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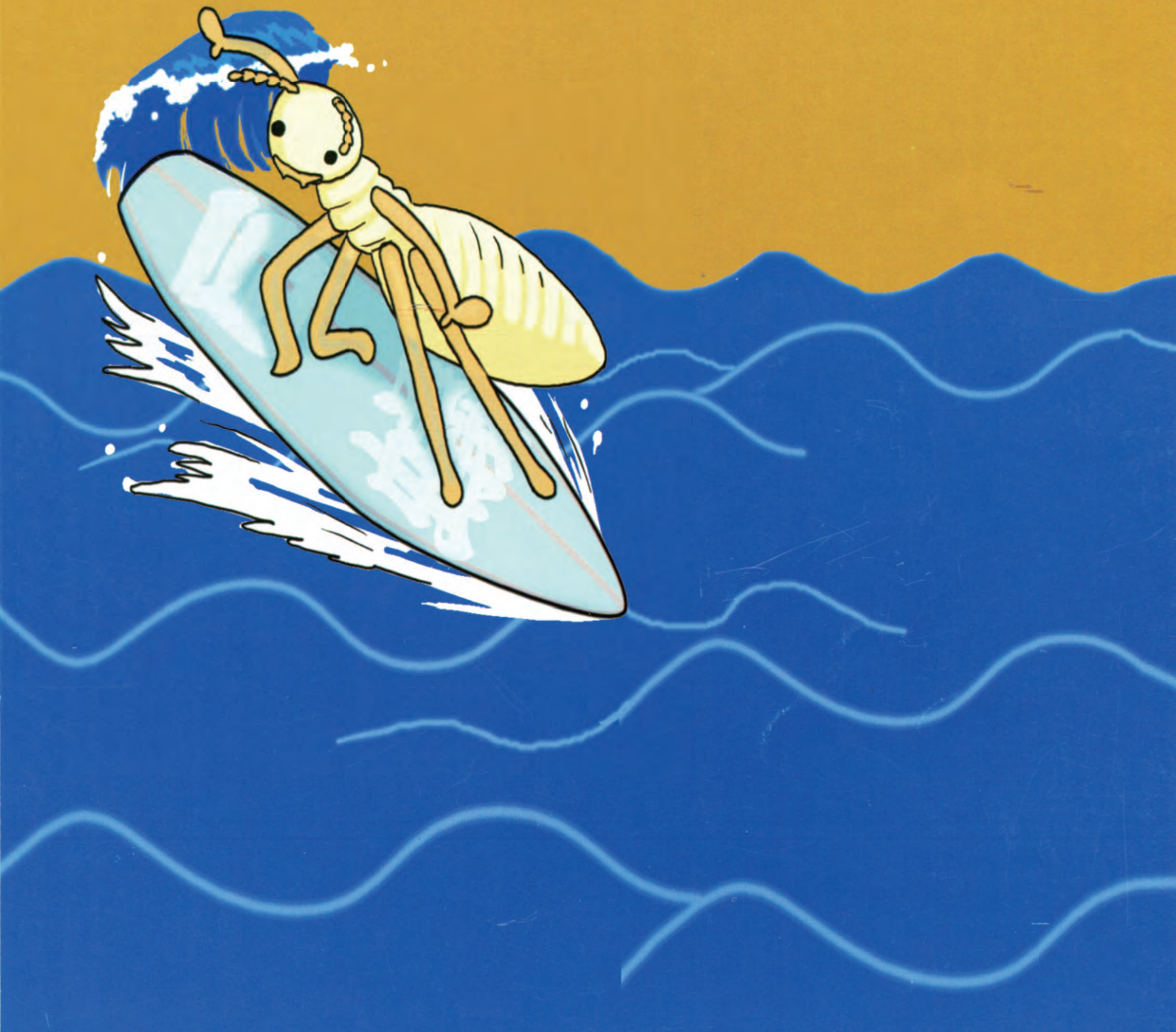

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Contents

History of Termite Management by Soil Treatment with Chemicals – Why Were Uses of Chlordane Banned?

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Abstract

We first started chemical protection of wood from termite in the late 19th century, although some termite control methods had been used locally. However, the early chemical treatments were actually limited to kill a part of termite foragers of a colony. Later in the 1940s some cyclodienes were widely commercialized for soil treatment to establish soil barrier. Because they were inexpensive and repellent, and suitable for a long-lasting formation of soil barrier against subterranean termites belonging to are most economically important pest group. With an increasing threats to human health and the environment, the most effective and widely used in the world, chlordane was banned in 1980s some countries. According to the Stockholm Convention in 2001, the use of 12 persistent organic pollutants (POPs) (aldrin, dieldrin, endrin, heptachlor, chlordane, DDT, mirex, dioxins, furans, hexachlorobenzene, toxaphene, PCBs) must be banned globally. Some POPs have been applied as termiticides, and unfortunately chlordane is still in use and/or in service in countries. More recently environmentally-acceptable carbamates, organophosphates, synthetic pyrethroids, neonicotinoides and others appeared as alternatives to persistent cyclodienes, and have been taking its market place in the world.

Key words: chlordane, cyclodienes, soil treatment, POPs (Persistent Organic Pollutants), environmental and human health risk

Introduction

It is believed that termites first appeared on the earth more than 130 millions of years ago and were distributed worldwide based on fossil records. Because termites are economically important urban, agricultural and forest pests, there is a long history combating termites in the world including Japan (Tsunoda 2003). Use of termite-resistant timber was quite reasonable when humans did not have any protective measure against termite attacks.

Termite control with inorganic chemicals started in the late 19th century, and then cyclodienes*[1]/ chlorinated hydrocarbons*[2] were introduced to the termiticide market in the mid-1940s. One of those chlorinated hydrocarbon insecticides, chlordane*[3] was inexpensive, effective and long-lasting.

*[1] cyclodiene: a term given to a sub-category of organochlorine insecticides

*[2] chlorinated hydrocarbon: a generic term given to compounds containing chlorine, hydrogen and carbon

*[3] a mixture of various chemical substances including α - chlordane and γ -chlordane as main isomers

The insecticide was first registered in the United States in 1948. All uses of chlordane were banned by US Environmental Protection Agency in 1983 with some exceptions (*e.g.* termite control). Finally, in 1988 all uses were voluntarily canceled in the United States except its use for fire ant control in power transformer (NPTN 2001). As shown in Table 1, chlorinated hydrocarbons including chlordane were introduced to treat soil against subterranean termites in Japan in 1947, and the use of chlordane was actually banned in 1986 because the termiticide was designated as a specific chemical substance (JTCA 1998).

Table 1 Recent history of chemical use for termite control in Japan

Year	Event description
1872～	Creosoted timber
1931	Inorganic salts (arsenic and copper compounds) introduced to treat timber
1947	Chlorinated hydrocarbons such as γ-BHC, DDT, aldrin, dieldrin and chlordane introduced into Japan
1969	Domestic production of γ-BHC and DDT banned
1983	Chlordane designated as a poisonous chemical substance
1986	Chlordane designated as a specific chemical substance*[1]
1986～	Organophosphates (chlorpyrifos, fenithrothion), synthetic pyrethroids (bifenthrin, permethrin, fenvalerate, cypermethrin, ethofenprox), carbamates (fenobucarb, carbaryl) , neonicotinoide compounds (imidacloprid), benzoylphenylurea compounds (hexaflumuron, noviflumuron)*[2]
Mid-1990s～	Phenylpyrrazole compound (fipronil)*[3], phenylpyrrolle compound (chlorfenapyr)*[3]

*[1] Annual consumption of chlordane exceeded 1,500 tons in the early 1980s in Japan
*[2] chitin synthesis inhibitor for bating application
*[3] nonrepellent termiticide

Why were uses of chlordane banned?

Prohibition or reduction in use of early termiticides was due to their relatively high acute and chronic toxicity. For example, organophosphates are generally acutely toxic, and their problem is labors' health, although they are readily degradable in the environment and can only afford a short service life. Chlordane that was the most extensively used chlorinated hydrocarbon as a soil termiticide, is not dangerous to use because it has moderate acute oral toxicity and low volatility (NPTN 2001), but harmful to the environment and human health through food chain. Acute oral toxicity (LD_{50}) of chlordane for rats ranges from 195 (～1950 early purified chlordane) to 590 mg/kg (1950～later purified chlordane)(Ingle 1965), while it is stable in the environment because it is not readily degraded by hydrolysis, oxidation, photolysis or microbial agents.

Early Chlordane (Octa-Klor or 1068) is more toxic to warm blooded animals than was Later Chlordane chiefly because the former contains a toxic unreacted intermediate, hexachlorocyclopentadiene which is negligible level in the latter (Ingle 1965).

The half-life of chlordane in water is comprehensively short, and for example, α-chlordane usually persists less than18 hours, whereas in soil some isomers remain stable for over 10 years (Eisler 1990). Another data indicate that the half-life of chlordane is estimated at 350 days (ranging from 37 to 3,500 days) (NPTN 2001). These figures meet definition of POP (ISSCPOPs 2001).Immobility or very low mobility of chlordane in soil is not likely to support the detection of the chemical in groundwater or terrestrial water. Contrary to our inference, the results of field survey demonstrated that chlordane and other organochlorines (chlorinated hydrocarbons) are detectable in samples from water, sediment and aquatic animals. Recent survey in two freshwater lakes in Vietnam revealed that although the amount of 13 chemicals analyzed varied with test sites, sampling seasons (dry or rainy season) and sample origins (water or sediment), some was recovered from almost every sample (Viet 2002). There are many studies which support bioaccumulation of chlordane and other cyclodienes and finally affect human health through food chain (*e.g.* Quinsey e*t al.* 1995; Kannnan *et al.* 1994).

As briefly reviewed, environmental and health risk is the key issue for chlordane to disappear from termiticidal market. This is quite common to other cases such as chromated copper arsenate (CCA). An increased public concerns about the use of unsafe toxic chemicals which had been instigated by mass media definitely accelerated the pace of banning chlordane.

Conclusions

The history of the use of termiticides clearly suggests that a new generation of termite control/management should be studied with awareness of environmental soundness and human health risk (see Table 2) by less or no chemical use. Early and conventional termiticides generally have repellent property and termites can not gain access to wooden structures beyond treated soil zone. This is desirable for effective prevention, while a remedial control with these termiticides does not always afford structures with a long-term immunity against termites. Because it is quite possible that termite activity under and around structures is partly suppressed by chemical soil-poisoning, when a target colony is bigger than our estimate.

Development of bait system seems to well meet the requirement. The baiting application is composed of monitoring devices and bait toxicants which are expected to interrupt physical functions of termites (*e.g.* chitin synthesis inhibition). Trophallaxis among colony mates is thought to play an important role in application efficiency. Another potential approaches are likely to derive from nonrepellency of newly introduced termiticides (Tsunoda 2005). When termites unconsciously pass the soil treated with nonrepellent termiticides, they take some active ingredient(s) [toxic materials(s)] and a tiny amount of toxic materials tend to adhere to the surface of termite bodies at the same time. Exposed termites transfer a part of these combined amounts to their colony mates before they dies (*e.g.* Tsunoda 2006; Tsunoda & Yamaoka 2007), and a whole colony will be eliminated as similar to bait application.

The Pacific Rim Termite Research is a forum to facilitate the exchange of information regarding:

(1) Development of termite management – alternative to existing termiticides and new concept/ approach.
(2) The risk and safety of the new termite management technology, including economic and environmental impacts.
(3) Sharing experience and knowledge among countries and relevant scientists.

We have to realize the significance of international cooperation to complete research subjects until everybody is pleased to share the obtained results for contribution to local/regional/global development and prosperity, because termite management has been becoming a global environmental concern in recent years.

Table 2 Comparison of acute toxicity of a few selected termiticides

Termiticide name	Oral LD_{50} (mg/kg)	Dermal LD_{50} (mg/kg)	Inhalation LC_{50} (mg/L)
Chlorinated hydrocarbon-chlordane*[1]	150-700 (rats)	590-840 (rats)	0.56->200 (rats 4-hr exposure)
Organophosphate-chlorpyrifos*[2]	135 (rats ♀) 163 (rat ♂)	202 (rats)	*[7]
Synthetic pyrethroid-bifenthrin*[3]	53.4 (rats)	>2,000 (rabbits)	*[7]
Carbamate-fenobucarb*[4]	425 (rats ♀) 524 (rat ♂)	>5,000 (rats)	>2.5 (rats 4-hr exposure)
Neonicotinoide-imidacloprid*[4]	410 (rats ♀) 440 (rats ♂)	>2,000 (rats)	0.0053 (rats 4- hr exposure)
Benzoylphenylurea compound-noviflumuron*[5]	>5,000 (rats)	>5,000 (rabbits)	5.24 (rats)
Phenylpyrazole compound-fipronil*[6]	97 (rats)	354 (rabbits) >2,000 (rats)	0.39 (rats)
Phenylpyrrolle compound-chlorfenapyr*[4]	461 (rats ♀) 304 (rats ♂)	>2,000 (rabbits)	>2.7 (rats ♀) 0.83 (rats ♂)

*[1] NPTN 2001, *[2] ATSDR 1997, *[3] EXTOXNET 1995, *[4] JWPA 2005, *[5] CDPR 2003,
*[6] USEPA OPPTS 1996
*[7] No information available due to low vapor pressure of chlorpyrifos at room temperature

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Phylogenetic Relationships of Some *Reticulitermes* Species (Isoptera: Rhinotermitidae) in China

by
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Abstract

DNA sequence comparisons of the mitochondrial COII genes were used to infer phylogenetic relationships among the four morphologically similar *Reticulitermes* species (*R. flaviceps*, *R. affinis*, *R. fukienensis* and *R..guangzhouensis*). Pairwise Tajima-Nei distances range from 0.14% between *R. guangzhouensis* and *R. fukienensis* to 3.49% between *R. affinis* and *R. fukienensis*. The phylogenetic trees were constructed by the N-J method. The combined results suggest several phylogenetic relationships including: (i) *R. fukienensis* and *R. guangzhouensis* are possibly conspecific; (ii) *R. flaviceps* and *R.. guangzhouensis* are not synonymous but related species; This finding suggests a combination of molecular and morphological approaches are necessary for accurate species identification.

Key words: *Reticulitermes* termites; mtDNA; COII; Phylogenetics; Molecular systematics

Introduction

There are over 2600 described termite species in the world (Kambhampati and Eggleton, 2000). They are found naturally in a wide range of terrestrial environments, particularly in the tropics and subtropics where they are, in many cases, the dominant invertebrate decomposers. However, in urban environments, they are important destructive pests. The damage caused by termites to structures and buildings accounts for greater than US$20 billion annually worldwide (Su, 2002).

The *Reticulitermes* comprises 111 species and is by far the largest Chinese genus with a wide distribution in China. Some species of *Reticuliterme* are the most important economic group of termites in China and cause damage to timbers in buildings, to synthetic materials, to underground cables (Zhong et al, 2000).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 collect, and we have but only the soldiers (not numerous) and some workers in the majority of specimen sets. It is especially difficult for accurate species identification because this genus lacks of discrete morphological characters. For this reason, the taxonomy of *Reticulitermes* species in China sometimes remains obscure. Cuticular hydrocarbon (CHC) profiles have been used for taxonomic purposes (Bagneres et al, 1990; Haverty et al, 1996; Collins et al, 1997; Haverty and Nelson, 1997).However, there is also evidence indicating that changes in CHC composition can be influenced by environmental conditions such as food and habitat (Collins et al, 1997; Woodrow et al, 2000) . More recently, molecular approach has been widely used in taxonomy studies (Jenkins et al, 2001; Austin et al, 2002). Molecular phylogenetic studies are able to reveal relationship among the populations and differentiate species regardless of caste. The analysis of the genus, *Reticulitermes*, is mainly based on the mitochondrial genes (COI,COII,COIII,16SrDNA, 12SrDNA and ND5) and nuclear gene ITSII. In this study, we used COII mitochondrial gene to illuminate the relationships among the four *Reticulitermes* species(*R. flaviceps*, *R. affinis*, *R. fukienensis* and *R. guangzhouensis*) in China.

Materials and methods

Termites were collected from Guangdong and Zhejiang Province (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 ethidium bromide. 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 imported into Mega4 to construct phylogenetic trees based on neighbour joining analysis with Kimuura-2-parameter model (transition/transversion 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	Species Name	Collecting sites
01R.gz	*R. guangzhouensis*	Guangdong entomological insitute,Guangzhou
02 R.gz	*R. guangzhouensis*	Guangdong entomological insitute,Guangzhou
03 R.gz	*R. guangzhouensis*	Guangdong entomological insitute,Guangzhou
04 R.gz	*R. guangzhouensis*	Guangdong entomological insitute,Guangzhou
05 R.gz	*R. guangzhouensis*	Guangdong entomological insitute,Guangzhou
06 R.gz	*R. guangzhouensis*	Baiyun Mountain ,Guangzhou
07 R.gz	*R. guangzhouensis*	Baiyun Mountain ,Guangzhou
08 R.gz	*R. guangzhouensis*	Baiyun Mountain ,Guangzhou
09 R.gz	*R. guangzhouensis*	Baiyun Mountain ,Guangzhou
10 R.gz	*R. guangzhouensis*	Baiyun Mountain ,Guangzhou
11 R.gz	*R. guangzhouensis*	Heyuan ,Guangdong province
12 R.gz	*R. guangzhouensis*	Heyuan Guangdong province
13 R.gz	*R. guangzhouensis*	Heyuan, Guangdong province
14 R.f	*R. flaviceps*	Guangdong entomological insitute,Guangzhou
15 R.f	*R. flaviceps*	Guangdong entomological insitute,Guangzhou
16 R.f	*R. flaviceps*	Longdong, Guangzhou
17 R.f	*R. flaviceps*	Longdong, Guangzhou
18 R.f	*R. flaviceps*	Longdong, Guangzhou
19 R.F	*R. fukienensis*	Hangzhou,Zhejiang province
20 R.a	*R. affinis*	Shaoguan,,North of Guangdong

Results and discussion

Average amplicon size resulting from DNA sequencing was about 760 base pairs (bp). To make sure of the true results, 65 bp from the two ends of the amplicon was excluded, and the remaining 695-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-0.23,G-0.14, and T-0.24 by Arlequin311. The data set contains 29 polymorphics, including one transversions and 28 transitions. Pairwise Tajima-Nei distances (Tajima and Nei,1984) range from 0.14% between *R..guangzhouensis* and *R.. fukienensis* to 3.49% between *R. affinis* and *R.. fukienensis*.

In the Neighbor-joining tree , three distinct clades were obtained (see Fig 1.) , *R. affinis* was in a single clade ,whereas the *R. fukienensis* and *R. guangzhouensis* formed a monophyletic group, supported with bootstrap values of 100%. The results suggest the phylogenetic relationships: (i) *R. fukienensis* and *R. guangzhouensis* are possibly conspecific; (ii) *R. flaviceps* and *R. guangzhouensis* are not synonymous but related species.

Since the morphological characters of four *Reticulitermes* species (*R. flaviceps*, *R. finis*, *R. fukienensis* and *R. uangzhouensis*) are closely similar, It is controversial whether this four

Reticulitermes species are synonymous or not. 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.

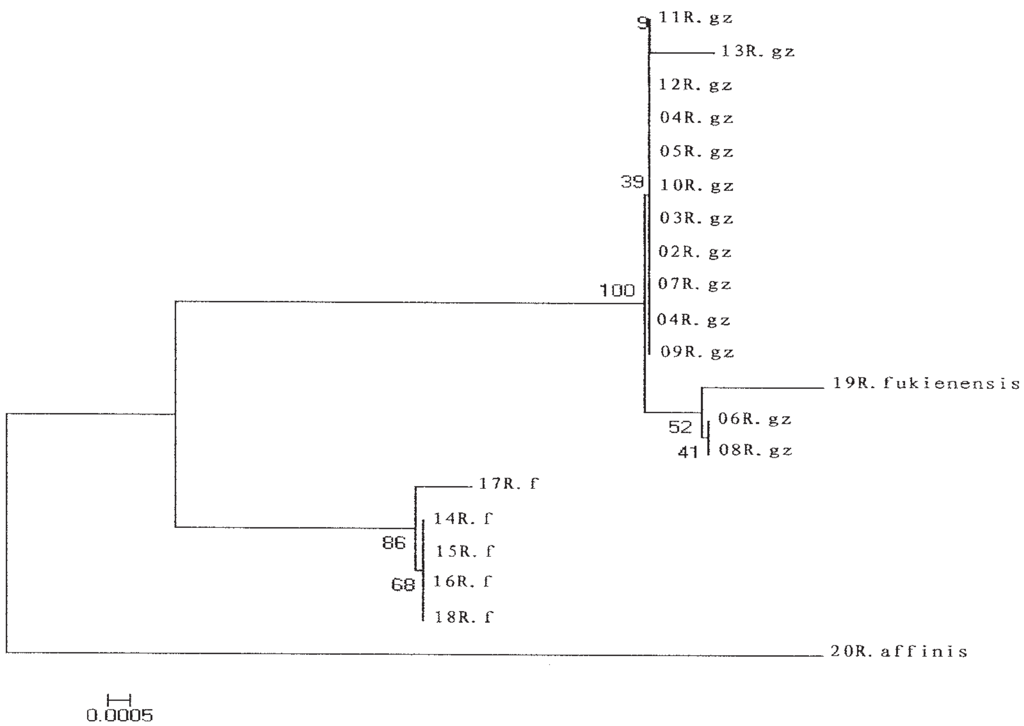

Fig.1.Neighbor-joining tree created with Mega4.

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Termite Rearing Using Mushroom Waste Medium

by
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Abstract

The distribution of ash, carbohydrate, dietary fiber, lipid, nitrogen and water was assayed in nymph and worker caste termites of *Reticulitermes speratus* (Kolbe). The percentage of total lipid versus dry weight of termites was 69.1 ± 0.7 in nymphs, and 61.2 ± 0.8 in workers. The nymphs and workers possessed oleic acid (~62 and ~69%), palmitic acid (~17 and ~11%), linoleic acid (~12 and ~8%), and stearic acid (~5 and ~8%) as major fatty acids, respectively. These results led us to the idea of termite rearing using mushroom waste medium for use in biodiesel fuel production. In this study, we attempted to feed worker caste termites of *R. speratus* on waste medium of *Hypsizigus marmoreus*, one of the most popular Japanese edible mushrooms. For an eight-week rearing period, egg-laying by neotenics developed from workers fed by a diet of waste medium of *H. marmoreus* was observed, whereas egg-laying did not occur by individuals fed by *Pinus densiflora* Sieb. et Zucc. as a control. The number of neotenics developed from workers fed by a diet of filter paper that included the water extract of waste medium of *H. marmoreus* was significantly larger than that fed by additive-free filter paper as a control.

Key words: termite rearing, mushroom waste medium, *Reticulitermes speratus*, *Hypsizigus marmoreus*, biodiesel fuel

Introduction

The amount of mushroom waste medium produced in Japan in 2004 was estimated at approximately 300,000 metric ton/year in dry weight (Matsumura *et al.*, 2006). Although a part of mushroom waste medium was reused as compost, most was dumped (Yoshida and Takahashi, 1994). Sawdust is used as one of substrates of medium, whereas most of sawdust remains intact in waste medium after the cultivation of mushrooms (Yoshida, 1994). In preliminary experiments rearing *Reticulitermes speratus* (Kolbe) and *Coptotermes formosanus* Shiraki using waste medium of *Hypsizigus marmoreus*, Bunashimeji in Japanese, as a diet of termites in our laboratory, it emerged that *R. speratus* individuals survived for an eight-week rearing period but that *C. formosanus* individuals died from the remaining fungus and/or growing mold on the waste medium. The results indicated that mushroom waste medium could be used as a diet for rearing of *R. speratus*. We determined nutritional value of nymph and worker caste termites of *R. speratus* and *C. formosanus* (Itakura *et al.*, 2006). The percentage of total lipid versus dry weight of termite was 64.3 ± 1.2 in nymphs and 46.5 ± 6.4 in workers of *C. formosanus*, in *R. speratus*, the percentage was 69.1 ± 0.7 in nymphs and 61.2 ± 0.8 in workers. Major constituent fatty acids were oleic ($C_{18:1}$) acid, palmitic ($C_{16:0}$), linoleic ($C_{18:2}$), and stearic ($C_{18:0}$) acids in nymphs and workers of both termite species. The composition of major fatty acids in termites was similar to that of olive oil (Table 1). These results led us to attempt to rear *R. speratus* using waste medium of *H. marmoreus* for a raw material to produce biodiesel fuel.

Materials and methods

Termites: *R. speratus* individuals were collected from a wild colony located in an infested wood in the Wakayama Prefecture, Japan, in April 2006, 2007 and were maintained in our laboratory at 26°C with their nest materials and with blocks of *Pinus densiflora* Sieb. et Zucc as the food source. Workers were collected from the colony maintained in the laboratory.

Waste medium of *H. marmoreus*: Waste medium of *H. marmoreus* was a gift from Hearts (Nagano, Japan). Medium to produce *H. marmoreus* was composed of sawdust of *Cryptomeria japonica* D. Don and *Pinus densiflora* Sieb. et Zucc. (≈15%), rice bran (≈8%), corncob meal (≈4%), milo grain (≈2%), additives (≈0.5%) and water (≈66%).

Determination of water extract, lignin, and monosaccharide composition of waste medium of *H. marmoreus*: A portion of oven-dried waste medium was weighed (W_1) and stirred in 100 ml of distilled water for 16 h. The waste medium residue was collected by filtration, oven-dried, weighed (W_2), and used for the determination of lignin and monosaccharides. The water extract (%) was calculated according to the following equation: $(W_1-W_2)/W_1 \times 100$. Klason lignin included in the waste medium was determined according to JIS (Japan Industrial Standard) P8008-1976, except that the hydrolysis with dilute sulfuric acid at the reflux temperature for 4 h was replaced by that at 120°C for 40 min in an autoclave. Monosaccharides included in the hydrolysate obtained during the assay of Klason lignin were determined according to the alditol acetate method modified by Misaki. Determination of alditol acetates derived from the monosaccharides was carried out using a GC-14A gas chromatograph (Shimadzu, Kyoto, Japan), with flame ionized detector (FID) and an injector at 200°C. The carrier gas was nitrogen (50 ml/min). A glass column with dimensions of 2.1 m x 3 mm id (Nihon Chromato Works, Tokyo, Japan) packed with ECNSS-M (GL Science, Tokyo, Japan) was used. The oven temperature was maintained at 190°C (Itakura *et al.*, 1995).

Termite feeding for determination of variations in constituents of waste media of *H. marmoreus* during rearing of *R. speratus*: Five round holes of 5 mm were made in the bottom of each cylindrical plastic container (80 mm in diameter x 75 mm in height). Dental plaster (25 g) with 26 % moisture content was uniformly spread on the bottom of each container and air-dried. About 6.5 g of compressed waste medium in the shape of cylinder (≈28 mm in diameter, ≈10 mm in height) was placed on the dental plaster in each of the containers. The containers were placed on dampened cotton, and two hundred workers were kept in each one at 26°C for eight weeks.

Termite feeding with waste medium of *H. marmoreus* for observation of egg-laying : A mixture of sand and water was poured into a polyplopylene container (Glad®, 130 mm x 90 mm x 65 mm in height, Clorox, NSW, Australia). About 6.5 g of compressed waste medium in the shape of cylinder (≈28 mm in diameter, ≈10 mm in height) was placed on sand at the center of a container. Five hundred workers were placed in a container. After each container was covered with a polyethylene sheet (Aisaika®, Nipro, Osaka, Japan) to keep high moisture, vented lid was placed on the container. Containers were kept at 26°C for eight weeks.

Solvent extraction of waste medium of *H. marmoreus*: A bottle of waste medium (≈500 ml) was poured into 3000 ml of Erlenmeyer flask. Waste medium was stirred in 50% ethanol, 50% ethanol at 60°C, ethanol, ethyl acetate, and chloroform for 20 h, sequentially. After removal of chloroform from waste medium, the dried up medium was stirred in distilled water at room temperature and 60°C for 20 h, respectively. Each solvent extract was concentrated using a rotary evaporator.

Termite feeding with each solvent extract: Two cylindrical containers (polyplopylene, 55 mm in diameter, 35 mm in height, Sansyo, Tokyo, Japan) were drilled a hole in and joined by a tygon tubing of 7 cm in length (2.4 mm id, 5.5 mm od, Saint-Gobain Performance Plastics Corporation, Akron, OH, USA). Sand was poured into each container. Distilled water was added to one of containers. A filter paper (70 mm, Advantec, Tokyo, Japan), to which each solvent extract was added to 0.05 or 0.5% [w/w] , was placed on sand of another container as food for termites. One-hundred fifty workers were placed in a container with a mixture of sand and water. After each container was covered with a polyethylene sheet (Aisaika®), vented lids were placed on both of the containers. Egg-laying and workers' development were observed 1, 2, 4, and 8 weeks by inverting both containers to remove all materials and termites from both containers. All experiments were carried out at 26°C.

Isolation of active components from concentrated water extract of waste medium: Three ml portions of the concentrated water extract were eluted by high-pressure liquid chromatography (HPLC) on a TSK gel ODS-80Ts column (21.5 x 300 mm) (Tosoh, Tokyo, Japan) at 40°C. Isocratic elution was carried out with 0.05% trifluoroacetic acid for 46 min at a flow rate of 3.0 ml/min. The absorbance at 254 nm of eluate was monitored. Elution was repeated 34 times. Each of four fractions was collected (Fig. 2) and lyophilized, respectively.

Identification of active components that could affect caste differentiation and/or egg-laying: Four lyophilized fractions were dissolved in 70 μl of deuterium oxide (Wako Pure Chemical Industries, Osaka, Japan), respectively. ^{1}H-NMR spectrum (ppm, *J* Hz) was obtained at

270 MHz using a JNM EX270 spectrometer (JEOL, Tokyo, Japan). Tandem mass spectrometry (MS) was carried out in the negative mode with a Q-TOF Premier (Waters, Milford, MA, USA). The capillary voltage was 3.1 kV and the corn voltage was maintained at 35 V. Each sample, the four fractions dissolved in deuterium oxide for ^{1}H-NMR, was subjected to MS by infusion at a flow rate of 1.0 μl/min. The collision-induced dissociation MS/MS analysis was conducted with argon as the collision gas and the collision energy was adjusted to 20-25 eV to optimize the sequence information for each sample. The mass range was *m/z* 50-1000. Ultraviolet (UV) spectrum was recorded from 220 to 380 nm using a UV-2400PC spectrophotometer (Shimadzu, Kyoto, Japan).

Results and discussion

Fatty-acid composition of total lipid extract of nymph and worker termites of *R. speratus*, together with that of olive oil, was shown in Table 1. It was evident that the composition of major fatty acids in *R. speratus* was similar to that of olive oil.

Table 1. Fatty-acid composition of total lipid extract of *R. speratus* (n = 2) (Itakura *et al.*, 2006)

Carbon number: double bonds	Fatty-acid distribution (g/100g of fatty acid)		
	Nymph[a]	Worker[b]	Olive oil[c]
$C_{14:0}$	1.5	1.9	-
$C_{16:0}$	16.9	10.6	12
$C_{16:1}$	1.8	1.5	-
$C_{18:0}$	5.2	8.1	2
$C_{18:1}$	62.3	68.8	72
$C_{18:2}$	12.0	8.4	8
$C_{18:3}$	0.03	0.05	1
$C_{20:0}$	0.3	0.7	-

[a] The percentage of total lipid versus dry weight of workers was 69.1 ± 0.7 (n = 3).
[b] The percentage of total lipid versus dry weight of nymphs was 61.2 ± 0.8 (n = 3).
[c] Fatty-acid distribution of olive oil (Shindo and Hirano, 1981).

As shown in Table 2, the hydrolysate of waste medium of *H. marmoreus* contained more than 50% of monosaccharides like arabinose, galactose, glucose, mannose, and xylose. Therefore, polysaccharide like cellulose and hemicellulose remained considerably in waste medium. Termites should feed waste medium and digest remaining polysaccharides. Indeed, workers of *R. speratus* survived on waste medium for 8 weeks. For eight-week rearing period, monosaccharides in the hydrolysate decreased from ≈52% to ≈34%, but water extract that contained mainly monosaccharides and disaccharides (surveyed by TLC, data not shown) and Klason lignin increased by ≈10%, respectively. In control waste medium placed on the dental plaster in a cylindrical container without termites in the same condition as rearing termites for 8 weeks, *H. marmoreus* that subsisted in waste medium caused decreases in monosaccharides in the hydrolysate like arabinose, galactose, mannose, and xylose by ≈7 to 38%, and increases in water extract and Klason lignin by ≈5%, whereas glucose remained at similar level. It seemed that surviving *H. marmoreus* digested mainly hemicellulose in waste medium.

The percentage of waste medium consumed by *R. speratus* termites for 8 weeks was 47.4 ± 4.9 (n = 5). Variations in Klason lignin, water extract, and monosaccharide were shown in Fig. 1, where constituents in waste medium recovered at 8 weeks were described versus the remaining mass of waste medium (52.6%). Obviously, termites digested polysaccharides in waste medium. Amount of glucose in the hydrolysate of waste medium was 32.8% (52.4% × 164.1 mg / 261.8 mg) at 0 week and 11.6% (17.8% × 109.8 mg /168.5 mg) at 8 weeks, respectively. Therefore, 35.4% of glucose remained in the hydrolysate of undigested waste medium, in other words, about two-thirds of glucose that composed cellulose and hemicellulose in waste medium was digested by *R. speratus* for 8 weeks. These results were not inconsistent with the presence of cellulases of termite origin and of protists origin as well as xylanases of termite and protists origin in *R. speratus* (Inoue *et al.*, 1997; Watanabe *et al.*, 1998).

Table 2. Variations in constituents of waste medium of *H. marmoreus* during rearing of *R. speratus* for 8 weeks (n = 5)

	Blank medium (0 week)	Residual medium[a] (8 weeks)	Control[b]
Water (%)	56.6 ± 1.4	-	-
	(vs. dry mass of medium)		
Water extract (%)	21.9	29.5	25.5
Klason lignin (%)	24.4 ± 1.1	35.1 ± 2.1	30.0 ± 0.1
Monosaccharide (%)	52.4	33.7	40.3
Arabinose (mg/500mg)	11.8 ± 1.1	5.6 ± 0.5	4.1 ± 0.2
Galactose (mg/500mg)	13.3 ± 2.1	10.7 ± 1.8	3.4 ± 0.1
Glucose (mg/500mg)	164.1 ± 10.9	109.8 ± 10.4	175.4 ± 16.1
Mannose (mg/500mg)	27.0 ± 1.9	21.9 ± 5.1	11.7 ± 0.6
Xylose (mg/500mg)	45.6 ± 3.4	20.5 ± 3.9	7.1 ± 0.5
Total of monosaccharides (mg/500mg)	261.8	168.5	201.7

[a] Waste medium, on which *R. speratus* fed for 8 weeks.
[b] Waste medium kept on dental plaster in the bottom of a cylindrical container in the same condition as rearing *R. speratus* termites for 8 weeks.

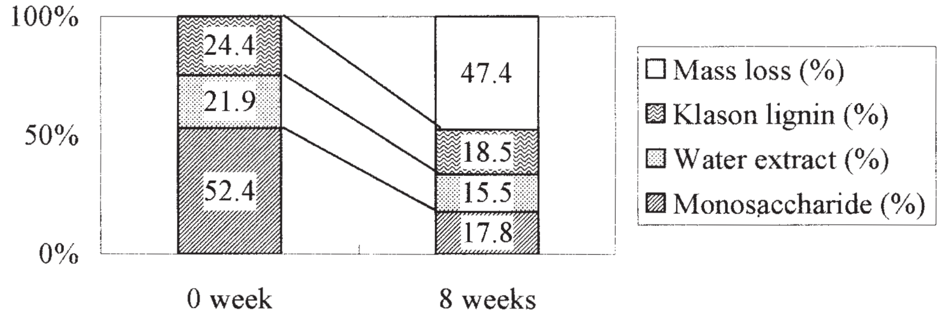

Fig. 1. Variations in constituents of waste media considering mass loss (47.4 ± 4.9 %) caused by termites' digestion for 8 weeks

Mostly hydrophilic compounds were extracted from waste medium as shown in Table 3. Waste medium was extracted with high yields by water (≈22%) and ethanol (≈10%). Mortality of *R. speratus* fed a filter paper with 0.05% of each solvent extract was in the range of 45 to 52% (Table 3), whereas that with 0.5% was in the range of 49 to 69% (data not shown). Egg-laying was observed in containers where termites fed filter paper with 0.05% of each solvent extract as well as control filter paper without solvent extract. Therefore, number of neotenics emerged from 150

Table 3. Number of neotenics emerged from 150 workers fed a filter paper with 0.05% of each extract and egg-laying by neotenics for 8 weeks

Solvent	Yield (%)	Worker	Neotenic	Mortality (%)	Containers involving eggs
50% Ethanol	4.5	70.8 ± 19.0	3.0 ± 0.3a,c	50.8	1/5
50% Ethanol (60°C)	6.0	74.2 ± 31.2	2.4 ± 0.5a,c	48.9	2/5
Ethanol	1.0	77.8 ± 14.9	2.8 ± 0.7a,c	46.4	2/5
Ethyl acetate	0.6	77.8 ± 19.2	3.3 ± 0.9a,c	45.9	3/5
Chloroform	0.1	77.3 ± 38.0	3.5 ± 1.0a,c	46.1	3/5
Water	19.4	68.7 ± 28.9	3.5 ± 0.3b,c	51.9	3/5
Water (60°C)	2.4	75.4 ± 28.4	4.2 ± 0.6b,c	47.2	2/5
Control	-	60.9 ± 10.9	1.3 ± 0.6a	59.1	1/5

Means followed by the same letter within each column are not significantly different from each other, $P > 0.05$ (n = 5).

workers was compared to evaluate effect of each solvent extract on caste differentiation of workers to ergatoids. The numbers of neotenics developed from workers that fed filter paper with 0.05% of water extract at room temperature (3.5 ± 0.3) and 60°C (4.2 ± 0.6) were significantly larger than that fed control filter paper (1.3 ± 0.6). To isolate and characterize the active components affecting caste differentiation, compounds included in water extract was separated by HPLC.

Water extract of waste medium was separated into four fractions as shown in Fig. 2. Each of four fractions was collected and lyophilized. From ≈100 ml of water extract, 3.3 mg of fraction 1, 2.1 mg of fraction 2, 2.0 mg of fraction 3, and 0.7 mg of fraction 4 was obtained, respectively, after lyophilization.

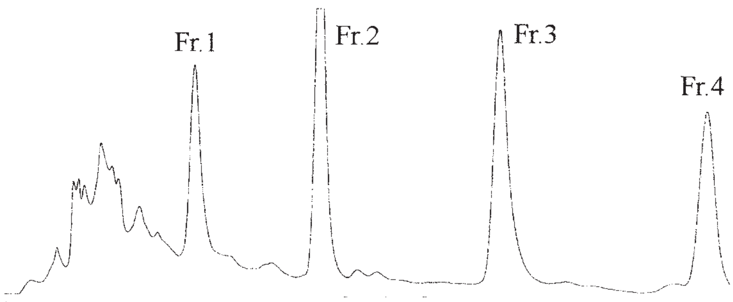

Fig. 2. HPLC of the water extract of waste medium of *H. marmoreus*

^{1}H-NMR spectra showed that 1-phenylpropene unit and saccharides could be included in fractions 1 and 2. Absorbance at 270 to 280 nm that originated from benzene nucleus was recorded in UV spectra of fractions 1, 2, 3, and 4. Molecular weight of 630 was deduced from TOF-MS of fraction 2.

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Chemical and Mechanical Properties of Termite Mandibles

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Abstract

Most insects, including termites, have tough mandibles for biting and carrying their food and fighting against their enemies. In order to clarify the chemical and mechanical properties of termite mandibles, elemental analyses and evaluation of compressive strength were examined in this study. Termite mandibles were found to be composed of highly oriented □-chitin fibrils, which oriented perpendicular to the cutting edge. The larger the surface of the mandible, the larger the compressive strength is, with the exception for two kalotermitids, *Incisitermes minor* and *Cryptotermes domesticus*. By elemental analyses using micro-beam scanning PIXE, only kalotermitids had Zn as a predominant metal on their mandibles, especially in the cutting edges, although all termites had Mn on the pigmentation area on the mandibles.

