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Oral toxicity of chitin synthesis inhibitors (CSIs) and plant extracts to rubber termite, *Coptotermes curvignathus* Holmgren (Rhinotermitidae: Isoptera)

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Abstract

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Coptotermes curvignathus (Holmgren) causes serious damage to live rubber trees in Thailand. Chemicals have been commonly used to control this termite. The control of this subterranean termite by soil treatments remains problematic, so the oral toxicities in a bait formulation of chitin synthesis inhibitors (CSIs), plant oils and crude extracts were evaluated to find environmentally friendly and safe products for the control. Baits made of cardboard mixed with various concentrations of the tested solutions of CSIs, plant oils, and crude extracts were ingested by worker termites. Ten worker termites were used for each concentration with five replications. Mortality was recorded and LC_{50} was estimated by probit analysis at 24, 48, 72, 96 and 120 h after treatment. Lufenuron was the most toxic to *C. curvignathus* with the lowest oral LC_{50} of 323.7 ppm at 120 h. The oral LC_{50} values of flufenoxuron, buprofezin, chlorfluazuron and novaluron were 505.6, 830.6, 1054.3 and 2 939.8 ppm, respectively. Oils from long pepper (*Piper retrofractum* Vahl), black pepper (*Piper nigrum* L.), and thiam [*Azadirachta excelsa* (Jack) Jacobs] were highly toxic to *C. curvignathus*, reaching 100% mortality after exposure for 120 h at the concentrations of 2 000, 2 500 and 3 000 ppm, respectively. Plant oils exhibited greater toxicity than plant crude extracts. Oral LC_{50} values of the plant oils were 4 382.6, 7 507.6 and 5 618.3, ppm, while those of plant crude extracts were 12 032.4, 12 630.6 and 23 860.6 ppm, in the same order. In conclusion, the long pepper oil and lufenuron have potential for the control of this termite species, to reduce or replace synthetic chemicals in future soil treatments. However, field trials should be done to confirm the efficacy of the product in fully realistic conditions.

Keywords: plant oils, plant crude extracts, *Coptotermes curvignathus*, oral LC_{50}, baits

Introduction

The rubber termite, *Coptotermes curvignathus* Holmgren (Rhinotermitidae: Isoptera), is a serious insect pest attacking fresh wood in rubber trees. After the root and the stem are infested, yellowing and wilting of leaves are initial signs of the infestation. Symptoms of brown leaf, leaf fall, and deaths of the trees subsequently take place in further advanced stages (Tho and Kirton, 1992; Kirton and Wong, 2001). Besides the rubber trees, also other plant species including conifers, monocotyledonous and dicotyledonous plants are reportedly attacked by *C. curvignathus*. In particular, coniferous species such as *Pinus* spp., *Araucaria* spp., and *Agathis* spp. are most susceptible to *C. curvignathus* (Thapa and Shim, 1971; Tho, 1974; Tho and Kirton, 1992, 1998). Damages by this termite in some eco-

