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RESEARCH

Chemical Composition, Antifeedant, Repellent, and Toxicity Activities of the Rhizomes of Galangal, *Alpinia galanga* Against Asian Subterranean Termites, *Coptotermes gestroi* and *Coptotermes curvignathus* (Isoptera: Rhinotermitidae)

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ABSTRACT. Dual choice bioassays were used to evaluate the antifeedant property of essential oil and methanolic extract of *Alpinia galanga* (L.) (locally known as lengkuas) against two species of termites, *Coptotermes gestroi* (Wasmann) and *Coptotermes curvignathus* (Holmgren) (Isoptera: Rhinotermitidae). A 4-cm-diameter paper disc treated with *A. galanga* essential oil and another treated with either methanol or hexane as control were placed in a petri dish with 10 termites. Mean consumption of paper discs (miligram) treated with 2,000 ppm of essential oil by *C. gestroi* was 3.30 ± 0.24 mg and by *C. curvignathus* was 3.32 ± 0.24 mg. *A. galanga* essential oil showed significant difference in antifeedant effect, 2,000 ppm of *A. galanga* essential oil was considered to be the optimum concentration that gave maximum antifeedant effect. The essential oil composition was determined using gas chromatography-mass spectrometry. The major component of the essential oil was 1,8-cineol (61.9%). Antifeedant bioassay using 500 ppm of 1,8-cineol showed significant reduction in paper consumption by both termite species. Thus, the bioactive agent in A. *galangal* essential oil causing antifeeding activity was identified as 1,8-cineol. Repellent activity shows that 250 ppm of 1,8-cineol caused 50.00 ± 4.47% repellency for *C. gestroi*, whereas for *C. curvignathus* 750 ppm of 1,8-cineol was needed to cause similar repellent activity (56.67 ± 3.33%). *C. curvignathus* is more susceptible compare to *C. gestroi* in Contact Toxicity study, the lethal dose (LD_{50}) of *C. curvignathus* was 945 mg/kg, whereas LD_{50} value for *C. gestroi* was 1,102 mg/kg. Hence 1,8-cineol may be developed as an alternative control against termite in sustainable agriculture practices.

Key Words: *Alpinia galanga*, *Antifeedant*, *Coptotermes gestroi*, *Coptotermes curvignathus*, 1,8-cineol

Biodegradation of wood caused by termites is recognized as one of the most serious problems for wood utilization, causing greater than USD 20 billion annually in damage, control, and repair costs worldwide (Su 2002). In Malaysia, the cost of termite control was estimated at USD 10–12 million for the year 2003 and the total repair cost was 3–4 times higher (Lee 2002a). Among the genera of termites, *Coptotermes* spp. was responsible for >90% of total damages in buildings and structures in West Malaysia (Lee 2002b).

The two most effective control options for subterranean termites are soil treatment and baiting (Su and Scheffrahn 2000). Soil treatments are typically made with large volumes of liquid termiticides that are either neurotoxins or inhibitors of mitochondrial respiration. It is also known that termites can cause damage to a variety of materials ranging from paper fabrics to even noncellulosic materials such as asbestos, asphalt bitumen, lead, and metal foils (Bultman et al. 1979). Phytophagous insects use plant volatiles to recognize their host plants. Therefore, the use of essential oils as a nonhost volatile emission to repel insect pests is a viable alternative for control (Mauchline et al. 2005). The high toxicity of the biocides and unacceptable environmental consequences has resulted in severe restrictions. The use of synthetic pesticides for control has led to many problems such as toxicity to nontarget organisms and exposure to pesticides and residue in food (Arnason et al. 1989). In the search for alternatives, the use of natural plant products for the protection of wood has attracted much attention (Nunes and Nobre 2004).

In recent years, it is increasingly recognized that, as a result of public opinion and environmental laws, the new and safer alternatives to traditional synthetic pesticides are both desirable and mandated. Secondary metabolites with no known function in photosynthesis, growth or other fundamental aspects of plant physiology provide a new source of natural pesticide and antifeedant (Arnason et al. 1989; Coats 1994). Green plants are widely used in traditional cultures worldwide and increasing drastically in most of the developed and developing countries as natural alternatives to synthetic chemicals (Ramesh et al. 2011).

The three essential oils obtained from clove, *Syzgium aromaticum*, West African black pepper (WABP), *Piper guineense*, and ginger, *Zingiber officinale* significantly reduced the percentage of *Callosobruchus maculatus* adults that emerged from the bambara groundnut cultivars in the F_1 generation and the number of adult offspring that developed in the cultivars during the 3-month storage period (Ajayi and Lale 2000).

Alpinia galanga (L.) (locally known as lengkuas), an aromatic herb from the family Zingiberaceae has been used for various purposes including insecticidal activity (Sukhirun et al. 2009), stomachache (Jirovetz et al. 2003, Ibrahim et al. 2004), antibacterial (Janssen and Scheffer 1985, Jirovetz et al. 2003), antifungal (Janssen and Scheffer 1985), antitumor (Yang and Eilerman 1999), antiulcer (Yang and Eilerman 1999), antiallergic (Matsuda et al. 2003), antioxidant (Juntachote and Berghofer 2005), and food condiment (Jirovetz et al. 2003, Ibrahim et al. 2004).