Key words: termite, mandible, elemental accumulation, chitin fibril, mechanical property

Introduction

Termites live in soil or trees, and eat dead wood, fallen leaves and branches, but some species chew holes in wood and non-wood materials such as concrete, tiles, and plastics by using their mandibles. Termites use their mandibles for the following purposes: (1) biting and swallowing food (2) fighting against enemies (3) carrying soil/sand particles for making nests (4) carrying eggs and younger larvae and so forth. They control the force and speed of the mandible movement by an instant recognition of the targets. Therefore, the elucidation of the mutual relation among the various behaviors, the force of biting and the properties of mandibles is expected to be useful in establishing novel methods for protection of building materials and structures.

Similar to termite mandibles, various organic-inorganic tissues are known in other animals, for example, (sea) shells, stingers, teeth, nails and bones, which support and protect their bodies. Recently, the functions of such unique biomineralized tissues have gathered interest, and the process and formation is gradually being elucidated (Stepen, 2001).

We had already revealed that a species-dependent existence of special elements, *i.e.* Mn was a common element through termite species and that only kalotermitids had Zn (Ohmura et al., 2007a,b).

In this study, we investigate the chemical and mechanical properties of termite mandibles, and discuss the contribution of metal accumulation to these properties.

Materials and methods

Insects

Five species of wood feeding termites were used: *Zootermopsis nevadensis* (Hagen), *Cryptotermes domesticus* (Haviland), *Incisitermes minor* (Hagen), *Coptotermes formosanus* Shirki, *Reticulitermes speratus* (Kolbe). A longicorn beetle *Monochamus alternatus* and a predator *Trogossita japonica* were also used for comparison. Matured worker and adult mandibles were dissected from their heads by using a pair of fine forceps and subjected to several analyses as mentioned below.

Observation of chitin fibrils in mandibles

Mandibles of matured workers of *C. formosanus*, *R. speratus*, *I. minor*, and *C. domesticus* were dipped in 5% KOH solution for overnight at room temperature, and then transferred into mixed solution of 1.7% NaOH: 7.5% CH_3COOH: distilled water = 1 : 1 : 3 (V/V) for 5 h at 70°C for protein removal. Upon washing with distilled water, the samples were sonicated for 10 min in 0.2 ml distilled water. After drying at room temperature, the samples were observed under a digital microscope (VHX-900, Keyence, Japan) and a scanning electron microscope (SEM) (JSM-5310, JEOL, Japan). For the SEM observation, a mandible was placed on a transparent plastic tape, compressed by a stainless steel hammer, and was gold-coated for the observation.

Structural analysis of chitin by electron diffractiometry

Mandibles of matured workers and soldiers of *C. formosanus* were removed and treated by the same method described above. After the sonication, a drop of the aliquot was pipetted onto the carbon membrane on the 200-mesh grid. After drying at room temperature, the samples were observed under transmission electron microscopy (TEM) and the electron diffraction analysis (JEM-2000EX, JEOL, Japan).

Compression test

Each dissected mandible was epoxy- or paraffin-embedded, and was compressed using a horizontal material testing machine (Takachiho-Seiki, Japan) with a load of 50 kgf at 0.1 cm/min. During the measurement of the strains, the processes of their compressive transformations were monitoring with a CCD-camera (Nikon, Japan) and were recorded on the DVD-video system.

Micro-beam scanning PIXE analysis

PIXE measurements were performed in the Particle Analysis System at Tandetron Accelerator facility (PASTA) at National Institute of Radiological Sciences (NIRS) using a micro-beam scanning PIXE system. Each mandible was fixed onto a thin film to obtain a geometrical image of the samples by STIM, a complementary method of micro-beam scanning PIXE along with the elemental distribution in the sample (Imaseki et al., 2003). All samples were set in a vacuum chamber 22 mm apart from a Si-Li detector (GRESHAM Airius80). The analysis was performed with a 2.6 MeV proton beam, which was adjusted to have a beam current of 50 pA. The accumulated charge produced in each sample was preset to be 40 nC, and monitored to ensure that all samples were irradiated to the same dose. Elemental map images of termites were achieved in 8-bit images along with the energy spectrum of X-ray emission from the elemental components. Arthropods' mandibles contain Zn, Mn, Fe, Cu and/or halogens (Hillerton and Vincent, 1982; Hillerton et al, 1984), with the accumulation of these metals acting to harden the cutting edges. Therefore, eleven elements were selected as target elements (Al, Si, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn).

Results

Chitin fibrils in mandibles

Chitin fibrils in mandibles showed radial orientation from the basal area to the cutting edge. The same orientation was observed in all the termite species.

By the SEM observation, the multiple-layered structure of chitin was found in the mandible exoskeleton of *C. formosanus* (Fig.1). Each layer had 1-3 μm thickness, and the outer layers were generally thinner than those of the inners. Chitin microfibrils of less than 100 nm width were observed in the cracked areas (Fig. 2).

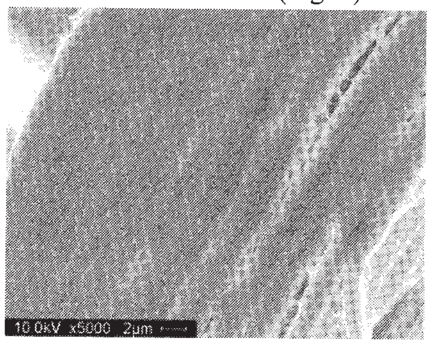


Fig. 1 A multiple-layered structure in the mandible exoskeleton of a worker of *C. formosanus*.

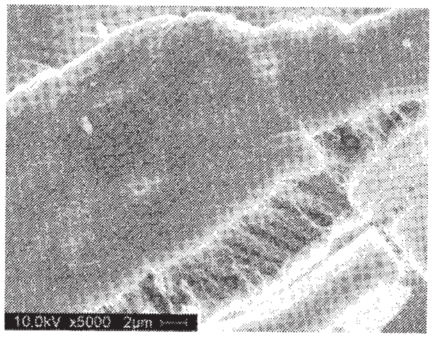


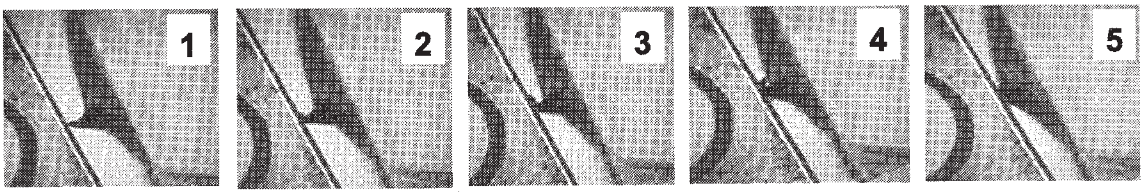
Fig. 2 Chitin microfibrils in the mandible exoskeleton of a worker of *C. formosanus*.

Structure analysis of chitin by electron diffractiometry

The same electron diffraction pattern was obtained from all the samples, showing that all chitin microfibrils had the highly oriented α-chitin structure.

Mandible strength

Insects generally have several teeth in the cutting edge of their mandibles. During compression, each tooth was broken and bucklinged with increasing the load. The compression process of *Z. nevadensis* and the relation between the load and displacement are shown in Fig. 3. The maximum load (Table 1) was measured when a tooth was just broken. The larger the surface area of mandibles, the larger the maximum load except for two kalotermitids, *I. minor* and *C. domesticus*.



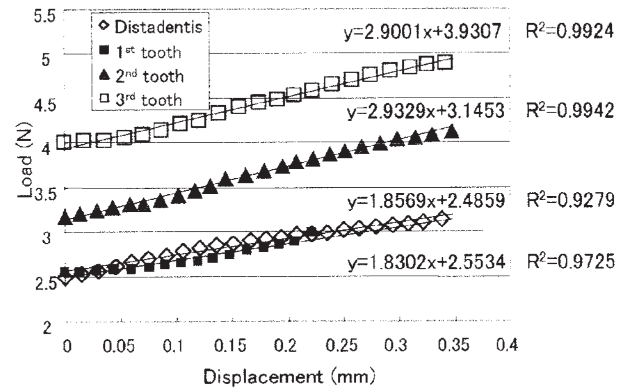


Fig.3 Compression process of the mandible of *Zootermopsis nevadensis* and the load-displacement relationships

Table 1 Maximum load and surface area of insect mandibles

Species Name	Maximum load (N)	Surface area (mm^2)
Zootermopsis nevadensis	2.0	0.84
Incisitermes minor	2.8	0.14
Cryptotermes domesticus	2.2	0.07
Coptotermes formosanus	1.8	0.12
Reticulitermes speratus	0.8	0.08
Monochamus alternatus	5.0	2.34
Trogossita japonica	2.2	0.89

Elemental contents and distribution

The differences in the element types and their distributions were observed with different termite species on 5 species of termite mandibles and two coleopterans through PIXE analyses. P, S, Cl, K and Ca were generally found, on the other hand, Mn distributed in the pigmentation area on the mandibles of all termites, and two coleopterans. Zn was detected in two kalotermitids, *C. domesticus* and *I. minor*, and a cadelle, *T. japonica* predominantly in their mandibles along with Cl (Table 2).

Table 2 Predominant elements detected on the mandibles

Species Name	Predominant elements
Zootermopsis nevadensis	Mn
Incisitermes minor	Zn,Mn,Cl
Cryptotermes domesticus	Zn,Mn,Cl
Coptotermes formosanus	Mn
Reticulitermes speratus	Mn
Monochamus alternatus	Mn
Trogossita japonica	Zn,Mn,Cl

Discussion

The perpendicular orientation of mandible chitin microfibrils to the cutting edge found in the present study is reasonable in resisting against the biting force when termites cut the wood fragments.

Two kalotermitids, called as dry-wood termites, *C. domesticus* and *I. minor*, showed the higher maximum loads than the other termites, which prefer wet wood as their food. This result may indicate that dry-wood termites have a special mechanism to enforce their mandibles.

The reason for metal accumulation was thought to add strength and toughness to the mandibles (Hillerton and Vincent, 1982; Hillerton et al, 1984; Schofield and Refevre, 1992). It was reported that there was a relationship between the Zn contents and the hardness of mandibles in a leaf-cutting ant, *Atta sexdens* (Edward et al, 1993; Schofield et al, 2002) and several termite species (Cribb et al, 2008). Our data also support the role of Zn on investing the hardness to the mandibles. Furthermore, the strength of *C. domesticus* mandible was larger than that of *T. japonica*, both of which have the same surface area and accumulate Zn, indicating the differences in mandible shapes and/or the chitin fibril orientations also reflected to the differences in the strength of mandibles. Despite the widely distribution of Mn in termite species and other insects, the meaning of Mn accumulation and its existence form are still unclear. Morgan et al. (2003) suggested that Mn might contribute to increase cuticle density and/or resistance to fracture. We have revealed that Mn distributed only on the pigmentation area (Yoshimura et al. 2005, Ohmura et al., 2007 a, b) suggesting that Mn might induce the pigmentation of mandibles.

Acknowledgements

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Preliminary Studies on Flight Activity of *Macrotermes gilvus* (Hagen) and *Macrotermes carbonarius* (Hagen) (Blattodea: Termitoidae: Termitidae)

by
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Abstract

Synchronised release of alates from parental colonies (swarming) is the main mechanism for outbreeding. Flight activity of subterranean termite is under the influences of extrinsic factors (season, specific climatic conditions for actual flights) and intrinsic factors (colony size, pheromone, nutrition, social facilitation and aggression of worker caste). Swarming activity of the subterranean, mound-building termites, *Macrotermes gilvus* (Hagen) and *Macrotermes carbonarius* (Hagen) was observed in Universiti Sains Malaysia, Minden Campus, Penang, Malaysia. Swarming activity differed significantly between both species and could be characterized by season, weather patterns, flight behaviours and sex-ratio. Alates of *M. gilvus* swarmed before dawn (0300 – 0430 hour) from April to September. However, alates of *M. carbonarius* dispersed from November, and then at dusk (1900 hour). The swarming of *M. gilvus* usually occurred after rain at a temperature of 25 ± 1°C and relative humidity of 96 ± 1 %. Notably, rain was not necessary in triggering the swarming of *M. carbanarius* and occurred at a temperature of 26 °C ± 1 °C and 77 % ± 1 % relative humidity. The limited results in this study indicated that the sex ratio of alates was female-skewed in *M. gilvus* and male-skewed in *M. carbonarius*.

Key words: extrinsic factors, intrinsic factors, swarming season, weather pattern, sex-ratio, flight behaviours.

Introduction

The fungus-growing genus *Macrotermes* is widely distributed in Africa, South and Southeast Asia. Of 45 species of *Macrotermes*, 11 species can be found in the Oriental region (Roonwal 1969). *Macrotermes gilvus* and *Macrotermes carbonarius* are common mound-building termites in Southeast Asia, notably in Malaysia, Singapore, Thailand, Indonesia and the Philippines.

Species of *Macrotermes* are serious pests of agricultural crops such as sugar cane which is planted on land formerly covered by rainforest, and in wooden structures (Roonwal 1969; Harris 1969; Cowie et al. 1989). The economic importance of *M. gilvus* has increased after the introduction of termite baiting against the dominant species of *Coptotermes*. *M. gilvus* has become a secondary pest in buildings and wooden structures once *Coptotermes are* suppressed or eliminated by baiting. *M. gilvus* can appear in buildings and structures several months after the *Coptotermes* infestation in those has been controlled. However, *Macrotermes* and other termitids do not respond well to baits (Lee et al. 2007).

The alate flight season varies depending on termite species and localities. In regions with cold winter and evenly distributed rainfall, the production of winged termite and flight activity are often restricted to warmer summer months, however, there are exception, e.g. for *Reticulitermes kanmoensis* that swarm during winter time (T. Yoshimura, personal communication) unlike in tropical regions where swarming coincides with the rainy season (Nutting 1969). Swarming can take place in one or over a few successive months. The flight season may vary from a period of a few days (*Odontotermes assmuthi*; Sen-Sarma 1962) to an entire year. Some termite species, e.g. *Macrotermes natalensis* (Ruelle 1964), *Odontotermes obesus* (Arora & Gilotra 1959), and *Porotermes adamsoni* (Hill 1942) show one flight per year but a number of successive swarms may happen in large colonies of *Reticulitermes flavipes* (Tang & Li 1959). Some species disperse either diurnally or nocturnally. These are just some examples of the diversity of termite flight behaviour.

The production of alates is under the influence of pheromones and nutrients. These are distributed by grooming and trophallaxis among members of a colony. Environmental conditions may affect the rate of alate production. Development of alates can be accelerated during winter in temperate zones or, in the wet or dry season in the tropics and at high humidity especially in rainy season (Brian 1965; Noirot 1961; Sands 1965b). Seasonal changes may have a lesser effect on alate production in Kalotermitids under some conditions; alates may be produced continuously throughout the year (Kalshoven 1930; Wilkinson 1962; Castle 1934).

Termite management focuses only on foraging workers. Alates, despite their potential to establish new incipient colonies yearly and start building mounds in months, are very difficult to manage let alone preventing their settlement and initial colony foundation. The flight behaviour of a variety of termite species has been studied extensively (Nutting 1969; Su & Scheffrahn 1987; Henderson & Delaplane 1994; Costa-Leonardo & Barsotti 1998). However, to date, only limited information is available on the flight activity of *M. gilvus* and *M. carbonarius*. We discuss in this paper the flight season, weather patterns at the time of swarming, flight behaviour and the alate sex-ratio of these two subterranean termites.

Materials and methods

Alate Swarming location: The study was conducted at the Universiti Sains Malaysia, Minden Campus, Penang, Malaysia (2°59'N and 102°18'E). A total of 29 sticky traps, measuring 28 cm x 19 cm were set up on fluorescent lights (Height: 3 m) (Phillips, Thailand) with 36 watt and light output 2600 lm in three localities. All traps were placed outdoors, usually along building veranda. Traps were checked daily. The collected alates were then identified using a stereo microscope Olympus SZ61 with IC Imaging Standard V2.1 (Olympus Co. Japan). Identification was based on the descriptions in Thapa (1981), Huang et al. (2000) and Tho (1992).

Field Observations: Swarming activity was observed at four colonies of *Macrotermes gilvus* in April 2007 and two colonies of *M. carbonarius* in November 2007. A routine field trip was held at selected areas. Once dispersal openings of alates were observed, new sticky traps were placed on fluorescent lights along a building veranda that is located close to the mounds (Distance: 9.0 – 41.5 m) prior to the nuptial flights.

Temperature and Humidity Data: Temperature, relative humidity, atmospheric pressure and precipitation data were obtained from the Malaysian Meteorological Department (MMD), Bayan Lepas station (5° 18'N and 100° 16'E, altitude 2.8 m), Penang, Malaysia. For the field observations, temperature and wind speed were recorded with a Thermo-Anemometer (AZ Instrument R.O.C, Taiwan) and relative humidity was checked with a Whirling Psychrometer (G.H.ZEAL LTD, London, England) hourly.

Results and discussions

Flight season: Flights of *M. gilvus* started to occur from April and lasted until September 2007 (Table 1). The flight was at the peak in the middle of June. We also observed some sporadic flights in July and recorded another peak in the middle of August. Swarming of *M. carbonarius* began much later than in *M. gilvus*, namely in November.

Time and duration of flight: Flight time appears to be correlated as a rule with specific meteorological conditions (Nutting 1969). In the *M gilvus* mounds, the dispersal holes could be observed as early as 2030 hour especially after rain. They might be stimulated by the mechanical disturbance of raindrops on the mound wall or due to the soaking of the soil (Ruelle 1964; Harris 1958). Swarming typically occurred between 0300 to 0430 hour before dawn and lasted for one hour to two and half hours. This timing of alate release might be a strategy to reduce exposure to predation. In the US, *Coptotermes formosanus* started to swarm close to 2030 and ended by 2130 hour (Henderson & Delaplane 1994), while in Japan, this species swarmed at dawn (T. Yoshimura, personal communication). The swarming of *M. carbonarius* occurred at dusk and lasted only for 4 to 10 minutes between 1900 to 1910 hour. The flight holes could be observed in the afternoon and only on days without rain. The swarming pattern of *M. carbonarius* showed a close similarity to that of *Coptotermes gestroi* (*havilandi*) in Brazil (Costa-

Leonardo & Barsotti 1998).

Table 1. Comparison of flight activity of *M. gilvus* and *M. carbonarius*

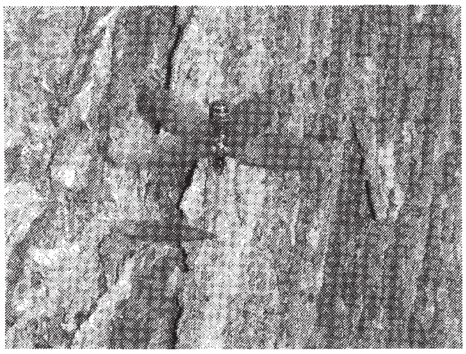
	M. gilvus	*M. carbonarius*
Flight season	April - September	November
Time of swarming	0300 – 0430 hour	1900 – 1910 hour
Duration of swarming	1 hour – 2.5 hours	4 min – 10 min
Humidity (%)	96 ± 1%	77 ± 1%
Temperature (°C)	25 ± 1°C	26 ± 1°C
Environmental condition	Swarmed after rain	Avoidance of rain
Sex ratio (F:M) (n)	(a) 2.53 : 1 (n = 39) (b) 3.62 : 1 (n = 1054) (c) 6 : 1 (n = 70) (d) 0.88 : 1 (n = 312)	(a) 1 : 1.67 (n = 192) (b) N.A

Climatic condition: Temperature and relative humidity appear to play an important role in determining the daily flight period. *M. gilvus* swarmed at a temperature of 25 ± 1 °C and relative humidity of no lower than 96 ± 1%. Rain was necessary to trigger swarming. In contrast, *M. carbonarius* appeared to be more tolerant of low levels of relative humidity. The swarming occurred typically at 77 ± 1%, and a temperature of 26 ± 1°C. This species avoided rain. In *Odontotermes formosanus*, swarming was observed in the early evening after rain with a relative humidity of more than 80% and temperature of at least 25°C (Hu et al. 2007). Temperature, humidity, rainfall, light intensity, wind velocity, atmospheric pressure and amount of electrical charging of air particles should be taken into account in determining a colony's flight. However, Martius (2003) reported that the swarming of alates was not significantly correlated to relative humidity, rainfall, evaporation and barometric pressure. Swarming events remained unpredictable as no obvious climatological differences between days with or without flights could be noted.

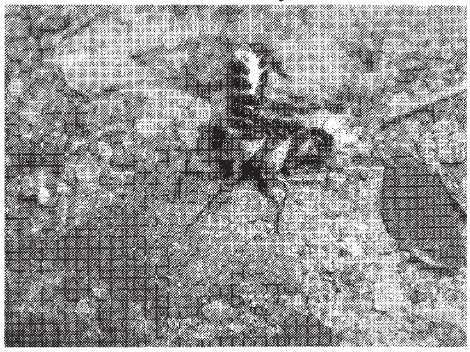
Behaviour of flight: Basically, both species of *Macrotermes* had similar flight behaviour. Swarming activities can be divided into three stages: (1). Pre-flight activity (protective behaviour of soldiers) (2). The peak of flight (emergence of alates from the mound) (3). Post-flight activity (de-alation, calling behaviour and tandem running). Females of *M. gilvus* usually landed on the bark of trees (Height: 0.5 – 1.5 m) and "called" males. Such behaviour was not exhibited by females of *M. carbonarius*. Females of *M. natalensis* and *Odontotermes badius* called from certain grasses and other plants several inches above the ground (Fuller 1915). Females of *M. gilvus* extended their wings and distended segments of their abdomen when in calling position (Plate 1 [A]). In contrast, *M. carbonarius* females attracted males by distending segments of their abdomen after shedding their wings (Plate 1 [B]). Calling behaviour was not present in *C. gestroi* (*havilandi*) (Wilkinson 1962; Costa-Leonardo & Barsotti 1998).

Alate sex-ratio: The sex-ratio in *M. carbonarius* was male-skewed. However, this finding must be treated with caution because it was only based on one colony. The sex ratio of collected alate populations of *M. gilvus* deviated significantly from the expected male-bias. A female-skewed sex-ratio was observed during most flights except in one colony which had a relatively balanced sex-ratio (Table

1). This result differs from those reported for other termite species (*Coptotermes formosanus*; Su & Scheffrahn 1987; *Nasutitermes corniger*; Thorne 1983). Thorne (1983) argues that male progeny is an "economy progeny" compared to female progeny since males have a smaller body size and lower weight.



(A)



(B)

Plate. 1. Positions of calling behaviour in *M. gilvus* (A) and *M carbonarius* (B)

Predators: During swarming or subsequent behaviour while alates were still in the open, alates of both species were attacked by predators, for example geckos (Gekkonidae), frogs, birds, ants (*Anoplolepis gracilipes*, *Tapinoma sp.*, *Monomorium sp.*, *Oecophylla smaragdina*, *Paratrechina longicornis*, etc.). When attacked by ants, the termite alates ran swiftly away to avoid getting attacked. The female alates positioned themselves again soon after such ant attacks and recalled male alates when those had lost their way after the attacks. Simulation of death (Nutting 1969) was not performed in *M. gilvus* and *M. carbonarius*.

Conclusions

This study provides information on the flight activity of *M. gilvus* and *M. carbonarius* in relation to environmental conditions and describes their flight behaviour. *M. gilvus* prefers to swarm in humid and dark condition while *M. carbonarius* swarms under drier conditions and in the twilight. In this study, population the sex ratios of alates were skewed, in favour of females in *M. gilvus* and in favour of males in the case of *M. carbonarius* but further studies are required to substantiate current findings.

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Dispersal Distances of Alates *Odontotermes formosanus* (Isoptera: Termitidae)

by
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Abstract

Alates of the bllack-winged subterranean termite, *Odontotermes formosanus* (Shiraki) were marked with paint and checked with fluorescence microscopy. The maximum horizontal flight distances for the test 3 colonies were 319 m, 1070 m and 888 m, while the maximum vertical flight distances were 21 m, 64 m and 14 m, and the dispersal territories were 0.143 km^2, 0.422 km^2 and 0.159 km^2. These results could help to determine area-wide management strategy against *O. formosanus*.

Key words: Dispersal distances, *Odontotermes formosanus*, area-wide management

Introduction

The black-winged subterranean termite, *Odontotermes formosanus* (Shiraki), is the most economically important dike-and-dam pest species in Southern China (Cai et al., 1965; Huang et al., 2000). It also attacks living plants and is a serious pest of crops. Although baiting or soil insecticide treatment was available for the control of *O. formosanus*, area-wide management of this species was lack of theoretical basis of termite biological information. Understanding the behavior of termite alate flight can provide an insight into the dispersal capabilities of the species as well as demonstrates possible niches that may be exploited by individual groups among native subterranean species (Haverty et al., 2003).

In the past tens of years, foraging territories of many termite species have been estimated to better understand its impact and to determine the effectiveness of control tactics (Crosland and Su, 2006; Evans et al., 1999; Lee et al., 2003; Tsunoda et al., 1999). However, little information is available concerning the magnitude of the other important aspect of termite behavior information, i.e. flight territories of alates.

Swarm or flying is a special behavior of social insects and it in response to weather condition of hot, stuffy and big rainfall, usually over two month period, May and June, during in the early summer (Li et al., 2004; Liu et al., 1998). Adult termite alates were known as weak dispersal insects (Pan, 1999; Shi et al., 1987) but movement patterns and dispersal distance have not been well documented, although preliminary work suggests that alates of Formosan subterranean termite, *Coptotermes formosanus* Shiraki, were capable of flying a horizontal flight distance of 100 m (Higa and Tamashiro, 1983), 460 m (Ikehara, 1966) and as far as 892 m (Messenger and Mullins, 2005), and a vertical flight distance of 40 m (Su et al., 1989). Shelton et al. (2006) estimated that the maximum distance flown by alates of the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar) was 458.3 m, which test by flight mills in the laboratory. *O. hainanensis* alates was reported to fly 50-400 m and move a height of 10-30 m (Shi et al., 1987).

It has been estimated that *O. formosanus* alates perhaps travel 100-600 m and the height between several meters to tens of meters from the colony (Li, 2002; Pan, 1999). However, many of dispersal studies were carried out by naked eye observation (He and Wang, 1986; Liu et al., 1998; Pan, 1999; Shi et al., 1987), possibly limiting the reliability of the results. An earlier study Hu et al. (2007) presented a feasible method, fluorescent spray paint/fluorescence microscopy technique, to mark termite alates, and was the basis for the follow-up study. The current study investigated dispersal distance of alates *O. formosanus* and discussed the factors which affected the distances. We used an improved mark-recapture method, in which termite wings were marked with fluorescent spray paints and checked with fluorescence microscopy technique. A similar approach was employed by Forschler (1994) to study foraging territories of termite *Reticulitermes Virginicus* (Banks).

Materials and methods

Colony A, B and C were selected on the test field at Tropic Forest Institute (23°11'N, 113°23'E), Guangzhou, China. Colony A swarmed on May 4, 2007. Weather conditions were recorded with a hand-held weather meter (AZ Co., Taipei, Taiwan): Temperature 21.7 °C, relative humidity 94 %, SE wind with a velocity of 0.2 m/s, air pressure 1.0053×10^5 Pa and rainfall of the day was 16.8 mm. Red fluorescent spray paint (Baocili Color Co., Guangzhou, China) was chosen as the marker for the alates of colony A.

Colony B and C swarmed on May 26, 2007. Temperature 21.7 °C, relative humidity 92 %, SE wind with a velocity of 0.7 m/s, air pressure 1.0029×10^5 Pa and rainfall of the day was 29.7 mm. Green and Red fluorescent spray paint were separately chosen as the marker for the alates of colony B and colony C.

The spray paint can was held 60 cm away from and 30 cm above the alates. Spray nozzle was depressed when they emerged from the exit holes of swarming and allowed the paint to follow a downwind, so that the aerosol of the spray paint would drift down onto the wings. The spray nozzle was pressed 3 times for 1 sec each time at each exit holes of swarming (Forschler, 1994). Shed wings were collected under lights of buildings in the vicinity of the test colony the next day. We recorded locations where the wings were collected with GPS Map 76 (Garming, Taipei, Taiwan) and used the GPS's average location function. Wings were collected within an 1×1 m square area at each GPS recording point, and were dried in an air-conditioned room for 48 hours (27°C, dehumidified). Wings were checked for fluorescent marks under a Zeiss Axioplan 2 fluorescence microscope under UV illumination (Zeiss, Jena, Germany).

Results and discussion

Dispersal distances and dispersal territories for each colony are present in Fig. 1-Fig. 3. The maximum horizontal dispersal distances of the three colonies were 319 m (from exit holes of swarming to light trap point NO. 28, Fig. 1), 1070 m (from exit holes of swarming to light trap point NO. 24, Fig. 2) and 888 m (from exit holes of swarming to light trap point NO. 8, Fig. 3), and the maximum vertical dispersal distances were 21 m (from exit holes of swarming to light trap point NO. 38, Fig. 1), 64 m (from exit holes of swarming to light trap point NO. 26, Fig. 2) and 14 m (from exit holes of swarming to light trap point NO. 26, Fig. 3), and the dispersal territories were 0.143 km^2, 0.422 km^2 and 0.159 km^2 (Fig. 1-Fig. 3, Table 1).

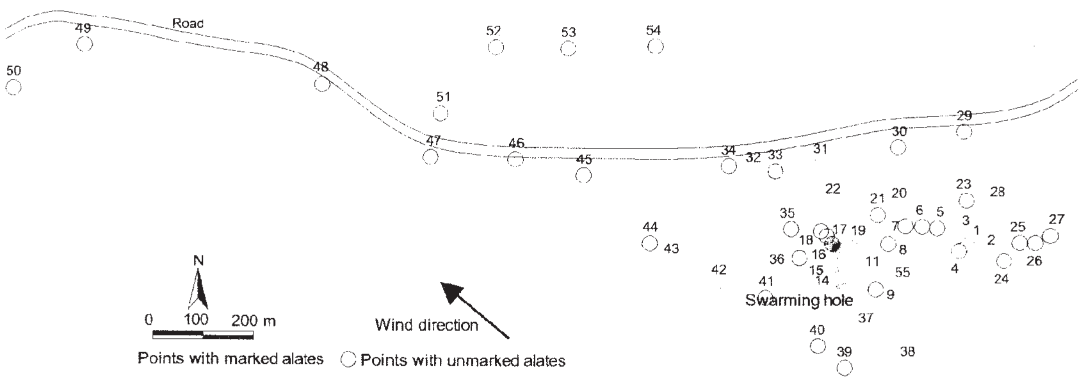

Fig. 1. Horizontal dispersal distance and territory of *Odontotermes formosanus* alates (colony A). The maximum horizontal dispersal distance of colony A: 319 m

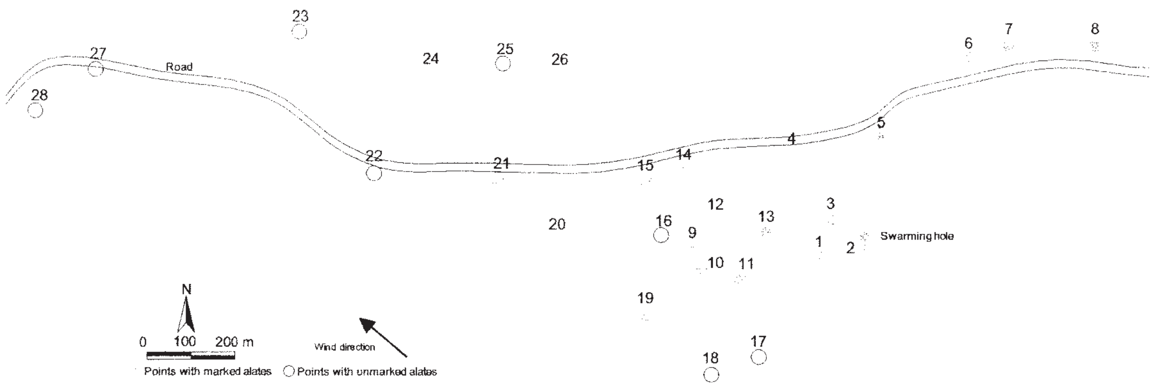

Fig. 2. Horizontal dispersal distance and territory of *Odontotermes formosanus* alates (colony B). The maximum horizontal dispersal distance of colony B: 1070 m

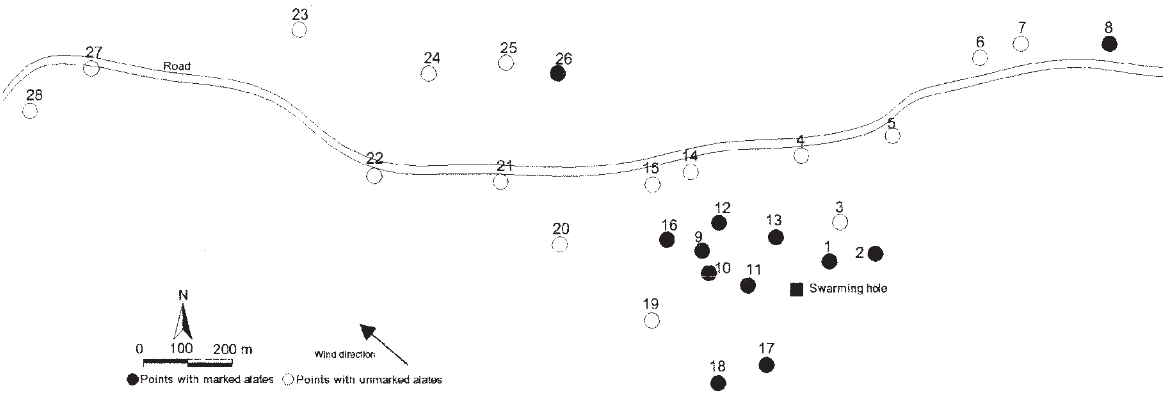

Fig. 3. Horizontal dispersal distance and territory of *Odontotermes formosanus* alates (colony C). The maximum horizontal dispersal distance of colony C: 888 m

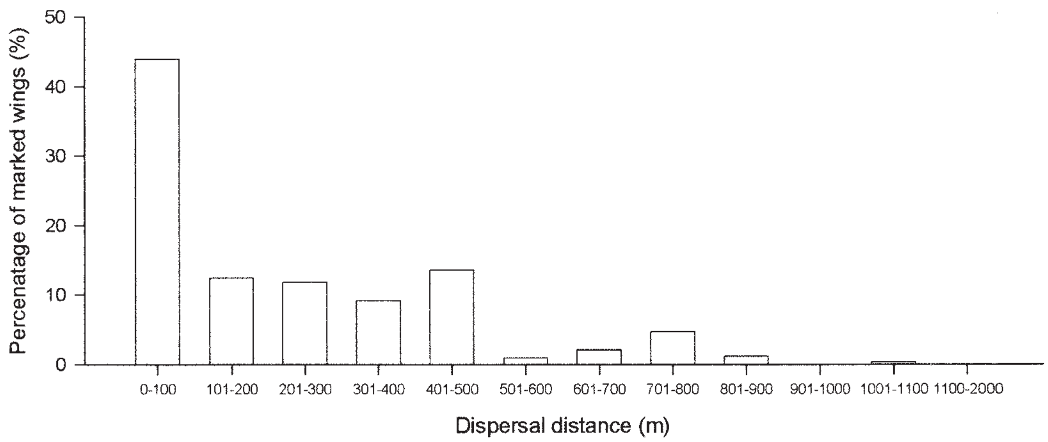

Fig. 4. The percentage of *Odontotermes formosanus* alates wings at different horizontal dispersal distance dispersal distance.

The results of the experiment indicated that *O. formosanus* alates were capable of flying 1070 m. In Southern China, some important dikes and dams were required to be no termite infested. In thus, how large of the region where exterminate all colonies around the dikes and dams depends on the maximum dispersal distance of alates. And there was often a debate about the magnitude of the area. We suggest that *O. formosanus* colonies should be treated inside an at least 1000 m wide corridor on all land-facing sides of dikes and dams. The data of the 3 colonies plus another colony data (Hu et al., 2007) were combined to analysis. Most alates traveled within 500m, accounting for the 90.9% of wings collected (Fig. 4). So, the region within 500 m distance from dikes on is the most important area to eliminate *O. formosanus* colonies.

Termite colony origin appeared to affect dispersal distance, with colony B alates flied farther than other colonies and have significance compared with colony A or colony C (Table 1). Alates of colony B also flied higher than other colonies and have significance compared with the colony C (Table 1). This was not directly related to differences in the physical size of the alates, because individuals in colony B were of equivalent size to those from other colonies. It is possible that intercolony differences in the position of exit hole for swarming or flight behavior may exist.

Termite alates of *Coptotermes formosanus* Shiraki were reported to have flown as far as 892 m (Messenger and Mullins, 2005), and could initiate colonies at heights of up to 40 m in high-rise buildings (Su et al., 1989). Shi et al. (1987) estimated that alates of *Odontotermes hainanensis* (Light) could reach a height of 10-30 m and travel 50-400 m from the colony. The maximum distance flown by alates of *Reticulitermes flavipes* (Kollar) was estimated as far as 458.3 m (Shelton et al., 2006). Liu (1998) estimated that *Reticulitermes flaviceps* (Oshima) disperse as far as 50-80 m and as high as 20-30m. He (1986) estimated that alates *Macrotermes barneyi* Light could travel 150 m and reach a height of 25 m. Our result showed that horizontal flight distance of alates *Odontotermes formosanus* (Shiraki) were able to fly to 1070 m and vertical flight distance could reach to 64 m. It indicated that alates of *O. formosanus* fly further than alates of *C. formosanus*, *O. hainanensis*, *R. flavipes*, *R.flaviceps* and *M. barneyi* and higher than alates of *C. formosanus*, *O. hainanensis*, *R.flaviceps* and *M. barneyi*, which in the historical document. So, it is possible that interspecies differences in dispersal distance exist.

Flight distance of alates depends on many factors, such as wind speed, wind direction, light disturbance, rain fall condition and other environmental variables during the swarming. We suggest that territories are mainly affected by wind direction and light disturbance. Dispersal behavior of alates was affected by wind direction because the flight direction followed wind direction in our field observed swarms every year. Flying in the wind direction may be that the strategy of the termite is to increase distribution capabilities.

Clearly circumstance light has some effect on the dispersal distance of alates. Alates of black-winged subterranean termites, *O. formosanus* exhibited a strong tendency to light. In particularly, if a special bright light positioned at the flight distance limit, alates will try their best to congregate around the light destination. In the experiment of Colony B, bright light trap of point NO.8 and NO.26 have attractive effect on the alates flight. We suggest that brighter light trap were attributable to enhanced alates attraction.

In summary, our result document *O. formosanus* alates were capable of flying horizontal distance of 1070 m and vertical distance of 64 m. Questions that remains to be investigated include the swarm intelligence, laboratory test on tethered mill, influence of different circumstances and the basis of differences in colony dispersal distances.

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Table 1. ANOVA result of alate horizontal dispersal distances and vertical dispersal distances for different colonies.

Colony	n	Horizontal distance			Vertical distance		
		Means	Minimum	Maximum	Means	Minimum	Maximum
A	121	46.2±6.0a	7	319	14.3±0.8y	-11	21
B	74	281.8±31.2b	18	1070	16.9±1.3y	-21	64
C	55	183.5±18.7c	96	888	-29.4±1.4z	-45	14
Total	250	146.1±12.3	7	1070	6.58±1.3	-45	64

Different letters following means are significantly different at the α=0.05 level by Tukey test. Comparisons are made between colonies means (a, b, c and y, z) for horizontal dispersal distances and vertical dispersal distances.