nomic crops have been reviewed in some Asian countries. It is a serious pest of oil palm in Malaysia (Lim and Bit, 2001), coconut in Indonesia (Mariau et al., 1992), and of forests and rubber trees in Thailand (Thanee, 2014). While there are several ways to control this termite, chemical control is the preferred method due to effectiveness and ease of application. Various insecticides have been recommended for soil treatment, such as chlorpyrifos, cypermethrin, alpha-cypermethrin, fipronil, and imidacloprid (Tho and Kirton, 1992). The toxicity of soil-treated insecticides against subterranean termites is significantly affected by characteristics of the treated soil, such as its clay and cellulose contents (Smith and Rust, 1993). Pyrethroids, such as cypermethrin and alpha-cypermethrin, function as repellants of subterranean termites, rarely making contact and subsequently this insecticide group has little toxic effects (Lenz et al., 1990; Su and Scheffrahn, 1990; Su et al., 1993; Kard, 1999). In addition, intensive use of these synthetic chemicals has been toxic to humans, domestic animals, other non-targeted organisms, and the environment. Baiting systems are a potentially ecosystem friendly alternative to these soil-treatment chemicals. Such systems are commercially available for controlling subterranean termites in buildings, but their application to control termites in agriculture and plantations has not been well developed. Worker termites carry the chemical back to the nest where it is passed along to other castes via mutual food exchange or grooming, resulting in the eventual elimination of the colony, although with a long delay. An advantage of baits is that they require significantly less chemicals than soil treatment and colony elimination of termites. However, the chemicals used in baits have to be slow-acting and non-repellent, to allow the worker termites to uptake large amounts of the chemical and transfer it to the colony. Baiting systems with chitin synthesis inhibitors (CSIs) and naturally occurring chemicals, used in insecticides derived from plants, and are generally considered an alternative way of insect control reducing health and environmental impacts, due to their rapid degradation and low toxicity to mammals (El-Wakeil, 2013; Chouvenc, 2018; Iqbal and Evans, 2018). Bioassay tests of plant extracts on termites have been widely documented (Serit et al., 1992; Moein, and Farrag, 2000; Chen et al., 2002; Doolittle *al*., 2007; Owusu et al., 2008; Nisar et al., 2012 and Osipitan and Oseyemi, 2012). The previous few reports on plant extracts against *C. curvignathus* have focused on contact toxicity, rarely on stomach poisons in baits (Abdullah et al., 2015; Roszaini et al., 2013). Therefore, this study aimed to evaluate the oral toxicity of some CSIs and plant extracts, including oils and crude extracts, in baits against *C. curvignathus,* in a laboratory. This study will be used to support development of poisonous bait products to achieve effective control of *C. curvignathus,* as alternatives to soil-treatments with insecticides.

Materials and Methods

Rubber termite, Coptotermes curvignathus Holmgren for bioassay tests

Workers of *C. curvignathus* were collected from a rubber plantation in Songkhla province, Thailand. Species identification was confirmed following the book "The insects of Australia" (Watson and Gay, 1970). The rearing of worker termites was done according to the methods described by Owusu (2008). Termite workers were transferred into a plastic box (18×20×10 cm^3) which was overlaid with 5 layers of 1.5 cm thick ground and 450 g of rubber sawdust. The rearing was in the dark, at 25–28°C and 70–75% R.H in the entomological laboratory at the Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University.

Preparation of chitin synthesis inhibitors (CSIs) and plant extracts

The five CSIs used in this study were buprofezin (Glozin® 40% W/V WSC), chlorfluazuron (Atabron® 5% W/V EC), flufenoxuron (Cascade® 5% w/v EC), lufenuron (Match® 5% W/V EC) and novaluron (Rimon® 10% W/V EC). Different dried plant parts (rhizome, fruit/seed and flowers) of the ten plant species *Azadirachta excelsa* (Jack) Jacobs Z (seed), *Zingiber montanum* (J.Koenig) Link ex A. Dietr. (rhizome), *Zingiber zerumbet* (L.) Smith. (rhizome), *Boesenbergia rotunda* (L.) Mansf. (rhizome), *Zingiber officinale* Roscoe. (rhizome), *Alpinia galanga* (L.) Willd. (rhizome), *Piper nigrum* L. (fruit), *Piper retrofractum* Vahl (fruit), *Amomum krervanh* Pierre. (fruit) and *Syzygium aromaticum* Merr. et Perry (flower) were purchased from a traditional pharmacy, except for the seeds of *A. excels* that were collected from agroforestry area in Songkhla province, Thailand. The plant materials were ground in a blender and subsequently macerated with *n*-hexane for 7 days. The plant extracts were filtered prior to concentration in a rotary evaporator. Each extraction was repeated four times. All the filtrates (referred to as plant oils) from the four extractions were combined or pooled together. The residual plant filter cakes were then macerated with ethanol similarly as when macerated with *n*-hexane, but these filtrates are referred to as plant crude extracts. The plant oils and the plant crude extracts were kept in amber reagent bottles in a refrigerator until use in bioassay tests.

