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Crude extracts of two different leaf plant species and their responses against subterranean termite *Coptotermes formosanus*

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Abstract. *Indrayani Y, Muin M, Yoshimura T. 2016. Crude extracts of two different leaf plant species and their responses against subterranean termite Coptotermes formosanus. Nusantara Bioscience 8: 226-231.* Crude extracts of plants consist of various chemical compounds useful for many purposes and their effects depend on the comprising active ingredients and applied concentrations. This study was conducted to determine the chemical compounds of plant leaf extracts and examine their responses against subterranean termite *Coptotermes formosanus* Shiraki. Two tropical plant species [clove (*Syzygium aromaticum*) and cajuput (*Melaleuca leucadendra*)] were extracted with 70% ethanol solvent. As the two different plant crude extracts contain eugenol, though to have termiticidal performance, various concentrations (0.1%, 0.05%, and 0.01%) based on their eugenol contents were prepared with filter paper samples for the test. The termite mortality and mass loss of extract-treated and untreated control filter paper samples were determined after four weeks of exposure. Results showed that crude extracts of different plant species showed different responses against the subterranean termites although they were applied at the same eugenol-based concentration. At the concentration of 0.1%, the crude extract of *S. aromaticum* caused termite mortality and consumption rates of 100% and 0.3%, respectively. Whilst, the crude extract of *M. leucadendra* caused significantly lower termite mortality (36.8%) and higher consumption (9.1%). Samples with lower eugenol-based concentration showed lower termite mortality and may have attractive effects on termites as indicated by higher consumption at the concentration of 0.01% compared to untreated controls. These results suggested that crude extracts of different two leaf species had different effects on subterranean termite *C. formosanus* even though they were applied at the same eugenol-based concentrations.

Keywords: *Coptotermes formosanus*, crude leaf extracts, *Melaleuca leucadendra*, *Syzygium aromaticum*, termite responses

INTRODUCTION

Plant species diversity in tropical areas has been an intense interest for researchers in various fields including its implication for restoration in forest ecosystem (Kamo et al. 2002) as well as its utilization for livestock feed and essential oils (Tawata et al. 2008). Very little is known on their potential use, particularly in direct relation to their chemical compounds. The use of bio-based materials in controlling subterranean termites has been observed by many researchers due to the economic and environmental reasons as well as their potential availability and variability (Ohmura et al. 2000; Sajap and Aloysius 2000; Sajap et al. 2006; Safian et al. 2011; Rodrigues et al. 2012; Alshehry et al. 2014; Ahmed et al. 2015). It is seemingly due to their extract ingredients potentially affecting the lives of many kinds of insects, including termites. The plant derivatives such as pyrethrins, terpenoids, azadirachtin, and flavanoids have been reported to have excellent termiticidal performance (Sharma et al. 1994; Cornelius et al. 1997; Chen et al. 2001; Nisar et al. 2015). These facts may contribute to the changes of termite control systems, application techniques, and products in the future.

The termiticidal performance of plant extracts was mainly examined using termite mortality and consumption rates as indicators. By this way, many plants have been recognized to have anti-termitic activities such as *Ruta graveolens* or *Cuminum cyminum*, *Annona squamosa*, *A. muricata*, *Rollinia mucosa*, *Rhazya stricta*, *Lantana camara*, *Ruta chalepensis*, *Heliotropium bacciferum*, *Dodonaea viscosa*, *Eucalyptus globules, lemongrass, clove bud and vetiver grass* (Zhu et al. 2001; Ahmed et al. 2011; Acda 2014; Alshehry et al. 2014; Nisar et al. 2015). Extracts of some tropical plants including *Antiaris toxicaria*, *Picrasma javanica*, *Eugenia caryophyllata*, *Piper cubeba*, and *Cymbopogon wintwrianus* have also been reported to have repellent effects to subterranean termites (Prianto 2010; Setiawan et al. 2010). However, termites may have various responses to crude extracts of plants containing a number of active compounds. The termite response may be positive (attractive), negative (repellent), or both positive and negative to a crude plant extract. Therefore, it is very important to aware that the use of leaf extracts should be carefully adjusted to meet the need for termite control systems, particularly for extracts with positive and negative termite responses.

