Mechanisms of Essential Oils from Citronella (*Cymbopogon winterianus* Jowitt) Against Siamensis Subterranean Termite Workers (*Coptotermes gestroi* Wasmann) and Mice (*Mus musculus* L.)

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ABSTRACT

This work aimed to evaluate the *in vivo* and *in vitro* toxicity of essential oils from citronella (*Cymbopogon winterianus* Jowitt) extract by tasting as contact and fumigation methods against Siamensis termites (*Coptotermes gestroi* Wasmann). The *in vivo* and *in vitro* acute oral toxicity were evaluated with mice (*Mus musculus* L.). The CRD with 5 replicates for all experiments was designed. Probit analysis was used to elucidate LC_{50} for termites and LD_{50} for mice. The *in vivo* LC_{50} on the 14^{th} day using the contact method showed ca. 0.54 mg/g and the fumigation method showed ca. 0.02 mg/g of essential oils against termite workers. This is an indication that the *in vivo* fumigation method showed significantly more toxic against termites than the contact method by 27.02 fold. The *in vivo* oral LD_{50} of essential oils showed ca. 5.81 g/kg body weight for male and ca.17.42 g/kg body weight for female at the 24^{th} hour. The *in vitro* fumigation method also exhibited ca. <2-3 fold inhibited acetylcholinesterases (AChE) and glutathione-S-transferases (GSTs) and ca. <1.5 fold inhibited esterases (ESTs) against termites compare to the untreated termites. While the *in vitro* in liver of mice exhibited ca. < 2 fold AChE and GSTs and ca. < 0.5 fold inhibited ESTs compare to the untreated mice control. But the contact method was likely nonsignificant *in vitro* enzymes activity compare to untreated mice control for all enzymes assays.

Key words: *Coptotermes gestroi* Wasmann, *Mus musculus* L., Mechanisms, Citronella essential oils

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INTRODUCTION

Termites have been seriously damaging a variety of materials, not only wooden products but also noncellulosic materials such as gypsum, plastics, cement and steel. The damage caused by termites has been estimated to exceed 3 billion dollars annually in the United States (Siramon *et al.*, 2009). Among species of termites, *Coptotermes gestroi* Wasmann (Isoptera: Rhinotermitidae) is the subterranean termite responsible for most destruction of wooden materials in countries such as Japan, Taiwan, and the southeastern Asia including Thailand. Synthetic pesticides are currently used to control termites such as cypermethrin, chlorpyrifos and chitin inhibitor, triflubenzuron (Scheffrahn *et al.*, 1997). All of them are the chemicals identified as toxic compounds. To avoid those impacts, this research focuses on alternatively friendly pesticides.

The catabolizing enzymes called detoxification enzymes, esterase, acetylcholinesterase (AChE) and glutathione-S-transferase (GSTs) are mainly responsible for their detoxification processes. The elevation or inhibition of detoxification enzyme activities after administering the xenobiotic including plant alleochemicals reveal the chance for survival or resistance to those particular compounds (Visetson, 1991). In mammals, all metabolized substances, mostly water soluble could be excreted through kidney, liver, lung, skin and alimentary canal depending on their structures (Shalaby *et al.*, 2011). Most invertebrates especially insects excrete the metabolizes via the mulphigialtubes, alimentary canaland their exuvy (Visetson, 2003).Essential oils are natural volatile substances found in a variety of odoriferous plants. They consist of mixtures of many compounds mainly terpene, organic compounds consisting of multiples of isoprene units dominated by constituents of essential oils (Cassel and Vargas, 2006). In Thailand, citronella (*Cymbopogon winterianus* Jowitt) which constitutes a high amount of essential oils, is the most well-known grass for cultivation. It is mainly planted for the production of cosmetics, odorants, flavor enhancers in many food products, as pharmaceuticals, fragrant perfume and mosquitoes repellent (Suwansirisilp *et al.*, 2013). Unfortunately, the vital data of essential citronella oils against subterranean termites is limited. This research was carried on to evaluate the toxicity mechanisms of essential oils from citronella (*C. winterianus* Jowitt). Thus, this study concentrates on the bioassays of essential oils from citronella grass extracts against termite workers (*C. gestroi* Wasmann). The contact and fumigation

methods which were the most useful tools for evaluation of toxicity were investigated. In the meantime, the main biochemical mechanism assays namely, esterase, GSTs and AChE were performed to reveal the possibility of detoxification mechanisms after exposure to the extracts. Finally, the stomach poison against ICR mice as well as detoxification mechanisms in mouse liver, were fruitful for evaluation of safety of mammals as well as the chance of producing resistance. All the results mentioned above would point out the potential of the essential oils from citronella grass for friendly-sustainably pesticide alternatives against termite in the future.