Oral toxicity test of chitin synthesis inhibitors (CSIs) against Coptotermes curvignathus Holmgren

Stomach poison activity was evaluated by using baits prepared by using a 200 g piece of cardboard mixed together with 500 ml of distilled water and a CSI, to attain the concentrations 0, 50, 100, 500, 1 000, 1 500, 2 000, 2 500, 3 000, 4 000 and 5 000 ppm. The poisonous baits were placed within a bamboo tube (∅ 4 cm and 30 cm length) prior to placing twenty worker termites/tube. The bamboo tubes were kept in dark with temperature at 25–28°C and 70–85% RH, in a laboratory. Each concentration level in the test bait was repeated five times. Mortality was recorded and LC_{50} values were calculated by probit analysis at 24, 48, 72, 96 and 120 h after treatment. Analysis of variance of percent mortality for various concentrations at 120 h, and for 5 000 ppm at different times after treatment, was performed. Means were also compared buy using Tukey's test.

Oral toxicity test of plant extracts to Coptotermes curvignathus Holmgren

Baits prepared for oral toxicity testing were made of 200 g cardboard pieces that were each mixed with 500 ml of distilled water, and with plant oils or crude extracts for final concentrations of 0, 50, 100, 500, 1000, 1500, 2000, 2500, 3000, 4000, 5000 and 10 000 ppm. The mixture was blended for 30 s to obtain a poisonous bait. The poisonous baits were placed within a bamboo tube (∅ 4 cm and 30 cm length) prior to placing in ten worker termites/tube. The bamboo tubes were kept in dark at 25–28°C and 70–85% RH, in a laboratory. Each concentration was tested in five replicates. Mortality and LC_{50} values were calculated by probit analysis at 24, 48, 72, 96 and 120 h after treatment. Analysis of variance of mortality was applied to different times after exposure to 10,000 ppm plant oil or plant crude extract bait. Means were also compared buy using Tukey's test.

Results and Discussion

Toxicity of chitin synthesis inhibitors (CSIs) to Coptotermes curvignathus Holmgren

Oral LC_{50} values of CSIs to *C. curvignathus* at different times of exposure are shown in Table 1. Lufenuron was the most toxic with the lowest LC_{50} of 323.7 ppm at 120 h after treatment. The CSI toxicity ranked by LC_{50} values in descending order is lufenuron > flufenoxuron > chlorfluazuron > buprofezin > novaluron. The toxicity gradually increased with CSI concentration (Figure 1A) and significantly increased with exposure duration, particularly for lufenuron (Figure 1B). Mortality with all CSIs was below 20% at 24 h, but it significantly increased at 48 h, and particularly lufenuron reached over 80% mortality (Figure 1B).

At the low concentrations of 1 000 ppm and 1 500 ppm, lufenuron showed significantly greater mortality than the other CSIs (Figure 1A). Our results agree with other reports, in which lufenuron has been documented as effective against subterranean termites. Fed lufenuron (250, 500, 1,000 ppm) to *C. formosanus* in 30–32 d significantly reduced survivorship, running speed, consumption and tunneling (Wang *et al*., 2014). Gautam and Henderson (2014) evaluated three CSIs as termite baits against *C. formosanus.* Lufenuron treatment gave significantly higher mortality than diflubenzuron or noviflumuron after six weeks of treatment. Protist population in higut of *Reticulitermes flavipes significantly decreased after 3d exposure to lufenuron, as compared to diflubenzuron, hexaflumuron, noviflumuron and novaluron (Lewis and* Forschler, 2010). Vahabzadeh et al. (2007) provided paper discs treated with diflubenzuron, hexaflumuron, lufenuron, and triflumuron to the Eastern subterranean termite *Reticulitermes flavipes* in a laboratory. Lufenuron was highly toxic to *R. flavipes*, causing high rates of mortality at all concentrations tested. Hexaflumuron and triflumuron showed a similar toxicity, while diflubezuron was the least toxic to the termites among the chemicals tested. Sajab et al. (2000) evaluated cellulosic baits containing 0.5% hexaflumuron against *C. curvignathus* in field conditions. The results showed that termite colonies ceased their activities at 25–44 d after baiting had commenced. Chouvenc (2018) evaluated the impact of a non-repellent liquid termiticide (fipronil) and a chitin synthesis inhibitor (CSI) termite bait (noviflumuron) on whole colonies of *Coptotermes gestroi* (Wasmann) in laboratory conditions, over a 12-m foraging distance. At the end of week 12, colo-

Table 1. Oral LC_{50} values of chitin synthesis inhibitors against *Coptotermes curvignathus* Holmgren at 24, 48, 72, 96 and 120 h

