.858	Durable	62
Sengon	1.109	Durable	84

Commonly explained that durability wood very determination of extractive. Fengel dan Wegener (1995) explained that extractiv in wood many vary quantity, used and function, that is determined by variety species and places. Many extractiv function like: poison, color compound, specific smell, protein to attractive for destroyed bioorganism and common extractive is non-function in wood. Natural durability of wood caused many factor that focused internal dan exsternal factor from wood.

Internal factor is chemical compound and physic properties. Chemical compound of natural wood like; celulose, hemiselulosa, lignin and exstractiv directly can wood to weak or contrary (dislike) by destroy organism, whatever in physically wood is places organism habitat and growth. Extractive and durability of wood depend on concentration compound that poison in wood that formed in growth heart wood from sapwood. Extractive group fenolic, alkaloid, flavonoid, saponin and steroid/triterpenoid with at concentration make function for anti-fungi, anti-microorganism, and anti-viruses etc. (Syafii, 1996, Harbone,1987 in Syafii, 1999).

External factor can influence durability factor is environment (habitat, used factor and organism) where determine by factor climatology: temperature, humidity, oxygen, CO2, pH, moisture water content of wood etc. Padlinurjaji (2003) explained that wood composition (cellulose, hemi cellulose and lignin) its to influences of growth fungi moisture water wood, oxygen enough and weather. Hunt and Garrat. (1986), correspondent that optimum temperature for growth fungi different for every species, but commonly between 75^0C and 90^0 F. Durability wood so determine by factor organism, because species wood durability with one organism, not yet resistant to organism other, on the other hand toxicity extractive for organism selective (Martawijaya, 1983)

Mortality termite

Indicator durability wood is mortality termites after application with term period one (1) month. Results mortality termites six species wood can see at Table 3, that tend to mortality termites vary between from 12 % - 84 %, low in balsa (12 %) and high in sengon (84 %). Mortality termites high in sengon, prediction not caused by durability wood in high but by any factor that un-controlled, Martawijaya (1983) declare that sengon wood in to classes low durability (durability class III), for further see Figure 1.

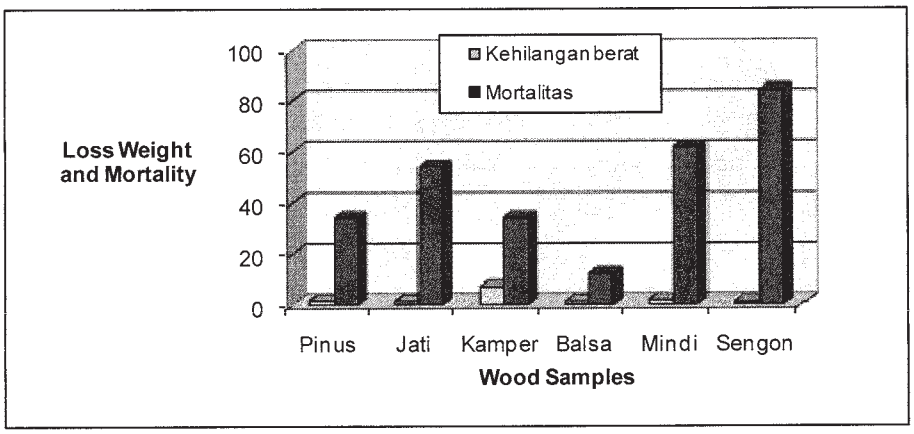

Figure 1. Weight loss of six species of wood samples and termites mortality in laboratory test

Graveyard Test

The data was gained in graveyard test was quantitative data, it was scoring data from against by termite from some species of wood have taken in field. Scoring data from graveyard test shown in Table 4.

Table 4. Means weight loss three species of wood samples in Graveyard Test

Species	Replications			Means
	I	II	III	
Balsa	80.86	96.493	9.57	62.24
Pinus	8.662	2.460	6.307	5.809
Sengon	13.993	27.152	11.166	17.437
	34.505	42.035	9.014	

In Table 4. Shown that the all species with grave under ground for two months was against by subterranean termite, weight loss between species was vary, The highest weight loss was balsa species (62.24 %) and than followed by sengon (17.437 %) and pinus (5.809 %). This is tend to level of weak six species wood for disturb of subterranean termite.

Highly defensive termite to balsa wood compare sengon and pinus wood, prediction caused by extractive in balsa wood not toxic for subterranean termite, see Figure 2.

Conclusions

1. In laboratory test, species wood *kamper* (*Dryobalanops* sp.) loss weight is 7.087 % grouping in low wood, other spescies: pinus (*P. merkusii*), *jati* (*T. grandis*), *balsa* (*Ochroma* sp.),

mindi (*M. azedarach* L.) and sengon (*P.falcataria*) grouping in good wood loss weight between 1.109 % - 1.858 %.

2. Mortality termites hight at sengon (*P.falcatari*) (84 %), and than mindi (*M. azedarach* L.) (62 %), jati (*T. grandis*) (54 %), pinus (*P. merkusii*) and low kamper (*Dryobalanops* sp.) (34 %).

3. Test in field wood *balsa* (*Ochroma* sp.), loss high with scoring 62.24 % and folowing sengon (*P.falcataria*) (17.437 %) and pinus (*P. merkusii*) (5.809 %).

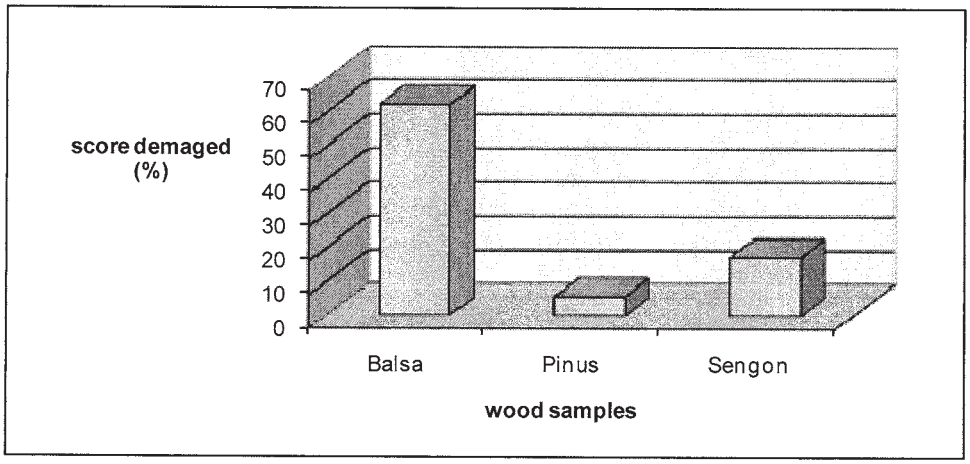

Figure 2. Weight loss three species of wood samples with graveyard test

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The Natural Durability of Several Wood Species against Subterranean Termite in Indonesia and Malaysia

by

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Abstract

Durable woods from natural forest are in limited amount now because of illegal logging, and forest land conversion due to oil palm plantation. On the other hand, many types of lesser use and lesser known woods are still in plentiful supply in the tropical forest, and potential to be new timber materials. The purpose of this study is to evaluate the durability of lesser use and lesser known against termite in laboratory test. The result indicated that lesser use wood species, *Sandorikum koetjape* (Kecapi), was more durable against termaite compared to other species in this experiment. The natural durability of wood species caused by most extractive content compared to other components of wood.

Key words: natural durability, tropical wood, *Coptotermes gestroi*, JIS Standard.

Introduction

The increasing of human population have increased the demand for timber as building material, house and office develoments, etc. Nowadays, wood still play an important role as building materials because of their advantages over other synthetic and natural materials. These include renewable resources, ease to work, easily joint, available in many forms and sizes, can utilized as isolator on dried weather and also have high aesthetic value. In Indonesia, these building timber normally come from wood species with high natural durability, and have good persistence towards weather, patogen microbe, and insect attacks. However, durable woods from natural forest are in limited amount now because of illegal logging, and forest land conversion due to oil palm plantation. On the other hand, many types of lesser use and lesser known woods are still in plentiful supply in the tropical forest, and are potentially new material timbers. Unfortunately, their durability had not been studied, especially with regards against termite attacks. Based on these background, the research on natural durability of lesser use and lesser known woods in Indonesia, Malaysia and Thailand is urgently required.

Objectives

The durability test against termite will be conducted in the laboratory, jointly between LIPI (Indonesia), USM (Malaysia) and Royal Forest Department (Thailand). Five types of wood samples will be taken from each country and will be tested to several termite species which are destructive to buildings and structures in Indonesia, Malaysia and Thailand.

Materials and methods

Materials

Each wood species with sized 2 cm x 2 cm x 1 cm (R x T x L) were cut from log with separated by sapwood part and heartwood part, except for teak wood. Sample from teak wood was use only from heart woiod because did not have sap wood on teak. Termite Species was use in this experiment is *Coptotermes gestroi* WASMANN

The Standard Methods of TermiteTest in Indonesia.

The Standard Methods for TermiteTest in Indonesia *was according to Japan Industrial Standard (JIS) No JIS. K 1571 , 2004*. The test blocks were subjected to subterranean termites *Coptotermes gestroi* with forced-feeding test. One each of the untreated and treated test blocks were placed at the bottom of a test container, an acrylic vessel with one end sealed with hard plaster of Paris to form a 5 mm thick bottom (Fig.1). One hundred and fifty workers of termite were subjected to the forced feeding test. Fifteen soldiers were introduced together with workers in the container. The containers were gathered in a large covered case with moist cotton wool at the bottom to keep the container

humid. This case was kept at room temperature in dark. The test continued for 3 weeks. Termite mortality (TM) and weight loss (WL) were recorded after finished the test.

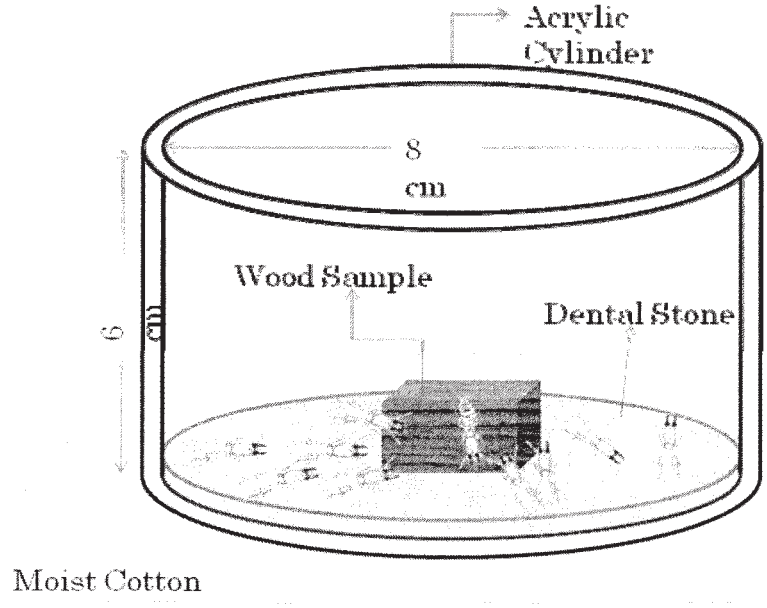

Fig. 1. An acrylic test container for wood block test (JIS)

The Standard Methods of TermiteTest in Malaysia

The Standard Methods for TermiteTest in Malaysia was use as ussually done in University Sain Malaysia. The Asian subterranean termite *Coptotermes gestroi* was collected from underground monitoring stations in the university campus.

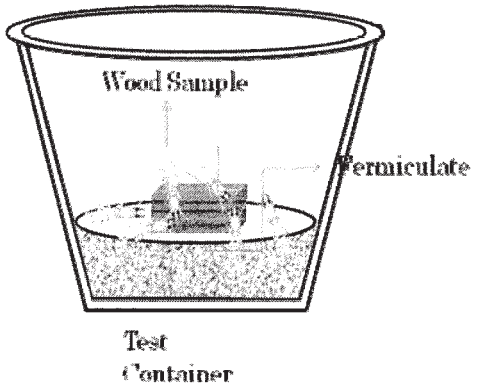

Fig. 2. Test container for wood block test in Malaysia

One each of the untreated and treated test blocks were placed at the top of vermiculate on the bottom of a test container. Vermiculate with 200% moist put on the bottom of test container to form a 5 mm of thickness (Fig. 2). Two hundred workers of termite were subjected to the forced feeding test and twenty soldiers were introduced together with workers in the container. The containers were gathered in a large covered case. This case was kept at room temperature (28 C) in dark. The test continued for 3 weeks. On the end of experiment were calculate the termite mortality (TM) and weight loss (WL) of samples after drying on the oven at 105 C for 24 hours.

Result and discussion

Weight loss of wood after exposed to termite attack at different locations (Malaysia and Indonesia), density and chemical composition of wood samples were shown in the Table 1. Base on the weight loss, this table indicate that teak wood was very durable compared that other species in both location of test and Kecapi wood on moderate durable however other sample were not durable.

Table 1. The Weight loss of wood separated in heartwood and sapwood after exposed to termtite attack and physical and chemicals properties wood samples.

Species	Part of Wood	WL (%)		Density	Extractive (%)	Lignin (%)
		Indonesia	Malaysia			
Paraserianthes falcataria (Albizia)	Sapwood	14.56	12.61	0,34	2,29	24,61
	Heartwood	14.04	12.78			
Maesopsis emanii (Manii)	Sapwood	12.22	12.86	0,5	1,67	24,70
	Heartwood	11.02	11.86			
Sandorikum koetjape (Kecapi)	Sapwood	8.23	7.30	0,53	4,14	23,52
	Heartwood	7.40	6.59			
Hevea brasiliensis (Rubber wood)	Sapwood	14.98	12.57	0,7	1,35	23,33
	Heartwood	14.81	12.36			
Durio zibethinus (Durian)	Sapwood	10.00	8.5	0,45	1,28	28,83
	Heartwood	8.09	5.94			
Tectona grandis (Teak)	Heartwood	2.28	0.91	0,75	4,6	29,9

In generally, the natural durability was cause by the content of extractive on the wood. Base on extractive content, teak wood and Kecapi wood were the higher extractive content compared that that of other species, however the type content of extractive from both species (Teak wood and Kecapi) were relative different. The extractive content of teak wood was most efective than that of Kecapi, the extractive on teakwood contain the active component of tectoquinon which very effective againts termite attack. The lignin content of all species are not different, and on the other hand, the specific gravity of rubber wood was higher compare than that of of teak wood however the durability of rubber wood was lower compare than teak wood. It is indicate that lignin and specific gravity are not affect on durability of wood atacke by termite (Fig. 3).

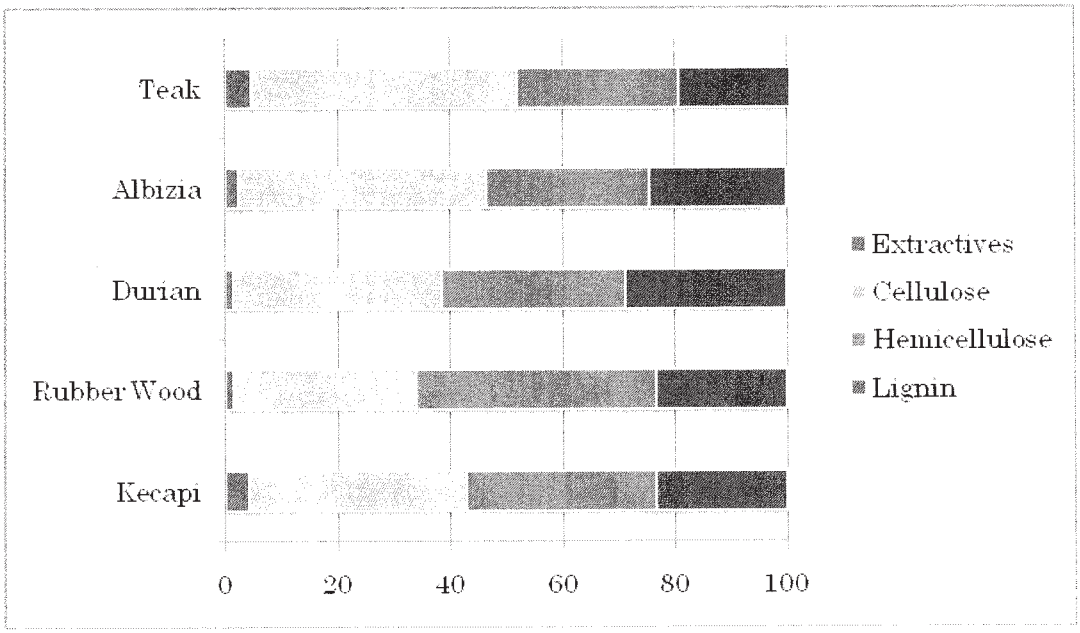

Fig. 3 The chemical composition of tropical wood species.

The average weight loss of after attack by *C. gestroi* in Indonesia in each same wood species were higher compared than in Malaysia. And the termite mortality in Malaysia was higher than in Indonesia. It is indicated that method which use in Indonesia (JIS K 1571, 2004) was most suitable for *C. gestroi*.

The durability of natural wood is caused by extractives content and ussuallydistributed in heart wood and sapwood. The weight loss of heartwood in each the same species were lower compared with sapwood even not so significants. It is indicate that extractive on heartwood was higher compared with sapwood.

Conclussion

Teak wood was very durable againts termite attach and Kecapi was moderate level, however other two species wre not durable. The natural durability was most caused by the extractive content rather than others chemical components. The methods of JIS K 14571 was more suitable for C. gestroi in South East Asian Countries (Malaysia and Indonesia).

Acknoledgements

We would like to many thank to EC of Pacific Rim Termite Research Group (PRTRG) to give the reaserach fund for conducting this research. We would like to thank also to Mr. Ikhsan Guswenrivo in USM, Mr. Didi Tarmadi and Mr. Khoirul Himmi Setiawan fromLIPI, for the help to do this experiment.

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The Termite Attack on Degradation of Yellow Bamboo (*Bambusa vulgaris* Schard var. *vitata*) and Green Bamboo (*Bambusa vulgaris* Schard var. *vulgaris*) in the Field Test

by

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Abstract

The culm and leaf of *Bambusa vulgaris* Schard var. *vitata* (yellow bamboo) and *Bambusa vulgaris* Schard var. *vulgaris* (green bamboo) were tested to identify their durable towards fungi degradating in the field. This research was carried out at the laboratory of Microbiology and Biochemis, Centre of Life Science and Faculty of Forestry, Bogor Agricultural University, Indonesia. The colonization and biodegradation process of culm of the both bamboos that used in this experiment was trapping method. The degradation level was measured based on the decreasing of dry weight of culm before and after trapping, and isolated and identified the tipe of the fungi. The incubation periods of trapping method were investigated every week for 12 weeks. The experiment was conducted in open field under bamboos plantation.

The results showed that *Schizopyllum commune* was dominant to colonize and to degrade culm of yellow and green bamboos. And *Trichoderma* sp. was dominant to colonize and to degrade leaf of yellow and green bamboos. The degradation levels of culm yellow bamboo were 19.9%. The degradation levels of culm green bamboo were 45.7%. The total degradation level of bamboo reeds on the 12th week was also caused by the termite attack, this is guessed for causing the high degradation level (45.7%). Yet the termite can't be monitored when the sample is being taken every week. Commonly, the highest degradation level of culm with the fungi in this experiment was occurred 5-6 weeks after incubation on yellow bamboo (3.6%), and 11-12 weeks after incubation on green bamboo (4.3%).

Key words: *B. vulgaris* var. *vitata* (yellow bamboo), *B. vulgaris* var. *vulgaris* (green bamboo), *S. commune*, *Trichoderma* sp., degradation, termite attack

Introduction

The bamboo is known to have a very important role for the development in Indonesia. Bamboo can be found in natural forest, plant forest and inside forest society area in various region in Indonesia. The use of bamboo is very wide, the reeds can be used for hing quality construction materials, either for the tools or the buildings which are earthquake resistant, whereas the shoot of bamboo can be consumed as food substance which is called "rebung" (Nasendi, 1995). In addition, bamboo is the prime raw material for the pulp industries in Asia (Monahan, 1998). A great number of bamboo reeds is also used in the traditional industries in villages in Indonesia (Rifa'I, 1994 *in* Widjaja *et* al., 1994).

The characteristic and species of bamboo is very various. It is known there are 22 species which are planted in Manglayang Barat, Bandung and other collections in Haur Bentes, Jasinga Bogor in a 5 Ha area. Accroding to Widjaya et al. (1994 *in* Nasendi, 1995) there are 56 original species from Indonesia with high economic potential. Whereas, over all there are 120 original species from Indonesia. Globally there are about 1500 species, about 10 species with high priority, 4 of them are from Indonesia. Ampel Bamboo (*Bambusa vulgaris*) is one of the bamboo with high priority in Indonesia. The bamboo which are used in this research are Ampel Bamboo consisting Yellow Bamboo (*Bambusa vulgaris* Schard var. *vitata*) and Green Bamboo (*Bambusa vulgaris* Schard var. v*ulgaris*).

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Biophisycally, bamboo can produce selulosa per Ha 2 - 6 times more than pine tree. The biomass increase per hour for bamboo is 10-30 %, compared to 2.5% for trees. Bamboo can be harvested in 4 years, compared to 8-20 years for species of tree which grows fast and *softwood* (Nasendi, 1995). According to Han (1998 *in* Feng & Gou, 2000) bamboo have the following chemical compositions : 26-43 % selulosa, 21-31% lignin, 15-26% pentosan, 1.7-5% dust and 0.7% silicon.

Bamboo, even known as one of the strongest structural material, often attacked by weathering fungi and biodeterioration during its storage. Monahan (1998) based on various references (Purushotham *et al.*, 1954; Chandra & Guha, 1979a,b, 1981; Tewari & Singh, 1979; Guha *et al.*,1980; Liese, 1980; and Kumar *et al.*, 1994) reported that the natural power of bamboo is variated between 1-36 months, it depends of the species and climate condition. In wet tropic area, bamboo reeds which ore on the storage experience decaying and biodeterioration in a big number.

Weathering and biodeterioriation on bamboo is generally caused by fungi, includes *soft rot, white rot dan brown rot.* Bacteria also caused bamboo reeds deterioration on the storage, with one or more bamboo destroyer organism on succession. Colonialization by the microorganism and the attack speed is depended on the water amount and the nutrient contens of the body, environment temperature, humidity, etc.. From the bamboo chemical composition, 90 % hemiselulosa are *xylan*, with structure between wide leaved wood *xylan* and needle leaves. Bamboo are rich in silicon (0.5-4%), especially inside the epiderm layers and cell walls. Monahan (1998) based on various references (Harmada, 1962; Mathew & Nair, 1990; dan Gnanaharan *et al.*, 1993) propose that even bamboo also have some resin content, waks, and tannin, there are no toxin enough to support its natural endurance, especially, the high content of starch on bamboo caused them to be very susceptible against *staining fungi* and weathering fungi. Skelernkim fibers which have the role to be the power bamboo are attacked by fungi and its power dropped eventually.

This research was conducted to determine the types of fungi and degradation level of bamboo reeds and yellow leaves bamboo (*Bambusa vulgaris* var Schard. *Vitata*) and green bamboo *(Bambusa vulgaris* var Schard. *Vulgaris*) which had been left on the ground floor beneath the open bamboo cluster naturally for 12 weeks. In addition to understand the role of termites in the succession of reeds and bamboo leaves degradator organisms.

Materials and methods

This research was conducted over four months from February to June 2003, at the Laboratory of Microbiology and Biochemistry, Center for Life Science Studies (PSIH), IPB, and in the back yard of Forest Management Department, Faculty of Forestry IPB.

Bamboo in use is the yellow bamboo (Bambusa vulgaris var Schard. Vitata) with a diameter of reeeds about 10 cm and green bamboo (Bambusa vulgaris var Schard. Vulgaris) with a diameter of reeds about 7 cm.

Plot consisted of thirty pieces of each reed bamboo type (size 2x3 cm^2) is placed in the hole (size 20x10x5 cm) on the ground floor beneath the open bamboo cluster naturally. Two plots are used for observation of fungi on bamboo strips which are taken every week (for 12 weeks). Two other plots are used to observe the total degradation level of the samples taken after 12 weeks. Observations and analysis of the dry weight measurements include initial and final samples every week, and the isolation and identification of the type of fungi that grow on these samples. Besides bamboo reed, also observed the types of fungi and degradation levels in bamboo leaves (3x15 cm^2 size) of each type with the number of pieces and the same method on a bamboo reed. So the total number of observed plots amounted to 16 for both types of bamboo. Also observed that there are termite attack, which can be seen from its bite marks.

The level of degradation of samples each week, obtained by the initial dry weight minus the dry weight of the reed end, the result is then divided by initial dry weight and then multiplied by 100%. Comparison of degradation level (%) for a week for twelve weeks of observation of yellow bamboo reeds and green bamboo, which is a way to find the difference in the level degradari week x the level of degradation weeks x-1, so there is the possibility of negative values obtained.

Results and discussion

From the observations obtained that after 12 weeks of yellow bamboo reeds on average experienced a total degradation level of 19.90%. Whereas on the green bamboo reed experienced a total

degradation level of 45.73%. The total level of degradation occurs in the green bamboo reed is caused also by the existence of termite attack, which appear with the termites bite, so the level of degradation to increase sharply. However, termites are not observed when the sample is taken every week. The level of degradation (%) of yellow bamboo reeds and green bamboo reeds every week and the total degradation level after 12 weeks can be seen in Figure 1.

Bamboo is in contact with the ground at the early stage of observation is commonly found infected by several types of bacteria, but this research do not identify the bacteria. Then in addition to bacteria in the early stages will usually have molding fungi and staining fungi. After a few weeks decaying fungi Basidiomycetes and Ascomycetes classes usually begin to attack by damaging the cell wall structure of bamboo so then happens the weathering (rot)

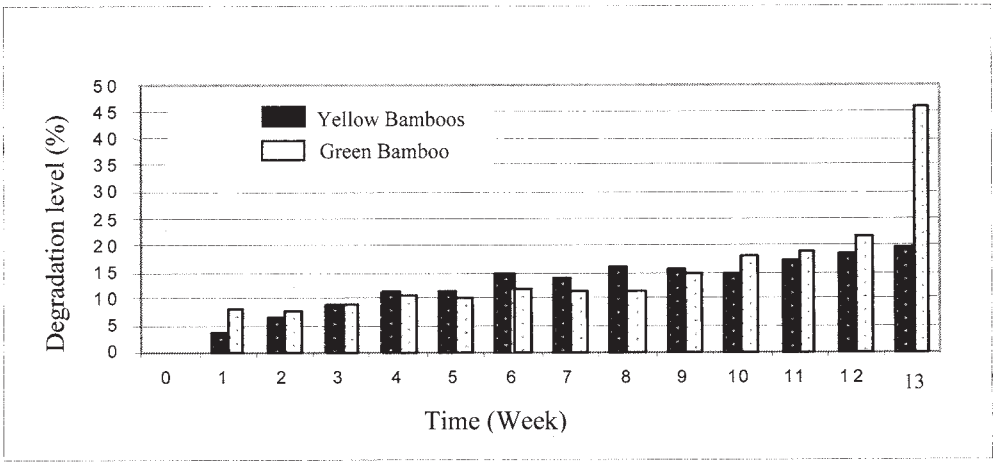

Figure 1. The level of degradation (%) of yellow bamboo reeds and green bamboo reeds and total degradation level after 12 weeks (12T)

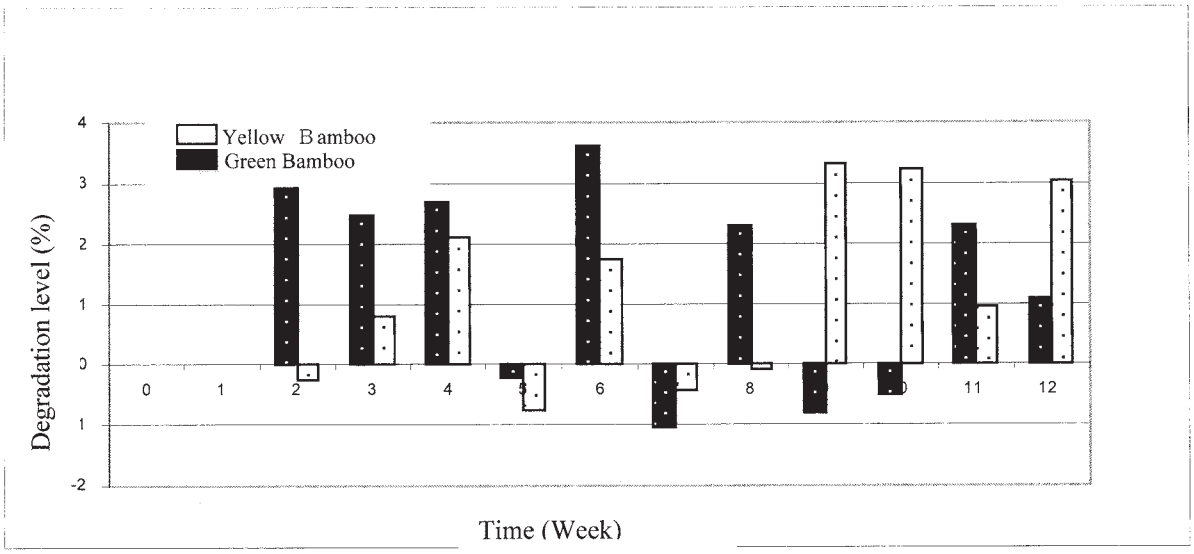

Figure 2. Comparison of degradation level (%) for a week for twelve weeks of observation on bamboo reed yellow and green

In figure 1 seen that the degradation level of bamboo reeds for every week is not increased consistently over the length of storage. This is thought to be caused by environmental factors and also by the factors of fungal species which colonialize the bamboo reed.