Studies had been conducted to explore the performance of many crude extracts that show different effects on termites. Since a crude extract contains a mixture of some compounds that likely to have different performance compared to that of single active compound, the effect of a crude extract on termites may depend on the concentration, composition, or synergism of its active ingredients. In other words, comprising active ingredients of crude extracts should be taken into account for their deleterious effects. This study considers the assessment of crude extracts of clove (*Syzygium aromaticum*) and cajuput (*Melaleuca leucadendra* L.) as potential bio-based materials for termite control. A preliminary test indicates that termites have not only positive responses (non-repellent) to clove and cajuput extracts, but also negative responses (repellent) to clove extract. It was probably due to their eugenol contents. The current study was aimed at determining the toxicity effects of the plant crude extracts applied at different concentrations based on their eugenol contents to termites *Coptotermes formosanus* Shiraki.

MATERIALS AND METHODS

Extraction method

Leaf extracts of two tropical plant species [clove (*S. aromaticum*) and cajuput (*M. leucadendra*)] were collected from Pontianak city, West Borneo of Indonesia. The plant leaves were air-dried in the shade for three days at ambient temperature then crosscut into small parts prior to the extraction. The extraction was conducted according to Anas et al (2013), with macerating 200 g of each of the dry plant materials in 400 ml of 70% ethanol solution for 72 hours and then rotary evaporated at 60°C until the extract changed to gel condition. The final yield was then analyzed for their comprising compounds using GC-MS.

Identification of components using GC-MS

The chemicals compound of crude extract was performed using a Shimadzu Gas Chromatography-Mass Spectrometry (GC-MS) type GCMS-QP 2010 with a Flame Ionization detector using a fused silica capillary column type phase Rtx-5MS with ID measuring 60 m x 0.25 mm. Helium was used as a carrier gas at a flow rate of 0.85 ml per minute. The column temperature at first was set at 50ºC for 10 min, then increased 3ºC per minute to 280ºC and kept for 1 min. The temperature of 280ºC was set up for injector port and interface of mass spectrometry (MS).

Treatment of filter papers

Based on the GC-MS analyses, all of the leaf extracts were provided at the same and equal concentration based on their eugenol contents of 0.01, 0.05, and 0.1% using ethanol 70% as the solution. Filter papers (Whatman No. 2: 70 mm in diameter) were dipped in the solution extracts of each plant for 30 seconds with five replications of each concentration. Untreated filter papers were also prepared with distilled water for controls.

Termite response test

Matured workers of the subterranean termites *Coptotermes formosanus* Shiraki available in the termitarium of the Deterioration Organisms Laboratory, Research Institute for Sustainable Humanosphere, Kyoto University, Japan were used for bioassay test. The termites were not feeding for 24 hours prior to the test. Response test was performed according to the method developed by Abdullah et al (2015) with some modifications. Dry treated and untreated filter papers were placed separately at the center of a Petri dish (8 cm in diameter). Distilled water (200µl) was dropped on the filter papers to moisten the papers. A total of 50 workers and 5 soldiers of *C. formosanus* were introduced into each test dish with 5 replicates. All treatment units were maintained at 28±2°C and 80% relative humidity in the dark for 4 weeks. Termite mortality was periodically observed (1, 3, 5, 7, 14, 21, and 28 days). At the end of test period, the percentage mass loss of each test unit was calculated based on the difference in oven-dried weight before and after the termite test. Results were statistically analyzed by Dunnett's test to compare between untreated and treated materials.

RESULTS AND DISCUSSION

Identification of crude extract components using GCMS

Figure 1 and 2 shows the gas chromatogram of *S. aromaticum* and *M. leucadendra* respectively. A total of 20 compounds were identified in the *S. aromaticum* crude extract and 13 compounds were found in *M. leucadendra*.