	LC_{50} (ppm)				
CSI	24 h	48 h	72 h	96 h	120 h
Buprofezin	522 441.6	307 838.2	19 041.6	4137.4	830.6
Chlorfluazuron	66 314.0	17 038.5	8053.7	2846.2	1054.3
Fluflenoxuron	346 298.0	6993.8	2447.5	1057.3	505.6
Lufenuron	21 862.6	4450.9	1920.5	718.9	323.7
Novaluron	2 093 899.7	767 467.5	21 5004.7	14 370.2	2939.8

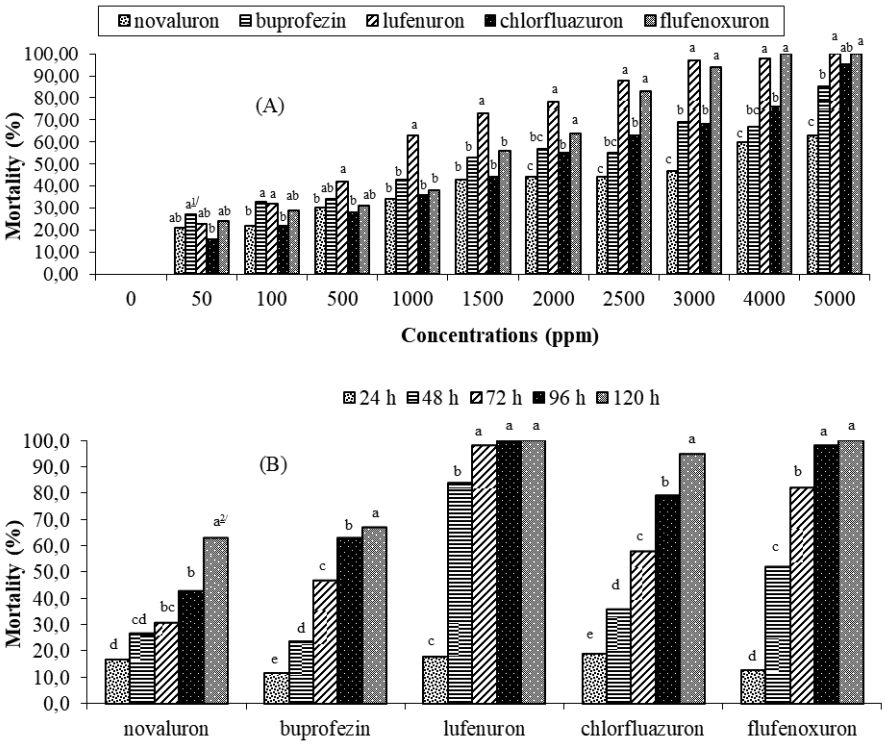

Fig. 1. Percent mortality of *Coptotermes curvignathus* Holmgren after fed with various concentrations (A), at different times fed with 5 000 ppm of chitin synthesis inhibitors for 120 h (B)

$a^{1/}$ and $a^{2/}$ = means with different letters in the same concentration and the same CSI, respectively are significantly different ($p < 0.05$) by Turkey test

nies exposed to fipronil did not have any difference in population size compared with control colonies. Comparatively, the colonies exposed to noviflumuron had no change in foraging activity for the first ≈40 d, but then the termites progressively ceased their activity throughout their foraging territory. This study suggests that subterranean termite colonies with access to CSI baits are inevitably eliminated, regardless of the position of the bait, while colonies exposed to fipronil are only locally excluded from the area near the treatment, but may maintain their foraging activity in untreated areas, and retain their potential for causing structural damage in the long term. Evan (2010) tested efficacy of three concentrations (0, 0.5 and 1%) of bistrifluron in cellulose bait pellets on the mound-building subterranean termite, *Coptotermes acinaciformis* (Froggatt) over an 8 wk period. Both doses of bistrifluron bait eliminated (viz. termites absent from nest or mound) termite colonies: 83% of colonies (10 of 12) were either eliminated or moribund (viz. colony had no reproductive capacity and decreased workforce) after 8 wk, while none of the control colonies experienced similar loss of function.