Distribution of the Termite *Odontotermes formosanus* in the Ryukyu Archipelago: A Possible Explanation for the Isolation of Okinawa Island

by
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Abstract

Subterranean-nesting termites do not colonize across the sea by rafting, and at the same time their winged-termites are also unlikely to fly such a long distance. The subterranean-nesting termite *Odontotermes formosanus* is common from Southeast Asia to the subtropics of East Asia, and is well-known as being discontinuously distributed in the Yaeyama Islands and the Shuri district of Okinawa Island; therefore, it may be hypothesized that *O. formosanus* was artificially introduced to Okinawa Island. Here we observed for the termites the vicinity of the Shuri district as well as islands of the Ryukyu Archipelago, and discussed the origin of the termites of Okinawa Island. Although we confirmed the presence in the Yaeyama Islands, we did not find the termites in the Miyako Islands, which situate between the Yaeyama Islands and Okinawa Island. In Okinawa Island, the termites exclusively occurred within a limited area that centers on the Shuri district (the old capital city area in the age of the Ryukyu Dynasty). In addition, by comparing their gene sequences deposited in the database, the populations in the Yaeyama Islands and Okinawa Island were estimated to have been separated relatively recently. We suggest that *O. formosanus* was brought into Shuri from the Yaeyama Islands in the age of the Ryukyu Dynasty in order to provide for the people the termite mushrooms that have been a valuable foodstuff for the local peoples.

Key words: fungus-growing termites, alates, termite mushrooms, artificial introduction, the Ryukyu Dynasty

Introduction

Distribution of animals is basically separated by the sea. For example, Wallace's line, which situates at the west of Sulawesi Island, is a clear boundary between Australasian and Southeast Asian animal fauna. The termite *Odontotermes formosanus* (Shiraki), which is one of fungus-growers (family Termitidae, subfamily Macrotermitinae), is a ubiquitous soil insect in Southeast Asia as well as the southern part of East Asia. The north-eastern limit of the range is Okinawa Island in the Ryukyu Archipelago, while the population of Okinawa Island appears distantly isolated from the other populations (Ahmad 1965, Huang *et al.* 1989, Shiraki 1909, Nawa 1914, Hozawa 1915, Ikehara, 1966, Yasuda et al. 2000, Katoh et al. 2002).

Termites sometimes colonize across the sea via driftwood or by winged-termites (alates). Such termites that make nests in wood can immigrate into and colonize other lands by rafting (Abe 1984, Gathorne-Hardy *et al.* 2000b), whereas *O. formosanus*, which makes subterranean nests, obviously does not flow on the sea. Furthermore, although only the sterile castes (workers/soldiers) of Macrotermitinae are found in wood and would be in a position to raft to other lands, they would be unable to produce reproductives and so could not colonize there (Gathorne-Hardy *et al.* 2000b). On the other hand, colony foundation by alates could be a possible explanation for the origin of *O. formosanus* in Okinawa Island; however, the flight ability of alates is generally supposed to be low (Nutting 1969, Abe 1984). Likewise, the alates of *O. formosanus* are shown to take a flight for at most only 1 km by field observations (Ikehara 1966, Hu *et al.* 2007). In fact, Okinawa Island is located 400 km from the nearest places (the Yaeyama Islands) where *O. formosanus* has been found so far (Nawa 1914, Hozawa 1915, Ikehara 1966, Yasuda *et al.* 2000, Katoh *et al.*2002).

As a possibility other than rafting or flight, we will think that *O. formosanus* was introduced to

Okinawa Island by man, while, due to the nests being in the soil, this could not have occurred inadvertently by import/export of wood (*c.f.* Huang *et a*l. 1989). Alternatively, we may have to think that the termites were brought into the island by intention, though *O. formosanus* is actually one of serious pest species (Huang *et al.* 1989). In the case of fungus-growers, the termites bring a valuable foodstuff—the so-called termite mushrooms—to the local peoples. The mushrooms grow up exclusively from the nests of fungus-growers. Therefore, in order to cultivate the termite mushrooms, the people may have intentionally introduced and rooted *O. formosanus* in Okinawa Island, where there have not been any fungus-growers other than *O. formosanus* (Hozawa 1915, Ikehara 1966, Yasuda *et al.* 2000). Here we clarified the distribution of *O. formosanus* and examined the origin in Okinawa Island by comprehensively observing the termites in islands of the Ryukyu Archipelago.

Study sites and methods

We examined major forest areas for *O. formosanus* in the islands of the Ryukyu Archipelago, namely Yonaguni Island, Iriomote Island, Kohama Island, Taketomi Island, Ishigaki Island, Tarama Island and Miyako Island (for Okinawa Island, see below). Forest areas were identified based on vegetation maps and aerial photographs. To find subterranean nests of *O. formosanus* is quite time-consuming work; we searched the forest areas for the termites foraging on/in litter on the forest floor.

In Okinawa Island, we carried out more detailed observation in scattered forest areas in the vicinity of the Shuri district in Naha city. On the basis of maps and aerial photographs, all the forest areas that are larger than 0.1 ha were observed for *O. formosanus*, except for such forest areas that we could not enter by some reasons such as surrounding-fences. We checked the termites as described above, while the maximum observation effort was determined as 30 minutes per person per ha. The forest areas where we did not find *O. formosanus* within the maximum observation effort were judged to be those where the termites are not distributed. Although the foraging activity of other fungus-growers changed in periods of time (Sugio 1995), such changes were not found in our preliminary observations at least for a period from 7:00 am to 6:00 pm.

Results

In the islands where we made observations, *O. formosanus* was found in Iriomote, Ishigaki and Taketomi Islands (for Okinawa Island, see below) (**Fig. 1**). In Iriomote Island, where almost the entire island is covered by natural forests, we examined a total of seven sites along Prefectural Road 215 and found the termites at all the sites. In Ishigaki Island, there are two major forest areas, Banna Forest Park and Kabira Park, and the termites were distributed both in the parks. In Taketomi Island, we searched the forest area located in the east part of the island as well as another area, and found the termites in the former. On the other hand, we did not find the termites at all in Yonaguni, Kohama, Tarama and Miyako Islands. In Yonaguni Island, the observations were conducted along Aragabana Natural Trail, in the forests of Mount Inbi, Mount Urabu, Mount Yonaguni and Mount Kubura, and at other two areas. In Kohama Island, we surveyed the forest near golf links in the northeast part of the island as well as the forest of Ufudaki. In Tarama Island, there were forest areas only along the coast. A total of six sites along Prefectural Road 223 were observed. In Miyako Island, we carried out the observations in a total of six areas, especially in the large forest area near Hirara City Tropical Park. In Okinawa we investigated a total of 108 forest areas in the vicinity of the Shuri district of Naha and found the termites at 48 areas usually within less than 20% of the maximum observation efforts (**Fig. 2A**). The results indicate that the maximum observation effort defined here is enough to judge the presence or absence of the termites in a certain forest area. The range of the size of the forest areas where the termites were found clearly overlapped with those where we did not find the termites within the maximum observation efforts (**Fig. 2B**); thus, the size of forest areas probably does not affect the presence or absence of the termites.

The distribution of the forest areas inhabited by *O. formosanu*s was concentrated in a round area with the diameter of approximately 4 km, which includes almost the entire Shuri district (**Fig. 3**). Within this round area, the termites were found 46 of 51 forest areas, whereas the termites were found only 2 of 41 forest areas within approximately 1-km outsides of the circle.

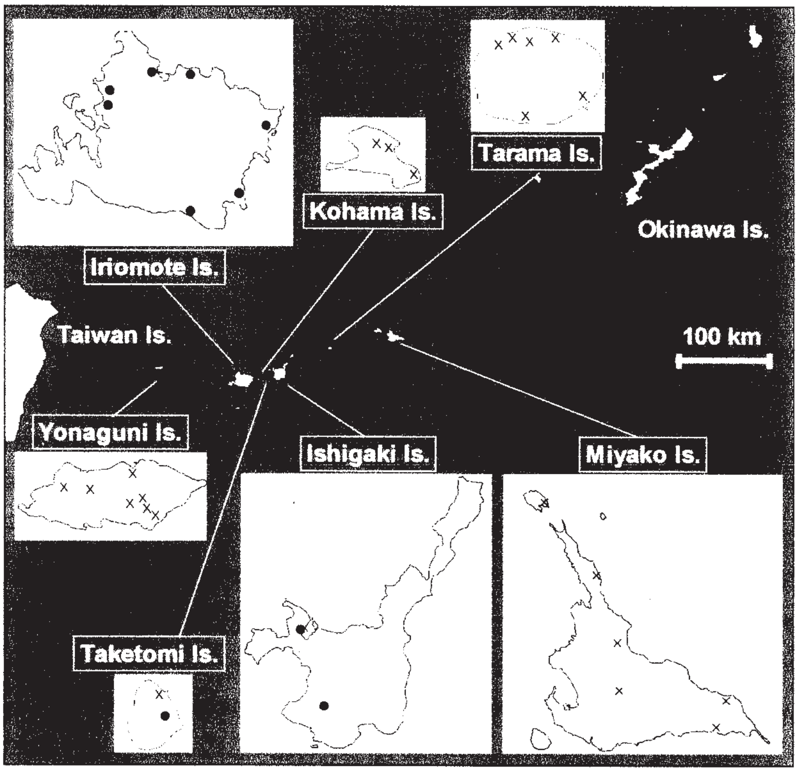

Fig. 1. Distribution of *Odontotermes formosanus* in the Yaeyama Islands and Miyako Islands. Closed circles and crosses indicate the forest areas where the termites were found or not, respectively. The Yaeyama Islands include Yonaguni, Iriomote, Kohama, Taketomi and Ishigaki Islands, and the Miyako Islands include Tarama and Miyako Islands. *O. formosanus* has been found also in Taiwan Island (Shiraki 1909, Hozawa 1915, Katoh *et al.* 2002), Chinese mainland (Huang *et al.* 1989), and Thailand (Ahmad 1965).

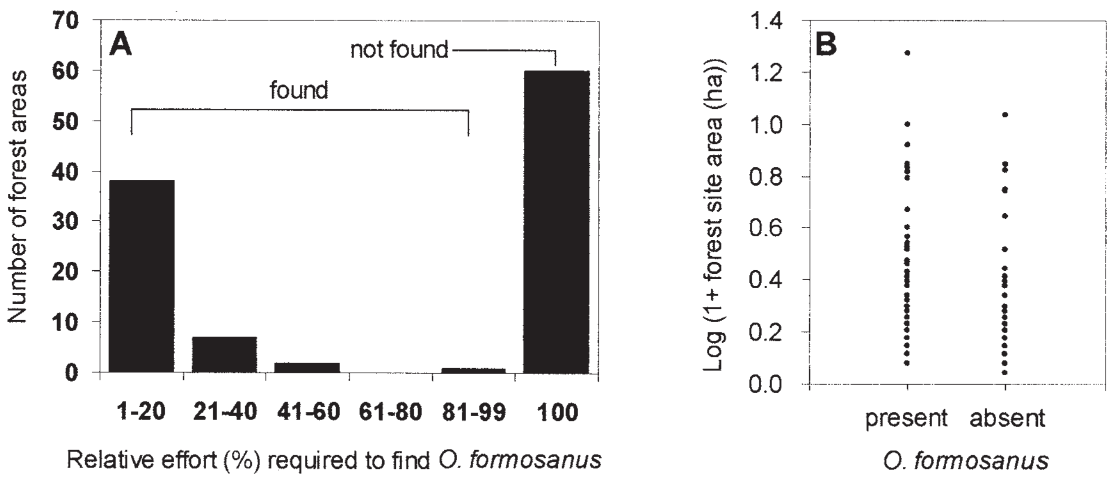

Fig. 2. Frequency of relative time (percentage of the maximum observation time) required to find *Odontotermes formosanus* in the forests sites around the Shuri district of Naha in Okinawa Island (A) and the size of forest area between the presence and absence of *O. formosanus* (B). (A) The maximum observation time was calculated based on the size of each forest area. (B) Each symbol indicates a forest area. Values shown are being log (x+1)-transformed.

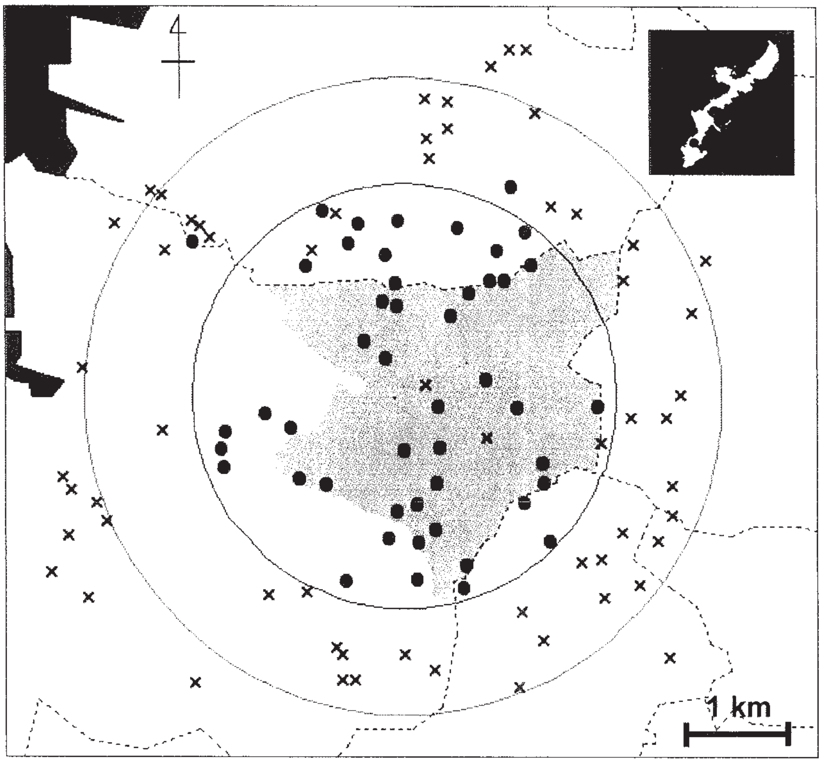

Fig. 3. Distribution of *Odontotermes formosanus* around the Shuri district of Naha in Okinawa Island. The grey area shows the Shuri district. Closed circles and crosses indicate the forest areas where the termites were found or not, respectively. Yasuda *et al.* (2000) have comprehensively observed Okinawa Island for *O. formosanus* and found the termites only in the Shuri district.

Discussion

Subterranean-nesting termites of the southern part (the Yaeyama Islands) of the Ryukyu Archipelago are a derivative of those of Taiwan Island and Chinese mainland (Hozawa 1915, Ikehara 1966, Huang *et al.* 1989, Yasuda *et al.* 2000). In Iriomote Island, which is the largest island of the Yaeyama Islands, we can usually find the three subterranean species of the family Termitidae, *O. formosanus*, *Pericapritermes nitobei* (Shiraki) and *Sinocapritermes mushae* (Oshima et Maki); these species are common to Taiwan Island and Chinese mainland. As mentioned above, subterranean-nesting termites do not cross the sea by rafting (Abe 1984; Gathorne-Hardy *et al.* 2000b). Therefore, in the ordinary course of events, it is likely that the three species went up north into the Yaeyama Islands from Chinese mainland via Taiwan Island in the ages when the sea level was much lower than the present level and there were land bridges among the present dry lands (see below). In line with the history, it has been reported that fungus-growers such as *O. formosanus* originated in Africa and moved toward the east (Aanen & Eggleton 2005). Note that the absence of *O. formosanus* from Yonaguni Island is exceptional, even though our observation covered all the major existing forest areas. Ikehara *et al.* (1966) reported the presence (**Table 1**), but did not provide the sampling sites and any other detailed information to check their claim. The present study showed that the subterranean-nesting termite *O. formosanus* is absent from the Miyako Islands, which situate between the Yaeyama Islands and Okinawa Island (**Fig. 1**). This suggests the land-bridged immigration of the subterranean-nesting termites did not occur up to not only the Miyako Islands, but also Okinawa Island. Supporting this, within the mid and northern parts of the Ryukyu Archipelago, there has been no record of the other subterranean-nesting species that are found in the Yaeyama Islands. Although Ikehara *et al.* (1966) have reported the presence as in the case of Yonaguni Island (**Table 1**), the possibility that *O. formosanus* in the Miyako Islands disappeared relatively recently by extensive predation or environmental changes would be very low due to the following facts: there seems to be few so strong predators that make termites extinct (*c.f.* Deligne et al. 1981) and the forests in the Miyako Islands have been maintained at least as long as those in Okinawa Island (Guidance for Natural Environment Conservation, Okinawa Prefecture Environmental Preservation Division), where we found the termites.

Separation of the populations of *O. formosanus* could be traced by calibrating their gene sequence differences with the molecular clock. Katoh *et al.* (2002) have provided the COII (mitochondrial cytochrome oxidase subunit II) gene sequences of *O. formosanus* collected from Taiwan, Iriomote, Ishigaki and Okinawa Islands (GenBank Accession No.: AB051867–AB051876), and Brower (1994) estimated the molecular clock calibration of mitochondrial DNA to be 2.3% per million years for insects. Subsequently, the separation between the population in Taiwan Island and the others can be calculated to have occurred approximately 0.96 million years ago (MYA). This value completely agrees with the possible geographical histories of the Ryukyu Archipelago (Kizaki & Oshiro 1980, Kimura 2000), which are summarized as follows: a land bridge among Chinese mainland, Taiwan Island and the Ryukyu Islands might have existed from the early to the middle Pleistocene (1.5–1.0 MYA). Given the fact that there is no difference in COII sequence among the populations of *O. formosanus* in Ishigaki, Iriomote and Okinawa Islands, we can calculate that these populations have been separated within the recent 64,000 years or maintain continuous genetic exchanges (alate exchanges). Taken all together, it is strongly suggested that an immigration of *O. formosanus* into Okinawa Island did not occur along the land bridge during from the early to the middle Pleistocene.

Table 1. A summary of the studies on the distribution of *Odontotermes formosanus* in the major (more than 20 km^2) islands of the Ryukyu Archipelago.

Reference	Yaeyama Islands			Miyako Islands			MD	Okinawa Islands			
	YN	IO	IS	TR	IB	MY		KM	IE	IH	OK
Nawa 1914	–	–	–	–	–	–	–	–	–	–	●
Hozawa 1915	–	–	●	–	–	–	–	–	–	–	–
Ikehara 1966	?	●	●	–	×	?	–	×	×	–	●
Yasuda *et al.* 2000	×	●	●	–	–	×	×	×	×	×	●
Kato *et al.* 2002	–	●	●	–	–	–	–	–	–	–	●
Present study	×	●	●	×	–	×	–	–	–	–	●

●: present; ×: absent; –: no observation or no information; "?": see text; YN: Yonaguni; IO: Iriomote; IS: Ishigaki; TR: Tarama; IB: Irabu; MY: Miyako; MD: Minamidaito; KM: Kume; IE: Ie; IH: Iheiya; OK: Okinawa.

Judging from the presence of fungus-growing species in Sulawesi and Madagascar Islands, a long-distance (up to 300-km) flight by alates has been thought not to be impossible due to no alternative explanation (Emerson 1955, Gathorne-Hardy *et al.* 2000a), though the frequency should be extremely rare due to the species having being endemic in the islands (Paulian 1970, Gathorne-Hardy *et al.* 2000a). This can be the case for the termite populations in Iriomote and Ishigaki Islands. Between these two islands, at the interval of approximately 10 km there are two small islands (Taketomi and Kohama Islands, see **Fig. 1**) possibly functioning as "stepping-stones" for the alate exchanges. In fact, we found the termites in Taketomi Island. Although the termites were not found in Kohama Island, Ikehara (1966) reported the presence; they may repeat invasion and extinction due to the small size of the island. Considering the case of Sulawesi and Madagascar Islands, 10-km flights by alates could occur much more frequently (*c.f.* Gathorne-Hardy *et al.* 2000b). Likewise, we might not rule out the occurrence of a long-distance (400-km) flight between the Yaeyama Islands and Okinawa Island within the recent 64,000 years. However, another possibility (*i.e.* an artificial introduction) should be considered due to the absence of *O. formosanus* from the Miyako Islands, which apparently situate on the way to Okinawa Island from the Yaeyama Islands (**Fig. 1**). Furthermore, it seems difficult to assume that a 400-km flight had occurred within such a short period.

Interestingly, the distribution of *O. formosanus* in Okinawa Island has been reported to be limited to the Shuri district of Naha City (Yasuda *et al.* 2000) and furthermore was shown to be highly concentrated in a small round area by the present study (**Fig. 3**). Yasuda *et al.* (2000) observed a total of 60 sites throughout Okinawa Island and found the termites from only one site that was set within the Shuri district, where the first report about the presence of the termites was made approximately 100 years ago (Nawa 1914). As far as we know, there is not any document that reports the presence of *O. formosanus* in Okinawa Island other than the Shuri district. The distribution pattern we showed can imply that the termite population started increasing from around the center of the round area relatively recently (between 64,000 to 100 years ago), because, among all the forest areas we examined, there is not clear difference in the environmental factors that possibly affect the termite distribution: surface-soil types (National Land Survey Division, Land and Water Bureau, Ministry of Land, Infrastructure, Transport and Tourism) and vegetations (The national survey on the natural environment, Biodiversity Center of Japan, Ministry of the Environment). Furthermore, the distances that alates usually fly range from several meters to 1 km and the directions of their flights seem to become random due to the wind direction (Ikehara 1966, Hu *et al.* 2007); the distribution area of *O. formosanus* would increase gradually and radially. Also, the distances among the forest areas are usually within the range of their flight distances (**Fig. 3**). Therefore, the first *O. formosanus* termites may have colonized around the center of the present distribution area, where we can find an important place—Shuri Castle, the royal palace of the Ryukyu Dynasty—in the recent history of Okinawa Island.

The present Shuri district was the capital city area (Shuri) in the age of the Ryukyu Dynasty (from 1429 to 1879 A.D.), when the Yaeyama Islands were under the rule of the Ryukyu Dynasty and various products and gifts, such as dugong meat, were brought to Shuri from the Yaeyama Islands. As mentioned above, termite mushrooms have been a premium foodstuff for local peoples; those of *O. formosanus* should be a candidate of the gifts from the Yaeyama Islands. However, the mushrooms cannot be kept fresh for a so long time that they would be shipped to Shuri in that age. So, the people might have brought the whole nests of *O. formosanus* or pairs of young king and queen to Shuri and rooted them. Unfortunately, there are few documents about the dietary life and classic foods of that age (Kinjo 1995). Also, as far as we know, there is no such evidence that shows the transportation of *O. formosanus* termites or their mushrooms. Nevertheless, on reconsidering the matter, the present study may provide a clue to know the past physical distribution and exchanges of the local peoples. Our results illustrate the discontinuous distribution of *O. formosanus* in the Ryukyu Archipelago and the highly concentrated distribution around the Shuri district of Okinawa Island, indicating possible avenues for further study.

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Biomass of Soil Termite *Macrotermes gilvus* Hagen in Natural Forest Ecosystem

by
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Abstract

Termite plays a very important role in ecosystem, especially in nutrient cycle through its functions as decomposers of organic material in natural forest (Vasconcellos *et. al.* 2007). No studies have ever been conducted to look at the biomass of *Macrotermes gilvus* Hagen in natural forest ecosystem. Biomass is a quantitative measurement of total mass of an organism from a part or all members of a population in a certain place and time (Lincoln *et al.* 1988). Biomass is possible to be used as an appropriate indicator to measure the number of food consumed by a species, hence it can be predicted the effects of the species in an ecosystem (Meyer *et al.* 2005).

The objective of the research was to study soil termite biomass *Macrotermes gilvus* Hagen in natural forest and to evaluate the need of food of the species as well as factors affecting it. The research was focused to observe various variables including wet and dry weight, ratio of dry weight/wet weight, and average of biomass based on nest size of *Macrotermes gilvus* Hagen. Research was conducted in Yanlappa Sanctuary, Bogor West Java. Termites were surveyed by collecting termite individual *Macrotermes gilvus* Hagen at different size of nest, large 0 – 0.99 m, middle (1 - 1.99 m), and small (≥ 2 m). Wet and dry weight, and data analyses were calculated by using SPSS ver. 13. Results indicated that mean of wet weight of the termite's colony *Macrotermes gilvus* Hagen for queen was about 1082,30 ± 6670,60 g, king was 1038.80 ± 1075.40 g, major sodier 33.30 ± 39.31 g, minor soldier was 8.43 ± 20.69 g, and nymph was 8.67 ± 14.84 g. Mean of dry weight of *Macrotermes gilvus* Hagen for queen was approximately 444.10 ± 1401.10 g, king was 654.20 ± 675.40 g, major soldier was 14.37 ± 16.89 g, minor soldier was 6.60 ± 13.76 g, and nymph was 6.23 ± 7.16 gr. Mean of ratio wet weight/dry weight of queen was about 41.03 ± 17.78%, king was 62.98 ± 61.99%, major soldier was 21.14 ± 31.20%, minor soldier was 9.63 ± 14.24%, and nymph was 7.50 ± 9.52%. Mean of soil termite biomass *Macrotermes gilvus* Hagen was approximately 9.36 kg/ha (936 kg/km^2). Average of termite biomass *Macrotermes gilvus* Hagen collected from large nest was about 4.4 kg/ha (440 kg/km^2), middle nest was 4.3 kg/ha (430 kg/km^2) and small nest was about 0.8 kg/ha (80 kg/km^2). Factors affecting soil termite biomass *Macrotermes gilvus* Hagen are source of food, energy efficiency, predators, and environment.

Key words: Biomass, colony, *Macrotermes gilvus* Hagen.

Introduction

Termite plays a very important role in an ecosystem especially in nutrient cycling through its function as decomposer of organic material in natural forest. In ecosystem, Termite is a very important component of biogeochemical cycles. (Vasconcellos *et. al.* 2007). No studies have ever been conducted to look at the biomass of *Macrotermes gilvus* Hagen in ecosystem. Biomass is a quantitative measurement of total mass of an organism from a part or all member of a population in a certain place and time (Lincoln *et al.* 1988). Biomass is possible to be used as an appropriate indicator to measure the number of food consumed by a species, hence it can be predicted the effects of the species in an ecosystem (Meyer *et al.* 2005).

The objective of the research was to study soil's mite biomass *Macrotermes gilvus* Hagen in natural forest and to evaluate the need of food of the species as well as factors affecting it. The research was focused to observe various variables including wet and dry weight, ratio of dry weight/wet weight, and average of biomass based on nest size of *Macrotermes gilvus* Hagen.

Methods

Research was conducted between December 2006 and April 2007 in Yanlappa Sanctuary, Bogor District, West Java. Yanlappa sanctuary is about 32 ha and located between 6°40' S and 106°45' E., with rainfall of about 2,399 mm/year. Soil is recorded as grey alluvial association and primer sediment was clay and sand with waved phisiography. Vegetation of the area was dominated by *Dipterocarpus hasseltii, Hopea sangal* Korth, *Schima walichii* Korth (Puspa), *Altingia excelsa* Noronhae (Rasamala), *Litsea spp, Lagerstroma spp* (Bungur) and *Ficus septica* (Awar-awar).

Termite Collection

Soil termites *Macrotermes gilvus* Hagen were surveyed by collecting 100 individuals from different castes (worker, major soldier, minor soldier, nymph. queen, and king) of different size of nest: small, middle, and large nests. Insect samplings were conducted by using colony classification (Meyer 2001) *i.e* small nest (0 – 0,99 m), middle nest (1 – 2,99 m) and large nest (≥ 3 m).

Wet weight measurement

1) Approximately 300 individual termites (worker, major soldier, minor soldier, nymph), queen, and king from different size of nest were put inside glass container.
2) Weight of each of 30 termite specimens of each caste was measured by using microbalance. Measurement was replicated for 10 times.

Dry weight measurement

1) Approximately 300 individual termites (worker, major soldier, minor soldier, nymph), queen, and king from different size of nest were put inside glass container
2) All specimens were then dried in an oven under 100 °C for 24 hours. Dried specimens were then measured for each caste.
3) All materials were cooled and stored in desiccator and weighed.
4) Replication was about 10 times.

Data analyses

Data were analysed by using SPSS version 13 (*Statistical Product and Service Solution*).

Results and discussion

In the study area there were 43 points of termite colonies found consisted of 15 spots of large nests, 23 spots of middle nests, and 5 spots of small nests. Termite biomass *Macrotermes gilvus* Hagen found in the study area was approximately 9.36 kg/ha (936 kg/km^2) with average of biomass for small nest was 5.37 kg (537 kg/km^2), middle nest was 6.05 kg (605 kg/km^2), and large nest was 9.49 kg (949 kg/km^2).

Wet weight of termite colony of *Macrotermes gilvus* Hagen in natural forest was recorded to higher in reproductive caste (queen and king), 1082.30 ± 667060 g and 1038.80 ± 1075.40 g, however major soldier was about 33.30 ± 39.31 g, minor soldier was 8.43 ± 20.69 g, and in contrast nymph was found to have smallest weight about 8.67 ± 14.84 g.

Average of dry weight of *Macrotermes gilvus* Hagen in natural forest was found to be higher in reproductive caste (queen and king) 444.10 ± 1401.10 g and 654.20 ± 675.40 g, dry weight for major soldier was recorded about 14.37 ± 16.89 g, minor soldier was 6.60 ± 13.76 g, and nymph reached 6.23 ± 7.16 g.

Average of wet weight /dry weight was recorded to be higher in reproductive caste (queen and king) about 41.03 ± 17.78% and 62.98 ± 61.99% respectively, ratio wet weight /dry weight of major soldier was 21.14 ± 31.20%, minor soldier was 9.63 ± 14.24%, and nymph was about 7.50 ± 9.52%.

Table 1. Wet and dry weight of *Macrotermes gilvus* Hagen in natural forest. Significant different was computed by using analyses of variance (ANOVA)

Caste/Sub Caste	Wet Weight (g)	Dry weight(g)	Ratio Dry weight/Wet Weight (%)
Worker	6.08 ± 18.06	3.63 ± 7.17	6.15 ±10.27
Major soldier	33.30 ± 39.31	14.37 ± 16.89	21.14 ± 31.20
Minor soldier	8.43 ± 20.69	6.60 ± 13.76	9.63 ± 14.24
Nymph	8.67 ± 14.84	6.23 ± 7.16	7.50 ± 9.52
Queen	1082.30 ± 6670.60	444.10 ± 1401.10	41.03 ± 17.78
King	1038.80 ± 1075.40	654.20 ± 675.40	62.98 ± 61.99

Mean of biomass between caste/sub caste in the colony of soil termite *Macrotermes gilvus* Hagen in natural forest based on the characteristic of nest, for small nest (S), worker was approximately 2.458 ± 2.788, major soldier was 9.058 ± 10.375, minor soldier was 2.575 ± 3.153, and nymph was about 0.764 ± 1.361. For middle nest, biomass of worker was about 2.524 ± 3.104 (n=300), major soldier was 10.135 ± 11.261, minor soldier was 3.175 ± 3.431, and nymph was about 1.192 ±1.614. For large nest (L), worker was about 5.517 ± 8.666, major soldier was 11.414 ± 13.164, minor soldier was 3.681 ± 4.033, and nymph was about 5.517 ± 8.666. With respect to total biomass, small nest reached 5,375, middle was about 6,052 and the large one was approximately 9,498 kg. Mean number of biomass can be seen in Table 2.

Tabel 2. Mean number of Biomass for each Case based on the size of nest *Macrotermes gilvus* Hagen in Natural forest (computed by using ANOVA)

Caste /Sub Caste	Small Nest (kg)	Middle Nest (kg)	Large Nest (kg)
Worker	2.458 ± 2.788 (n=300)	2.524 ± 3.104 (n=300)	5.517 ± 8.666 (n=300)
Major soldier	9.058± 10.375 (n=300)	10.135 ±11.261 (n=300)	11.414 ±13.164 (n=300)
Minor soldier	2.575 ± 3.153 (n=300)	3.175 ± 3.431 (n=300)	3.681 ± 4.033 (n=300)
Nymph	0.764 ± 1.361 (n=300)	1.192 ± 1.614 (n=300)	5.517 ± 8.666 (n=300)
Total	**5.375**	**6.052**	**9.498**

Highest Biomass of *Macrotermes gilvus* Hagen was recorded in major soldier, and then followed by minor soldier, worker, and nymph. Highest number of biomass of major soldier may be related to its contribution to the colony that is smaller compared with minor soldier and worker caste. Number of worker was found to be higher compared with other castes with its portion was about ± 80% of the total colony. Meanwhile, individual shifting was faster in worker caste compared with soldier. Soldier was mostly longer lifespan (Meyer *et al.* 2001). In a colony, worker is responsible for various kind of tasks including food foraging and feeding another caste, repairing the nest, and also building a new nest. It also helps to control air circulation especially for CH_4 and CO_2, maintaining mushroom plantation, as well as controlling nest humidity. (Turner, 2006). Smallest biomass was recorded for nymph, this may be related to phenology of termite since nymph is immature phase that still growing become mature termite with certain function for the colony. .

Foods consumed by termites are not only used for growth and energy but they are also stored inside termite's gut.. Feces and water are secreted from the body are converted into energy, tissue, organ, and another use (Strassmann & Queller, 2007).

Environment factor has a strong relationship with termite biomass.. Higher number of *Macrotermes gilvus* Hagen biomass might be related to their habitats in the tropic with abundant food around it and high level of decomposition, which result in low level of energy utilization.. Less predator around the colony will result in more efficient of energy utilization compared with lots of predator living around it. (Meyer *et. al.* 1999).

Conclusions

From the research on termite biomass of *Macrotermes gilvus* Hagen in natural forest ecosystem, there were some conclusions::

1. Mean of wet weight of colony of *Macrotermes gilvus* Hagen was 1082.30 ± 6670.60 g, raja 1038.80 ± 1075.40 g, major soldier was 33.30 ± 39.31 g, minor soldier was 8.43 ± 20.69 gr, and nymph was 8.67 ± 14.84 gr.
2. Mean of dry weight of *Macrotermes gilvus* Hagen for queen was 444.10 ± 1401.10 g, raja 654.20 ± 675.40 g, major soldier was 14.37 ± 16.89 g, minor soldier was 6.60 ± 13.76 g, and nymph was 6.23 ± 7.16 g.
3. Mean of ratio wet weight/dry weight for queen was 41.03 ± 17.78%, king was 62.98 ± 61.99%, major soldier was 21.14 ± 31.20%, minor soldier was 9.63 ±14.24%, and nymph was 7.50 ± 9.52%.
4. Mean of termite biomass of *Macrotermes gilvus* Hagen reached 9.36 kg/ha (936 kg/km^2). Mean of termite biomass *Macrotermes gilvus* Hagen for large nest was 4.4 kg/ha (440 kg/km^2), middle nest was 4.3 kg/ha (430 kg/km^2) and small nest was 0,8 kg/ha (80 kg/km^2).

5. Factors affecting soil termite biomass *Macrotermes gilvus* Hagen are source of food, energy efficiency, predators, and environment.

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Effects of *Eucalyptus* Plantation on Termites in Dead Wood on the Forest Floor

by
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Abstract

Expansion of *Eucalyptus* plantation is considered to be one of the major human disturbances to tropical forests, where termites are superabundant and an important decomposer. Here we studied the biomass of dead wood and the abundance and species composition of termites in dead wood in *Eucalyptus* plantation forest (EF) in Thailand and compared the data with those of the neighboring dry evergreen forest (DEF), which has been previously studied. The biomass of dead wood was much lower in the EF, especially for the dead wood with large diameters ($\geq$ 5 cm). Although the abundance of termites (termites m^{-2}) was quite different, the abundance of termites per unit weight of dead wood (termites kg^{-1}) was not different between the two forests, suggesting that the importance of termites in the decomposition is same in these forests. In the EF, however, we did not find the termite species that have been collected in the DEF from the dead wood with large diameters ($\geq$ 5 cm). *Eucalyptus* plantation may affect termite assemblages through the changes of dead wood biomass on the forest floor both quantitatively and qualitatively.

Key words: wood-feeding termites, Eucalyptus plantation, species diversity, food preference, resource competition, diameter of dead wood, abundance

Introduction

Tropical forests are widely accepted as the ecosystem that harbors the most diverse species of organisms on the earth, while human disturbance, such as plantation, is well-known to negatively affect and change the abundance and diversity of the organisms (*e.g.* Lawton *et al.* 1998). The plantation of *Eucalyptus* trees, which grow eminently fast and are of value as a pulp material, is now remarkably increasing all over the world and has replaced a considerable area of the tropical forests (Cossalter & Pye-Smith 2003). In tropical forests, termites are one of the most abundant animals and greatly contribute to the maintenance of the forests by decomposing mass of litter and dead wood on the forest floor (Wood & Sands 1978, Martius 1994, Eggleton *et al.* 1999, Yamada *et al.* 2005, 2006). So far, termite assemblages in tropical forests have been shown to be significantly affected by a variety of human disturbance (Eggleton *et al.* 1996, Jones *et al.* 2003); the comparison of termite fauna between *Eucalyptus*-tree plantation and the original forest will lead us to better understanding of the effects of the plantation on the species diversity and nutrient cycling. Here we focused on the dead wood accumulated on the forest floor and observed termites in a *Eucalyptus* plantation forest. Then, we compared the biomass of dead wood, the abundance and species composition of the termites in dead wood, and their role in the decomposition of the litter and dead wood between the *Eucalyptus* plantation forest and the neighboring natural forest.

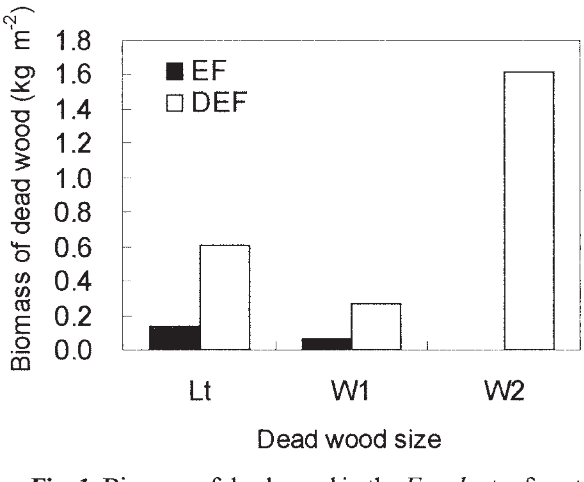

Fig. 1. Biomass of dead wood in the *Eucalyptus* forest (EF) and dry evergreen forest (DEF) in Thailand. The values shown are the arithmetic means of 7 and 10 quadrats (2 m x 2 m) in the EF and DEF, respectively. Lt in the DEF includes leaves. For dead wood size, see the "Study site and methods" section. Data of the DEF were given by Yamada *et al.* (2003)

Fig. 2. Abundance of termites in dead wood per unit weight of dead wood in the *Eucalyptus* forest (EF) and dry evergreen forest (DEF) in Thailand. The values shown were calculated by dividing a total individual number of termites by a total weight of dead wood (Lt in the DEF includes leaves). For dead wood size, see the" Study site and methods" section. Data of the DEF were given by Yamada *et al.* (2003).

Study site and methods

Observation was carried out in a 30-year-old *Eucalyptus* plantation forest (EF) at Sakaerat Environmental Research Station (SERS) in northeast Thailand. According to the vegetation map of SERS, originally the EF seems to have been a dry evergreen forest (DEF). In the DEF at SERS, the biomass of dead wood and the abundance and species composition of the termites in dead wood have been studied by Yamada *et al.* (2003). The same methods were employed here, except for the number of 2 m × 2m quadrats; we collected all the dead wood from within a total of seven quadrats set in the EF. The dead wood collected was divided into the following three groups: Lt: 1cm < diameter, W1: 1 cm ≤ diameter < 5 cm, and W2: 5 cm ≤ diameter. These pieces of dead wood were individually examined for termites by breaking them into small pieces by hatchet, and at the same time we picked up all the termites that were found in the dead wood.

Results and discussion

Table 1. List of the termite species collected from dead wood in the *Eucalyptus* forest (EF) and dry evergreen forest (DEF) in Thailand. The values shown are the arithmetic mean abundances per unit area (termites m^{-2}). For dead wood size, see the "Study site and methods" section. Data of the DEF were given by Yamada *et al.* (2003).

