BIOTOXICITY OF TROPICAL PLANT ESSENTIAL OILS AGAINST SUBTERRANEAN TERMITES *COPTOTERMES CURVIGNATHUS*

Roszaini K*, Mailina J, Shaharuddin H, Zaihan J & Nor-Azah MA

Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia

**roszaini@frim.gov.my*

Submitted Mei 2021; accepted September 2021

This study was established to evaluate the efficiency of essential oils from tropical plants such as *Cinnamomum zeylanicum*, *Cinnamomum rhyncophyllum* and *Litsea elliptica* in preserving susceptible timber against a subterranean termite named *Coptotermes curvignathus*. Two different tests mainly the toxicity and repellent tests were conducted in five concentrations (0.5%, 1.0%, 2.0%, 3.0% and 4.0%) to observe the repellency, feeding response and mortality of the termites. A no-choice test was conducted in the laboratory to investigate the effectiveness of essential oils in protecting *Hevea brasiliensis*. Based on the findings, *C. zeylanicum* essential oil showed high antifeedant (56.2%–96.2%) and repellent (41.0%–62.0%) activities. The oil was also very effective in no-choice test with a lower percentage of wood use (3.5%–5.9%), a higher percentage of *C. curvignathus* mortality (90.2%–100%) and a higher visual assessment (8.9–9.2). The performance was followed by *C. rhyncophyllum,* while *L. elliptica* essential oil had the highest repellent activity at each level of concentration. The results suggested that these essential oils had beneficial activities of economic value for the development of new or latest termite control product.

Keywords: Subterranean termites, essential oil, termite assays, gas chromatography, *Hevea brasiliensis,* wood protection

INTRODUCTION

Essential oils are complex mixtures of volatile and semi-volatile organic compounds originating from a single botanical source (Tisserand & Young 2013). About 300 species or 10% of 3,000 species (Muturi et al. 2017) were reported to have commercial value and recognised as environmentally friendly, easily biodegradable, minimally toxic to mammals, have shown repellent (Gleiser et al. 2011), toxicant (Conti et al. 2010), insecticidal effects (Islam et al. 2009) and biological activities (Mehdizadeh & Moghaddam 2018).

Previous studies reported that essential oils from *Lippia sidoides* and *Pogostemon cablin* guaranteed to control *Nasutitermes corniger* termite in the field (Janaína et al. 2013). The essential oils of *Cassia* leaf, cedarwood, *Eucalyptus globules, E. citrodora,* lemongrass and geranium had less effect of repellency and toxicity on *Coptotermes formosanus* termites (Zhu et al. 2001). The essential oils from linseed, jatropha, neem and jojoba gave complete mortality of *Odontotermes obesus* termite and were good in feeding resistance in both the choice and no-choice tests (Ahmed et al. 2020).

Cinnamomum or locally known in Malay as kayu manis in Malaysia, are small evergreens trees with aromatic bark and leaves. Several species of *Cinnamomum* were studied for their chemical components and tested against 21 species of fungi (Ibrahim & Goh 1992). However, only a few studies on the effectiveness of essential oils from the plant family were conducted on termites or other insects. A study by Roszaini et al. (2013) reported the toxicity and repellent properties of four plants essential oils including *Cinnamomum camphora* against *Coptotermes curvignathus* termites. Paranagama et al. (2004) reported the repellency and toxicity of essential oils from the *Cinnamomum zeylanicum* leaves on *Sitophilus oryzae* rice weevil. Kartal et al. (2006) studied the effect of essential oils compounds on decay and termite resistance of wood, while Chang and Cheng (2002) and Cheng et al. (2004) found that *Cinnamomum osmoploeum* showed good insecticidal activity against mosquito larvae and termites. Another previous study also revealed that *Cinnamomum* species essential oils had toxicity and insecticidal activities against other insects (Fichi et al. 2007).

Litsea elliptica, or also known in Malay as medang pepijat and was known for its aromatic bark, wood, leaves and fruits. Several previous studies had shown that the plant showed potential insecticidal activity against vector mosquitoes (Rohani et al. 1997), repellence activity against *Aedes aegypti* (Ibrahim & Zaridah 1998) and was toxics against termites (Hidayatulfathi et al. 2003). However, there were not many published studies regarding *L. elliptica* against subterranean termites except one by Arbain et al. (1990).

Many wood preservatives in the market were banned because of improper usage procedures and negative impact on human health and the environment due to their heavy metals content. Therefore, many requests and studies on these antitermitic novels were conducted and focussed on the search for effective new materials with fewer side effects. Findings from Koul et al. (2008) and Upadhyay et al. (2011) had suggested that excellent alternatives to synthetic pesticides could originate from natural products, in which they could reduce negative impacts on human health and the environment. In addition, the usage of the natural plant-derived pesticides in small quantities was sufficient to reduce the population of various pest species.

This study aimed to examine the acute toxicity effects of tropical plant essential oils on termites and to be used as potential wood preservative. Therefore, a preliminary toxicological investigation on three essential oils was performed against the *C. curvignathus* subterranean termites.

MATERIALS AND METHODS

Termites

Coptotermes curvignathus soldier and worker termites were collected from an active colony on *Hevea brasiliensis* or rubberwood logs in Field 51of the Forest Research Institute Malaysia (FRIM). The wooden logs were cut and transported to the laboratory to isolate termites according to the soldier and worker castes. Soldiers and workers were removed from the logs by placing several pieces of filter paper moistened with water and left on the log for a few minutes. The filter papers filled with termites were placed into a basin to separate active soldier and worker termites.

Plant material

The essential oils from *L. elliptica, C. rhyncophyllum* and *C. zeylanicum* were obtained from the FRIM essential oils collections at FRIM's Natural Products Division. All essential oils were collected from the leaf part of each tree. The plants went through hydrodistillation process for 6 hours in a clavenger device (Nor-Azah et al. 2005). The product oil layer was separated from water and dried over anhydrous sodium sulphate. All oils were stored at 4 °C until further use. Methanol (CH_3OH) was used as the solvent to dilute each stock solution.