MATERIALS AND METHODS

1. Termite collection and maintenance

Thai termites, *C. gestroi* were cultured and collected in June 2014 to August 2016 from the field at Kasetsart University, Kampangsan campus, Nakhon Pathom province. Every other month, the termites were put in filter papers moistened with distilled water used as foodstuffs. They were kept in plastic cages (1.50×1.0 × 0.50 m) incubated in a mobile refrigerator set at 25 °C. All samples were transported in to the laboratory at Zoology department, faculty of Science, Kasetsart University, Bangkhen on the same day and reared at 25 °C and 80% relative humidity before trials commenced on the following day.

2. Extraction and identification of compounds from citronella of essential oils.

Fresh leaves of citronella grass were cut ca. less than 1 cm. long weighted 10 kg. and put in the steam distillation machine described by Koul *et al.* (2004). The condensate was directly collected using a 500 ml beaker and then poured into a separating funnel. This formed two layers of oil and water. The tap of the separating funnel was opened to let out the water while the oil was immediately collected into 100 ml stoppered bottle. The essential oils were isolate using column chromatography as described by Tirimanna (1983). Moisture was removed by sodium sulphate anhydrous and stored in sealed vials at low temperature before analysis. The moisture content of the leaves was determined by the weights before and after drying at 105°C for 10 hours in an oven. The essential oils were analyzed by gas chromatography-mass-spectrometry analysis (GC-MS). The analysis used capillary GC/MS on an Agilent 6890/HP 5973 mass selective (MS) detector system operating at 70 eV. The chromatographic column used was a fused silica capillary column with stationary phase HP-5MS (cross-linked 5% phenylmethylsiloxane),

30 m length, 0.25 mm i.d. and 0.25 micron coating thickness. Injector and transfer line temperature were set at 200 ℃ and 280 °C, respectively; the oven temperature was programmed from 40 ℃ – 220 °C, at 3 °C/min. Helium was employed as carrier gas (1 Ml/min); injection of 1 ml of a 1% solution of whole essential oil in ethyl acetate, split 1:50, scan range 41-300 amu and scan time 1.0 sec (Adam, 1995).The essential oils of leaves citronella (*C. winterianus* Jowitt) were obtained with yields ranging from 2.20 to 2.55% based on dry leaves within 3 hours. The essential oils from samples after GC/MS analysis of compositions (citronellal, trans-geraniol and beta-citronellol in the crude ca. 30.79, 18.72 and 11.72, respectively) were selected for used in all experiments. The amount of 2.50 g was dissolved in 5% v/v aqueous ethanol prior mixed with a 5 % v/v emulsifier, Triton X-100. This solution was mixed and stirred using a stirred plate (Fisher Scientific) at room temperature for at least 24 hours to reach solubilization equilibrium. The solution was served as the stocks for all experiments.

3. The *in vivo* toxicity against termites and mice

3.1 Contact method

The contact method modified from Ohtani *et al.* (1997) was employed to evaluate the contact toxicity. Each oil sample was dissolved in 1 ml of ethanol and then applied to 1 g of filter paper (Advantec No.1, 9.0 cm in diameter). A piece of filter paper treated with ethanol only was used as a control. After the solvent was removed from the treated filter papers by air drying at ambient temperature, 30 active termites (28 workers and 2 soldiers) above the third instar were placed on each piece of filter paper in a petri dish (9.0 cm in diameter × 1.5 cm in height). Distilled water was added every six hours onto each filter paper and the covered petri dishes were incubated in darkness at 25 ℃.

3.2 Fumigation method

The fumigation method modified from Park and Shin (2005) was employed to evaluate antitermitic activity. A paper disk (8 mm, Advantec) treated with the essential oils was placed in the bottom of a glass cylinder (5 cm diameter × 10 cm) with a wire sieve fitted 3.0 cm above the bottom. Then the glass cylinders were closed with the glass lid. The 30 active termites (28 workers and 2 soldiers) were placed on the sieve. This prevented the direct contact of the termites with the test plant oils. Filter paper soaked with water was supplied as food. The termite workers were maintained at 25 ± 1 ℃ and 80% relative humidity. The adult termites were considered to be dead if appendages did not move when prodded with a brush.

The 5 concentrations of 1.0 – 5.0 mg/g essential oils from extract, control treatments (5% ethanol) were treated with adults *C. gestroi*. All concentrations of the compounds varied depending on the discriminating doses that exhibit 10-90 % linear mortality. The mortality of the termite workers were counted for 7 and 14 days.