Termite species	EF			DEF		
	Lt	W1	W2	Lt	W1	W2
Kalotermitidae						
Glyptotermes brevicaudatus						0.7
Incisitermes sp.1					3.0	0.4
Neotermes sp.1					1.1	
Neotermes sp.						0.0
Rhinotermitidae						
Schedorhinotermes medioobscurus						76.5
Schedorhinotermes spp.					0.1	
Macrotermitinae						
Odontotermes formosanus					2.7	
Odontotermes proformosanus-L					6.1	
Hypotermes makhamensis				0.3	0.0	1.3
Ancistrotermes pakistanicus	6.9	2.1			2.3	2.8
Microtermes obesi		3.0			0.3	
Termitinae						
Globitermes sulphureus					0.1	357.9
Microcerotermes crassus	29.4	112.2		50.5	615.3	148.4
totals	36.3	117.4	0.0	50.8	630.9	587.8

In the DEF, Yamada *et al.* (2003) have shown the biomass of dead wood (kg m^{-2}) on the forest floor, and the abundance of each termite species in dead wood both per unit area (termites m^{-2}) and per unit weight of dead wood (termites kg^{-2}). Compared to these data, the biomass of dead wood was extremely low in the EF (**Fig. 1**). In particular, the large dead wood (W2) was so scarce that we did not find such a piece of dead wood within our quadrats. Therefore, it is suggested that the EF provides only a small amount of food and habitats for wood-feeding termites. As predicted by Yamada *et al.* (2005, 2007), if there is a resource competition among wood-feeding termites, the termites should decrease in proportion to the biomass of dead wood. More precisely, the abundance per unit weight of dead wood (termites kg^{-1}) in the EF should have a similar value to that of the DEF. In fact, our results showed that the abundance as well as species composition was almost same in the dead wood of Lt and W1 between the EF and DEF (**Fig.** 2). From the aspect of the role of termites, this may imply that termites equally contribute to the decomposition of such relatively small dead wood in the two forests, though the apparent abundances (termites m^{-2}) are largely different (**Table 1**). On the other hand, the fauna of termites in dead wood in the EF was a small part of that of the DEF (**Table 1**). An explanation could be found in possible food preference of wood-feeding termites. Recent studies (Yamada *et al.* 2003, Evans *et al.* 2005) have reported that at least some wood-feeding species tend to attack specific size of dead wood. In the present study, the termite species that have been collected mainly from the W2-sized dead wood of the DEF, such as *Schedorhinotermes medioobscurus* and *Globitermes sulphureus*, were not found in the dead wood of the EF. Therefore, we can suggest that termite fauna is strongly affected by qualitative as well as quantitative changes in the dead wood accumulated on the forest floor. As in the case of the present study, *Eucalyptus*-tree plantation could decrease the abundance and species diversity of wood-feeding termites in tropical forests through the changes of food and habitat resources.

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Study about Intestinal Protozoa of Subterranean Termite, *Coptotermes* sp.

by
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Abstract

All species of the lower termite genera harbor throughout most of their lives faunules of flagellate protozoa in the hindgut. Mostly, protozoan fauna in termite gut are very complex and have its specificity. *Coptotermes* sp. mainly has three kinds of protozoan fauna in its intestinal. This study objects to collect the information about characterization and distribution of intestinal protozoa inhabited termite gut of subterranean termite (*Coptotermes* sp) collected from different places, as well as to determine the influence of biotermiticide to protozoa population.

The observation showed that protozoa occurrence in termite gut were identified as *Pseudotrichonympha grasii* Koidzumi, *Holomastigotoides hartmanni* Koidzumi and *Spirotrichonympha leidy* Koidzumi. The total number of protozoan fauna of *Coptotermes* sp. collected from laboratory colony was much higher than in the field colonies. The existence of protozoan fauna in termite gut did not decrease by the fungal and plant extract treatment.

Key words: *Coptotermes* sp., flagellate protozoa, hindgut

Introduction

Termites have an important place in economic entomology, with the cost of damage to buildings, especially in developed countries in America and Asia. Damage to houses by termites can, in some countries, exceeds that caused by natural disasters and fires in a single year (Pearce 1997). In developing countries, they have even more impact, destroying local huts, crops, housing, and public buildings. Much of history of humankind has been lost due to termites eating books, buildings and even destroying cave painting and archaeological remains. The first indication of termite infestation often comes too late, when doors and roofs fall down or floors collapse. This kind of condition generates difficulties to detect and control termite.

Termites can devour any material that incorporates cellulose. In the lower termites, flagellate protozoa are important for breaking down of cellulose in cellulose-based materials (Pearce 1997). The lower termites and the wood-feeding roaches depend upon the flagellates for digestion of sound wood (Krishna and Weesner 1970). Gut protozoa take up wood particles by phagocytosis in the hindgut where most of the food absorption takes place. Protozoa that convert hemicellulose and some extent cellulose into acetate, carbon dioxide, hydrogen and methane ferment the processes anaerobically. The cellulose fermentation is conducted by enzymes called cellulase in the gut than can originate from microorganisms present, such as protozoa in the lower termites. Termite cellulose is also produced in the midgut and in minor portions of the foregut (Pearce 1997).

Coptotermes sp., classified in Rhinotermitidae, is economically important termite in Indonesia that produce serious damage. Some scientists have involved in investigation of protozoa population in termite gut. All species of the lower termite genera harbor throughout most of their lives faunules of flagellate protozoa in the hindgut (Krishna and Weesner 1970). Mostly, protozoan fauna in termite gut are very complex and have its specificity. The degree of host specificity is not uniform among the genera and species of intestinal flagellates harbored by the lower termites. Mostly, *Coptotermes* sp. has three kinds of protozoan fauna in its intestinal; *Pseudotrichonympha grasii* Koidzumi (150-250 μ m), *Holomastigotoides hartmanni* Koidzumi (50-150 μ m) and *Spirotrichonympha leidy* Koidzumi (20-50 μ m) that found in *Coptotermes formosanus* Shiraki (Yoshimura 1995).

Study about characterization of protozoa in termite gut is able to give essential contribution for termite control in the future. By considering the phenomenon of protozoa in termite gut, it is expected to understand the digestion mechanism of termite as well as its important roles in termite feeding behavior. This study objects to collect the information about characterization and distribution of intestinal protozoa inhabited termite gut of Subterranean termite (*Coptotermes* sp) collected from different places. Besides, the influence of biotermiticide to protozoa population will be examined in this research.

Materials and methods

Termites

Termites used were workers of *Coptotermes* sp. that collected from termite colony in R & D Unit for Biomaterials, Indonesian Institute of Science and field colony in Cibinong neighborhood at July 2007.

Biotermitice Treatment

Here, the kinds of biotermiticide used in this investigation were fungal spores of *Humicola* sp. and extractive of neem plant (*Azadirachta indica*). First, the two kinds of biotermiticide were dissolved in sterilized aquadest in order to make biotermiticide solution. Then, termites were sprayed with biotermitice solution and then incubated for 1 – 3 days before being dissected in order to measure the protozoa population in its gut.

Dissection Process of Termite

Dissection process purposed to acquire termite gut in order to collect flagellate protozoa. Termite gut was pulled out from the posterior end with a pair of fine forceps. About 50 μl NaCl 0,6 % solution was prepared and dropped onto glass slide, then the termite gut or intestinal contents were placed on that glass slide to extend their survival. The gut was cut into three parts, anterior, middle and posterior portion then placed and mixed on different glass slide in order to expose and measure the population of protozoa contained in termite gut. The following figure show the portion division of termite gut.

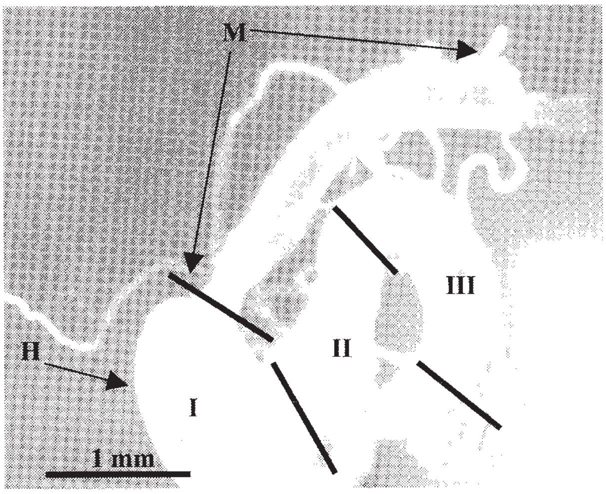

Figure 1. Division portion of termite gut of worker of *Coptotermes* sp.
M: Midgut, H: Hindgut, I : anterior, II: middle, III: posterior.

Measurement of Protozoa Population

A 2 μl of suspension was taken randomly from each slide and transferred onto different glass slide. Then, the number of protozoa in this solution was measured by observing under microscope.

Results and discussion

Population of Protozoa

Observation of protozoan fauna in termite gut showed three types of protozoa (Fig. 2). Individual faunas of flagellates in the more primitive termites from two to as many as ten species, but the extreme number are unusual. Sometimes there are three species of a genus in one host, more frequently there are two, but usually there is only one. Here, the protozoa occurrence in termite gut were identified as *Pseudotrichonympha*, *Holomastigotoides* and *Spirotrichonympha* (Fig.3). These protozoa are commonly found in termite gut. The termite *Hodotermopsis sjoestedti* harbours a symbiotic fauna of protests comprising at least 11 genera and a much larger number of species, including *Pyrsonympha*, *Dinenympha*, *Trichomitopsis termopsidis*, *Spirotrichonympha*, *Holomastigotes lanceolata* n. sp., *Holomastigotes elongatum*, *Trichonympha* and so on (Brugerolle and Bordereau 2004). Yoshimura 1995 had investigation on *Coptotermes formosanus* Shiraki that indicated three protozoa in termite gut of; *Pseudotrichonympha grasii* Koidzumi, *Holomastigotoides hartmanni* Koidzumi and *Spirotrichonympha leidy* Koidzumi.

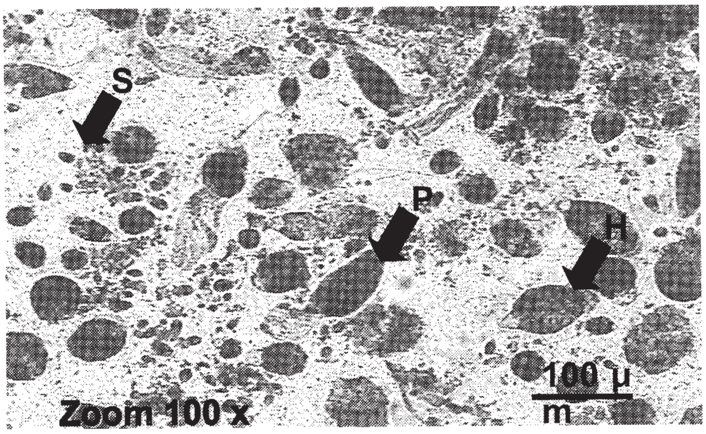

Figure 2. Distribution of three types of protozoan fauna in hindgut of *Coptotermes* sp.

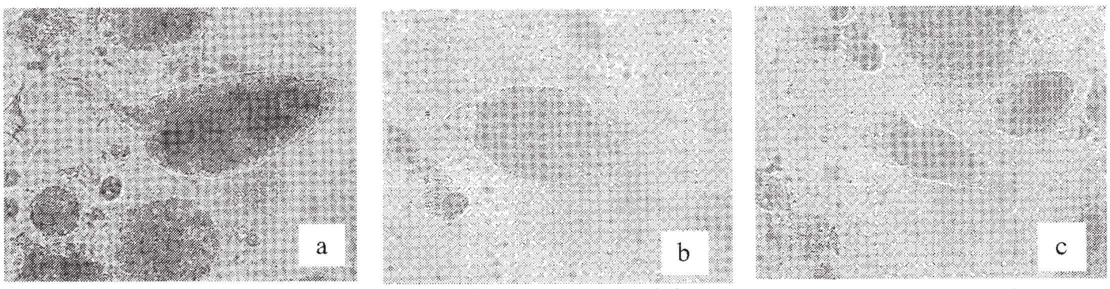

Figure 3. Three protozoan species in the hindgut of *Coptotermes* sp. (400 x, 50 µm).
(a). *P. grasii* Koidzumi, (b). *H. hartmani* Koidzumi, (c) *S. leidy* Koidzumi

The distribution and average number of protozoan fauna in hindgut of worker of *Coptotermes* sp. is shown in table 1. The total number of protozoan fauna in laboratory colonies was much higher than in the field colonies. Total number of protozoa per worker of laboratorium colony was more than 7000, while the total number of protozoa per worker of field colony was less than 2000. This numerical difference might be caused by the fluctuating activity of termites through the year (Yoshimura 1995).

Table 1. Average number of protozoa in the hindgut of worker *Coptotermes* sp.

Location	Protozoa*	Portion			Total	Total/worker
		I	II	III		
Lab	P	389	1297	667	2.353	7.533
	H	456	764	756	1.975	
	S	486	1.464	1.256	3.206	
Field	P	283	328	78	689	1.817
	H	253	150	147	550	
	S	256	150	172	578	

Note: P: *Pseudotrichonympha grasii*, H: *Holomastigotoides hartmanni*, S: *Spirotrichonympha leidyi*

This condition might be caused by the difference condition of laboratory and field. Laboratory colonies generally have shown a stable protozoan fauna throughout the year because were kept under constant laboratory condition (Yoshimura 1995).

The humidity and temperature of laboratory are relatively stabile compared with field circumstance. In this case, termites were collected in July (dry season) that has high temperature and lower humidity than other season so that this condition generate to suppress feeding activity, then finally influence the total number of protozoan fauna in hindgut.

Effect of Biotermiticide to Population of Protozoan Fauna

The following table (Table 2) illustrates the number of intestinal protozoa of *Coptotermes* sp. influenced by fungal and plant extractive treatment.

Treatment	Protozoa*	Portion			Total	Total/worker
		I	II	III		
Control	P	400	578	207	1.185	3.831
	H	434	336	328	1.098	
	S	473	575	500	1.548	
Fungal	P	470	720	267	1457	5.377
	H	634	620	406	1660	
	S	750	962	548	2260	
Extractive	P	412	695	359	1466	4.800
	H	562	542	345	1449	
	S	564	884	437	1885	

Note: P: *Pseudotrichonympha grasii*, H: *Holomastigotoides hartmanni*, S: *Spirotrichonympha leidyi*

The number of protozoan fauna treated with fungal or plant extractive do not show considerable variation, furthermore the total number of protozoan fauna treated with fungal and plant extract indicated escalation rather than untreated termites.

In the intestinal tracts of *C. formosanus* a different complex of fungi were present, some of which were antagonistic to brown rot *Gloeophyllum trabeum*. *Aspergillus flavus*, *Hypocrea virens* Chavarri, Samuels and Steward, *T. asperillum*, along with *Penicillium janthinellum* Biourge and *Cladosporium cladosporioides* (Fres.) de Vries were the fungi isolated from the guts. When these fungi were tested against *C. formosanus*, only *A. flavus* was found to be toxic to termites (Jayasimha 2002). This might indicated that termite have its defense system by having some microorganisms in its gut. Insect intestinal fluid of *Galleria mellonella* also had bacterium *Enterococcus faecium* that produced a bacteriocin and lysozyme that suppressed *Pseudomonas aeuruginosa* and some yeasts (Cole and Hoch 1991). The fermentation activities of the gut microbiota in some insects lead to the production of significant quantities of volatile acids that are thought to contribute to colonization resistance in guts of vertebrates (Rolfe 1984, Walden and Hentges 1975 in Cole and Hoch 1991) and to inhibit germination of entomopathogens (Smith and Orula 1982 in Cole and Hoch 1991) and might therefore help to prevent colonization and infection by fungi in insect guts.

Conclusion

Protozoa occurrence in termite gut were identified as *Pseudotrichonympha grasii* Koidzumi, *Holomastigotoides hartmanni* Koidzumi and *Spirotrichonympha leidy* Koidzumi.The total number of protozoan fauna of *Coptotermes* sp. collected from laboratory colony was much higher than in the field colonies. The existence of protozoan fauna in termite gut did not decrease by the fungal and plant extract treatment.

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Utilization of Soldier Defensive Secretions from Subterranean Termites *Coptotermes curvignathus* Holmgren (Isoptera: Rhinotermitidae) to Inhibition Damping-off on Pine Seed (*Pinus merkusii* Jungh et de Vriese)

by
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Abstract

Termite soldier of the genus *Coptotermes* (Isoptera: Rhinotermitidae) eject a viscous, white and sticky defense secretion. These soldier defensive secretions used to overcome their enemy. In this research, soldier defensive secretions (SDS) from *Coptotermes curvignathus* Holmgren was isolated and extracted in polar and non-polar solution and used to inhibition fungi which caused damping-off on Pine seed (*Pinus merkusii* Jungh et de Vriese). The solution used for extraction was ethyl acetate, ethanol, aquabidestilata and n-hexane. The fungi strains used are *Rhizoctonia solani*. Evaluation of inhibition growth of fungi *R. solani* is conducting with in vitro methods. The result showed that extract from ethyl acetate shown the highest inhibition zone to *Rhizoctonia solani* growth. The compound then purified by GCMS and the result showed that mainly bioactive compound is Tetradecanal $C_{14}H_{28}O$. This is the first research about possibilities of soldier defensive secretions from *Coptotermes curvignathus* Holmgren as antifungal against damping-off fungi on Pine seed.

Keywords: soldier defensive secretions, *Coptotermes curvignathus* Holmgren, damping-off, *Rhizoctonia solani*, pine seed, *Pinus merkusii*

Introduction

The demand of wood showed increase day by day. However, supply wood from natural forest showed decreased caused by over-cutting, land clearing, plantation and urban area. Plantation forest is one solution to overcome wood supply for many purpose in Indonesia. One of the woods which used on plantation forest is Pine. Pine seed almost damage by damping-off which caused by *Fusarium* sp, *Rhizoctonia* sp and *Rhizopus stolonifer*. Meanwhile the majority of termites societies possess a soldier caste which is morphologically specialized to perform a defensive function. Defense may be effected by crushing, biting, snapping, hole-plugging, rushing, squirting, defecating, and exploding or by combinations of these strategies. Soldier termites of the advanced subfamilies Rhinotermitidae, especially on genus Coptotermes, eject a viscous, white colour and sticky defensive secretions from frontal gland which act as contact insecticides (Prestwich, 1984, Lamberty *et al*, 2001, Da Silva *et al*, 2003). These soldier defensive secretions used to overcome their enemy. In this research, soldier defensive secretions from *Coptotermes curvignathus* Holmgren were used to inhibition the damping-off fungi, *Rhizoctonia solani*, on Pine seed.

Materials and methods

Collection and Isolation of soldier defensive secretions

Colonies of subterranean termites *C. curvignathus* Holmgren were obtained from dead Pine tree in Yanlappa Jasinga, Bogor, West Java and rearing on Laboratory of Forest Biology IPB (Bogor Agricultural University) for one year (*Laboratory reared termites colony)*. Soldiers were removed from the colony and the defensive secretions were collected according to Chuah *et al* (1990) methods. The soldier defensive secretions (SDS) the dilution with polar and non polar solution. The solution used is ethanol, ethyl acetate, aquabidestilata, and n-hexane. Each solution was mix with defensive secretions from 4000 termite soldiers.

Collection and Isolation of damping-off fungi

Damping-off fungi were isolation from the soil of pine plantation in Cianjur, West Java. The soil was dilution with water, and then poured to PDA medium. From collection and isolation, this research used fungi *Rhizoctonia solani* and *Fusarium oxysporum*.

Bioassay in-vitro

Bioassay in-vitro was conducted with Mori *et al* method (1997). The medium used is PDA and mix with soldier defensive secretions (SDS) extract (ethanol, ethyl acetate, aquabidestilata, and n-hexane), with concentration 10% (w/w). Fungi *R. solani* (5 day old, ∅ 6 mm) were put in the center of medium and incubation for 7 day (26°C – 28°C). The same treatment was conducted to *F. oxysporum*. The inhibition percentage is measured with formula:

$$\text{Percentage of growth inhibition} = \frac{R_1 - R_2}{R_1} \times 100\%$$

R_1 = ∅ growth of control fungi (mm)

R_2 = ∅ growth of treatment fungi (mm)

Concentration Minimum of SDS Extract to Inhibition Damping-off Fungi

From the bioassay in-vitro test, the result shows that only extract from ethyl acetate can inhibition
the growth of *Rhizoctonia solani* fungi. This evaluation is aimed to find the minimum concentration of SDS extract to inhibition the damping-off fungi. The bioassay was conducted with Mori *et al* method (1997). The medium used is PDA and mix with soldier defensive extract (ethyl acetate), with concentration 2%, 4%, 6%, 8% and 10% (w/w). Fungi *Rhizoctonia solani* (5 day old, ∅ 6 mm) were put in the center of medium and incubation for 7 day (26°C – 28°C). The inhibition percentage is measured with formula:

$$\text{Percentage of growth inhibition} = \frac{R_1 - R_2}{R_1} \times 100\%$$

R_1 = ∅ growth of control fungi (mm)

R_2 = ∅ growth of treatment fungi (mm)

Toxicity of SDS Extract on Pine seed

This evaluation aimed to find the effect of SDS extract on germination of Pine seed. Twenty-five pine seeds were diluted on SDS extract ethyl acetate with concentration 8% for one hour and then dry on filter paper. Pine seed were incubation on merang paper and treatment with 12 hour light NUV then follow with 12 hour dark for 14 day. After 14 day, the percentage of seed germination is measured with formula:

$$\text{Percentage of seed germination} = \frac{\text{Number of germination seed}}{\text{Number of total seed}} \times 100\%$$

Analysis the bioactive compound

The bioactive compound on extract ethyl acetate soldier defensive secretions was conducted with GCMS (Shimadzu), with GC : gc17a, MS : 5050qp, Column : DB5 MS.

Results and discussion

Characterization of soldier defensive secretions

Soldier defensive secretions were characterized on colour, viscosity, pH and temperature. Characterization of SDS was shown in Table 1.

Table 1. Characterization of soldier defensive secretions from *C. curvignathus* Holmgren

Solution	pH	Temperature (°C)	Viscosity (poise)	Colour
Ethyl Acetate	4	27	0,0005	White
Ethanol	5	27,5	0,00002	White
n-hexane	5,5	27	0,00003	White
Aquabidestilata	4,5	27	0,0002	Grey

Inhibition growth on damping-off fungi

Inhibition growth on damping-off fungi *R. solani* and *F. oxysporum* was conducted with bioassay in-vitro. The results showed that SDS extracts both from polar and non polar solution have no activity to inhibition the growth of *F. oxysporum.* Meanwhile on *R. solani* only SDS extract on ethyl acetate solution can inhibition the growth of fungi (Figure 1). Roseangus *et al* (2000) reported that SDS from Nasutitermes have an antiseptic role which can inhibited the growth of *Metarhizium anisopliae* fungal. The result of this research is an evidence of the possibilities of SDS from *C. curvignathus* as biopesticide.

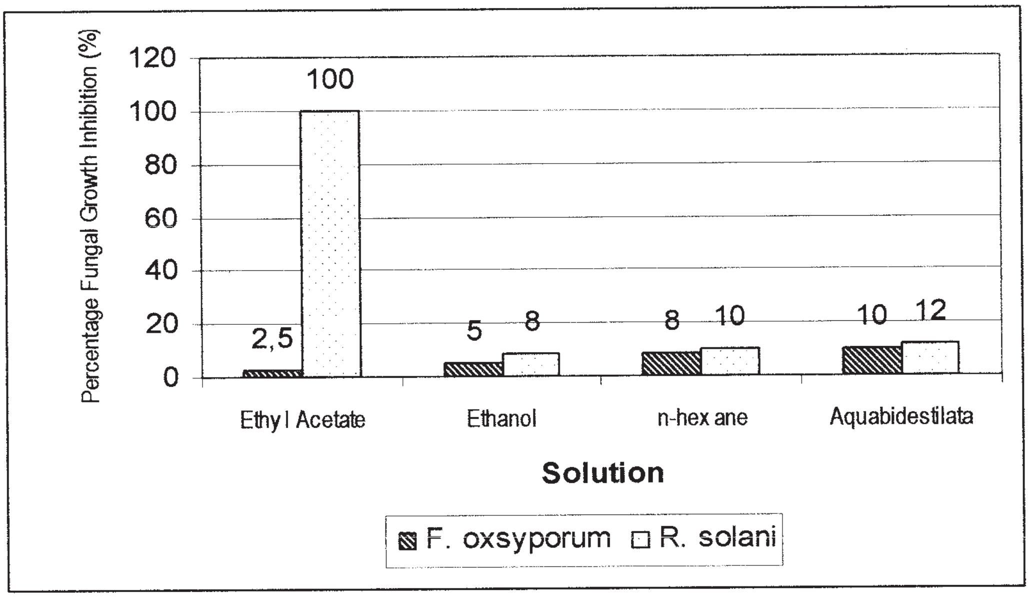

Figure 1. Inhibition growth of *F. oxysporum* and *R. solani* fungi on medium PDA with 10% extract of soldier defensive secretions *C. curvignathus*

Minimum Concentration to inhibition *R. solani* damping-off fungi

Result of evaluation the minimum concentration to inhibition the growth of *R. solani* fungi is shown in Figure 2. Concentrations 8% and 10% of extract ethyl acetate SDS can inhibition the growth of *R. solani* fungi until 100%.

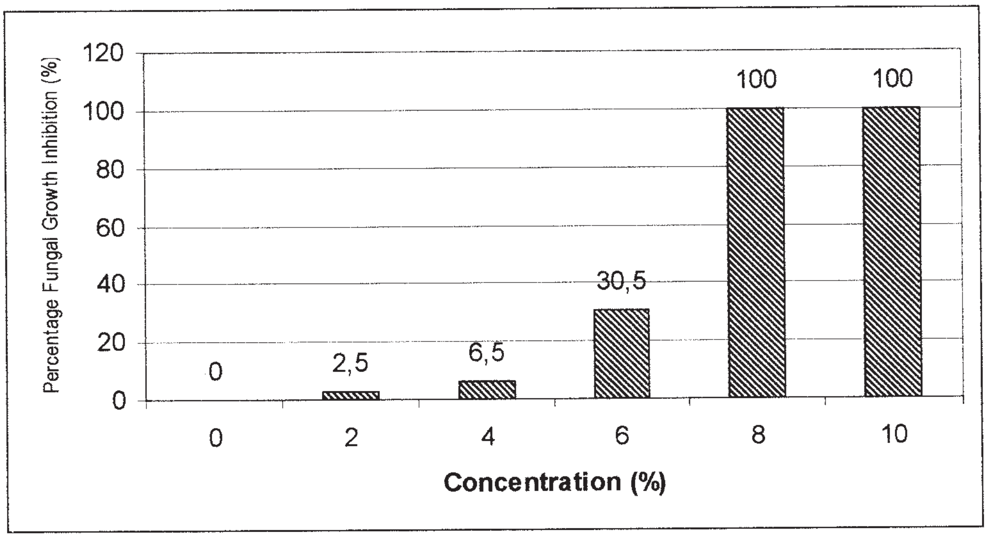

Figure 2. Inhibition growth of *R. solani* fungi on medium PDA with extract ethyl acetate soldier defensive secretions *C. curvignathus*

Toxicity of SDS Extract on Pine seed

SDS from *C. curvignathus* is non toxic and didn't influence the germination of Pine seed. Result of the evaluation on toxicity of extract ethyl acetate soldier defensive secretions on Pine seed showed that 60% of Pine seed is germination as shown on Figure 3.

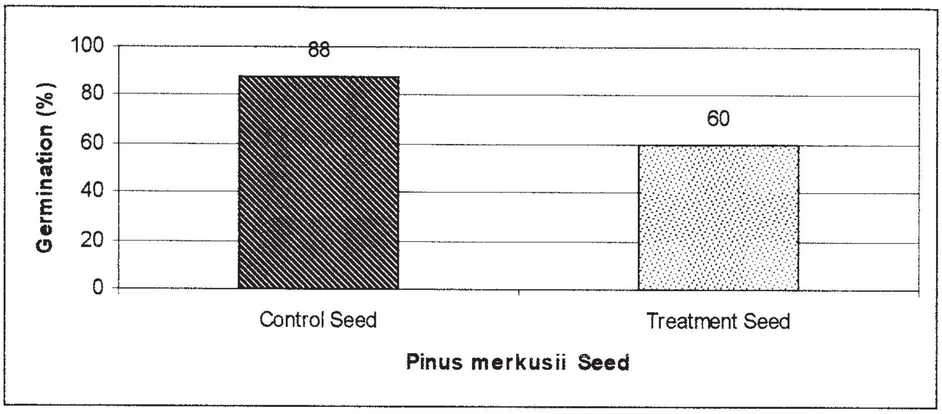

Figure 3. Germination of Pine seed after dilution on SDS *C. curvignathus* extract in ethyl acetate solution with 8% concentration for one hour

Bioactive compound of extract ethyl acetate soldier defensive secretions

Extract ethyl acetate soldier defensive secretions was analysis with GCMS. The result is shown on Figure 4. The major component of bioactive compound is Tetradecanal $C_{14}H_{28}O$ (content 60.17%).

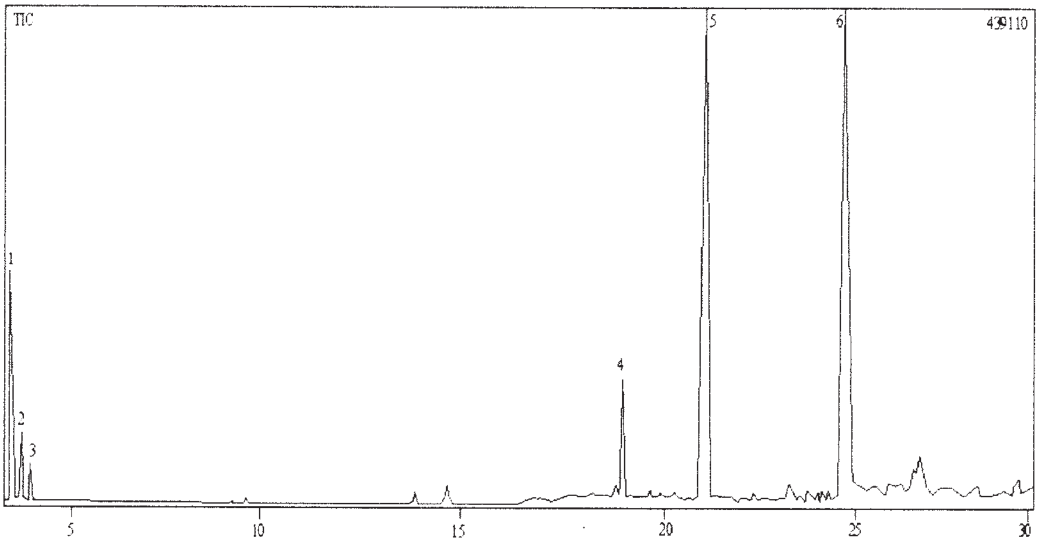

Figure 4. Chromatogram for Ethyl acetate extract of soldier defensive secretions *C. curvignathus* Holmgren

Table 2. Chemical Compound and Retention Time from Ethyl acetate extract of soldier defensive secretions *C. curvignathus* Holmgren

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

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

Conclusions

Extract Ethyl Acetate soldier defensive secretions of subterranean termites *C. curvignathus* Holmgren have strong activity to inhibition damping-off fungi *R. solani* with concentration 8%. The extract Ethyl Acetate SDS of subterranean termites *C. curvignathus* Holmgren also non toxic on Pine seed. The germination seed of Pine seed after dilution on extract Ethyl Acetate SDS of subterranean termites *C. curvignathus* Holmgren for one hour is 60%, meanwhile on control seed is 88%. The bioactive compound on extract Ethyl Acetate SDS of subterranean termites *C. curvignathus* Holmgren is Tetradecanal $C_{14}H_{28}O$.

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Aggressiveness between Colony and Species in *Coptotermes curvignathus* Holmgren and *Coptotermes gestroi* Wasmann (Isoptera: Rhinotermitidae)

by
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Abstract

Implementation of termite control method using bait system needs to consider any possible interaction among termite colonies around a building. It has been long time known that aggressive or agonistic behavior involved in this interaction. Such a behavior may vary among species, environmental condition and maturity of the colony, season, and genetic relatedness. This research was intended to evaluate aggressive behavior expressed by two *Coptotermes* species in Indonesia, *C. gestroi* and *C. curvignathus*. Bioassay conducted in this evaluation involving pairing of 10, 20, 40, and 80 termite workers (colored with red, blue, and no colored) within and between colony and between species. Mortality and consumprion data 24 hours after pairing showed that body coloring did not affect termite mortality but affected their consumption. Workers aggressiveness in pairing between colonies and species' caused higher mortality than those within colony pairing. Workers of *C. gestroi* were more active and aggressive than those of *C. curvignathus*.

Key words: Aggressive behavior, agonistic, *C. gestroi, C. curvignathus*, mortality, consumption

Introduction

Termite is one of the oldest insect groups on the planet earth. Termite fossil discovered in Amazon showed that termites have been existing on the planet earth since 220 years ago (Borror *et al.* 1996). This group of insect posses a close relationship with cockroaches. *Mastotermes darwiniensis* is a lower termites that has a lot of similarities with primitive cockroach from the family of Cryptocercitidae (Thorne, Grimaldi, and Krishna 2000). Termites are also called white ants, even though there are a lot of morphological differences between them.

Termites could digest cellulose, a natural product that they found either naturally in a forest like fallen log, leaf litter, tree trunk, humus, or after processed or manufactured like furniture, door and window frame, paper, and carton. Therefore, in natural setting termites play an important role as decomposers, but on the other hand, in urban setting, they become very destructive pests. Report from the year of 1996 stated that during the year of 1995 termites caused around 1,67 trillion rupiahs loss to the home owmer in Indonesia (Rakhmawati 1996).

Efforts to control termite problems have been done in this country especially using termiticide in form of soil treatment, wood preservation, and incorporated in cellulose baits. Control method using cellulose bait conducted by installing the station contain toxic bait in an area where termite active. In installing bait station we need to consider the number of termite colonies around that area and the possibility of interaction among them.

One possible interaction between termite colonies is agonistic or aggressive interaction. This is especially occurs between species, however in certain species the same behavior will also occur between colonies within species. King (1973) stated that agonistic behavior is a response in defensive or offensive among individual in a competition. However, there is a variation among species', colonies, or individual in expressing this behavior. Therefore, this research was aimed to assess the possibility of agonistic expression shown by two species of *Coptotermes*.

The objective of this research was to study the effect of encountering of two colonies or species of termites at the same source of food on their mortality, cellulose consumption, and other behavior. This research was also conducted to observe the effect of body coloring on termite activities and mortalities.

Materials and methods

Termite Collection

Termites used in this research were one colony of *C. curvignathus* (collected from Yanlappa Experimental Forest in Jasinga, Bogor, and already kept for about one year in the Laboratory of Forest Biology, Center for Life Sciences, Bogor Agricultural University) and two colonies of *C. gestroi* (collected from houses in Jakarta).

Termite Body Coloring

Nile blue A 0.05% (w/w) and Neutral red 0.25% (w/w) in distilled water (Su *et al.* 1991) were used to color termite bodies in order to recognize individual from different colonies or species that were paired in an arena. To color termite bodies, paper towels were dipped around 5 minutes in those dyes, squeezed them up, and fed to the termites for one week.

Bioassay

The effect of body coloring to mortality and consumption: For this bioassay, 10, 20, 40, and 80 termites (90% third instar workers and 10% soldiers) from each colony and species were kept in 10 cm petri dish lined up with 9 mm moist Whatmann filter paper at the bottom. Mortality and consumption of termites were observed after 24 hours.

The effect of body coloring on within colony aggressiveness: In this bioassay, termites within each colony at the amount of 10, 20, 40, and 80 red individuals were paired with 10, 20, 40, and 80 uncolored individuals. The same things were done with blue individuals versus uncolored ones. In a period of 24 hours, termite mortality were observed every 2 hours. After 24 hours filter papers consumed by both group of termites paired in the arena were calculated.

Aggressiveness of termite workers within and between colony within species and between species: In this bioassay, 10, 20, 40, and 80 termites were paired within colony, between colony, and between species (blue versus red, red versus uncolored, and blue versus uncolored) in 10 cm petri dishes lined up with 9 cm moist filter papers. Mortalities were observed within a period of 24 hours in the interval of 2 hours (Fisher and Gold 2003). After 24 hours, filter papers were oven dried and weighed to find the amount of termite consumption. This bioassays were replicated 3 times.

Data Analysis: Evaluation of the effect of body coloring on termite mortality and consumption were conducted in 24 treatment combination and replicated 3 times in Completely Randomized Design of Experiment with 3 factors, meanwhile evaluation of the effect of body coloring on aggressiveness within colony condcted in 16 treatment combination, and evalution of aggressiveness behavior within and between colony and between species conducted in 12 treatment combination and replocated 3 times in Completely Randomized Design of Experiment with 2 factors. Analysis of Variance (ANOVA) were analyzed with SPSS program vesion 13 and means were separated with Duncan's Multiple Range Test with $\alpha = 0.5$.

Results and discussion

The Effect of Body Coloring on Termite Mortality and Consumption

Coloring termite bodies with 0.05% natural red and 0.25% Nile blue did not affect the mortality of *C. curvignathus* and *C. gestroi* (Table 1). After 24 hours most of the termites in the arena were still alive and actively fed on the filter papers provided. Different body color did not cause the termites aggressive each other within colony. However, those two dyes seemed to affect their consumption (Table 2). Red and blue termites consumed less filter papers than uncolored ones.

Natural red and nile blue A normally used to color animal tissue specimens in the laboratory and could be toxic for living insects at certain amount. However, Su *et al.* (1991) showed that both dyes could be withheld in the body of *R. flavipes* and *C. formosanus* for 15 days without causing any mortality. These dyes could be absorbed by most of termite body parts (head, thorax, and abdomen) and form a chemical bond with body fat, thus the color couldn't taken out by faeces (Santi 2003). Both dyes affected termite consumption, because the dyes that formed a chemical bond with body fat could influence termite feeding behavior (King 2000).

Table 1. Termite mortality in the evaluation of the effect of body coloring against termite mortality and consumption

Termite colony	Color	Mortality (%) ± SE			
		10	20	40	80
C. gestroi colony 1	Red	0.00 ± 0.00 a	1.67 ± 2.89 a	1.67 ± 1.44 a	0.42 ± 0.72 a
	Blue	3.33 ± 5.77 a	1.67 ± 2.89 a	2.50 ± 2.50 a	1.67 ± 1.91 a
	Uncolored	0.00 ± 0.00 a	1.67 ± 2.89 a	1.67 ± 1.44 a	1.25 ± 1.91 a
C. gestroi colony 2	Red	0.00 ± 0.00 a	1.33 ± 2.89 a	2.50 ± 0.00 a	0.83 ± 1.44 a
	Blue	0.00 ± 0.00 a	1.67 ± 2.89 a	1.67 ± 2.89 a	1.25 ± 2.16 a
	Uncolored	3.33 ± 5.77 a	1.67 ± 2.89 a	0.00 ± 0.00 a	0.83 ± 1.44 a
C. curvignathus	Red	0.00 ± 0.00 a	0.00 ± 0.00 a	0.83 ± 1.44 a	0.83 ± 0.72 a
	Blue	0.00 ± 0.00 a	3.33 ± 2.89 a	1.67 ± 1.44 a	1.25 ± 2.16 a
	Uncolored	0.00 ± 0.00 a	3.33 ± 2.89 a	0.00 ± 0.00 a	1.67 ± 1.44 a

Mean ± SE: Means with the same letter were not significantly different using Duncan's Multiple Range Test ($\alpha = 0.05$)

The Effect of Body Coloring on within Colony Aggressiveness

Body coloring did not cause significant mortality effect on *C. curvignathus* and *C gestroi* workers when they were paired in the arenas (Table 3). Worker mortality for all pairing was less than 3%. The amount of filter papers consumed by termites was also not significantly different at any level of pairing (Table 4). These were indicated that both dyes used to color termite bodies did not affect feeding activity of termite workers when they were paired in the arenas.

Isolation parts of colony members during the process of body coloring did not affect the aggressiveness among colony members within a colony because when they were reencountered in the arena they showed docile behavior (Thorne and Haverty 1991). Research conducted by Harahap (2005) showed that separating colony members of *R. flavipes* and *R. virginicus* for 6 weeks did not convert the behavior of termite workers within a colony. Colony of *Zootermopsis nevadensis, N. angusticolis,* and *Z. laticeps* that were kept in the laboratory for more than 21 months did not show aggressive behaviors toward their nestmates (Thorne and Haverty 1989). Separating termite colony members for very long time could cause them develop new colony with secondary reproductive (Harris 1971), so when they were reencountered in an arena they will show aggressive behavior toward their ex nestmates.

Termite mortality in within colony pairing could be due to the stress condition because they were transferred into new environment (Uva *et al.* 2004). Other factor that could make termite stress, among others, are disturbance on their nest, exposing them to open air and sunlight, placing them to unfavorable environment, and confrontation against their enemies without any barrier that normally occur in the nature (Thorne and Haverty 1991).