Antitermitic bioassay for toxicity determination

The bioassay method used in the previous study (Roszaini et al. 2013) was used to evaluate the antitermitic activity of the essential oils against *C. curvignathus* termite. Samples of 5.0 mg, 10 mg, 20 mg, 30 mg and 40 mg of essential oils from each plants were dissolved in 100 µl of CH_3OH to obtain solutions (m/v) of 0.5%, 1.0%, 2.0%, 3.0% and 4.0%, respectively. Subsequently, a volume of 20 µl of the solutions was applied to each 30 mg filter paper sample using a micropipette and dried in a vacuum desiccator for 24 hours. The paper discs were weighed before and after oven-dried at 60 °C. The untreated and paper discs treated with solvent only were used as test controls. Twenty active worker termites were placed in each petri plate of 90mm in diameter and 16 mm depth containing 3 g of sterile sand. A few drops of water were added periodically to each petri dish. All petri dishes with lids were placed in an incubator and kept in dark at a temperature of 22 ± 2 °C and 65 ± 5% relative humidity. The mortality of the termites was counted and recorded every 24 hours for 10 days. Each test contained 3 replicates including control. The difference in dry weight before and after the exposure was used to calculate the consumption of the filter papers. A dose-mortality line was developed depending on the exposure times and the lethal concentration (LC_{50}) of wood extracts were determined using the probit method (Finney 1971).

Repellency assay

The ability of the essential oils to repel termites was assessed according the method used by Roszaini et al. (2020). A filter paper with 9 cm diameter was cut into two. The first part of the paper was treated with 1 ml of essential oil at different concentrations after diluted with CH_3OH and the second part was treated with 1 ml CH_3OH using a micropipette. Three replicates for each test and control experiment were also prepared. The dish was dried in a laminar flow hood for one hour. Half of the treated and control dish was attached with sticky tapes. Each dish was later placed in a 9.1 cm diameter petri dish and 50 worker termites were put in the centre of each dish. The number of termites presents in the control and the treated halve samples were assessed hourly for 4 hours.

No-choice tests (wood block bioassay against subterranean termites)

Hevea brasiliensis wood blocks (25 mm × 25 mm × 6 mm) were pressure-treated with the same concentration levels of 0.5%, 1.0%, 2.0%, 3.0% and 4.0% of essential oil as toxicity and repellency tests. All samples were subjected to no-choice feeding tests according to the AWPA E1-17 (AWPA 2017) standard methods with slightly modified. The untreated *H. brasiliensis* wood blocks were used as controls.

Each screw bottle with 8 cm diameter and a height of 13 cm was filled with 200 g of sterilised sand and 30 ml of distilled water. The bottles were left overnight to equilibrate at laboratory conditions before testing. One wood block was placed on the surface of moist sand and 400 termites (360 workers and 40 soldiers) were added to each bottle. All bottles were stored in an incubator and maintained at 22 ± 2 °C and 65 ± 5% relative humidity for 28 days. Within this period if all termites were found dead, the bottle was removed and the number of days until 100% mortality was recorded. At the end of the test after 28 days, the blocks were taken out from the test bottle, cleaned, dried overnight and weighed again. The remaining living termites were weighed and recorded for each bottle. The condition of the specimen blocks was observed and rated visually based on a scale of 1 to 10 where 0 denoted total failure and 10 denoted sound (no attack) (AWPA 2017).

Statistical analysis

The effects of essential oil concentration weight loss and feeding inhibition were determined according to a completely randomized design with a 95% confidence level. One-way ANOVA was used to check the effect of the treatment and Duncan's test (Duncan Multiple Range Test) was used for ranking the average values of the measured property. Abbott's formula (1925) was used to connect mortality for the controls and probit analyses.

RESULTS AND DISCUSSION

Toxicity test

The previous study by Nor-Azah et al. (2000) reported the chemical composition of the essential oils from the three species (Table 1). The essential oils were composed of different compounds and normally abundant in terpenes and their oxygenated derivatives called terpenoids (Baser & Buchbauer 2015). Some of the chemical components observed in the oils were eugenol, cinnamyl acetate, eugenyl acetate, β-caryophyllene, benzyl benzoate, β-phellandrene, (E)-methyl cinnamate and 2-undecanone. The presence of these chemical components in the oils could be one of the factors that contributed to the toxicity effects of the oils on t he subterranean termites.

Table 1 Major chemical constituents of the three selected tropical plant essential oils (Nor-Azah et al. 2000)

Species	Local name	Chemical constituents
Litsea elliptica	Medang pepijat	2-undecanone (70.89%)
Cinnamomum rhyncophyllum	Kayu manis	Benzyl benzoate (77%), ß-phellandrene (6.3.0%) and (E)-methyl cinnamate (4.2%)
Cinnamomum zeylanicum	Kayu manis	Eugenol (84.35%), cinnamyl acetate (0.85%), eugenyl acetate (0.52%), β-caryophyllene (3.08%)

Toxicity of the three plants essential oil against *C. curvignathus* was presented in Table 2. All essential oil was strongly toxic toward *C. curvignathus* at all concentrations for the ten days observation. At the end of the exposure time of 10 days, the average consumption of filter paper was significantly lower than in their corresponding controls. This effect was more pronounced for filter papers treated with *C. zeylanicum* essential oil than those treated with *C. rhyncophyllum* and *L. elliptica* essential oil. The lowest weight loss was observed in higher concentrations at 4.0% for every essential oil at 0.26% in *C. zeylanicum*, 0.33.0% in *C. rhyncophyllum* and 2.59% in *L. elliptica*, respectively. The statistical analysis also indicated that the toxicity activity of *C. curvignathus* essential oil was significantly higher except for *C. zeylanicum* at 0.5% concentration than control and CH_3OH ($F = 8.567$, $p = 0.001$).