Riyanto and Oshawa (1998) and Yamahara (1998) reported the insect antifeedant and acaricidal properties of *A. galanga* gainst bean weevil, moth, and mite. Paranagama et al. (2004) reported the repellency and toxicity of four essential oils from the leaves of

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Cymbopogon citratus, *Cymbopogon nardus*, *Cinnamomum zeylanicum,* and rhizome of *Alpinia calcarata* to *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Paranagama et al. (2004) found that *S. oryzae* L. or known as rice weevils were tolerant to the essential oil of the rhizomes of *A. calcarata,* and result of mortality test conducted on the *S. oryzae* L. using essential oil of the rhizomes of *A. calcarata* was similar with the control. Up to date, there is no report on antifeedant activity studies using essential oil or extracts of *A. galanga* against termites.

This study aims to determine the antifeedant properties of the essential oil and methanolic extract of the rhizomes of *A. galanga* against two termite species, namely *Coptotermes gestroi* (Wasmann) and *Coptotermes curvignathus* (Holmgren), for its potential use as a biopesticide. This study also attempts to determine the bioactive compounds that contribute to the antifeedant properties of the essential oil and methanolic extract of the rhizomes of *A. galanga* against *C. gestroi* and *C. curvignathus*.

Materials and Methods

Plant Material. The rhizomes of the *A. galanga* were supplied by an organic vegetable farm situated at Hulu Langat, Selangor, Malaysia (latitude 3.112063, longitude 101.816311). The identification was confirmed by Professor Dr. Halijah Ibrahim, Institute of Biological Sciences, Faculty of Science, Universiti Malaya. A voucher specimen (HI 1423) was deposited at the herbarium, Rimba Ilmu, Institute of Biological Sciences, Faculty of Science, Universiti Malaya, Malaysia. Rhizomes (2 kg) were dried in the shade for 3 days at ambient temperature then cut to small pieces for further use.

Termites. *C. gestroi* were collected from rotten wood, whereas *C. curvignathus* were collected from living rubber tree in Tropical Botanical Garden, Rimba Ilmu, University of Malaya, Malaysia (latitude 3.12479, longitude 101.652846) during daytime. The termites were then kept at 25°C and 98% relative humidity in the dark. For feeding bioassay, the termites were not supplied with water or food for 24 h prior to experiment. For identification, the termites were kept in 70% ethanol and were sent to Dr. Shawn Cheng, Forest Research Institute of Malaysia (FRIM), Kepong, Malaysia. The specimens were deposited in the Entomology Lab, FRIM. Specimen number of *C. gestroi* is ENT 130 and *C. curvignathus*is ENT 131.

Extraction Method

Methanolic Extraction. Extraction method was modified from the method used by Messer et al. (1990). Dried rhizomes (100 g) were soaked in 1,000 ml of methanol for 24 h. The mixture was filtered and the filtrate was concentrated to 20 ml using a rotary evaporator at 40°C. The concentrated methanol extract was used as the stock extract.

Extraction of Essential Oil. Extraction of essential oil was done according to the method used by Ibrahim et al. (2009). Fresh rhizomes (1 kg) were cut into small pieces and subjected to hydro distillation for 8 h using the Clevenger type apparatus; the oil obtained was dried over anhydrous sodium sulfate. The concentrated essential oil was used as the stock extract.

Dual Choice Bioassay Using Crude Extract. Two paper discs (4.0 cm diameter, ~19.5 mg dry weight) were placed in petri dishes (9 cm diameter). One disc treated with 25 µl of *A. galanga* essential oil was left to dry then weighed and moistened with 15µl of distilled water (Messer et al. 1990). For the control, a disc was treated with 25 µl of hexane and moistened with 15µl of distilled water. Ten petri dishes were prepared. Both paper discs were arranged side by side in each petri dish, and 10 *C. gestrois* were placed in each petri dish. The experiment was run for 3 days.

At the end of the experiments, the paper discs were dried at room temperature and reweighed to calculate the paper consumption. Paper consumption is the amount of paper consumed by the termites (initial weight minus final weight of paper disc) at every 24 h for three consecutive days. The average weight consumed was calculated. The results were then analyzed using *t*-test. The bioassay was repeated with *C. curvignathus.* The experiments were repeated with different concentrations (500, 1,000, 2,000, and 5,000 ppm) of *A. galanga* essential oil.

Identification of Components Using Gas Chromatography and Gas Chromatography Mass Spectrometry. The oil was analyzed on a shimadzu gas chromatography (GC) Q2010 with a Flame Ionization (FID) detector using fused silica capillary column HP-5ms, 5% phenylethylsiloxane (30.0 m by 0.25 mm ID by 0.25-µm film thickness) with helium as carrier gas at a flow rate of 1 ml per minute. The column temperature was programmed initially at 60°C for 10 min, then increased 3°C per minute to 230°C and was kept isothermally for 1 min. The temperature of injector port and interface of mass spectrometry (MS) was 230 and 250°C, respectively.

