

## Efficiency of *Litsea cubeba* essential oil and citral against *Lasioderma serricorne* and *Stegobium paniceum* adults

Suthisut, D.\*#, Noochanapai, P., Kengkanpanich, R., Pobsuk, P., Potikan, S.

Postharvest and Processing Research and Development Division, Department of Agriculture, 50 Phaholyothin Road, Ladyao, Chatuchak, Bangkok, Thailand 10900

\*Corresponding author, Email: dsuthisut@yahoo.com

#Presenting author, Email: dsuthisut@yahoo.com

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### Abstract

Mature fruits of *Litsea cubeba* were extracted by water distillation. The compounds in the essential oil were identified by GC-MS. E-citral (50.0%) and Z-citral (35.2%) were the major constituents. Contact and fumigation toxicity of *L. cubeba* essential oil and its major compound (citral) were investigated in the laboratory with adults of two stored-product insects, *Lasioderma serricorne* and *Stegobium paniceum*. Contact toxicity on filter paper with *L. cubeba* essential oil was over 70% at highest dose (2.54  $\mu\text{L}/\text{cm}^2$ , 24 h) for both insects. The  $\text{LC}_{50}$  values at 24 h were 1.6 and 0.8  $\mu\text{L}/\text{cm}^2$  for *L. serricorne* and *S. paniceum* respectively. Citral did not control *L. serricorne* adults (5.0% mortality), but *S. paniceum* adults were susceptible (94.0%) to citral at 2.54  $\mu\text{L}/\text{cm}^2$ , with  $\text{LC}_{50}$  value of 1.2  $\mu\text{L}/\text{cm}^2$ , 24 h after exposure. In fumigation tests, *L. serricorne* (53.3% mortality) were more resistance to *L. cubeba* essential oil than *S. paniceum* (98.9%) at 242  $\mu\text{L}/\text{L}$  in air, 48 h after exposure. The  $\text{LC}_{50}$  values of *L. serricorne* and *S. paniceum* adults were 130.0 and 2.9  $\mu\text{L}/\text{L}$  in air, respectively. Similar trends were seen with citral, *L. serricorne* and *S. paniceum* adults with 89.0 and 100% mortality at 242  $\mu\text{L}/\text{L}$  in air after 48 h exposure. The  $\text{LC}_{50}$  values at 48 h were 20.2 and 4.2  $\mu\text{L}/\text{L}$  in air, respectively.

Keywords: essential oil, *Litsea cubeba*, citral, *Lasioderma serricorne*, *Stegobium paniceum*

### 1. Introduction

Currently, the common methods to eliminate stored-product insect populations are: chemical control, cold treatment, heat treatment and fumigation with methyl bromide and phosphine (Fields and White, 2002; Abdelghany et al., 2009; Subramanyam et al., 2011.). However, uses of these chemical insecticides are declining because of environmental impact, insect resistance or concerns over worker safety and residues on grain. In the near future, these current methods may not be available to control stored-product insects, thus replacement control methods are needed.

Essential oils are secondary metabolites which can be extracted from many parts of plant; such as leaves, flowers, roots or fruits. There are many effects of essential oils to stored-product insects (Stefanazzi et al., 2011; Suthisut et al., 2011a, b). Some of the more effective essential oils are from neem (*Azadirachta indica* a. Juss.) (Nukenine et al., 2011) and eucalyptus (*Eucalyptus* sp.) (Gusmão et al., 2013). Therefore, essential oils could be the replacement method to control the stored-product insects.

May Chang (*Litsea cubeba* (Lour.) Persoon) is a perennial plant which can be found in many parts of Thailand (Ko et al., 2009). Its essential oils can be extracted from leaves, bark or fruit. *Litsea cubeba* essential oil is toxic to many insects and acts as repellent to mosquitoes (*Aedes aegypti* (L.)) (Noosidum et al., 2008), controls maize weevil (*Sitophilus zeamais*

Motschulsky) and red flour beetle (*Tribolium castaneum* (Herbst)) (Ko et al., 2009). Additionally, it also has a very interesting property as biochemical control on the other stored-product insects. For this experiment, the efficacy of *L. cubeba* essential oil and its major compound (citral) was investigated on cigarette beetle (*Lasioderma serricorne* (F.)) and drugstore beetle (*Stegobium paniceum* (L.)) by contact and fumigant toxicity.