Body coloring gave the same effect to both termite species tested in this bioassay when colored individuals were paired with uncolored ones. The differences of consumption level between termite species possibly due to a variation of their habitat and food sources in nature (Harahap *et al.* 2005). *Coptotermes curvignathus* and *C. gestroi* normally live in similar habitat so their consumption level was not significantly different.

Aggressiveness within and between Colony and between Species

Pairing termite workers within colony, between colony, and between species at any level of density did not cause significantly different in mortality (Table 5). However, further data analysis on overall mortality data, regardless of level of worker density paired, showed that mortality in different colony and different species pairing were significantly higher than within colony pairing (Table 7). This fact indicated that there were aggressiveness occurred among termite workers, even though not so obvious, when they encountered in the same arena.

Table 2. Termite consumption in the evaluation of the effect of body coloring against termite mortality and consumption

Termite colony	Color	Consumption (mg) ± SE			
		10	20	40	80
C. gestroi colony 1	Red	20.08 ± 5.09 cd	28.62 ± 1.91 defgh	29.95 ± 14.23 defgh	36.38 ± 7.61 ghijk
	Blue	19.42 ± 0.95 cd	27.48 ± 4.14 defgh	33.28 ± 5.63 efghi	50.05 ± 3.11 ijklm
	Uncolored	40.95 ± 14.33 hijk	69.02 ± 19.59 lmn	71.48 ± 27.57 lmn	85.95 ± 42.76 mnop
*C. gestroi*colony 2	Red	20.92 ± 2.15 cdef	17.05 ± 6.37 bc	20.75 ± 6.24 cde	41.35 ± 4.74 hijkl
	Blue	17.25 ± 0.44 bcd	17.75 ± 4.65 bcd	34.12 ± 5.88 fghij	41.68 ± 1.80 hijkl
	Uncolored	53.15 ± 1.97 ijklm	55.88 ± 2.12 jklm	85.78 ± 28.55 mnop	117.15 ± 11.48 op
C. curvignathus	Red	28.22 ± 6.49 defgh	21.72 ± 1.53 cdefg	34.05 ± 9.92 efghi	59.92 ± 6.00 klm
	Blue	6.75 ± 1.64 a	11.12 ± 2.39 b	14.62 ± 7.96 bc	67.92 ± 1.30 lmn
	Uncolored	57.52 ± 5.70 klm	105.85 ± 23.05 nop	141.78 ± 33.04 p	158.48 ± 89.58 p

Mean ± SE: Means with the same letter were not significantly different using Duncan's Multiple Range Test (α = 0.05)

Table 3. Termite mortality in the evaluation of the effect body coloring on within colony aggressiveness

Termite pairing	Mortality (%) ± SE			
	10 vs 10	20 vs 20	40 vs 40	80 vs 80
C. gestroi colony 1 vs colony 1	1.67 ± 2.58 a	0.83 ± 1.29 a	1.67 ± 1.02 a	1.67 ± 1.02 a
C. gestroi colony 2 vs colony 2	1.67 ± 2.58 a	2.50 ± 3.16 a	1.88 ± 7.94 a	1.46 ± 0.65 a
C. curvignathus colony 1 vs colony 1	1.67 ± 2.58 a	1.67 ± 2.04 a	1.25 ± 0.79 a	1.35 ± 1.28 a

Mean ± SE: Means with the same letter were not significantly different using Duncan's Multiple Range Test (α = 0.05)

Table 4. Termite consumption in the evaluation of the effect body coloring on within colony aggressiveness

Termite pairing	Consumption (mg) ± SE			
	10 vs 10	20 vs 20	40 vs 40	80 vs 80
C. gestroi colony 1 vs colony 1	23.45 ± 4.28 a	32.97 ± 6.90 a	42.33 ± 9.26 a	69.62 ± 8.22 a
C. gestroi colony 2 vs colony 2	26.95 ± 5.91 a	31.95 ± 8.33 a	42.20 ± 7.94 a	72.68 ± 8.09 a
C. curvignathus colony 1 vs colony 1	11.05 ± 5.30 a	20.97 ± 4.71 a	33.33 ± 8.02 a	54.68 ± 5.47 a

Mean ± SE: Means with the same letter were not significantly different using Duncan's Multiple Range Test (α = 0.05)

Table 5. Termite mortality in the evaluation of the effect body coloring on aggressiveness within and between colony and between species

Treatment	Termite pairing	Mortality (%) ± SE			
		10 vs 10	20 vs 20	30 vs 30	40 vs 40
Within colony	*C. gestroi* colony 1 vs 1	0.00 ± 0.00 a	0.83 ± 1.44 a	1.67 ± 1.91 a	1.46 ± 0.36 a
	C. gestroi colony 2 vs 2	1.67 ± 2.88 a	0.83 ± 1.44 a	1.67 ± 0.72 a	1.46 ± 0.36 a
	C. curvignathus colony 1 vs 1	1.67 ± 2.89 a	2.50 ± 2.50 a	1.25 ± 2.16 a	1.67 ± 0.95 a
Between colony	*C. gestroi* colony 1 vs 2	3.22 ± 2.88 a	5.83 ± 3.81 a	6.25 ± 2.16 a	2.29 ± 0.95 a
Between species	*C. gestroi* colony 1 vs *C. curvign.* colony 1	6.67 ± 7.63 a	2.50 ± 0.00 a	4.17 ± 4.01 a	2.71 ± 1.30 a
	C. gestroi colony 2 vs *C. curvign.*colony 1	5.00 ± 5.00 a	5.00 ± 4.33 a	9.58 ± 12.22 a	1.88 ± 1.08 a

Mean ± SE: Means with the same letter were not significantly different using Duncan's Multiple Range Test (α = 0.05)

Tabel 6. Termite consumption in the evaluation of the effect body coloring on aggressiveness within and between colony and between species

Treatment	Termite pairing	Consumption (mg) ± SE			
		10 vs 10	20 vs 20	30 vs 30	40 vs 40
Within colony	*C. gestroi* colony 1 vs 1	51.78 ± 6.15 bc	88.12 ± 9.02 def	162.65 ± 39.11 gh	191.98 ± 31.68 h
	C. gestroi colony 2 vs 2	19.02 ± 5.80 a	43.52 ± 4.71 b	40.95 ± 4.33 b	68.98 ± 7.83 cde
	C. curvignathus colony 1 vs 1	57.72 ± 11.42 bc	95.62 ± 29.99 ef	126.52 ± 3.69 fg	169.85 ± 27.06 gh
Between colony	*C. gestroi* colony 1 vs 2	66.65 ± 10.25 cde	91.92 ± 10.80 ef	123.65 ± 22.91 fg	171.58 ± 11.02 gh
Between species	*C. gestroi* colony 1 vs *C. curvign.* colony 1	45.15 ± 10.97 b	64.92 ± 14.06 cd	95.75 ± 18.93 ef	114.15 ± 23.22 f
	C. gestroi colony 2 vs *C. curvign.*colony 1	58.92 ± 9.83 bc	58.75 ± 17.33 bc	55.12 ± 12.75 bc	109.85 ± 12.72 f

Mean ± SE: Means with the same letter were not significantly different using Duncan's Multiple Range Test (α = 0.05)

Table 7. Means of overall worker mortality

Treatment	Termite pairing	Mortality (%)
Within colony	*C. gestroi* colony 1 vs colony 1	0.99 a
	C. gestroi colony 2 vs colony 2	0.41 a
	C. curvignathus colony 1 vs colony 1	1.77 a
Between colony	*C. gestroi* colony 1 vs colony 2	4.43 b
Between species	*C. gestroi* colony 1 vs *C. curvign.* colony 1	4.01 b
	C. gestroi colony 2 vs *C. curvign.*colony 1	5.36 b

Mean ± SE: Means with the same letter were not significantly different using Duncan's Multiple Range Test (α = 0.05)

Consumption level were significantly higher at the higher density level of termite workers paired (Table 6). However there was no certain pattern observed in the amount of filter paper consumed among the termite workers pairing; within colony, between colony, or between species. This fact indicated that there was no relationship between mortality and consumption.

In pairing of less density termite workers (10 vs 10 and 20 vs 20) they tended to avoid encountering non-nestmate individuals so fighting between colony or species members could be avoided. However, at the higher density levels of pairing (40 vs 40 and 80 vs 80) they did not have enough room so fighting between colony or species members were inevitable.

At the first time two different colonies or species' put together at the same arena, all individuals ran around the petri dish with random pattern. When two or more individuals from different colonies or species encountered, fighting among them were inevitable. Aggressive behavior within colony (shown by individuals with different body colors) observed after 4 hours, meanwhile mortality in different colony and species paring have been observed since 2 hours after pairing. Termite colony members have the ability to recognize their nestmates at very short time, so they will express aggressive behavior soon after they were encountered (Thorne and Haverty 1991).

Limited sources of food seemed to be the factors of aggressive behavior expressed by termite workers (Thorne and Haverty 1991). The other sources of foods for temites, besides cellulose, are weak and dead workers from the same colony (Nandika *et al.* 2003). In this bioassay, individuals in pairing within colony ate their weak or dead nestmates, even though they had different body colors. The same thing is likely to occur when these termites were paired between colony and between species. The only body part left from this cannibalism activities were worker's head.

Termites could recognize their nestmates by detecting body odor of their nestmates, cuticular hydrocarbon, and other chemical compunds contained in the body walls (Shelton and Grace 1996). Termite body odor in a colony affected by the type of food they consume. Wood eaten by a termite colony could alter chemical compunds in their bodies, and as consequence individuals from different colonies or species will show aggressive behavior against their non-nestmates when they explore same source of food (Florane *et al.* 2004). Cuticular hydrocarbon could be detected by one termite by touching their antenna to the body wall of other termites. In this bioassay termites observed to show "jerking" activity after their antenna touching the body wall of their non-nestmates. This behavior was expressed by shaking their bodies when they met individuals from different colony, any kind of moving things, and when their nests were disturbed (Howse 1970).

In pairing between species, workers of *C. gestroi* were observed more active and aggressive in attcaking workers of *C. curvignathus*. Those workers attacking their non-nestmates by chewing the body part of their enemies, starting from the abdomen. Indication of aggressiveness observed from this research was broken antenna and broken legs.

Conclusions

Body coloring did not affect termite mortality but affected their consumption. In pairing within colony, these dyes did not affect both mortality and consumption. Aggressiveness within colony, different colonies, and different species pairing were not significantly different, however termite mortality in different colonies and different species' pairing were significantly higher than those in within colony pairing and affected their feeding pattern.

Workers of *C. gestroi* were more active and aggressive than those of *C. curvignathus*. In expressing this aggressive behavior they attacked each other. Termites could recognize their nestmates by antenna touching. Some termites observed to show "jerking" behavior when they met their non-nestmates at the bioassay arena.

Tabel 6. Termite consumption in the evaluation of the effect body coloring on aggressiveness within and between colony and between species

Treatment	Termite pairing	Consumption (mg) ± SE			
		10 vs 10	20 vs 20	30 vs 30	40 vs 40
Within colony	*C. gestroi* colony 1 vs 1	51.78 ± 6.15 bc	88.12 ± 9.02 def	162.65 ± 39.11 gh	191.98 ± 31.68 h
	C. gestroi colony 2 vs 2	19.02 ± 5.80 a	43.52 ± 4.71 b	40.95 ± 4.33 b	68.98 ± 7.83 cde
	C. curvignathus colony 1 vs 1	57.72 ± 11.42 bc	95.62 ± 29.99 ef	126.52 ± 3.69 fg	169.85 ± 27.06 gh
Between colony	*C. gestroi* colony 1 vs 2	66.65 ± 10.25 cde	91.92 ± 10.80 ef	123.65 ± 22.91 fg	171.58 ± 11.02 gh
Between species	*C. gestroi* colony 1 vs *C. curvign.* colony 1	45.15 ± 10.97 b	64.92 ± 14.06 cd	95.75 ± 18.93 ef	114.15 ± 23.22 f
	C. gestroi colony 2 vs *C. curvign.*colony 1	58.92 ± 9.83 bc	58.75 ± 17.33 bc	55.12 ± 12.75 bc	109.85 ± 12.72 f

Mean ± SE: Means with the same letter were not significantly different using Duncan's Multiple Range Test ($\alpha = 0.05$)

Table 7. Means of overall worker mortality

Treatment	Termite pairing	Mortality (%)
Within colony	*C. gestroi* colony 1 vs colony 1	0.99 a
	C. gestroi colony 2 vs colony 2	0.41 a
	C. curvignathus colony 1 vs colony 1	1.77 a
Between colony	*C. gestroi* colony 1 vs colony 2	4.43 b
Between species	*C. gestroi* colony 1 vs *C. curvign.* colony 1	4.01 b
	C. gestroi colony 2 vs *C. curvign.*colony 1	5.36 b

Mean ± SE: Means with the same letter were not significantly different using Duncan's Multiple Range Test ($\alpha = 0.05$)

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Some Data on Termites Damaging Green Trees in Hanoi city

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Abstract

Researches show that out of over investigated 60 species of green trees in Hamoi city and one place of historical interest, 44 species have been damaged by termites at different levels. Eight detected species of termites that damage green trees in Hanoi are: *Coptotermes travians, C. formosanus, C. emersoni, Odontotermes angustignathus, O. hainanensis, O. proformosanus, Cryptoterme domesticus, Prorhinotermes* sp. Each of these termites can damage one or several species of trees, for example: *C. travians* attacks 7 species of trees; *C. emersoni*- 6 species; *O. angustignathus*- 6 species... Vice versa, each species of tree might be attacked by one or several species of termites such as *Khaya senegaensis Juss*: by 5 species; *Delonix regia* Raf - 4 species, *Dacotomelum duperreanum* Perr - 4 species. Damaged trees all have diameters of 0.3m or above. The highest percentage of damage belongs to following species of trees: *Celtis sinensis* Perr, *Bischofia javanica* Bl., *Hopea odorata* Roxb, *Casurina equisetifolia* Port, *Bischofia javanica* Bl, , *Erythina indica* Lamk, *Ficus benamica, Cinamomun camphora(L)* Purl. Comparing some trees concurrently available within the area of the place of historical interest with trees on the street, we can see that, for the same species of trees, those living in the place of historical interest are attacked at a higher rate, due to a more favourable environment to the termites at the place. Comparing the street green trees damaged by termites, and those damaged by other pests, we found that on the same species of tree, the termite-attacked percentage is lower. Tree-destroying termites have relations with termites attacking houses and buildings. In most cases, tree-eaters are *Coptotermes*, which often penetrate from trees and destroy neighbouring construction works. Therefore, the recognition of attacked trees are not only practically meaningful for the prevention and control of tree-termites, but also have remarkable significance to the treatment of construction works' culprits.

Key words: termite attack on living trees, *Coptotermes*, *Odontotermes*, *Cryptotermes*, *Prorhinotermes*

Introduction

With a view to assessing the damage status of green trees in the streets of Hanoi, in the past several years, we were assigned to investigate termite situation in order to provide necessary scientific data for managers in their duty of protecting street trees and preventing accidents caused by fallen/broken trees etc. This assignment is part of a municipal-level research theme on tree's pest, chaired by the Green Tree and Park Company of Hanoi, in which our Centre for the Treatment of Termites and Subsurface Defects is responsible for the termite subject. The result of such research at that time mainly presented statistic tables on the percentage of termites damaging on each species of tree, and initiated control solutions.

Now, we studied and supplemented more data, including termite status in a place of historical interest, and this report was enriched by some new information.

Materials and methods

-The investigation has been conducted on 20,000 green trees of 45 species on streets in 8 districts in the inner city of Hanoi, and 570 trees of 35 species in an important place of historical interest, and some other trees in parks. Some species of trees were investigated with limited figure, of which we have marked separately.

- The investigation was made at all trees from young newly planted to perennial ones
- Signs to determine if a tree is damaged by termites are mud tunnels, termite nests, termite individuals...

- Diameter D 1.3 means that the diameter of tree body at the height of 1.3 m from the ground surface.

Results and discussion

1 Species of trees infested and destroyed by termites: Of 45 species of street trees and 35 species of trees in one place of historical interest, the following is attacked and destroyed by termites(Table 1)

Table 1: List of termite-damaged trees in the city

No	Name	Street trees Damage percentage	Place of historical interest Damage percentage	Remarks
1	*Aphanamixis palystachya* Parker	*3.9*		
2	*Dalbergia* sp	*4.2*		
3	*Dimocarpus longan* Lour	*8.3*	*72.58*	
4	*Eucalyptus camuldulensis* Del.	*12.3*	*100*	
5	*Chucrasia tabularis*	*4.5*		
6	*Cinamomun camphora(L)* Purl	*9*	*25*	
7	*Casurina equisetifolia* Port.	*33.8*	*65.2*	
8	*Bauhimiapurpurica* L.	*2.5*		
9	*Allospondia lakonensis* Staft	*1.7*		
10	*Ficus religiosa* L.	*15.3*		
11	*Erythina indica* Lamk	*6.5*		
12	*Broussonetia papyrifera* Vent	*1,4*		
13	*Mangifera foctida* Lour	*9*	*79.5*	
14	*Delonix regia* Raf	*6.6*	*66*	
15	*Terminalia catoppa*	*4.6*	*50*	
16	*Celtis sinensis* Perr	*36.2*	*100**	
17	*Dacotomelum duperreanum* Perr	*5*		
18	*Allospondia lakonensis* Staft	*0.4*		
19	*Lagerstroemia speciosa* Fers	*0.6*	*55*	
20	*Alotonia scholaris* Br	*2.6*		
21	*Khaya senegaensis* Juss	*1.9*		
22	*Swietenia macrophylla* King.	*1.5*		
23	*Bischofia javanica* Bl	*21.2*	*50*	
24	*Hopea odorata* Roxb	*19*		
25	*Peltophorum pterocarpum* Hlyn	*0.8*		
26	*Cassia siamea* Lamk	*2.4*		
27	*Ficus elastica* Roxb	*2.3*	*66 **	
28	*Ficus benamica*	*12.5**	*100**	
29	*Nephelium lappaceum* L.		*100*	
30	*Tanarindus indica* L.		*46*	
31	*Persea americana* Mill.		*75*	

No	Name	Street trees Damage percentage	Place of historical interest Damage percentage	Remarks
32	*Spondiaspinatta*		*50*	
33	*Calamus* sp		*100*	
34	*Chrysophyllum cainito* L.		*25**	
35	*Taxodium ditichum* Rich.		*100**	
36	*Grevillea robusta* Cunn.		*100*	
37	*Salix babilonica* L.		*100*	
38	*Livistona cochinchinensis*		*82*	
39	*Roystonea regia* Cook		*69*	
40	*Ptychosperma macarthuri* H.		*100*	
41	*Cocos nucifera* L.		*78**	
42	*Pouteria sapota* H.		*60**	
43	*Bougainvillea spectabilis* Willd		*53*	
44	*Mangifera indicaL*		*100**	

Note: * indicates the trees of which the investigated number is less than 10 trees.

In fact, the number of affected species of trees are higher than 44, and the percentage of damage is likely higher than the above-shown figure, as some unclear signs of termite activities at the time of investigation were left unrecorded.

If comparing the percentage of trees on streets damaged by termites with those in a place of historical interest, we found that the percentage of damage in this place is much higher. It is because, that the environment for them is different: trees in the place are planted on natural earth ground, which is easier for termites to survive and make nests, whereas the penetration of termites is much difficult in streets where the grounds around stumps are paved with bricks or asphalt.(Table 1)

On the other hand, in parallel with our own investigation on termite damage status to trees, some other scientists made a survey too on the affect of other pest on street trees. Generally speaking, trees are much less damaged by termites than by other pests. Below is some examples (Table 2)

In many cases, a tree is destroyed both by termites and other pests, such as rots, fungi. When studying on tree's pests, professional scientists found that a tree's disease grows with the tree's age: the older the tree, the bigger its diameter is, the more likelihood the disease happens. In the meantime, in the process of investigation on termite damage on both perennial and young newly-planted trees, we found also that most of attacked trees have the D 1.3 diameter of 0.3 m up, except one tree of *Allospondia lakonensis* Staft of only several centimeters in diameter which was attacked as it was planted right on the position of another tree cut away due to be heavily damaged by termite , but its own root system was not chopped off, and the earth was not treated before planting a new tree.

The lowest percentage of damage belongs to *Peltophorum pterocarpum Hlyn* 0.8%, *Lagerstroemia speciosa* Fers 0.6%, *Allospondia lakonensis* Staft 0.4%, *Khaya senegaensis* Juss 1.9%. The highest percentage of damage belongs to *Casurina equisetifolia* Port 33.8%, *Celtis sinensis* Perr 36.2%, *Bischofia javanica* Bl 21,2%. The above percentage has a scientific and practical significance in the selection of green trees for planting on streets, places of historical remain and parks.

Table 2: Comparison of the percentage of damage caused by termites with those by other pests

No	Name	Percentage of damage caused by other pests %	Percentage of damage caused by termites %
1	*Khaya senegaensis* Juss	*43.77*	*1.9*
2	*Bischofia javanica* Bl.	*75.53*	*21*
3	*Celtis sinensis* Perr	*87.69*	*36.2*
4	*Ficus benemica*	*62.85*	*12.5*
5	*Hopea odorata* Roxb	*88.88*	*19*
6	*Eucalyptus camuldulensis* Del.	*43.47*	*12.3*
7	*Casurina equisetifolia* Port	*72.72*	*33.8*
8	*Ficus religiosa* L.	*84.21*	*15.3*

2 Termite species that damage green trees

2.1 List of damaging species detected in the course of study

In the process of study, 4 genera of termites were detected which include 8 following species:

- *Coptotermes travians*
- *C.formosanus*
- *C. emersoni*
- *Prorhinotermes sp.*
- *Odontotermes hainanensis*
- *O.angustignathus*
- *O.proformosanus*
- *Cryptotermes domesticus*

2.2 Some characteristics of termites that destroy green trees in Hanoi, and the affected trees

Coptotermes travians: This species makes nest in big trees. Their nest is a shining black porous beehive block. The nest's shape depends on the shape of the empty hole where termites build their nests. This species is the culprit that empties tree bodies most. In some cases, this species is found making their nest in warehouses, buildings and destructed the architecture. For green trees, they are harmful to the following species: *Dimocarpus longan, Delonix regia, Terminalia catoppa, Celtis sinensis, Dacotomelum duperreanum, Khaya seneggaensi, Swietenia macrophylla...*

Coptotermes formosanus: this species destroys warehouses, buildings and often makes nest in an architectural building, and also inside the tree body and is very dangerous to the trees. The termite nest is a grey or dark brown porous beehive block, the shape of which follows the hollow chamber where it stays in. For green trees, they attack the following: *Delonix regia, Celtis sinensis, Docolomelum duperreanum, Alotonia scholaris, Khaya senegaensis*

Coptotermes emersoni: This species has a small-sized individual, the nest of which often lies deeply inside the root system, sometimes even right beneath the layer of tiles around the tree's stump. For green trees, this species is caught to do harm to such trees as *Delonix regia, Terminalia catoppa, Celtis sinensis, Dacototmelum duperreanum, Khaya senegaensis, Ficus elastica.*

***Prorhinotermes* sp**: This species is not often met in Hanoi. Their nest is small, black, having few individuals. It does not cause as much damage as above three species. We can catch this species in *Mangifera indica*.

Odontotermes angustignathus: this is a fungus- growing species which can often be seen in dikes and dams of Vietnam in general and in Hanoi in particular. Their nest is located deeply under the ground. This species destroys a number of green trees, in such cases, their nest may be situated under the earth next to the tree's stump, or in hollow chamber created by other cause inside the tree's body. Places of historical remain and parks are more favourable environment for these species to reside and develop. This species attacks the following trees: *Aphanamixis palystachya, Delonix regia, Khaya senegaensis, Bischofia javanica, Hopea odorata ,Casia siamea.*

O. hainanensis: With a nest structure similar to *O. angustignathus*, *O. hainanensis* is also a fungus-growing species that often lies more deeply under the ground surface. They sometimes make nest in hollow chamber inside tree body or even right in the parterre, or in holes of architectures. It causes damages to the following green trees: *Eucalyptus camuldulensis, Dacotomelum duperreanum, Allospondia lakonensis.*

O. proformosanus: This is a fungus-growing species too, generally building hidden nest, but sometimes we can see the nest emerge from the ground surface next to the tree stump. The nest, if hypogenous, is not as deep as *O. hainanensis*' nest. The nest of this species can be also seen in empty holes in architectural buildings. They damage *Khaya senegaensis and Bichofia javanica.*

Cryptotermes domesticus: this is a dry-wood species, so cannot be found in living trees, but only in dead or dry trees.

Hence, C. travians endamages 11 species of trees; *C. formosanus* – 7; *C. emersoni* – 6; *O. angustignathus* – 7; *O. hainanensis* – 6; *O. proformosanus* – 4; *Prorhinotermes* sp – rarely; and *Cryptotermes domesticus* – only in dry trees. Generally speaking, for destroyed trees, the *Coptotermes* group is often present, except some trees where it is not yet found in, such as *Eucalyptus camuldulensis, Aphanamixis palystachya, Chrysophyllum canito, Mangifera indica.*

On the other hand, the same species of tree is mostly not only attacked by one but also by several species of termites such as *Delonix regia* is attacked by 5 termites species like *C. travians*, *C. formosanus*, *C. emersoni*, *O. angustignaths*, *O. hainanensis*; *Khaya senegaensis* is attacked by 5 species: *C. travians*, *C. formosanus*, *C. emersoni*, *O. angustignaths*, *O. proformosanus*. This fact has a scientific and practical meaning in the establishment of preventive measures to protect trees from termite attacks.

Above is the data of survey on green trees in Hanoi. Comparing it with other provinces, we can see the difference. For example: *Eucalyptus camuldulensis* in Hanoi is just detected being attacked by 2 species of termites (*O. angustignathus* and *O. hainanensis*), while in other provinces of Northern Vietnam, up to 8 species of termites are detected to damage this kind of tree: *Macrotermes annandalei, M. barneyi, Odontotermes formosanus, O. hainanensis, O. pahamensis, O. angustignathus, Microtermes dimorphus*. This distinction has a relation with the various zoogeographical characteristics of the locations. This situation shows the diversity and complicacy of green tree's termite destruction status, and result of the above research shown above is just initial inadequate data.

2.3 Relationship between tree- damaging termites and building-destruction termites

From the list of species attacking green trees of Hanoi, with the exception of 2 rarely-met species (*Prorhinotermes* sp and *Cryptotermes domesticus*), then two major damaging genera are *Coptotermes* and *Odontotermes*. While the damages caused by *Odontotermes* to constructions are not so big, the consequence caused by *Coptotermes* is really enormous. Many researches show that foraging territory of many species in *Coptotermes* group is very wide, they can forage out up to 50 m away from their nest. Meanwhile, due to a very narrow space from the street trees to the houses in Vietnam, roots of green trees on the pavement can easily go through a nearby house-foundation, making up a tunnel to lead termites to the building. Here are some examples: a building of Embassy of Czech Republic in Hanoi was heavily ruined by *C. formosanus*. By investigation, we found that this colony came from a nest inside a *Casurina equisetifolia* planted several meters away from the building. Alongside the West Lake of Hanoi, there is a road with so many *Casurina equisetifolia*, and houses and buildings along this road are heavily attacked by termites. Any example: Victoria hotel on Vo Van Tan street, Ho Chi Minh City, was damaged by *C. travians* that came from a tree on the pavement 5 meters away from the hotel.

Therefore, in the control of termites for construction buildings, the recognition of termites in trees in the surrounding environment has a very important role in practice.

Conclusions

Above is the initial data of a survey on termite damaging green trees in the city, including green trees on streets and in a place of historical interest of the Hanoi capital. These data are not yet adequate and surely should be supplemented by subsequent researches, however, they have important value in terms of science and practice in the prevention and control of termites for green trees and constructions in the city.

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Termite Damage Survey and Control on 14 Species of Street Trees at Sun Yat-sen University, Guangzhou City

by
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Abstract

A survey was conducted on termite damages on 14 species of street trees on campus at Sun Yat-sen University, Guangzhou from April to October of 2005. The results showed that all the 14 tree species were damaged to a different degree by termites. Four species of termites were identified, and they fell into 4 genera and 2 families. The dominant species included the *Coptotermes formosanus* Shiraki and *Odontotermes formosanus* Shiraki. A total of 3422 trees were surveyed, 19.43% of them were damaged by *Coptotermes formosanus*, and 10.32% by the *Odontotermes formosanus*.

Key words: Termite, street trees, damage rate, tree species, baiting system

Introduction

Guangzhou City (22°35′-23°35′N, 112°57′-114°03′E) is located in southern China, and near the South China Sea. Guangzhou is in southern subtropical area with monsoon climate, mean temperature is 21.4-21.9°C in a year, and mean yearly rainfall is 1,623.6-1,899.8 mm. Guangdong Province, where Guangzhou is located, is one of the provinces with most severe termite infestations, and has a total of 69 species of termites (Li, 2002). While the greening area in Guangzhou increases, the number of incidents of broken branches and fallen trees has been increasing because of termite damage in recent years.

Sun Yat-sen University is located in Haizhu District of Guangzhou City with an area of about 1.17 km^2. It was founded by Mr. Sun Yat-sen in 1924, and is a famous institution with advanced education in southern China. We surveyed termite damages on 14 species of street trees with an emphasis on the Sun Yat-sen Univerity campus in 2004, in order to provide suggestions and guidance for selection and planning of street trees species in Guangzhou. For the *Coptotermes formosanus* Shiraki on street trees, the control effect was satisfactory when using a baiting system.

Materials and methods

Termite damages were checked visually on trees with the diameter at breast-height above 15 cm during clear days with frequent termite activities from April to October in 2005. A tree species was identified using characteristics of leaf, flower, and fruit, and the number of trees by species was recorded. Stem surfaces, branches, tree holes and other parts of trees were inspected to find the occurrence of mud covering, mud tubes, exit holes of swarming, and other signs of termite activities. If termites were found alive, soldiers and workers of various castes were collected and stored in 75% ethanol, and the specimens were identified to species in lab.

The bait system 'Bai Ting Yi', with the active ingredient being 0.1% Triflumuron (W/W), was produced by the Guangdong Kejian Termite Prevention Co. Ltd., and used to control *Coptotermes formosanus*. 'Bai Ting Yi' was composed of attractants, lures with termiticides, and bait station. When the exit holes of *Coptotermes formosanus* swarming were found on stems, 100g lures was placed directly for feeding termites. When only mud tubes of *Coptotermes formosanus* were found, bait stations were established near the tree base, and attractants were then put in the bait stations. The bait stations were inspected once every two weeks with termite activity and death recorded. When termites were found to enter a bait station to feed on the attractants, 100g lures was then added into the bait station. Lures were refilled after over ¾ was lost from termite feeding. The bait station was serviced until termites stopped feeding lures.

Results and analysis

Termite species on street trees

Collected specimens from street trees were identified to species, and four species of termites were found, including *Coptotermes formosanus* Shiraki, *Odontotermes formosanus* Shiraki, *Macrotermes barneyi* Light, and *Reticulitermes flaviceps* Oshima.

Species and proportions of damaged trees

All the 14 species of trees surveyed were damaged by termites to a certain degree. 3422 trees were inspected, 31.41% of them (i.e., 1075 trees) were damaged by termites. The most susceptible tree species for termites was camphor tree (*Cinnamomum camphora* (L.) Presl.), 278 of 298 of them (i.e., 96.19%) were damaged. The 2nd most susceptible tree was broadleaf trees *Melaleuca leucadendron*, 67.75% of trees inspected were damaged, and all four termite species were found on this tree. *Ficus virens*, *Ficus benjamina*, and *Roystonea regia* showed the lowest proportions of damaged trees, and their infestation rate were less than 10%. Termite damages on different tree species were shown in Table 1.

Different damaged parts of street trees by termites

Coptotermes formosanus fed primarily in heartwood (part of xylem) of trees. During the inspection of trees of *Melaleuca leucadendron*, exit holes of swarming *Coptotermes formosanus* could only be found on bark surfaces from late April to early June, and it was difficult to find external signs of termite activities such as mud tubes and etc. in other times. In September of 2006, we found a stump of a *Melaleuca leucadendron* tree blown down by typhoon, the diameter of the stump was over 40 cm, but the xylem was gone due to boring of *Coptotermes formosanus*. Mud tubes of *Coptotermes formosanus* were rarely found on bark surfaces of tree species such as *Ficus microcarpa*, *Magnolia alba*, and *Bauhinia purpurea*, and only exit holes of swarming termites and frass were found on the cut surfaces of pruned branches in most cases. However, mud tubes of *Coptotermes formosanus* were frequently observed on bark surfaces of trees like *Bombax malabarcum*, *Delonix regia*, and *Cinnamomum camphora* from May to November of the year.

Nests of *Odontotermes formosanus* and *Macrotermes barneyi* were built in soil, and they damaged street trees mostly through building mud covering on bark surfaces and feeding the bark. Both species of termites did not establish nests inside trees, and therefore had little negative impacts on tree growth, but affected the looks of some places on campus negatively. *Cinnamomum camphora* was the most susceptible tree for *Odontotermes formosanus* and *Macrotermes barneyi*, and the proportions of damaged trees could be up to 67.47% and 11.42% respectively. Both termites usually built mud covering on bark surfaces of *Cinnamomum camphora* from July to October, and mud covered stem from the ground surface up to 4 m high.

Reticulitermes flaviceps could cause light damages to trees, and was only found in rotten holes at the tree base on *Melaleuca leucadendron* for occasional times. The activities of *Reticulitermes flaviceps* were usually found in rotten stumps of trees and bamboo according to our observations.

Effect of *Coptotermes formosanus* control

'Bai Ting Yi' was used to control *Coptotermes formosanus* on street trees in Sun Yat-sen University continuously from April 2006 to May 2007. The street trees were inspected again in July 2007, and the proportion of damaged trees by *Coptotermes formosanus* was reduced to 3.91% from 19.43% two years ago.

Discussion

Damage rate of street trees by termites

Coptotermes formosanus feeds primarily on xylem of trees. Therefore, determination of its damage can not depend on external signs on bark surfaces only. Since we can not inspect trees through drilling holes, and we also lack the relevant equipment, the resulting damage rate of *Coptotermes formosanus* on street trees from this investigation should be an underestimation.

The base of street trees along municipal roads is encircled by hardened concrete surfaces of sidewalk or roads, and this is not a favorable environment for nesting by soil-dwelling termites. In addition, termite behaviors on bark surfaces of street trees can be affected by pedestrians. So, termite damage rate of street trees along municipal roads should be lower than that on campus.

Species selection of street trees

Selection of street trees by the Garden and Greening Department of a city usually is based on tree shape, blooming date, growth rate and pest (insects and diseases) resistance. But the resistance of trees to termites, esp. *Coptotermes formosanus*, is rarely considered. If *Coptotermes formosanus* establishes nests in a street tree, it's possible falling in storms and will present a big hazard to pedestrians and vehicles. Therefore, tree species such as *Ficus virens*, *Ficus benjamina*, and *Roystonea regia*, which are not preferred by *Coptotermes formosanus*, should be selected when planting street trees. Susceptible trees such as *Melaleuca leucadendron*, *Cinnamomum camphora*, and *Syzygium jambos*, which grow big, and are preferred by *Coptotermes formosanus*, should be avoided.

However, common termite species can be variable in different areas. According to the investigations by Li et al. (2001), *Odontotermes formosanus* is the most common termite on garden plants in Wuhan City, Hubei Province; *Coptotermes formosanus* causes no damage to 52 species of trees except *Ginkgo biloba* (Li et al., 2001)

Methods of *Coptotermes formosanus* control on street trees

The emphasis of street tree termite control should be *Coptotermes formosanus*, and its control needs to take into consideration the following aspects:

(1) Termite inspection and prevention should be conducted before transplanting street trees.

(2) Timely inspection and control of *Coptotermes formosanus* on growing street trees is recommended in the swarming season when frequent termite activities can be easily identified using external signs.

(3) If exit holes of swarming *Coptotermes formosanus* are identified on bark surfaces, termicides can be injected directly into termite nests inside trees through the exit holes.

(4) If only mud tubes are found on bark surfaces with few termites, lure stations can be set up around tree base, and inspected periodically. When termites are found to feed on the lures, termicides can be added to the lure stations. Baiting systems are recommended, and gradually adopted in recent years. The active ingredients are mostly growth regulation hormones, and they can be transferred effectively among individuals in a colony. Therefore, satisfactory control effects of *Coptotermes formosanus* can be expected using the baiting systems.

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Table 1 Termite damages on 14 common street trees in Sun Yat-sen University, Guangzhou

species	inspected	damaged	percentage (%)	Number and percentage of damaged trees							
				Coptotermes formosanus		*Odontotermes formosanus*		*Macrotermes barneyi*		*Reticulitermes flaviceps*	
				No.	percentage	No.	percentage	No.	percentage	No.	percentage
Ficus virens	209	16	7.66	11	5.26	2	0.96	3	1.44	0	0
Ficus microcarpa L.	165	29	17.58	26	15.76	2	1.21	1	0.61	0	0
Ficus benjamina	253	17	6.72	14	5.53	3	1.18	0	0	0	0
Bombax malabarcum	130	30	23.08	10	7.69	15	11.54	5	3.85	0	0
Delonix regia	62	20	32.26	12	19.35	7	11.29	1	1.61	0	0
Magnolia alba	254	36	14.17	12	4.72	21	8.27	3	1.18	0	0
Bauhinia purpurea	379	85	22.43	63	16.62	20	5.28	2	0.53	0	0
Mangifera indica L.	153	44	28.76	17	11.11	19	12.42	10	6.54	0	0
Roystonea regia O.F. Cook	452	29	6.42	10	2.21	16	3.54	3	0.66	0	0
Caryota mitis	216	47	21.76	23	10.65	18	8.33	6	2.78	0	0
Cinnamomum camphora	289	278	96.19	60	20.76	195	67.47	33	11.42	0	0
Lagerstroemia speciosa	171	23	13.45	13	7.60	9	5.26	1	0.58	0	0
Syzygium jambos (L.) Alston	103	24	23.30	13	12.62	8	7.77	3	2.91	0	0
Melaleuca leucadendron	586	397	67.75	381	65.02	18	3.07	10	1.71	6	1.02
Total	3422	1075	31.41	665	19.43	353	10.32	81	2.37	6	0.18

Note: some trees of *Mangifera indica*, *Cinnamomum camphora*, and *Melaleuca leucadendron* were simultaneously damaged by both *Coptotermes formosanus* and *Odontotermes formosanus* (or *Macrotermes barneyi*).

Evaluations of Minimum Perimeter Treatments by Using Imidcloprid for Controlling Subterranean Termites in Selected Premises in Penang, Malaysia (*Coptotermes gestroi* Wassmann) (Isoptera: Rhinotermitidae)

by

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Abstract

To minimize pesticide use and intrusion to homeowners, we evaluate the efficacy of an innovative minimum perimeter treatment application of imidacloprid (18% w/w imidacloprid) as an IPM option in post-construction termite control. This study was undertaken to evaluate the success of an innovative approach to treat structures with non-repellent termiticides using minimum perimeter treatment. Four private houses were chosen for the research. Meanwhile one site was chosen to be as control where the house receive full perimeter treatment (conventional method). Imidacloprid treatments controlled all initial interior termite activities within three weeks and fully controlled after five weeks of treatment.

Key words: imidacloprid, minimum perimeter treatment, *Coptotermes gestroi*

Introduction

Termites or white ants, in some countries, were from the Order Isoptera. Culliney & Grace (2000) reported that there are more than 2700 species of isopteran in the world. In Peninsular Malaysia alone, there are about 175 species of termite from 42 genera (Tho, 1992). Termites can be classified under three main groups; dampwood termites, drywood termites and subterranean termites. The most destructive is subterranean termites and termite control is generally focused only on to this group (Abdul Hafiz & Mohd Hadzri, 2007).

In addition, subterranean termite is a cryptic insect. Knowledge and understanding of their biology, foraging area and the ecology help in their detection and control. Additionally, the choice of termiticide and the proper way in termiticide application technique are important to ensure the job efficacy. Recently, some of the pest control companies are proposing minimum internal drilling liquid termiticide application method. Normally, the traditional application technology associated with soil barrier treatment is destructive to property owners and needs the use of a high amount of finished product because it specifies a thorough and uniform application to the interior and exterior of the building by drilling holes through the slab near to the beam foundation (Potter and Hillery, 2003; Hu and Hickman 2006; Abdul Hafiz & Abu Hassan, 2006; Abdul Hafiz *et al*, 2006; Abdul Hafiz *et al*, 2007).