GC-MS analysis was performed using an Agilent Technologies 6890N gas chromatograph equipped with a 5,975 inert mass selective detector (70 eV direct inlet) on fused silica capillary column HP-5ms (30.0 m by 0.25 mm ID by 0.25-µm film thickness). The carrier gas was helium (99.999%) at a flow rate of 1 ml per minute and a split ratio of 1:10. The column temperature was programmed as follows: the temperature was initially set at 60°C and was kept isothermally for 10 min, then increased at 3°C per minute to 230°C and held for 1 min.

Retention indices were determined from the gas chromatogram by logarithmic interpolarization between bracking alkanes using a homologous series of *n*-alkanes as standards and in accordance with established method. The total ion chromatogram obtained as auto integrated by ChemStation software, and the compounds were identified by comparison with Wiley 9th edition NIST 11 Mass Spectral Library.

Dual Choice Bioassay Using Synthetic Compound. One disc was treated with 25 µl, 1,000 ppm of 1,8-cineol; the disc was left to dry then weighed and moistened with 15 µl of distilled water (Messer et al. 1990). For control, a disc was treated with 25 µl of hexane and moistened with 15 µl of distilled water. Ten petri dishes were prepared and both paper discs were placed in each petri dish. Ten termites were released in each petri dish. The experiment was run for 3 days at room temperature. The experiment was also repeated with 2,000, 5,000, and 10,000 ppm of 1,8-cineol. At the end of the experiment, the paper discs were reweighed to calculate the paper consumption by the termites. The results were analyzed using *t*-test.

Repellent Activity. Repellent activity of the identified active compound, 1,8-cineol was obtained using the method adopted by Ramamurthy et al. (2012). Ten cm diameter petri dish was used for repellency test. Nine centimeter filter paper was divided into two halves with each half treated and another half was untreated (control). For the treated filter paper, 1 ml of 250 ppm of 1,8-cineol was applied. For control or untreated filter paper, 1 ml of hexane was applied. Both treated and control filter papers were air dried. Ten termites were released at the centre of each petri dish and then covered with porous mesh cloth. The bioassay was repeated for nine times. The numbers of termite present in each portion of the petri dish were recorded. The termite presence was recorded at 2-h intervals such as 2, 4, and 6 h. Then the repellent test was conducted with different concentrations of 1,8-cineol, that is 500, 750, and 1,000 ppm. The percentage of repellency (PR) was calculated using the formula below:

$$PR = \frac{NC - NT}{NC + NT} \times \ 100$$

Where, NC = Number of termite on the control portion and NT = Number of termite on the treated portion.

Toxicity Test. Toxicity effects of essential oil of *A. galanga* and the identified active compound on *C. gestroi* and *C. curvignathus* were carried out in the laboratory according to the methods of Zapata and Smagghe (2010) with some modifications. A series of dilutions of essential oils extracted from the rhizomes of *A. galanga* and the identified active compound was prepared using hexane as the solvent. Aliquots of 0.5 ml of the dilutions were topically applied onto the thorax of the *C. gestroi* with a micropipette. Controls were treated with hexane. Ten *C. gestroi* were used for each concentration and control,

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Table 1. Mean consumption of paper disc treated with methanolic extract and essential oil of *A. galanga* by *C. gestroi* and *C. curvignathus* in dual choice bioassay for 24, 48, and 72 h

Termite species	Extract	Mean consumption of paper disc (mg)					
		24 h		48 h		72 h	
		Treated	Control	Treated	Control	Treated	Control
C. gestroi	Methanolic ext.	7.31 ± 0.42^a	7.17 ± 0.28^a	12.41 ± 0.69^a	12.21 ± 0.61^a	15.41 ± 0.89^a	15.77 ± 0.99^a
	Essential oil	3.25 ± 0.22^b	6.99 ± 0.35^a	5.10 ± 0.24^b	12.20 ± 0.58^a	8.10 ± 0.65^b	15.85 ± 0.75^a
C. curvignathus	Methanolic ext.	7.34 ± 0.36^a	7.30 ± 0.29^a	12.36 ± 0.84^a	12.24 ± 0.68^a	15.56 ± 0.98^a	15.70 ± 1.07^a
	Essential oil	3.24 ± 0.23^b	6.96 ± 0.32^a	5.22 ± 0.18^b	12.28 ± 0.76^a	8.16 ± 0.55^b	15.98 ± 0.74^a

Means within the same column followed by the same letter are not significantly different by *t*-test ($P < 0.05$).