3.3 Mice oral toxicity test

The 500 individuals of male and female each of 10 day old ICR mice (average weight 25.00- 30.00 g. for male and 30.00-35.00 g. for female) were purchased from the Mahidol Laboratory Mice Department, Mahidol University, Salaya. One mouse was set in each cage. Male and female were set aside. The temperature was set at 25°C. Mouse food was purchased from the same institution and the mice we fed once a day (4-5 g/ mouse/day). The base material for mouse was changed weekly. Water for mouse was changed daily. The females and males of 3 week old ICR mouse unit of 5 replicates were carried out. The room for the mice experiments was controlled day/night period (16:8) with 25°C and 80% RH. In each experiment, 1.00 - 8.00 g/kg body weight essential oils were used against 1 ml water to control treatment. The effect of the essential oils was recorded as acute oral toxicity analysis after 24 and 48 hours, the mortalities of animals are assumed as toxicity. The force feeding of essential oils at high doses (6.00-8.00 g/kg body weight) were separately done then followed by *in vitro* detoxification enzyme assays using their liver tissues after 24 hr administrations.

Probit analysis were conducted with modification of Finney (1978).The Abbott' s formula (Abbott, 1925) was carried out in case of mortality in untreated control was detected. The toxicity in terms of LC_{50} against termites and LD_{50} against mice was compared. The toxicity index (TI) of each experiment was determined according to Sun (1950). The correction factors (CF) followed Visetson and Milne (2001) were quantified to measure the effectiveness and changes in enzyme levels, respectively.

4. Detoxification mechanisms of treated termites and mice with essential oils from citronella extracts

The methods were modified from Visetson and Milne (2001). The survival adult termites (130 sampling in each replicate) or 1 g. of mice liver from toxicity experiments in *in vivo* assays were used in this *in vitro* assays to optimize enzyme activity of esterase, AChE and GSTs. After the assays conditions were finalized, the samples were immediately placed in ice and were homogenized on cool mortar in the suitable homogenization

buffer. This condition contained 1 ml, 0.1 M potassium phosphate, containing 1 mM EDTA, pH 7.5 and 1 ml, 0.1 M potassium phosphate, containing 10 mM glutathione reduced form, pH 7.5. These samples were centrifuged at 4 °C, 18,000 rpm for 5 minutes. The supernatant were decanted into a clean 1.5 ml microtube, placed on ice and use immediately for enzyme assays. The detoxification enzyme assays were done with the pH optimum of potassium phosphate buffer (pH 4-13). The paranitrophenylacetate, acetylthiochorineand 1,2-dichloro-4, nitrobenzene (DCNB) were used as substrates for analyses of esterase, AChE and GSTs, respectively. The determination of protein content of the termites followed the Lowry *et al.* (1951) using bovine serum albumin as a standard to quantify all enzyme activities. The spectrophotometer and Printer used for all enzyme assays were from Perkin Elmer-Lamda 25 with Winlab program.

5. Statistical analysis

This research used Completely Randomized Design (CRD) with 5 replicates. This type of design brought about effective and conveniences for counting *in vivo* mortality and *in vitro* detoxification enzyme assays on both contact and fumigation methods at consecutive time. The analysis of variance was used with SPSS, V.11.5 and comparisons from each treatment were used DMRT at $P<0.05$. This research was done at the Department of Zoology, Faculty of Science, Kasetsart University, Bangkhen Campus between January 2013 to July 2017.

RESULTS AND DISCUSSION

1. Chemical composition of citronella essential oils

The chromatogram of the raw essential oils of citronella obtained by GC/MS analysis (described in the texts) yielded the peaks identified by the mass spectra library, which are presented in table 1. The Three highest concentrations of the all compositions in citronella were citronellal, trans-geraniol and beta-citronellal which showed the percentage in the crude of 30.79, 18.72 and 11.72, respectively. The other compounds such as delta-cadinene and cyclohexanemethanol, 4-ethyl-alpha, alpha,4-trimethyl-3-(1-methylethenyl),[1R-(1 alpha,3 alpha 4 beta) were ca. 5 percent. Moreover, the trace compounds, 1-limonene, cis=2,6-dimethyl-2,6-qctadiene, geranyl acetate, beta lemene, geramacrane-D were ca. 3 percent. The lowest compound concentration was t- muurolol which indicated of ca. 2 percent and the longest retention time (Table 1).