Materials and methods

Site 1 (Mengkuang), Site 2 (Gelugor), Site 3 (Kg. Guar Perahu), Site 4 (Bayan Lepas) receive minimum perimeter treatment. Meanwhile, Site 5 (Taman Rupawan) internal and external perimeter of the wall was fully treated with imidacloprid. The structures were chosen base on the following criteria

1. Slab on grade construction
2. Clear evidence of an interior infestation *of Coptotermes* spp. on the structures. (This criterion was met after confirming the presence of any one of or a combination of exposed structural damage, exit holes, mud foraging tubes, foraging territory and population estimation)
3. The owner agreed to a reasonable access to both the interior and exterior of the structure for the duration of the study.

Effectiveness of tested termiticides on termites was measured by the amount of materials (pine billets or tissue paper) in the monitoring stations eaten by the colony. In all trial sites the amount of food consumed by termites were weighed biweekly before and after treatment.

Infested blocks from the underground monitoring stations of the chosen sites were brought to the Medical Entomology Laboratory, School of Biological Sciences, Universiti Sains Malaysia every two weeks and carefully disassembled. Termites were removed by gently tapping the stakes over a plastic tray. The termites were separated from the remaining debris by allowing access to a stack of five pine blocks (20 cm x 10 cm) that had been soaked in water for 24 hours. The termites were allowed to aggregated in the stacks of pine blocks within 4-6 h and were removed and weighed (Tamashiro *et al.*, 1973). All woods were washed and oven-dried for 48 hours at 80 ° C and weighed to compute the wood consumption before and after feeding (Sajap *et al.*, 2000, Abdul Hafiz & Mohd Hadzri, 2007).

Meanwhile, for the above ground monitoring station, the weight of the toilet roll tissue paper was predetermined before being feed by the termites. They were reweighed biweekly. The infested toilet roll tissue paper was then brought back to the laboratory, School of Biological Sciences, Universiti Sains Malaysia. The infested toilet tissue paper was unrolled and cleaned by hand and the termites were separated. Then it was oven-dried for 48 hours at 50°C and reweighed to compute the amount toilet roll tissue paper consumed before and after feeding (Sajap *et al.*, 2000, Abdul Hafiz & Mohd Hadzri, 2007).

Sites 1, 2, 3, and 4 were treated with minimum perimeter treatment technique with imidacloprid (Fig.1). In this technique, the structure was treated with 0.05% imidacloprid solution by conducting minimum exterior perimeter wall plus limited interior perimeter wall of building treatments in the selected infested area where the termite are forage. The technique was modified to the current practice used by the "Pest Control" company called as "Conventional Corrective Treatment". The main purpose of this treatment specifications was to minimize treatment by minimize drilling through concrete slabs. Site 1 was treated in week 13. For Site 2, the treatment was conducted in week 16. Site 3 was treated in week 35 and Site 4 was treated in week 32.

As comparison Site 5, (Taman Rupawan) was fully treated interior and exterior perimeter of the wall where the current practice was used by the pest control applicators. The site was treated in week 14. Holes were drilled around the perimeter wall of the buildings and the distance between each drilled hole was 45 cm. The solution of imidacloprid was diluted in the ratio of 1:400 (0.05%) in the 200 liter drum. Five liter solution was injected using an injector slab with a flow meter attached to it into each. For the minimum control using imidacloprid, holes were drilled either at the exterior or the interior or at both places in the limited infested area where the termite forage. The sites were not given full external and internal perimeter treatment.

Results and discussion

Site 1 (Mengkuang), the total of 64 holes were drilled. Nevertheless, after the treatment in week 13, the population was still active with mean wood consumption of about 25g. Five weeks later no termites were detected in any of the underground monitoring station (UMS) planted around the building. The total amount of mean feeding consumption before the treatment was 58.66 g. However, a total of mean food consumption after the treatment was 4.48 g before the termite consumption began to cease (Fig.1)

In Site 2 (Gelugor), the total of 75 holes was drilled. Two weeks after the treatment, the wood feeding consumption by the termites decreased as shown by the aboveground and underground monitoring stations observed. By the week 18^{th}, 4 weeks after the treatment there was no longer food consumption in all the four underground monitoring stations and the two aboveground monitoring stations. The total of mean feeding consumption before the treatment was 85.1 g. However, the total of mean food consumption after the treatment was 5.08 g before the termite feeding activities ceased (Fig. 2).

In Site 3 (Kg.Guar Perahu), the total of 56 holes was drilled. One week after the treatment the consumption rate decreased for the above ground monitoring station. Eventually, three weeks after the treatment (week 40), the feeding activity completely stopped in all above ground monitoring stations. The total of mean feeding consumption before the treatment was 9.56 g. Meanwhile a total of mean food consumption after the treatment was 6.39 g before the termite feeding activities completely stopped (Fig.3)

In Site 4 (Bayan Lepas), the total of 56 holes was drilled. Two weeks after the treatment (week 34), the food consumption decreased. On the week 36, and 38 weeks the food consumption of the termites decreased and later ceased feeding (Fig.4). The termite feeding consumption ceased on the week 40. The total of mean termite feeding consumption before the treatment was 17.94 g. Meanwhile a total of mean food consumption after the treatment was 5.41 g before the termite consumption ceased.

Site 5 (Taman Rupawan), the total of 271 holes was drilled. Two weeks after the treatment on week 16, the amount of food taken by the termites decreased in the above ground monitoring station (Fig.5). Meanwhile, for the underground monitoring stations there was a slight increase in the food consumed. However, 4 weeks after the treatment (week 18th), no feeding activity was detected in from all the above ground monitoring station. In underground monitoring stations, termites feeding had decreased completely. On week 20th, six weeks after treatment, the termites stopped eating in all aboveground and underground monitoring stations (Fig.5). Interestingly, from this treated site, the underground monitoring station number four was located at house number one (untreated). Meanwhile the treated house was at house number three. Therefore, from the result showed that there was a transfer effect from the treated to the untreated house where from the foraging study it was confirmed that only one termite colony was found at this site. The total of mean feeding consumption before the treatment was 77.32 g. Meanwhile a total of mean food consumption after the treatment was 66.93 g before the termite consumption ceased away.

According to Potter and Hillery (2003), it is believed that the application of repellent termiticide can be more destructive to property due to the traditional application method. With the new non-repellent termiticide in the market, the termiticide can be applied as minimum as possible solely around the exterior building perimeter plus limited interior, especially at the termite infestation area. The method is practice by some of the pest control due to the unique character of the termiticide. The delayed mode of action permits transfer of this toxicant from exposed termites to unexposed nest mates through social interactions including mutual grooming, thus causing secondary mortality in subterranean termite population (Potter & Hillery 2003; Hu & Hickman 2006; Gurbel *et al.* 2006; Abdul Hafiz *et al.* 2007a, b). From the result, all the sites that received minimum perimeter treatment controlled termite

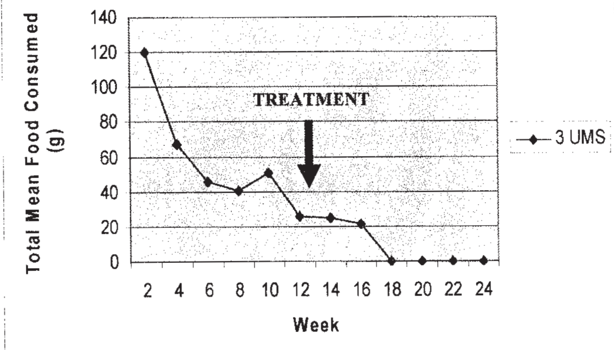

Figure 1: Mean food consumption before and after treatment at Site 1 (Mengkuang)

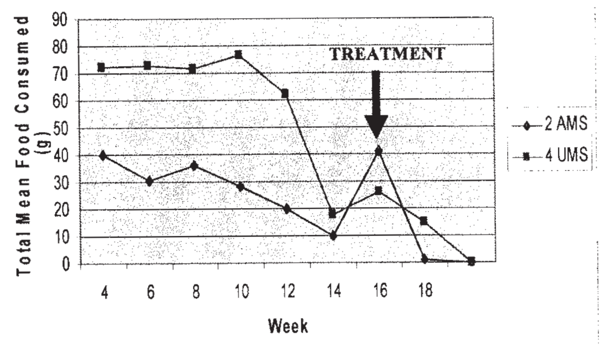

Figure 2: Mean food consumption before and after treatment at Site 2 (Gelugor)

successfully within 3 weeks to 5 weeks. Meanwhile Site 5 (Taman Rupawan) that received full treatment successfully controlled termites within 8 weeks. Therefore, those sites that used lesser amount of imidacloprid solution for minimum perimeter treatment controlled successfully termite infestation than the site which used more imidacloprid solution for full perimeter treatment. Thus, with the innovative in perimeter treatment (minimum internal and minimum external perimeter treatment) reduced time and amount of termiticide resulted into economic benefit that can allow the pest management professionals to charge less to property owners for termite treatments. Additionally, environment hazards are also minimized with less usage of termiticide.

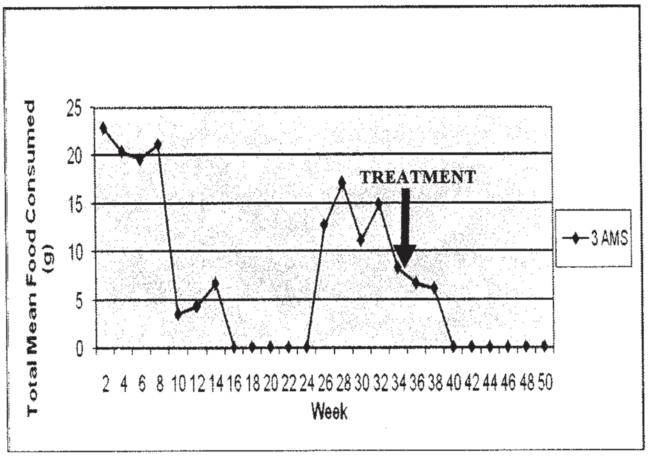

Figure 3: Mean food consumption before and after treatment at Site 3 (Kg.Guar Perahu)

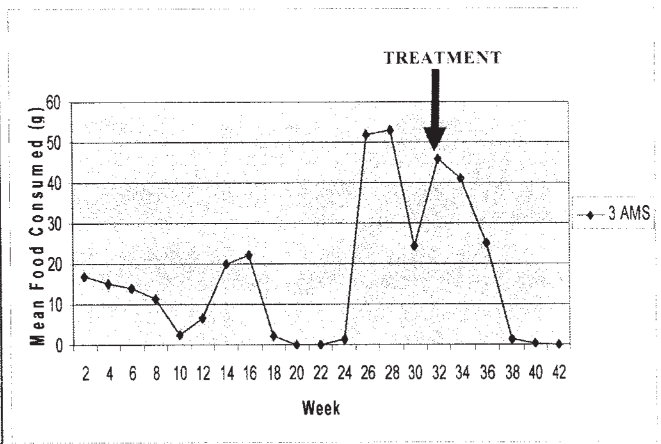

Figure 4: Mean food consumption before and after treatment at Site 4 (Bayan Lepas)

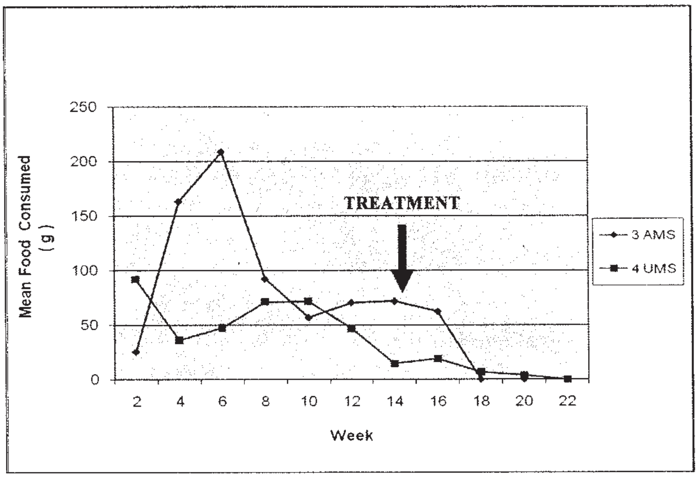

Figure 5: Mean food consumption before and after treatment at Site 5 (Taman Rupawan)
UMS = Underground monitoring station
AMS = Above ground monitoring station

Conclusions

The studies show that after the treatment the colony will ceased within 3-5 weeks. As a conclusion from this study demonstrates that the minimum perimeter treatment minimizing the termiticide usage, less number of holes on the floor, less chemical & labor cost and more time saving. More important is less chemical into the environment but still provide effective and longer protection where it is a good option to pest control.

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A Laboratory Study on the Transfer Effect of Some Termiticides by Subterranean Termite *Coptotermes gestroi* (Isoptera: Rhinotermitidae)

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Abstract

The transfer effect of three slow-acting termiticide, Fipronil formulated as AGENDA ™ 2.5EC, Imidacloprid formulated as PREMISE 200SC® and Indoxacarb formulated as STEWARD SC, were tested under laboratory condition among workers of subterranean termite, *Coptotermes gestroi*. The concentrations which were tested were (0.001, 1.00, 10.0, 100.0, 1000.ppm for each termiticide. The donor and recipient ratio were either (10:50 or 1:5). The donor termites were exposed to above termiticide of different concentrations for 24 hours. The transfers of lethal concentrations from the donors to the recipients were observed on the mortality of recipient termites.

Key words: Slow-acting, Termite, Termiticide, laboratory, Malaysia

Introduction

The cost of termite control in Malaysia was estimated at a US$ 10-12 million (Lee, 200b; Ngee *et al.*, 2004) and 90% of the infestation on structure and buildings were causeed by several species of *Coptotermes* (Lee, 2002a). Subterranean termite *C.gestroi* (Wasmann 1896) (Rhinotermitidae: Isoptera) is very considered a pest species in South East Asia and Brazil and causes more damage in these countries (Kirton and Brown, 2003; Costa-Leonardo *et al.*, 2004). New generation termiticides which contain *Imidacloprid*, *Fipronil*, and *Indoxacarb* as their active ingredient were used as soil – applied or by direct injection in the colony, posed lethal effects to subterranean termite (Ramakrishna *etal.*,2000; Osbrink, 2001; Kamble and Davis, 2005; Ping *et al.*, 2005) . The advantages of these new termiticide are slow-acting and non repellent. Thus termite can not detect these termiticide when apply on soil or when use in laboratory on filter paper. The transfer effect of *Imidacloprid, Fipronil*, and *Indoxacarb* among termite workers was studied by researchers, and the results showed that the termite ability to transfer termiticide from the treated termites (donors) to the untreated termites (recipients). (Ferster *eta*l., 2001; Thorne and Berisch, 2001; Delgarde and Rouland-Lefervre,2002; Parman and Vargo, 2005; Tomalski and Vargo,2005; Ping *et al.*,2005; Shelton *et al.*, 2006). In this study, the transfer effect of *Imidacloprid, Fipronil*, and *Indoxacarb* was tested among subterranean termite, *C. gestroi* and compared the transfer efficacy of concentrations from exposed termite to unexposed termites.

Materials and methods

Subterranean termites *C.gestroi* were collected from the under ground monitoring stations, which were established in / out side USM Campus. Pine stakes (*Pinus cariba* (2.5x2.5x15 cm) were used and placed in the hollow plastic containers (16x18 cm). These plastic containers were buried at a depth of high size of it (18 cm) below the ground. After stakes being infested (7-14day) by the termites-depend on colony active, the infested stakes were brought back to the Entomology laboratory. The termites were removed from stakes as described by Tamashiro *et al.*, 1973). Healthy and active of third instars of *C.gestroi* were chosen in this study to test transfer effect between termites.

Blue termite

Approximately 1000 worker were stained with 0.1 %(wt /wt) Nile Blue A by forced feeding of stained filter paper (Whatman No.1, 9.0cm dimater) for 4-7days. The termites were colored blue and, used as the donors.

Treated filter paper

The filter papers were treated with concentrations (1, 10, 100, 1000. ppm) with Premise ® SC 200 (Active ingredient: Imidacloprid 18.3% wt/wt) Agenda ™ 2.5 EC (Active ingredient: Fipronil 2.92% wt/wt) and Steward (Active ingredient: Indoxacarb14.5% w/w). Blue termites were directly introduced into Petri dish and allowed to crawl on the treated filter paper for 4 hours under laboratory condition.

Donor (10) + Recipient (50)

After that 10 blue termites (donors) were removed and reintroduced into new Petri dish containing fifty untreated worker termites called (recipients) with untreated filter paper. Each concentration replicated 5 times. Daily observations were recorded until experiment end. Recipient (untreated termite) mortality was used as evidence to provide that the transfer of lethal concentrations occurred among termite. Data analysis: Termite mortality examined by analysis of variance (ANOVA) at 0.05.

Results and discussion

The results are indicated that the transfer of lethal concentration from the donor termites occurred among termites (recipient), and there are significant differences among treatments for each termiticide against subterranean termite *C.gestroi* (Table 2). Percentage of recipient mortality and lethal time was fast in fipronil concentration (28.4, 35.6, 43.6, and 51.2) for 1, 10,100 and 1000 ppm respectively than Imidacloprid (0.0, 13.6, 14.8, 22) and than Indoxacarb (11.6, 24, 30, 37.2) after 48h (2days) after treatment. These results were similar to that of Shelton and Grace (2003). Fipronil was affecting faster on the termite mortality than Imidacloprid, and these results were similar to those indicated by Henderson (2003 a & b). The donors termite were affecter faster by lethal concentration than recipient(Table 1), this because the donor termites exposed directly on termiticide than recipient, thus there are significant differences between donor mortality and recipient mortality accepted Fipronil concentration. The advantage (slow- acting is very important to the success of transfer termiticide from donor termite to recipient termite (Thorne and Berisch, 2001). The percentage of recipient mortality was significant between concentrations; at high concentration (1000 ppm) mortality percentage increase. (22, 37.2, 51.2) for Imidacloprid, Indoxacarb, and Fipronil, respectively after 2 days. So the transfer effect of lethal concentration may occur but in limited area of foraging termites. Generally the transfer effect or the transfers of lethal concentration possibility occur successfully at 10 and 100 ppm during termite grooming or by trophallaxis, in the laboratory as (Sheltom and Grace, 2003).

Conclusions

Termite ability to transfer chemicals (termiticides) to the nestmates in the colony: The transfer effect of the chemicals will be more successfully at low concentrations. If we treat limited area or few holes (1-2) in the field of one colony of termite, it will eliminate the termite (*Coptotermes gestroi*) colony by transfer effect.

With the use of chemicals in this study at low concentrations, termite behavior (walking, grooming, trophallaxis) is not affected by the termiticide promptly, and termites can move around in the colony and the nest mates get contaminated and died later on.

Table 1. Percentage mortality and survival of donor termites.

Treatment	Number		1day		2day		3day		4day		5day		6day	
Imidacloprid	N	%	Mor %	Sur %	Mor %	Sur %	Mor %	Sur %	Mor %	Sur %	Mor %	Sur %	Mor %	sur %
0	250	100	0	100	0	100	0	100	0.8	99.2	1.2	98	0	98
1	250	100	0	100	0	100	16.4	83.6	28	55.6	44.4	11.2	11.2	0
10	250	100	0	100	13.6	86.4	21.6	64.8	36	28.8	28.8	0	0	0
100	250	100	2	98	14.8	83.2	22.8	60.4	35.2	25.2	25.2	0	0	0
1000	250	100	6	94	22	72	28	44	44	0	0	0	0	0
Fipronil														
0	250	100	0	100	0	100	0.8	99.2	0	99.2	0.4	98.8	0	98.8
1	250	100	11.2	88.8	28.4	60.4	32.8	27.6	27.6	0	0	0	0	0
10	250	100	26.8	73.2	35.6	37.6	28.8	8.8	8.8	0	0	0	0	0
100	250	100	38.8	61.2	43.6	17.6	17.6	0	0	0	0	0	0	0
1000	250	100	48.8	51.2	51.2	0	0	0	0	0	0	0	0	0
Indoxacarb														
0	250	100	0	100	0	100	1.2	98.8	0.4	98.4	0	98.4	0.4	98
1	250	100	0	100	11.6	88.4	23.2	65.2	31.6	33.6	33.6	0	0	0
10	250	100	11.2	88.8	24	64.8	34.4	30.4	30.4	0	0	0	0	0
100	250	100	24.8	75.2	30	45.2	34.8	10.4	10.4	0	0	0	0	0
1000	250	100	29.2	70.8	37.2	33.6	33.6	0	0	0	0	0	0	0

Table 2. Percentage mortality and survival of recipient termites.

Treatment	Number		1day		2day		3day		4day		5day		6day	
Imidacloprid	N	%	Mor %	Sur %	Mor %	Sur %	Mor %	Sur %	Mor %	Sur %	Mor %	Sur %	Mor %	sur %
0	50	100	0	100	4	96	0	96	0	96	2	94	0	94
1	50	100	0	100	6	94	38	56	44	12	12	0	0	0
10	50	100	14	86	34	52	38	14	14	0	0	0	0	0
100	50	100	18	82	34	48	30	18	18	0	0	0	0	0
1000	50	100	28	72	40	32	32	0	0	0	0	0	0	0
Fipronil														
0	50	100	0	100	0	100	4	96	0	96	0	96	2	94
1	50	100	26	74	28	46	42	4	4	0	0	0	0	0
10	50	100	36	64	34	30	30	0	0	0	0	0	0	0
100	50	100	50	50	50	0	0	0	0	0	0	0	0	0
1000	50	100	78	22	22	0	0	0	0	0	0	0	0	0
Indoxacarb														
0	50	100	2	98	0	98	0	98	4	94	0	94	0	94
1	50	100	18	82	24	58	24	34	34	0	0	0	0	0
10	50	100	16	84	22	62	28	34	34	0	0	0	0	0
100	50	100	22	78	32	46	32	14	14	0	0	0	0	0
1000	50	100	32	68	34	34	34	0	0	0	0	0	0	0

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Durability of Treated *Eucalyptus camaldulensis* Dehn. Wood against Subterranean Termite in Field Test

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Abstract

Eucalyptus camaldulensis Dehn. roundwoods were treated with CCA and CCB by using three preserving methods; soaking , hot and cold bath, and vacuum-pressure method before subjection to field test for proving their durability against subterranean termite by using choice test method. This experiment was conducted in two conditions; above-ground and in-ground condition within 30 months exposure period. Results revealed that, the treated roundwoods showed better performance, very durable and durable to the attack of subterranean termite, than the untreated ones in both conditions. For the untreated roundwoods, their durability was classified as perishable in the in-ground condition, and non durable in the above-ground condition. Types of wood preservatives and preserving methods of treatment indicated no significant difference in durability improvement of treated roundwoods within 30 months.

Key words: *Eucalyptus camaldulensis*, treated roundwood, water borne wood preservatives, subterranean termite resistance

Introduction

Because of logging ban in 1989, wood production in Thailand was shifted from natural forests to planted forests. *Eucalyptus camaldulensis* Dehn. is one of the most famous fast growing species planted in Thailand. Eucalyptus wood is versatile and can be used for multiple purposes including pulpwood, plywood, particle board, posts, fuelwood, and sawnwood. Thinning small logs from *E. camaldulensis* plantation, especially during 3-5 years, have to be done for optimum management for higher economic yields.

Because of small in diameter and unreasonable price, these thinned logs are always judged as waste, or only making charcoal and firewood. To optimize wood utilization, these thinned logs can be manufactured for solidwood products such as furniture, or for construction, which will take more benefit.

Termites, nowadays, are still playing an important role in destroying wood components and wood products in tropical region, particularly subterranean termites. The fast growing wood, like thinned *E. camaldulensis* wood, generally contains high percentage of sapwood, is reported that eases to damage by wood destroying organisms, especially by termite.

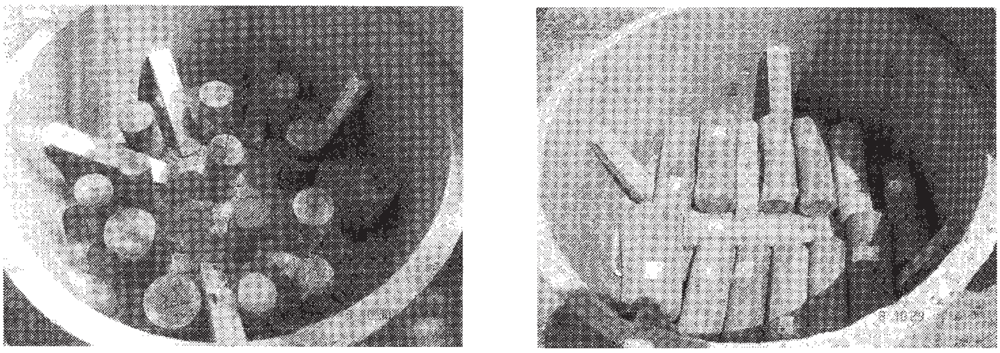
Therefore, to overcome these problems, this experiment was set in order to find out techniques to prolong durability of *E. camaldulensis* thinned wood against subterranean termites and to expand for diverse and optimum utilization of these thinned wood .

Materials and methods

Treated wood specimens: Five years air-dried of *Eucalyptus camaldulensis* Dehn. roundwoods from Burerum province, the north-eastern part of Thailand , were cut to 25 cm. in length and treated with two types of water borne wood preservatives; CCA and CCB, by using three processes of treatments; soaking, hot & cold bath and vacuum-pressure. The retention of each roundwood specimen was also evaluated and then air-dried to 20 percents of moisture content.

Test methods: This field test was carried out in Khonkhan province, also in the north-eastern part of Thailand. The treated roundwood specimens, comparing with the untreated ones (control) were tested for subterranean termite resistance in two conditions; above-ground and in-ground condition by using choice test method. There were 5 replications in each condition arranged in randomized completely block design (Anantachai, 1995).

Above-ground condition: A circular cement tube (80 cm. in diameter and 60 cm. in height) was installed on the planned ground and thickened the ground inside with 5 cm. of sand. Cement blocks were put horizontally on the sand in the center of the circular cement tube. Each group of treated roundwood specimens and control (untreated) were laid on the cement blocks. Rubberwood (5 X 2.5 X 30 cm.), using as bait, were leaned on the wall of the circular cement tube. Closed the cement tube with a lid (Figure 1).



A) In - ground condition B) Above - ground condition

Figure 1. Field test for durability of water borne treated *E.calmaldulensis* roundwoods against subterranean termites.

In - ground condition: For setting up the in-ground durability test, this condition was similar to the above – ground test, except no cement blocks. Each group of treated roundwood specimens and control (untreated) were vertically 5 cm. submerged in the sand inside the circular cement tube.

Inspection and Evaluation: All of roundwood specimens were inspected and visual rated on percentage of damage caused by termite in every 6 month. Results were classified for level of termite resistance (durability) according to the rate as shown below (Sornnuwat, 1996 a).

Percentage of damage	Visible attack on wood	Classified durability
0	None	Very durable (VD)
1-15	Hardly visible damage	Durable (D)
16-40	Superficial and slightly damage	Moderately durable (MD)
41-75	Moderately damage	Non durable (ND)
> 75	Heavy damage	Perishable (P)

Results and discussion

Termites: *Microcerotermes crassus* was mainly found attacking untreated wood specimens (control) in both conditions, meanwhile, *Macrotermes gilvus* had damaged those in some replications of the in – ground condition test. These results corresponded with Sornnuwat (1996 b) which had reported that *Microcerotermes crassus* was the most popular specie which always found in rural area of Thailand.

Untreated wood: Percentage of damage of untreated roundwood specimens caused by termite were shown in Figure 2. Results proved that the untreated ones in the above-ground condition presented better resistance to termite attack than the in-ground condition. In the in-ground condition test, *E. calmaldulensis* untreated roundwoods was classified as non durable in 12 months, while, the above-ground condition could be extended to 30 months.

Treated wood: In the period of 30 months exposure (Table 1 and Table 2), the treated roundwood specimens indicated excellent durability in both conditions. They were classified as very durable level for CCA preservative treatments and durable level for CCB preservative treatments. Since, period of exposure to subterranean termite in this experiment was quite short to identify which treatment methods would perform the best, it needs a long time to continuous closely inspection. According to its highest retention obtained in wood specimens, however, it could intended to state that vacuum–pressure treatment method would show better durable to the attack of subterranean termite than hot & cold bath and soaking methods (Eaton and Hale, 1993).

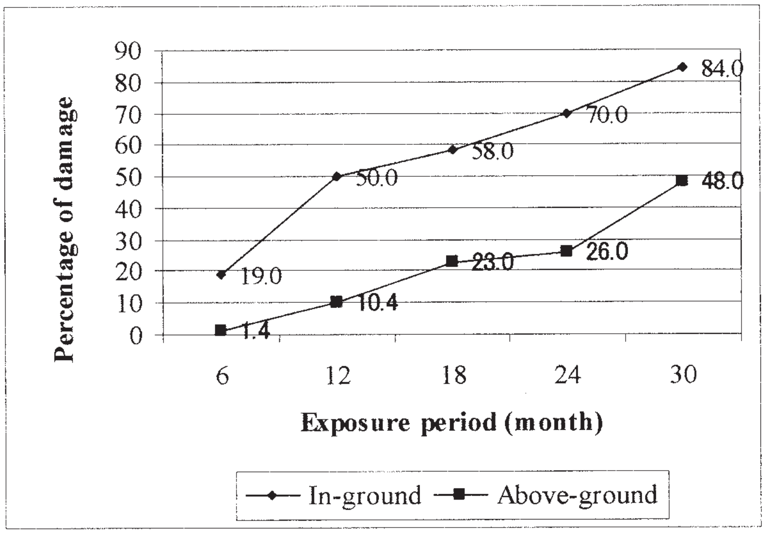


Figure2. Average percentage of damage of untreated roundwoods (control) in "in-ground" and "above-ground" conditions.

Table 1. Percentage of damage of *E. camaldulensis* roundwoods in "above-ground"condition within 30 months exposure.

Wood preservatives	Treatments	Retention (kg/m^3)	Damage (%)	Classified durability
CCA	Soaking	4.7	0.0[1/] a[2/]	VD
	Hot and Cold Bath	6.2	0.0 a	VD
	Vacuum-Pressure	14.3	0.0 a	VD
CCB	Soaking	4.3	2.0 a	D
	Hot and Cold Bath	6.5	1.0 a	D
	Vacuum-Pressure	12.7	0.2 a	D
Control (none)	None	-	48.0 b	ND

1/ = Mean of 5 replications.
2/ = Mean followed by the same letter are not significantly different at 95% level of confidence.

Table 2. Percentage of damage of *E. camaldulensis* roundwoods in "in –ground" condition within 30 months exposure.

Wood preservatives	Treatments	Retention (kg/m^3)	Damage (%)	Classified durability
CCA	Soaking	4.5	0.0[1/] a[2/]	VD
	Hot and Cold Bath	6.6	0.0 a	VD
	Vacuum-Pressure	14.4	0.0 a	VD
CCB	Soaking	3.6	8.2 a	D
	Hot and Cold Bath	6.8	0.1 a	D
	Vacuum-Pressure	12.0	0.4 a	D
Control (none)	None	-	84.0 b	P

1/ = Mean of 5 replications.
2/ = Mean followed by the same letter are not significantly different at 95% level of confidence.

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Resistance of Smoked Wood to Termite Attack

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Abstract

Mindi wood (*Melia azedarach*) and sugi wood (*Cryptomeria japonica*) were smoked during four hours using mangium (*Acacia mangium*) wood. Smoked and control woods were tested to (1) dry wood termite (*Cryptotermes cynocephalus* Light) in laboratory, (2) subterranean termite in the field or graveyard test, and (3) subterranean termite (*Coptotermes curvignathus* Holmgren) in laboratory. Wood sample sizes for smoking process were 0.8 cm by 2 cm in cross section by 5 cm in longitudinal direction for dry wood termite test, 20 cm for graveyard test, and 2.5 cm for subterranean termite test. Replication of the wood sample was ten for graveyard test and five for the other tests. The results showed that wood species affected wood resistance to subterranean and dry wood termites attacks, whereas mindi wood had better resistance than sugi wood, and referring to National Standardization Bureau (2007) mindi wood belongs to termite attack resistance class III and sugi wood class IV-V. The smoke treatment could increase wood resistance to termite attack which was indicated by lower attack degree, lower wood weight loss, and higher termite mortality, and the treatment could increase resistance class to be one class better compared to the control.

Key words: Smoked wood, dry wood termite, subterranean termite, laboratory test, field test, and resistance class.

Introduction

In Indonesia during three decades logs supply has been reduced from 19-30 million m^3 per year during 1980-1999, which was mostly supplied from natural forest, to be 9-14 million m^3 per year during 2000-2004 which was about 60 % from plantation forest. In 2005 logs production reached 24.2 million m^3 consisting of 9.3 million m^3 from natural forest, 0.8 million m^3 from Perhutani or stated-owned enterprise for plantation forest, 12.8 million m^3 from industrial plantation forest, and 1.3 million m^3 from community forest, it means all plantation forests supply 14.9 million m^3 or 61 % of total logs supply (calculated data from Ministry of Forestry, 2006). In the future plantation forest will be more important supplying logs demand, and concerning to this matter Ministry of Forestry has been developing industrial plantation forest of fast growing species.

About two million hectares of fast growing species has been developed with cutting cycle of 10-15 years, e.g. mangium (*Acacia mangium),* sengon (*Paraserianthes falcataria),* tusam (*Pinus merkusii),* and mindi *(Melia azedarach)* (Nurrochmat and Hadi, 2005 and Anonim, 2005). Mindi tree has been planting by community and Perum Perhutani because of fast growing, for example in Bogor the tree of 10 years age has 10 meters height branch free and 38 cm diameter at breast height (Balitbang Kehutanan, 2001). The specific gravity of mindi wood is 0.53 (0.42-0.65), shrinkage from green to oven dry weight is 3.3 % in radial and 4.1 in tangential directions, strength class II-III equal to mahagony, sungkai and red meranti woods, and durability class IV-V or not resistant.

Wood from plantation forest generally has a lot of juvenile wood and the wood is inferior in physical-mechanical properties and durability comparing to mature wood. Even the existing housings in Indonesia were built with mature wood, but in 2000 the economic loss of various buildings by termite attack was around US$ 200-300 million (Yoshimura and Tsunoda, 2005), apparently the loss will be increased in the future if the juvenile wood from plantation forest is not preserved prior to use for building materials. Furthermore Yoshimura and Tsunoda stated that the loss was happened also in the other countries, e.g. in China US$ 300 million, Japan US$ 0.8-1 billion, Korea US$ 200 million and Malaysia US$ 10-12 million.

To extend service life of timber, it can be done through wood preservation, i.e. fill in poison chemical or preservative to the wood. Currently this technique is very populer, but it has side effect to contaminate environment and poison for the human being and other living organisms. Hadi *et al.* (2005) stated that CCA (Chromated Chlor Arsen) was very effective for wood preservation purpose before 2000 era, but in early 21st century it was banned in almost any country because of poison to living organisms and environment, and the subtitute was looked for even it is not equal effectiveness.

Wood smoke contains a large number of polycyclic aromatic hydrocarbons, and it is composed mainly of phenols, aldehydes, ketones, organic acids, alcohols, esters, hydrocarbons and various heterocyclic compounds (Stołyhwo and Sikorski, 2005). Supriana (1999) mentioned that smoke method was the traditional way to preserve wood in Indonesia, the wood is placed above wood stove for very long duration and the wood will be more dry and more resist to bio-deterioration attacks.

The purpose of this work is to determine resistance of smoked mindi *(Melia azedarach)* and sugi (*Cryptomeria japonica*) woods to subterranean termite attack in the field and laboratory tests, and dry wood termite attack in laboratory test.

Materials and methods

Materials

The wood species of mindi *(Melia azedarach)* from Bogor Indonesia and sugi (*Cryptomeria japonica*) from Japan were used to determine termite attack resistance. The burnt wood to produce smoke was mangium (*Acacia mangium*) and smoking period was four hours. The sample size of mindi and sugi woods for smoking process was 0.8 cm by 2 cm in cross section, and the length of the samples was according to the test purpose, and amount of samples were ten for field test and five for laboratory tests.

Dry wood termite test

Five wood samples sized 5 cm by 2 cm by 0.8 cm (length, width and thickness), on the center of it sample a glass tube (3 cm height by 1.8 cm diameter) was placed, and 50 worker termites were introduced in the glass tube. The samples were then put in a dark room for 12 weeks. At the end of the test, wood failure, wood weight loss and termite mortality were determined, the test procedures were according to National Standard Bureau (2007).

Graveyard test

Ten wood samples sized 20 cm by 2 cm by 0.8 cm (length, width and thickness) were vertically buried at 15 cm depth to the soil ground in Bogor during three months. At the end of the test, sample failure was determined (modified from ASTM, 1995).

Laboratory test for subterranean termite

Five wood samples sized 2.5 cm by 2 cm by 0.8 cm (length, width and thickness) were put in and touched to the jam pot, and in each jam pot was put 200 g of sand (7 % moisture content) and 200 healthy and active worker termites, and the jam pots were put in the dark room for six weeks. Each week the bottles were weight and if moisture content of the sand reduced 2 % or more, water was added to reach moisture content standard. At the end of the test, wood failure, wood weight loss and termite mortality were determined (modified from National Standard Bureau, 2007 and AWPA, 1972)

Results and discussion

Dry wood termite test

After twelve weeks test period the percentages of wood failure, wood weight loss, and termite mortality are shown at Table 1.

Table 1. Failure and weight loss of wood sample, and mortality of dry wood termite.

Wood	Treatment	Attack Degree (%)	Weight Loss (%)	Mortality (%)
Mindi	Control	25.0	6.87	46.4
	Smoked	25.6	4.21	68.4
Sugi	Control	57.0	16.88	22.0
	Smoked	35.0	7.18	59.6

According to Table 1, mindi was different from sugi regarding to wood resistance to dry wood termite attack, mindi had better resistant than sugi which was indicated by lower wood failure, lower wood weight loss, and higher termite mortality. Regarding to percentage of wood weight loss, mindi belongs to resistance class III and sugi IV (National Standard Bureau, 2007).

Smoke treatment significantly affected wood resistance to dry wood termite attack, and it could increase the resistance which was indicated by wood failure 35 % lower compared to the control, wood weight loss 52 % lower, and termite mortality 47 % higher. Regarding to percentage of wood weight loss, the resistance class of both woods increased one class compared to the control.

Graveyard test

After three months test, the percentage of wood failure is shown at Table 2.

Table 2. Wood failure of graveyard test.

Wood	Treatment	Attack Degree (%)
Mindi	Control	40.0
	Smoked	13.5
Sugi	Control	100
	Smoked	58.5

According to Table 2, mindi was more durable than sugi, and smoke could increase durability level comparing to control wood which was indicated by wood failure of smoked wood less about 50 % comparing to the control.

Subterranean termite in laboratory test

After six weeks test period the percentages of wood failure, wood weight loss, and termite mortality are shown at Table 3.

Table 3. Failure and weight loss of wood sample, and mortality of subterranean termite.

Wood	Treatment	Attack Degree (%)	Weight Loss (%)	Mortality (%)
Mindi	Control	14.0	10.34	61.3
	Smoked	6.0	5.95	100
Sugi	Control	100	45.36	3.8
	Smoked	94.0	21.36	7.2

According to Table 3, mindi was different from sugi regarding to wood resistance to subterranean termite attack, mindi had better resistant than sugi which was indicated by lower wood failure, lower wood weight loss, and higher termite mortality. Regarding to percentage of wood weight loss, mindi belongs to resistance class III and sugi V (National Standard Bureau, 2007).

Smoke treatment significantly affected wood resistance to subterranean termite attack, and it could be increase the resistance which was indicated by wood failure 12 % lower compared to the control, wood weight loss 51 % lower, and termite mortality 39 % higher. Regarding to percentage of wood weight loss, the resistance class of mindi wood increased one class compared to the control.