Toxicity of plant extracts to Coptotermes curvignathus Holmgren

All the plant oils and the crude extracts were toxic to *C. curvignathus* to various extents dependent on plant species, concentration and duration of exposure. The plant oils were more toxic to *C. curvignathus* than the plant crude extracts (Figure 2). Four plant species, *P. retrofractum, A. excelsa, P. nigrum* and *Z. montanum* were highly toxic to *C. curvignathus* with the low LC_{50} values of 4758.8, 5658.9, 5907.3 and 6 941.1 ppm, respectively, at 120 h (Table 2). The mortality increased with concentration and with time of exposure (Figure 2 and Figure 3).

Doolittle et al. (2007) reported that 1 ppm of neem (*Azadirachta indica*) extract reduced the number of *Pseudotrichonympha grassii* and spirochaetes inhabiting in the hindgut of *C. formosanus*, resulting in 100% mortality of the termite. Chieng and Assim (2008) reported 100% mortality of *Coptotermes* sp. after treatment with *Piper sarmentosum* oil at 10 000 ppm for 2 days. These findings agree with our study results, in which the oils from *P. retrofractum, A. ex-*

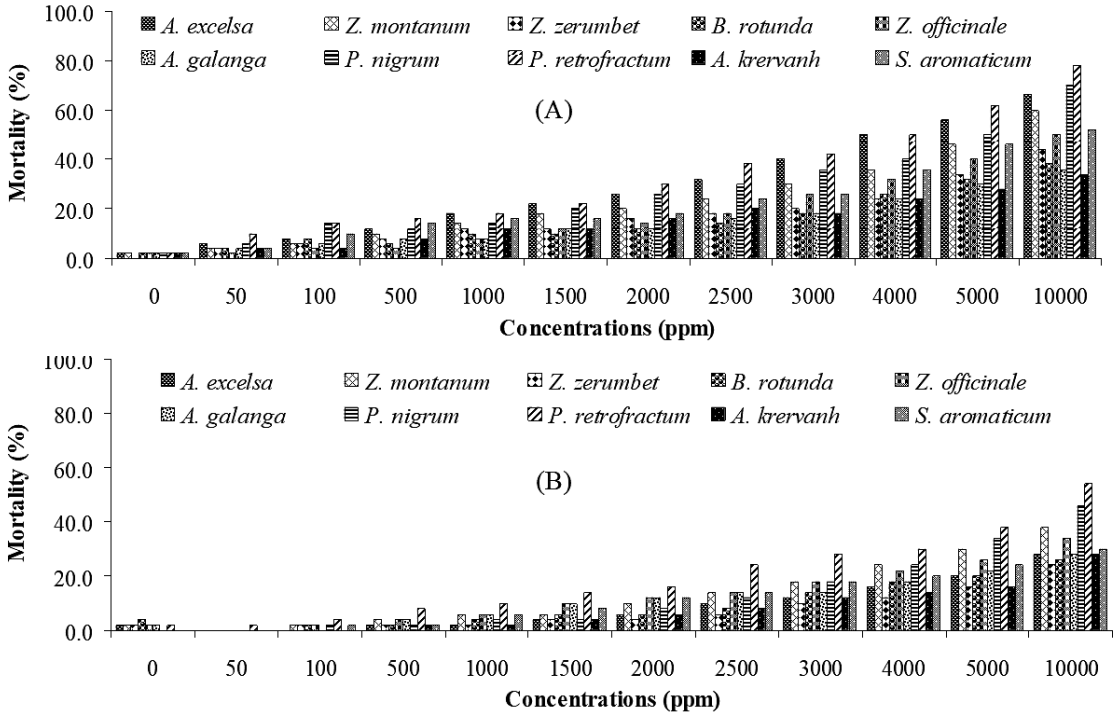

Fig. 2. Percent mortality of *Coptotermes curvignathus* Holmgren after fed with various concentrations of plant oils (A) and plant crude extracts (B) for 120 h