Table 1 Chemical compositions in citronella essential oils from the GC/MS described in the texts used in these experiments

Identified compound[(1)]	RT[(2)](min)	Percent area[(3)]
l-Limonene	6.57	3.46 ± 0.42d
Citronellal	11.22	30.79 ± 2.54a
beta-Citronellol	14.69	11.72 ± 2.37c
trans-Geraniol	15.88	18.72 ± 2.31b
cis-2,6-Dimethyl-2,6-octadiene	20.52	3.94 ± 0.32d
Geranyl acetate	22.14	3.42 ± 0.40d
Beta-Elemene	22.67	3.27 ± 0.22d
Germacrene-D	29.50	3.61 ± 0.52d
delta-Cadinene	32.91	4.28 ± 1.03d
Cyclohexanemethanol,4-ethyl-alpha, alpha,4-trimethyl-3-(1-methylethenyl), [1R-(1 alpha,3 alpha 4 beta)	35.39	5.59 ± 0.01d
t-Muurolol	41.41	2.22 ± 0.05d

(1) Method of identification of active compounds from essential oils was followed Adam (1995) described in texts.

(2) Retention time was classified as the time in minute after the sample was injected.

(3) Means ± SD, 5 replicates, Means in the same column followed by a common letter are not significantly different at 5 % level by DMRT.

2. Toxicity from citronella essential oils against termites and mice

Firstly, the LC_{50} value of contact method against termites (Table 2) on the 7th day of essential oils showed non significantly different toxicity, 1.10 fold compared to the contact method at P<0.05 DMRT. The contact LC_{50} value was ca. 0.99 mg/g essential oils while the fumigation LC_{50} value was ca. 1.04 mg/g essential oils on the 7th day. On the other hand, the LC_{50} value of fumigation method against termites on the 14th day of essential oils showed significantly higher toxicity, 27.02 fold, more than on the 14th day of contact method at P<0.05 DMRT. The fumigation LC_{50} value was ca. 0.02 mg/g essential oils while the contact LC_{50} value was ca. 0.54 mg/g essential oils on the 14th day. These were the indications

Table 2 Comparison of *in vivo* toxicity (LC_{50})[(1)], coefficient of correlation (r^2)[(2)]and slope[(3)]of *C. gestroi* affected by essential oils from citronella with contact and fumigation methods at the 7th day and the 14th day

Methods	Day	LC_{50} ±S.E	*P*-Value	Slope±S.E	Chi square	r^2	TI[(4)]	Fold[(5)]
Contact	7	0.99±0.19A	0.001	7.24±1.11A	59.26±11.23A	0.71±0.10B	95.11	-
	14	0.54±0.20a	0.039	6.49±2.11a	36.25±7.34a	0.82±0.11a	100.00	27.02
Fumigation	7	1.04±0.18A	0.388	4.23±1.11B	24.29±3.56B	0.91±0.01A	100.00	1.10
	14	0.02±0.21b	0.124	1.88±0.21b	30.95±5.44a	0.93±0.01a	3.70	-

(1) Means ± SD, 5 replicates each of 30 individuals were employed, 7 and 14 days check per batch for all experiments. Means followed by different identical letters within the same column at the same day are significantly different at 5 % level by DMRT.

(2) r^2 is correlation determination between treatments and mortality.

(3) Dose-response slopes of mortality (%) against concentration (mg/g)

(4) TI means toxicity index, the percentage of the higher LC_{50} value was divided by the lower LC_{50} value on the same day followed Sun (1950).

(5) Fold means the higher toxicity index was divided by the lower toxicity index on the same day.

that the essential oils works well at 14th day after application especially by the fumigation method. All correlation coefficients (r^2) were above 0.8 for all experiments except for the 7th day of contact method which exhibited around 0.7. The LC_{50} from both methods indicated that the citronella essential oils revealed more toxicity against this species of termites than the essential oils from eucalyptus leaf extracts done by Siramon *et al.* (2009) on Japanese termite workers (*C. formosanus* Shiraki) which showed LC_{50}ca.12.68 - 17.50 mg/g. These might come from the ρ-cymene and y-terpine in the eucalyptus leaves that gave lesser toxicity than constituents in citronella essential oils (citronellal, β-citronellol and geraniol). In addition, these essential oils also gave more toxicity against termite workers (*C. gestroi*) ca. 10 fold higher compare to Cheng *et al.* (2007) who utilized the contact method against the Japanese termite workers (*C. formosanus* shiraki) showing of LC_{50} ca. 10 mg/g of heartwood and sapwood essential oils of *Calocedrus macrolepis* var. Formosana and *Cryptomeria japonica* and leaves of *Chamaecyparis obtusa* var. formosanas. However, the citronella essential oils from these experiments were ca. 3-5 fold less toxic than of (+)-α-pinene,(-)-limonene, (-)-α-pinene,β-pinene and β-phellandreneagainst Japanese termite which showed IC_{50} ca. 0.03, 0.13, 0.41, 0.42 and 0.67 mg/mL. respectively (Seo *et al.*, 2014).