Table 2. Mean consumption of paper disc treated with different concentrations of *A. galanga* essential oil by *C. gestroi* and *C. curvignathus* in dual choice bioassay for 24, 48, and 72 h

Termite species	Concentration	Mean consumption of paper disc (mg)					
		24 h		48 h		72 h	
		Treated	Control	Treated	Control	Treated	Control
C. gestroi	500 ppm	6.99 ± 0.37^a	7.09 ± 0.35^a	12.02 ± 0.57^a	12.08 ± 0.56^a	15.85 ± 0.60^a	15.74 ± 0.75^a
	1,000 ppm	4.57 ± 0.40^b	6.99 ± 0.35^a	8.10 ± 0.49^b	12.20 ± 0.58^a	12.87 ± 0.47^b	15.85 ± 0.75^a
	2,000 ppm	3.30 ± 0.24^b	6.90 ± 0.38^a	5.09 ± 0.22^c	12.22 ± 0.58^a	8.14 ± 0.70^c	15.95 ± 0.75^a
	5,000 ppm	3.25 ± 0.22^b	6.93 ± 0.26^a	5.08 ± 0.24^c	12.34 ± 0.51^a	8.21 ± 0.61^c	16.01 ± 0.75^a
C. curvignathus	500 ppm	6.90 ± 0.37^a	7.12 ± 0.41^a	12.04 ± 0.72^a	12.02 ± 0.42^a	15.84 ± 0.58^a	15.60 ± 0.82^a
	1,000 ppm	7.10 ± 0.34^a	7.06 ± 0.32^a	12.00 ± 0.45^a	12.14 ± 0.72^a	15.86 ± 0.69^a	15.88 ± 0.74^a
	2,000 ppm	3.32 ± 0.24^b	6.94 ± 0.38^a	5.20 ± 0.16^b	12.38 ± 0.76^a	8.16 ± 0.92^b	16.08 ± 0.74^a
	5,000 ppm	3.18 ± 0.19^b	6.92 ± 0.29^a	5.08 ± 0.26^b	12.36 ± 0.64^a	8.38 ± 0.36^b	16.04 ± 0.75^a

Means within the same column followed by the same letter are not significantly different by *t*-test ($P < 0.05$).

and the experiment was replicated ten times. Both treated and control termites were then transferred to glass petri dishes and kept under room temperature. Mortality percentages were recorded after 24 h of treatment, and lethal dose (LD_{50}) values were calculated according to Finney (1971). The test was repeated with 1,8-cineol. The whole procedure was repeated with *C. curvignathus.*

Results

Dual Choice Bioassay Using Crude Methanolic Extract and Essential Oil of *A. galangal*. *C. gestroi* consumption of paper treated with *A. galanga* methanolic extract of 7.31 ± 0.42 mg after 24 h was found to be insignificant as compared with the consumption of untreated paper 7.17 ± 0.28 mg (*t*-test, $P > 0.05$). Similarly the *C. gestroi* consumption of paper treated with methanolic extract was not significantly different after 48 and 72 h of treatment (Table 1).

However, the *C. gestroi* consumption of paper disc treated with essential oil showed significant reduction in comparison to control. *C. gestroi* paper consumption for essential oil-treated paper was 3.25 ± 0.22 mg and control was 6.99 ± 0.35 mg (*t*-test, $P < 0.05$). Similarly, the paper consumption by *C. curvignathus* for paper discs treated with essential oil was 3.24 ± 0.23 mg which was significantly less than that of the control, 6.96 ± 0.32 mg (*t*-test, $P < 0.05$) after 24 h of treatment. Similar results were obtained after 48 and 72 h (Table 1).

Table 2 shows the paper consumption by *C. gestroi* and *C. curvignathus* for paper discs treated with 500, 1,000, 2,000, and 5,000 ppm of *A. galanga* essential oil. Results showed that there was no significant difference in the paper consumption by *C. gestroi* for 500 ppm, whereas there was a significant difference in the paper consumption for 1,000, 2,000, and 5,000 ppm of *A. galanga* essential oil. Paper consumption by *C. gestroi* for paper discs treated with 500 ppm of *A. galanga* essential oil was 6.99 ± 0.37 mg and control 7.09 ± 0.35 mg (*t*-test, $P > 0.05$). Paper consumption for 1,000 ppm was 4.57 ± 0.40 mg and control 6.99 ± 0.35 mg (*t*-test, $P < 0.05$), 2,000 ppm was 3.30 ± 0.24 mg and control 6.90 ± 0.38 mg (*t*-test, $P < 0.00$), and 5,000 ppm was 3.25 ± 0.22 mg and control 6.93 ± 0.26 mg (*t*-test, $P < 0.05$; Table 2). Similar results were obtained after 48 and 72 h of treatment.

However, the paper consumption by *C. curvignathus* of papers treated with 500, 1,000, 2,000, and 5,000 ppm of *A. galanga* essential oil showed that there was no significant difference in the paper consumption for 500 and 1,000 ppm, whereas there was a significant difference in the paper consumption for 2,000 and 5,000 ppm of *A. galanga* essential oil. The paper consumption by *C. curvignathus* for paper treated with 500 ppm of *A. galanga* essential oil was 6.90 ± 0.37 mg and control was 7.12 ± 0.41 mg (*t*-test, $P > 0.05$). *C. curvignathus* paper consumption for treated paper with 1,000 ppm of *A. galanga* oil was 7.10 ± 0.34 mg and control 7.06 ± 0.32 mg (*t*-test, $P > 0.05$); treated paper with 2,000 ppm of *A. galanga* essential oil was 3.32 ± 0.24 mg and control 6.94 ± 0.38 mg (*t*-test, $P < 0.05$), and 5,000 ppm treated paper was 3.18 ± 0.19 mg and control 6.92 ± 0.29 mg (*t*-test, $P < 0.05$; Table 2). Similar results were obtained after 48 and 72 h of exposure.