The feeding inhibition was listed in Table 2 for all the different essential oil concentrations for 24 hours. All essential oil showed more than 60% of the feeding inhibition at 4.0% concentration level. *Cinnamomun zeylanicum* essential oil showed the highest percentage at 96.19% feeding inhibition, while *L. elliptica* showed the lowest inhibition at 61.43.0%. Essential oil of *C. zeylanicum* appeared to be the most toxic against *C. curvignathus* as indicated by its low LC_{50} values of 2.44.0% followed by *C. rhyncophyllum* at 4.20% and *L. elliptica* at 12.60%. The performance of *C. zeylanicum* essential oil was near twice toxic as that of *C. rhyncophyllum* and five times toxic than *L. elliptica* essential oil. The combination of major compounds such as eugenol, cinnamyl acetate, eugenyl acetate and β-caryophyllene in *C. zeylanicum* could be the reason for the best toxicity effect among the three plant species. The performances of *C. zeylanicum* oil were also known for its various pest control properties (Koul et al. 2008). A previous study by Isman et al. (2009) concluded that the significant effect of an essential oil was not contributed by the major constituents alone but was due to the synergistic effect of several chemical constituents. A study by Xie et al. (2015) suggested that eugenol

Table 2 Effect of essential oils on feeding and mortality of *Coptotermes curvignathus*

Treatment	Concentrations (%)	% paper consumption	% Feeding-Inhibition (FI%)	LC_{50} (%)
Control		5.75 (0.32)[a]		
Methanol		3.12 (0.35)[b]		
L. elliptica	0.5	3.25 (0.25)[c]	51.91 (3.56)[bc]	12.604[a]
	1	4.64 (0.32)[c]	46.19 (4.86)[c]	
	2	3.13 (0.18)[c]	53.81 (2.69)[bc]	
	3	2.66 (0.47)[d]	60.48 (2.73)[ab]	
	4	2.59 (0.27)[d]	61.43 (4.04)[a]	
C. rhyncophyllum	0.5	2.06 (0.09)[c]	70.48 (1.35)[c]	4.195[b]
	1	1.43 (0.32)[d]	79.05 (4.86)[b]	
	2	1.76 (0.42)[cd]	74.29 (2.17)[bc]	
	3	0.40 (0.28)[e]	94.29 (4.04)[a]	
	4	0.33 (0.19)[e]	95.24 (2.69)[a]	
C. zeylanicum	0.5	3.01 (0.47)[b]	56.19 (2.13)[c]	2.436[c]
	1	1.76 (0.57)[c]	74.29 (3.41)[b]	
	2	0.90 (0.08)[c]	86.67 (1.35)[b]	
	3	0.34 (0.10)[d]	95.24 (1.35)[a]	
	4	0.26 (0.09)[d]	96.19 (1.35)[a]	

Mean (± standard deviation) of 3 replicates for each species; percentage values followed by the same letter (superscript) were not significantly different in the same group vertical at the 0.05 level of probability; LC_{50} = Lethal Concentration which caused a 50% reduction in feeding as compared to the non-treated control exhibited the strongest antitermitic activity after it gave 100% mortality in *Reticulitermes chinensis* termites after 5 days of testing. In another situation in the current study, although the presence of eugenyl acetate was only 0.52%, it also had toxic effect on *C. curvignathus* as it had been reported that eugenyl acetate gave high toxicity against human cabbie mites (Pasay et al. 2010) and β-caryophyllene was one of the most toxic compounds against *Reticulitermes speratus* termites (Park & Chin 2005).

The toxicity of *C. rhyncophyllum* essential oil could be due to the high availability of benzyl benzoate at 77%. Benzyl benzoate had shown insecticidal activity against many organisms, such as *Dermatophagoides pteronyssinus* or house dust mite (Suhaili & Ho 2008), *Tyrophagus putrescentiae* mites (Harju et al. 2004), *Sarcoptes scabiei* mite (Dressler et al. 2016), ovicidal activity to *Sogatella furcifera* plant hoppers and reduced mite populations (Rebmann et al. 1996). The ß-phellandrene, which was belonged to the monoterpenes group, could also contribute to the toxicity of *C. rhyncophyllum* essential oil against termites. Evidences from several reports showed that monoterpenoid essential oil inhibited the activity of acetylcholinesterase, an enzyme which broke down (Houghton et al. 2006). When the acetylcholinesterase activity was inhibited, it caused the inability of acetylcholine to stimulate the muscle or to transmit signals from one cell to another and eventually led to death of an organism including termites.

Even though, *L. elliptica* showed less effective than *C. zeylanicum* and *C. rhyncophyllum*, it was also able to inhibit termite activity with the presence of 71% 2-undecanone (Table 1). A previous study showed that *L. elliptica* essential oil had insecticidal activity against *Aedes aegypti* mosquitoes (Ibrahim & Zaridah 1998). In addition, a large spectrum of pharmacological activity of *Litsea* spp. including insecticides could be obtained from coarse extracts, fractions and phytochemical constituents (Wang et al. 2016).

Essential oils from the three plant species showed different time for first mortality in *C. curvignathus* (Table 3). The time for first mortality also depended on the concentration of essential oils used. At the concentrations of 0.5%, 4.33 days for first mortality were recorded for *C. zeylanicum* essential oil and 6.33 days for first mortality for *C. rhyncophyllum* essential oil. However, *L. elliptica* essential oil showed almost 14 days for first mortality, which was about 3.16 times required at the same concentration. At

Table 3 Effects of exposure of subterranean termite, *Coptotermes curvignathus* to filter paper treated with essential oils at different rate of concentration

	Concentrations (%)	Termite mortality (%)			
		4 days	7 days	10 days	14 days
Control (untreated)	0	6[f]	10[h]	18[e]	24[e]
L. elliptica	0.5	46[e]	56[e]	62[d]	69[d]
	1.0	52[de]	48[f]	58[d]	65[d]
	2.0	40[e]	30[g]	74[cd]	83[c]
	3.0	58[d]	66[d]	90[b]	100[a]
	4.0	60[cd]	89[b]	100[a]	100[a]
C. rhyncophyllum	0.5	54[d]	63[d]	65[d]	88[bc]
	1.0	61[cd]	58[e]	73[cd]	84[c]
	2.0	69[bc]	74[c]	100[a]	87[bc]
	3.0	75[ab]	70[cd]	95[ab]	100[a]
	4.0	68[bc]	100[a]	100[a]	100[a]
C. zeylanicum	0.5	60[cd]	63[d]	77[c]	86[bc]
	1.0	55[d]	60[d]	75[c]	88[bc]
	2.0	65[c]	72[c]	84[bc]	92[b]
	3.0	78[a]	90[b]	100[a]	100[a]
	4.0	80[a]	100[a]	100[a]	100[a]

Mean of 3 replicates for each species; percentage values followed by the same letter (superscript) were not significantly different in the same group vertical at the 0.05 level of probability the highest concentration of 4.0%, *C. zeylanicum* essential oil needed only 1 day, while *C. rhyncophyllum* essential oil recorded 3 days and *L. elliptica* essential oil recorded almost 9 days for first mortality to be detected. Mortality by the essential oil of *C. zeylanicum* was significantly different from the treatment control ($p < 0.05$), while there was no significant difference from the control treatment of *C. rhyncophyllum* essential oil at 0.5% and 1.0% of concentrations.