Comparison of degradation level (%) for a week for twelve weeks of observation of yellow bamboo reeds and green bamboo can be seen in Figure 2.

From Figure 2 on the observations of yellow bamboo reeds obtained that the highest degradation level is 3.6%, occurred between 5^{th} and 6^{th} week. Figure 3 shows that the fungi isolated

in 5th and 6th week is *Schizophylum commune* (Sc), *Penicillium* sp.1 (Psp 1), sp.1 sterile *mycelium* (Ms 1) and *Penicillium* sp.2 (Psp 2). The level of degradation of this type are also quite important (2.9%) occurred between the first and second week (Fig. 2). Fungi are isolated in the first week and the second is a Trichoderma sp. (Tric), sp.1 sterile *mycelium* (Ms1), *Rhizopus* sp. (Rhi), *S. commune* (Sc) and *Penicillium* sp.1 (Psp1).

Table 1. The types of fungi which are isolated in the reeds and bamboo leaves yellow and green

No.	The name of fungi	Degradation types	Kelas Fungi
1.	Tri : *Trichoderma* sp.	Molding	Deuteromycetes
2.	Ms1 : Miselium steril sp.1	Decaying	Basidiomycetes
3.	Rhi : *Rhizopus* sp.	Molding	Deuteromycetes
4.	Sc : *Schizophyllum commune*	Decaying	Basidiomycetes
5.	Psp1 : *Penicillium* sp.1	Molding	Deuteromycetes
6.	Fus : *Fusarium* sp.	Molding	Deuteromycetes
7.	Bot : *Botryodiplodia* sp.	Staining	Deuteromycetes
8.	Ms2 : Miselium steril sp.2	Pelapukan	Basidiomycetes
9.	Psp2 : *Penicillium* sp.2	Molding	Deuteromycetes
10.	Gli : *Glyocladium* sp.	Molding	Deuteromycetes
11.	Mon : *Monilia* sp.	Molding	Deuteromycetes

The highest level of degradation in the green bamboo reed is 3.3% which occurred between 8th and 9th week (Fig. 2), fungi are isolated in 8th and 9th week *Trichoderma* sp. (Tric), *S. commune* (Sc), *Botryodiplodia* sp. (Bot). and *Monilia* sp. (Mon), and *Miselia* sterilia sp.1 (Ms 1) and sp. 2 (Ms 2). In Figure 2 also looks at the level of degradation of this kind are quite important (3.2%) occurred between weeks 9 and 10. In Figure 4 looks fungi are isolated in week 9 and 10 is *Trichoderma* sp. (Tric), *S. commune* (Sc), *Botryodiplodia* sp. (Bot) and *Monilia* sp. (Mon).

In addition to the above, the other fungal species isolated is *Glyocladium* sp. (Gli). The types of fungi found and the type of damage can be seen in Table 1.

To see the frequency of occurrence of each type of fungi during the observation, it can be seen in figure 3 and figure 4. Figure 5 are for the observations on bamboo reed bamboo reed yellow and green, whereas figure 4 are for observations on bamboo leaves yellow and green bamboo leaves. In yellow bamboo reed, *S. commune* (Sc) dominates with 27.12% frequency of occurrence, was the green bamboo reeds, fungi that dominate the *S. commune* (Sc) and *Penicillium* sp1. (Psp1) with each frequency 17.5% (Fig. 5). In this research, it is found that *S. communes* appear on reed dominant yellow and green bamboo. According to the report Martawidjaja (1965), S. commune can attack various types of wood in Indonesia, as well as a fierce wood mold fungi.

On bamboo leaves yellow and green, the dominant fungi found at it is the *Trichoderma* sp. (Tric) with frequency respectively 31.6% and 29.3% (Fig. 4). *Trichoderma* sp. a soil sabrop fungus that can take substances content of leaf cells litter and other organic materials for growth (Dix & Webster, 1994)

Some bamboo species are known to be durable, such as research findings Feng & Guo (2000), note that the bamboo *whangee* containing benzene-ethanol extractives that are anti-rotted higher than *yunnanicus* bamboo, so that in the process of architecture and its use is more resistant to obsolete.

From the result of the research on green bamboo, the difference between the degradation level after 12 week and the total degradation level is 23,73%, where on the total degradation level it is discovered termite attack. It is expected that in the field green bamboo reeds is allegedly preferred bysoil termite than of yellow bamboo.

Some of the known termite soil are *Captotermes curvignathus, Macrotermes gilvus* and *Microtermes* sp. According Arinana and Massijaya (2009), the results of testing the durability of composite boards LKAB (Waste wood and woven bamboo) betung bamboo is not preferred by termites with an average value of termite mortality of 61.21% and the percentage weight loss of composite boards for LKAB is 7. 72% with kontral (Sengon wood) of 21.23%.

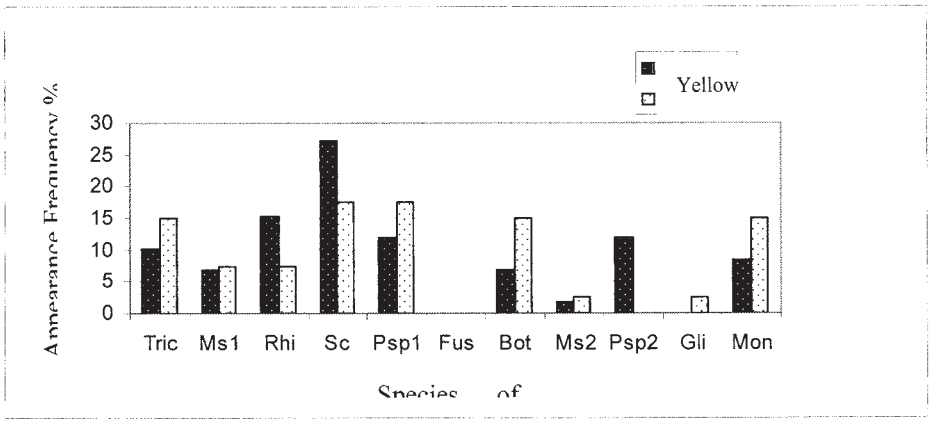

Figure 3. The frequency of occurrence (%) fungal species in the yellow bamboo and reed green bamboo reed

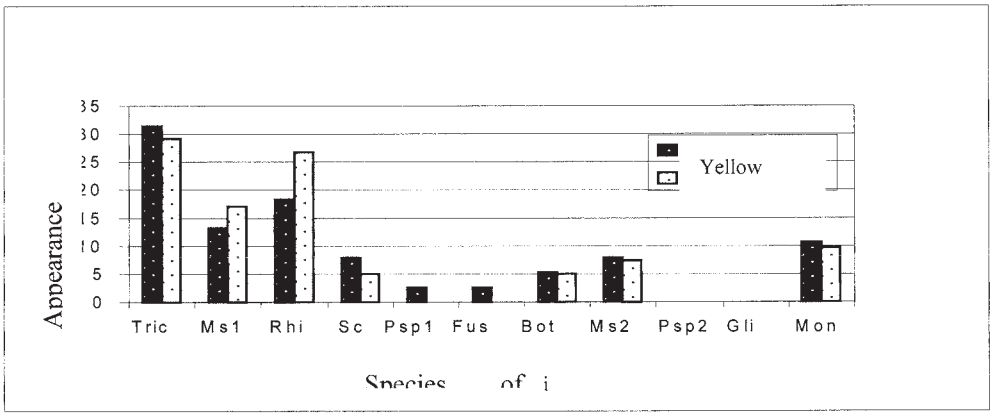

Figure 4. The frequency of occurrence (%) the kinds of fungi on bamboo leaves yellow and green bamboo leaves

The influence of climate and weather also affect the durability of bamboo in the field (Nandika et al., 1994). In the tropics, such as in Indonesia, storage of materials such as bamboo berligninselulosa outdoors damage caused by microorganism, especially fungi. Bamboo is in contact with the ground at an early stage will usually have molding fungi and Staining fungi. After a few weeks decaying fungi Basidiomycetes and Ascomycetes classes usually begin to attack by damaging the cell wall structure of bamboo that happens weathering (rot) (Dix & Webster, 1994). Macro climate data for the Darmaga Bogor can be seen in Table 2.

Table 2. Data temperature, humidity and rainfall and the long average exposures

Month	Temperature (°C)		Average Relative Humidity (RH) (%)	Average Rainfall (mm)	Average long of irradiation (hour)
	Maximum Average	Minimum Average			
March	31.1	23.1	87	471	
April	32.1	23.4	85	309	63
May	32.0	22.9	83	501	75
June	31.6	21.7	82	180	84
July	31.5	21.2	78	25	89
August	32.8	21.6	76	91.3	85.1

from March until July 2003 in the Darmaga, Bogor Source: Badan Meteorologi dan geofisika, Balai Wilayah II, Stasiun Klimatologi Klas I, Darmaga Bogor.

Table 2. Data temperature, humidity and rainfall and the long average exposure. Starch content of bamboo is also influence the durability of bamboo. Matangaran research results (in 1987 Nandika et al., 1994) showed that starch content Ampel bamboo (Bambusa vulgaris) is high enough that many types of bamboo powder attack dry wood. At the base of the intensity of the attack bamboo powder dry wood was always higher than the middle and end. In the book there is also a high-intensity attacks when compared with ruasnya. This is closely related to starch content in that section

Conclusion

Yellow bamboo reed having the highest degradation level (3.6%) in storage between 5^{th} and 6^{th} week. And green bamboo reed degrades the highest (4.3%) in storage between 11^{th} and 12^{th} week. The level of degradation of the bamboo reed is not consistent every week to increase as the length of storage. This is thought to be caused by environmental factors and also by the factors of fungal species which colonialize the bamboo reed.

In this research found that *S. commune* appears dominant on bamboo reed yellow and green, while *Trichoderma* sp. appear dominant in the leaves of yellow and green bamboo.

The level of degradation of yellow bamboo reed total is 19.9%, while the total degradation level of green bamboo reed is 45.7%. In green bamboo that are stored for 12 weeks (12T), also seen attacked by termites. However, there are no termites in each week of sampling.

Recommended for further research related to the diversity of fungi, types of bamboo with different ecological conditions.

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Effect of Weight Loss Attacked by Subterranean Termite on Mechanical Properties of Mangium Wood

by

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Abstract

Solid and laminated mangium wood were tested for their effect of weight loss attacked by *subterranean termite* on value of modulus of elasticity (*MOE*) and modulus of rupture (*MOR*). The adhesive was used *Polyurethane as Water Based Polymer Isocyanate*, which was used in cold press application. Manufacturer's instructions were followed for mixing ratio of hardener and resin. The ratio of resin and hardener was 100:15 by weight. The field test conducted at the arboretum for two and half months. The result showed that the weight loss of *subterranean termite* attack of solid wood less than 12%, and 10% for laminated wood; *MOE* and *MOR* of solid wood to *subterranean termite* attacks, which weight loss $\leq$ 12% were decreased but not significant; *MOE* and *MOR* of laminated wood to *subterranean termite* attacks, which weight loss $\leq$ 10% were increased but not significant; and it was not correlation between weight loss and mechanical properties of wood after subterranean attacks.

Keywords: laminated wood, modulus of elasticity, modulus of rupture, solid wood, subterranean termite

Introduction

The research on the degree of termite attack in laboratory and field scale have been carried out in Indonesia. Subterranean termites are an important group of urban insects pests in tropical countries (Lee 2002, Lee *et al.* 2007).

However, the research associated with the effect of the attack on the mechanical properties of solid wood and laminated wood have not been reported. And in many cases, this termite attack has caused a decrease in the strength of that allegedly can cause loss of ability to sustain the load on a building construction system. Biological degradation was the main factor that degrades the durability of wooden product. Among all factors leading of biodegradation, termite were the most damaging wooden products worldwide (Chang and Cheng 2002). It has been widely accepted that termite have different preferential level to attack every wood species, so affect the level of consumption (Indrayani *et al.*, 2007).

This research was conducted with the aim to analyze the correlation between weight loss of solid and laminated wood due to termite attack and decrease the mechanical properties of wood.

Materials and methods

Materials

The wood used in this research was Acacia mangium, family Leguminoseae obtained from the products of Plantation Forest Perhutani Unit III in Legok, Bogor, West Java. The wood is about 8 years old with log diameter between 22 – 28 cm.

The type of adhesive was *Polyurethane as Water Based Polymer Isocyanate*, which was used in cold press application. The adhesive for bonding consist of two parts. The first part of the adhesive is PI 3100 as a liquid resin, and H7 as a liquid hardener. The two parts are mixed in the ratio of 100 parts resin to 15 parts hardener (by weight).

The drying process was done by nature, the wood beams with cross-section dimension of 6.50 cm x 6.50 cm arranged in orderly pile, placed in a room with roof. When the moisture content reached about 15%, the wood beams were planed to obtain beam cross-section dimension of 3 cm x 6 cm (thickness and width), and 50 cm of length. Laminated wood was arranged of 3 cm of thickness, 3 x 2 cm width of laminated wood, and 50 cm of length. The number of the tested was 21 for solid and laminated wood respectively.

The examination of field testing

Arboretum Forestry Faculty of Bogor, where the population is found *Subterranean Termite macrotermes*. In place will be the location of first infestation the cleansing of the grass and litter. After that put the box without a cover top and bottom measuring 1.5 x 2 m as a barrier. Sample and then have placed horizontally on the box. The solid and laminated beam have placed in the position randomly. The box was then covered with plastic tarp, and then left for two and half month. Sampling is then carried out with different degrees of attack.

Samples that had been moved from the arboretum had been washed and then cleared of debris. After that, it had been conditioned at room temperature. Before testing, samples had been oven at 60^0 C for 48 hours to obtain uniform water content. The samples were than weighed to determine loss of weight and water content was measured by using a moisture meter.

The examination of bending test

The solid beam or laminated beam was placed on top of two placements which were given load in the centre of the span and the resulted deflection was measured. As the beam tested was defined the span tested is 42 cm. The equipment used in this bending test was Universal Testing Machine Instron 330 Type with loading capacity of 50 KN, and computer with supporting soft and hard ware.

Before the tests were conducted for each beam, measurement of the beam cross section dimension were done, i.e. the beam width and height on two tested spots, which were to be used further in the calculation of modulus of elasticity.

Additional load was given with prediction of bending stress within elastic limits. On every additional load, reading of the deflection measurement in the middle of the span was done. The speed of loading is 3 mm/minute. To estimate that the tests were still within the elasticity limits, it can be done by defining the largest loading by calculating the bending stress that might emerge. Based on the data, the correlation curve of force and deflection was illustrated; the slope angle of the curve was used to calculate the wood modulus of elasticity.

Results and discussion

Field observations showed that the behavior of termite attack on each different sample. Nevertheless, it appears that there is no special preference for termites to attack solid wood or laminated wood. Termites look more like attacking some samples together before then switch to the other samples. As a result, although the study sample removal was done in stages but it seeems a long infestation not correlate directly with the level or degree of weight loss attacks. Some samples are fed longer have to lose weight was smaller compared with the fed sample shorter.

Modulus of elasticity (*MOE*) is an indication of the material ability to bend or indicate the nature of stiffness. (*MOR*) reflects the maximum load carrying capacity of a member in bending and is proportional to maximum moment borne by the specimen (Anonim, 1999). The two value were the main indication of mechanical properties of wood that should be known.

Based on the results of bending tests to determine the mechanical properties of solid wood that the weight loss < 12%, showing that R^2 =0.032, *MOE* was decreased, but was not significantly, Figure 1. In contrast of solid wood, in laminated wood showed that weight loss < 10% seen increased in *MOE* value, with R^2 = 0.016 although it was not significant also, Figure 2.

Based on the results of bending tests until failured was happened, it showed that solid wood with the weight loss < 12%, showing that R^2 =0.04, modulus of rupture (*MOR*) wast decreased, but was not significantly, Figure 3. In contrast of solid wood, in laminated wood showed that weight loss < 10% seen increased in *MOR* value, with R^2 = 0.138 although it was not significant also, Figure 4.

One of the reason that weight loss and mechanical properties of wood as *MOE* and *MOR* were not significant, becauce of the samples test were not in uniform strength or stiffness.

The area of termite wood eaten also determines the value of its *MOE* and *MOR*. If the area consumed in areas where timber recieves greatest moments, it is resulting smaller *MOR*. Neither,

when the area consumed by termite was depper, it will result smaller *MOE* than it was compared termites consumed not to deep but wider.

To improve the resilience of the wood preservation, should be considered its impact on the prosess of gluing the laminated wood.

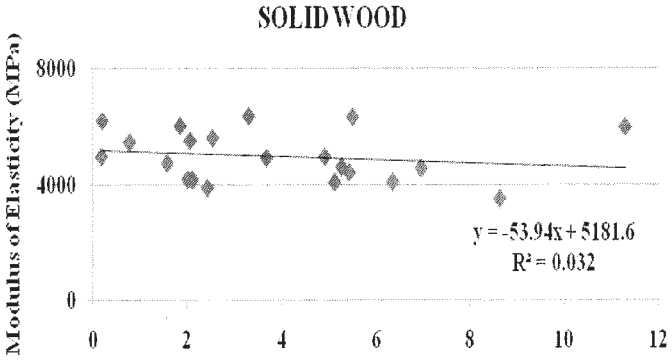

Figure 1. The correlation of solid wood weight loss and modulus of elasticity (*MOE*)

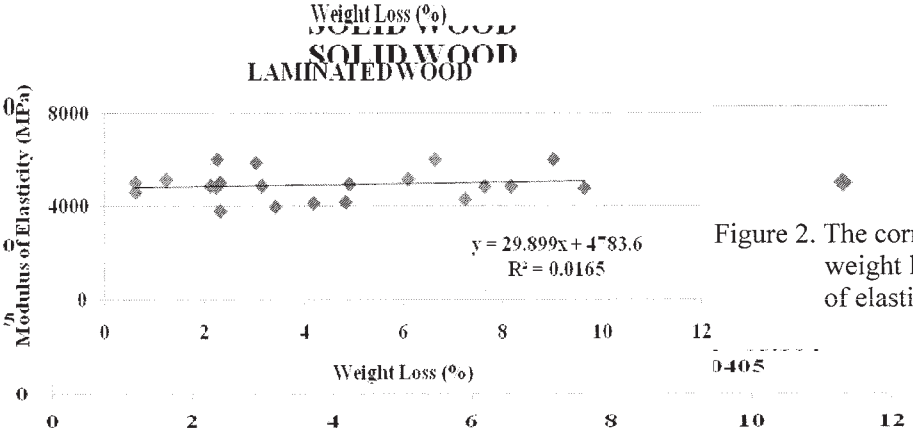

Figure 2. The correlation of laminated wood weight loss and modulus of elasticity (*MOE*)

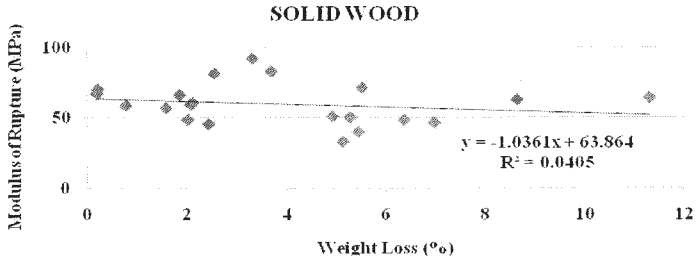

Figure 3. The correlation of solid wood weight loss and modulus of rupture (*MOR*)

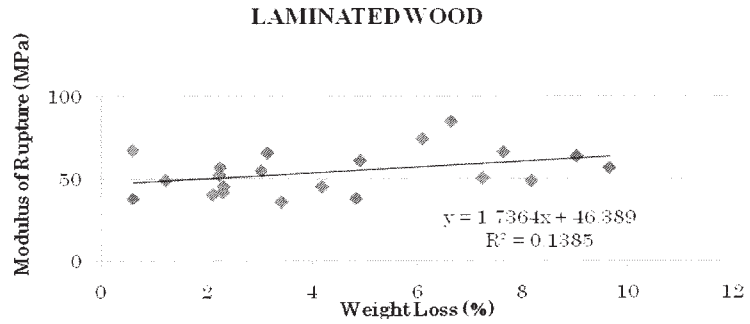

Figure 4. The correlation of laminated wood weight loss and modulus of rupture (*MOR*)

Conclusions

From discussion above, it could be conluded that:

1. The weight loss of *subterranean termite* attack of solid wood less than 12%, and 10% for laminated wood for two and half months.

2. *MOE* and *MOR* of solid wood to *subterranean termite* attacks, which weight loss ≤ 12% were decreased but not significant.
3. *MOE* and *MOR* of laminated wood to *subterranean termite* attacks, which weight loss ≤ 10% were increased but not significant.
4. No correlation between weight loss and mechanical properties of wood after subterranean attacks.

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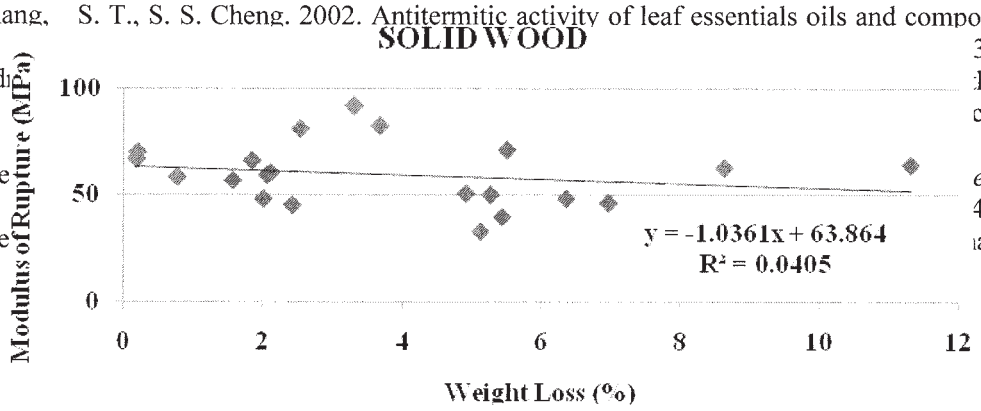

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Effect of Strand Treatment on Durability Properties of Oriented Strand Board Made from Sentang Wood

by

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Abstract

The objective of this research was to evaluated strand treatment effect to durability of oriented strand board made from *M.excelsa* wood. The resistance of OSB against subterranean termite (*C. curvignathus*) attack was determined through modified wood block test, the dimension of sample (l x w x t) was 20 by 20 by 10 mm^3 and exposured for 4 weeks to termites. Weight loss, antifeedant and termite mortality parameters were determined.The result in this research showed that strand treatment can improved durability of board. Preservative treatment on strand showed the best result in durability compared with untreated and other treatment. This is proofed by the value of weight lose and mortality which have a respectivelly lowest (0.61%) and bigest (100%) compared with untreated and other treatment.

Keywords: treatment strand, OSB, durability, termite

Introduction

Indonesia was one of the bigest megabiodiversity country in the world. As the tropical contry, Indonesia region which is suited to growth and propagation of fungi, microorganism, and organism that caused biodeterioration of wood. In the other hand, 80-85% the most of wood in our contry had low durability to wood destroying organism.

One of tropical wood as the subject in this research is sentang wood (*Melia excelsa* Jack). Sentang wood is one of the promising fast growing tree species that can be introduced in timber estate and community forest. *M excelsa* wood has bulk density around 0.42-0.52 and it is categorized as medium density wood. This wood belongs to non durable wood based on grave yard test performance and classified into non durable to moderat classes (Iswanto, 2008; Ching, 2003). The extractive content of *M. excelsa* after dissolved in cold water, hot water, 1% sodium hydroxide (NaOH 1%) and alcohol-benzene were in the range of 4.25-5.07%,7.39-7.83%, 9.29-11.19%, and 2.09-2.64%, respectively (Iswanto, 2008). In order to optimum utilization of this wood based on the properties of wood, *M. excelsa* wood can be used for light construction, furniture, panel and veneer. Hence, *M. excelsa* wood is also promising to be used as a raw material for OSB product.

Oriented strand board (OSB) is a structural panel suitable for a wide range of construction and industrial applications. It is a mat-formed panel made of strands sliced in the long direction from small diameter, fast growing round wood logs and bonded with an exterior-type binder under heat and pressure (Structural Board Association, 2005). OSB was manufactured in a cross-oriented pattern similar to plywood to create a strong, stiff structural panel (APA, 2009).

Subterranean termite is the most important agent of wood and wood products deterioration agents in tropical region. Subterranean termite utilizes wood both as a shelter and food sources. *M.excelsa* wood belongs to non durable wood (Iswanto, 2008). Hence in order to improve natural durability of OSB prepared from *M.excelsa* strands, the resistance of OSB against subterranean termite (*Coptotermes curvignathus* Holmgren) attack under various pre-treatment techniques was observed.

Materials and methods

Materials: The treated of OSB made from sentang wood. Treatment of strand as OSB materials were immersing in cold and hot water (for 72 hours, at 80°C for 2 hours are respectivelly),

preservative (2.5% CCB preservative solution for 48 hours) and steam (at 126° C at 1.4 kg/cm^2 for 1 hour).

Methods: The resistance of OSB against subterranean termite (*C. curvignathus*) attack was determined through modified wood block test. The dimension of sample (l x w x t) was 20 by 20 by 10 mm^3 and exposured for 4 weeks to termites. Weight loss, antifeedant and termite mortality parameters were determined. Before and after test, the samples were dried in the oven at 103 ± 2°C for determining of weight loss and antifeedant. Antifeedant was determined by calculating the ratio between weight loss of untreated and treated samples. Tables 1 and 2 showed the resistance level of wood against termite attack based on antifeedant and mortality classification.

Table 1. Resistance level of wood against termite attacked based on Antifeedant classification

Class	Antifeedant value (%)	Resistance level
IV	$75 \leq x < 100$	very strong
III	$50 \leq x < 75$	strong
II	$25 \leq x < 50$	moderately strong
I	$0 \leq x < 25$	weak

Source: Sornnuwat *et al.* (1995)

Table 2. Resistance level of wood against termite attacked based on termite mortality

Mortality (%)	Resistance level
≥ 95	very strong
$75 \leq x < 95$	strong
$60 \leq x < 75$	fairly strong
$40 \leq x < 60$	moderately strong
$25 \leq x < 40$	fairly weak
$5 \leq x < 25$	weak
< 5	in-active

Source: Sornnuwat *et al.* (1995)

Results and discussion

The mean value of weight loss of samples after baited for 4 weeks to subterranean termite (*C. Curvignathus*) ranged between 0.61~8.90%. The lowest and the highest values of weight loss were achieved on OSB prepared from preserved strands and steamed strands, respectively (Table 3). Subterranean termite utilizes wood both as a shelter and food sources (Bowyer *et al.* 2003). Preserved strand proof can increase durability of board to termite attack. CCB preservative as a toxic caused highly of termite mortality so it had a lower of weight lose.

Table 3. Resistance of OSB against termite attack based on antifeedant and termite mortality

Treatment	Weight Lose (%)	Antifeedant (%)	Mortality (%)
Untreated	7.57	-	40.00 (moderately strong)
Immerse in Cold Water	5.66	14.40	61.33 (fairly strong)
Immerse in Hot Water	5.54	15.46	64.67 (fairly strong)
Immerse in Preservative	0.61	85.13	100.00 (very strong)
Steam	8.90	8.10	52.00 (moderately strong)

Based on antifeedant criteria, the untreated, hot and cold water immersed and steamed treatments showed similar resistance to subterranean termite (*C. Curvignathus*). These boards belong to weak or less resistance. On the other hand, OSB prepared from preserved strands belongs to strong or resistance to subterranean termite (*C. Curvignathus*). Almost similar phenomenon was occured when the resistance of OSB was measured based on termite mortality criteria.

The lowest and the highest termite mortality were obtained on OSB prepared from untreated and preserved strands, respectively. Based on termite mortality criteria the untreated board, boards

prepared from steamed strands were categorized to moderately strong, while OSB prepared from hot water immersed strands and preserved strands were categorized to fairly strong and very strong resistance to subterranean termite (*C. Curvignathus*) under the adopted experimental condition.

Conclusion

The preservative treatment on strand can improved durability of board to subterranean termaite attack. This is proofed by strand with preservative treatment have a lowest and bigest are weight lose and mortality respectivelly if compared with untreated and other treatment. In general, all of boards in this research are classified into moderately strong to very strong.

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Durability of Cement-Gypsum Board Made from Kenaf-Core (*Hibiscus cannabinus* L.)

by

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Abstarct

This study deals with the effect of cement-gypsum and autoclave curing technology on the durability of cement-gypsum board to dry-wood termite and subterranean termite. Materials used in this research were core-kenaf particles as a reinforcement material, cement and gypsum as a binder, and calcium cloride ($CaCl_2$) as an accelerator. The target density of boards were 1,2 g/cm^3.