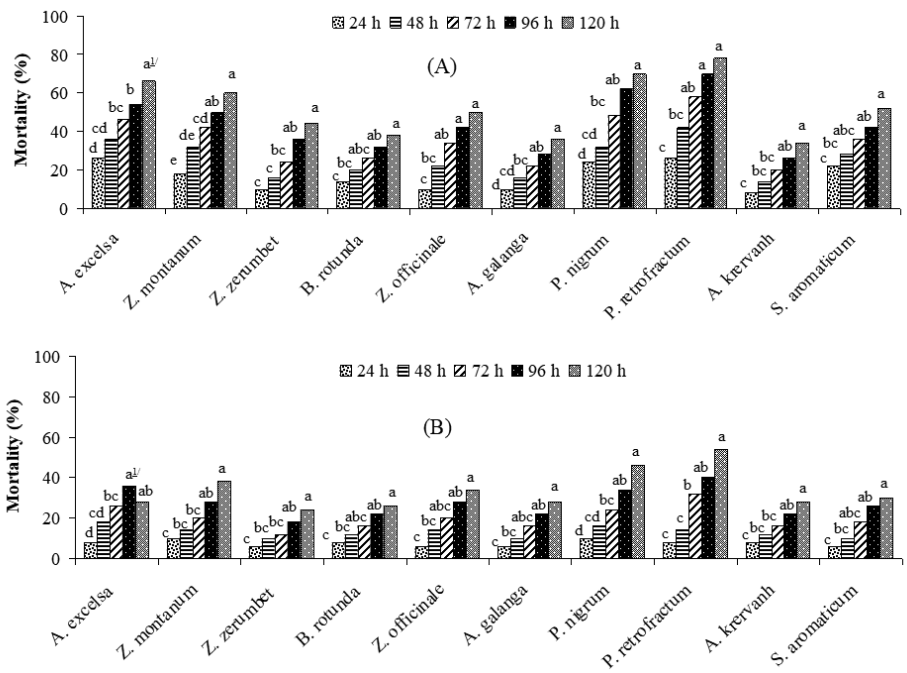

Fig. 3. Mortality of *Coptotermes curvignathus* Holmgren after exposure to baits with 10 000 ppm plant oils (A), or plant crude extracts (B), at different times after treatment

a[1/] = means with different letters in the same plant species are significantly different ($p < 0.05$) by Turkey test

Table 2 Oral LC_{50} values of plant oils and crude extracts against *Coptotermes curvignathus* Holmgren at 24, 48, 72 and 120 h

		LC_{50} (ppm)				
Plant extract	Plant species	24 h	48 h	72 h	96 h	120 h
Oils	*A. excelsa*	11867.2	9829.2	8256.2	7140.8	5658.9
	Z. montanum	14886.6	11795.6	9989.7	8506.8	6941.1
	Z. zerumbet	19396.9	16409.2	13698.3	11046.3	9453.3
	B. rotunda	16157.1	14185.9	12942.4	11794.9	10444.4
	Z. officinale	18220.2	13666.1	10795.9	9482.9	8117.0
	A. galanga	20532.0	17265.6	14479	12888.6	10905.3
	P. nigrum	13880.0	11808.6	8987.3	6950.8	5907.3
	P. retrofractum	12173.1	10228.5	7528	5831.5	4758.8
	A. krervanh	21163.9	18494.7	15072.1	13685.1	11354.6
	S. aromaticum	14067.3	12202.8	10488.5	9293.1	7724.7
Crude extracts	*A. excelsa*	156602.0	71773.2	25741.4	15423.8	11968.8
	Z. montanum	65381.4	54049.4	31824.9	18873.4	14377.4
	Z. zerumbet	135583.4	63360.1	75867.2	30643.9	24353.1
	B. rotunda	111105.2	62125.4	57614.3	28830.3	21663.3
	Z. officinale	116810.8	51796.8	39964.9	23429.8	19206.9
	A. galanga	364824.9	166748.6	95059.0	38057.3	35369.5
	P. nigrum	62248.0	37001.0	23405.6	15493.4	12630.7
	P. retrofractum	105013.1	104822.9	35369.5	34645.1	10173.5
	A. krervanh	81499.7	51207.3	49890.8	43589.9	27229.7
	S. aromaticum	150395.1	96872.9	72731.3	34020.1	29288.8