This was the first study in researching for the termiticides effect of *C. zeylanicum.* The good performance of *C. zeylanicum* was also reported against mange mite, *Psoroptes cuniculi* (Fichi et al. 2007). The research showed that after 24 hours of contact, all concentrations between 0.10% and 10% showed a good *in vitro* acaricidal efficacy ($p < 0.01$) against the mite. In another study, *C. zeylanicum* oil vapours had potential fumigant activity against *Ephestia kuehniella* (LC_{50}: 2.87 μL L^{-1} air) and its essential oil also had the highest detergency rate of 93.82% in oviposition deterrence experiment on *Callosobruchus maculatus* (Abbasipour et al. 2012). The effectiveness of these *C. zeylanicum* leaves essential oil could be due to the presence of eugenol, camphor, tetradecanal and cinnamyl caryophyllene as reported by Noor-Fasihah (2011). Previous studies showed that eugenol and camphor were toxic against subterranean termite such as *C. curvignathus* and *C. gestroi.* (Roszaini et al. 2013, Roszaini et al. 2020)

Although *L. elliptica* essential oil gave lower mortality rates than C. *zeylanicum* and *C. rhyncophyllum,* it still showed toxicity against *C. curvignatus* at over 75% of the mortality rate recorded (Figure 4). Research findings from Hussain et al. (2012) stated that most plants had biological activity against different insects and other organisms.

Repellent activity

Litsea elliptica essential oil had the highest repellent activity at each level of concentration compared to *C. rhyncophyllum* and *C. zeylanicum* (Figure 1). The high (> 80%) repellent activity was observed from the concentration of 2.0% and the activity increased with the increased essential oil concentration gradient. Both essential oils of *C. rhyncophyllum* and *C. zeylanicum* showed almost the same repellency. The high repellent activity at 4.0% of *L. elliptica* oil could be explained by the presence of high 2-undecanone at 70.89% (Table 1) but not in *C. rhyncophyllum* and *C. zeylanicum.* The content 2-undecanone which was also known as methyl nonyl ketone and IBI-246 was reported to possess good insect repellent activity (Bohbot & Dickens 2010).

Retention of treated wood samples

Figure 2 showed the retention values of samples treated with different concentrations of essential oils. The highest essential oil retention was at 21.7% for the *C. zeylanicum* sample treated with 4.0% oil retention and the lowest retention was 3.1% for the *L. elliptica* sample treated with 0.5% oil retention. The results also showed that all samples treated with *C. zeylanicum* essential oil had the highest retention value for all essential oil concentration levels tested, followed by *C. rhyncophyllum* and the lowest was in *L. elliptica.* The retention value also showed an increase with the increasing rate of concentration of essential oil applied. Statistical analysis showed that there was significant difference in the retention value at each concentration of essential oil applied except in some cases in *C. rhyncophyllum* and *L. elliptica.* Many factors could influence the permeability and absorption of solutions in wood such as wood structure, wood moisture content and impregnation methods (Nicholas & Siau 1973). In addition, the uptake of the solution was also better in the longitudinal direction than in the radial or tangential directions due to the natural longitudinal positions of the wood cells (Lahtela et al. 2014).

No-choice tests (Wood block bioassay against subterranean termites)

The experiments showed that the essential oil for all different concentrations affected the weight loss of the wood block samples (Figure 3). The highest weight loss of 12.47% for each treatment was found in the control untreated blocks which showed relatively high difference in value as compared to other treatments. The lowest weight loss at 3.53.0% was in the test block treated with *C. zeylanicum* essential oil of 4.0% concentration. The results showed the decrease in weight with the increase of oils concentration applied. The results of the analysis of variance on weight loss indicated that the treatment with different essential oil at various concentrations had significant effect on