The results shown that the proportions of cement-gypsum and autoclave curing time have influenced the physical and mechanical properties of cement-gypsum boards. The proportions of cement-gypsum of 60:40 with 8 hours autoclave curing time was fulfill all the test parameters of JIS A 5417 1992, JWPA: No.11-1992 and the AWPA: No.E7. Cement-gypsum board made from core-kenaf with autoclave curing technology more resistance to dry-wood termite and subterranean termite attack.

Key words: cement-gypsum board, kenaf, autoclave, termites

Introduction

The more rapid industrialization, especially the timber industry in Indonesia, causing the rate of destruction of natural forests is increasing. The search for alternative materials and innovative environmentally sound technologies expected to reduce such problems. The utilization of kenaf core in the manufacture of cement-gypsum board with autoclave curing technology is a promising effort and provides great opportunities for the wood processing industry as well as new products will be environmentally friendly. However, the evaluation on durability of cement-gypsum board to dry-wood termite and subterranean termite is needed.

The objective of this research is to evaluate the effect of cement-gypsum and autoclave curing technology on the durability of cement-gypsum board to dry-wood termite and subterranean termite

Material and methods

Cement-gypsum board with a target density of 1.2 g/cm^3 was made from core of kenaf (*Hibiscus cannabinus* L.) by using autoclave curing technology. The formulations of face and back of board made from a mixture of cement, kenaf particles and water in the ratio of 2.5: 1.0: 1.25. Whereas for the middle layer of board is made from a mixture of gypsum, kenaf particles and water in the ratio of 3.0:1.0: 1.5. The autoclave curing times were 2, 4, 8, and 16 hours. The weight proportion of cement-gypsum used for each layer was 40:60, 50:50, and 60:40.

The evaluation of board resistance to termites, include its resistance to dry wood termite and subterranean termite complies with Japan Wood Preserving Association (JWPA: No. 11-1992) for laboratory testing, and preserves the American Wood Association Standard (AWPA: No.E7-1993) for grave yard test.

Results and discussion

Board resistance to dry wood termite (Cryptotermes cynocephalus Light).

The mortality rate of dry wood termite in each week of observation shows that the mortality rate of dry wood termites in week IV likely to be high and it ranged from 18 to 46%, as shown in Figure 1.

The proportion of cement-gypsum board of 60:40 and 4-hour autoclave curing has a highest mortality rate of dry wood termites (46%), while the proportion of cement-gypsum board of 40:60 and 4 hours autoclave curing was the lowest mortality rate of dry wood termites (18%).

Mortality rate of dry wood termites tend to be high on the week III and IV caused by the cement and gypsum layering surrounding the wood particles.

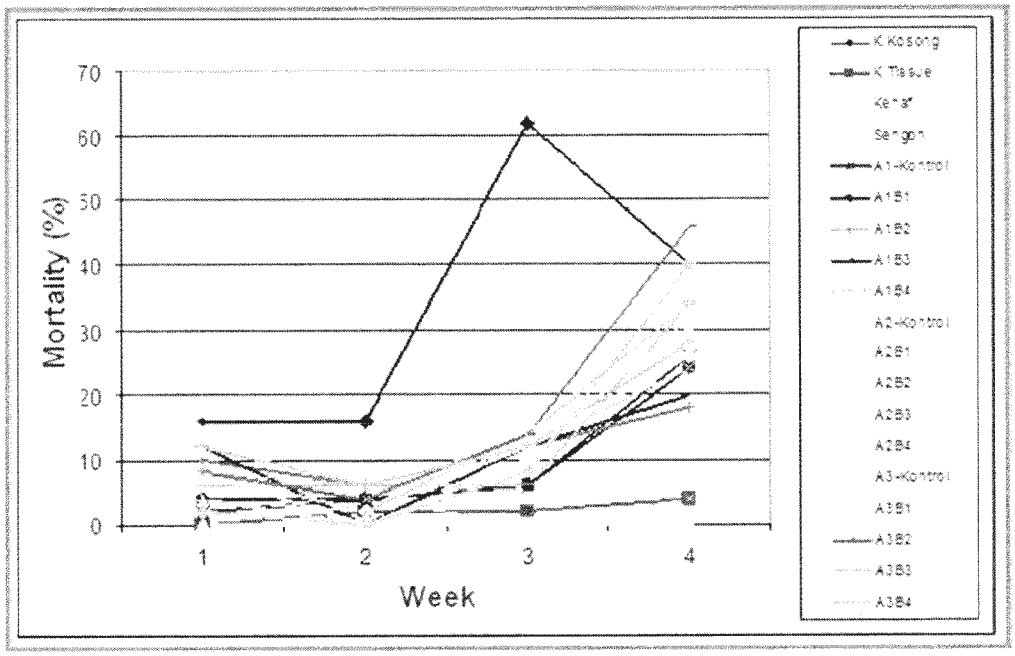

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Figure 1. Mortality of dry wood termite

Baesd on visual observations, the feeding behavior of dry wood termites are almost identical to the proportion of the three types of cement-gypsum board at various autoclave curing times. A lot of dry wood termites attacked the lining of the middle board (gypsum mixed-kenaf core particles), while face-back layers of cement mixed with kenaf core particles were not attacked. This shows the resilience of a layer of cement to the dry wood termite attack is high enough compared to the layer of gypsum board.

The average weight loss of the board due to the dry wood termite attack ranged from 2.57 to 4.86%, as shown in Figure 2. The proportion of cement-gypsum of 40:60 with conventional two weeks curing had a highest weight loss of 4.86%, while the lowest weight loss of 2.57% occurred on board with the proportions of cement-gypsum of 60:40 and 16 hours autoclave curing.

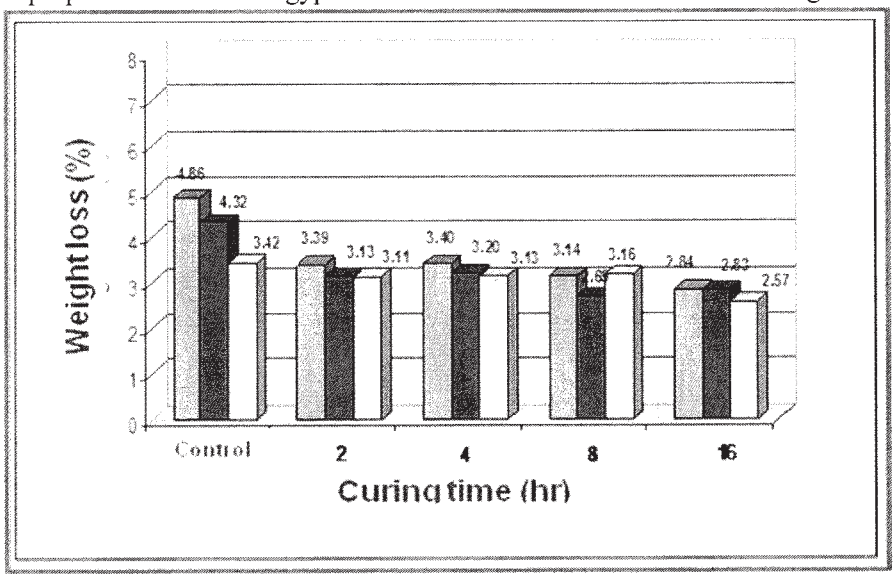

Figure 2. Weight loss of boards caused by dry-wood termite

Board resistance to Soil termite.

The weight loss of the cement-gypsum boards were 1.84 to 7.06%, while the weight loss of core-kenaf was 88.65%, as shown in Figure 3. The highest weight loss percentage of 7.06% occurred on board which the proportions of cement-gypsum of 40:60 with conventional 2 weeks curing, while the lowest weight loss of 1.84% occurred on cement-gypsum board proportion of 60:40 with 8-hour autoclave curing. In general, the weight loss of cement-gypsum boards made by autoclave curing were lower than the board made with conventional 2 weeks curing.

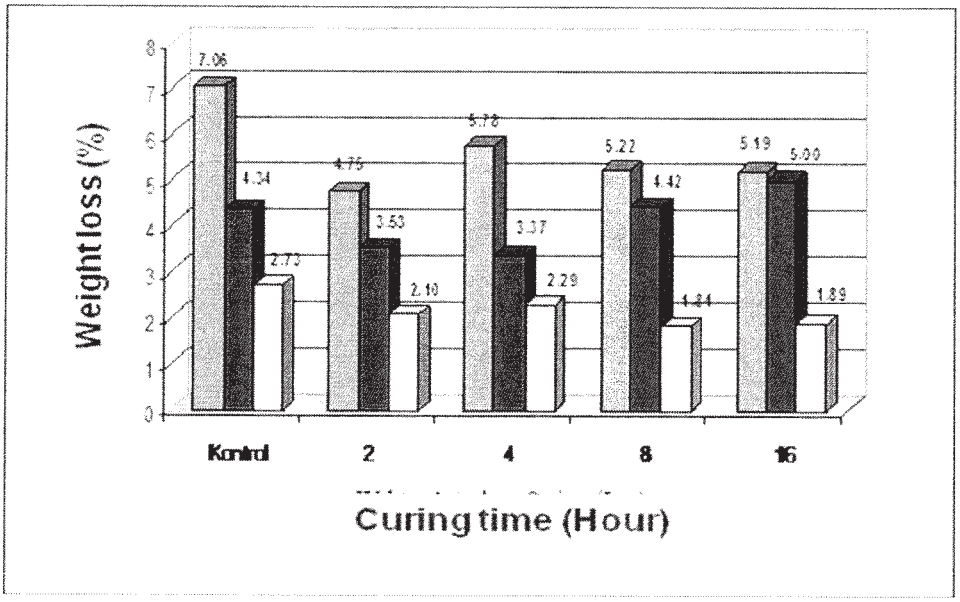

Figure 3. Weight loss of boards caused by subterranean termites

Conclusion

The proportions of cement-gypsum of 60:40 with 8 hours autoclave curing time was fulfill all the test parameters of JIS A 5417 1992, JWPA: No.11-1992 and the AWPA: No.E7, 1993. Cement-gypsum board made from core-kenaf with autoclave curing technology more resistance to dry-wood termite and subterranean termite attack.

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Composite Boards of Wood Waste and Betung Bamboo Woven (*Dendrocalamus asper*) Resistance from Subterranean Termite Attack (*Coptotermes curvignathus*)

by

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Abstract

In the future, raw materials for composite board are so many varieties as impact of lack of wood and increasing of wood need by society. The use of wood waste and bamboo is one alternative to solve the problem of high quality wood. Therefore, this research was designed to result high composite board in resistance to termites. To reach the goal, the adhesive modification was done with paraffin adding in making adhesive process. Comparative composition between Isocyanate : MF adhesive that used were 1:0 and 0:1 (control), 1:1, 1:2, 1:3, and 1:4. Paraffin content that added to composite board are 0% (control), 2%, 4%, 6% and 8%. Size of composite board was 30 cm x 30 cm x 1 cm with target density 0.66 g/cm^3. The resistance of product to subterranean termites *C. curvignathus* based on MWBT (modified wood block test). Results of the research showed that composite board which had the best resistance to subterranean termites *C. curvignathus* was board in composition of Isocyanate : MF adhesive 1 : 1 with 2% wax content. Percentages of weight loss and mortality that board were 4.45 % and 97.73 % respectively. A, B and C included in the group's moderately resistant, while the boards D, E, and F included in the non-resistant groups by Sornnuwat (1996) classification.

Keywords: *Dendrocalamus asper*, wood waste, *Coptotermes curvignathus*, melamin formaldehyde (MF), isocyanate, paraffin.

Introduction

As derived from wood, composite boards was developed in addition to increase the efficiency of the use of natural resources, and to cover some of the weaknesses of solid wood. Superior properties of composite boards compared with the solid wood is that the composite boards in size can be more flexible, density of boards can be made in accordance with the intended use, the existing timber defects can be distributed uniformly, and is homogeneous.

Raw materials of composite board in the future will be highly variable as a result of the shortage of wood raw material as long as the woods demand by the public. Utilization of wood waste and bamboo as raw material composite board is an alternative to solve the problem of raw material shortage of high quality wood. In some of the wood processing industry waste is usually used as furnace fuel, or burned out without the use of means, which can cause environmental pollution (Febrianto, 1999). By processing wood waste into raw material composite boards can improve the quality of the wood waste and produce high quality products. The use of bamboo woven as a layer of composite board is the other alternative to replace wood materials.

Series studies of the development of composite board from wood waste and bamboo woven that has been made during the last three years by Massijaya & Hadi (2008) has shown excellent results in terms of physical and mechanical properties. However, resistance to destructive biological factors (subterranean termite) is not known, so this further research is necessary to know the resilience of the resulting composite board of the destroyer biological factors such as subterranean termite.

The purpose of the study are to knowing the influence of adhesive composition of Isocyanate-MF and levels of paraffin to resistance of composite boards made from wood waste materials and woven of bamboo on the destroyer biological factors (subterranean termite) and determining the composition of Isocyanate-MF adhesive and best paraffin content in the manufacture of composite boards.

Materials and methods

Equipment and materials research

The instrument used in this study is the disk flaker, buckets, scales, spray gun, blender, wooden box measures 30 cm x 30 cm, teflon sheet, aluminium plate (*caul*), cold press, hot press, oven, desikator, aluminium foil, bowls porcelain, test tube, filter, water bath, gloves, and glass bottles for testing resistance to subterranean termite.

The material used was paraffin, wood waste from the species of Dipterocarpaceae, Sengon (*Paraserianthes falcataria*), Acacia (*Acacia mangium*), Betung Bamboo (*Dendrocalamus asper*) woven, melamine formaldehyde (MF) adhesives, Isocyanate adhesives, distilled water, sterile sand and subterranean termite *C. curvignathus.*

Creating composite board

Composite board was made in three layers composite board, 30 cm x 30 cm x 1 cm dimension with 1.3 compression ratios. Treatment given in this study focused on the composition of the mixture of MDI glue and MF and paraffin content provided on composite board made. Comparison of the composition of the mixture of Isocyanate and MF adhesives are set as 1:0, 1:1, 1:2, 1:3, 1:4, and 0:1.

While paraffin content that added to composite board are 0% (control), 2%, 4%, 6%, and 8%. Replications for each parameter were observed as many as 5 replications.

Resistance against subterranean termite *C. curvignathus* test

Testing of subterranean termite used Modified Wood Block Test (MWBT) standard. Testing composite board of termite attack is made by test sample is dried until a dry oven, then put into glass bottles containing 30 g of sterile sand and 6 ml distilled water. Into the glass bottle are inserted 200 worker and 20 soldier subterranean termite *C. curvignathus*. Glass bottles covered with aluminum foil and placed in a dark room. Weight loss and mortality was calculated after 21 days of baiting.

Weight loss and mortality of termites is the quality parameters of the composite boards against termite attack.

Percentage of weight loss due to termite attack is calculated by the formula:

Weight Loss = (W0 – W1)/W0 x 100%, Description: W0 = weight of oven dry test sample before being fed to the termites (g) and W1 = weight of oven dry test sample after being fed to the termites (g)

Percentage of individuals that termites die (mortality) was calculated using the formula:

Mortality rate = (N0 – N1)/ N0 x 100%, Description: N0 = number of individual termites before baiting and N1 = number of individual termites after the test

Data analysis

Data analysis is done by a simple descriptive analysis to determine the average value and RAL 2 factorial. Meanwhile, to see where the influence of different treatment to any of the responses that tested carried multiple Duncan test area.

Results and discussion

Resistance of composite board againstsubterranean termites *C. curvignathus*

Tests conducted with laboratory testing, which test samples of termites fed on subterranean termite *C. curvignathus* for 21 days. To see the resistance of the composite board against subterranean termite attack *C. curvignathus* based on the influence of adhesive composition of Isocyanate-MF and paraffin content. This can be seen from the percentage weight loss and mortality, composite board subterranean termite *C. curvignathus.*

Weight loss of test samples

The average percentage weight loss due to termite attack can be seen in Figure 1.

From Figure 1. can be seen that the large overall weight loss of test samples ranged from 4.45% - 12.78%. Also be seen from the histogram that the type of board B2 has the smallest percentage of weight loss in the amount of 4.45% and the highest percentage of weight loss are owned by the board of E2 by 12.78%. The smaller the percentage of weight loss test examples show that the more disliked by subterranean termites *C. curvignathus*. This may result from the influence of adhesive and the paraffin content of the composition in accordance with the conditions that are not favored by termites, so the test samples being eaten by termites is very little. Termites are not able to adjust to the new environment will die, while the remaining will be getting weaker and gradually get sick and die.

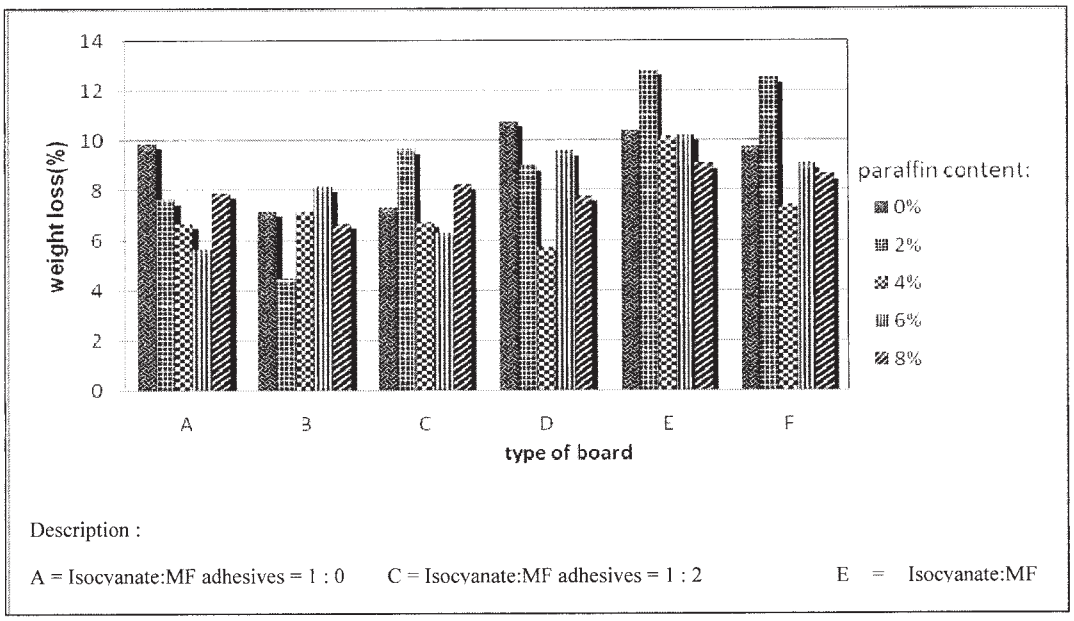

Figure 1. Percentage weight loss of test samples

Table 1 shows the percentage of weight loss relationship test samples with standard levels of resistance to MWBT (modified wood block test) followed the criteria of Sornnuwat (1996) that are grouped into five levels, they are highly resistant, resistant, moderately resistant, non-resistant, and susceptible. From the results of tests conducted with this MWBT standards, shown in Table 2 the percentage relationship to the level of weight loss endurance test sample and the level of resistance to *C. curvignathus* after 21 days of testing. Based on the composition of the adhesive, the type of board A, B, and C can be classified in the group's moderately resistant; while the board of D, E, and F can be classified in the non-resistant groups.

Table 1. Percentage average weight loss of standard test samples MWBT (modified wood block test) and level of resistance (Sornnuwat 1996)

Percent weight loss (%) *(No-choice test)*	Level of resistance
0	Highly resistant
1 – 3	Resistant
4 – 8	Moderately resist ant
9 – 15	Non-resistant
>15	Susceptible

Table 2. The average percentage of weight loss based on the adhesive test sample and the level of resistance to subterranean termite *C. curvignathus*

Type of board	Percent weight loss (%)	Level of resistance
A	8	Moderately resistant
B	7	Moderately resistant
C	8	Moderately resistant
D	9	Non-resistant
E	11	Non-resistant
F	9	Non-resistant

Mortality of subterranean termite (*C. curvignathus*)

Test results showed that the average mortality of subterranean termite *C. curvignathus* in all test samples were in the range of values above 60%. The average percentage of mortality from termite attack can be seen in Figure 2.

In the histogram shows that the percentage of termite mortality is high with a value range 65.91% - 97.73%. In general, based on test results, the greatest percentage of mortality is also owned by board type B2 (97.73%), but the smallest percentage of mortality on board type A2 (65.91%).

Supriana (1983) in Saragih (2009) also states that in a single food preference test in the laboratory, termites exposed to only one choice of food. In a special case, the termites eat the food or it will starve to death. Therefore, in this test, can be seen that the samples used test is the only food source for

termites test, so based on the percentage of lost weight (Figure. 1) indicates a high value. In addition, because the standard tests used are not standard for composite board so that termites can attack from the side of the test samples.

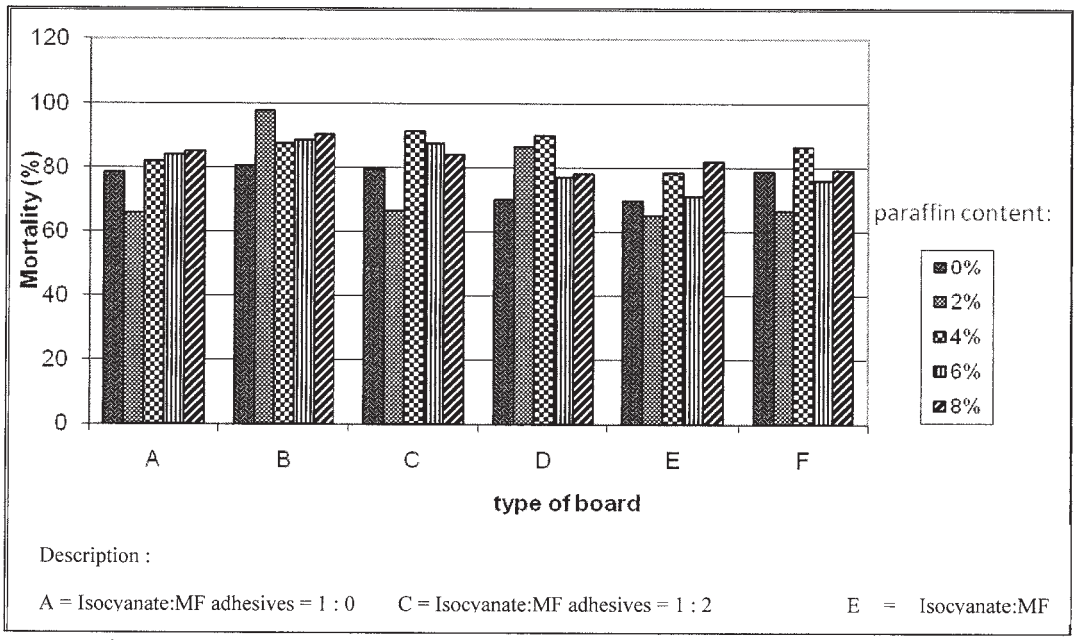

Figure 2 Percentage mortality rates of subterranean termite *C. curvignathus*.

The high value of mortality caused by the evaporation of gas from the formaldehyde emission in the test bottle from the test sample itself, especially those containing melamine formaldehyde adhesive, so that these emissions is possible termite attack nerves and cause of death in the termite *C. curvignathus* happens at the end of the test. Other causes of mortality of subterranean termites is the adhesive that content contained in these composite boards are toxic to flagellates whose live in the termite gut. And by utilizing the biological nature of termites themselves, namely the nature thropalaxis, the termites will distribute each other's food, pheromone, or flagellates through this thropalaxis behavior. Therefore, these flagellates activities attack that do not result in more active and eventually even termites will die.

The attack form of subterranean termites (*C. curvignathus*) to test samples

According to Krisna & Wesner (1971) *in* Rismayadi (1999), termites will tend to choose foods that contain lots of cellulose, easy to bite and destroyed. Ground termite attack (*C. curvignathus*) in the test samples occurs only on the side.

Demineralization test samples on the side, it is possible that part not covered by the adhesive and paraffin. Not coated by adhesive and paraffin caused during test samples, that cutting is located in the middle, so that the core containing wafers cut. Where the wafers are cut off part is not coated by the adhesive and paraffin, and then to the outer layer of woven bamboo was (face and back of board) are not given the adhesive and paraffin at all. This provides a big opportunity for termites to attack the test samples. And also mixing the powder paraffin with wafers done manually, so the mixing is also cause uneven presence of paraffin in the core part of the board. However, it is attacked by the termites stay focused on the side of the wafers of wood species sengon, acacia, and yellow shorea.

Conclusion

The conclusion of this research :

1. Use of Isocyanate-MF adhesive and the addition of paraffin to give tangible effect on weight loss test sample but not have real impact on mortality, mortality of termites with a value of more than 60%.
2. Composite board that has resistance to subterranean termite *C. curvignathus* attack it is best board type B2, the board that the adhesive composition of Isocyanate-MF 1: 1 with 2% paraffin

content, so the combination of the adhesive composition and content of this paraffin are optimum conditions for the manufacture of composite boards from wood waste and bamboo woven.

3. Adhesive composition affect the level of resistance of composite boards, which boards A, B and C included in the group's moderately resistant, while the boards D, E, and F included in the non-resistant groups by Sornnuwat (1996) classification.

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The Resistance of Oriented Strand Board from Betung Bamboo (*Dendrocalamus asper* (Schult.f.) Backer ex Heyne) Against Dry-wood Termite and Subterranean Termite

by

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Abstract

This study was aimed to analyze the resistance of Oriented Strand Board from betung bamboo (*Dendrocalamus asper* (Schult.f.) Backer ex Heyne) on dry wood termite and subterranean termite. Materials used in this study were strands from betung bamboo, isocyanate adhesive, dry wood termite (*Cryptotermes cynocephalus*) and subterranean termite (*Coptotermes curvignathus* Holmgren). The target density of board samples was 0.75 g/cm^3. Three-layer OSB was manufactured using four face-core ratio levels (40:60, 50:50, 60:40 and 70:30). The contents of isocyanate used were 7%, 6%, 5% and 4% based on the bamboo strand oven dry weight. Testing OSB against termite attack carried out on dry-wood termite and subterranean termite with laboratory technique. The research results showed that resistance of the produced OSB against dry-wood termite and subterranean termite attack was better compared to bamboo itself. The weight loss of OSB on dry-wood termite was 8.43%-16.93%, while on subterranean termite was 3.26-8.89%. The mortality on OSB more than bamboo.

Key words: bamboo, OSB, isocyanate, dry wood termite, subterranean termite.

Introduction

A way to overcome the shortage of wood raw material is the development of innovative products with utilizing lignocellulosic materials for wood substitution. OSB is a structural composite product and one of the wooden panels that are designed to replace the plywood (Nishimura *et al.* 2004).

One of lignocellulosic materials that have potential to be used as an alternative substitution for wood is bamboo. From several types of bamboo growing in Indonesia, betung bamboo (*Dendrocalamus asper* (Schult.f.) Backer ex Heyne) is the most common bamboo species used for construction materials and woven wall.

The previous study (Saad *et al.* 2008) showed that the ratio of strands in the face-core OSB and the use of isocyanate adhesive on a certain level can improve physical and mechanical properties of OSB. Like other wood products, OSB from bamboo also contains cellulose that can be attacked by biodeterioration agents especially termites. Indonesia is a paradise for the life of termites (Nandika 2008). High humidity and warm temperatures throughout the year is a popular for the termites. Termites are known to be the most important pest causing damage to wooden constructions and other wood products. Therefore, the resistance of OSB made from several face-core ratios with different levels of adhesive on dry-wood termite and subterranean termite attack also needs to be investigated.

Materials and methods

Materials

Materials used in this study were strands betung bamboo, isocyanate adhesive with the trademark Bond H3M PI produced by Poly Oshika Co. Ltd... Japan and distributed by PT. Polychemi Asia Pacific Indonesia with 99.13% solid content, dry-wood termite (*Cryptotermes cynocephalus* Light) and subterranean termite (*Coptotermes curvignathus* Holmgren).

Methods

To determine the resistance of OSB against termites attack, the OSB is made with 4 types of the core face ratio, namely: 40:60, 50:50, 60:40, 70:30 which is based on the weight ratio stands in percent (%). To determine the influence of adhesive levels, the variation levels of adhesive were 4%, 5%, 6% and 7%. Three-layer boards made where the strands in the face and core layers perpendicular to each other. Target board density was 0.75 g/cm^3 with a dimension of 30 cm x 30 cm x 0.9 cm.

OSB Production

Strand was made with the size of 60-70 mm (L) x 20-25 mm (W) x 0.6 mm (T). The strands were then air-dried prior to oven dried in oven at 60°C to achieve 6-7% moisture content. OSB was made from the face:core ratios of 40 : 60, 50 : 50, 60 : 40, 70 : 30 based on the weight of strands.

The content of isocyanate at various levels of 4%, 5%, 6% and 7% were applied. Strands were mixed with isocyanate adhesive in a rotary blender. Then, the strands were weighed to determine the weight of strands for each layer. Strands were then manually compiled and perpendicularly arranged. The hand-formed mats were pressed at 150°C, using a single step pressing with the specific pressure of 25 kg/cm^2 for 5 minutes. The produced OSB was then conditioned for two weeks at room temperature and cut into test samples.

Testing OSB Against Termite Attack

Testing OSB against termite attack carried out on dry-wood termite and subterranean termite with laboratory technique. In tests against termites was added as a control treatment is a betung bamboo sample.