Termite response test

Research results showed remarkable variation in percent mass loss and termite mortality on the tested samples (Tables 1 and 2). Termites *C. formosanus* consumed the extract treated filter papers up to 0.1% eugenol-based concentration of *M. leucadendra*, but not that of *S. aromaticum* (Table 1). The increased mass losses at the concentrations of 0.05 and 0.01% compared to 0% indicate that the higher eugenol content of both plant crude extracts in line with the higher toxicity effect. The termites showed significant avoidance behavior on filter papers treated with 0.1% extract of *S. aromaticum* as indicated by a very low mass loss of the treated papers. In contrast, paper consumption by termites at untreated and those treated up to 0.1% extract of *M. leucadendra* were not significantly different, indicating that the termites were healthy feeding on the treated filter papers. These results were in line with the termite mortality being not statistically different between untreated and treated up to 0.05% extract of *M. leucadendra* (Table 2).

The results also demonstrated that termite mortality with papers treated with 0.01 to 0.1% eugenol-based extracts of *M. leucadendra* was relatively low at about 16-40% after 28 days of exposure (Table 2). On the other hand, with the same eugenol-based concentration of 0.1%, all termites died at papers treated with extracts of *S. aromaticum*. These results indicate that the presence of eugenol compounds has a potential response for termite control because of its toxicity. It was found that the toxicity effects of crude extracts depend on the synergic interaction of comprising active ingredients. The threshold value of single compound such as eugenol could not be considered as an appropriate toxicity level in the use of crude extracts.

Table 1. Percent mass loss of untreated and crude extracts treated samples after exposure to *Coptotermes formosanus* for four weeks

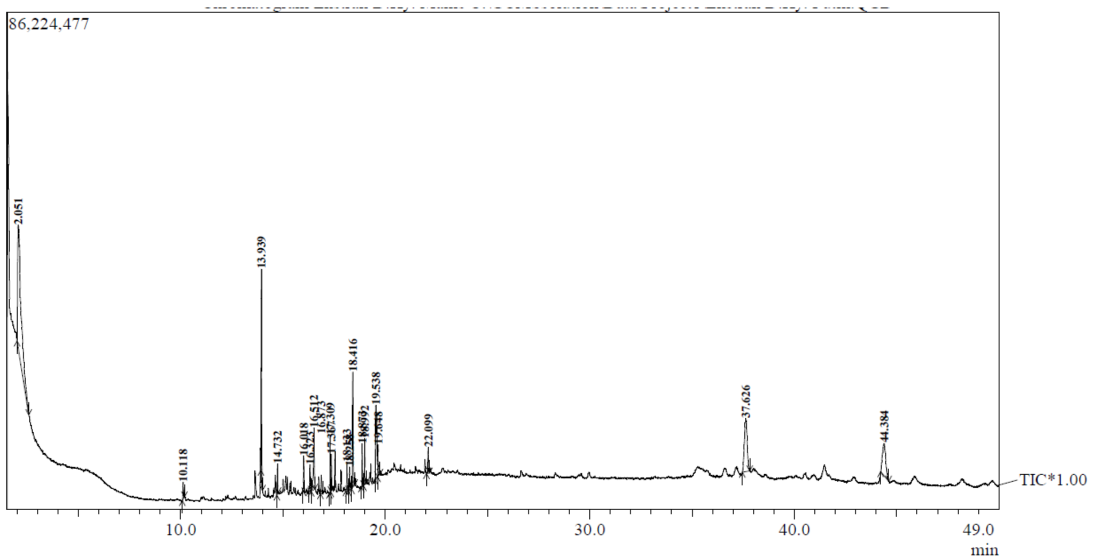
Extract Concentrations (%)	Mass loss (%) of samples treated with plant crude extracts[1, 2]	
	Syzygium aromaticum	***Melaleuca leucadendra***
0	14.65±2.57	14.65±2.57
0.01	26.27±2.79**	23.65±2.65**
0.05	17.13±5.41	18.81±2.61
0.1	0.26±0.09**	9.13±3.31*