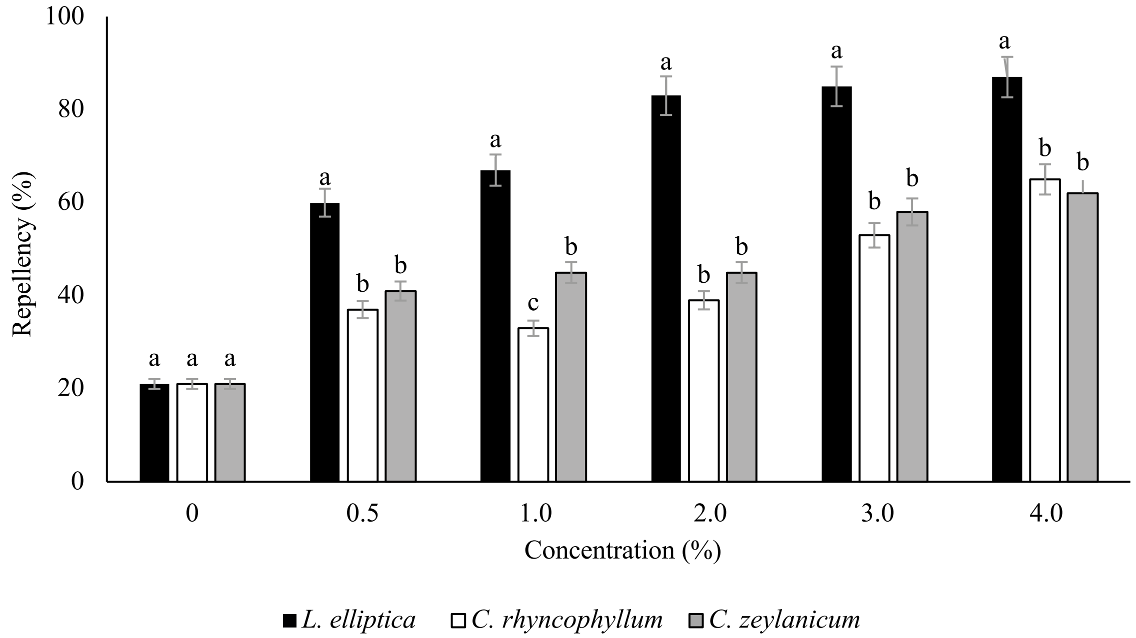

Figure 1 Mean percentage of repellent activity for *C. curvignathus* in a choice test using three replicates of each species and concentrations
Termite was treated either essential oil applied to filter paper with 0.5%, 1.0%, 2.0%, 3.0% or 4.0% concentration or control (methanol only) filter paper; different letters above bars indicated significant difference between treatments in the same group of concentration at $p < 0.05$

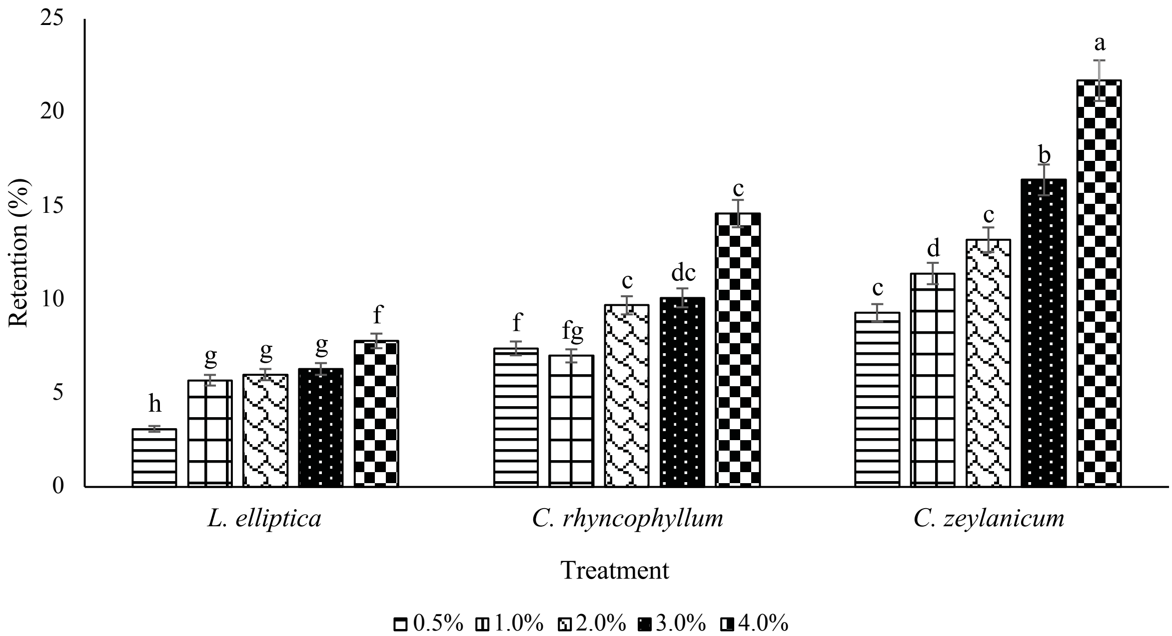

Figure 2 Retention of treatment solution in *H. brasiliensis* test blocks
Different letters above bars indicated significant difference between treatments in the same group at $p < 0.05$

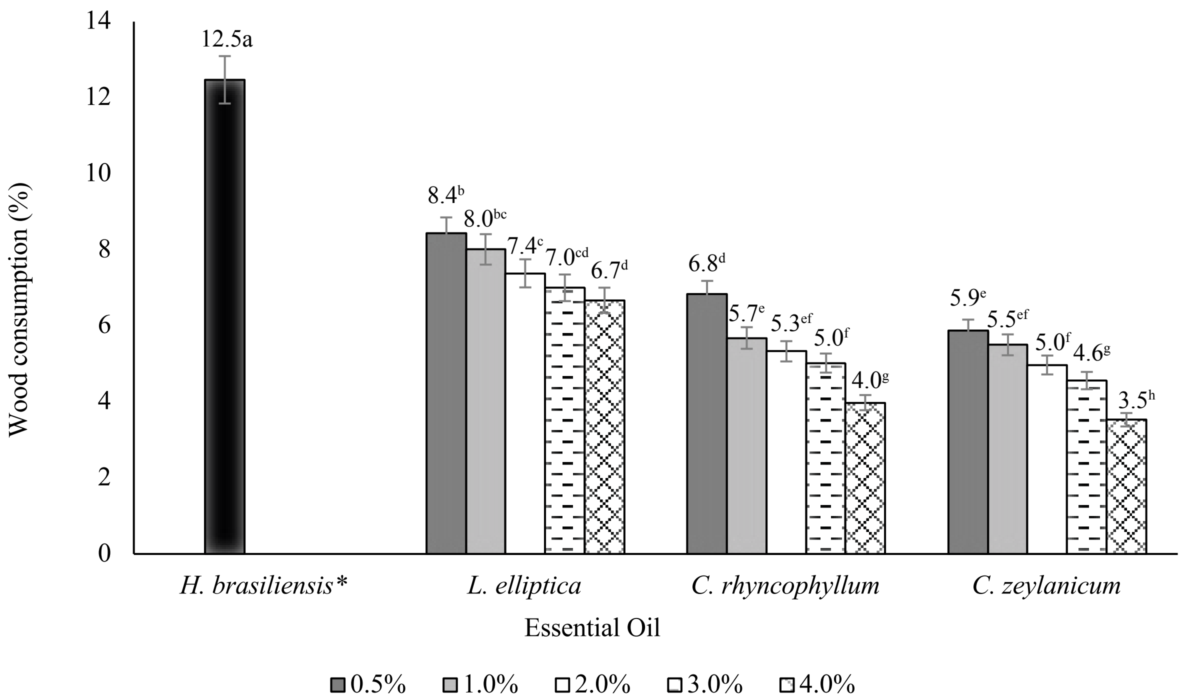

Figure 3 Mean wood consumption (%) of treated *H. brasiliensis* with different essential oils in no choice laboratory bio-assays against the termite *C. curvignathus*
Each value representd the mean of 5 replicates; *H. brasiliensis** = untreated sample block; mean weight loss (%) by the same letter was not significantly ($p < 0.05$) different between treatments in the same group of concentration