Conclusions

From discussion above, it could be concluded that:

1. Wood species affected wood resistance to subterranean and dry wood termites attacks, whereas mindi wood had better resistance than sugi wood which was indicated by lower attack degree, lower wood weight loss, and higher termite mortality. Referring to National Standardization Bureau (2007) mindi wood belongs to resistance class III and sugi wood class IV-V.
2. Smoke treatment during four hours to mindi and sugi woods could increase resistance level, which was indicated by lower attack degree, lower wood weight loss, and higher termite mortality. According to National Standardization Bureau (2007) the resistance class of both woods could increase to be one class better compared to the control.

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The Subterranean Termite Resistance of Composite Board Made from Indonesian Community Forest Tree Species

by
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Abstract

The resistance of composites board on termite attack is one of the important parameters on its quality. The objective of this study is to analyze the resistance to the subterranean termite attack of composite board made from Indonesian community forest tree species which was combined with cement sack waste paper as face and back layers. The samples of composite board fabricated from shaving particles of gmelina wood (*Gmelina arborea*), candlenut wood (*Aleurites moluccana*), and sengon wood (*Paraserianthes falcataria*) bonded by melamine formaldehyde resin at 10% based on oven dry weight of particles and face/back layers. The treatment of this study consists of three factors namely: wood species (3 levels), part of tree (2 levels: stem and branch), and layers (2 levels: no layers and using 4 layers each side). Each board type was tested in three replications, totally 36 boards were produced. The results obtained are as follows: 1) the higher of composite board subterranean termite resistant were composite board made of gmelina, sengon and candlenut, respectively. 2) the resistance of composite board made of stem part were not significantly different with those of branch part, 3) composite board made of gmelina wood without face and back layers were more resistance compared to those with cement sack face/back layers. However, composite board made of candlenut and sengon wood with cement sack face/back layers were more resistance compared to those of without face/back layers.

Key words: composite board, subterranean termite, gmelina wood, candlenut wood, sengon wood

Introduction

The resistance of composite boards on termite attack is one of the important parameters to evaluate the composite board quality. The composite board resistance on the termite attack is urgently required, especially when it will be used in the area of favorable termite habitat like Indonesia. Hence, it is very important to consider the resistance of composite board on the termite attack when it will be used.

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). Therefore, the resistance of composite board very determined by wood species raw materials used. Variability of each wood species resistance has been reported by some researcher, for example Wistara *et al.*, (2002); Tarmadi *et al.*, (2005). The report however used samples from stem of tree commonly, while the resistance variability in the tree, like stem and branch not yet been reported. Whereas, the branch of tree were very potential to develop as raw material of composite board caused by the high potency of this part. Suhasman *et al.*, (2007) have been reported the potency of branch of three wood species from community forest, namely 31.99% for gmelina wood, 18.09% for candlenut wood, and 15.63% for sengon wood compared to the stem part.

Due to the very limited supply of wood from natural forest, the utilization of raw materials alternative is very important to developed, like wood from community forest, and waste of lignocelluloses materials. Suhariyanto (2002) have been stated that the total area of community forest in Indonesia were 1,265,460 ha, with the estimate production potency was 9.3 million cubic a year. The dominant species from the forest were sengon, gmelina, teak, mahogany, acacia, and candlenut. Therefore, it is very important to develop utilization the raw material resources for composite board. Beside that, waste of non wood lignocelluloses materials like cement sack paper is one of the raw material alternative resources for composite board which can be used as face and back layers. As a cement producer country with 33 million ton a year production capacity (Tempo Newspaper, 2005), the potency of cement sack waste paper is very big.

The purpose of this study is to investigate the termite resistance variability of composite board made from three of Indonesian community forest tree species and cement sack waste paper. The focus of this study was to analyze the effect of wood species, the effect of stem and branch, and the effect of cement sack paper face/back layers on the termite resistance of composite board. In this study, the resistance of the composite board was conducted in the field test. The excess of the field test was the termite attack the product in the natural condition (Hadi, 1997), so its attack activity likes the actual condition. Beside that, the relationship of laboratory test and actual service environment is still questionable (Sekino and Suzuki, 2002).

Materials and methods

Preparation of Composite board sample

Sample used in this study were composite board made of shaving particles of gmelina wood (*Gmelina arborea*), candlenut wood (*Aleurites moluccana*), and sengon wood (*Paraserianthes falcataria*). The resin type was melamine formaldehyde. The board target density was 0.75 gcm^{-3}, while the resin solid content was 10% based on the oven dry weight of shavings particles and cement sack paper. The board construction consists of two types namely: board with four layer cement sack for each sides and no layer board. The type of composite board were: 1) Board made of gmelina wood stem and no layers (GSNL), 2) Board made of gmelina wood stem with layers (GSL), 3) Board made of gmelina wood branch and no layers (GBNL), 4) Board made of gmelina wood branch with layers (GBL), 5) Board made of candlenut wood stem and no layers (CSNL), 6) Board made of candlenut wood stem with layers (CSL), 7) Board made of candlenut wood branch and no layers (CBNL), 8) Board made of candlenut wood branch with layers (CBL), 9) Board made of sengon wood stem and no layers (GSNL), 10) Board made of sengon wood stem with layers (GSL), 11) Board made of sengon wood branch and no layers (GBNL), and 12) Board made of sengon wood branch with layers (GBL). Each board types were tested in three replications, totally 36 boards was produced in this study. The dimension of test specimen was 10 mm thickness by 25 mm width by 100 mm length.

Termite Resistance Test in the Field

The test specimens were set on the surface of soil containing termite colony in the field at the arboretum of Faculty of Forestry, Bogor Agricultural University, Indonesia, as shown in Fig. 1. The specimen were put randomly and covered with blockboard.

Figure 1. Subterranean termite resistance test of the composite board in the field

After one month, the weight loss percentage was determined using formula:

$$\text{Weight loss } (\%) = (W0 - W1)/W0 \times 100\%$$

Whereas,

Weight loss	=	Weight loss percentage
W0	=	Composite board weight prior to the test at oven dry weight condition
W1	=	Composite board weight after the test at oven dry weight condition

Based on the weight loss percentage, the resistance level of composite board on the subterranean termite then classification according to the scale as shown in Table 1.

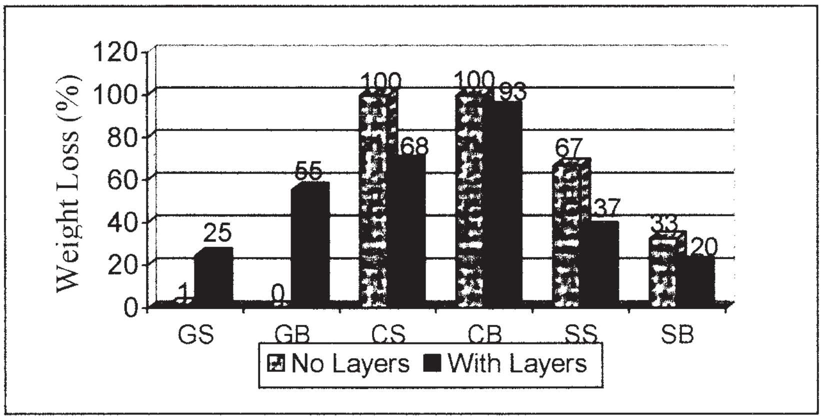
Table 1. The resistance of wood classification on the termite attack based weight loss

Class	Resistance	Weight loss (%)
I	Very resistant	<3.52
II	Resistant	3.52 - 7.50
III	Moderately resistant	7.50 - 10.96
IV	Non resistant	10.96 – 18.94
V	Susceptible	> 18.94

Source : National Standardization Board, 2007

Results and discussion

The results of this study showed that, there was weight loss variability due to the subterranean termite attack after exposure for one month in the field as shown in Fig. 2.



GS = Gmelina Stem GB = Gmelina Branch
CS = Candlenut Stem CB = Candlenut Branch
SS = Sengon Stem SB = Sengon Branch

Figure 2. Weight loss of samples due to the subterranean termite attack after exposure for one month in the field

Histogram in the Fig. 2 showed that there were variability resistances of composite board on the termite attack, especially in different wood species. Wood composite made of gmelina wood more resistant compared to those of others, while the composite board made of candlenut wood very susceptible compared to the others. This phenomenon showed that the resistance of the composite board on the termite attack is very dependent on the raw material used. The data is not remarkably different with the durability data of solid wood three species. According Mandang and Pandit (2002), the durability of gmelina wood are 4^{th} to 5^{th} class (not durable – extremely not durable). Furthermore the durability of sengon wood are 4^{th} to 5^{th} class, while the durability of candlenut wood is 5^{th} class (extremely not durable) (Martawijaya *et al.*, 1989).

Comparing of composite board resistance in terms of part of tree such as stem and branch showed that the resistance of gmelina and candlenut wood is not remarkably different, while the composite board made from branch of sengon wood more resistant compared to those of stem on termite attack. This fact indicated that utilization of tree branch have not been produced negative impact on the composite board resistance. The same results obtain from the mechanical properties of the composite board in the previous research. The composite board made of stem and branch parts of tree were not significantly different (Suhasman *et al.*, 2007). Thereby, potency of raw material from branch which during the time tend to disregarded in the reality can be used as raw material without any negative influence to the board properties, in terms of mechanical properties and the resistance of termite attack.

The resistance of composite board with layers and no layers showed that the different phenomena among of gmelina wood and both of candlenut and sengon wood. Composite board made from gmelina wood with no layer more resistant compared to those of board with layers, while the composite board made from candlenut and sengon wood with layers more resistant compared to

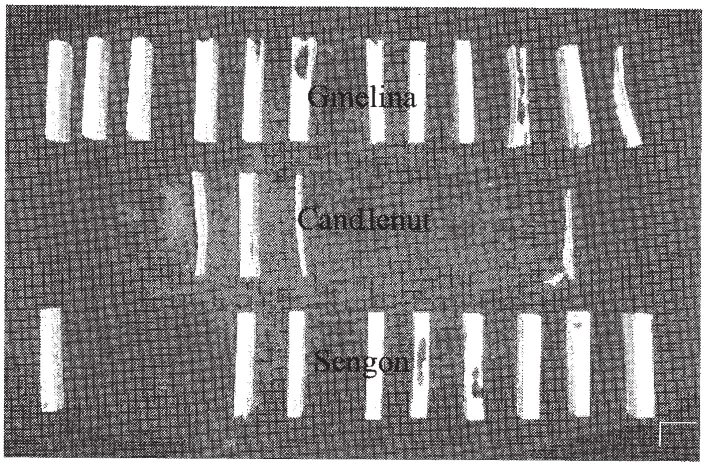


Figure. 3. Sample condition after exposure: Gmelina sample performed lightest termite attack, while a part of candlenut samples completely attack by termite.

those of board with no layers. This fact indicated that, gmelina wood more resistant compared to layer materials, while the candlenut and sengon wood more susceptible compared to the layer materials. This matter supported by observation result. The remained sample part in case of composite made from candlenut and sengon wood after exposure to the termite was cement sack layers. Generally, its wood used up attacked.

Based on the weight loss of composite board, the resistance of the board on termite attack can be classified as shown in Table 2.

Table 2. The resistance classification of composite board on the subterranean termite

No.	Board Types	Classification
1	Board made of gmelina wood stem and no layers (GSNL)	Very resistant
2	Board made of gmelina wood stem with layers (GSL),	Susceptible
3	Board made of gmelina wood branch and no layers (GBNL)	Susceptible
4	Board made of gmelina wood stem with layers (GBL),	Susceptible
5	Board made of candlenut wood stem and no layers (CSNL	Susceptible
6	Board made of candlenut wood stem with layers (CSL)	Susceptible
7	Board made of candlenut wood stem and no layers (CBNL	Susceptible
8	Board made of candlenut wood stem with layers (CBL)	Susceptible
9	Board made of sengon wood stem and no layers (GSNL),	Susceptible
10	Board made of sengon wood stem with layers (GSL).	Susceptible
11	Board made of sengon wood stem and no layers (GBNL)	Susceptible
12	Board made of sengon wood stem with layers (GBL).	Susceptible

The data in the Table 2 showed that most of the composite board types are susceptible on the subterranean termite attack. Therefore, improvement effort on the termite resistance properties of composite board is very important.

In the case of composite board resistance to the subterranean termite attack, there are not significant difference among composite boards made from stem part and branch part. In the case of part of tree, the phenomenon was not remarkably different in terms of mechanical properties. In contrast with wood species, the composite board made from candlenut wood more susceptible compared to those of others. However, in terms of mechanical properties, the composite board made from candlenut wood performed more superior compared to the others (Suhasman *et al.,* 2007). This matter indicate the importance of effort to improve the composite board resistance to the termite attack, especially candlenut wood and sengon wood for exploiting the wood species as composite board raw material.

Conclusions

The conclusions of this study are as follows:

1. Composite board made from gmelina performed more resistant on subterranean termite attack compared to those of others.
2. The resistance of composite board made from branch part of tree performed not remarkably different compared to those made from stem part of tree.
3. Composite board made from gmelina with no layer performed more resistant on termite attack compared to those from the same wood with cement sack face/back layers. However, the composite board made from candlenut and sengon with layers performed more resistant on termite attack compared to the board with no layers.
4. The resistance most of the board classified as susceptible, so creative efforts were extremely required to improve the composite board resistance properties on the subterranean termite attack.

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Antitermite Activities of Fraction Groups Derived from *Antiaris toxicaria* and *Azadirachta indica* Barks Extracts

by
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Abstract

The Research about the effect of *Azadirachta indica* and *Antiaris toxicaria* bark extract toward anti-termite activity had been conducted. Subterranean termites, especially *Coptotermes sp*, are destructive insects which attack both field and wood product, and cause high economic loss. Many researches were conducted to develop environmental friendly and non-toxic wood preservatives based on leaf, bark, and wood extractive compound. This research focus was analyzing anti-termite activities of fraction group derived from *Azadirachta indica* and *Antiaris toxicaria* bark extract by chromatography column and thin layer chromatography.

The result of fractionation process of both *Azadirachta indica* and *Antiaris toxicaria* bark extract was providing three fractions. Fraction 1 (Fr 1) showed higher rate of termite mortality than Fraction 2 (Fr 2) and Fraction 3 (Fr 3). Fr 1 from *Azadirachta indica* bark extract caused 100% termite mortality effect on sixth day observation, while *Antiaris toxicaria* bark extract caused 90% termite mortality effect in the end observation periods. In other hand, termite consumption rate to the paper disc of *Azadirachta indica* bark extract showed less consumption rather than *Antiaris toxicaria* bark extract.

Key words: Anti termite activity, *Coptotermes sp*, *Azadirachta indica*, *Antiaris toxicaria*

Introduction

Subterranean termites, especially *Coptotermes sp*, are destructive insects which attack both field and wood product, and cause high economic loss till 224-238 billion Rupiah per year (Prasetyo and Yusuf, 2004). Many researches were conducted to develop environmental friendly and non-toxic wood preservatives based on leaf, bark, and wood extractive compound, mostly high natural durable wood (Tarmadi *et. al.*, 2007). Syafi'i (2001) reported that extractive of several wood such as *Dalbergia latifolia* (Sonokeling), *Cassia siamea* (Johar), *Hopea spp* (Damar Laut), *Diospyros polisanthera* (Eboni), *Parinari corymbosa* (Kolaka), *Manilkara kanosiensis* (Torem), *Metrosideros petiolata* (Lara), *Palaqium gutta* (Nyatoh) and *Pterocarpus indicus* (Sonokembang) have anti-termite activity. Shibutani (2004) also reported that *Pterocarpus heterophylla* (Kayu Nangka) have extractive compound, *artocarpin*, which is very potential for anti-termite agent. Other report by Guswenrivo et. al. (2005) indicated that diethyl ether extract of *Piper betle* L. given significant rate of termite mortality.

Furthermore, as reported by Prianto *et. al.* (2005), *Azadirachta indica* bark extract with diethyl ether has given highest termite mortality rate compared to other solvents such as n-hexane, methanol, and acetone. Prianto *et. al.* (2006) also reported that *Antiaris toxicaria* bark extract with methanol also given highest mortality effect compared to other solvents. Based on this report, this research is directed to analyze anti-termite activities from fraction group derived from *Azadirachta indica* and *Antiaris toxicaria* bark extract by chromatography column and thin layer chromatography.

Materials and methods

Extraction and Fractionation

Several extract were studied to develop the most optimal extract for the natural-like anti-termite agent. In this research, methanol solvent was used in bark maceration of *Antiaris toxicaria* and diethyl ether solvent for *Azadirachta indica* bark maceration. Preparation treatment of extraction was done by powdering the air-dried bark of both wood barks to obtain wood meal passing through 60 mesh sieve. Then, one hundred grams of *Antiaris toxicaria* meal was extracted by 1.0 l of methanol, while *Azadirachta indica* meal by diethyl ether. Both extract result of them were evaporated by

Rotary Evaporator to get condensed extract solution. Furthermore, the separation of condensed extract was done by chromatography column, which silica gel as stationary phase and mobile phase using n-hexane, diethyl ether, and methanol solutes. For each 5 ml eluate fraction had been put in discrete numbered vials, and analyzed by Thin Layer Chromatography. The eluate with equal Rf value was combined as the same fraction.

Bioassay Test toward Subterranean Termite Species

Anti-termite activities of fraction group derived from *Azadirachta indica* and *Antiaris toxicaria* bark extract was done by force feeding test method, which fraction from both extract drops in paper disc, was fed to *Coptotermes sp*. Dry paper disc was treated by 10% (w.t.) extract fraction and vacuumed in desiccators for six hours to evaporate solvent. The bioassay test method against *Coptotermes sp* referred to Guswenrivo *et. al.* (2005) and Prianto *et. al.* (2005), where fifty tworkers and 5 soldiers of *Coptotermes sp*, and extract-treated paper disc 10% was entered in petri disc coated by plaster paris 3 mm. Termite mortality was observed per two days periods and in the final period observation, the mass loss of paper disc also determined.

Results and discussion

The result of fractionation process of Both *Azadirachta indica* and *Antiaris toxicaria* bark extract by chromatography column and TLC was providing three fractions; there was Fraction 1 (Fr 1), Fraction 2 (Fr 2) and Fraction 3 (Fr 3). From totally 1.5 g *Antiaris toxicaria* bark extract provided Fr 1 0.79 g, Fr 2 0.24 g, and Fr 3 0.15 g, while from 1.5 g *Azadirachta indica* bark extract provided Fr 1 0.87 g, Fr 2 0.29 g, and Fr 3 0.11 g.

Figure 1. showed the highest mortality rate of *Antiaris toxicaria* bark extract, about 90%, was given by Fr 1 in the final periods, while Fr 2 and Fr 3 just gave mortality rate under 70% in the final periods observation. The similar condition also showed by figure 2, where Fr 1 *Azadirachta indica* bark extract gave highest mortality rate, about 100%, on the day six observation period. The other fraction, Fr 2 and Fr 3, gave 100% mortality rate on the day eight. Based on this data, could be assumed that effective active compound was contained in fraction 1 of both extracts.

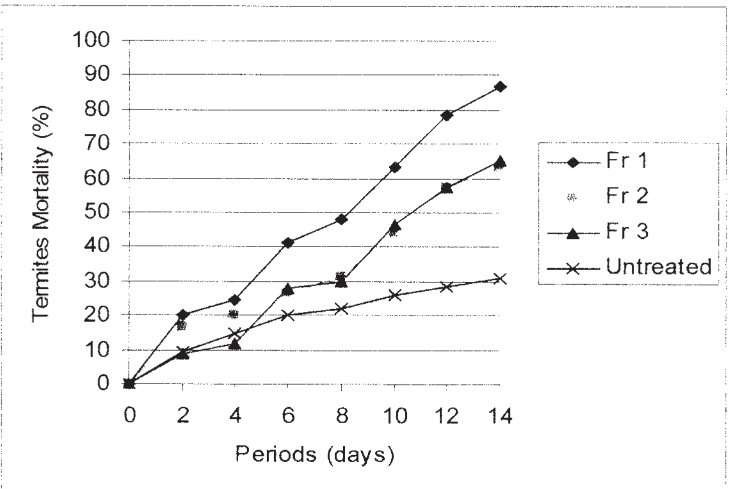

Fig. 1 Termite Mortality Rate of *Antiaris toxicaria* bark extract

Figures 1 and 2 also showed that fraction group compound from both bark extract were have very significant role on termite mortality rate. Fraction group compound of *Azadirachta indica* bark extract provided more mortality effect rather than *Antiaris toxicaria* bark extract. Based on this, could be concluded that fraction group compound of *Azadirachta indica* bark extract was very potential to be developed as natural wood preservatives rather than *Antiaris toxicaria* bark extract.

Termite's ability on consuming treated paper disc was depending on chemical compound's toxicity of fraction group of both extract above. Figure 3 points out that *Coptotermes sp* just only could consumed 7% paper disc treated by fraction 1 (Fr 1). It was less than Fr 2 about 13 % treated paper disc, and Fr 3 20% treated paper disc. The different result showed by mass loss observation of

Antiaris toxicaria bark extract (Figure 4.), where all treated paper disc consumed by *Coptotermes sp* until 60%.

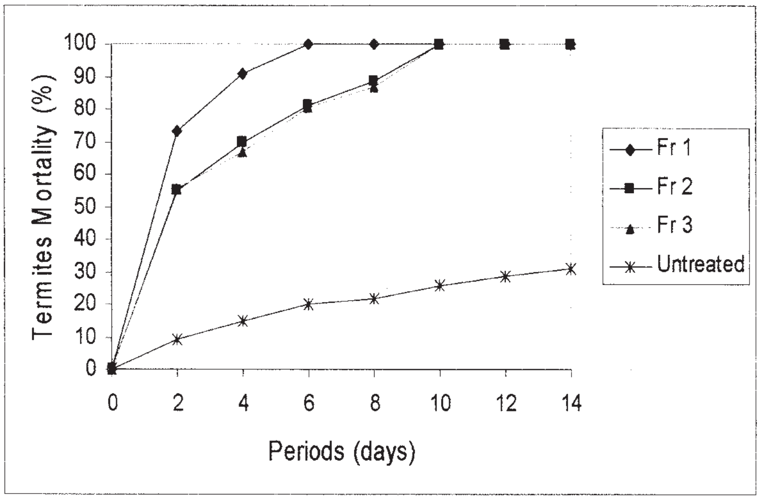

Fig. 2 Termite Mortality Rate of *Azadirachta indica* bark extract

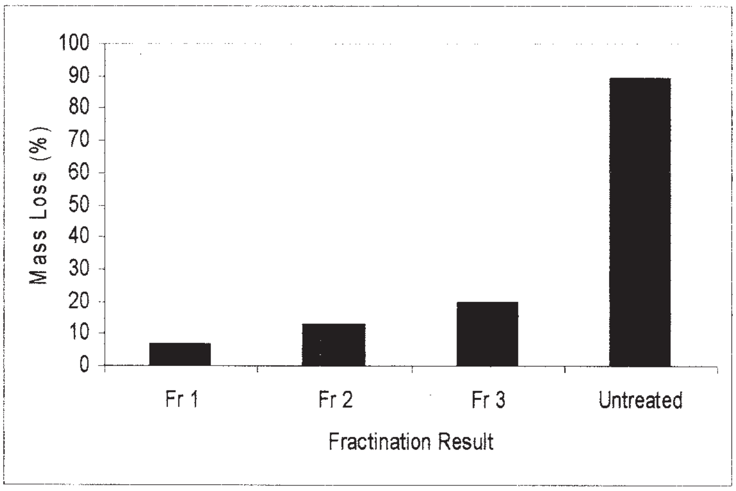

Fig. 3 Percent Mass Loss of Treated Paper Disc by *Azadirachta indica* bark extract

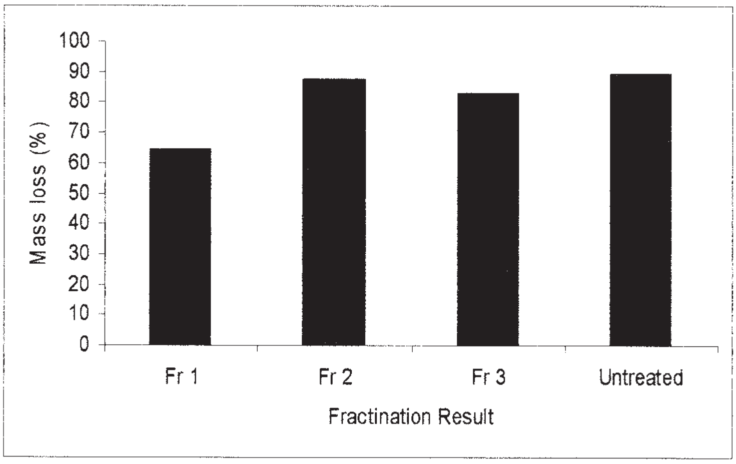

Fig. 4 Percent Mass Loss of Treated Paper Disc by *Azadirachta indica* ark extract

Figures 3 and 4 also showed that on the same concentration, treated paper disc of *Azadirachta indica* bark extract gave less consumption rate than treated paper disc of *Antiaris toxicaria* bark extract. Based on this data, could be concluded that active compounds of *Azadirachta indica* bark extract were more toxic than *Antiaris toxicaria* bark extract.

Conclusions

The result of fractionation process of both *Azadirachta indica* and *Antiaris toxicaria* bark extract was providing three fractions, which were tested for their anti-termite activities. Fraction 1 (Fr 1) from both bark extract showed higher termite mortality rate rather than Fraction 2 (Fr 2) and Fraction 3 (Fr 3). From percent mass loss observation, it could be concluded that paper discs treated with *Azadirachta indica* bark extract gave less consumption rates than those treated with *Antiaris toxicaria* bark extract. It showed that active compounds of *Azadirachta indica* bark extract were more toxic to termites than the other one.

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The Nature of Antitermite Fractionated-Compound of *Picrasma javanica* and *Nicotiana tabacum* with Column Chromatography

by
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Abstract

This study evaluated the effect of *Picrasma javanica* and *Nicotiana tabacum* compounds on termites. The result of fractionation processes of those compounds were subjected to bioassay test using *Coptotermes sp*. There were some compounds which were potential as anti-termites, namely P_2 and T_2. However, they still need to further be analyzed in such way that caused high level of termite mortality and in contrast with low level of mass losses. There were possibilities that those compunds had toxic character. Fraction P_1, P_5 dan P_6 gave 100% of termite mortality, but in a very long time duration and big mass losses. There were possibilities those compounds had the character of slow action. Character of active compound and toxic capacity could be used to determine the method of using the extract for termite control.

Key words : Extract, Antitermite, *Nicotiana tabacum, Picrasma javanica, Coptotermes sp*

Introduction

Requirement of wood for human being will always mount. Wood has natural characteristics which are difficult to be replaced by other materials, namely renewable, easy in workmanship, owning high esthetics value, easy to be jointed and relatively cheap. Nevertheless, wood also has weakness. Wood is biodegradable material, which means it is easily attacked by termite and fungi, especially wood that has low durability. In Indonesia, almost 80% of wood has pertained low durability, therefore strive of wood preservation is much needed for its usage efficiency (Tarmadi, 2007).

Generally, wood preservation is done by using chemical preservative that contains heavy metal and also synthetic materials. It has generated a new problem that is the existence of environmental contamination. It is also threatening the health of human being because the materials do not easy to ravel and have the character of poison, like Chrome-Copper-Arsenic wood preservative (CCA). In recent years, research has been focused on development of new wood preservative without any environmental concerns such as heavy metals (Hwang 2004).

Indonesia has high diversity of flora. It is estimated that there are 30.000- 40.000 plants species. The big potency has still not yet been exploited in an optimal fashion. It can be made as source of new seeking pharmacy, food, and also anti-insect. Many plants species have antibiotic and also poison-like chemical compound component. Indriyati (1987) reported that *Pangium edule* contain ginocardin, that is glucoside that can release cyanide acetate. It has toxic characteristic. Shibutani (2004) also reported that *Artocarpus heterophyllus* has artocarpin, which was active against both *Coptotermes formosanus* and *Reticulitermes speratus*. The exploration of natural compound which has anti-insect activity is expected to be a subtitute of chemistry-base wood preservation and the harmful affect can be avoided. Therefore, research enterprises about natural organic compound is still required in the future.

Materials and methods

Fractination Process

Two extract result of maceration method that most effective to termite attack, namely Ki Pahit (*Picrasma javanica*) bark by ethyl acetate solvent and Tobacco leaves (*Nicotiana tabaccum*) by methanol solvent were fractionationed with column chromatography. Silica was used as the stationary phase. The moving phase are used accordingly of its solvent. Every 5 ml of secretory extract from chromatography column was then collected in tubes.

The fractionated results were then analysed by Thin Layer Chromatography (TLC) to determine the extract purity. Stationary phase which used in TLC was silica and the moving phase were ethyl acetate for *Picrasma javanica* and methanol for the tobacco. Extract result of fractionation that have same Rf values on TLC were represented as one fraction, so it can be united. The fractions were then tested to termite by paper discs as bait.

Bioassay using *Coptotermes sp.*

Paper discs were used in forced-feeding termite tes. The paper discs has previously been given treatment by the compound from each fraction. Dry paper disc was dropped by 10% extract fraction and vacuumed in dessicator for six hours to evaporate the solvent. As a comparison paper disc without treatment was used to control. Untreated (control) and treated paper discs were placed at the bottom of petri discs that were coated by plaster paris 3mm. Fifty termites worker and five termites soldier of *Coptotermes sp* and extract-treated paper disc 10% were entered in it. Termite mortality was observed every day and by the end, the mass loss of paper discs was measured.

Results and discussion

According to Prianto (2006), base on the bioassay time, *Picrasma javanica* bark extract with ethyl acetate solvent has showed the best effectiveness. It has caused termite mortality 100% during six days by 5% extract concentration. The extract is then fractionated to investigate the compound which was responsible to termite mortality. Fractionation of *Picrasma javanica* bark extract yield 54 tubes (each 5 ml). TLC analysis has grouped those fractinaonated extracts into six fractions. Feeding-test result of sixth fraction shown at Figure 1. Fraction P_2 show significantly effect to termite mortality. It gave 100% termite mortality during 14 days. Compared to extract before fractionated, there is time-addition that required to get 100% termite mortality. This would be happened because of other toxic compounds were separated in other fractions. Fraction P_2 show termite mortality above 90%, while P_1, P_4, P_5 and P_6 gave termite mortality which are still good enough, that are above 80%. Generally, all of fractions still give significant effect to termite mortality compare to its control (untreated).

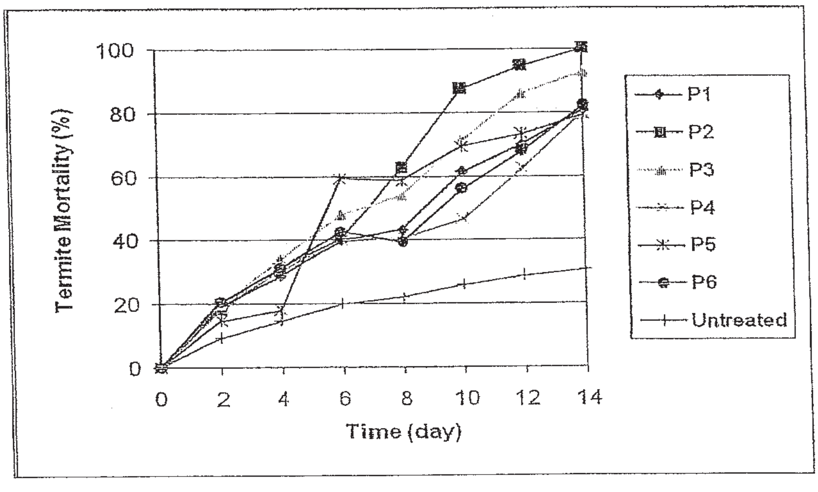

Figure 1. Termite mortality treated by *Picrasma javanica* fraction.

Fundamentally, each plants had toxic-character material to protect its against microorganisme with different level of toxicity (Prianto,2005). Tobacco leaves had reppelent and antifeedant character that would inhibite an insect growth, that is *Sitophilus zeamais*. Two fractionation results of tobacco extract were identified, namely T_1 and T_2. Both of this fractions had caused termite mortality that showed at Figure 2. The Figure showed that T_2 give 100%

termite mortality on the third days. This fraction seems more effective compared to *Picrasma javanica* fractionation. Result of bioassay also show the difference compared to extract before fractionation. It had caused time acceleration of 100% termite mortality on the third day, meanwhile before fractionation it had happened on seventh day. This matter is possible by the existence of the make-up of compound effectiveness after locked out from other compounds. According to Apipudin 2000, there were nicotin as character of poison found on leaves equal to 80% and 20% of the rest are in bar and root.

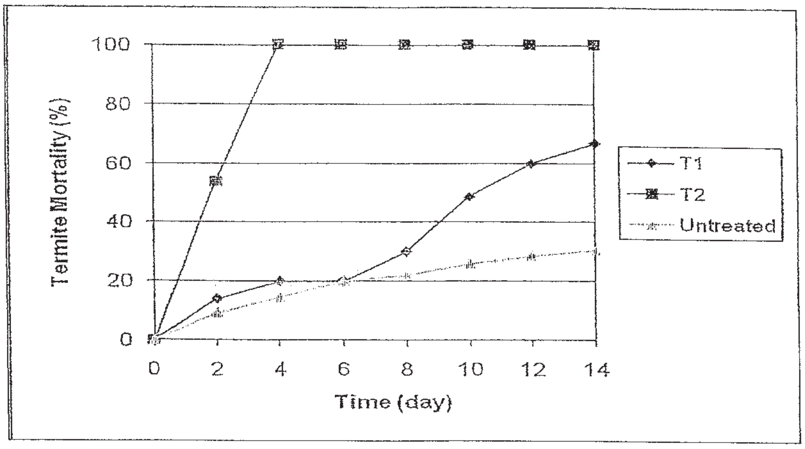

Figure 2. Termite mortality treated by *Nicotiana tabaccum* fraction

Percentage of mass loss from paper discs after baited to termite during 14 days was presented in Figure 3. Based on the figure, it can be seen the existence of difference of mass loss between fractions in each extract. In general, each extract with high termite mortality has resulted low mass loss. Fraction P_2 of *Picrasma javanica* extract gave the highest termite mortality and caused 6,8 % of mass loss. This value is still higher compared to mass loss of P_3 and P_4 fractions that caused smaller termite mortality (< 95%). That was possible due to termite different condition at the third test. The third fractions that had the same toxicity level but condition of healthier termite was more resistance to the toxic compound. Two fractions were identified from tobacco, namely T_1 and T_2. The T_1 fraction gives higher level of mass loss (95 %) than its control (89%). It showed that the chemical compound in T_1 do not have the character of toxicity. While T_2 fraction gives lower mass loss (8,6%) than its control (89 %) with significant termite mortality (100%).

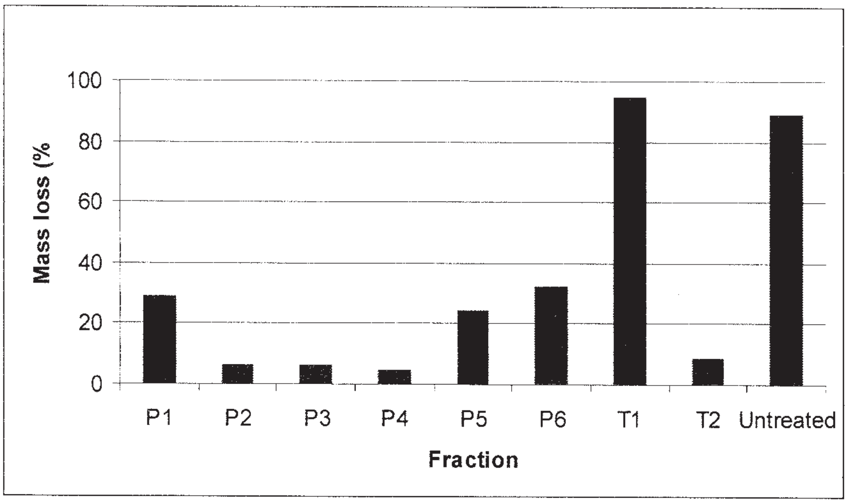

Figure 3. Percent mass loss of untreated and treated paper disc by *Picrasma javanica* and *Nicotiana tabacum*.

Conclusion

There were some compounds which were potential as anti-termites, namely P_2 and T_2. However, they still need to further be analyzed in such way that caused high level of termite mortality and in contrast with low level of mass losses. There were possibilities that those compunds had toxic character. Fraction P_1, P_5 dan P_6 gave 100% of termite mortality, but in a very long time duration and big mass losses. There were possibilities those compounds had the character of slow action. Character of active compound and toxic capacity could be used to determine the method of using the extract for termite control.

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The Virulence of Isolates of Entomopathogenic Fungus *Myrothecium roridum* Tode ex Steudel (Deuteromycotina: Hyphomycetes) against Subterranean Termites *Coptotermes* sp. (Isoptera: Rhinotermitidae)

by
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Abstract

Bioassay of two isolates of entomopathogenic fungus *Myrothecium roridum* Tode ex Steudel originated from different hosts: soil *(My-Soil)*, and sand (My-Sand) as bio-control for subterranean termites *Coptotermes sp.* have been conducted. For each isolate, the conidial concentration 10^7, 5.10^6, 10^6, 5.10^5 and 10^5 conidia/ml were used and the bioassay was repeated four times. The data were then tested further to determine the lethal concentration (LC) and lethal time (LT) using probit analysis. The result showed, Concentration-response test indicated that the all treatment using various concentration of both isolates until 5.10^5 conidia/ml could kill ≥ 90% termites *Coptotermes* sp. on 6 days after application. Based on the probit analysis, the value of LT_{50} and LC_{50} of *M. roridum* from sand were calculated at 1.64 days and 5.8 x 10^4 conidia/ml, and *M. roridum* from soil 2.61 days and 8.6 x 10^4 conidia/ml respectively.

Key words: virulence, isolate, *Myrothecium roridum*, concentration, Coptotermes sp.

Introduction

Termite is one of the many pests that causing seriously hazards for building. Controlling termites attack in Indonesia still mainly used chemicals treatments, now there are no termite's controls using microorganisms (bio-pesticide) yet. Pearce (1997) mentioned that biological control of termites currently involves the use of natural pathogens, that is those commonly associated with the termite, but not causing serious harm unless present in large number. These include nematodes, fungi, bacterium and viruses, but to use the fungal pathogens more successful for the control of termite.

The Entomopathogenic fungi *Myrothecium roridum* Tode ex Steudel not common yet to attack termite. Tulloch *et al.* 1970 in Domsch (1980) mentioned that *Myrothecium roridum* generic description are colonies reaching 4.0-6.0 cm diam in 14 days at 25°C on PDA, mycelium white to rosy buff; reverse rosy buff or yellow. Sporodochia 60-750 µm diam, olivaceous – black, flattened or convex, often formed in concentric zones. Phialides 11-16 x 1.5-2.0 µm. Conidia rod-shaped or narrowly ellipsoidal, mostly with both ends rounded but the basal one slightly more truncate, 5.5-7.0 x 1.5-2.0 µm.

The resulted in earlier research by Desyanti *et al.* (2005), *Myrothecium roridum* was isolated from soil is one of the Entomopathogenic fungi could kill the *Coptotermes gestroyi* WASMANN 100% after 1 week. The objective of this study was to evaluate the Entomopathogenic fungus *Myrothecium roridum,* an important natural control agent of the termites, and in this experiment, we would like to continuous the research about using Entomopathogenic fungus *Myrothecium roridum* from two hosts (soil and sand): we studied about their virulence to subterranean termites *Coptotermes* sp.

Materials and methods

Insects

The used termites in this research are worker and soldier castes from *Coptotermes* sp. They have been taken care in laboratory of Forest Product Biology Bogor Agricultural University Indonesia.

Fungus isolates and preparation

The tested fungus isolates and their hosts or origins shown in Table 1 and Figure 1. All fungal isolates were cultured on SDAY for laboratory experiments. A stock culture of each isolate was frozen at 4^0C and stored until used. Before used, the isolates were recultured on SDAY for no longer than two passages in 9 cm diameter Petri dishes and incubated at the incubator at 24 ^{0}C and 95 RH for 3 weeks for complete sporulation. A mixture of conidia and hyphae was harvested by flooding the Petri dishes with sterile distilled water containing 0.05 % Triton X-100. And agitating with a glass rod. Conidia were separated from hyphae and substrate materials by filtration of suspension through a filter cloth. The concentration of conidia was determined using a *haemocytometer*. For bio-assay (concentrations -response test) the all samples were properly adjusted to the final concentration were 10^7, 5.10^6, 10^6, 5.10^5 and-10^5 conidia/ml for the both isolates. The percentage of viable conidia was determined prior to the bioassay.