Secondly, the mice LD_{50} at 24 hours gave ca.5.81 g/kg. for males (slope = 20, r^2 = 82) and females indicated ca. 17.42 g/kg (slope= 17, r^2 = 92). The LD_{50}at the 48th hours revealed ca.5.53 g/kg. for the males (slope = 22, r^2 = 84) and the females indicated ca. 8.01 g/kg. (slope= 14, r^2 = 98) (Table 3). There were indications of ca. 2.17 fold higher toxic against males than females mice for all experiments. The toxicity of essential oils extracted from citronella extracts against mice were still lower than the toxicity of essential oils from eucalyptus and clove done by Shalaby *et al.* (2011) who showed LD_{50} ca. 2,334.4 and 3,597.5 mg/kg. body weight, respectively. The higher toxic in male than female was similar to the works of Rajeh *et al.* (2012) who indicated that male mice were more sensitive to the plant extracts than the female one. However, according to the following chemical labeling and classification of acute oral toxicity based on oral LD_{50} values as recommended by the Thai Department of Agriculture (Anonymous, 2015), the citronella essential oils are still determined safe to human being when used against termites infestation.

Table 3 *In vivo* toxicity of ICR mouse (LD_{50}) against essential oils force fed of essential oils from citronella extract after 24 and 48 hr under the laboratory condition

Compound	Time (Hour)	Slope[1]		LD_{50}[2],[3]		r^2[4]		TI[5]		Fold[6]	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Essential oils											
	24	20	17	5.81±2.10a	17.42±6.80a	82	92	100.00	100.00	1.05	2.17
	48	22	14	5.53±1.10a	8.01± 1.30b	84	98	95.20	45.98	1.00	1.00

[1] Dose-response slopes of mortality (%) against concentration (mg/g)

[2] Means ± SD, 5 replicates males and females each of 3 individuals were employed, 24 and 48 hr check per batch for all experiments. Means in the same column followed by a common letter are not significantly different at 5 % level by DMRT.

[3] LD_{50} is the amount of material (g/kg b.w.) which causes the death of 50% of a group of test animal

[4] r^2 is correlation determination between treatments and mortality.

[5] TI means toxicity index, the percentage of the higher LC_{50} value was divided by the lower LC_5 value on the same day followed Sun (1950).

[6] Fold means the higher toxicity index was divided by the lower toxicity index on the same day.

3. Effects of essential oils from citronella extract on esterase, acetylcholinesterase and glutathione-S-transferase activity of termites and mice

Firstly, the detoxification mechanisms in the contact method fluctuated (Table 4) while the fumigation method were reduced against all concentrations of essential oils (Table 5) The paranitrophenol generated against fumigation method showed ca. 10% reduced activity against untreated control(ca.20.54 nMparanitrophenol/min/mg protein) (ca. CF = -0.92). The AChE generated showed up to ca. 50% (ca. CF = -0.65) reduced activity against untreated control (ca. 4.44 acetylthiocholine generated/min/mg protein). The essential oils also showed AChE inhibition activity of the identified compounds from C. nobile, *E. punctulatus*, *O. multicaulis* and *S. chamaecyparissus*, against Japanese

termites. The identified compounds were tested individually for their fumigant toxicity against Japanese termites (Seo *et al.*, 2014). The accumulated acetylcholine brings about continuous stimulation of the cholinergic nerve throughout the central and peripheral nervous system, followed by paralysis and death (Visetson, 1991). In addition, the inhibition of cholinesterase from citronella essential oils was similar to the work of Rajendran and Sriranjini (2008) that applied essential oils from plant families Apiaceae, Lamiaceae, Lauraceae and Myrtaceae against the rust-red beetle and stated that the mode of action of the plant compounds indicated inhibition of acetylcholinesterase enzyme activity as the major site of action. This work showed reduction of glutathione conjugated products/mg protein/min ca. 65% against fumigation method (ca CF = -0.36). However, the activities of termite GSTs in cytosol toward the model substrate 1-chloro-2,4-dinitrobenzene also showed inhibition in termites by the works of Haritos *et al.* (1994) as well as Victoria *et al.* (1996) who stated thatglutathione-S-transferases(GSTs) protect the Australian termites from the toxic effects of plant chemicals. The change in activity might be occurred at the first time for their survival (Seo *et al.*, 2014). On the other hand, the contact method was likely non significantly *in vitro* enzymes activity compare to untreated mice control (ca. CF = for all enzymes assays).