the wood block weight loss. Essential oil from *C. zeylanicum* produced better termite resistance followed by essential oils from *C. rhyncophyllum* and *L. elliptica* in the laboratory conditions. Previous studies also showed that *C. zeylanicum* essential oil demonstrated insecticidal activity against *Tribolium castanuem* red flour beetle (Pugazhvendan et al. 2012), *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* mosquitoes (Prajapati et al. 2005), *Culex tritaeniorhynchus giles* and *Anopheles subpictus* mosquitoes (Govindarajan 2011), as fumigant against *C. maculatus* beetle, *T. castaneum* beetle and *E. kuehniella* moth (Abbasipour et al. 2012) and the oil with main constituents also showed ovidical and adulticidal effects against *Pediculus humanus capitis* human head lice (Yang et al. 2005). The increase in the concentrations of essential oil used to achieve 100% protection and 0% living termites was needed for further research.

The average percentage of *C. curvignathus* termite mortality was presented in Figure 4. It was evident that each concentration of the essential oil was lethal to the worker and soldier termites. The highest mortality at 100% was recorded at 4.0% of *C. zeylanicum* essential oil and the lowest was in the untreated control at 74.50%. The results of weight loss and mortality indicated that the addition of essential oil in the wood blocks had resulted in increased resistance of *H. brasiliensis* wood to termite attack. The findings indicated that the essential oils from the three plant species had potential antitermite activities.

The analysis of variance results showed that *H. brasiliensis* treated with different concentrations of essential oils did not show any significant differences in *C. curvignathus* mortality except between 0.5% with 3.0% and 4.0% in certain cases after the feeding, however they did show significant differences between the treated and control samples. Generally, the increase in essential oil concentrations caused significant increase in the mortality of *C. curvignathus* within 24 hours.

Visual-rating results showed that the addition of essential oil increased the durability of the treated wood from moderate or severe attack in untreated *H. brasiliensis* with a visual rating of 7.0 to slight attack with mean visual rating of 9.2 (Figure 4). The addition of essential oils

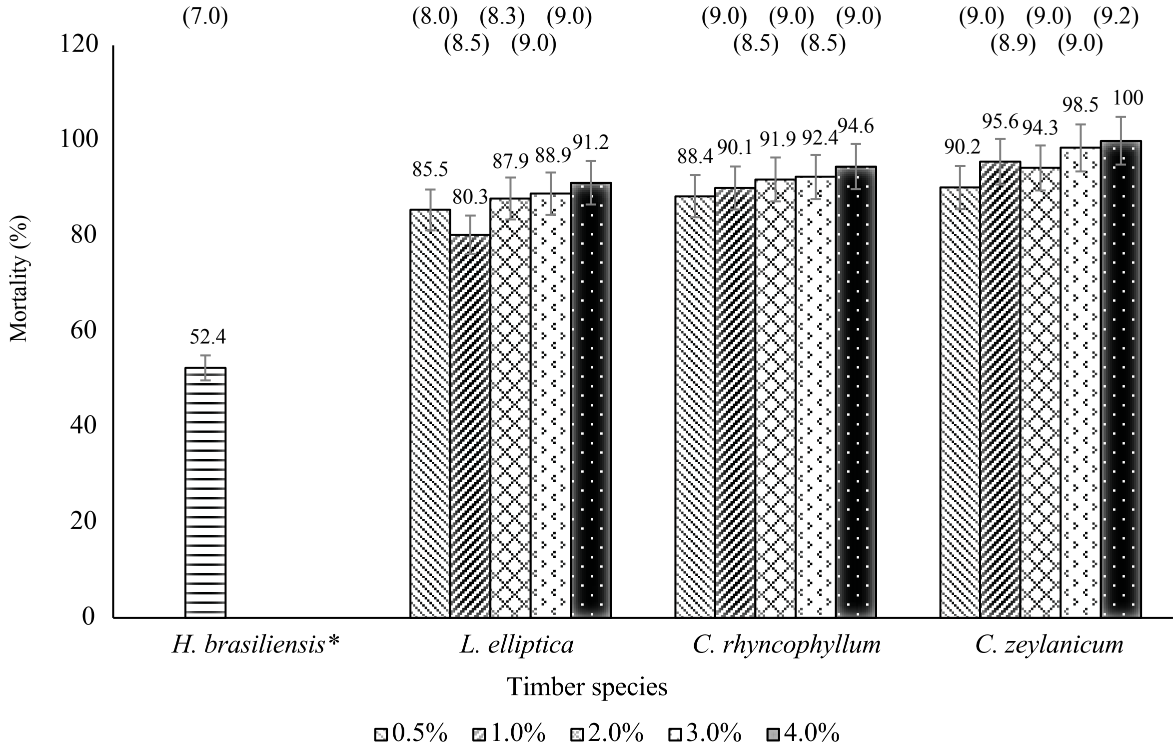

Figure 4 Mortality of *C. curvignathus* after 28 days exposed to sample treated with different essential oils at different concentration
*H. brasiliensis** = untreated sample block; each value represented the mean of 5 replicates; number in the bracket above the bar was the visual rating value

helped in controlling termite attack on wood. However, statistical analysis showed there were no significant differences except in few cases between the solvent used and the concentrations of essential oil in wood blocks but the treated blocks differed significantly from untreated blocks of *H. brasiliensis*. The observation might be related to the absorption of essential oil into the wood causing less weight loss and was supported by the lack of termite attack on the test block. As mentioned in early research by Tisserand and Young (2013), essential oil was a complex mixture of volatile and semi-volatile organic compounds and found little evidence in termite attack on the sample with the weight loss between 3.0% and 6.0% (Fatima & Morrell 2015). The trend was believed due to the volatilisation of the oils from the block rather than weight loss.