Testing of dry-wood termite carried out with one each of samples was placed in a glass container (5 cm x 4.5 cm x 3.5 cm). Fifty dry-wood termites were introduced into each container. Glass container and then covered with gauze and then placed in a dark room. Sample tests stored for 28 days and every 7-day is the number of individual observations of dead termites.

Testing of dry-wood termite used the glass bottles as a container. Thirty gram of sand was filled in the bottle and moistened with 6 ml of distilled water. One each of sample was buried in the sand of glass bottle. Two hundred workers and 10% numbers of soldiers were introduced into the bottle. Each bottle was covered with black cloth and placed in a dark room for 21 days and every 7 day is the number of individual observations of dead termites.

After trapped, samples were taken out from containers, cleaned, and oven-dried, and reweight to determine percentage weight loss from the equation:

$$\text{Weight Loss (\%)} = (\text{Wo-W1})/\text{Wo} \times 100\%$$

where, Wo = weight of sample prior to termite exposure (g)

W1 = weight of sample after termite exposure (g)

Percentage of dead termites (mortality) was determined from the equation :

$$\text{Mortality rate(\%)} = \text{N1} / \text{No} \times 100\%$$

where, No = number of initial termites

N1 = number of dead termites

Analysis

This research was determined using three replications for each type of board. The average value for the parameters were observed compared with each other to determine the resistance of each type of the board against termites.

Results and discussion

Density

Board density is one factor that can affect the feeding behavior of termites. With high density, the OSB more compact that may be inhibiting feeding behavior of termites. Treatment of pressing at high temperatures in the production of OSB will increase the density of the board that becomes harder than the density of the bamboo sample. Research results showed that the density of produced OSB ranged from 0.78-0.89 g/cm^3. The density value of the produced OSB is greater than the density of bamboo (0.64 g/cm^3).

The Resistance OSB against Termites

The resistance of bamboo OSB against termites is shown in Table 1.

Table 1. The weight loss of OSB and mortality of termite

Sample	Dry-wood Termites		Subterranean Termite	
	Weight loss (%)	Mortality (%)	Weight loss (%)	Mortality (%)
Control	11.90	1.25	15.52	6.82
A1B1	9.09	10.17	6.25	16.36
A1B2	8.92	11.83	3.92	15.91
A1B3	9.42	8.33	7.32	10.45
A1B4	9.10	8.67	3.26	11.21
A2B1	9.54	7.67	7.51	13.79
A2B2	9.18	7.33	7.01	14.7
A2B3	9.00	6.83	6.45	16.06
A2B4	8.84	7.83	6.95	16.52
A3B1	8.74	16.50	7.34	16.67
A3B2	8.59	13.67	8.89	17.73
A3B3	8.78	12.33	5.85	20.45
A3B4	8.70	6.83	6.53	20.45
A4B1	9.72	8.00	6.8	35.91
A4B2	16.93	5.50	3.86	39.39
A4B3	8.79	14.83	7.33	36.82
A4B4	8.43	16.83	4.83	33.03

where : sio face-core 40:60, resin content 7% — A3B1 : rasio face-core 60:40, resin content 7%
sio face-core 40:60, resin content 6% — A3B2 : rasio face-core 60:40, resin content 6%
sio face-core 40:60, resin content 5% — A3B3 : rasio face-core 60:40, resin content 5%
sio face-core 40:60, resin content 4% — A3B4 : rasio face-core 60:40, resin content 4%
sio face-core 50:50, resin content 7% — A4B1 : rasio face-core 70:30, resin content 7%
sio face-core 50:50, resin content 6% — A4B2 : rasio face-core 70:30, resin content 6%
sio face-core 50:50, resin content 5% — A4B3 : rasio face-core 70:30, resin content 5%
sio face-core 50:50, resin content 4% — A4B4 : rasio face-core 70:30, resin content 4%

control : sample of betung bamboo

The Resistance of OSB against Dry-Wood Termite

Research results showed that the weight loss of OSB ranged from 8.43%-16.93%. The OSB with highest weight loss was found at the board with face-core ratio 70:30 content of the adhesive 6%, while the lowest was found at the board with face-core ratio of 70:30 content of the adhesive 4%. The results of variance analysis shown that face-core ratio, content of the adhesive or their interaction are not significant influence on the weight loss. This means that regardless of the face-core ratio and content of adhesive, termites do not like the OSB has given isocyanate adhesive. The weight loss is one parameter to determine effectively of the materials tested. The greater weight loss means more material eaten by termites and materials tested less resistance against attack.

Test results shown that the mortality rate of the OSB below 50%. The results of variance analysis shown that the face-core ratio and their interaction are significantly influence, while content of the adhesive not influence. The highest mortality was found at the OSB with face-core ratio 60:40 with content of adhesive 7%, while the lowest at the OSB with face-core ratio 70:30 with content of adhesive 6%. On the OSB that the lowest mortality have the highest weight loss. Termite mortality rate in OSB higher compared with bamboo. The OSB that processed with pressing and use isocyanate was not favored by subterranean termites, although still occur damage in small number and mortality was not arrive 100%. Still the damage to the test sample because the termites eating in the conditions no other food choices.

The Resistance of OSB against Subterranean Termites

Research results showed that the weight loss of OSB ranged from 3.26-8.89%. The highest weight loss was found at the OSB with face-core ratio 60:40 content of adhesive 6%, while the lowest

was found at the OSB with face-core ratio 40:60 content of adhesive 4%. Based on the results of variance analysis, face-core ratio, content of adhesive and their interaction are not significantly influence.

The weight loss of OSB lower compared with the control of bamboo. The weight loss reduced a lot from 15.52% to 3.26%, more than 70% decrease. This shows that the use of isocyanate adhesive and pressing on OSB was significantly inhibiting feed activities of termites. These results indicated that the adhesive is very effective to protect OSB from the termite attack. According to Weaver and Owen (1992), one of the advantages used of isocyanate is increase resistance of wood against deterioration of biological factors. The eating behavior of termites shown that their attack on the center of the board where this section has lower density. The OSB that pressing processed at high temperature has different density level from the surface to the center of the board. The center has the lowest density, while the surface has the highest density. The low density resulting holes or cracks that can be entered by the termites to attack the board. The coating surface of bamboo strands by isocyanate can protect the OSB from termite attack, especially the parenchyma that softer than vascular bundles.

Results of mortality testing subterranean termite indicated that there are differences between types of OSB on the value of mortality. The value mortality of OSB greater than the value mortality of the control (6.82%).

The results of variance analysis shown that face-core ratio had significant influence on the value of termite mortality, while the content of adhesive and their interaction not significant influence Further tests are performed using Duncan multiple comparison shown that the face-core ratio of 40:60, 50:50 and 60:40 respectively are not significantly different but significantly different on ratio 70:30.The OSB with ratio 70:30 content of adhesive 6% has highest termite mortality rate but lowest weight loss. This is because the number of individual remaining termites is also the less. The highest mortality at the OSB with ratio 70:30, because boards have characteristic more hard and dense so termites weak and dead gradually. Possible healthy termites eat the weakly termites to survival of his life until the end of the period testing.

Conclusion

The resistance of OSB against Dry-Wood Termites and subterranean termites attack higher than the bamboo with the same species. The weight loss of OSB was not influenced by the face-core ratio and content of adhesive, while the mortality of termite was only influenced by the face-core ratio.

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The Resistance of Binderless Particleboard against Subterranean Termites *Coptotermes curvignatus* Holmgren Attack

by

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Abstract

The purpose of this research was to evaluate the resistance of binderless particleboard on subterranean termite attack. Material used in this study were andong bamboo (*Gigantochloa pseudoarundinaceae*) and three species of wood from community forests namely, Sengon (*Paraserianthes falcataria*), Gmelina (*Gmelina arborea*), and Mindi (*Melia azedarach*). Binderless particleboard were produced by oxidizing particles then hot pressed at a temperature of 180°C for 15 minutes. 150 workers and 15 soldier of Subterranenan termite (*Coptotermes curvignatus* Holmgren) collected from a nest then put in the container paralon pipe with 80 mm 60 mm in diameter and height respectively, and coated on the bottom 10 mm thick gypsum. Sample size tested were 20 x 20 x 7 mm, and exposed in container for three weeks. Parameter observed was the weight loss due to termite attack. For comparison, in this study also produced a conventional particle board using urea formaldehyde resin (UF). The results showed that the highest weight loss was found in bamboo particleboard, followed by Sengon, Mindi, and Gmelina. This phenomenon were found both in the binderless particleboard or particleboard with UF resin. Weight loss of binderless particleboard were higher (1.49% - 7.42 %) compared to those of particleboard using UF resin (0,01-1,64 %). Weight loss of binderless particleboard with various levels of hydrogen peroxide tend to increase when hydrogen peroxide content increases, but there are no particular trend related to catalyst ferrous sulphate content.

Keywords: Binderless particleboard, Coptotermes curvignatus, Hidrogen peroxide

Introduction

One effort to produce particleboard that have environmentally friendly characteristics is development manufacturing binderless particleboard technology. This attempt can be done through enzymatically activated of chemical components of particles (Hüttermann *et al.* 2001, Widsten *et al.* 2004, Müller *et al.* 2007), or oxidized of wood particles (Karlsson and Kandelbauer 2002, Widsten *et al.* 2003). Development of binderless particleboard through activation of wood particles using hydrogen peroxide and catalyst ferrosulphate also been done by Suhasman *et al.* (2009) and Suhasman *et al.* (2010). The results of these studies indicate that the mechanical properties of binderless particleboard were similar or even better than conventional particleboard, especially in terms of dimensional stability and modulus of elasticity. Seeing the promising characteristics of these products, so further research is needed to evaluate the product resistance against termite attack.

The resistance of particleboard against termite attack is one of the important parameters to assess the quality of the product because of the high levels of damage caused by this biodegradation agents. Losses caused by termite attack is estimated to reach Rp. 2.80 trillion in 2000 (Nandika *et al.* 2003), and is projected to continue to increase from year to year.

A numerous factors affect particle board resistance to termite attack such as raw material species, raw material treatment, and resin types. Particleboard made from different wood species will have different resistance to termite attack, even between the stem and branch have a different characteristics (Suhasman, *et al* 2008). Indrayani (2007) stated that it has been widely accepted that termite have different preferential level to attack every wood species, so affect its level of consumption. Treatment of raw materials such as chemical modification of wood by acetylation (Hadi *et al.* 1995), and preservation will also enhance the resistance of the particleboard against termite attack. The influence of adhesives type against termite attack has been suggested by Weaver and Owen (1992). He stated that one of the advantages of isocynanate utilization as an adhesive is

the increases the resistance of wood against deterioration due to biological factors. Nevertheless, the results of existing studies have not reported the resistance of binderless particleboard against termite attack. In addition, the use of ferrosulfat as a catalyst in the oxidation process suspected increasing resistance of the product against termite attack. Since Fe is the chemical that can serve as a wood preservative. This study was aimed to evaluate the durability binderless particleboard made from several species of raw materials, and the influence of $FeSO_4$ catalyst levels on resistance of binderless particleboard against subterranean termite (*Coptotermes curvignatus* Holmgren).

Materials and methods

Particleboard Manufacturing

Binderless particleboard made from 4 species of raw materials ie andong bamboo (*Gigantochloa pseudoarundinaceae*) and three species of wood from community forests namely, Sengon (*Paraserianthes falcataria*), Gmelina (*Gmelina arborea*), and Mindi (*Melia azedarach*). Bamboo and wood converted into a fine particle size that pass on 10 mesh sieve and then air dried. The particles are then oxidized using 20% hydrogen peroxide based on dry particle weight and 5 % ferrous sulphate (based on hydrogen peroxide weight) as a catalyst. Oxidized particles then compressed at a temperature of 180 °C for 15 minutes with a pressure of 25 kgf cm^{-2}. Target density of the boards were 0.75 g cm^{-3} with dimensions of 30 x 30 x 0.7 cm.

In addition, in this study also produced a board using oxidized sengon wood particles with various of hydrogen peroxide and ferrous sulphate levels. Levels of hydrogen peroxide treatment consists of 3 degree of 5, 10, and 20%, while the levels of ferrous sulphate consist of two stages namely 2.5 and 5%. Board were produced by hot compressed at the same conditions with the other boards, as well as the dimensions and density of the target. The boards were then conditioned for a month before being fed on termites

For comparison, in this study also made a conventional particle board using 10% Urea Formaldehyde resin based on dry particles weight. Target density and dimensions of the board were similar to the other boards, but the temperature pressing was 130 °C. The board then conditioned for 2 weeks before being fed to the termites.

Termite Test

Paralon pipe of 80 mm in diameter and 60 mm in height and at the bottom is covered with 1 cm thick gypsum prepared as a container test. In the container was then placed a corrugated plastic that have similar dimension with sample. Sample that had been dried in an oven at a temperature of 105 ± 3 °C then placed on the plastic. 150 workers and 15 soldier of *coptotermes curvignatus* then introduced in the container. Samples then covered with black cloth and placed in a dark room for 3 weeks. To maintain humidity, then every 3 days interval of water sprayed by using hand spray. At the end of the week-3 samples removed and cleaned then dried in an oven at a temperature of 105 ± 3 °C. Each sample is weighed and then calculated weight loss due to termite attack.

Results and discussion

Comparison of weight loss among type of binderles particleboard raw material are presented in Figure 1. Base on the data, it turns out that bamboo is the most vulnerable materials attacked by termites. Weight loss value is higher than all types of wood. Although bamboo has been known to the material more vulnerable to powdered bamboo than termites, but in the case of this research, bamboo was also prone to termite attack.

Furthermore, when viewed from the three wood species studied, shows that the highest weight loss occurred in sengon wood, followed by Mindi and Gmelina. There is a tendency that the wood have a lower density (sengon, 0.33 g cm^{-3}, Mindi 0.43 cm^{-3}, and Gmelina 0.5 cm^{-3}) is more easily attacked by termites.

When compared between binderless particleboard with conventional particle board is seen that the conventional particle board is more resistant to termite attack. Although Fe is known to increase the durability of wood, but low levels appear to be ineffective to increase the resistance of the boards against termite attack. The presence of formaldehyde compounds in conventional particle board tends to cause the board is more resistant to termite attack, moreover, the process of conditioning a short (2 weeks) led to concentration of free formaldehyde is still quite high.

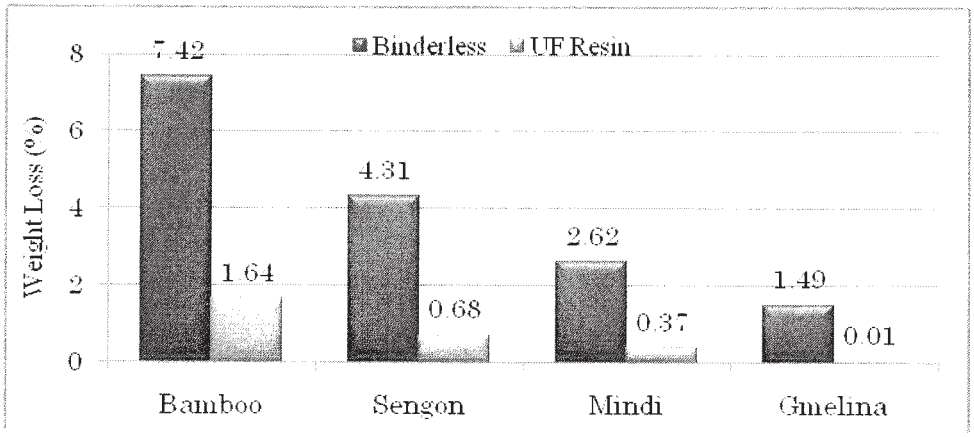

Figure 1. Weight Loss of Particleboard

Weight loss data of binderless particleboard at various levels of hydrogen peroxide and ferrosulphate are presented in Figure 2. The data in the figure shows that the weight loss of the sample tend to increase when levels of hydrogen peroxyde increases. However, low levels of ferrous sulphate tend to high weight loss result. This phenomenon especially occured in 10 % and 20% hydrogen peroxide levels.

High weight loss due to increasing hydrogen peroxide level can be caused by the degradation of cellulose by chemical component of the oxidation treatment. The results of X-Ray analysis showed a reduction in the degree of crystallinity, namely from 31.03% for particles without oxidation treatment to 25.81% of particles obtained from binderless particleboard made through oxidation treatment. Decreasing of the crystallinity degree can lead to more easily accessed and degrade the chemical components of materials by termite, especially cellulose. This indicates that elevated levels of hydrogen peroxide can cause increased degradation of chemical components that make cellulose more accessible to the termites.

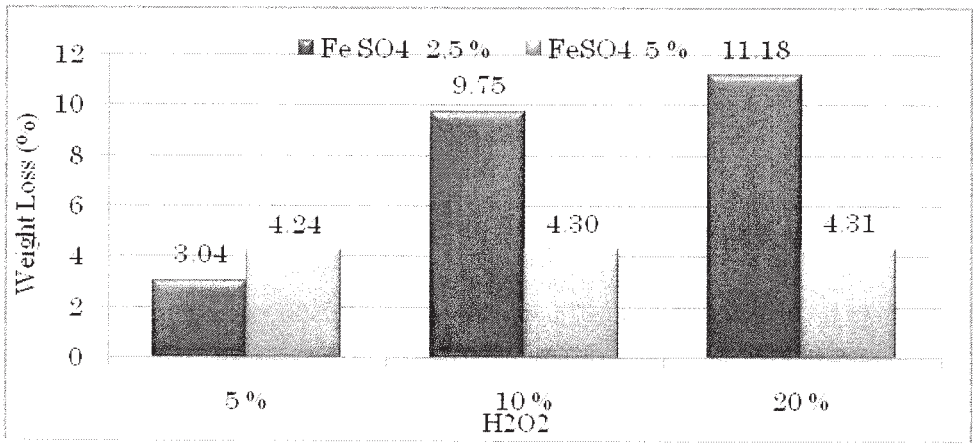

Figure 2. Effect of Hidrogen Peroxyde and Ferrosulphate Concentration on Weight Loss of Binderless Particleboard

Nevertheless, this phenomenon was only found in particle board with a 2.5% catalyst levels. This indicates that the levels of higher catalyst namely 5% were likely to produce boards that are more resistant to termite attack. As mentioned earlier, Fe can essentially act as a wood preservative, so the higher levels, can produce boards that are more resistant to termite attack.

Conclusions

The research results showed that bamboo tends to be more susceptible to termite attack compared with the particle board made from wood. This phenomenon is especially true in binderless particleboard. Particle board made from high density raw materials tends to produce particle board that is more resistant to termite attack. Increasing levels of hydrogen peroxide tends to decreasing of

resistance of binderless particleboard against termite attack, but higher levels of catalyst (5% based on hydrogen peroxide weight) produce boards that tend to be more resistant than the boards that use the catalyst 2.5%.

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Effect of Phenol Formaldehyde Content on Termite Attack Resistance of Particleboard Made from Oil Palm and Mangium Wood

by

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Abstract

This research provides results of a laboratory and field test study that evaluated the termite resistance of particleboard. The objective of this research was to evaluated phenol formaldehyde (PF) content effect to durability of particleboard made from oil plam (*Elaeis guineensis* Jacq.) and mangium wood (*Acacia mangium*). The PF adhesive level content for particleboard investigated were 7%, 10% and 15%. The dimension of sample was 200 mm by 50 mm by 8 mm and exposured for 1 weeks to termites, and then weight loss and attack level parameters were determined. The result showed that the level of PF content can improve the durability of particleboard. PF level content of 15% shows the best result in durability compared to other. This was showed by the value of particleboard weight loss and attack level on this PF level content is the lowest than to other. In generally, mangium particleboard was more resistance to termite attack than oil palm. This was showed by the value of weight loss and attack level of mangium particleboard lower than oil palm.

Keywords: phenol formaldehyde, particleboard, termite attack resistance

Introduction

Particleboard is an engineered wood product manufactured from wood particles, such as wood chips, sawmill shavings, or even saw dust, and a synthetic resin or other suitable binder, which is pressed and extruded. Particleboard is a composite material (Wikipedia 2010).

The main matter or particleboard was woods which taken from plants forest or other agriculture waste. Those matters usually has a low natural durability, so it was need a preservative that could increase its strength on biodeteriation. Meanwhile, the preservative was expensive and caused a bad impact to environment.

PF resin is an adhesive biokomposit used. PF is thermosetting resin type for exterior used, because it is more resistance to weathering and biodeterioration. In this research, particleboard of oil palm wood and mangium was made by using PF resin. PF content level were 7%, 10% and 15%. PF resin was used as the matrix on particel board and the preservative of wood biodeterioration attack, such as termite.

The objective of this research was to evaluated phenol formaldehyde (PF) content effect to durability of particleboard made from oil palm (*Elaeis guineensis* Jacq.) and mangium wood (*Acacia mangium*). The subject in this research is oil and mangium wood. Three level of phenol formaldehyde (PF) adhesive content used were 7%, 10% and 15%.

Subterranean termite is the most important agent of wood and wood products deterioration agents in tropical region. Hence in order to improve natural durability of particleboard, the resistance of particleboard against subterranean termite attack under various PF content was observed.

Materials and methods

Materials:

The particleboard made of oil palm and mangium wood. Three level of phenol formaldehyde (PF) adhesive content used were 7%, 10% and 15%.

Methods:

Particleboard was manufactured by mixing dry wood particles together with PF resin and forming the mix into a sheet. The sheets formed (250 mm by 250 mm by 8 mm) are then hot-compressed for 10 minutes, under pressures 25 psi and temperatures 160 °C. The boards are then cooled, conditioned and trimmed.

Test specimens (200 mm by 50 mm by 8 mm) were buried vertically in the ground for 1 week. Specimens were buried with 150 mm of their length below ground level. Test sites were located in the arboretums of Bogor Agricultural University, Bogor, Indonesia. The dominant subterranean termites are *Coptotermes* spp., *Macrotermes* spp., *Microtermes* spp., and *Nasutitermes* spp. (Nandika *et al.* 2003). At the end of the test, the condition and weight loss percentage of the specimens were determined. Before and after test, the samples were dried in the oven at 103 ± 2°C. Table 1 and 2 showed the resistance level of wood against termite attack.

Table 1. Resistance level of wood against termite attack based on weight loss

Weight loss (%)	Resistance level
0	very strong
1–3	strong
4– 8	moderately strong
9–15	weak
>15	vulnerable

Source: Sornnuwat *et al.* (1995)

Table 2. Resistance level of wood against termite attack based on attack level

Class	Attack level (%)
A	Tidak diserang (0) unattack
B	Sedikit terserang (~12.5) fairly attack
C	Serangan ringan (~25) light attack
D	Serangan berat (~37.5) heavy attack
E	Serangan hancur (~>50) strong attack

Source: Sornnuwat *et al.* (1995)

Result and ddiscussion

The reserach showed that PF content can improved durability of particleboard. PF level content of 15% showed the best result in durability compared with others. The mean value of weight loss of samples after baited for 1 weeks to subterranean termite ranged between 7.36~71.81% (Table 3). The lowest values of weight loss were achieved on mangium particleboard with PF content 15% (7.36%). The highest values of weight loss were achieved on oil palm particleboard with PF content 7% (71.81%).

The mean value of attack level of samples after baited for 1 weeks to subterranean termite ranged between 20.83~83.33% (Table 3). The lowest values of attack level were achieved on mangium particleboard with PF content 15% (20.83%). The highest values of attack level were achieved on oil palm particleboard with PF content 7% (83.33%).

Subterranean termite utilizes wood both as a shelter and food sources (Bowyer *et al.* 2003). Particleboard from a bio-based resources are degraded biologically because organisms recognize the carbohydrate polymers (mainly the hemicelluloses) in the cell wall and have both non-specific chemical and highly specific enzyme systems capable of hydrolyzing these polymers into digestible units (Rowell 1998). Addying PF content added can increase durability of particleboard to termite attack. PF as a toxic will caused a light of termite attack so it has a lower weight loss.

Table 3. Resistance of particleboard against termite attack based on weight loss and attack level

Wood	PF content (%)	Weight loss (%)	Attack level (%)
Oil palm	7	71.81 (vulnerable)	83.33 (strong attack/E)
	10	48.13 (vulnerable)	62.50 (strong attack/E)
	15	27.11 (vulnerable)	45.83 (strong attack/E)
Mangium	7	14.76 (weak)	41.67 (strong attack)
	10	12.09 (weak)	33.33 (heavy attack/D)
	15	7.36 (moderately strong)	20.83 (light attack/C)

Mangium particleboard more resistance to termite attack than oil palm particleboard. This is proofed by the value of weight loss and attack level mangium which have the lowest compared with

oil palm. The mean value of weight loss and attack level of mangium particleboard were lower than oil palm particleboard.

In oil palm particleboard, the lowest values of weight loss was achieved on particleboard with PF content 15% (27.11%) and the highest was with PF content 7% (71.81%). The lowest values of attack level was achieved on particleboard with PF content 15% (45.83%) and the highest was with PF content 7% (83.33%). In mangium particleboard, the lowest values of weight loss was achieved on mangium with PF content 15% (7.36%) and the highest was with PF content 7% (14.76%). The lowest values of attack level was achieved on particleboard with PF content 15% (20.83%) and the highest values was with PF content 7% (41.67%).

Based on Sornnuwat *et al.* (1995) criteria, oil palm particleboard belongs to vulnerable (weigt loss) and strong attack/E criteria (attack level). In other hand, weight loss of mangium particleboard belongs to weak (PF content 7 and 10%) and moderately strong (PF content 15%), and attack level of mangium particleboard belongs to strong attack/E (PF content 7%), heavy attack/D (PF content 10%) and light attack/C (PF content 15%).

Conclusion

1. PF content can improved durability of particleboard. PF content level of 15% showed the best result in durability compared with others. This is proved by the value of weight loss and attack level which have the lowest value compared with other PF content level.
2. Mangium particleboard more resistance to termite attack than oil palm particleboard. This is proofed by the value of weight loss and attack level of mangium which more lower than oil palm value.

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Comparison of the Termite Test Methodology of Japanese and Indonesian National Standards*

by

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Abstract

Termite test methodologies of two national standards, Japanese standard JIS K 1571-2004 and Indonesian standard SNI 01.7207-2006 were compared by exchanging termite and wood species to discuss and improve the validity of test results. The comparative tests were conducted in RISH Kyoto University (Japan) using *Coptotermes formosanus* Shiraki and in Bogor Agricutural University (Indonesia) using *Coptotermes curvignathus* Holmgren. Wood specimens were untreated *Cryptomeria japonica* sapwood blocks measuring 20 mm (T) x 20 mm (R) x 10 mm (L) and *Hevea brasiliensis* heartwood blocks measuring 5 mm x 25 mm in cross section x 25 mm (L) for JIS and SNI, respectively. Test durations were three and six weeks in JIS and SNI, respectively. Although mean mass losses of wood specimens were not much different between the two methods: 16.4% by *C. formosanus* and 16.7% by *C. curvignathus* in the JIS test, while those in the SNI test were 25.7% and 26.0%, mortalities of termites in the JIS method were 14.7% for *C. formosanus* and 74.9% for *C. curvignathus*, and those were 32.4% and 100% in the SNI method for the two termite species, respectively. These findings suggested that SNI test method should be modified to reduce temite mortality for the reliable comparison of the test results.

Key words: Indonesian standard SNI 01.7207-2006, Japanese standard JIS K 1571-2004, forced-feeding subterranean termite test, *Coptotermes curvignathus* and *Coptotermes formosanus*.

Introduction

In 2008 Indonesian log production was 31.9 million m^3, consisting of 23% and 77% from natural and plantation forests, respectively (Ministry of Forestry 2009). This was a great change in comparison with that in 1997 when the logs from natural forest accounted for more than 90% of the total production 29.5 million m^3 (Ministry of Forestry 2002). In the last eight years most of the logs have been produced from fast-growing tree species at a 10-15 year harvest cycle, and those generally contain juvenile wood portion.

Indonesian Wood Atlas volumes 1-3 (Abdurrohim *et al.* 2004, Martawijaya *et al.* 1984, 1989) refer to the natural durability of Indonesian woods mostly from natural forest and only some from plantation forest. Since the durability of Indonesia wood species is determined by termite test using *Coptotermes curvignathus* Holmgren according to the Indonesian Standard SNI 01.7207-2006, it is difficult to directly compare the Indonesia test results with those obtained by different test methods such as Japanese standard JIS K 1571-2004 which designates *Coptotermes formosanus* Shiraki as a test termite species.

The purpose of this study was to compare the results of termite tests conducted according to the two national standards by exchanging termite and wood species.

Materials and methods

Termite tests were carried out at both RISH of Kyoto University and Bogor Agricultural University according to JIS K 1571-2004 and SNI 01.7207-2006, respectively.

Indonesian standard method

Untreated wood specimens were prepared from *Hevea brasiliensis* Muell. Arg. (rubber wood) heartwood measuring 5 mm x 25 mm in cross section x 25 mm (L). Individual specimen was

* This study was carried out as a part of collaborative research project financially supported by RISH Kyoto University Japan and the Ministry of National Education of Indonesia.

put in the glass jam pot to contact with an inside wall with 200 g of sand at 7% moisture content of water holding capacity of the sand and 200 sound worker termites of *C. curvignathus*. The assembled jam pots were placed in the dark room for six weeks. Each pot was weekly weighed to adjust the moisture content of the sand with water. At the end of the test, mass loss of each wood specimen and termite mortality were determined (SNI 01.7207-2006). Five replicates were tested. Test results were analyzed by t-test.