Secondly, the detoxification enzyme activity, ESTs, AChE and GSTs in the mice liver exhibited low levels of change (ca. 1.32- 1.75 fold, ca. CF = +1.40) depending on types of enzymes and the concentrations of compounds (Table 6) The esterase was elevated ca. 1.32-1.40 fold while AChE and GSTs were ca. 1.28-1.75 fold inhibition (ca. CF = -0.78 and -0.67). The small reduced activity of these enzymes were consistent with the works of (Li *et al.*, 2017). In addition, Yang *et al.* (2013) stated that the change in detoxification enzymes in rats against citral extracts increased the ability to renoprotective and hepatoprotective through its antioxidative and anti-inflammatory effects. On the contrary, the citronella essential oils exhibited very different hepatotoxicity compare to the eucalyptus essential oils. Shalaby *et al.* (2011) stated that the eucalyptus essential oils caused relatively moderate pathological changes in the liver as congestion of blood vessels in the portal area associated with inflammatory infiltration while the citronella essential oils in these works did not show any toxic symptom. This is another indication of the safety use of citronella essential oils against these termites

Table 4 *In vitro* effects of essential oils from citronella extract by contact method on esterase (ESTs), acetylcholinesterase (AChE) and glutathione-S-transferase (GSTs) activity of *Coptotermes gestroi*

Concentration (mg/g)	24 hr					
	ESTs	CF	AChE	CF	GSTs[(1),(2)]	CF[(3)]
1.0	26.46±2.55a	+1.29	4.01±1.46a	-0.90	3.03±0.16a	+1.01
2.0	28.88±1.73a	+1.41	3.92±0.63a	-0.88	3.03±0.12a	+1.01
3.0	25.27±7.65a	+1.29	3.61±0.96a	-0.81	3.10±0.13a	+1.03
4.0	18.58±1.39b	-0.90	2.35±0.71b	-0.53	1.08±0.10b	-0.36
5.0	15.89±1.87c	-0.77	2.05±0.25b	-0.46	1.10±0.10b	-0.37
Control (5% ethanol)	20.54±1.07b		4.44±2.74a		3.00±0.52a	
CV(%.)	18.34%		20.0%		16.23%	

[(1)] Means ± SD, 5 replicates each of 130 individuals were employed, 24 hr check per batch for all experiments. Means in the same column followed by a common letter are not significantly different at 5 % level by DMRT.

[(2)] Enzyme assays were followed Visetson, 1991; Visetson and Milne, 2001, the unit of esterase, acetylcholinesterase and glutathione-S-transferase are nM paranitrophenolgenerated/ min/mg protein, nM acethylethiocholine generated/min/mg. protein and nM DCNB-conjugated products/min/mg. protein

[(3)] CF is a correction factor (enzyme activity in treatment / enzyme activity in control) with +(elevated activity) and - (inhibited activity) compare to untreated control followed Visetson and Milne (2001)

Table 5 *In vitro* effects of essential oils from citronella extract by fumigation method on esterase (ESTs), acetylcholinesterase (AChE) and glutathione-S-transferase (GSTs) activity of *Coptotermes gestroi*

Concentration (mg/g)	24 hr					
	ESTs	CF	AChE	CF	GSTs[1],[2]	CF[3]
1.0	18.96±0.21b	+0.92	1.70±1.14c	-0.38	1.01±0.02b	-0.34
2.0	18.53±2.22b	+0.90	1.18±0.38c	-0.27	1.02±0.12b	-0.34
3.0	16.46 ±0.11c	-0.80	1.54±0.11c	-0.35	1.02±0.12b	-0.34
4.0	18.65 ±1.11b	-0.90	2.91±0.36b	-0.65	1.08±0.10b	-0.36
5.0	17.65± 4.22bc	-0.86	2.35±0.22b	-0.53	1.02±0.01b	-0.34
Control (5% ethanol)	20.54±1.07a		4.44±2.74a		3.00±0.52a	
CV (%.)	16.06%		16.13%		10.32%	

[1] Means ± SD, 5 replicates each of 130 individuals were employed, 24 hr check per batch for all experiments. Means in the same column followed by a common letter are not significantly different at 5 % level by DMRT.