CONCLUSION

This study examined the efficacy of essential oils from three species of plants as a protective ingredient for *H. brasiliensis* against termites. High essential oils toxicity on subterranean termites, *C. curvignathus* suggested that the essential oil might be a potential source of bioactive substances for wood preservatives. The rapid but reliable toxicity study served as an initial screening of bioactive compounds that could then underwent a more detailed bioassay for a particular wood treatment activity. Significant effects of essential oils especially from *C. zeylanicum* on termites suggested that the oil could be used as wood preservative or termiticide and active compounds responsible for antitermitic activity required further investigation.

ACKNOWLEDGEMENTS

The authors acknowledged the financial support from FRIM Young Scientist Grant No. 41310404005. The authors expressed their gratitude to the staff of the Wood Entomology Laboratory and Herbal Product Development Laboratory of FRIM for termite collection and essential oils preparation.

REFERENCES

Abbasipour H, Mahmoudvand M, Rastegar F et al. 2012. Chemical composition, fumigant toxicity and oviposition deterrence of *Cinnamomum zeylanicum* essential oil to three stored-product insects. *Archives Des Sciences* 65: 535–542.

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265–267.

Ahmed S, Fatima R & Hassan B. 2020. Evaluation of different plant derived oils as wood preservatives against subterranean termite *Odontotermes obesus. Maderas Ciencia y tecnologia* 2291: 109–120.

Arbain D, Dasman-Ibrahim S & Sargent MV. 1990. The constituents of the bark of *Litsea elliptica* (Lauraceae). *Australian Journal of Chemistry* 43: 1949–1952.

AWPA (American Wood Preserver's Association). 2017. Laboratory methods for evaluating the termite resistance of wood-based materials: Choice and no-choice tests. AWPA E1-17. Pp. 1–6 in *AWPA Book of Standards.* AWPA, Birmingham.

Baser KHC & Buchbauer G. 2015. *Handbook of Essential Oils: Science, Technology, and Applications*, Second Edition. CRC Press, Boca Raton, FL.

Bohbot JD & Dickens JC. 2010. Insect repellents: Modulators of mosquito odorant receptor activity. *PLoS ONE* 5: e12138.

Chang ST & Cheng SS. 2002. Antitermitic activity of leaf essential oil and components from *Cinnamomum osmophleum. Journal of Agricultural and Food Chemistry* 50: 1389–1392.

Cheng SS, Liu JT, Tsai KH et al. 2004. Chemical composition and mosquito larvicidal activity of essential oils from leaves of different *Cinnamomum osmophloeum* provenances. *Journal of Agricultural and Food Chemistry* 52: 4395–4400.

Conti B, Canale A, Bertoli A et al 2010. Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitology Research* 107: 1455–1462.

Dressler C, Rosumeck S, Sunderkötter et al. 2016. The treatment of scabies: A systematic review of randomized controlled trials. *Deitsches Arzteblatt International* 113: 757–762. http://doi.org/10.3238/arztebl.2016.0757

Fatima R & Morrell JJ. 2015. Ability of plant-derived oils to inhibit dampwood termite (*Zootermopsis augusticollis*) activity. *Maderas Ciencia y Tecnologia* 17: 685–690.

Fichi G, Flamini G, Zaralli LJ et al. 2007. Efficacy of essential oils of *Cinnamomum zeylanicum* against *Psoroptes cuniculi. Phytomedicine* 14: 227–231.

Finney DJ. 1971. *Probit analysis.* 3rd Edition, Cambridge University Press, London.

Gleiser RM, Bonino MA & Zygadlo JA. 2011. Repellence of essential oils of aromatic plants growing in Argentina against *Aedes aegypti. Parasitology Research* 108: 69–78.

Govindarajan M. 2011. Larvicidal and repellent properties of some essential oils against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine* 4: 106–111. http://doi.org/10.1016/S16995-7645(11)60047–3

Harju ATK, Pennanen SMA & Liesivuori J. 2004. The efficacy of benzyl benzoate sprays in killing the storage mite *Tyrophagus putrescentiae* (Acari: Acaridae). *Annals of Agricultural Environmental Medicine* 11: 115–119.

Hidayatulfathi O, Sallehudin S, Ibrahim J et al. 2003. Evaluation of methanol extracts of some Malaysian plants for larvicidal activities. *Tropical Biomedicine* 20: 153–157.

Houghton PJ, Ren Y & Howes MJ. 2006. Acetylcholinesterase inhibitors from plants and fungi. *Natural Product Reports* 23: 181–199.

Hussain MS, Fareed S, Ansari S et al. 2012. Current approaches toward production of secondary plant metabolites. *Journal of Pharmacy and Bioallied Sciences* 4: 10–20.

Ibrahim J & Goh SH. 1992. The essential oils of Cinnamomum species from Peninsular Malaysia. *Journal of Essential Oil Research* 4: 161–171.

Ibrahim J & Zaridah MZ. 1998. Development of environment-friendly insect repellents from the leaf oils of selected Malaysian plants. ASEAN Review of Biodiversity and Environmental Conservation (ARBEC). 7 pages http://www/arbec.com.my/pdf/art8novdec99.pdf

Islam MS, Hasan MM, Xiong W et al. 2009. Fumigant and repellent activities of essential oil from *Coriandrum sativum* (L.) (Apiaceae) against red flour beetle *Tribolium castaneum* (Herbst) (Cleoptera:Tenebrionidae). *Journal of Pest Science* 82: 171–177.

Isman MB, Machial CM, Miresmailli S et al. 2007. *Essential oil-based pesticides: new insights from old chemistry.* Wiley-VCH, Weinheim, Germany.

Janaína KAL, Albuquergue ELD, Santos ACC et al. 2013. Biotoxicity of some plant essential oils against the termite *Nasutitermes corniger* (Isoptera: Termitidae). *Industrial Crops and Products* 47: 246–251.