Note: [1] Mean and standard deviation of five replicates; [2] Values in columns with one and two asterisks are significantly different from the control at $P < 0.05$ and $P < 0.01$, respectively, by Dunnett's test

Table 2. Mortality of *Coptotermes formosanus* fed extract-treated and untreated control filter paper samples after three weeks exposure

Extract Concentrations (%)	Termite mortality (%) on samples with plant crude extracts[1, 2]	
	Syzygium aromaticum	***Melaleuca leucadendra***
0	18.40±4.77	18.40±4.77
0.01	24.00±6.32	16.40±4.34
0.05	28.40±5.18*	20.80±3.03
0.1	100.00±0.00**	36.80±17.30*

Note: [1] Mean and standard deviation of five replicates; [2] Values in columns with one and two asterisks are significantly different from the control at $P < 0.05$ and $P < 0.01$, respectively, by Dunnett's test



Peak Report TIC

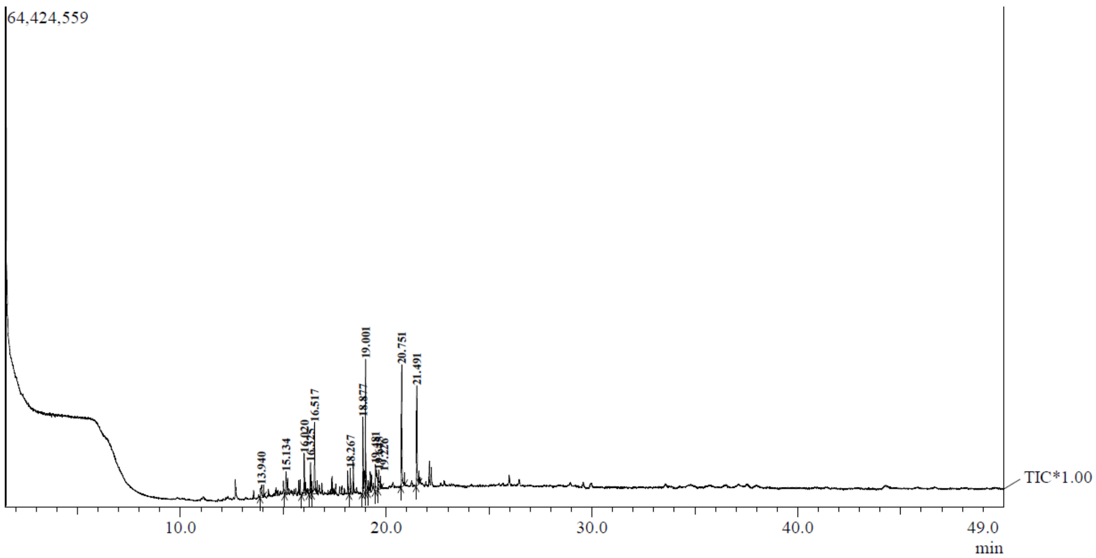
Peak#	R.Time	Area	Conc%	Name
1	2.051	245731140	35.46	Propane, 2-fluoro- (CAS) 2-Fluoropropane
2	10.118	6675181	0.96	Cyclohexene, 1-methyl-4-(1-methylethenyl)- (CAS) 1-P-MENTHA-1,8-DIENE
3	13.939	54851031	7.92	Phenol, 2-methoxy-4-(2-propenyl)- (CAS) Eugenol
4	14.732	7604224	1.10	Benzene, 1,2,3-trimethoxy- (CAS) 1,2,3-Trimethoxybenzene (CAS) Methylsy
5	16.018	11823600	1.71	Guaiol
6	16.323	8642122	1.25	Guaiol
7	16.512	31218595	4.51	.beta.-Eudesmol
8	16.873	6006466	0.87	2,5-Dimethyl-3,4,6-trimethoxybenzaldehyde
9	17.309	12082544	1.74	Benzyl benzoate
10	17.367	8425715	1.22	2',3',4' Trimethoxyacetophenone
11	18.133	7379705	1.06	Pentadecanoic acid, 14-methyl-, methyl ester (CAS) METHYL 14-METHYL-P
12	18.258	9057546	1.31	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl- (CAS) 2-METHYL-5
13	18.416	33139356	4.78	Hexadecanoic acid (CAS) Palmitic acid
14	18.873	10859092	1.57	1,4-Naphthalenedione, 5,8-dihydroxy-2-methoxy- (CAS) 1,4-Naphthoquinone
15	18.992	12688220	1.83	4H-1-benzopyran-4-one, 2,3-dihydro-5-hydroxy-7-methoxy-2-methyl-6-(3-me
16	19.538	54309086	7.84	9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid
17	19.648	14844139	2.14	9-Octadecenoic acid (Z)- (CAS) Oleic acid
18	22.099	9028883	1.30	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate
19	37.626	85837182	12.39	Olean-12-en-28-al, cyclic 1,2-ethanediyl mercaptal (CAS)
20	44.384	62744333	9.05	
		692948160	100.00	