Identification of *A. galanga* Components Using GC-MS. Figure 1 shows the gas chromatogram of *A. galanga* essential oil. The volatile constituents identified in the rhizome oils of *A. galanga* are given in Table 3. The results of this research showed that the yield of essential oil is 0.32% of fresh rhizome. A total of 21 compounds were identified in the oil, comprising 87.8% of the total oil. The rhizome oil consists of 7 monoterpenes (9.2%), 11 monoterpenoids (74.5%), one sesquiterpene (3.2%), and two sesquiterpenoids (0.9%). The peak at retention time 17.021 min corresponded to 1,8-cineole which was identified as the major compound contributing to 61.9% of the total oil.

Identification of Positive Active Compounds From *A. galanga* After TLC. The major organic compound that was found in the TLC fraction of *A. galanga* by GC-MS was 1,8-cineol as shown in the result (Fig. 2). 1,8-cineol was resolved as a single peak at retention time of 14.119 min. The percentage of area was 99.99%.

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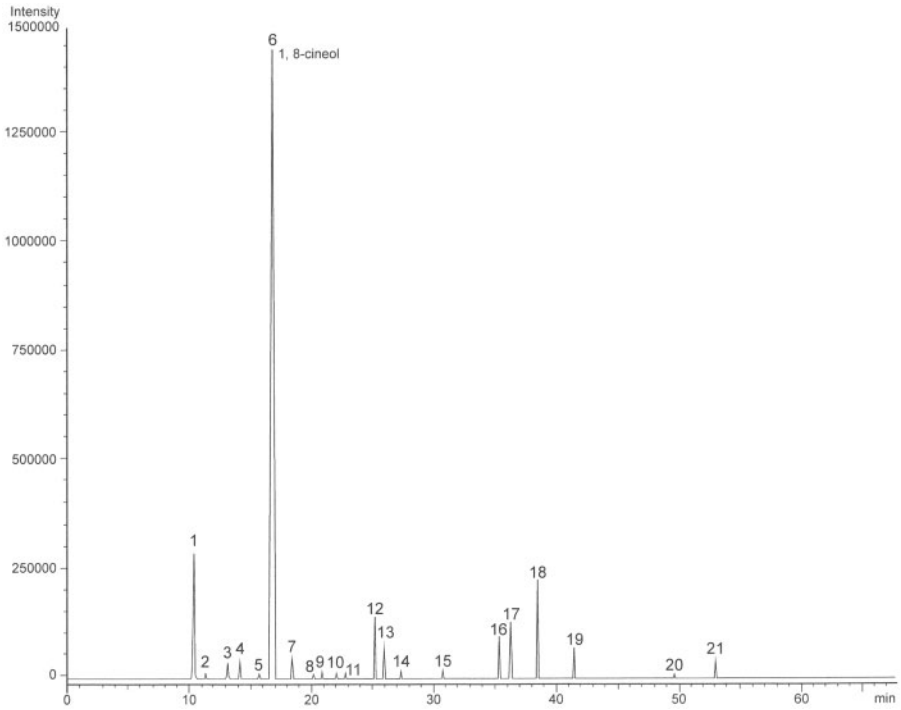


Fig. 1. GC chromatogram of rhizomes of *A. galanga* essential oil.

Table 3. Chemical composition of *A. galanga* rhizome essential oil

Peaks no.	Chemical constituents	R.T	R.I.		Percentage (%)		Method of identification
			A. galanga	Reference*	*A. galanga*	Reference**	
1	α-pinene	10.487	926	939	5.7	2.0	MS
2	Camphene	11.357	939	954	0.2	0.1	MS
3	β-pinene	13.155	966	979	0.9	0.6	MS
4	β-myrcene	14.180	982	991	1.0	0.1	MS, RI
5	α-terpinene	15.779	1,004	1,017	0.6	t	MS
6	1,8-cineole	17.021	1,027	1,031	61.9	40.5	MS, RI
7	γ-terpinene	18.422	1,050	1,060	0.6	0.3	MS, RI
8	α-terpinolene	20.161	1,078	1,089	0.2	0.1	MS
9	Linalool	20.943	1,091	1,097	0.3	0.1	MS, RI
10	(E)-p-mentha-2,8-dien-1-ol	22.066	1,110	1,123	0.3	0.1	MS
11	Cis-p-mentha-2,8-dien-1-ol	22.891	1,126	1,138	0.2	0.1	MS
12	4-terpineol	25.259	1,170	1,177	2.1	1.3	MS, RI
13	α-terpineol	25.985	1,184	1,189	1.6	1.1	MS, RI
14	Trans-carveol	27.435	1,212	1,217	0.4	–	MS, RI
15	Bornyl acetate	30.779	1,280	1,289	0.5	0.1	MS, RI
16	Lavandulyl acetate	35.340	1,377	1,290	1.8	–	MS
17	Methyl eugenol	36.320	1,399	1,404	3.2	1.5	MS, RI
18	Trans-beta-farnesene	38.528	1,451	1,443	3.2	3.2	MS
19	Eugenol acetate	41.511	1,522	1,523	2.2	–	MS, RI
20	Farnesal	49.631	1,736	–	0.2	–	MS
21	Farnesyl acetate	53.096	1,834	–	0.7	1.7	MS