## 2. Materials and Methods

### 2.1. Insect cultures

Adults (0-14 days) of *L. serricorne* and *S. paniceum* were used in this study. Both species were obtained from Department of Agriculture, Bangkok, Thailand. *Lasioderma serricorne* was reared on wheat flour and *S. paniceum* was reared on aniseed seed. All rearing and experiments were conducted at room temperature ( $30\pm 2^{\circ}\text{C}$ ) with  $70\pm 20\%$  rh.

### 2.2. Extracted *L. cubeba* essential oil

Mature fruits of *L. cubeba* were collected from Chiang Rai province, Thailand during 2011. The essential oil was extracted by water distillation (Suthisut et al., 2011b). The voucher specimen (BK 263502) was deposited at the Bangkok Herbarium, Botanical Research Unit, Department of Agriculture, Bangkok, Thailand. Citral (natural  $\geq 95\%$ , mixture of E and Z isomers) was purchased from Sigma-Aldrich, USA.

The major components of *L. cubeba* essential oil were determined by GC-MS (Agilent model 6890N (GC) and 5973 (MS)), equipped with a DB-5MS capillary column (0.25m (diameter), 30 m (length), 0.25  $\mu\text{m}$  (film thickness)). The column temperature was programmed initially at  $75^{\circ}\text{C}$ , then increased at  $2^{\circ}\text{C}/\text{min}$  to  $100^{\circ}\text{C}$ , and then increased at  $3^{\circ}\text{C}/\text{min}$  to  $120^{\circ}\text{C}$  and held for 10 min, then increased at  $2^{\circ}\text{C}/\text{min}$  until  $134^{\circ}\text{C}$  increased at  $5^{\circ}\text{C}/\text{min}$  until the final temperature of  $240^{\circ}\text{C}$  was reached, where it was held for 15 min.

The injector and detector temperatures were set at  $280^{\circ}\text{C}$  and helium was used as the carrier gas at flow rate of 1 mL/min. Two  $\mu\text{L}$  of the essential oil was injected in the splitless mode. GC retention time and their mass spectra presented in MS library were used for identifying the compounds in the essential oil.

### 2.3. Contact toxicity

*Litsea cubeba* essential oil and citral were diluted with ethanol 99% into 0.25, 0.5, 1, 2 and 4% which were equivalent to 0.16, 0.32, 0.64, 1.27 and 2.54  $\mu\text{L}/\text{cm}^2$ , respectively. The solutions (1,000  $\mu\text{L}$ ) were spread onto a filter paper (9 cm diam: Whatman no. 1), while ethanol served as a control. Both were allowed to dry for 10 min (5 replicates/dose). The filter papers were placed in a Petri dish, twenty unsexed adults released into the dishes and the dishes covered. Mortality was checked after 1, 3, 6 and 24 h.

### 2.4. Fumigant toxicity

The essential oil of *L. cubeba* and citral were placed onto a filter paper (2 cm diameter) at 0, 0.1, 0.5, 1, 1.5, 2, 4 or 8  $\mu\text{L}$  corresponding to 0, 3, 15, 30, 45, 60, 121 or 242  $\mu\text{L}/\text{L}$  in air (5 replicates/dose). Solutions of *L. cubeba* essential oil and citral were allowed to evaporate for 5 min and the filter papers, and then they were attached to the underside of lid of glass vial (33 mL). Adults of *L. serricorne* and *S. paniceum* (20 insects/vial) were put into the vial without food, the lid screwed tight and parafilm was wrapped around the lid. Mortality was checked after 12, 24 and 48 h.

### 2.5. Statistical analysis

Mortality data was corrected by using Abbott's formula (Abbott, 1925) and Tukey's multiple test was used to compare treatment means at  $p < 0.05$  (R Core Team, 2012). The lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) were estimated using probit analysis (POLO-PLUS program version 2.0, LeOra Software, Petaluma, CA, USA).