Virulence bioassays

A conidial suspension of each isolate was prepared as described above. 80 *Coptotermes* sp. workers and 8 soldiers were dipped in 0.50 ml of the conidium's suspension. Before used, conidium's suspension was agitated. The control termites were dipped in 0.05 % Triton X-100 only. After allowing the excess liquid to drip off, a filter paper was placed in the petri dishes as food source and then the treated termite of sum 20 workers and 2 soldiers were placed in Petri dishes. The Petri dishes were maintained under dark condition. Termites' mortality was assessed every day for 6 days. Dead termites were incubated at 24^0C and 95 % RH for 5-7 days. The cause of death was confirmed by examination of the fungus outgrowth of the cadaver. Each treatment consisted of 20 termites per replication, and the bioassay was replicated four times.

Table 1. The Entomopathogenic fungi *Myrothecium roridum* were found from hosts or source (soil and sand) in Indonesia.

Isolates	Isolates Source	Species of Fungi	Geographic Origin (years)
1.My-Soil	Soil	*Myrothecium roridum*	Cibodas (2004)
2.My-Sand	Sand	*Myrothecium roridum*	Padang (2006)

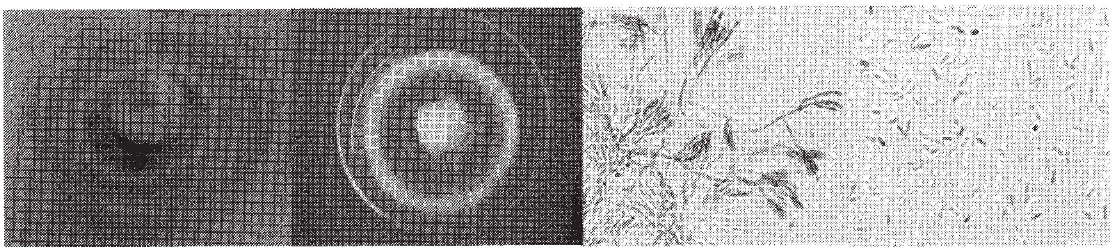
A B C D

Figure 1. Colonies of *M. roridum* isolates [My-Sand (A), My-Soil (B)] in SDAY culture medium after tree weeks, their micro culture (C) and conidia (D).

Statistics

For the group mortality data in the virulence studies were analyzed using Probity Analysis (Finney, 1971.) for getting lethal time of mortality (LT) and lethal concentration of mortality (LC) were determined.

Results

Concentration-response test

Mortality: The mortality of treated termite by various concentration (10^7, 5.10^6, 10^6 and 5.10^5 conidia/ml) of both isolates *M. roridum* were not significantly different until 6 days application, but different with concentration more lower. It means the fungal isolates (*M. roridum* from sand and *M. roridum* from soil) did not show the difference in their virulence against *Coptotermes* sp. The treatments with concentration $\geq 5.10^5$ conidia/ml could kill the termites $\geq$ 90%, however, their virulence in lowest concentration also showed by lowest mortality (< 65%). In general the complete mortality (100%) has been reached after 6 days (Fig. 2). The result indicated that generally *M. roridum* isolates effective to control *Coptotermes* sp.

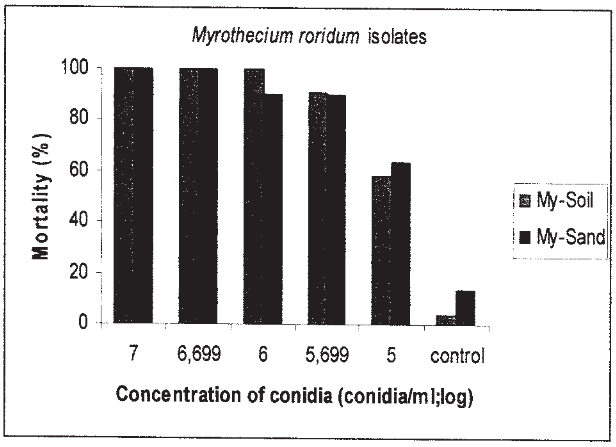

Figure. 2. Mortality of termites *Coptotermes* sp.(%) treated by various concentration (conidia/ml; log) of *M. roridum* isolates after 6 days .

Lethal time (LT) and concentration (LC): The Lethal Time ($LT_{25,50 \text{ and } 95}$) and Lethal Concentration ($LC_{25,50 \text{ and } 95}$) of infected termite by *M. roridum* isolates is shown in Table 2.

Table 2. Lethal time (LT) an Lethal concentration (LC)of termites treated with conidia suspension of *M. roridum* isolates after 6 days

Species	Lethal Time (days)			Lethal Concentration (conidia/ml)		
	25%	50%	95%	25%	50%	95%
M. roridum from sand	1.24	1.64	3.30	1.6×10^4	5.8×10^4	1.2×10^6
M. roridum from soil	2.37	2.61	3.29	3.9×10^4	8.6×10^4	5.7×10^5

Discussions

Virulence is the disease producing power of a microorganism. This is the ability of a microorganism to invade and cause injury to the host. It is the relative capacity of a microorganism to overcome the host defense mechanism and is often defined in relation to the resistance of the host. A pathogen may be highly virulence because of the low resistance or high susceptibility of the host, and conversely, a pathogen may have low virulence because of the high resistance or low susceptibility of the host. Pathogenicity is nearly synonymous to virulence in that it refers to the disease producing ability of the microorganism. The difference is that pathogenicity is applied to groups or species of microorganism; whereas, virulence is used in the sense of degree of pathogenicity within the group or species. Pathogenicity is sometimes regarded as a genetically determined ability to produce disease, and virulence as not being genetically produced. Thus, we may say that the pathogenicity of *M. roridum* is high for *Coptotermes* sp., but its virulence may differ depending on conditions, such as methods of cultivation, storage, formulation, and environmental factors. The example under certain nutrient conditions, the virulence of a pathogen is higher than with other nutrients, *M. roridum* maybe has many strains, which at one time were considered to be distinct species because of different morphological characteristics. The strains, however, vary in their virulence depending on the susceptible insect species (Tanada & Kaya, 1993).

Significantly different of Lethal Time (LT) and Lethal Concentration (LC) treated by different isolate caused by originated of their sources, this conditions causing they have different characterization to attack termites. Beside that also depends on the environmental in their origin area, the characteristics of the spores and their structures also effect spore germination. Successful germination and penetration on their hosts depend, not necessarily, on the total percentage of germination but also on the duration of the germination time, mode of germination, aggressiveness of the fungus, the type of fungal spore, and host susceptibility (Samson et al. 1988 in Tanada & Kaya 1993).

The development of mycosis can be separated into three phases: (1) adhesion and germination of the spore on the insect's cuticle, (2) penetration into the hemocoel, and (3) development of the fungus, which generally results in the death of an insect. Fungal species have numerous strains that differ in their virulence and pathogenicity. In general, the strains of species isolated from a specific host are more virulent for that host than those isolated from other host. Successive transmission within a hose may also result in the enhancement of virulence or the isolation of a more virulent strain

Beside virulence, sporulation ability in surfaces of their hosts and optimally concentration in application to control of termites can become very important when they are for purpose transmission in their colony. The fungus is conspicuous macroscopic growth on the surfaces of their hosts. Some entomopathogenic fungi, however, form no superficial growth or produce sparse, inconspicuous or minute external structures that are difficult to detect by the inexperienced investigator. Most entomopathogenic fungi are obligate or facultative pathogens and some are symbiotic. Their growth and development are limited mainly by the external environmental conditions, in particular, high humidity or moisture and adequate temperatures for sporulation and spore germination (Prior and Perry 1980 in Butt *et al.* 2001).

Conclusions

Concentration-response test indicated that the isolates of *Myrothecium roridum* from sand and soil used concentration $\geq 5.10^5$ conidia/ml could utilization as bio-control of termites in laboratory, the treatments caused mortality of termites $\geq 90\%$. And the other, their mortality were highest (100 %) after 6 days. Lethal Time of 50% mortality by *M. roridum* from sand was 1.64 days and by *M. roridum* from soil was 2.61 days. And Lethal concentration of 50% mortality by *M. roridum* from sand was 5.8×10^4 conidia/ml and by *M. roridum* from soil was 8.6×10^4 conidia/ml

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Mortality Rate of Infected Termites by Secondary Metabolite of *Humicola* sp.

by
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Abstract

Fungi spore can be used as biotermiticide, since they have high pathogen properties and were environmentally friendly. However, the utilization of an extract of secondary metabolite from fungi can be developed as an alternative way to minimize the impact caused by the spore when they are used as biotermiticide. This research was done to observe the ability of secondary metabolite extract from *Humicola* sp. The fungus was inoculated in sorghum substrate and grown in it by adding either colloidal chitin or skim milk to the media as variation of the medium composition. Extraction was done after 4, 5, 6, and 7 days of incubation time. Then the metabolite extract was infected to termites through spraying method and the observation was conducted every third day for eight days.

Key words: secondary metabolite, *Humicola* sp., mortality rate

Introduction

Indonesia is one of a country that supplies many microbe isolate source with high economic value since it has high biodiversity resources. Therefore, some researches need to be done in order to find new isolate which can produce commercial enzymes to be used in industries. Recently, the enzyme industry has rapidly developed and become important.

People's high attention toward environment contamination, have raised enzyme process technology become one of alternative technology process on industrial applied, which are more environmental friendly rather than chemical process (Falch, 1991). The secondary metabolites are organic compounds that are not directly involved in the normal growth, development reproduction of organisms (Lawrence, 1995). The secondary metabolite is formed after the primary metabolism or growing phase has already finished. The ability to utilize large molecules ultimately depends on the ability of the fungus to digest them, which in turn depends on the enzymes with which the fungus is equipped. Fungi typically have a large number of enzymes but, for the most part, many of them are inactive until the fungus comes into contact with a substrate on which particular enzymes can act (Moore-Landecker, 1996).

One of approach method to explore metabolism capacity is studying about gene which encode typical enzyme. There are many organism such as bacteria, fungi, plants and marine organism which able to produce enzyme to convert polymer compound to be their monomer and oligopolymer. Usually, these organisms have various enzyme gene or secondary metabolite where their gene expressions are inducted by extra cellular protein or its derivate (Dawes, I.W; I.W. Sutherland, 1992). Bacteria use chitinase to assimilate chitins as carbon and nitrogen sources. While in the plants, this enzyme is utilized against the attack of pathogen organism containing chitins. Fungi and Insect used this enzyme for cell wall morphogenesis and cuticle building. Considering the great amount of chitins as major component of organism's cuticle, which is produced continuously, so chitins is widely reliable to utilize as substrate of microorganism growth.

According to the previous research (Guswenrivo *et al.* 2007), *Humicola* sp. fungi could be used as termites bio-control. The ability of the fungi to utilize substrate or growth media for its metabolism depends by its ability to digest the media in the influence of enzyme. The absorption of nutrient is influenced by the enzyme activity. Basically, synthesis of certain enzyme was induced by the existence of certain nutrient in the growth media though the fungi are unable to digest the nutrient (Moore-Landecker 1996). Thus, *Humicola* sp. will produce enzymes that play important role in termite degradation if it grows in termite substrate, (Guswenrivo et al, 2007).

In their life cycle, termites is getting molted off where the major component of molting cuticle is protein and chitins compounds. This molting process will continuously do by termites till complete cuticle has build divinely. So, the addition of chitin and milk skim in fungi growth media are done in order to induce chitinase and protease synthesis which may play an important role in termite infection. Therefore, the objectives of this research are: (1) to evaluate the destructive power of secondary metabolite extract from *Humicola* sp. fungi, so it can be used as one of biotermiticide alternative formula; (2) to observe the effect of chitin induction and skim milk to the secondary metabolite extract; (3) to observe the pathogen level to *Coptotermes* sp.

Materials and methods

The fungus as used in this research is *Humicola* sp. that from previous research and it has been Research and Development unit for Biomaterial Collection.

Fermented Medium

Fungi fermentation is doing with sorghum medium. The variations of sorghum medium are sorghum, sorghum by adding colloidal chitin, and sorghum by adding skim milk. The sorghum was blended until size 20 mess. Sorghum culture was prepared by added sorghum in to Erlenmeyer, and then added colloidal chitin and skim milk to other Erlenmeyer. After all of material for medium was added onto Erlenmeyer, than add aquadest to Erlenmeyer. Then it was sterilized with autoclave for 15 minutes at 121^0C.

Fermentation Process

Fermentation procedure was done in room condition. Inoculated media were incubated for 4, 5, 6, and 7 day.

Extraction of Secondary Metabolite

Extraction was done by added sterilized water onto sorghum medium that has colonized by fungi, and then leaved around 2 hour at 4^0C, after that, separated between solution and solid to get extract of secondary metabolite. Separated was done with gauze and filter paper. Furthermore, extract of secondary metabolite bait to termite. Temperature of storage of extract is at 4^0C.

Bioassay

Bioassay was used by contact (spraying). The specimen test consist of fifty workers of *Coptotermes* sp. that were sprayed with extract of secondary metabolism *Humicola* sp.. The specimen test was placed in petri dish and kept at 28^0C under humid condition in the dark place for 8 days. Each treatment carried out in three-replicates. The untreated termites were used as a control. To examine the pathogenic effect of secondary metabolite against termite, mortality rate of termite was observed every two days during 8 days observation.

Results and discussion

Humicola sp. from the collection was grown on the fermentation medium, incubated it for a several days and extract with cold sterilized aquadest to get secondary metabolite of fungus. The purpose of the extraction of secondary metabolite is to found the influence of the metabolite that produce by *Humicola* sp. against *Coptotermes* sp. The test of the extracted are used spraying method and following figure show the mortality of termite after fungal infection during 8 days observation.

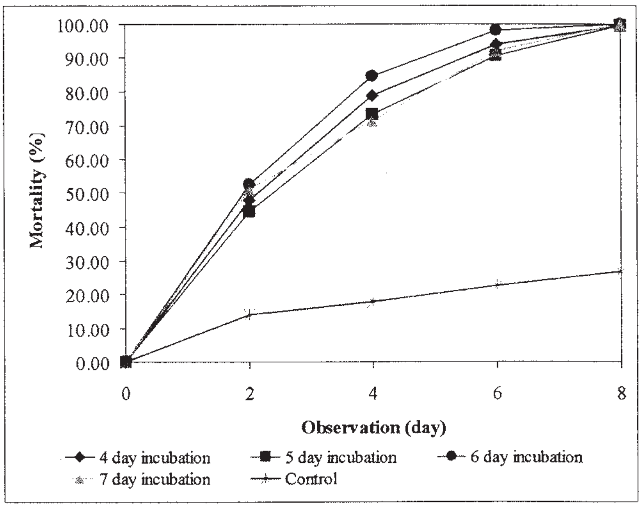

Figure1. Termite mortality during incubation

This figure shows the effect of secondary metabolite of fungi it grew on sorghum medium, to termite mortality compared with control. It is may be caused extract secondary metabolite have the toxic quality. Fungus is ascertainable to produce toxic substance as used to killing nurse insect pass through enzymatic activity. Bidochka and khachatourians (1987) reported that *Beauveria bassiana* is able to destroy insect cuticle with enzymatic activity. These enzymes include protease, chitinase, and extracelluler lipase.

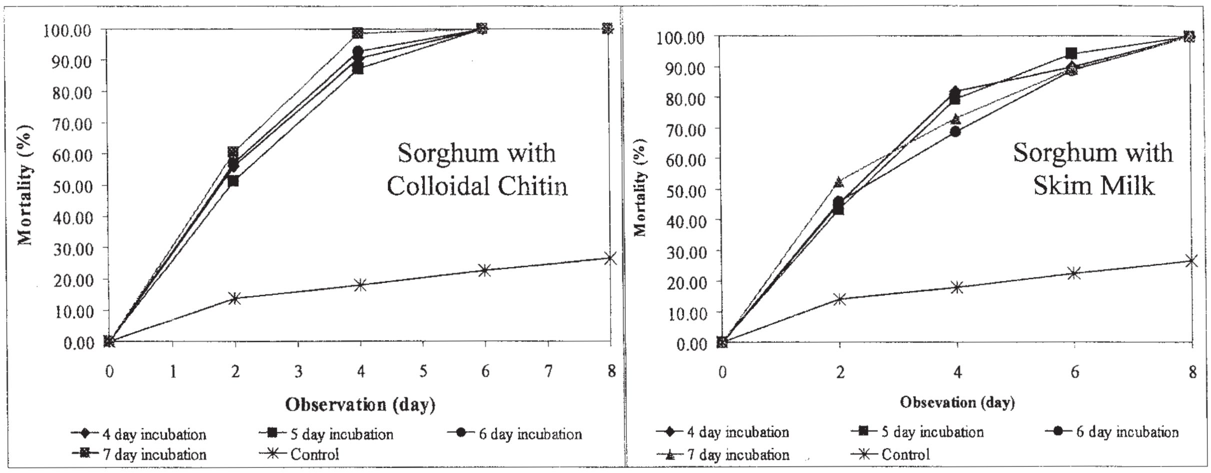

Figure 2. Termite mortality during incubation at different medium

In figure 2 shows the effect of secondary metabolite of fungi grew on sorghum medium added with colloidal chitin, to termite mortality and compared with extract secondary metabolite grew on medium added with skim milk. Where medium added with colloidal chitin can cause termite mortality until 100% in just four days, whereas medium with skim milk can cause termite mortality until 100% in 8 days observation. It be caused of adding colloidal chitin can increase enzyme chitinase produce. So in the baiting process, chitinase was bulked large on upper leather degradation of termite body consisted of chitin component

Secondary metabolite extract from the medium added with colloidal chitin can cause termite mortality higher as compare to the extract from sorghum medium. In the cased showed that addition colloidal chitin gave positive influence concerning to fungus development and secondary metabolite synthesis. The extract produce from medium added with colloidal chitin can cause termite mortality until 100% in just four days, whereas medium without colloidal chitin can cause termite mortality until 100% in 8 days observation. It is caused of adding colloidal chitin to sorghum media can be inducted synthesis of enzyme chitinase. So it can increase termite mortality compared with the sorghum medium. Thereby fungus was grown in the medium added with chitin, will be adapting with that condition and produce a lot of chitinase earlier. Despitefully, the fungi ability to used the growth medium in their metabolism process depend on absorb nutrition ability and activity of enzymes.

Meanwhile, treatment extract secondary metabolite of fungi grower at sorghum medium added with skim milk caused termite mortality did not give a marked difference compared with extract secondary metabolite grown at sorghum without added with skim milk. This is because the content of skim milks much of the protein and it does not give an effect to produce toxicity secondary metabolite.

The incubation time of fungus at treatment with colloidal chitin, shows rate of termite mortality did not give a marked difference between each variation time. But, the best termite mortality shows at 7 days incubation time where the mortality achieved 100%. It allied to synthesis secondary metabolite of fungi, happened when the grown achieved stationer phase and the growing was done because ran out of nutrition.

Conclusion

1. The extract secondary metabolite of *Humicola* sp. has potential as bio-termiticide.
2. Added colloidal chitin can be stimulating production of toxic secondary metabolite it shows with termite mortality achieved 100% in just four days.

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Pest Control Industry in Indonesia

by
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Termites' ability to utilize cellulose as food source and their unique life as social insects makes termites able to adapt themselves to various environmental conditions including manmade settlement (urban) environment. In the urban environment termite is well know as a pest because it causes a great deal of havoc upon buildings as well as other lignocellulosic materials.

At present, termite attack is increasing in not only low-rise but also high-rise buildings. The latter cases had been far beyond our experience. Termite attack raised more public concern after the Indonesian President's Palace and several multi-storied buildings such as offices and apartments in Jakarta and other big cities suffered significant termite attack. Nandika *et al.* (2000) stated that the potential termite attack in Indonesia is very high for several reasons, *e. g.* variety of termite species, climatic and environmental conditions conducive to easy proliferation of termite and use of nondurable constructional woods.

The coming into existence of termite control enterprises is the sign of the ever-increasing demand for termite control in the buildings. The promising demand appeared to begin in the mid-1970s when several sate-owned buildings were severely attacked by termites. At that time the number of termite control enterprises was small, being more or less 7. Among them there were a few foreign enterprises from the Philippines and Rentokil from UK and newly established Indonesian enterprises. The ever-increasing cases of termite attack and subsequent demand threat have contributed to the incredible booming of termite control industry in Indonesia.

Pest control enterprises

As yet there are already some 281 pest control enterprises in 11 provinces in Indonesia, They deal with rodent control, pest (insect) control, termite control and fumigation. In consideration of the geographical area, the number of population and the potential pest attack, the number of such enterprises is still quite small. In the future it is expected that there should be more pest control enterprises in each province.

Thus, the pest control enterprises have significantly contributed to the national development, pointing out at least in the form of protection to the development assets of buildings and of increased quality of public health and sanitation. Meanwhile, no less than 6,000 workers rely on such enterprises for their lives – exclusive of those working in formulation industries and pesticide distributors.

Indonesian pest control enterprises have established Indonesian Pest Control Enterprise Association ("IPPHAMI"). This entity is the only one association of pest control enterprises in Indonesia. This association was established out of great concern for and awareness of the importance of increased capacity of pest control enterprises in the country. During more than 30 years IPPHAMI has been concocted a membership certification system. This system is intended to zealously maintain standard quality of pest control enterprises. Every pest control enterprise is required to have the service of entomologists, medical supervisors, certificated pest control supervisors and technicians who have secured operational license from the Department of Health. That requirement has to be satisfied by any enterprises that are desirous of membership in the association.

It follows that every member of IPPHAMI is demanded to be capable of vouchsafing the best pest control services in order to protect, maintain and improve human health and environment qualities.

Legal aspect and termite control standard in Indonesia

Controlling of termite in buildings in Indonesia has been under the government policy in the form of law. IPPHAMI has contributed largely in the conception and the birth of the law on termite control in the discharge of building construction.

In the beginning of year 2000, IPPHAMI together with the state Public Works and Expert Council of IPPHAMII vouchsafing valuable input for Building and Construction Bill regarding he urgency of materialization, in the bi;; then being deliberated, of termite control for the reliability of buildings. The logical cause of the proposal is the highly potential threat of termite attack in Indonesia. The Government and the Legislative Body eventually assented to the coverage of termite control in a specific law, viz. the Act No. 28/2002 on Building Construction. The birth of the act on building construction has legally justified any measure of termite control in buildings.

The implementation of termite control thus far conducted by the members of IPPHAMIS is based on the Indonesian National Standard ("SNI"), viz. SNI No. 03-2404-2000 concerning the Procedure for Termite Control on Houses and Buildings by termiticides in conjunction with SNI No. 03-2405-2000.

Challenge ahead

IPPHAMI is in the position and is ready to encounter the future anent the challenge of the need for undoubtedly increasing demand for termite control. Even termite control upon dwelling houses will grow annually averagely by 800,000 units – let alone the current housing backlog which nears 5.8 million units. Additionally, there are still some 13-14 million existing houses which are poor and uninhabitable and in dire need of protection against termite attack.

In order to encounter such challenge, cooperation with various parties including researchers is strongly recommended. Demand for better quality services and more environmentally friendly technology will be satisfied by the Association through establishment of collaboration and liaison with knowledge and technology centers. Therefore, every termite control enterprise is urged to participate in any national, regional and international events, seminars or training. As for IPPHAMI, it will even discharge Pest Summit in 2010.

Digestibility of Cellulosic Hydrocolloid by Termite *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae)

by
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Abstract

Forced-feeding of worker termites of *Coptotermes formosanus* Shiraki with cellulosic hydrocolloids from *Ocimum americanum* L. and *Salvia viridis* L. was carried out to analyze their digestibility. The present results indicate that both termites and symbiotic protozoa in their hindgut could not utilize the cellulosic hydrocolloids as their adequate nutrients. Since *Pseudotrichonympha grassii* Koidzumi, the largest protozoa, could utilize fibrous cellulose in woody materials, inability of utilization of high molecular weight gellous cellulose contained in the cellulosic hydrocolloids indicates importance of solid structure of cellulose as nutritional sources.

Key words: *Coptotermes formosanus*, digestibility, cellulosic hydrocolloid, forced-feeding

Introduction

Lower termites gave serious hazards to wooden materials in Japan and subtropical countries in participation with symbiotic protozoa in their hindgut. However, lower termites participate important role in eco-system on earth and contribute partly in its carbon recycling system. In order to know more about the importance of lower termites as recycler of biomass on earth and to prevent attack of termites, thorough investigation of digestive system of lower termites including symbiotic system with protozoa in the hindgut of lower termites is necessary. Previously, contribution of woody polysaccharides including various model celluloses on digestive system of *C. formosanus* Shiraki was analyzed (Kanai et al. 1982; Yoshimura et al, 1993a,b). A key result given in the previous reports was importance of cellulose, as a food, having relatively high degree of polymerization to maintain the largest protozoa, *Pseudotrichonympha grassii* Koidzumi, while the fauna of the other two types of protozoa, *Holomastigotoides hartmanni* Koidzumi and *Spirotrichonympha leidyi* Koidzumi could be maintained with low-molecular weight cellulose (degree of polymerization of 17-27) (Yoshimura et al., 1993a).

Several authors of this report also found that hydrocolloids containing cellulose are produced from exocarp of seeds from a group of plants (Azuma et al., 2000; Yudianti et al., 2004). *Ocimum americanum* L. and *Salvia viridis* L. are typical representative origins of plants belonged to Lamiaceae family which produce cellulosic hydrocolloids. A characteristic property of the hydrocolloids isolated from seeds of these plants is presence of amorphous cellulose having degree of polymerization of about 4,000 and formation of complex composite with hemicellulose and pectinic polysaccharides (Azuma et al., 2000). Since the seeds of these plants are distributed at the surface of soil in vegetable farms or gardens not in woody land and/or inside of houses, *C. formosanus* may not have a chance to use this kind of cellulosic hydrocolloid as food stuff.

In this report, we investigated effects of cellulosic hydrocolloids on digestive system of a termite, *C. formosanus*.

Materials and methods

O. americanum L. were cultivated at a farm of Kyoto University in 2006 and its seeds collected on September to November in the same year were used for this study. Weight of a seed of *O. americanum* was in the range of 1.1 ± 0.3 mg and content of the cellulosic hydrocolloid was determined to be 23.7 ± 0.4 (SD) % (w/w). The numerical size of a seed was 2.23 ± 0.16 (SD) mm in length, 1.00 ± 0.1 (SD) mm in height and 1.34 ± 0.09 (SD) mm in width. Seeds of *Salvia viridis* L.

were purchased from Richters Herbs Co. Ltd., Ontario, Canada. Weight of a seed of *S. viridis* was in the range of 3.0 ± 0.4 mg and content of the fibrous material was determined to be 15.45 ± 0.32 (SD) % (w/w). The numerical size of a seed was 2.94 ± 0.19 (SD) mm in length, 1.11 ± 0.05 (SD) mm in height and 1.64 ± 0.12 (SD) mm in width. Their properties including neutral sugar compositions were listed in Table 1.

Cellulosic hydrocolloids were isolated from exocarp layers of the seeds of *O. americanum* and *S. viridis* as described previously (Azuma et al, 2000). Briefly, dried seeds were soaked in distilled water for about 30 min and treated with electric mixer for 10sec. Then the hydrocolloids were squeezed out through 50 mesh screen by filtration, freeze-dried and used as representative cellulosic hydrocolloidal diets for forced-feeding experiment.

Termites used were undifferentiated mature larvae (workers) and soldiers of *C. formosanus*, maintained at 28°C with pieces of pine wood (*Pinus densiflora* Sieb. et Zucc.) in RISH. Forced-feeding experiments were carried out using cellulosic hydrocolloids from *O. americanum* and *S. viridis* wetted with tap water placed separately on small aluminum specimens inside acrylic test cylinders (80mm in diameter and 60mm in height) whose bottom were sealed with hard medicinal plaster as described previously (Kanai et al, 1993). Fifty workers and 5 soldiers of *C. formosanus* were kept in the acrylic containers to feed on cellulosic hydrocolloids. Three replicates were done for each sample. Similar sets of containers were prepared for starvation experiment, sapwood powder of *P. densiflora* (about 24mesh) containing 30w% of boric acid as a model for poisonous food and sapwood powder alone of *P. densiflora* as a control. All containers were set

Table 1. Properties of cellulosic hydrocolloids from the seeds of *O. americanum* and *Salvia viridis*

Origin of hydrocolloids	Cellulose content (%)	Neutral sugar composition (%)					
		Arabinose	Rhamnose	Galactose	Glucose	Xylose	Mannose
O. americanum	23.5	10.1	2.4	26.7	51.0	6.3	3.5
Salvia viridis	36.0	0.4	0.3	25.7	55.4	17.6	0.7

on wet cotton pads inside plastic containers to supply water from the plaster bottom and kept under dark at 26 °C for 30 days. Then mortality and weight of termites were daily measured and activities of endogenous β-glucosidase localized in the midgut of *C. formosanus* (Itakura et al, 1998) and cellobiohydrolase localized in its hindgut were analyzed by using *p*-nitrophenyl β-D-glucopyranoside and *p*-nitrophenyl cellobioside as substrate, respectively.

Results and discussion

1. Effects of cellulosic hydrocolloids on mortality and weight loss of *C. formosanus*

Mortality of *C. formosanus* fed on cellulosic hydrocolloids from seeds of *O. americanum* and *S. viridis* selected as representative origin of hydrocolloids was analyzed together with boric acid as a famous poisonous material and sapwood meal of pine wood (*P. densiflora*) as an ideal food. The results shown in Figure 1 indicate that both cellulosic hydrocolloids effect on worker termites of *C. formosanus* similar to starvation and not poisonous like boric acid. The results also indicate that both hydrocolloids are not so suitable foods as pine wood powder. The results of weight loss during forced-feeding shown in Figure 2 also indicate that behavior of both cellulosic hydrocolloids from seeds of *O. americanum* and *S. viridis* was similar to that of starvation. Boric acid (30w%) rapidly increased mortality and termites mostly died within 12 days accompanied with rapid loss of weight. Similarity of the behavior of the cellulosic hydrocolloids to that of starvation indicate that the cellulosic hydrocolloids were not used as nutrients for workers. Since the result of *O. americanum* was much closer to that of the starvation condition, utilization ability of the hydrocolloid from *S. viridis* as nutrient was expected to be lower than the hydrocolloid from *O. americanum*.

Weight loss of about 30% induced higher than 80% mortality. During feeding for 14-16 days, behavior of both cellulosic hydrocolloids were closely similar to that of starved condition, but extension of feeding indicates that both cellulosic hydrocolloids act as foods unsuitable for worker termites of *C. formosanus*, even if they contained cellulose whose degree of polymerization as high as 4,000 (Azuma et al., 2000). The gellous property of both cellulosic hydrocolloid produced by wrapping cellulose with amorphous hemicellulosic and pectic polysaccharides may inhibit digestion.

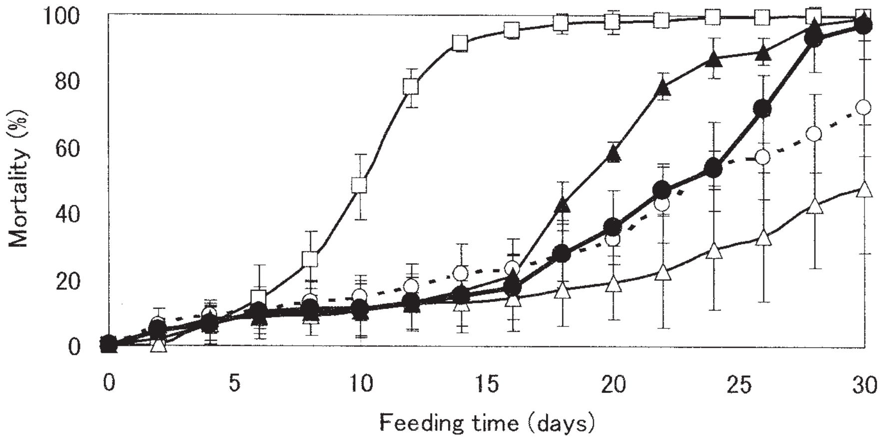

Figure 1. Mortality of workers of *C. formosanus* fed on the cellulosic hydrocolloids from *O. americanum* and *S. virides*.
Symbols: —●—, cellulosic hydrocolloid from *O. americanum*; —▲—, cellulosic hydrocolloid from *S.viridis*; —□—, 30w% of boric acid. incorporated in sapwood powder of *P. densiflora*; --○--, starvation; —Δ—, sapwood of *P. densiflora* (control).

2. Effects of cellulosic hydrocolloids on protozoan fauna

Figures 3-5 indicate the effects of feeding of the cellulosic hydrocolloids on fauna of three types of protozoa inhabited in the hindgut of *C. formosanus*. The result shown in Figure 3 showed the change of the largest protozoa, *P. grassii*. Rapid disappearance of *P. grassii* within a week like starvation suggests that *P. grassii* could not utilize both cellulosic hydrocolloids as effective nutrients. Remaining of *P. grassii* was detected only in the case of pine wood powder. Previously, it was found that relatively high degree of polymerization of cellulose as nutrients were necessary to maintain *P. grassii* (Yoshimura et al.,1993a).

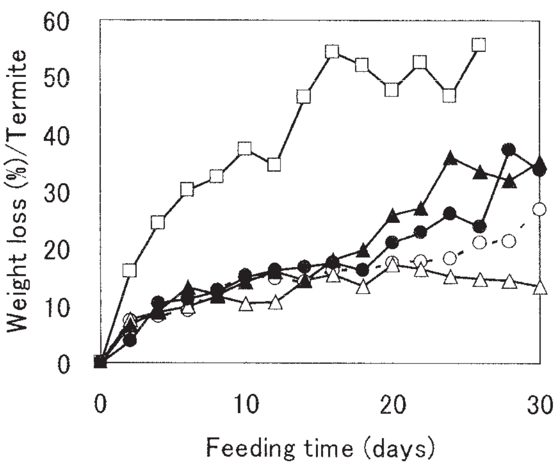

Figure 2. Weight loss of termite after forced feeding with cellulosic hydrocolloids including the results of 30w% boric acid, starvation and pine wood powder (control). Symbols: The same as in Figure 1.

In the case of the small size protozoa, *H. hartmanni*, substantial number of protozoa survived during forced-feeding for 20 days (Figure 4), while the smallest size protozoa, *S. leidyi*, mostly disappeared within a week, but still remained 10-20% of the original state after forced-feeding for 5-15 days (Fig. 5). Remaining of *H. hartmanni* and *S. leidyi* indicates that these protozoa can utilize the cellulosic hydrocolloids as their nutrients.

Disappearance of *P. grassii*, however, indicates that *P. grassii* could not utilize the cellulosic hydrocolloids as their nutrients although the degree of polymerization of the cellulose contained in the cellulosic hydrocolloids may be sufficiently high for their utilization. In addition, *P. grassii* could utilize amorphous cellulose prepared from Avicel SF (Yoshimura et al., 1993b). The discrepancy may be due to gellous property of cellulose in the present cellulosic hydrocolloids, or in the other words, lack of solid property of the cellulose in the cellulosic hydrocolloids.

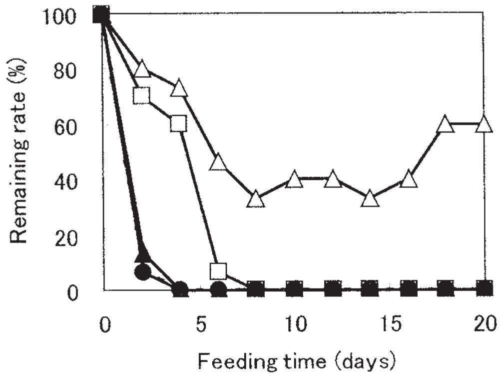

Figure 3. Effects of cellulosic hydrocolloids on *P. grassii*. Symbols: The same as in Figure 1.

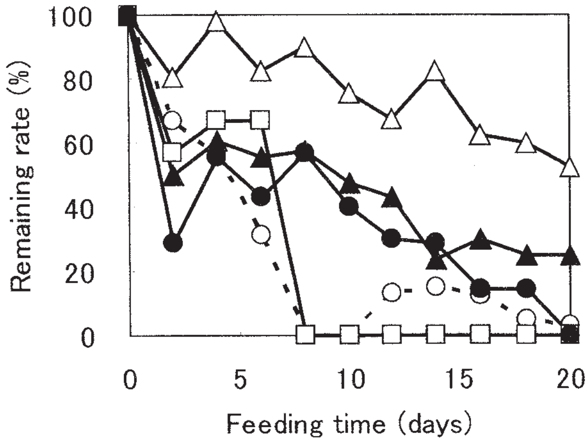

Figure 4. Effects of cellulosic hydrocolloids on *H. hartmanni*. Symbols:The same as in Figure 1.

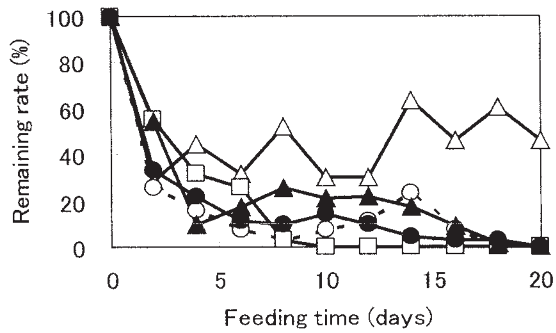

Figure 5. Effects of cellulosic hydrocolloids on *S. leidyi*. Symbols: The same as in Figure 1.

3. Effects of cellulosic hydrocolloids on enzyme activities

There are two location specific enzymes in relationship with cellulose degradation; one is the endogenous β-glucosidase in the midgut (Itakura et al, 1998), and the other is cellobiohydrolase originating from protozoa in the hindgut. The results of effects of cellulosic hydrocolloids on these enzyme activities are shown in Figures 6 and 7. Both enzyme activities gradually decreased during 10 days feeding, and their behaviors are related to starvation, indicating that both midgut and hindgut were damaged by ingestion of cellulosic hydrocolloids. The results suggest that the cellulosic hydrocolloids are not adequate foods for both termites and protozoa.

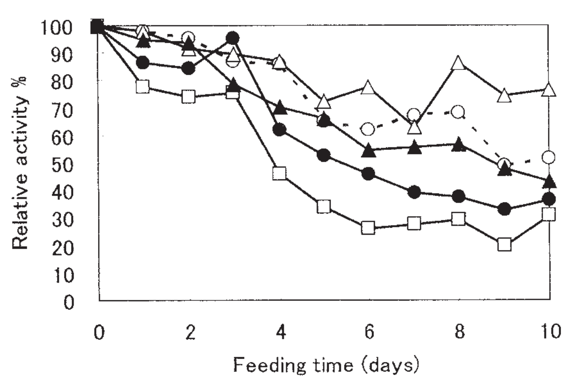

Figure 6. Effects of cellulosic hydrocolloids on β-glucosidase acitivity.
Symbols: The same as in Figure 1.

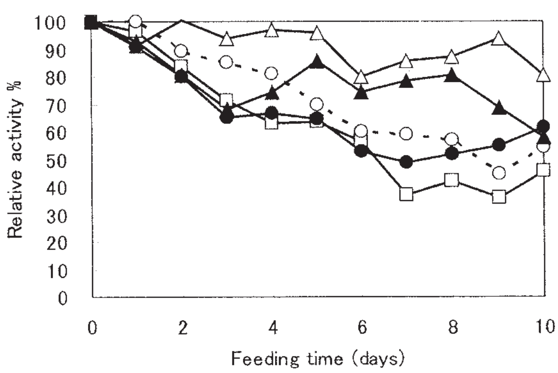

Figure 7. Effects of cellulosic hydrocolloids on cellobiohydrolase activity.
Symbols: The same as in Figure 1.

Conclusions

The present study dealing with the effects of cellulosic hydrocolloids on digestive system of worker termites of *C. formosanus* indicate that both termites and symbiotic protozoa in their hindgut could not utilize the cellulosic hydrocolloids as their adequate nutrients. Since *P. grassii*, the largest protozoa, could utilize fibrous cellulose in woody materials, inability of utilization of high molecular weight gellous cellulose contained in the cellulosic hydrocolloids indicate importance of solid structure of cellulose as nutritional sources.

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