Japanese standard method

Untreated wood specimens were prepared from *Cryptomeria japonica* D. Don sapwood measuring 20 mm (T) x 20 mm (R) x 10 mm (L). Individual specimen was exposed to 150 workers and 15 soldiers of *C. formosanus* in an acrylic cylindrical container [60 mm in height and 80 mm in diameter] of which one end was sealed with plaster to form a bottom, and maintained at 28 ± 2 °C and > 75% RH in the dark for three weeks. At the end of the test, mass loss of each wood specimen was determined together with mortality of worker termites. Test results were analyzed by t-test.

Differences of the two methods are summarized in Table 1.

Table 1 Differences of termite test methods between JIS K 1571-2004 and SNI 01.7207-2006

Item	Japanese Standard JIS K 1571-2004	Indonesian Standard SNI 01.7207-2006
General	Forced-feeding test	Forced-feeding test
Test termite species	*Coptotermes formosanus* Shiraki	*Coptotermes curvignathus* Holmgren
Number of termites used per test unit	150 workers and 15 soldiers	200 workers
Wood specimens	Species: *Cryptomeria japonica* D. Don or *Pinus densiflora* Sieb. et. Zucc. sapwood Annual rings/10 mm: 3-5 Oven-dried density: 0.25-0.32 g/cm^3 Size: 20 mm (R) x 20 mm (T) x 10 mm (L) for pressure treatment (dressed sawn), 10 mm (R) x 10 mm (T) x 20 mm (L) for superficial treatment (dressed sawn)	Species: Not specified Size: 2.5 cm (L) x 2.5 cm (T) x 0.5 cm (R) (dressed sawn)
Pre- and post-conditioning of wood specimens	Oven-drying at 60 ± 2 °C for 48 h prior to weighing individual specimen	Oven-drying at 102 ± 3 °C until no weight change detected
Weathering procedure	Treated wood specimens to be weathered. Pressure-treated specimens: A group of specimens of the same treatment is placed in a 500 ml beaker with deionized water 10 times as much as the volume of wood specimens, stirred at 400-450 rpm for 8 h at 25 ± 3 °C, and dried at 60 ± 2 °C for 16 h. The cycle is repeated 9 times.	Not specified
Test container	Acrylic cylindrial container (60 mm in height and 80 mm in diameter) with a 5-10 mm thick bottom of plaster of Paris (designated in JIS T 6605 for dental use)	Round jam pot (450-500 ml capacity) with a wide-mouth and a bottom area of 25-30 cm^2
Test assembly	An individual wood specimen placed on a 1 mm thick plastic net (mesh) at the center of bottom to avoid excessive water absorption by a direct contact of the specimen and plaster	An individual wood specimen buried in the 200 g sand at 7% MC under water holding capacity of the sand (or moist sand) in the jam pot so that one of the largest areas of the sample (the widest side) allowed to contact inside vertical wall of the pot

Results and discussion

As shown in Table 2, there was no big difference in mass losses of rubber wood specimens after 6-week exposure to two termite species, *C. formosanus* and *C. curvignathus* when the tests were conducted separately at RISH of Kyoto University and Bogor Agricultural University according to the Indonesian standard SNI 01.7207-2006. However, the significant difference was seen between termite mortalities of the two termite species by t-test.

Table 2 Results by Indonesian standard test

Item	By *Coptotermes formosanus* at RISH of Kyoto University		By *Coptotermes curvignathus* at Bogor Agricultural University	
	Mass loss (%)	Mortality (%)	Mass loss (%)	Mortality (%)
Mean	25.7	32.4	26.0	100
Standard deviation	4.46	10.01	4.26	0
t-test*	A		A	
		B		C

* The same letters of t-test indicate no significant difference.

Since the wood specimens sustained more than 18.94% mass losses in both tests in Japan and Indonesia, the test wood species wass classified in class V or the lowest durability class as designated in SNI 01.7207-2006 (Table 3), the exchange of termite species was thought to have negligible effect on the test results.

Meanwhile, 100% mortality of *C. curvignathus* was unexceptionally recorded in 5 replicates after 5 weeks' exposure, and much lower mortality 32.4% was produced by *C. formosanus*. Although there is no test validity criterion on the mortality, it is strongly recommended to modify test methodology to constantly obtain lower termite mortality. Maintenance of the moisture content of the sand, temperature and humidity of the dark test room should be considered.

Table 3 Resistance classes against the subterranean termites, *Coptotermes curvignathus* (Indonesian Standard SNI 01.7207-2006)

Resistance class	Mass loss (%) range
I: Very resistant	< 3.52
II: Resistant	3.52 – 7.50
III: Moderate	7.30 – 10.96
IV: Poor	10.96 – 18.94
V: Very poor	> 18.94

The test results obtained by the Japanese standard JIS K 1571-2004 were similar to those by Indonesian Standard SNI 01.7207-2006: mass losses were 16.4% by *C. formosanus* and 16.7% by *C, curvignathus*, and a large difference in mortality was seen between the two termite species (Table 4).

Table 4 Results by Japanese standard test

Item	By *Coptotermes formosanus* at RISH of Kyoto University		By *Coptotermes curvignathus* at Bogor Agricultural University	
	Mass loss (%)	Mortality (%)	Mass loss (%)	Mortality (%)
Mean	16.4	14.7	16.7	74.9
Standard deviation	4.03	5.58	5.35	24.44
t-test*	A		A	
		B		C

* The same letters of t-test indicate no significant difference.

Conclusions

The unexpectedly high mortality of *C. curvignathus* occurred, regardless of test mehods used in the current study. It still remains questionable what caused such a remarkable difference between test termite species, although the Indonesian test conditions are not clearly difined. It is, therefore, needed to examine the effects of test conditions including the regulated moisture content of sand where a wood specimen is buried, during the test duration, *etc.* and to determine the incidence of undesirable microbial growth specific to the termite species during the test duration.

Acknowledgement

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About the Detection of Hypogenous Termite Nests by Geophysical Methods

by

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Abstract

Due to the needs of sub-surface treatment of termite nests inside dike and damp bodies, it is necessary to detect hypogenous termite nests, but the target of the detection is main chambers which are the residence of the king and queen and most of the colony's individuals, and also the crossroad of the gallery network that causes infiltration of water from the reservoir or river leading to dam and dike collapse .However, the main chambers are normally located deep under the ground, thus difficult to be detected. By electrical resistance method, strange bodies that have resistivity different from the surrounding environment can be detected. Ground penetration radar method works basing on the reflection of electromagnetic waves on strange bodies under the ground. Gamma-absorption method works basing on the ability to find holes in the investigated environment. The first two methods can measure quickly, but often meet with false anomalies as the consequence of the non-homogeneity of the medium and the termite nest structure. The gamma-absorption method is able to distinguish the main chamber from the auxiliary ones, but the measurement speed is low because hole drilling should be done first .No of these 3 methods can respond sufficiently all the requirements of the detection.

Introduction

From 80s years of the previous century, the application of geophysical methods to detect termite nests inside dikes and dams in Vietnam had been studied and duly appraised by some authors within the framework of state-level study topics or scientific thesis. Geophysical equipment in the above cases were designed aiming to investigate geological objects of big sizes, while, the targetted termite nests in delta dikes of Northern Vietnam are small-sized and deeply located, therefore above methods are of limited effiiency and so far only used where applicable only

However, recently, somewhere in Vietnam that provide control services to dike-and-dam termites have over-appreciated the efficiency of some geophysical methods, considering them as being able to settle all detection requirements.For this reason , our article is written with the aim to clarifying such viewpoint.

Materials and methods

This article gathers our results of studies carried out in the last 3 decades, and also quotes some extracts of study works of some other authors.

Equipment and measuring methods used for detecting termite nests were all those used for geological investigations. For gamma- absorption methods used for this target, we modified slightly measuring methods as compared with those used in the geological investigations

Results and discussion

I. Why is it necessary to find termite nests in dikes and dams?

There are many species of termites insdie dikes and dams in Vietnam, among which, Odontotermes is the most popular and dangerous genera in the delta dikes of Northern Vietnam. Finding termite nests by digging method is simple, but not efficient and dangerous as it will break dike and dam's structure. The needs of underground control of termite nests to eradicate the whole colony and fill back all holes created by them therefore arise. For that purpose, many people had tried to pump mortar and termiticide into auxiliary-chambers or swarming holes, then press them to run into the main chamber, but in vain. This proves that finding out the main chamber in order treat directly into them is an absolutely must, because the main chamber is the residence of the king and queen, most of individuals of the colony, and also the crossroad of all the gallery network that cause danger to dikes and dams. Target for the detection is therefore the main chamber, not auxiliary-chambers.

Anyway, for Odontotermes species in this region the main chamber has the diameter of 0.5m more or less, locates from 1m to several meters under the ground, very difficult to detect, while there are plenty of auxiliary-chambers with the diameter of +/- 0.1m, being located right under the soil surface (0.1-0.2m), very easy to discover.

II. Detection by electrical method (EM)

II.1. Basis

When a power supply with unchanged current is put onto the ground, if the ground's environment is homogenous and isotropic, the electric field created in this condition is called normal field and be allocated in accordance with a certain rule.

But, as far as some strange objects (termite nest) appear in the investigated invironment with their electric resistivity different from the resistivity of the surrounding environment, then the normal electric field shall be altered. It is the detection and appraisal of this anomaly which is the basis for the application of EM in the detection of termite nests in dike and dam

II.2. Model test carried out by Lam Quang Thiep (1972)

This author put an electric-insulated ball deep under a water tank,performed an electrical measurement along a line on the water surface crossing over the ball and concluded that it was able to detect the ball (the termite nest) under the depth of h not exceeding 3-4 times of the ball's diameter. The author also found out the most appropriate measuring method.

II.3. Field survey: Within 3 years, the author found out 6 anormalies of promise along the Red River dike of Hanoi. By digging some points of anormaly , 2 termite nests were found out and both were big nests locating near the surface, while deep and small nest were not detected.

II.4. Tests conducted by ourselves: Several years later on, we studied again dike sections that were earlier investigated by the above author, and found that:

- Different from the water environment in the model test, the environment of earth in dikes is quite non-homogenous in terms of density, moisture and is mixed with foreign matters like sand, stones, construction materials... that certainly cause many false anomalies in the course of innvestigation (Later on, when we studied dam environment, we found that the dam environment is even more non-homogenous than the dike's).
- termite species located in these sections are normally Odontotermes hainanensis and O . angustignathus of which the ratio between depth and diameter h/d of their nests often exceeds the range determined by the above model test. On the other hand, in terms of nest structure, these species often have plenty of auxiliary-chambers right below the surface, which create a "barrier" to prevent the electric current from going downward to the main chamber, thus make it more difficult to find out the main chamber locating below
- if the measuring path does not cross over the center of the nests, the ratio h/d will incraese ,so influences on the possibility of detection

From our experience after field surveys, we see that, with the concurrent influence of the above 3 factors, this method is not so much efficient. Even today, when the electric resistance technology has been much improved, but the efficiency of this method of detection is not ameliorated.

III. Detection by Ground penetrating radar (GPR M)

III.1. Basis:

This method is based on the studies on the transmission characteristics of electromagnetic waves under the ground. A transmiting antenna put on ground will transmit electromagnetic waves into the ground invironment. As far as in the underground environment there is a strange object (termite nest), with different transmission characteristics from that of the environment ,then the electromagnetic wave reflection will happen. These waves will partly return to the ground's surface and be recorded by receiving antenna. This is the basis for the original usage of GPRM in the detection of termite nests.

III.2. Model tests carried out by author Ngo Chi Coi (2003).

At the "Vietnamese-German workshop on dike monitoring" in Hanoi in 2003, Ngo chi Coi and his group had reported results of model tests of GPRM In a soil environment, the authors made holes with the diameter of 20 cm to 100cm at the depth of 100 cm to 300 cm, then measured by the GPRM equipment via which, they could detect the holes via curves displayed on the screens of the measuring equipment. From this, the authors concluded that this method can detect termite nests,

without clearly mentioning limitation in terms of the homogeneity of the environment or structures of the termite nests. The report also said that they detected so many termite nests.

III.2 .Evaluations of other scientists

-However, many scientists said they also carried out some test and found that the result of field survey on dikes was not as clear and simple as in the model test,because in may cases the anormalies are not caused by termite nests but by the non- homoneity of the environment , and it's even more difficult to distinguish the main chamber with the auxiliary ones.

-Nguyen Van Giang (2003), in an article about the usage of GPRM to survey the dike situation, also mentioned about the possibility of using this method for detection of termite nests, and said that he was able to find out a termite nest which was 2m long, 0.8m wide and 1m deep.

-In 2008, in a report about the detection of termite nests by using GPRM on a dam in the Central area of Vietnam, author Tran Thien Nhien said that the termite nests were too small to find out by this method, even there were 2 cases when he put a radar detector right on the top of the nests already determined by biological method, he was unable to detect the signals reflected from the termite nests laying below.

III.3. Our remarks

+ Similar to the EM above,due to the non-homogeneity of the environment in dikes and dams, therefore, among the curves seen on the sreen of the radar equipment, there must be many false anomalies , not corresponding to termite nests.

+ Auxiliary chambers of Odontotermes species often lay largely near the ground surface, so the EM and the GPRM can mostly detect auxiliary chambers rather than the main chambers, and are even unable to distinguish these two sorts of chambers ..Regarding the main chambers ,GPRM can discover only large and shallow ones ,like the above mentioned result obtained by Nguyen van Giang ,but these good conditions are not numerous .Please also be noted that to discover the auxiliary chambers, it's not necessary to use complicated equipments.

IV. Gamma absorption method (GAM) carried out by Vu van Tuyen (1977)

IV. Basis:

In a homogenous environment where the absorbability is μ, if we radiate a bundle of radioactive rays with the initial intensity of I_o, then at the distance of l from the radiating source, the radioactive intensity I_n to be measured will be:

$$I_n = I_o . e^{-\mu l} \qquad (1)$$

If within the distance l of radiation, there's an empty hole (termite nest) with the diameter of d, then the radioactive intensity I_a measured will be:

$$I_a = I_o . e^{-\mu (l - d)} \qquad (2)$$

If we compare these two above equations, we can see that Ia >> In. This means that when a radioactive ray is transmitted via an environment having termite nests, the intensity of the radioactive ray measured within the distance of l is much higher than normal. This is the basis for the application of the GAM in the detection of termite nests.

IV.2. Model tests

In a homogenous earth environment, we make an empty hole, then measure the radioactive intensity by GAM. As far as the empty hole get bigger and bigger, the respective radioactive intensity measured is increasing correspondingly by exponential growth . Therefore, the dimension of the empty chamber (termite nest) can be determined

IV.3. Field survey:

- The GAM is very sensitive to even small pores of the soil, then the detection of holes gets result evidently , the diameter of this hole can be measured and thus, we can know where the main chamber is ,if we compare the diameter of detected chambers
- A big disadvantage of this method is that it takes more time than other methods as we need to drill holes before measuring.

Conclusion

The target of detection is to find out the main chamber of termite nests, then, we can see via the advantages and disadvantages of these three methods :

+ Most of already located termite nests by EM and GPRM are often auxiliary-chambers near the surface, while main chambers can only be detected as far as it is big and located shallowly.

+ With the GAM, the detection of pure holes or termite nests are both useful for the dike and dam manager , and it's possible to detect the main chamber, determining their dimension , but the disadvantage is their lower speed.

+The detection of all these methods must be performed at the area where swarming holes and waiting chambers of termites were observed .. But these signals appear only within the mature period of the colony development .This means that newly-built nests and old nests which have no signals like this ,can be passed over during the detection

+By all reasons above mentioned ,we can even say that inside dikes and dams, the total number of nests already detected may take only a portion as compared with the unknown termite nests still existing in dike and dam body

+ Therefore, by our opinion ,the most important point and also the key basis to find out termite nest is to study thoroughly biological and ecological characteristics of termites species, whilst geophysical methods play only a kind of supporting role.

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Distribution of Mound Subterranean Termite *Macrotermes gilvus* Hagen (Blattodea:Termitidae) in Yanlappa Nature Reserved, Indonesia - the Effect of Land Characteristics

by

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Abstract

Macrotermes gilvus Hagen (Blattodea: Termitidae) is a common mound-building termite species found in South East Asia regions, particularly in Indonesia, Malaysia, Singapore, Philippines and Thailand. However, scientific information on their demography and mound construction in Indonesian natural forests had not yet reported. A study was conducted to investigate demography and mound construction of subterranean termite *Macrotermes gilvus* Hagen (Blattodea:Termitidae) in Yanlappa Nature Reserved, West Java, which represents low-land natural forest in West Java, Indonesia. Termite mound distribution survey was conducted in strip transect, 50 m width interval, and supported by *Global Positioning System*. Termite population in each mound was determined after dismantted of the mounds, meanwhile mound construction were observed vertical as well as horizontal section. The mound of *M. gilvus* was distributed clusterly with density of 5 mound/Ha, density mainly located at elevation 3% - 5%, and under *Leaf Area Index* of 0-2. We concluded that *M. gilvus* is primary decomposers and contribute to litter fragmentation and the recycling of nutrient into the soil. The important role that termites play as primary decomposer. *M. gilvus* play an important role as a source of heterogeneity in this nature reserved ecosystem. This role is particularly important in ecosystem under stresses. The density and dynamic of *M. gilvus*, be taken into account in the global strategy of the forest resources management and conservation.

Keywords : Distribution, nature reserved, mound, *Macrotermes gilvus*.

Introduction

Macrotermes gilvus Hagen (Blattodea: Termitidae) is a common mound-building termite species found in South East Asia regions, particularly in Indonesia, Malaysia, Singapore, Philippines and Thailand. However, scientific information on their demography and mound construction in Indonesian natural forests had not yet reported. A study was conducted to investigate distribution of mound subterranean termites *Macrotermes gilvus* Hagen (Blattodea:Termitidae) in Yanlappa Nature Reserved, Indonesia.

M. gilvus are recognized as ecosystem engineer because they promote soil transformations by disturbance processes. This species collect organic matter and mineral particles from different depths and deposit them in mounds, enhancing the content of organic C, clay and nutrient. Also, pH and microbial population are higher in termite mound than in adjacent soil. The material accumulated is redistributed by errosion, affecting soil microstructure and fertility. Termites also build galleries that increase soil porosity and water infiltration and these galleries may be filled up with top soil material, with rainfall contributing to the process of formation of deep, uniform latosol (Schaefer 2001). The main factors for this trend are related to the scarcity and abnormal seasonal distribution of rains, to the increasing demographic pressure and to an overexploitation of natural resources (Traore *et al.* 2008).

Materials and methods

The study site was located in Yanlappa sanctuary, Bogor, West Java, Indonesia. Field colony mound of *Macrotermes gilvus* Hagen on the natural forest of Indonesia, was selected for the object of this study. Termite mound distribution survey at least 32 Ha was conducted in strip transect, 50 m width interval, and supported by *Global Positioning System* (Turner 2000). These zonations were digitized and the mound termite data from the survey transects were overlaid using GIS prosedures. Leaf area index was done using a hemyphot method, vegetation analisis was transect, elevation class was GPS facilities (Macquire & Goodchild 1991). Data processing and analysis

were conducted using ANOVA. In order to normalize the data, counts were transformed using the natural logarithm (Steel & Torrie 1980).

Results and discussion

Yanlappa natural reserve is about 32 Ha and located between 6°40' S and 106°45' E, with rainfall was about 2399 mm/year. Mounds building by *Macrotermes gilvus* Hagen are quite common in the natural forests, especially in Yanlappa natural reserve. They are irregularly dome-shaped to sub conical structure of brownish earth, and are not infrequently found near base of trees and often with some grass and other bushy vegetation growing on them.

The mound of *M. gilvus* was distributed clusterly with density of 5 mound/Ha, density mainly located at elevation 3% - 5%, and under *Leaf Area Index* of 0-2. It was shown in the present study that a number of interrelated environmental factors influence termite distribution. Major parameters such as geology, terrain morphology and woody vegetation are reflected in land type. The land shaped by forces best described by local relief, slope class. Major parameter distribution mound termites *M. gilvus* can be seen in Table 1.

Tabel 1. Major Parameter distribution mound termites *Macrotermes gilvus* in Yanlappa, nature reserve, Indonesia

Parameter	Mound density	Area (m^2)
Class elevation (m dpl)		
50-100	75	258.124
100-150	80	80.835
Leaf Area Index		
0-1	128	333.217
1-2	27	5.743
Slope class (%)		
3-5	155	288.026
6-17	0	16.707
18-20	0	34.228

According to the results, the Macrotermes mounds rest of subplot appeared as 3 different areas for tree species richness but still having the same site condition like parent soil and climatic factors. Compare the species diversity between the different areas mounds termite *M.gilvus* can be seen in Table 2.

Table 2. Compare the species diversity between the different areas in Yanlappa Nature Reserve, Indonesia

Higher density		**Low density**		**No density**	
Tree					
Artocarpus elastic	34,33	*Artocarpus elastica*	57,41	*Uncaria gambir*	37,21
Pentace polyantha	33,28	*Mallotus oblongifolius*	24,65	*Diospyros frutescens*	32,58
Knema intermedia	29,17	*Knema intermedia*	23,23	*Chrysophyllum roxburghii*	29,43
Vitex quinata	18,38	*Chrysophyllum roxburghii*	21,50	*Planchonia valida*	25,38
Euonymus javanicus	17,37	*Polyalthia lateriflora*	20,20	Kihuut	23,09
Ixora grandifolia	*14,38*	*Croton argyratus.*	20,12	*Artocarpus elastica*	22,33

A total of 226 spesies and 169 families were identified in the study subplot. These families, Myrtaceae 47,47%, Moraceae 34,33%, Tiliaceae 33,28%, Rubiaceae 21,34% and Verbenaceae 18,38% were the most represented families. To compare the species diversity between the different areas, the specific density was calculated as species richness at the unit of 100 m^2 of area for the mounds and surroundings. The mean density of tree community showed no significant difference by distribution mound termites in our study subplots ($P > 0.05$).

M. gilvus are primary decomposers and contribute to litter fragmentation and the recycling of nutrients into the soil. The important role that termites play as primary decomposer. Decomposing microbes are secondary receivers of carbon compounds fragmented by the termites. *M. gilvus* are less dependent on these factors because of mound architecture and fungal symbiosis. Termites are

also able to patchily changes soil properties in the environment. The interaction of passing on of nutrient rich particles across a decreasing size spectrum enables the movement of nutrients through the terrestrial ecosystem.

In modifying the distribution and availability of soil nutrients, soil engeener influence ecosystem services such as maintenance of biodiversity, stability and nutrient cycling. It is therefore necessary to study the links between their impact on ecosystem functioning and their ecological requirements, their ability to respond to their environment, as well as their relationships with other soil engineers in order to understand the structure of heterogeneity and then the functioning of ecosystem (Jouquet *et al.* 2006). These result of data that can used to evaluate the role that a particular species of termite plays in an important natural ecosystem. This is major contribution to providing data on an invertebrate component of the ecosystem.

Conclution

From the research on distribution of mound termites *Macrotermes gilvus* Hagen in natural forest ecosystem, there were some conclusions: The mound of *M. gilvus* was distributed clusterly with density of 5 mound/Ha, density mainly located at elevation 3% - 5%, and under *Leaf Area Index* of 0-2. *M. gilvus* is primary decomposers and contribute to litter fragmentation and the recycling of nutrient into the soil. The important role that termites play as primary decomposer.

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How Much Area Is Foraged by Termites in Tropical Forests?

by

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Abstract

Termites are well known for their contribution to removal and decomposition of litter in tropical forests. Although the quantitative importance has been repeatedly documented, the qualitative features, such as intensity of litter foraging in a small area and its frequency, still remain unclear. Here we observed litter removal by termites in a dry evergreen forest, Thailand, by using coarse and fine wire-mesh cages allowing and not allowing termite accesses to the inside litter samples and evaluated the intensity and frequency. Naturally accumulated litter samples were used to avoid possible effects of litter quality on litter removal by termites. Litter samples were put in coarse and fine cages and collected bimonthly for more than one year. Termites were shown to have intensively removed litter samples by comparing the litter weight remaining between coarse and fine cages. Frequency of occurrence of intensive litter removal by termites was estimated from a curve of the percentage of the total collected cages along the sampling times. A simple model with a constant rate of litter removal by termites in a cage per day was well-fitted to the curve of the observed percentages in each sampling time, resulting in the rate of 0.0031. Accordingly, termites are suggested to remove litter from around 0.3% of the forest floor per one day, and are roughly estimated to forage more than half of the forest floor per one year.

Key words: Keywords: Sakaerat Environmental Research Station, litter cages, removal of litter, model analysis

Introduction

Tropical forests harbor a great diversity of plants and animals, which contributes to the highest productivity levels in the terrestrial ecosystems. Extremely abundant plants provide a mass of food and habitat for animals, especially for decomposers. Dead leaves and wood continuously fall onto the ground, and open-air litter foraging in some termites is known for a conspicuous feature in tropical forests (e.g. Sugio 1995, Miura & Matsumoto 1998), while actually many termite species forage the ground surface and remove litter to their nests (Traniello & Leuthold 2000).

In order to quantify the amount of litter removed by termites, not a few previous studies have been carried out under various methodologies. Collins (1983) calculated consumption of litter by termites in Malaysian tropical forests to be from 0.9 to 16.3% of litter production. In Collins (1983), the calculation was done by using consumption rates by various termite species, which had been measured in laboratory and field trials. Although Collins (1983) has given a fairly good estimation to the question how much litter termites remove from the forest floor, the methodology is rather indirect, and some problems have been pointed out for feeding experiments to measure consumption rates (Bignell & Eggleton 2000). Matsumoto & Abe (1979) developed more direct methods and measured the loss of leaf area (i.e. removal by termites) after they marked newly (intact) fallen leaves and distributed them on the forest floor, showing 22 to 32% of daily fallen leaves to be

consumed by certain termite species. A precise estimation could be made by using this methodology, while apparently it can be applicable only for specific termites.

Litter-bag methods have been employed to evaluate litter removal by termites and other soil macrofauna, such as ants and earthworms, in the field (e.g. Yamashita & Takeda 1998, Mando & Brussaard 1999, Höfer et al. 2001, Ouédraogo et al. 2004). Litter removed by soil macrofauna has been determined on the basis of the difference of litter weight remaining between the litter-bags allowing and not allowing termite accesses. Yamashita & Takeda (1998) used fine- and coarse-mesh litter bags and found a more weight loss of leaf litter in the coarse litter bags in a Malaysian tropical forest. Since termites were a representative group of soil macrofauna in the forest, Yamashita & Takeda (1998) implied that termites mainly contributed to the high decomposition rate in the coarse litter bags.

Together, the previous studies have quantitatively clarified the role of termites in litter removal; however, there are, as far as we know, very few studies on its qualitative aspects, for examples, intensity of litter removal in an actual foraging area and its frequency. Such knowledge will lead us better understand the decomposition processes in tropical forests. Here we observed removal of litter by termites in a dry evergreen forest in Thailand by using a modified litter-bag method, which allows direct access to litter samples, and highlighted the qualitative features of litter removal by termites.

Study site and methods

Our study was conducted in the dry evergreen forest (DEF) at the Sakaerat Environmental Research Station (14°30' N, 101°56' E; c.a. 550 m above sea level) in Nakhon Rachasima Province, Northeast Thailand. The mean annual temperature and rainfall are 27.5 °C and 1144 mm, with typical monthly rainfall less than 40 mm during dry season from November to March. We set up the main plot (40 m × 40 m) in the dry evergreen forest, and randomly selected 13 subplots (2 m × 5 m each) within the main plot.

To examine the removal of litter by termites (and partly by other soil macrofauna) we used wire-mesh open-bottom and closed-bottom cages. The size of cages was 10 cm × 10 cm × 10 cm (in height). Open-bottom cages were made from general 2 mm-wire mesh, which allow termites to enter from the bottom as well as the sides. Closed-bottom cages were made from the 0.2 mm wire-mesh, Termimesh (Termimesh Japan), to prevent termite attacks. In October 2008, naturally accumulated litter was collected from 1 m × 1 m-area neighboring to each subplot, and brought back to the laboratory and oven-dried at 75°C for 48 hours. Dry weight of the litter sample was from 300 to 500 g in each 1 m^2-area. A total of seven pairs of the coarse (2 mm mesh) and fine (0.2 mm mesh) cages were set in each subplot in November 2008. Approximately 3 to 5 g dry weight of the litter sample (c.a. 1% of the total litter collected from the neighboring area) was put in a 10 cm × 10 cm-area where the accumulated litter was remove, and covered by a coarse cage and fixed to the soil by wire. In the case of a fine cage, after the accumulated litter was removed from a 10 cm × 10 cm-area, the surface soil layer (c.a. 3 cm) was put into the cage with keeping it intact. A 3 to 5g-litter was put on the surfaced soil layer in the cage, and the top of the cage was closed to be fixed to the original place. Usually bimonthly from November 2009 to January 2010, the litter samples were carefully collected from the cages, and brought back to the laboratory, washed, dried at 75°C for 48 fours, and weighted. During the incubation period, the total rainfall was 843 mm and there was less than 40 mm monthly rainfall from November 2008 to February 2009, June 2009, and November and December 2009.