celsa, and *P. nigrum* were highly toxic to *C. curvignathus. Alpinia galangal* oil in our tests showed low toxicity with an oral LC_{50} of 10 905.3 ppm. However, Abdullah et al. (2015) reported the oral LD_{50} of *Alpinia galangal* oil to *C. curvignathus* at 72 h as 3 456 mg/kg. This is possibly due to the different extraction methods used in these two experiments. The essential oils in our study were obtained by maceration with *n*-hexane, whereas the Abdullah et al. (2015) study used aqueous distillation. Sajap and Aloysius (2000) tested the leaf extracts of *A. excelsa*, extracted with the alternative solvents acetone, hexane and methanol, on subterranean termite, *C. curvignathus* in field conditions. The responses of termite workers depended on concentration of extract used to treat the soils and pine blocks. The termite workers became insensitive for a longer period after exposure to an extract. The results showed that extracts of *A. excelsa* leaf had an inhibitory effect on subterranean termites. The soil treated with extracts reduced tunneling activities of the termites. Umeh and Ivbijaro (1999) investigated the efficacy of insecticide derived from two local plants, ripe seeds of neem (*Azadirachta indica*) and the crude seed oil of *Piper guineense,* each at 10% concentration, dosed at 390 l/ha and 18 liters/ha, respectively, against the termites infesting maize plots. The crop damage by the termites (*Microtermes spp, Mecrotermes bellicosus* and *M. subhyalinus*) was significantly reduced and maize yield increased in the treated plots relative to the control plots.

The solvent used in plant extraction plays an important role to the toxicity against *C. curvignathus*. Plant oils obtained with *n*-hexane extraction were more toxic to *C. curvignathus* than plant crude extracts obtained with ethanolic extraction (Table 2 and Figure 2). Moein and Farrag (2000) evaluated toxicity of pepper oils extracted using the alternative solvents hexane, ethanol, and petroleum ether, against the termite *Cryptotermes brevis*. The results showed that hexane extract was the most toxic to the termite, reaching 50% mortality at 5000 ppm for 48 h, while ethanolic and petroleum ether extracts exhibited 4.7% and 14.3% mortalities, respectively. Cabrera *et al.* (2001) assayed the chloroformic extracts of the woods Ipe (*Tabebuia sp.)* and Itauba (*Mezilaurus sp.)* against the dry-wood termite, *Cryptotermes brevis,* in a laboratory. Both extracts were applied at the rate of 0.1 g/mL on filter paper prior to feeding the termites. The results showed significant reduction in feeding rates as well as increased mortality after 30 days. Ogunsina *et al.* (2009) used hexane and ethanol extracts of *Lantana camara* and *Euphorbia laterifloria* against subterranean termite workers. 50% and 90% mortalities were recorded with 0.50 g/100 mL and 0.90 g/100 mL of hexane extract, *for L. camara* and for *Enuopiri,* respectively. According to Tellez *et al.* (2001)

tarbush (*F. cernua*) leaves were fractionated by extracting successively with hexanes, diethyl ether, and ethanol. The hexane fraction contained monoterpenoids, while the ethanol fraction was primarily sesquiterpenoids. The crude fractions were tested against fungi, algae and termites (*Reticulitermes* sp.) for potential activity. Application of 1 µg of the essential oil from the hexane fraction was sufficient for visible antifungal activity in bioautography assays. Other plant species have been documented for insecticidal activity against *C. curvignathus.* Ding and Xing (2010) reported that soil mixed with fresh leaves and stem of lantana (*Lantana camara* L.) caused significant reduction in tunneling of *Coptotermes formosanus* and *Reticulitermes flavipes,* but had no effect on mortality. Leaves, stems and flowers of *Lantana camara* were more repellent than its roots.

Conclusions

Among the five chitin synthesis inhibitors used in this study, lufenuron was the most toxic to *C. curvignathus* by oral exposure. Chemicals used together with baits have to be slow-acting to allow the worker termites to transport large amounts of them to the termite colony. Lufenuron meets this requirement by reaching 100% mortality only 96 h after exposure. Regarding oral toxicity to *C. curvignathus*, the plant oils extracted with *n*-hexane were markedly more toxic than the plant crude extracts extracted with ethanol. Oil from the long pepper, *P. retrofractum,* possessed the greatest toxicity to workers of this termite species. Therefore, lufenuron and long pepper oil could be further studied in order to develop poisonous bait products for effective control of *C. curvignathus,* as alternatives to soil-treatments with synthetic insecticides.

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