Reference* = Adams 2001; Reference** = Jantan et al. 2004. MS = mass spectrometry, NIST 05 Mass Spectral Library. RI = retention indices. "-" = not reported. "t" = trace.

Feeding Bioassay Using Synthetic Compound, 1,8-cineol. Feeding bioassay was conducted to compare the paper consumption of *C. gestroi* and *C. curvignathus* with 100, 200, 500, and 1,000 ppm of the synthetic compound 1,8-cineol and control. Regardless of time, there was no significant difference in the paper consumption of *C. gestroi* for 100 ppm, whereas regardless of time, there was a significant difference in the scores for 200, 500, and 1,000 ppm of 1, 8-cineol treated paper. Paper consumption of *C. gestroi* treated with 100 ppm of 1, 8-cineol was 5.05 ± 0.25 mg and control was 4.98 ± 0.24 mg (*t*-test, $P > 0.05$), *C. gestroi* paper consumption for 200 ppm treated paper was 2.53 ± 0.35 mg and control 4.87 ± 0.40 mg (*t*-test, $P < 0.05$), 500 ppm treated paper was 1.90 ± 0.25 mg and control 4.88 ± 0.24 mg (*t*-test, $P < 0.05$), and 1,000 ppm was 1.99 ± 0.23 mg and control 4.84 ± 0.22 mg (*t*-test, $P < 0.05$; Table 4). Similar results were obtained after 48 and 72 h.

However, paper consumption of *C. curvignathus* with 100, 200, 500, and 1,000 ppm of 1,8-cineol shows there was no significant difference in the scores for 100 and 200 ppm, whereas there was a significant difference in the scores for 500 and 1,000 ppm of 1,8-cineol. Paper consumption of *C. curvignathus* treated with 100 ppm of 1, 8-cineol was

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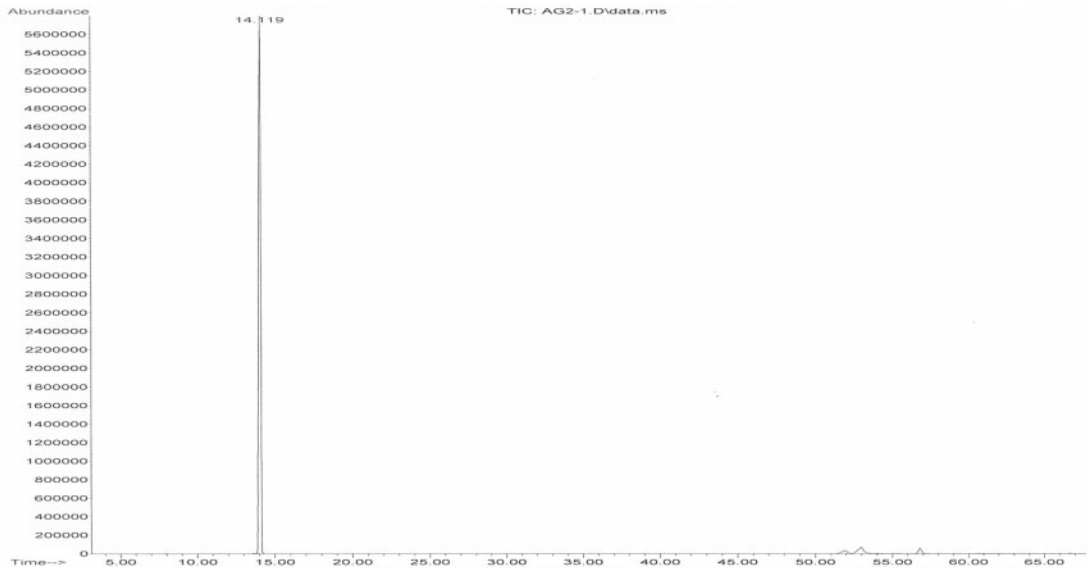


Fig. 2. Chromatogram of positive active compound from *A. galanga* after TLC.