[2] Enzyme assays were followed Visetson, 1991; Visetson and Milne, 2001, the unit of esterase, acetylcholinesterase and glutathione-S-transferase are nMparanitrophenol generated/min/mg protein, nMacethylethiocholine generated/min/mg. protein and nM DCNB-conjugated products/min/mg. protein

[3] CF is a correction factor (enzyme activity in treatment / enzyme activity in control) with +(elevated activity) and - (inhibited activity) compare to untreated control followed Visetson and Milne (2001)

Table 6 Some *in vitro* detoxification enzyme activity, esterase (ESTs), acetylcholinesterase (AChE) and glutathione-S-transferase (GSTs) activity in mice (*Mus musculus*) liver after force feeding essential oils from citronella extracts at high dose

Compound	Concentration (g/kg body weight)[1]	24 hr [2]					
		ESTs	CF	AChE	CF	GSTs	CF[3]
Essential oils	6.0	427.35±44.38a	+1.32	57.24±10.31ab	-0.78	138.08 ±10.10b	-0.67
	8.0	450.12±63.57a	+1.40	48.32±23.21b	-0.66	118.02±28.41b	-0.57
	Control (1 ml. of water)	322.31±23.69b		73.10±12.21a		206.01±32.43a	
CV (%)		15.76%		12.12%		25.35%	

[1] Means ± SD, 5 replicates males and females each of 3 individuals were employed, 24 hours check per batch for all experiments. Means in the same column followed by a common letter are not significantly different at 5 % level by DMRT.

[2] Enzyme assays were followed Visetson, 1991; Visetson and Milne, 2001, the unit of esterase, acetylcholinesterase and glutathione-S-transferase are nM paranitrophenol generated/min/mg protein, nM acethylethiocholine generated/min/mg. protein and nM DCNB-conjugated products/min/mg. protein.

[3] CF is a correction factor (enzyme activity in treatment / enzyme activity in control) with + (elevated activity) and – (inhibited activity) compare to untreated control followed Visetson and Milne (2001).

CONCLUSION

This work has been found that the fumigation method showed ca. 0.02 mg/g of citronella essential oils with ca. 27.0 fold compare to the contact method at the 14th day after application. These citronella essential oils showed no toxicity against ICR mice both *in vivo* and *in vitro*. The LD_{50} showed ca. 5.81g/kg body weight for males and ca.17.42 g/kg body weight for females at the 24th hr and 5.53 g/kg body weight for males and 8.01 g/kg body weight at the 48th hr, respectively. Moreover, they exhibited low levels of inhibited AChE and GSTs but small induction of ESTs against both termites and mice. It could be concluded that the fumigation method for citronella essential oil in this study could be used against termite infestation that no one has been reported.

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REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of a insecticide. *J. Econ. Entomol.* 18: 265-267.

Adam, R.P. 1995. *Identification of essential oil components by gas chromato graphy/mass spectrophotometry.* Allured:Illinois.

Anonymous. 2010. *Recommendation against Insects and Pest Control in Thailand,* Entomology and Zoology Division, Office of Research and Development for Plant Protection, Department of Agriculture, Ministry of Agriculture and Co-operative, ISBN 978-974-436-743-3, 303 pp.

Cassel, E. and R.M.F. Vargas. 2006. Experiments and modeling of the *Cymbopogon winterianus* essential oil by steam distillation. *J. Mex. Chem. Soc.* 55: 57-60.

Cheng, S.S., H.T. Chang, C.L. Wu and S.T. Chang.2007.Anti-termitic activities of essential oils from coniferoustrees against *Coptotermes formosanus. J. Bioresource Technology.* 98: 456-459.

Finney, D.J. 1978. *Statistical method in biological assay.* 3rd ed. Charles Griffin &Company Limited, England.

Haritos, V.S., J.R.J. French and J.T. Ahokas. 1994. Cytochrome P450 monooxygenase and glutathione S-transferase of two Australian termites: *Mastotermes darwiniensis* and *Coptotermes acinaciformis*. J. *Insect Biochem. Molec. Biol.* 24(9): 929-935.

Koul, V.K., B.M. Gandotra, S. Koul, S. Ghosh, S. Tikoo and A.K. Gupta. 2004. Steam distillation of lemon grass (Cymbopogon spp.).*Indian J. Chem Technol*. 11: 135-139.

Li, C.C., Fu.Y. Hsiang, H.C. Chun, T.L. Yun and T.Y. Hsien. 2017. Effects of lemongrass oil and citral on hepatic drug-metabolizing enzymes, oxidative stress, and acetaminophen toxicity in rats. *J. Food and Drug Anal.* 25: 1-7.

Lowry, H.O., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Bio.Chem.* 193: 265- 275.

Matsumura.1975. Botanical insecticides, pp. 94-99. *In Matsumura (ed.). Toxicology of Insecticides.* Plenum, New York.

Ohtani, Y.,M. Hazama and K. Sameshim. 1997. Crucial chemical factors of the termiticidal activity of hinoki wood (Chamaecyparis obtusa) III. Termiticidal extractives romhinaki wood. *Mokuzai Gakkaishi.* 43: 1022-1029.