Figure 1. GCMS analyze of *S. aromaticum* crude extract

A previous study showed that eugenol isolated from distilled clove leaf oil, purified to have 96.7% eugenol content, and used to treat filter papers at the concentration of 1-10% using hexane solvent was toxic to termites *Coptotermes gestroi* (Setiawan et al. 2010). In contrast, a study using 10-50% crude extract of clove (equal to eugenol content of 0.008-0.04%) was found to attract subterranean termites (Indrayani et al. 2015, unpublished data). The current study indicates that eugenol present in plant crude extracts may be either toxic or attractive to termites, depending on its concentration and the plant species. The crude extract of *S. aromaticum* was toxic to termites *C. formosanus* at the eugenol-based concentration up to 0.1%. A much higher eugenol-based concentration than 0.1% was required for the crude extract of *M. leucadendra* to be toxic to the termites.

Table 1 also showed that the crude extracts of the tested plant species showed higher consumption of filter paper samples treated with 0,01% eugenol-based concentration and no significant termite mortality compared to untreated control. The results indicate that the crude extracts are not toxic when applied at the low concentration level, even may perform an attractant effect to termites. This finding was supported by Machado et al. (2013) who examine oil of the neem tree (*Azadirachta indica*) showing a poor performance against termites. It is the fact that the repellent or non-repellent of crude extracts are also a function of concentration, not only the achievement of any particular compounds.

Figure 3 and 4 represent mortality rate of *Coptotermes formosanus* exposed to crude extract of *S. aromaticum* and *M. leucadendra*. Mortality rates of termites were directly related to their feeding activities and results of this study clearly showed that each of the extract treated sample had a different level of toxicity. The extract of *S. aromaticum* generally showed a higher level of toxicity to termites compared to that of *M. leucadendra* at the same eugenol-based concentrations. This fact indicates that the toxicity of crude plant extracts is related to the compound diversity associated with the origin of the material, not only to a single active ingredient such as eugenol content. These factors may partly account for the results obtained in terms of termite mortality rates (Figures 3 and 4). Samples treated with the crude extract of *S. aromaticum* at the eugenol-based concentration of 0.1% showed sharp increase in termite mortality toward the end of the exposure period (Figure 3). At the same eugenol-based concentration, the crude extract of *M. leucadendra* showed a slower rate of termite mortality (Figure 4).



Peak Report TIC

Peak#	R.Time	Area	Conc%	Name
1	13.940	6799739	3.36	Phenol, 2-methoxy-4-(2-propenyl)- (CAS) Eugenol
2	15.134	9188052	4.54	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS) ar-Curcumene
3	16.020	12085258	5.97	Guaiol
4	16.325	6859341	3.39	.alpha.-Gurjunene
5	16.517	22725186	11.22	Guaiol
6	18.267	7149964	3.53	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl- (CAS) 2-METHYL-5
7	18.877	21016358	10.38	1,4-Naphthalenedione, 5,8-dihydroxy-2-methoxy- (CAS) 1,4-Naphthoquinone
8	19.001	27491771	13.58	4H-1-benzopyran-4-one, 2,3-dihydro-5-hydroxy-7-methoxy-2-methyl-6-(3-me
9	19.226	11411867	5.64	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl- (CAS) 2-METHYL-5
10	19.481	16323310	8.06	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-methyl- (CAS) 2-METHYL-5
11	19.645	9624680	4.75	9-Octadecenoic acid (Z)- (CAS) Oleic acid
12	20.751	28102959	13.88	1-Phenyl-3-(p-chlorophenyl)-1,3-propandione
13	21.491	23687037	11.70	2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)- (CAS) Pi
		202465522	100.00	