Results and discussion

Time courses of litter weight loss in the cages are shown in Figure 1. There were significant differences among the sampling times as well as between fine and coarse cages by two-way ANOVA after converting the percentages to degrees by angular transformation (arcsin square root) (total: $d.f. = 126$; time: $d.f. = 6$, $F = 17.869$, $P < 0.01$; cage: $d.f. = 1$, $F = 135.674$, $P < 0.01$; time × cage: $d.f. = 6$, $F = 1.288$, $P = 0.27$). Decomposition rate consants (k $year^{-1}$, see Olson 1963) were 1.79 and 0.69 in the coarse and fine cages, respectively. Yamada et al. (2003) have reported an extreme abundance of termites in the forest, and we often observed clear evidence of termite attack (e.g. foraging termite individuals and/or trails); we suppose that the weight loss was caused by termites. As is evident from the model (single exponential decay model, Olson 1963), theses values, determined based on the decomposition of naturally accumulated litter (i.e. a mixture of fresh and

rotten litter), should be underestimated compared to those obtained in previous studies, where only fresh litter has been used for litter bag experiments. Nevertheless, Yamashita and Takeda (1998) have reported a very similar constant, 2.15 for fresh leaf litter in coarse mesh (2 mm) litter bags. Takeda (1996) has also reported a mean decomposition rate constant of fresh leaf litter in tropical forests being 1.85. It may be suggested that termites attacked the litter samples in the cages more intensively than those observed in the previous studies.

Distribution of termites is usually aggregated (Bignell & Eggleton 2000, Inoue et al. 2001), and their foraging is generally done in a large group within a relatively small area basically because of their sociality. On this basis, we can expect the pattern of litter removal by termites to be much more intensive that that by microorganisms such as bacteria and fungi. To evaluate the intensity, we calculated an index (intensity index) as the ratio of the litter weight remaining in a coarse cage to that in the paired fine cage (Figure 2). We calculated the values for 86 pairs out of a total of 91 pairs,

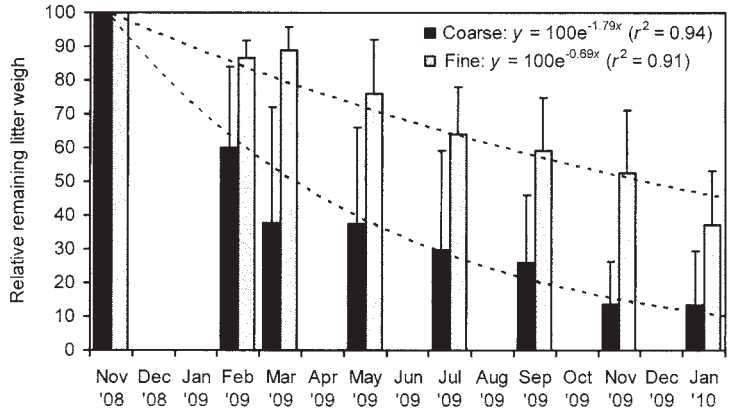

Figure 1. Time course of litter weight remaining in coarse and fine cages. The values show are arithmetic means and standard deviations.

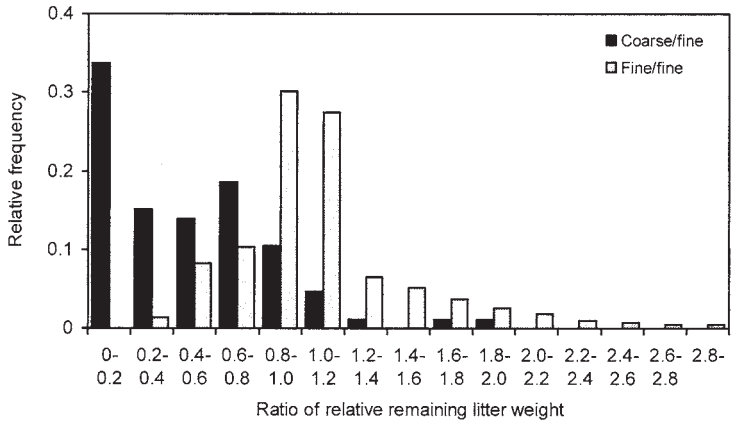

Figure 2. Distributions of the ratio of litter weight remaining between pairs of coarse and fine cages and between fine cages. The former is an index to represent the intensity of litter removal by termites.

because litter samples in five fine cages were apparently damaged by some animals, which probably dwelled in the surface soil layers put into the cages. The intensity index will be more close to 0 when termites remove more litter from the coarse cage, while around 1 when termites scarcely remove litter from the coarse cage. In order to know the dispersion of the index in the case of only microbial decomposition, we calculated the ratio of the litter weight remaining in the fine cages between all pairings separately in each sampling time (a total of 976 pairs, Figure 2). There was a significant difference between the two distributions by Kolmogorov-Smirnov test ($D = 0.634$, $P < 0.01$). Judging from the distribution of the ratios between fine cages, the distribution of intensity index seems to be a mix of two distributions: one is the similar distribution to that between fine cages, but slightly shifted to left, and the other is the distribution highly skewed to low levels (i.e. 0 to 0.4). The

former distribution probably means the absence of intensive foraging by termites, while usually higher water contents in coarse cages (data not shown) may have accelerated litter decomposition by microorganisms. The latter distribution clearly indicates the evidence of intensive litter-removal by termites. A total of 48.8% of the coarse cages were within the intensity index from 0 to 0.4.

The percentage of the coarse cages with the intensity index from 0 to 0.4 does not directly reflect a rate of intensive litter removal by termites, because the coarse cages incubated for different periods are combined together in Figure 2. To estimate a rate of intensive litter removal (foraging) by termites per day, we calculated the percentage of the integrated number of the collected cages, which was intensively foraged by termites (Figure 3), and constructed a model to explain the curve as follows:

$$p(t) = 1-(1-k)^t$$

$$N_t = \sum_{i=1}^{t} n(i)$$

$$P(t) = \frac{1}{N_t}\sum_{i=1}^{t} p(i)n_i$$

where, k is the constant rate of intensive foraging by termites per day in a cage, t the incubation time (days), p the probability of a cage to be foraged by termites, n the number of samples collected in a time, N the integrated number of cages collected, and P the percentage of the total cages, which was foraged by termites. We used the observed percentage at the end point (t = 430) to fix the k value, and calculated 95% confidential intervals of the percentage of the total cages, which was foraged by termites, from 1000 resamplings by using the p value given by the model for each internal point (Figure 3). The calculations resulted in the k of 0.0031, and the model was well-fitted to

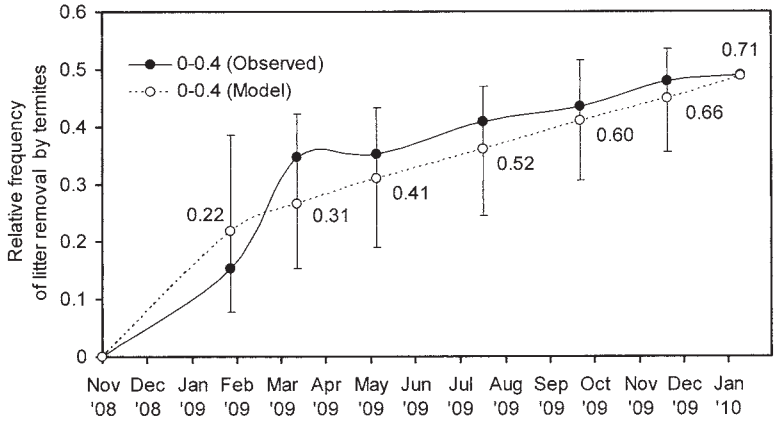

Figure 3. Time course of the percentage of litter removal by termites in the accumulated coarse cages. The observed values were calculated by adding newly collected cages in each sampling time. The values on the points of the model curve indicate the probability that termites have removed litter in a coarse cage by the point. For the model, see text.

the observed percentages at the 95% confidence level. We also considered a time-depended model, where k is a function of time and decreases along incubation period, while the shape of the curve was quite similar and seemed to explain the observed data more considerably without considerable precision (data not shown). Although it will most likely that termites less frequently or do not attack highly rotten litter in a cage due to the long-time incubation, such a case was apparently not observed during the study period. In fact, termites are known to utilize quite a wide range of organic matter from fresh to highly rotten one (Donovan et al. 2000), and the termite fauna in the studied forest consist of various types of feeding group (Inoue et al. 2001, Yamada et al. 2003). In addition, the natural litter composition on the forest floor is more or less constant. Therefore, our results strongly suggest that approximately 0.3% of the forest floor is foraged by termites per day in the tropical forest, and that roughly more than half of the forest floor is foraged by termites per year (Figure 3).

Conclusions

Termite foraging is very intensive and sometimes completely removes the litter in a small area. Such intensive removal of litter by termites seems to daily occur in 0.3% of the forest floor in the dry evergreen forest at the Sakaerat Environmental Research Station. It roughly means that termites forage more than half of the forest floor in one year.

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Sex ratios of field termite populations of *Reticulitermes hageni* (Isoptera: Rhinotermitidae)

by

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Abstract

Nowhere is sexual division more striking than in social insects. Thirty termite inspection ports consisting of a 10-cm ID by 16-cm L section of PVC pipe containing at least 60 g of wood were sampled every three months from September 2008 through July 2009. In that time, 90 *Reticulitermes hageni Banks* collections were obtained that contained sufficient numbers of termites (N>400) to examine sex ratio's of several castes. The range of sex ratio's (Male to Female) observed was 0.49-3.50 for workers, 0.75-5.25 for soldiers and 0.02-26.00 for nymphs. These results are the most comprehensive research of sex ratio based on extensive sampling of field populations and reveal that there is a great deal of variation in sex ratios, with the potential to provide insights into colony-level sex allocation and seasonal variation in *R. hageni*.

Keywords: Isoptera, *Reticulitermes hageni*, sex ratio, sample size, fluctuations of sex ratios

Introduction

The study of sex ratio evolution is an active area of evolutionary biology (Bourke and Franks 1995). Social Isoptera display a remarkably complex and diversified caste system where worker and other castes are diploid (Roisin 2000), but Fisher's (1929) sex ratio hypothesis cannot be easily applied to termites. Recent advances in sexual selection in termites has created growing interest in using the Isoptera as models for sex ratio research (Crozier and Schluns 2008, Hayashi et al. 2007, Korb et al. 2009, Lo et al. 2009, Matsuura 2006).

Previous studies of sex ratios give important baseline data (Darlington 1986, Dean and Gold 2004, Henderson and Rao 1993, Howard and Haverty 1980, Jones et al. 1988, Pawson and Gold 1996, Roisin and Lenz 2002, Thorne 1983, Zimet and Stuart 1982), but a variety of sample sizes have been utilized and a recent census suggested a sample sizes of at least 100 individuals per caste are necessary to actually represent a population's sex ratio (Hu and Forschler 2009. The work on sample size improved precision in estimates and proposed a standard sample size for further studies.

The application of a G-test for heterogeneity has been used to identify departures from a 1:1 sex ratio (Matsuura 2006, Muller and Korb 2008). The simpler definition of neural (0.67-1.50), female-skewed (<0.67), and male-skewed (>1.50) offers advantages over G-test for describing individual collections. For example, a G-test of two collections, one male-skewed accompanied by an equal number of female-skewed individuals are recorded as 1.00 or equal despite the fact that the two collections were heavily biased.

This paper reports sex ratios from 30 collection sites of *Reticulitermes hageni* Banks for a year, using a sample size of 100 individual in each caste. We anticipate that these results will serve to illustrate the tendency of each sexual bias within a caste and seasonal dynamics of sex ratios of *R. hageni*.

Materials and methods

Termite Collections. A single field site on Sapelo Island in MacIntosh County, Georgia, USA, was sampled every 3 month for a year - from September 2008 to July 2009. Termite inspection ports consisted of a PVC pipe receptacle that was 16-cm long by 10-cm diameter. Termite sandwiches were placed in each inspection port and were composed of 9 pieces of pine wood (2 cm ×2 cm × 12 cm) separated by wooden dowels (0.02 cm ×0.5 cm) and held together with a plastic cable tie (18-cm length). Termite inspection ports were capped using a 10-cm-diameter plastic knock-out plug (Forschler 1996). Termites collected on each sampling date included 90 samples with > 100 termites

that were stored in 100% ETOH for use in this study as well as being placed in our voucher collection.

Sex and Species Identification. Sex was determined by the arrangement of sternal plates as described by Zimet and Stuart (1982). The sternal plate character was verified as a correct indication of sex by dissection (Roisin and Lenz 1999). Species identification was made using soldier characters in published keys (Scheffrahn and Su 1994) and worker live weight. The weight was calculated using the mean of 5 groups of 10 workers. If the species remained the same and weight data were consistent (±10% error) from a site over the 12 month, we assumed that termites were representative of the same population over time.

Sample Size. We examined 100, randomly selected, termites of every caste (worker, soldier and nymph) for each collection. Obtaining a sample size of 100 for each caste per collection site/date was not always possible, we report the number of individuals used to obtain each sex ratio estimate. A sample size smaller than 100, i.e. 50 (±14% error) and 25 (±20% error) provides a less reliable estimate than 100 (Hu and Forschler 2009). Allowing for the estimation of error caused by sample size, a sex ratio within 0.67-1.50 (male to female) was defined as neutral, <0.67 described as female-skewed and >1.50 named male-biased (Hu and Forschler 2009).

Results and discussion

Sex Ratio of Caste. The sex ratios for worker samples ranged from 0.49-3.50 (N=90). Of the worker samples, 3.3% were female-skewed, 64.4% were neutral and 32.2% were male-skewed. Sex ratios for soldiers ranged from 0.75-5.25 (N=19). The soldier collections were classified as neutral 47.4% of the time and 52.6% were male-skewed. Sex ratios for nymphs samples ranged from 0.02-26.00 (N=16) with 62.5% female-skewed, 25.0% neutral and 12.5% male-skewed (Table1). All of the three classes have no significant difference from 1.00 (G-test), and no statistical effect among worker and soldiers and nymph class (One-way ANOVA test).

A variety of sex ratios has been reported in *Reticulitermes*. Zimet and Stuart (1982) found that sex ratios from field populations were 1.33 for workers, 0.76 for soldiers, and 2.70 for nymphs in *R. flavipe*. Jones et al.(1988) and Matsuura (2006) recorded that the sex ratios of workers and soldiers of *R. flavipes* were essentially 1:1. Dean and Gold (2004) reported sex ratios of 0.97 for workers, 0.79 for soldiers and 0.88 for nymphs of *R. flavipes*. Our data range (0.49-3.50 for workers, 0.75-5.25 for soldiers and 0.02-26.00 for nymphs) covered all previous sex ratio data and provided new sex ratio limits for each class in *Reticulitermes*. The data from this field survey revealed that there is great deal of variation in sex ratio strategies in subterranean termites.

Table 1. Sex ratios of 3 class in termite *Reticulitermes hageni*.

Class	Mean±SE (N)	Range	Female-skewed	Neutral	Male-skewed
Worker	1.41±0.05 (90)	0.49-3.50	3.3%	64.4%	32.2%
Soldier	1.70±0.24 (19)	0.75-5.25	0	47.4%	52.6%
Nymph	3.15±1.80 (16)	0.02-26.00	62.5%	25.0%	12.5%

No significant difference between classes by One-way ANOVA test at the 0.05 level of probability.
N, number of colonies studied.

Hayashi et al. (2007) used experimental laboratory pairings of neotenic phenotypes then separated the eggs to be reared by workers. They concluded the sex ratio's of nymphs and workers followed a single X-linked locus genetic model, later further supported by Crozier (2008) and Lo et al. (2009). The model predicts sex ratio of offspring workers in fNmN, fEmN and fEmE paired neotenic crossings (f: female, m: male, N: nymphoid and E: ergatoid) would be 1.00, and offspring workers in fNmE would be male exclusively. However, data in Fig.1 showed the workers' sex ratio can be differentiate greatly between 0.49-3.50, and only 22.4% of worker collections (N=90) fit the Hayashi's model.

The model also predicts nymph's sex ratio from fNmE, fN, fE to be exclusively female, from fEmN to be exclusively male, and from fEmE to be neutral. The sex ratio of our field data showed a range from 0.02-26.00 for nymphs. The x-linked model can explain the 3 of the ratios observed the two outer limits (sex ratio=0.02, 16.00, 26.00) and one neutral sex ratio (sex ratio=0.94), but the remaining 75% of our nymph collections (n=16) do not fit the model.

Sex Ratio Regulation. It has been suggested that the overall sex ratio of a termite colony is neutral (sex ratio=1.00) (Henderson and Rao 1993). If one caste within the colony were male-skewed another must be female-skewed to make sex ratio of the colony neutral. However, recent work has showed that workers sex ratios from 8 laboratory *R. flavipes* colonies were all male skewed (Hu and Forschler, in review) revealing that colony-level sex ratios are not necessarily 1:1. Therefore several questions remain to be answered, such as, do reproductives lay eggs that are predetermined to be one sex or another resulting in skewed sex ratios? Are all eggs of equal sex ratio and then regulated by workers (Lenz and Runko 1993)? Several factors have been reported could affect change in a population's sex ratio, such as sex-linked gene (Hayashi et al. 2007), the death of primary of queen (Lenz and Runko 1993), trophallaxis behavior (Muller and Korb 2008), but none of them are universal.

Seasonality change of sex ratios of field termite population appeared to be far more complicated (Figure 1). The mechanism(s) involved in caste sex ratio variation from month to month and even between populations is poorly understood. Based on our observational data, we believe that there are numerous factors affecting the dynamics of sex ratios other than a sex-linked gene that would include nutrients, temperature, juvenile hormone titer, colony size, and resource level. A comprehensive understanding of the mechanisms resulting in skewed sex ratios in termites is needed in future investigations.

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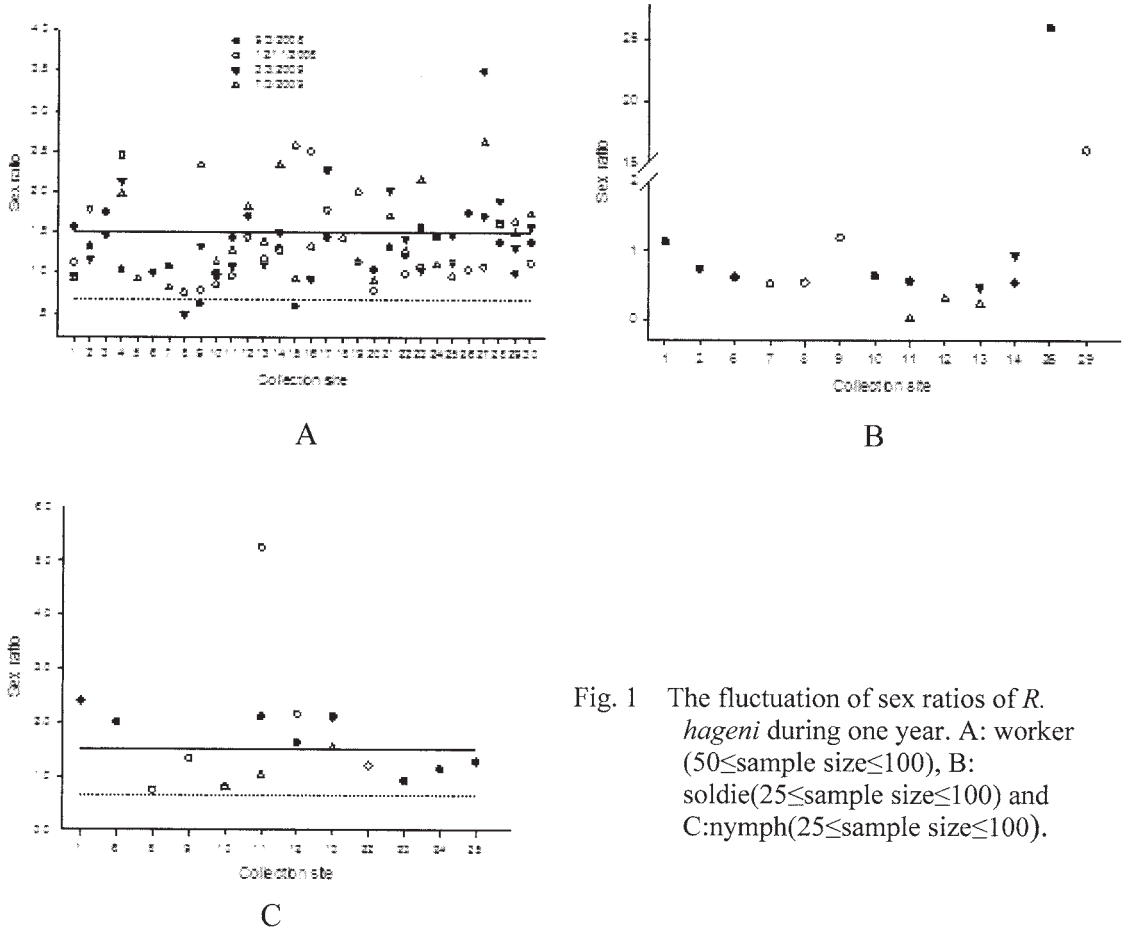

Fig. 1 The fluctuation of sex ratios of *R. hageni* during one year. A: worker (50≤sample size≤100), B: soldie(25≤sample size≤100) and C:nymph(25≤sample size≤100).

Colony Age Impacts on Neotenic Differentiation of *Cryptotermes domesticus* (Isoptera: Kalotermitidae)

by

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Abstract

The *Cryptotermes domesticus* orphaned colonies at ages of 1-4 years were raised for 3-6 months and examined once a week for the presence of neotenics after removal of primary reproductives. The observation data indicated that no neotenic production for 1-year-old orphaned colonies within 6 months. In contrast, 86.67% of 2-year-old orphaned colonies produced a pair of neotenics, and all 3-year-old and 4-year-old orphaned colonies gave out a pair of neotenics within 3 months after primary reproductive removal. 4-year-old orphaned colonies give out neotenics in fewer days than 2-year-old orphaned colonies. The results reported here provide a new understanding of neotenic differentiation in *C.domesticus* .

Key words: *Cryptotermes domesticus*, primary reproductives, neotenic production, colony age, orphaned colony

Introduction

Termites *Cryptotermes domesticus* (Haviland) is a serious pest causing wood damage. It is one of the most important termite species in China. *C. domesticus* is spread-abroad and difficult to control (Gay 1969, Huang et al. 2000, Li 2002, Ping and Xiu 1997, Zhu et al. 1994) because it easily produces neotenics and rebuild colonies. Huang et al. have studied the colony formation, population development, foraging, swarming behavior and life cycle of *C. domesticus* (Huang and Dai 1989, 1990, Huang et al. 1997, Huang et al. 1994, 1995, Huang et al. 2009). The results indicated that *C. domesticus* finish a life cycle at indoor temperatures required seven years in Guangzhou (23.1° N) and six or seven years in Zhanjiang (21.2° N). But at a constant temperature of 27℃ and relative humidity of 80%, the cycle can be completed in two or three years. In the absence of Queen and/or King, some individuals (termed neotenic) take over the role of reproduction. Yet not every colony gives out neotenics. Clearly, how old the colony needed to be for individuals to develop into neotenics is not a process that is well understood. Thus, we raised orphaned colonies at ages of 1-4 years after removal of primary reproductives to investigate the colony age effects on neotenic differentiation. The paper is helpful for a comprehensive understanding of reproduction, dispersal and formulating strategies against this species.

Materials and methods

Termites Breeding. Alates swarmed from lab-cultured populations of *Cryptotermes domesticus* was employed as primary reproductives in this study. After swarming, paired alates went into pre-bored holes (¢ 0.3cm×1.5-2cm) by itself in uninfested blocks (*Pinus massoniana* Lamb) (4cm×4cm×3cm). About 100 paired colonies manipulated between 2002-2004 were raised in glass tank (¢ 30cm×50cm). The tank was covered with a net to prevent other insets to get in. *C. domesticus* colonies at ages of 1-4 years were broken up, and the Queen and King was located and removed. Orphaned colonies were cultured in *Castanopsis* sp block chambers (¢ 2.5cm × 1cm) and examined the caste composition once a week through the transparent plastic tape which covered on the chambers.

Statistical Analysis. Colony age effects on neotenic production were analyzed by One-way ANOVA and LSD test which performed with software SAS.

Results and discussion

During 3-6 months observation after experimental orphaned, the neotenic production is significant different within orphaned colonies at ages of 1-4 years after removal of both primary Queen and

King (Table 1). There was no neotenic production in 1-year-old orphaned colonies within 6 months after removal of primary productives. However, 86.67% of 2-year-old, 100% of 3-year-old and 100% of 4-year-old orphaned colonies produced a pair of neotenics within 3 months.

Table 1. Colony age effects on neotenic production percentage for orphaned colonies

Colony age(year)	Number of test Colony	Colony size	Number of Colony with neotenic	Neotenic output percentage	Observation month	Date of Removal primary reproducitves
1	20	5-6	0	0	6	28/6/2003-2/7/2004
2	15	7-13	13	86.67	3	10/6/2004-9/8/2006
3	12	13-20	12	100	3	10/5/2002-22/8/2005
4	12	20	12	100	3	28/6/2003-26/6/2007

From the data in Table 1, it indicates that there is no neotenic production ability for 1-year-old orphaned colony, while orphaned colonies older than 2 years can produce a pair of neotenics in the case of lost of Queen and King to rebuild the colony.

After removal of the primary productives, the 1st neotenic differentiated at 16.8±4.65 days and 2nd neotenic differentiated at 34.6±6.27 days in the 2-year-old orphaned colonies, 1st neotenic was produced at 9.9±0.53 days and 2nd neotenic was produced at 21.3±2.01 days in 3-year-old orphaned colonies, while 1st neotenic was produced at 9.5±0.72 days and 2nd neotenic was produced at 18.42±1.56 days in 4-year-old orphaned colonies. There was a great statistically difference for the 1st neotenic and 2nd neotenic differentiation between different ages of orphaned colonies (Table2). 4-year-old orphaned colonies tend to produce both 1st neotenic and 2nd neotenic in less days than 2-year-old orphaned colonies. But no significant difference between 2-year-old and 3-year-old orphaned colonies, or between 3-year-old and 4-year-old orphaned colonies.

Table 2. Colony age effects on 1st neotenic and 2nd neotenic differentiation after remove of primary reproductive.

Colony age(year)	Days needed for the 1st neotenic output	Days needed for the 2nd neotenic output	Days between 1st neotenic and 2nd neotenic	N
1	-	-	-	20
2	16.8±4.65	34.6±6.27a	17.7±3.42x	10
3	9.9±0.53	21.3±2.01ab	11.4±1.80xy	10
4	9.5±0.72	18.4±1.56b	8.9±0.98y	10

Different letters following means± standard error are significantly different at the α=0.05 level by One-way ANOVA and LSD test. Comparisons are made between colonies ages (a, b, c and x, y, z) impact on the days needed for the 1st and 2nd neotenic. N, number of colonies studied.

Conclusion

In *C. domesticus*, orphaned colonies is prone to produce neotenics and fund new colonies to infest (Huang et al. 1997, Huang et al. 2003, Huang et al. 2004, Huang et al. 2005, Huang et al. 2007, Qian et al. 2005). There are two important factors determine the differentiation of neotenics. One is extrinsic, in the absence of Queen and/or King, some individuals take the role of reproduction and become neotenics. The other factor is the intrinsic factors. 1-year-old colonies do no have the ability to give out neotenic while 2-year-older orphaned colonies have the potential to produce neotenics. Then colony age is an important intrinsic factor to determine the output of neotenics. There are certainly needs more detailed experimentation than what was presented herein to find out the neoteny differentiation mechanisms in *C. domesticus*.

Acknowledgments

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Replacement of Reproductives in Orphaned Field Colonies of *Macrotermes gilvus* and *Macrotermes carbonarius* (Blattodea: Termitidae)

by

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Abstract

In experimentally orphaned field nests of *Macrotermes gilvus* (Hagen) and *Macrotermes carbonarius* (Hagen), we found 15 colonies of *M. gilvus* re-established by containing replacement reproductives (adultoids), which accounts for 39.5%, while in *M. carbonarius*, three orphaned colonies (15%) were re-established. On the other hand, we found that high prevalence of re-colonization by other termite species after the dead of *M. gilvus* (18.4%) and *M. carbonarius* (20.0%) colonies.

Key words: Orphaning, *Macrotermes*, fungus-comb, adultoids, developmental pathway, replacement reproductives.

Introduction

Macrotermes gilvus (Hagen) is widely distributed in Southeast Asia. There are nine subspecies which vary in ecological and biological aspects (Roonwal 1970). Generally, it can be found in the lowland of altitude (> 160 m above sea level). *M. gilvus* soldiers can be distinguished from all other *Macrotermes* by its anterior region of the hyaline tip of the labrum broadly conical, whereas another sibling species, *Macrotermes cabonarius* (Hagen), have a trilobed hyaline tip. *M. carbonarius* is mainly located in lowland forest (< 160 m above sea level), agricultural and rural areas (Abe 1979; Abe and Matsumoto 1979; Tho 1992). It is heavily chitinized with orange-red to black-coloured body.