Table 4. Consumption of paper disc treated with 1, 8-cineol by ten *C. gestroi* and *C. curvignathus* in dual choice bioassay for 24, 48, and 72 h

Termite species	Concentration	24 h		48 h		72 h	
		Treated	Control	Treated	Control	Treated	Control
C. gestroi	100 ppm	5.05 ± 0.25^a	4.98 ± 0.24^a	10.06 ± 0.37^a	9.41 ± 1.57^a	15.06 ± 0.56^a	14.88 ± 0.77^a
	200 ppm	2.53 ± 0.35^b	4.87 ± 0.40^a	4.63 ± 0.91^b	9.94 ± 0.50^a	7.28 ± 0.64^b	14.92 ± 0.76^a
	500 ppm	1.90 ± 0.25^b	4.88 ± 0.24^a	3.82 ± 0.43^b	9.88 ± 0.49^a	5.73 ± 0.67^b	14.65 ± 0.74^a
	1,000 ppm	1.99 ± 0.23^b	4.84 ± 0.22^a	3.80 ± 0.53^b	9.75 ± 0.46^a	5.84 ± 0.64^b	14.59 ± 0.68^a
C. curvignathus	100 ppm	4.98 ± 0.32^a	5.02 ± 0.22^a	10.02 ± 0.42^a	9.96 ± 0.52^a	15.02 ± 0.64^a	14.96 ± 0.77^a
	200 ppm	5.02 ± 0.19^a	5.14 ± 0.21^a	10.12 ± 0.35^a	10.22 ± 0.44^a	15.12 ± 0.53^a	15.34 ± 0.67^a
	500 ppm	1.90 ± 0.29^b	4.92 ± 0.28^a	3.82 ± 0.52^b	9.86 ± 0.62^a	5.74 ± 0.78^b	14.78 ± 0.90^a
	1,000 ppm	2.08 ± 0.28^b	4.76 ± 0.18^a	4.04 ± 0.49^b	9.56 ± 0.40^a	6.04 ± 0.74^b	14.32 ± 0.58^a

Means within the same column followed by the same letter are not significantly different by *t*-test ($P < 0.05$).

4.98 ± 0.32 mg and control was 5.02 ± 0.22 mg (*t*-test, $P > 0.05$), paper consumption for 200 ppm was 5.02 ± 0.19 mg and control 5.14 ± 0.21 mg (*t*-test, $P > 0.05$), 500 ppm was 1.90 ± 0.29 mg and control 4.92 ± 0.28 mg (*t*-test, $P < 0.05$), and 1,000 ppm was 2.08 ± 0.28 mg and control 4.76 ± 0.18 mg (*t*-test, $P < 0.05$; Table 4). Similar results were obtained after 48 and 72 h.

Repellent Activity. The identified active compound, 1,8-cineol was strongly repellent to *C. gestroi* and *C. curvignathus* (Table 5). The repellent activity of 1,8-cineol were significantly influenced by the concentration applied and, interestingly, the activity was also increased when insects were exposed to 1,8-cineol for a longer time. 1,8-cineol showed a higher repellent activity to *C. gestroi* compared with *C. curvignathus*. When applied at concentrations ranging from 250 to 1,000 ppm during 6 h of exposure the PR values observed ranged from 63 to 100% for *C. gestroi*. A similar tendency was observed for *C. curvignathus*: that is, the PR values at concentrations of 250–1,000 ppm ranged from 50 to 90%. *C. gestroi* was particularly sensitive to the 1,8-cineol since >90% repellency was obtained with 750 ppm, while for the *C. curvignathus* 1,000 ppm was needed to achieve similar PR values.

Toxicity Test. The essential oil extracted from the rhizome of *A. galanga* and the identified active compound (1,8-cineol) were found to cause toxicity to *C. gestroi* and *C. curvignathus* adults (Table 6). On the basis of LD_{50} values, *C. gestroi* was more susceptible toward 1,8-cineol ($LD_{50} = 1{,}102$ mg/kg) than the oils extracted from *A. galanga* ($LD_{50} = 5{,}407$ mg/kg). Similarly, for *C. curvignathus*, 1,8-cineol (945 mg/kg) was more susceptible compared with the essential oil of *A. galanga* (3,456 mg/kg). In comparison to *C. gestroi*, *C. curvignathus* was more susceptible toward the essential oil and 1,8-cineol.

Discussion

1,8-cineole was identified as the most abundant compound (61.9%) in the rhizome of *A. galanga* used in this experiment, which is much higher than that reported (40.5%) in the oil of *A. galanga* obtained from Tenom, Sabah, Malaysia (Jantan et al. 2004). The high content of 1,8-cineole obtained in this study maybe due to the use of fresh rhizome for extraction and the immediate analysis of the essential oil using GC-FID and GC-MS. This study showed that *A. galanga* is a good source of 1,8-cineole.