Park, I.K. And S.C. Shin. 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). *J. Agric. Food Chem.* 4388-4392.

Rajeh, M.A., Y.P Kwan, Z. Zakaria, L.Y. Latha, S.L. Jothy and S. Sasidharan. 2012. Acute toxicity impacts of Europhiahirta L extract on behavior, organs body weight index and histopathology of organs of the mice and Artemiasalina. *Pharmacognosy Res*. 4(3): 170-7.

Rajendran, S. and V. Sriranjini. 2008. Plant products as fumigants for stored-product insect control. *J. Stored Prod. Res.* 44: 126-135.

Robert, W.D., T.K. Shripat and K.P. Suresh.1995. Characterization of general esterase in workers of the eastern subterranean termite (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 88 (3): 574-578.

Scheffrahn, R.H., N.Y. Suand P. Busey. 1997. Laboratory and field evaluations of selected chemical treatments for control of drywood termites (Isoptera: Kalotermitidae). *J. Econ. Entomol*. 90: 492-502.

Seo, S.M., J., Kim, J., Kang, S.H Koh, Y.J.Ahn, K.S. Kang and I.K. Park.2014.Fumigant toxicity and acetylcholinesterase inhibitory activity of 4 Asteraceae plant essential oils and their constituents against Japanese termite (Reticulitermes speratus

Kolbe).US National Library of Medicine National Institutes of Health. 113: 55-61.

Shalaby, A., N. Presneau and H. Ye. 2011. The role of epidermal growth factor receptor in chordoma pathogenesis: a potential therapeutic target. *J. Pathol.* 223: 336-46.

Siramon, P., Y. Ohtani and H. Ichiura. 2009. Biological performance of eucalyptus camaldulensis leaf oils from Thailand against the subterranean termite Coptotermes formosanus Shiraki. *J. Wood Sci.* 55: 41-46.

Sun, Y. P. 1950. Toxicity index: an improved method of comparing the relative toxicity of insecticides. *J. Econ. Entomol*, 34: 45.

Suwansirisilp, K., S.Visetson, A. Prabaripai, S. Tanasinchayakul, J.P. Grieco, M.J. Bangsand T. Chareonviriyaphap. 2013. Behavioral responses of Aedesaegypti and Culex quinquefasciatus (Diptera: Culicidae) to four essential oils in *Thailand. J. Pest Sci.* 86: 309-320.

Tirimanna, A.S.L. 1983. Surveying the chemical constituents of the neem leaf by two dimensional thin-layer chromatography. *Proc. Int. Neem Conf.* 2: 67-74.

Victoria, S., B. Haritos, S.Jill,E.B.Sue, R.J.F. John and T.A. Jorma.1996. Glutathione Transferases and Glutathione Binding Proteins of Termites: Purification and Chatracterisation. *Insect Biochem. Mol. Biol*. pp. 617-625

Visetson, S. 1991. *Insecticide Resistance Mechanism in the Red Rust Flour Beetle (Tribolium castaneum* Burst). PhD. Thesis.The University of Sydney, Australia.

Visetson, S. 2001. Effect of Azadiractin from Various Thai Neem Extracts on Some Detoxification Enzyme Activity in Callosoruchus maculates F., pp. 38-46. *20th ASEAN/ 2nd APRC Seminar on Postharvest Technology,* September 11-14, 2001. Lotus Hotel Pang Suan Kaew, Chiangmai Thailand.

Visetson, S. and M. Milne.2001. Effects of root extract from derris (Derris elliptica Benth) on mortality and detoxification enzyme levels in the diamondback moth larvae (plutella xylostella Linn.). *Kasetsart J. (Nat. Sci.)*. 35: 157-163.

Visetson, S. 2003. Synergistic effects of sesamine Oil with cypermetrin on the survival and detoxification enzyme activity of plutella xylostella L. Larvae. *Kasetsart J. (Nat. Sci.).* 37: 52-59.

Yang, Y., Y. Wu, S. Chen, G.J. Devine, I. Denholm, P. Jewess and G.D. Moores. 2004. The involvement of microsomal oxidases in pyrethroid resistance in Helicoverpa armigera from Asia. *Insect Biochem. Mol. Biol.* 34: 763-773.

Yang, S.M., K.F. Hua, Y.C. Lin, A. Chen, J.M. Chang and C.L. Kuoping. 2013. Citral is renoprotective for focal segmental glomerulosclerosis by inhibiting oxidative stress and apoptosis and activating Nrf2 pathway in mice. https://doi.org/10.1371/journal.pone.0074871.