Macrotermes gilvus and *M. carbonarius* display forked developmental pathways, which is in line with those of in subfamily Macrotermitinae (Neoh and Lee 2009). The forked developmental pathway separates two developmental lines (neuter and sexual) at the first larval moult. The developmental pathway of neuter castes is genera-specific. In contrast, the developmental pathways of the sexual (nymphs and alates) is constant (Noirot 1969). The alates go through one undifferentiated larval instars and followed by five immature nymphal instars.

Under normal circumstances, a mature female alates and male alates pair and mate during flight activity to found a new colony. If the primary reproductives become moribund or die, termite societies develop various strategies to prolong the longevity of the colony: (1) the primary pair is replaced by new reproductives from one of potential developmental pathways within colony; (2) the diversion effort of workers to take care of new reproductives or nymphs. Replacement reproductives can develop from both immature apterous and brachypterous castes of termite. For example, apterous castes – larvae, pseudergates and workers give rise to larvoids, pseudergatoids and ergatoids, respectively; brachypterous castes – alates that retain in the parental colony and nymphs become adultoids and nymphoids, respectively (Myles 1999). There are three types of adultoids: (1) microimagos – dwarf alates which derived from young nymphs possess a pair of shorten wings; (2) pseudoimagos – show a pair of irregular broken wings and poor pigmentation; (3) normal adultoids – resemble closely to primary imago.

The occurrence of replacement reproductives in experimentally orphaned colonies have been extensively reported (e,g., *Coptotermes lacteus* (Frogatt): Lenz and Runko 1993; *Microcerotermes papuanus*: Roisin 1990; *Nasutitermes princeps*: Roisin and Pasteels 1986). In this study, the origin of replacement reproductives in queenless colony and the mechanism of colony re-establishment in *M. gilvus* and *M. carbonarius* were investigated.

Materials and methods

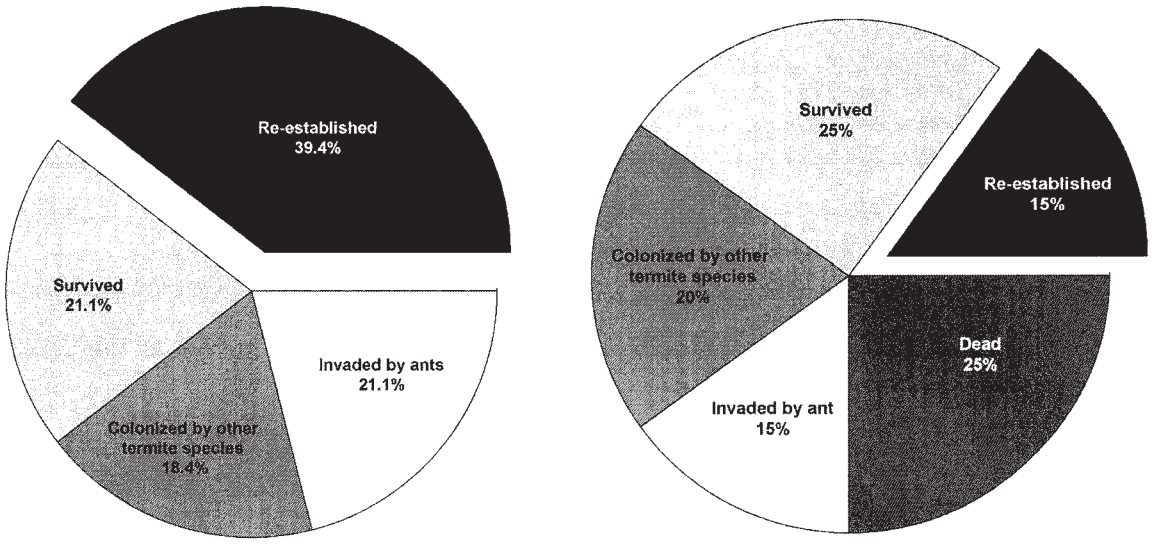
Study sites were located at the Minden Campus of Universiti Sains Malaysia (USM), Penang, Malaysia (5° 21' N and 100° 18' E). In the orphaning experiments, 39 colonies of *M. gilvus* (Height: 0.05–0.50 m; Diameter: 0.60–1.80 m) and 20 colonies of *M. carbonarius* (Height: 0.10–0.54 m; Diameter: 0.65–1.90 m) were selected. The mounds were orphaned by removing the royal chamber. After removal of the royal chamber, fragments of mound material, fungus-combs and outer casing were piled back onto the original mound site to avoid predation or damage through extreme weather events.

The orphaned colonies were investigated only once at selected time intervals: three (only *M. gilvus*), six, nine or 12 months after orphaning.

Results and discussion

Of the 38 orphaned colonies of *M. gilvus*, 15 (39.4%) colonies re-established (Fig. 1A) by using adultoids. Eight colonies survived and contained workers and soldiers. These could also have the chance to re-establish. However, subsequent examinations were not attempted since the mound was taken apart very thoroughly at the first inspection; seemingly there was no chance that this colony would survive any further. Fifteen colonies had died out. In seven (18.4%) of those cases, other termite species were present in the mounds at the time of inspection, e.g., *M. carbonarius*, *Globitermes sulphureus* Havilandi, *Amitermes* sp., *Odototermes* sp., *Pericapritermes* sp. and *Microtermes pakistanicus* Ahmad. Only workers and soldiers of the invading species were found. It would appear that these termite species have used the mounds just as feeding sites. Eight colonies were invaded by ants. Usually, dead termite bodies and fungus comb overgrown by a fast-growing fungus that were observed in the dead mounds.

In contrast, *M. carbonarius* only registered 15% success rate (3 out of 20 orphaned colonies) of colony re-establishment (Fig.1B). The orphaned colonies were headed by normal adultoids. Four colonies were invaded by other termite species.



(A) (B)

Fig. 1. (A) *M. gilvus*; (B) *M. carbonarius*. Frequency of orphaned colonies re-established after selected time intervals.

In this study, we found that the origin of replacement reproductives in both species studied is straightforward, which reproductives can only develop from alates to become adultoids. The occurrences of adultoid replacement are consistent with those of *Macrotermes bellicosus* Smeathman (Bordereau, 1975), *Macrotermes natalensis* (Havilandi) (Coaton, 1949 cited in Sieber and Darlington, 1982), *Macrotermes michaelseni* (Sjöstedt) (Sieber and Darlington, 1982) and

Odontotermes sp. (Noirot, 1956 cited in Myles, 1999).

Among the higher termites, Termitinae undergoes various potential developmental pathways to produce replacement reproductives. As in the *Microcerotermes*, workers are sexualized (ergatoids) once primaries are removed and gradually superseded by nymphoids in the nymph production season (Noirot, 1956, *M. parvus*; Roisin, 1990, *M. papuanus*; Leponce et al., 1996, *M. biroi*). The genus *Nasutitermes* is highly variable across species. Adultoid replacement reproductives present in queenless colonies in *Nasutitermes coxipoensis* (Lefeuve, 1987), *Nasutitermes princeps* (Desneux) (Roisin and Pasteels 1986a,b), *Nasutitermes costalis* (Roisin and Pasteels, 1986a), while *Nasutitermes novarumhebridarum* is replaced by ergatoids (Roisin and Pasteels, 1987). On the other hand, Costa-Leonardo et al. (1998) reported that the presence of nymphoid replacements in field colonies of *Amitermes euamignathus* and laboratory sub-colony of *Embiratermes festivellus*.

In all survived colonies, fungus-combs were largely consumed. This might suggest that workers direct more colony energy investment from foraging and mound fortification towards caring for the brood and reproductives (retained alates).

Acknowledgement

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Role of Antennae in the Detection of Ambient Humidity by the Termite, *Coptotermes formosanus*

by

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Abstract

Termites prefer high humidity habitat. Previously we reported electroantennograms (EAGs) to humidity changes of the termite, *Coptotermes formosanus* Shiraki and the putative hygroreceptive sensillum on the antenna. In this study, to demonstrate the antennal function of searching water source, group and individual behavioral tests were conducted. The results indicate that termite antennae play an important role in the quickness of reaction and to search environmental water source.

Key words: *Coptotermes formosanus* Shiraki, antenna, water detection, Y tube test, Container test

Introduction

The subterranean termite, *Coptotermes formosanus* Shiraki is a social insect and one of the most destructive insects of houses and wood structures in Japan and United States (Yamano, 2000; Mulrooney et al., 2007). As the surface-to-volume ratio is large in small sized animals, environmental humidity is particularly important for survival in all insects. Termites live in high humidity habitat and their survival is largely dependent on moisture (Nakayama et al., 2005; Kulis wt al., 2008). It is essential to find a water source in the nature for their high water demands. Therefore, sensory signals about environmental humidity change probably modulate the behavior patterns of the termites. Previously we reported EAGs to humidity changes of the termite, *C. formosanus* Shiraki and showed that the termite antennae responded increasing humidity with increasing depolarizing potential. Furthermore, we found the putative hygroreceptive sensillum on the antenna using SEM (scanning electron microscope), and characterized its external structure and distribution.

However, the behavioral modulation by environmental humidity or antennal mechanism underlysing such humidity-dependent or humidity related modulation of behaviors remains unclear now in termites. In this study, in order to show the detection ability of humidity change or graduation of habitat environment in the termite, we have demonstrated behavioral study using two termite groups; one group with intact antennae and the other lost both antennae. The container test was conducted to examine the group level reaction and the Y tube test was introduced to examine the individual level behavior. These studies on antennal hygroreception of the termite will bring about better understanding of the humidity-dependent behavioral modulation and will be useful to prevent their invasion to houses and wood structures.

Materials and methods

Insects

The termites, *C. formosanus*, were obtained from a laboratory colony maintained in the dark at 28 °C and more than 85% R.H. in Kyoto University, Japan. Worker termites were collected and placed into 90 × 15 mm Petri dishes containing a wet paper disc (a Whatman No. 1 filter paper) in a dark chamber at 25 °C for 1 to 3 weeks before use. As for the termites without antennae, termite antennae were excised at scape with a small scissor after termites had been cold anesthetized on ice for 30 minutes, then placed in a dark chamber at 25 °C for 1 to 3 weeks before use.

Group test

Group test was conducted in the container (21cm d.d., 20cm i.d., 12.5cm high), which was connected with two chambers (10 cm d.d., 9.3 cm i.d.) by a 8.5 cm silicone tube (d.d. 7.5 mm, i.d. 6 mm). One chamber contained a wet paper disc (a Whatman No. 1 filter paper) and the other contained a dry paper disc (a Whatman No. 1 filter paper). A small plastic cylinder (5.5 cm i.d.,

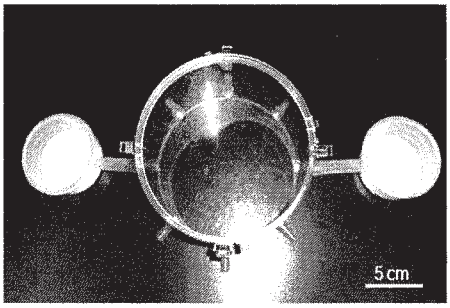

Fig.1 Container test

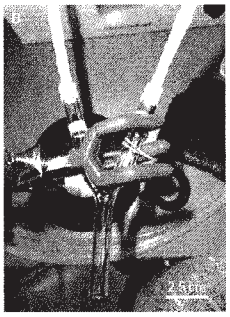

Fig.2 Y-tube test

5.8cm high) was put in the middle of chamber as an initial enclosure and 100 termites were placed in the cylinder for 15 minutes to reduce the influence of replacement. After this treatment, then the cylinder was removed and the termites were allowed to move freely in the container. The number of termites in the container, two connecting tubes and two chambers was counted at 3, 6, 12 and 24 hours after termites were released. In addition, during the initial 4 hours, the number of termite was counted at every 15 minute. The container and chambers were hermetically sealed by lids and closed well during the test. Temperature was kept at 25 ℃.

Individual test

Y maze made of glass tube was used in this experiment, since it had been verified that termite could walk normally in a preliminary experiment.

The wet and dry air were prepared as follows. Fresh air was taken by a diaphragm pump (AP-115 Iwaki air pump, IWAKI CO. LTD, Tokyo) from the outside. The air was desiccated with silica gel, molecular sieve 3A, 5A (1-4896-01 and 1-4896-02, respectively, Shinwa Chemical Industries LTD, Kyoto) and then cleaned by passing through active carbon. The cleaned air was then fed to a two way by connecting Y shaped connector. The flux was controlled at 400 milliliter/minute by a flowmeter en route. One of the outlets was connected to an empty glass bottle (30 ml) for a dry stream and another was divided into a glass bottle (30 ml) containing 25 ml distilled water as a source of water vapor. The air passing through these bottles was fed to a glass y-tube (5 cm for each arm and base, 6 mm i.d., 9 mm o.d.) for the behavioral test.

Termites were put in desiccated container for 5 hours before use. Then, one termite was released at the base of the glass y-tube and the brightness acted as a 'push' set of visual stimuli to induce the termites to move away from the release point toward the Y tube junction (Mburu et al., 2009). The time, which a released termite reached the half point of an arm was calculated. Temperature was kept at 25 ℃. 100 termites were used in the y-tube test.

Results and discussion

It is reported that termites are more sensitive to water loss of their cuticular when they live in low moisture content conditions (McManamy, 2008). Ambient humidity is one of very important factors in determining survival of the subterranean termite. At the beginning of each experiment, the most termites, which have intact antennae reacted more quickly than the termites, which lost their antennae. It seems that antennae help the initial perception of the humidity change in surrounding environment.

In the group test, both group of termites with/without antennae moved actively in the container after they were released as if they examined their surrounding environment. Termite group with antennae moved to the wet chamber whiten 15 minutes after they were released in the container (Fig. 3 A). The termites moved backwards and forwards between the wet chamber and the dry chamber during the 24 hour-observation. On the other hand, approximately 50% of termites could not move out from the container in the group without antennae during the observation in spite of their dedicated searching (Fig. 3 B), and the individuals, which could not find the way to the wet chamber were dead in 24 hours.

In the individual test, 80 % of termites with antennae chose the wet air coming arm immediately. The average time for making a decision of direction was 12.68 ± 9.83 seconds in the group with antennae. While, it took more time to decide the moving direction to the termites without antennae. It was 105.74 ± 91.11 seconds and 36% of them chose the dry air

coming direction. Termites with antennae ran toward the wet air coming arm directly or they sometimes came back to the base, the release point, however, did not go to the dry air coming direction. The movement of the termites without antennae was slow compared with the termite with antennae and they walked back and forth basically between the wet air coming arm and dry air coming arm, but seldom came back to the release point.

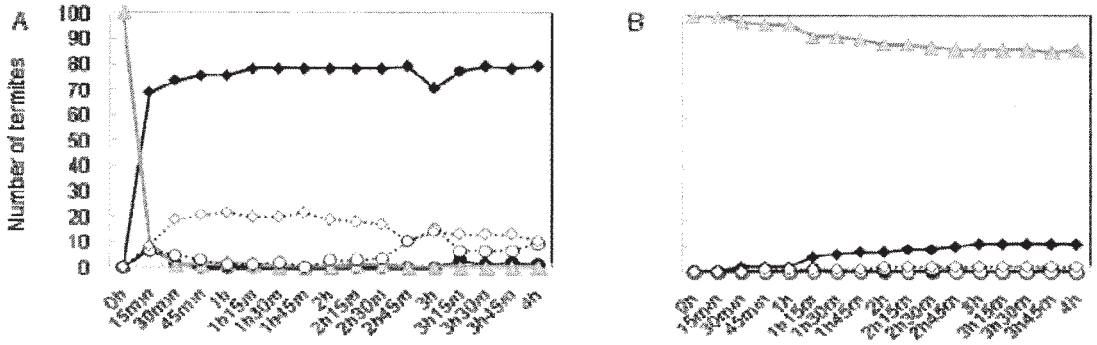

Fig. 3. Typical reaction of termites, *C. Formosanus* in the container test.
A: Termite group with antennae. B: Termite group without antennae. ◆ indicates that termites in wet chamber. ● indicates that termites in the tube of the way to wet chamber. ▲ indicates that termites in the middle container, where termites release. indicates that termites in the tube of the way to dry chamber. ◇ indicates that termites in dry chamber.

Termite antenna is moniliform composed by scape, pedicel and 11-13 segments and there is a hygrothermo-receptor like structure on each segment in our observation. Besides, we previously reported that termite antenna electrophysically reacted to the humidity change. According to those study and the results of these behavioral tests, it seems that termite antennae play an important role to detect a water source in the surrounding environment.

Conclusion

Termite *C. formosanus* with intact antennae made behavioral decision more quickly than those without antennae in both group and individual tests. In addition, when termites lost their antennae, many of them failed to find water source and died eventually in group test. While in individual test, the termites with antennae tend to choose the direction of high humidity air coming but the termite without antennae showed smaller tendency in decision depending on the humidity.

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Influence of Wavelength on Phototaxis in the Termite, *Coptotermes formosanus* Shiraki

by

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Abstracts

Phototaxis of workers and soldiers of *Coptotermes formosanus* Shiraki was investigated under exposure to UV radiation (350–375 nm) and visible light (400–650 nm) with narrow band gaps (25 nm) at given photon flux densities (3.6–60 μmol/m^2/s) for 30 and 300 sec. No positive phototaxis was observed in this experiment. On the other hand, negative phototaxis occurred at wavelengths shorter than 550 nm, and became more apparent at wavelengths shorter than 450 nm. The effect of photon flux density on this phenomenon was assessed using visible light at wavelengths of 400 and 425 nm. Negative phototaxis was still observed with photon flux density down to 3.6 and 23 μmol/m^2/s for wavelengths of 400 and 425 nm (violet–blue light), respectively. These results suggest that visible light may possibly be utilized to repel termites.

Key words: Phototaxis, *Coptotermes formosanus*, wavelength, behavior

Introduction

Over the last decade, physical and mechanical pest control measures have undergone dramatic changes to diminish the impact of pesticides on the environment. Among these measures, pest control using artificial light has attracted interest, and yellow light is already used to control nocturnal insects such as the noctuid moth (Goseki *et al.*, 2004). The use of green light has also been investigated in terms of determining the wavelength range that is effective for controlling the noctuid moth without damaging agricultural crops (Yamada *et al.* 2005).

Another accepted method is termite control via physical barriers such as graded stone and stainless steel mesh to prevent penetration (Yanase *et al.*, 2005; Murao *et al.*, 2006). Termites are known to avoid bright light, but the phototaxis mechanism is not sufficiently well understood to be used for termite control. Alates are attracted to light, and most of them are trapped on a night with a full moon rather than a new moon (Pearce, 1997). Yamano (1973) tested the phototaxis of *Coptotermes formosanus* Shiraki alates using various light sources, and reported that light at wavelengths of 400–420 nm was most effective, but longer or shorter wavelengths showed a sharp decrease in attracting alates. Park & Raine (2005) examined the response of *C. formosanus* to light using an incandescent lamp and found that termite workers, presoldiers and soldiers exhibited negative phototaxis over 0.6 lux. *Incisitermes minor* (Hagen) nymphs clearly showed negative phototaxis when exposed to fluorescent and incandescent lamps, but they did not respond to red light, indicating that nymphs could sense light even before their eyes had fully developed (Cabrea & Rust, 1996). Three Rhinotermitid species, *Reticulitermes tibialis* Banks, *R. flavipes* (Kollar), and *R. virginicus* (Banks), were killed when exposed to UV lamp at 30 and 60 W/m^2 (Siderhurst *et al.*, 2006). *Coptotermes* sp. died when irradiated at light intensity above 100 candela (light source was not mentioned) (Pearce, 1997). Patents have been submitted for termite control methods using UV radiation (Hand & Hand, 1992; Zaizen & Kobayashi, 2001).

Despite the interest in termite control using artificial light, there have been relatively few studies that have examined the effects of wavelength of light on phototaxis of termites, and none that have elucidated such effects of wavelength on termite workers and soldiers. In this study, workers and soldiers of *C. formosanus*, which causes severe damage to wood structures and living trees, were studied to determine the effects of wavelength on phototaxis using monochrome light source with narrow band gaps and constant photon flux density. The effect of photon flux density on phototaxis was also studied.

Materials and methods

Termites

Termites were collected from a laboratory colony of *Coptotermes formosanus* Shiraki in late June of 2008. The nest was dug up in Kurashiki, Okayama Prefecture in February 2003, and was moved to the Forestry and Forest Products Research Institute (FFPRI), Tsukuba, Japan, to a chamber maintained at 26 ± 2°C, 65% RH. Alates have not been observed since 2005. Young larvae, but no nymphs, have been captured in rearing timber, indicating that the nest has adapted well to laboratory conditions, producing new individuals.

Test methods

A transparent polystyrene container (60×100×26 mm in height) was used for the tests (Fig. 1). A hole (26×20 mm) was made in a narrow side of the container and equipped with a quartz cover glass for guiding the light inside the container. The bottom of the container was covered with black paper. The container was left in a dark room under 23 ± 3 °C, 65 ± 5% RH for over 10 min, after which 150 workers and 15 soldiers of *C. formosanus* were introduced into the container and then left for 5 min to adapt to the dark.

Irradiation was carried out at wavelengths ranging from 350 to 650 nm with a 25 nm band pass width and an interval of 50 nm (350–450 nm) or 100 nm (450–650 nm) using Hypermonolight® (SM-25, Bunkoh-Keiki Co.,LTD.). Photon flux density was maintained by using ND filter at 60 μmol/m^2/s behind the quartz cover glass of the container perpendicular to the light beam (position ①, Fig. 1). At that time, the photon flux density at position ② was 17.6 μmol/m^2/s. The effects of changes in photon flux density on phototaxis were also studied at 400 and 425 nm.

The position of termites at 30 and 300 s after the start of irradiation was recorded using a digital camera (EXILIM® Series, Casio Electronics Co. Ltd.), and the number of termites in an irradiated area was counted (N = 3). After irradiation, termites were kept under a daylight fluorescent lamp for over 5 min, and then in the dark for 5 min to start irradiation at another wavelength.

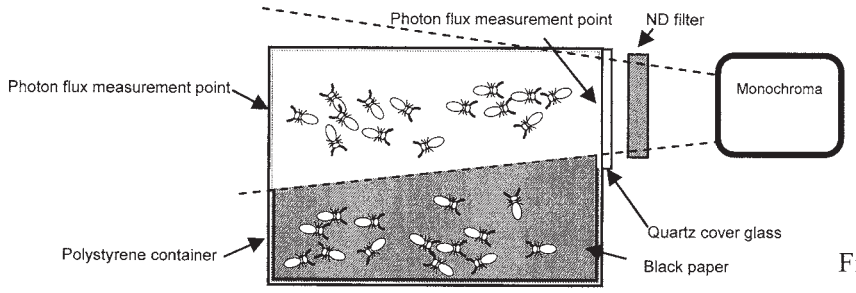

Fig.1 Apparatus

Results and discussion

Table 1 shows the ratio of termites in the irradiated area at a photon flux density of 60 μmol/m^2/s. Photon flux density was measured before and after irradiation at each wavelength to confirm that it was constant.

The ratio of termites in the irradiated area was highest at the wavelength of 650 nm (red) at all irradiation times (Table 1). The ratio of 45.9 % for 30-s irradiation at a wavelength of 650 nm indicates that the number of termites in and outside the irradiated area was almost the same, suggesting that workers and soldiers of *C. formosanus* did not show negative phototaxis. The ratio of termites in the irradiated area at a wavelength of 650 nm decreased slightly with increasing irradiation time, although there was no significant difference between the irradiation times (30, 300 s) ($p > 0.05$) (Table 1). Such low sensitivity to red light has also been reported in honeybees (von Helverson, 1972; Tominaga, 1995) and the nymphs of *I. minor* (Cabrea & Rust, 1996).

At wavelengths of <550 nm, the ratio of termites in the irradiated area decreased with decreasing wavelength, and finally became constant at about 20-25% (Table 1). Each ratio of termites at <550 nm was significantly lower than that at 650 nm ($p < 0.05$, Table 1), indicating negative phototaxis. Some of the ratios at <450 nm were lower than that at 550 nm. This clearly suggests negative phototaxis at <450 nm. It was also observed at <550 nm that soldiers tended to stay within the boundary area between irradiated and non-irradiated areas. The ratio of termites in

the irradiated area tended to decrease with increasing irradiation time at all tested wavelengths, and this tendency was clearly seen at <375 nm. Previous studies have reported negative phototaxis in the UV-radiation of termites (Siderhurst *et al.*, 2006), and patents using this phenomenon have already been published (Hand & Hand, 1992; Zaizen & Kobayashi, 2001). Negative phototaxis was also observed in the radiation mainly consists of visible light (incandescent or fluorescent lamp) (Park & Raine, 2005). However, the effect of wavelength on phototaxis was not clear, and the effect of UV-radiation in incandescent light was not considered. In this study, the effects of wavelength using monochrome light resulted in negative phototaxis in workers and soldiers, especially at 400–450 nm (blue-violet).

The ratio of termites in the irradiated area decreased with decreasing wavelength at 300-s irradiation (Table 1). However, there was no clear tendency for short-time irradiation (30 s). Wavelengths of 400–450 nm visible light at 30-s irradiation showed negative phototaxis more clearly than that at 350–375 nm. These results suggest that termites may easily respond to wavelengths of 400–450 nm. We then investigated the minimum light intensity on negative phototaxis by using an ND filter to apply a graded decrease in photon flux density at the wavelength of 400 nm (violet) and 425 nm (blue) for 30-s irradiation (Tables 2 and 3). At a wavelength of 400 nm, negative phototaxis was still observed even when photon flux density was decreased from 60 to 3.6 μmol/m^2/s ($p > 0.05$) (Table 2). At a wavelength of 425 nm, the ratio of termites in the irradiated area increased with decreasing photon density and finally negative phototaxis became unclear when photon density was decreased to 12 μmol/m^2/s or less ($p < 0.05$) (Table 3). These results suggest that higher photon flux density is needed at the wavelength of 425 nm to maintain high negative phototaxis compared to that at 400 nm.

Alates of *C. formosanus* showed positive phototaxis at wavelengths of 400–420 nm (Yamano, 1973). In our study, we revealed that workers and soldiers of *C. formosanus* showed negative phototaxis at the same wavelength range as alates. This indicates a difference among the castes of *C. formosanus* in response to light. Maekawa *et al.* (2008) reported that compound eyes develop in third instar larvae of *R. speratus*. It is necessary to study the light-sensitive parts of the workers and soldiers of *C. formosanus* to clarify the function of the organs. We plan to examine the phototaxis of other termite species taking into consideration the differences among castes.

Table 1 Mean percentage of termites ± S.D. (%) in the irradiated area under different wavelength //at photon flux density of 60 μmol/m^2/s

	Wavelength (nm)						
Ti	350	375	400	425	450	550	650
30	27.7±10.	31.9±8.9	21.0±4.0	26.7±5.0	21.4±6.4	33.3±3.7	46.9±4.5
300	12.7±2.2	13.7±2.7	17.0±4.2	17.6±4.6	17.4±2.5	25.1±6.5	39.8±4.

Means in the same column followed by the same capital letters, and those in the same line followed by the same uncapitalized letters are not significantly different ($p > 0.05$, Tukey-Kramer HSD test). Photon flux density was measured at ① point in Fig.1.

Table 2 Mean percentage of termites ± S.D. (%) in the irradiated area under different photon flux densit at wavelength of 400 nm after 30 second-irradiation

Photon flux densit	3.6	8.4	17	34	60
Mean ± S.D.	29.7±8.7a	30.7±14.2a	26.1±3.5a	21.0±6.7a	28.7±9.0a

Means followed by the same letters are not significantly different ($p > 0.05$, Tukey-Kramer HSD test). Photon flux density was measured at ① point in Fig.1.

Table 3 Mean percentage of termites ± S.D. (%) in the irradiated are under different photon flux density at wavelength of 425 nm after 30 second-irradiation

Photon flux dens	2.4	6.6	12	23	43	60
Mean ± S.D.	38.0±9.5a	42.6±5.1a	36.4±10.4a	26.7±8.2b	18.4±2.1b	18.4±2.1b

Means followed by the same letters are not significantly different ($p > 0.05$, Tukey-Kramer HSD test). Photon flux density was measured at ① point in Fig.1.

Acknowledgement

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Cryptotermes Eavesdrop to Avoid *Coptotermes* Competitors

by

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Abstract

Termites communicate with vibro-acoustic signals; because they travel over long distances in wood they are vulnerable to eavesdropping by other species. This possibility was investigated using choice experiments with live termites and recorded signals of the drywood termite *Cryptotermes secundus* and the subterranean termite *Coptotermes acinaciformis*, which is the dominant competitor in their habitat. *Cryptotermes* was attracted to its own vibration signals, shown by tunnelling preferentially into wooden blocks receiving these signals, but was repelled by those of the *Coptotermes*, shown by avoiding tunnelling into wooden blocks receiving these signals. This response increased with decreased wood size, which corresponded with increased risk and signal strength. Avoiding confrontation by eavesdropping explains why two or more species with very different competitive abilities can feed in the same wood. These results provide further evidence that vibro-acoustic signals are important for termite sensory perception and communication.

Key words: aggression; competition; communication; eavesdropping.