The result of this study showed that the methanolic extract of *A. galanga* did not give positive result and was not significantly different, whereas the essential oil from *A. galanga* gave positive result and showed significant difference in the antifeedant effect of the termites investigated. The bioassay with different concentrations of essential oil of *A. galanga* against *C. gestroi* showed that 2,000 and 5,000 ppm gave similar antifeedant effect. At 2,000 ppm, the mean consumption of paper disc was 8.14 ± 0.70 mg after 72 h and at 5,000 ppm gave mean consumption of paper disc of 8.21 ± 0.61 mg after 72 h. No significant difference between 2,000 and 5,000 ppm (*t*-test, $P > 0.05$) was observed. Similar results were obtained for *C. curvignathus*. Hence, 2,000 ppm of *A. galanga* essential oil was considered the optimum concentration that gave the maximum antifeedant effect, whereas for the

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Table 5. Repellent activity of 1, 8-cineol against ten *C. gestroi* and *C. curvignathus* in dual choice bioassay for 2, 4, and 6 h

Termite species	Concentration	Repellency (%)		
		2 h	4 h	6 h
C. gestroi	250 ppm	50.00 ± 4.47 [a]	56.67 ± 4.17 [a]	63.34 ± 6.15 [a]
	500 ppm	60.00 ± 8.03 [b]	63.34 ± 6.15 [b]	70.00 ± 4.47 [b]
	750 ppm	76.67 ± 3.33 [b]	83.34 ± 6.15 [b]	93.34 ± 4.22 [b]
	1,000 ppm	83.34 ± 6.15 [b]	96.67 ± 3.33 [b]	100.00 ± 0.00 [b]
C. curvignathus	250 ppm	36.67 ± 3.33 [a]	40.00 ± 5.16 [a]	50.00 ± 4.47 [a]
	500 ppm	43.34 ± 6.15 [a]	53.34 ± 6.67 [a]	63.34 ± 8.03 [a]
	750 ppm	56.67 ± 3.33 [b]	60.00 ± 8.03 [b]	70.00 ± 6.83 [b]
	1,000 ppm	63.34 ± 8.03 [b]	73.34 ± 4.22 [b]	90.00 ± 4.47 [b]

Means within the same column followed by the same letter are not significantly different by *t*-test ($P < 0.05$).

Table 6. The 50% of LD_{50} values of essential oil and synthetic compound (1,8-cineol) against *C. gestroi* and *C. curvignathus*

Termite	Essential oil of *A. galanga* (mg/kg)			Synthetic compound, 1,8-cineol (mg/kg)		
	LD_{50}	Regression line	95% confidence interval (lower to upper)	LD_{50}	Regression line	95% confidence interval (lower to upper)
C. gestroi	5,407	$Y = 1.87x - 2.139$ $R^2 = 0.803$	2,027 to 7,086	1,102	$Y = 1.732x - 0.342$ $R^2 = 0.720$	253 to 1,952
C. curvignathus	3,456	$Y = 2.71x - 4.871$ $R^2 = 0.909$	2,493 to 4,235	945	$Y = 2.443x - 2.292$ $R^2 = 0.915$	491 to 1,417

synthetic 1,8-cineol, 200 ppm concentration is considered as minimum concentration that can cause antifeedant effect on *C. gestroi*.

A. galanga essential oil was active against *C. gestroi* and *C. curvignathus*. This oil had antifeedant effects on *C. gestroi* and *C. curvignathus*. Therefore, *A. galanga* essential oil which has a high percentage of 1,8-cineol can be used to control *C. gestroi* and *C. curvignathus* due to its antifeedant action. Similarly, a study conducted by Abeywickrama et al (2006) also confirmed that 1,8-cineol was the major constituent in the essential oil of *A. calcarata.* 1,8-cineole is known as the marker compound for *Alpinia* spp. Extract of ginger, *Z. officinale* was shown to possess insecticidal activity (Oparaeke et al. 2005, Singh et al. 2005).

Several reports have shown that essential oils are effective against several insect species with varying potencies (Ho et al. 1996, Huang et al. 1999, Tunc et al. 2000, Zhu et al. 2001, Kostyukovsky et al. 2002, Garcia et al. 2005) acting as toxins, growth inhibitors, development disruptors, deterrents or repellents. Essential oil components of *Flourensia* spp. have been reported as having insect antifeedant (Faini et al. 1997), phytotoxic (Mata et al. 2003), antifungal, antialgal, and antitermite properties (Tellez et al. 2001). Orange oil extract contains 92% D-limonene, was also known to be toxic to insects in laboratory experiments. Stohs (2010) reported that 68% of termites were killed by the orange oil extract.

The essential oils extracted from the rhizome of *A. galanga* were shown here to possess contact toxicity as well as repellent activity toward *C. gestroi* and *C. curvignathus*. Essential oils from orange have been screened for their bioactivity against Formosan subterranean termite, *Coptotermes formosanus* (Raina et al. 2007). Other studies have shown that *A. galanga* can also used to control *Bactrocera dorsalis*. (Sukhirun et al. 2009), *Plutella xylostella and Callosobruchus chinensis* (Riyanto and Ohsawa 1998) and *Sitophilus zeamais*, *Tribolium castaneum,* and two parasitoids (Suthisut et al. 2011). *A. galanga* seed extracts showed acaricidal activity exhibiting mortality against *Tyrophagus putrescentiae* and *Dermatophagoides pteronyssinus* (Yamahara 1998).

In this study, the bioactive component in *A. galanga* was identified as 1,8-cineol and the results showed that it exhibited antifeedant activity, repellent activity, and toxicity effect toward the termites. In conclusion, the essential oil of *A. galanga* has the potential to be used as an alternative control in sustainable agriculture practices.

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