Kartal SN, Hwang WJ, Imamura Y et al. 2006. Effect of essential oil compounds and plant extracts on decay and termite resistance of wood. *Holz als Roh-und Werkstoff* 64: 455–461.

Koul O, Walia S & Dhaliwal GS. 2008. Essential oils as green pesticides: Potential and constraints. *Biopesticides International* 4: 63–84.

Lahtela V, Hämäläinen K & Kärki T. 2014. The effects of preservatives on the properties of wood after modification (Review paper). *Baltic Forestry* 20: 189–203.

Mehdizadeh L & Moghaddam M. 2018. Essential oils: Biological activity and therapeutic potential. Pp 167–179 in *Therapeutic, Probiotic and Unconventional Foods.* Elsevier, Amsterdam. http://doi.org/10.1016/B978-0-12-814625-5.00010-8

Muturi EJ, Ramirez JL, Doll KM et al. 2017. Combined toxicity of three essential oils against *Aedes aegypti* (Diptera: Culicidae) larvae. *Journal of Medical Entomology* 54: 1684–1691. http://doi.org/10.1093/jme/tjx168

Nicholas DD & Siau JF. 1973. *Factors influencing the treatability of wood. In Wood deterioration and its prevention by preservative treatments.* Vol. II. Preservatives and Preservative Systems, Syracuse University Press, New York.

Noor-Fasiha H. 2011. Chemical composition and biological activity of essential oil from *Cinnamomum* spp. and *Litsea* spp. BSc with Honours thesis. Universiti Malaysia Sarawak, Malaysia.

Nor-Azah MA, Mastura M, Vimala S & Abu-Said A. 2000. Aromatic plants: properties and potential uses of selected species. Pp 56–62 in Chang YS, Mastura M, Vimala S & Zainon AS (eds). *Towards bridging science and herbal industry. Proceedings of the Seminar on Medicinal & Aromatic Plants.* 12–13 September 2000, Kuala Lumpur.

Nor-Azah MA, Sam YY, Mailina J & Chua LL. 2005. (E)-Methyl cinnamate: the major component of essential oils of *Alpinia malaccensis* var. *nobilis*. *Journal of Tropical Forest Science* 17: 631–633.

Paranagama PA, Abeysekera KHT, Nugaliyadde L et al. 2004. Repellency and toxicity of four essential oil to *Sitophils oryzae* L. (Coleoptera: Curculionidae). *Journal of Natural Science Foundation Sri Lanka* 32: 127–138.

Park IK & Chin SC. 2005 Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitermes speratus* Kolbe). *Journal of Agricultural and Food Chemistry* 53: 4388–4392. http://doi.org/10.1021/jf050393r

Pasay C, Mounsey K, Stevenson G et al. 2010. Acaricidal activity of eugenol-based compounds against scabies. *PLoS ONE* 5: 1–9. http:// doi.org/10.1371/journal.pone.0012079

Prajapati V, Tripathi AK, Aggarwal KK et al. 2005. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus. Bioresource Technology* 96: 1749–1757. http://doi.org/10.1016/j.biortech.2005.01.007

Pugazhvendan SR, Ronald RP, & Elumalai K. 2012. Insecticidal and repellent activities of four indigenous medicinal plants against stored grain pest, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Asian Pacific Journal of Tropical Disease* 2: S16–S20. http://doi.org/10.1016/S2222-1808(12)60116-9

Rebmann H, Weber AK, Focke I et al. 1996. Does ben-zyl benzoate prevent colonization of new mat-tress by mites? *Allergy* 5: 876–882.

Rohani A, Nazni WA, Ngo LV et al. 1997. Adulticidal properties of the essential extracts of some Malaysian plants on vector mosquitoes. *Tropical Biomedicine* 14: 5–9.

Roszaini K, Nor-azah MA, Mailina J et al. 2013. Toxicity and antitermite activity of the essential oils from *Cinnamomum camphora, Cymbopogon nardus, Melaleuca cajuputi* and *Dipterocarpus* sp. against *Coptotermes curvignathus. Wood Science and Technology* 47: 1273–1284. http://doi.org/10.1007/s00226-013-0576-1

Roszaini K, Nashatul-Zaimah NA & Nor-Azah MA. 2020. Potentials of *Upuna borneensis* and *Shorea longisperma* wasted seeds extractives against subterranean termites *Coptotermes gestroi* Wasmann. *Journal of Tropical Forest Science* 32: 83–91.

Suhaili ZA & Ho TM. 2008. Residual activity of benzyl benzoate against *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *Southeast Asian Journal of Tropical Medicine and Public Health* 39: 507–510.

Tisserand R & Young R. 2013. *Essential Oil Safety: A Guide for Health Care Professionals.* Elsevier Health Sciences, United Kingdom.

Upadhyay RK, Jaiswal G, Ahmad S et al. 2011. Antitermite activities of *C. decidua* extracts and pure compounds against Indian white termite *Odontotermes obesus* (Isoptera: Odontotermitidae). *Psyche* 820245: 1–9. http://doi:10.1155/2012/820245

Wang YS, Wen ZQ, Li BT et al. 2016. Ethnobotany, phytochemistry and pharmacology of the genus *Litsea*: An update. *Journal of Ethnopharmacology* 2: 181–186 http://doi.org/10.1016/j.jep.2016.01.032

Xie YH, Yang Z, Cao D et al. 2015. Antitermitic and antifungal activities of eugenol and its congeners from the flower buds of *Syzgium aromaticum* (clove). *Industrial Crops Products* 77: 780–786.

Yang YC, Lee HS, Lee SH et al. 2005. Ovicidal and adulticidal activities of *Cinnamomum zeylanicum* bark essential oil compounds and related compounds against *Pediculus humanus capitis* (Anoplura: Pediculidae). *International Journal of Parasitology* 35: 1595–1600. http://doi.org/10.1016/j.ijpara.2005.08.005

Zhu BC, Henderson G, Chen F et al. 2001. Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite. *Journal of Chemical Ecology* 27: 1617–1625. http://doi.org/10.1023/a:1010410325174