Introduction

Different species of termites are often found feeding in the same wooden food, even though different species will fight aggressively, often to the death when they meet (Thorne & Haverty 1991). It seems plausible that the less competitive species may avoid the dominant one; if so then the mechanism for avoiding competitors is unknown. Eavesdropping is the detection of heterospecific signals, studied primarily in vertebrates (e.g. Seyfarth & Cheney 1990; Rainey et al. 2004), and has been reported in bees (Nieh et al. 2004; Yokoi et al. 2007), and it offers an explanation how less competitive species avoid exclusion.

Eavesdropping is more likely to evolve when differences between competitors are large. The difference in competitive ability between drywood termites such as *Cryptotermes* and the subterranean termites *Coptotermes*, often found feeding in the same tree, is large *Cryptotermes* queens do not become physogastric and so lay few eggs, producing colonies of 200-300 individuals, with very few soldiers (Gay & Watson 1982). In comparison, *Coptotermes* queens become physogastric, producing colonies of one million or more individuals, with tens of thousands soldiers (Evans et al. 1999). More than 85% of trees are hollowed out by *Coptotermes* in northern Australia (Werner et al. 2008), but somehow *Cryptotermes* live in *Coptotermes* infested trees (Gay & Watson 1982). This study aimed to test the hypothesis that *Cryptotermes* were detecting *Coptotermes* and avoiding them using vibration signals normally used determine wood size and the quality of the food source (Evans et al. 2005, 2007).

Materials and methods

Test species. *Cryptotermes secundus* and *Coptotermes acinaciformis* were collected near Darwin, Australia. Experiments were performed constant temperature rooms (28°C & 80% RH) in Canberra; 5,070 *Cryptotermes* from 31 colonies and ~16,800 *Coptotermes* from five colonies were used.

Laboratory bioassays. The test unit had (almost) identical wooden blocks (cut sequentially from *Pinus radiata*, 20 x 20 mm) of 20, 160 or 400 mm length. Test *Cryptotermes* (15) were held in central cell (15 mm) made from tape, aluminium foil and/or plastic between the ends of the paired blocks; thus test termites had the choice of two almost identical ends (Figure 1). There were four treatments: live *Cryptotermes*, recorded signals of *Cryptotermes*, live *Coptotermes*, and recorded signals of *Coptotermes*, plus a no signal control. The signal treatment was applied to one wooden block in each pair. For the live termite treatments, cages (20 mm diameter acrylic tube, aluminium foil and tape) were attached to the wooden blocks; one cage with either 15 *Cryptotermes* or 300 *Coptotermes* (due to higher mortality; nb recorded signals were controls – see below). For

the recorded signals, Philip-Harris vibration generators (# F4H31134, Leicester, UK), were attached to one wooden block in each pair, and played the recorded signals continuously from CD players (Sony D-EJ100). All signal amplitudes were similar and negligible signal was transmitted through the central **cell. The experiment ran for two weeks. Tunnelling data were analysed by ANOVA with Bonf**erroni corrections (Systat 9, SPSS), with preference determined from 95% confidence intervals.

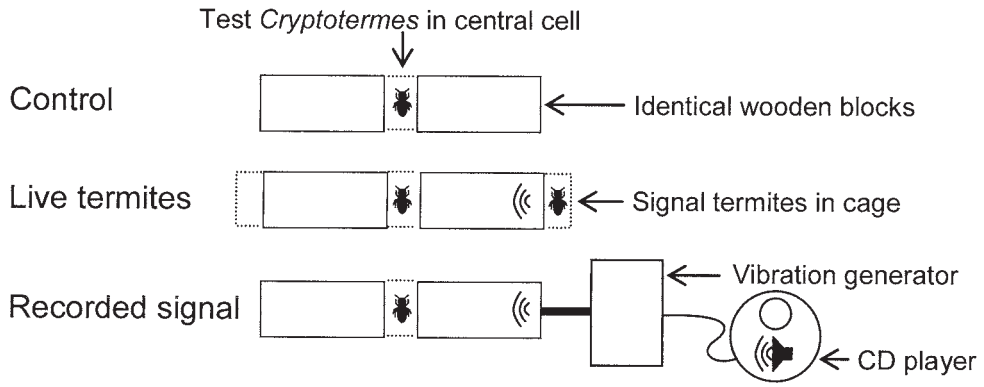

Figure 1. Schematic diagram of experimental apparatus. Wooden blocks were cut sequentially from the same length of wood and paired to be as similar as possible. There were three sizes: 20 mm, 160 mm and 400 mm. Signal termites in the live termite treatment were either *Cryptotermes* or *Coptotermes*.

Recorded signals. The recorded signal treatments controlled for the different numbers in the live termite treatments. Six *Cryptotermes* and three *Coptotermes* colonies were recorded in *Pinus radiata* wooden blocks 20, 160 and 400 mm long in an anechoic chamber. A Brüel & Kjaer accelerometer (#4370) and charge amplifier (#2635) were used with signals recorded directly to a computer soundcard. Ten minute composite recorded signals were assembled from the longer recordings, one for each species-block size combination. Recorded signals had the same level of activity (mandibles 'scratches' and breaking wooden fibre 'snaps') and were not significantly different: mean ± se scratches/min, *Cryptotermes* 71.1 ± 1.8 and *Coptotermes* 70.7 ± 1.7 ($F_{1,54}$ = 0.018, p= 0.894); snaps/min *Cryptotermes* 8.8 ± 1.0, and *Coptotermes* 9.2 ± 0.5 ($F_{1,54}$ = 1.658, p = 0.203).

Results and discussion

Test *Cryptotermes* termites tunnelled a similar total distance (sum of tunnel lengths in both paired blocks) between signal treatments ($F_{4,275}$ = 0.188, p = 0.944), but tunnelled significantly further in the longest blocks (length treatment $F_{2,275}$ = 6.621, p = 0.002); thus signal treatments did not affect termite feeding but amount of food did. Therefore to control for the effect of wood block size proportional data were used (proportion tunnelling = tunnel length in block receiving signal / sum of tunnel lengths in both blocks; a random block was chosen for controls). The two way ANOVA found a significant interaction ($F_{4,271}$ = 2.066, p = 0.039) between signal type and block length, therefore each block size was analysed separately. For 20 mm long wooden blocks, signal treatments were significantly different ($F_{4,59}$ = 14.755, p < 0.001); with live *Cryptotermes* not significantly different from recorded *Cryptotermes* (p > 0.05); with live *Coptotermes* not significantly different from recorded *Coptotermes* (p > 0.05); but with both *Cryptotermes* treatments significantly different from both *Coptotermes* treatments (p < 0.05). The same situation was observed for 160 mm long wooden blocks: signal treatments were significantly different ($F_{4,115}$ = 5.590, p < 0.001); with *Cryptotermes* treatments not significantly different (p > 0.05); with *Coptotermes* treatments not significantly different (p > 0.05); but with *Cryptotermes* treatments significantly different from *Coptotermes* treatments (p < 0.05). However, for 400 mm long wooden blocks no treatments were significantly different ($F_{4,97}$ = 0.283, p = 0.889) (Figure 2).

The preference of the test *Cryptotermes* was confirmed with 95% confidence intervals; when the 95% confidence intervals did not overlap 0.5, the test termites were determined to have a preference. *Cryptotermes* preferred 20 mm and 160 mm blocks with live *Cryptotermes* and

recorded *Cryptotermes* signals; they avoided 20 mm and 160 blocks with live *Coptotermes* and recorded *Coptotermes* signals (Figure 3). Test *Cryptotermes* had no preference in all control block pairs and all 400 mm blocks.

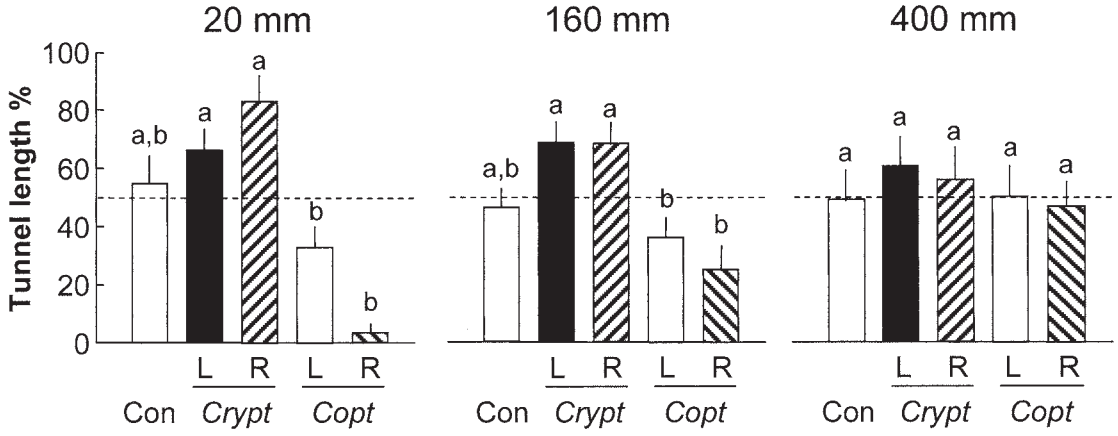

Figure 2. Proportional tunnelling by test *Cryptotermes secundus* in the signal block (random for controls). Con = control, L = live signal termites, R = recorded signal, *Crypt* = *Cryptotermes*, *Copt* = *Coptotermes*. Columns with the same letter are not significantly different; blocks of different length analysed separately.

These results show clearly that the test *Cryptotermes* were using vibration signals to distinguish the signal termites, with attraction to their own species and avoidance of the other species, at least at shorter distances. The detection of chemical signals was unlikely because the test *Cryptotermes* were completely contained and distant from the signal termites, either way, the recorded signals produced the same pattern as the live termites, thus confirming vibration signals alone could produce the different responses. Interestingly, the recorded signals produced a greater response than the live termites, perhaps because there the amount of signal was greater: the vibration signals were played continuously, whereas live termites may have chewed on the wooden blocks less constantly.

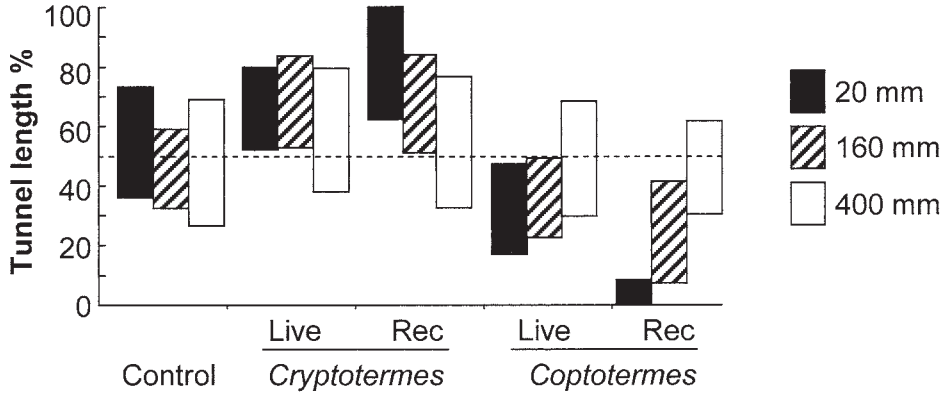

Figure 3. 95% Confidence intervals of the proportion of the total tunnel length in the block receiving the signal. Rec = recorded signal. A preference was observed when the 95% CI did not include 50% (dashed line); attraction to the signal block when the 95% CI was above 0.5, avoidance when below.

The ability to identify species using vibrations made from feeding could be considered a form of eavesdropping. This is distinct from eavesdropping in the usual sense of the term, when one prey species listen to the alarm calls of other prey species to detect the presence of predators (Seyfarth & Cheney 1990; Rainey et al. 2004), but it is analogous to the eavesdropping on foraging signals of competitors observed in bees. Various bee species mark flowers they have visited, to warn their nestmates the flower has been emptied of nectar; and stingless, sweat, bumble and honeybees have detect and respond to heterospecific signals (Nieh et al. 2004; Yokoi et al. 2007).

This is the first time the ability to identify other termite species from vibration signals has been demonstrated. There are several advantages to vibration signals: they are fast, operate over distance and do not require direct contact – particularly useful for such poorly armed insects that rely on crypsis for defence. There are two possible explanations for the decrease in response with the increase in wooden block size. First, the termites may be capable of evaluating risk, i.e. by avoiding competitors only when the threat is imminent. Second, signal perception may diminish with signal strength as the block size increases. The benefit in avoiding *Coptotermes* was clear, as *Coptotermes* will kill the *Cryptotermes* if they come into contact (and did so in some 20 mm replicates through which they had tunnelled; these were not used in the analysis). However the benefit in being attracted to conspecifics is less clear. Outbreeding may be one possible explanation: should *Cryptotermes* colonies be orphaned (i.e. the mother queen or father king or both die), the workers mature into neotenic replacement reproductives; newly reproductive brothers and sisters will be forced to mate if they do not encounter another colony. This suggestion gains support from the peaceful acceptance of unrelated individuals in *Cryptotermes secundus* colonies, and genetic data shows 25% of field colonies are the product of a merger (Korb & Schneider 2007).

Will ability to eavesdrop be widespread in termites? The medium in which they live may play a role. Soil dampens vibration signals much more than wood (Liu & Nagel 1993), reducing the detection range to less than five centimetres (Mankin et al. 2000). Thus subterranean termites may be less attuned to vibration signals than drywood termites, and if so, then drywood termites can exploit the same food resources as subterranean termites by being even more cryptic than subterranean species. While it has been shown here that drywood termites use vibrations to identify their own from other species, exactly which features of these signals the termites use to extract the information and how they process such information are yet to be determined.

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Temperature and Humidity Preference of Three Dry-Wood Termite Species

by

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Abstract

Three experiments were designed to evaluate the influence of temperature and humidity preference for three dry-wood termite species, *Cryptotermes domesticus* (Haviland), *Incisitermes minor* (Hagen), and *Cryptotermes cynocephalus* (Light). For experiment I, nymphs of *C. domesticus* were loaded in to a holed specimen of Indonesian pine and kept in a test chamber under various relative humidity (60, 70, 80, and 90%) and temperature (15, 25, 35, and 45 °C)conditions. The propagation of acoustic emission (AE) signals due to the feeding activity of termites in the specimen was monitored. As the experiment II, twenty-four different combination of six temperatures (15, 20, 25, 30, 35, and 40 °C)and four relative humidity (60, 70, 80, and 90%) conditions were used for nymphs of the western dry-wood termite *Incisitermes minor* (Hagen). The feeding activities of the termites were monitored by the detection of generated acoustic emission (AE) events from feeder wood blocks of Douglas fir in a test chamber. In the last type of experiment, Type III, nymphs of *C. cynocephalus* were loaded in to a holed specimen of Sengon and put in to Tupperware than kept at different temperature and relative humidity for eight weeks. Relative humidity was maintained within Tupperware using saturated salt solutions of NaCl, NH_4Cl, and KCl for 75, 80 and 85% RH respectively. Temperatures of 25, 30, 35 °C were maintained by placing the Tupperware in temperature-controlled in chamber. Feeding activity of termites in the specimen was determined by survivorship and wood weight loss. Our results indicated that termite feeding activities vary with changes in RH and temperature. The optimal temperature and RH conditions for the feeding activities of both of dry-wood termites, *C. domesticus* and *I. minor* were 35°C-70%, while the optimal temperature and RH conditions for *C. cynocephalus* were 25 °C-75%.

Key words: Dry-wood termite, Feeding activity, Temperature, Relative Humidity (RH)

Introduction

One of the most important wood-destroying insect groups in the world is the dry-wood termite. Dry-wood termites differ from other termites in their ability to live within structural timbers or furniture inside buildings, feeding on wood with low moisture content. This species was reported to be much more tolerant of high temperatures and arid conditions than other termite species. Temperature is one of the most important environmental factors affecting wood consumption by termites. Rudolph *et al.* (1990) stated that dry-wood termites (Kalotermitidae) obtained their favorable relative humidity (RH) conditions indirectly via the available moisture content from wood, because they live in dry wood. Both temperature and humidity are likely to play important roles in the survival of termites and influence their feeding activities.

Some of dry-wood termite species such as, *Cryptotermes domesticus* (Haviland), a dry-wood termite which was found throughout the tropics and which causes serious damage to houses; *Incisitermes minor* (Hagen), categorized as a serious pest in the United States; and *Cryptotermes cynocephalus* (Light) is the native pest in Indonesia.

The purpose of this study was to investigate the temperature and RH preference of *C. domesticus*, and *I. minor* by monitoring feeding activity, which was defined as generated AE event, from feeder wood blocks in a test chamber (experiment I and II). In the experiment III, we determine how temperature and RH affected *C. cynocephalus* as measured by survivorship and wood consumption.

Materials and methods

Experiment I

Wood specimen, measuring 30 (R) x 30 (T) x 50 (L) mm, with one hole in the center, 5 mm in diameter and 30 mm in depth to accommodate the termites, were prepared from an air dried a specimen of Indonesian pine (*Pinus merkusii* Jungh et de Vriese). Twenty-five nymphs of *C. domesticus* with no external evidence of wing buds or eyes were loaded into the hole which was then covered with a piece of transparent glass. A piezoelectric AE sensor with a resonant frequency of 140 kHz was attached to the top surface of the wood specimen using silicon grease and a rubber band (Fig.1). The signal from the sensor was amplified by about 66dB, filtered by a high-pass filter with a cutoff frequency of 100 kHz, and discriminated at a threshold voltage of 0.1 V with the AE apparatus. The observation units were kept in a test chamber (Eyela KVL 1000; Tokyo Rikakikai, Tokyo, Japan) at steady temperature of 15°C, 25 °C, 35 °C, and 45 °C at 70% RH for 12 hours and the feeding activities were monitored by the detection of generated AE events. For the purpose of the fixed-RH test, the observation units were kept in the test chamber at RHs of 60%, 70%, 80%, and 90% at 27 °C.

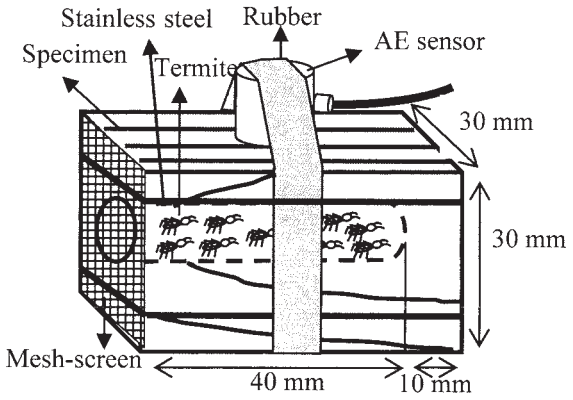

Fig. 1. Test apparatus for AE monitoring of the feeding activity of *C. cynocephalus* and *I. minor*.

Experiment II

For experiment II, an experimental setup similar to that used for the experiment I was employed, except for the number of nymphs (ten individuals), species of wood specimen (spruce, *Picea abies* Karst.), measuring of hole (40 mm in depth and 10 mm in diameter), and the specification of AE sensor (resonant frequency of 150 kHz, amplified by 85dB, filtered by a high-pass filter with a cutoff frequency of 50kHz and discriminated at a threshold voltage of 0.6 V).

Experiment III

Twenty nymphs of *C. cynocephalus* were placed in laminated block of sengon (*Paraserianthes falcataria* L. Nielsen). Each of these blocks was constructed from three wafers measuring 3 cm x 5 cm x 1 cm. A hole was drilled in the center of each of the three wafers and the hole with termites inside was than covered with a fine mesh screen to allow each replicate to be enveloped in the desired RH and temperature. A rubber band was treated to hold the 3 wafers together. Twelve experimental treatments consist of all of the possible combinations of the following temperatures and RHs: 25, 30, 35°C and 75, 80 and 85% RH. Relative humidity was maintained within Tupperware using saturated salt solutions of NaCl, NH_4Cl, and KCl for 75, 80 and 85% RH respectively (Winston & Bates, 1960). Temperatures of 25, 30, 35°C were maintained by placing the Tupperware in temperature-controlled in chamber. Feeding activity of termites in the specimen was determined by survivorship and wood weight loss. Every two weeks, for a period of eight weeks, the laminated block were removed and the number of dead and live termite was counted and the laminated block was weight.

Results and discussion

Experiment I: *C. domesticus*

Figure 2 shows the changes in AE event rates generated by termite attacks under various temperature conditions. Temperature was found to have a significant effect on termite feeding activities. At 15°C

and 45°C, no significant effect AEs were detected other than electric noises. The highest temperature (45°C) caused some of the nymphs to become moribund, and their feeding activity decreased rapidly. However, the coolest temperature (15°C) did not kill the termites, but did reduce the feeding activity. The thermal limits of other *Cryptotermes*, *C. brevis* (Walker) (scheffrahn *et al.*, 1997) were 48°C for

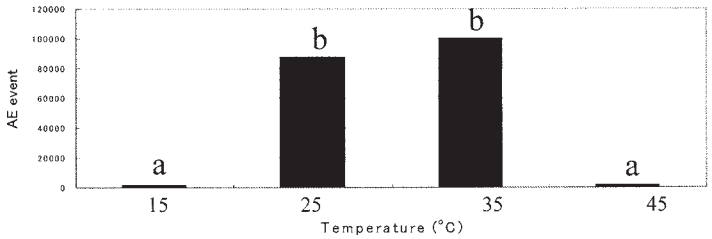

Fig. 2. Temperature effect on *C. cynocephalus* feeding activity. The same letter indicates no significant difference at $P = 0.05$ (Tukey's

10 min. Given the wide distribution of *C. domesticus* in the tropics, this noteworthy result deserves further investigation. As shown in Fig.2, no significant difference was observed between the cumulative AE events for 24 hours at 25°C and those at 35 °C Turkey's test: P=0.05). The lowest rate of AE event was obtained at 15°C and 45°C, while the maximal rate was observed at 25 °C and 35 °C.

The total numbers of AE events during a monitoring period of 12 hours under various humidity conditions are shown in Fig.3. Our results indicate that RH condition have a significant effect on the feeding activity of *C. domesticus*, specifically, cumulative AE events were significantly higher at 70% and 80% RH than at 60% and 90% RH (Tukey's test:P = 0.05). There was no significant difference between the cumulative AE events for 12 hours at 70% RH and those at 80% RH, nor between those at 60% RH and those at 90% RH (Tukey's test:P = 0.05). The present results clearly indicate that 70 – 80% is the optimal RH condition for the feeding activity of *C. domesticus*.

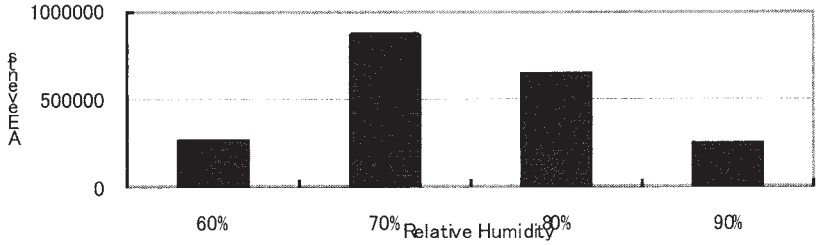

Fig. 3. Humidity effect on *C. cynocephalus* feeding activity. The same letter indicates no significant difference at P = 0.05 (Tukey's test)

Experiment II: *I. minor*

The feeding activities of *I. minor* monitored by the AE events under various temperature and humidity combination are summarized in Fig.4. In general, the average number of AE events generated from the wood specimens steadily increased with temperature up to 35 °C, regardless of RH with an exceptionally high value for the 30 °C -70% RH condition.

The results of two-way 4 x 6 factorial ANOVA indicated that temperature had an independent significant effect on the feeding activity of termite (ANOVA: $P < 0.01$). No significant differences were observed among temperatures of 25, 30 and 35°C at 60, 70 and 80% RHs with an exceptionally higher feeding activity at the condition of 30°C-70%, while at 90% RH temperature of 35°C gave a significant effect on the feeding activity of termite (Tukey's test: $P < 0.05$).

The results of the ANOVA indicated that RH had an independent significant effect on the feeding activity of termite as well (ANOVA: $P < 0.01$). The Tukey's test showed that RH had significant effect at 15°C, 20°C, and 40°C ($P < 0.05$), and no significant effect was seen at 25°C, 30°C, and 35°C with an exceptionally higher feeding activity at the condition of 30°C-70%. The optimal RH was noted at 70% but no significant differences were observed between RHs of 70% and 80% at all temperature conditions.

The combination of temperature and RH also significantly affected the feeding activity of termite. Combinations of 35°C-70% and 35°C-80% resulted in higher average AE events. Combinations of 30°C-90%, 25°C-90%, and 20°C-80% showed lower average numbers of AE events than combinations of 30°C-60%, 30°C-80%, 35°C-60%, 25°C-70%, 25°C-80%, 35°C-90%, 20°C-70%, 25°C-60%, but no significant differences were observed between these two groups (Tukey's test: $P < 0.05$) (Fig. 4). The lowest feeding activity occurred in the combination of 40°C-90%; however, there were no significant differences in average AE events between this combination and the combinations of 40°C-60%, 20°C-90%, 40°C-80%, 15°C-90%, 40°C-70%, 15°C-60%, 15°C-70%, 15°C-80%, and 20°C-60%, (Tukey's test: $P < 0.05$).

At the end of the monitoring process, the survival rates of the termites at the temperatures of 15, 20, 25, 30, and 35°C were 100% regardless of the RHs. However, a temperature of 40°C caused some of the nymphs to become moribund.

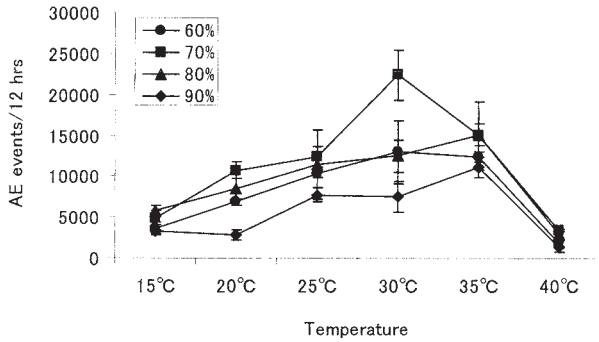

Fig. 4. Cumulative AE events for 12 hrs generated from wood specimens with *I. minor* nymphs under various temperature-RH conditions. Error bars represent standard deviations.

Experiment III: *C. cynocephalus*

In general, mortality of *C. cynocephalus* nymphs was higher at 85% RH than it was at 80% or 75% RH at all three temperatures (Table 1). Mortality was also highest at 35°C and lowest at 25°C. At the end of eight weeks, mortality was greatest at 35°C – 85% RH. After eight weeks, the greatest amount of weight loss occurred at 25°C at all three relative humidity, although there were no significant differences among those three treatments. Weight loss was greatest at 75% RH and least at 85% RH. This may explain that mortality was lower at 25°C and 75%RH, resulting in more termites feeding over time, even though their level of activity was reduced compared with termites at higher temperature.

Table 1. Mean percent survival of *C. cynocephalus* and mean percent weight loss of wood held at different combinations of temperature and RH over eight weeks

No.	Treatment		Mortality (±SD)*	Weight loss (±SD)*
	°C	% RH		
1	25	75	30.00±15.16a	1.26±0.70a
2	25	80	39.58±3.145a	1.04±0.72a
3	25	85	45.00±12.05ab	1.02±0.31a
4	30	75	46.25±2.504ab	0.78±0.10b
5	30	80	57.08±13.78b	0.73±0.12b
6	30	85	59.58±13.77b	0.51±0.24b
7	35	75	95.87±1.72c	0.66±0.33b
8	35	80	94.58±0.89c	0.64±0.08b
9	35	85	98.33±0.72c	0.53±0.27b

* Means followed by the same letter are not significantly different (Tukey's test: $P < 0.05$).

In the present investigation, 35°C was the optimal temperature for both *C. domesticus* and *I. minor* (Figs. 2 and 4), while the optimal temperature for *C. cynocephalus* was 25°C. This may indicate a preference for higher temperature in those of *C. domesticus* and *I. minor* when compared

with *C. cynocephalus*. The fact that the majority of both *C. domesticus* and *I. minor* attacks are found in the upper parts of houses (roofing materials) (Indrayani *et. al*, 2004), while *C. cynocephalus* mainly attack furnitures seems to support this assumption. The optimal temperatures for the feeding activity of the other dry-wood termite species were 25-30°C for *Kalotermes flavicollis* (Sen Sarma, 1965), 28.3-29.1°C for *C. brevis*, 31.8-32.2°C for *C. dudleyi*, and 29.7°C for *C. havilandi* (Steward, 1981).

The results from the present study show that temperature has a strong effect on the feeding activities of the dry-wood termite, relating to its infestation manner. On the other hand, RH has a slight effect on the feeding activity. This information is essential for gaining a better understanding of the feeding behavior of the pest dry-wood termite and may contribute to the improvement of termite control measures with less or no use of chemicals such as remedial control of this termite by heat treatment, a method that has already been promoted in the US for dry-wood termite species (Su, 2000).

Conclusion

Environmental RH and temperature influence the feeding activities of the dry-wood termite. The optimal RH and temperature conditions for *C. domesticus* and *I. minor* were estimated at 35 ºC and 70%, while the optimal RH and temperature conditions for *C. cynocephalus* was at 25 ºC and 75%. At 15 ºC, feeding activities decline to dormancy and at the other extreme of 40-45 ºC, termites die within a few minutes. These results suggest the possibility of heat treatment as a method of controlling a species which causes enough damage to create serious economic problems in the tropics.

Acknowledgment

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