Figure 2. GCMS analyze of *M. leucadendra* crude extract

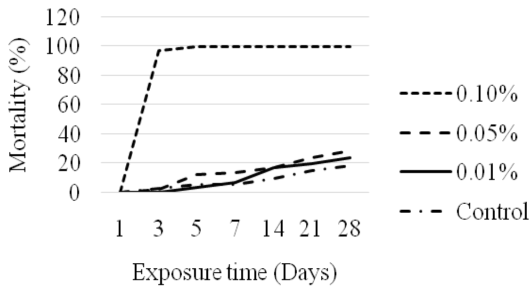


Figure 3. Mortality rates of termites, *Coptotermes formosanus*, exposed to untreated and *S. aromaticum* extract-treated filter papers for four weeks

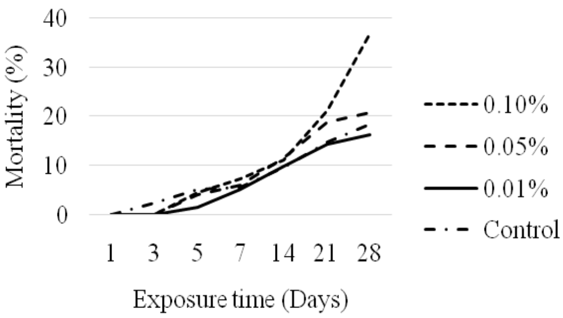


Figure 4. Mortality rates of termites, *Coptotermes formosanus*, exposed to untreated and *M. leucadendra* extract-treated filter papers for four weeks

In all cases of the different plant extracts at the eugenol-based concentration up to 0.05% caused no significant mortality rates of the termites during the exposure period. However, when 0.1% eugenol-based concentration of *S. aromaticum* was used, termite mortality was 100% only after three days of exposure. A slower toxicity effect was detected in samples treated with 0.1% eugenol-based concentration of *M. leucadendra*, showing the average termite mortality of around 40% after four weeks of exposure. This might be caused eugenol content in *S. aromaticum* crude extract was higher (7.92%) than that of *M. leucadendra* (3.36%) (Figures 1 and 2). This finding supported by Cornelius et al (1997), who stated that eugenol was significantly more toxic than the other semiochemicals such as citral, citronellal, geraniol, nerol, and pyrrolidine and found that mortality subterranean termite *Coptotermes formosanus* reach 100% after exposed to eugenol at concentration as low as 0.06 µl/g of sand.

The current study showed that ethanol crude extracts of *S. aromaticum* and *M. leucadendra* had different toxicity effects on subterranean termite *C. formosanus* although they were used at the same eugenol-based concentrations. The crude extract of *S. aromaticum* totally prevented termite feeding on filter papers treated with 0.1% eugenol-based concentrations and caused 100% termite mortality, while that of *M. leucadendra* showed lower termite mortality (20-55%). Filter papers treated with 0.01% crude extracts of the two plant species demonstrated attractive effects on termites as indicated by higher consumption and insignificantly different mortality compared to untreated controls. These results indicate that crude extracts of the two plant species may be potentially useful in the development of termite control products in the future.

To avoid environmental pollution, it is indispensable to seek bio-pesticide which could produce a synergistic effect to improve the control effects on termite. This experiment shows that extracts from *S. aromaticum* leaves contain biologically active compound with demonstrable effects on subterranean termite *C. formosanus*.

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