## 3. Results and Discussion

### 3.1. Chemical analysis of *L. cubeba* essential oil by GC-MS

There were 10 compounds found in *L. cubeba* essential oil (Table 1), with most of the essential oil being either E-citral (50.0%) or Z-citral (35.2%). This is similar to previous studies. Yang et al. (2014) found that *L. cubeba* essential oil from China has 33 compounds and E-citral, Z-citral and D-limonene were the most common compounds. Ko et al. (2009) collected and extracted *L. cubeba* mature fruits from Chiang Mai province and found 17 compounds: E-citral (41.3%), Z-citral (30.1%) and methylheptenone (5.6%). While, Si et al. (2012) reported that the composition of *L. cubeba* essential oil from 8 locations in China contained up to 59 compounds and the major components were E-citral and Z-citral. The chemical constituents of essential oil may vary in the components and amounts due to the season and location (Bakkali et al., 2008) and extraction method.

**Table 1** Chemical compounds of essential oil from *Litsea cubeba* mature fruits collected from Doi Phangknon, Muang District, Chiang Rai province, Thailand.

Compound	Retention time (min)	Composition (%)
E-Citral	23.12	50.0
Z-Citral	21.38	35.2
D-Limonene	9.85	2.0
Bicyclo(3.1.0) hex-2-ene, 4-methyl-1-(1-methylethyl)	7.56	1.8
L-Linalool	13.33	1.0
6-Octenal,3,7-dimethyl	16.27	1.0
beta-Myrcene	8.15	0.7
Geraniol	22.08	0.6
1,8-Cineole	9.98	0.5
(1R)-2,6,6,-Trimethylbicyclo(3.1.1) hept-2-ene	6.25	0.4

### 3.2. Contact toxicity

Contact toxicity on filter paper of *L. cubeba* essential oils and citral were investigated with *L. serricornis* and *S. paniceum* adults (Tables 2 and 4). Increased durations cause greater mortality. The mortalities of *L. serricornis* and *S. paniceum* adults were similar; 73.0 and 83.3% at 2.54  $\mu\text{L}/\text{cm}^2$  after 24 h exposure, respectively. However, the LC<sub>50</sub> of *L. serricornis* and *S. paniceum* adults tested with *L. cubeba* essential oil were 1.6 and 0.8  $\mu\text{L}/\text{cm}^2$  after 24 h

exposure. Therefore, *L. serricornis* was approximately 2 times more tolerant to *L. cubeba* essential oil than *S. paniceum*.

Similar studies were conducted with other essential oils. Cassia (*Cinnamomum cassia* (L.) and cinnamon oil (*Cinnamomum zeylanicum* Blume) were tested with *Sitophilus oryzae* (L.) and the results showed that the LC<sub>50</sub> values of *S. oryzae* were 100 mg/cm<sup>2</sup> and 20 mg/cm<sup>2</sup> after 48 h (Lee et al., 2008). Tapondjou et al. (2005) found that the essential oils from *Eucalyptus saligna* Sm. and *Cupressus sempervirens* L. were toxic to *S. zeamais* and *T. castaneum* but *E. saligna* oil was more toxic to both insects than *C. sempervirens*. The LC<sub>50</sub> value of *S. zeamais* and *T. castaneum* were 0.36 and 0.48 µL/cm<sup>2</sup> at 3 days after exposure, which is more toxic than *L. cubeba* essential oils in this study. Furthermore, microapplicator was also used to apply on insect thorax for contact toxicity. *Litsea cubeba* essential oil was reported against *S. zeamais* and *T. castaneum* adults (Ko et al., 2009). *Alpinia conchigera* Griff., *Zingiber zerumber* Smitt, *Curcuma zedoaria* (Berg.) Roscoe are all from the Zingiberaceae. The essential oils were extracted from rhizomes and the essential oils were tested with *S. zeamais* and *T. castaneum* adults. The three essential oils and their major compounds (terpinen-4-ol and isoborneol) were toxic to both insects. (Suthisut et al., 2011a) It can be seen that essential oils could be used to control the insects, but the effectiveness depends on the insect species.

Even though *L. cubeba* essential oil was toxic to *L. serricornis*, citral was not (Table 2). In contrast, *S. paniceum* had similar susceptibility to *L. cubeba* essential oil and citral (0.8 to 1.2 µL/cm<sup>2</sup>, LC<sub>50</sub>, Table 4). Hence, *L. cubeba* essential oil and citral could control *S. paniceum*, but *L. cubeba* essential oil could only control *L. serricornis*. This suggests that the one or more of the minor compounds in *L. cubeba* essential oil are the active compounds against *L. serricornis* or they synergize citral. This result is similar with Hori (2003) that *L. cubeba* essential oil could repel *L. serricornis*, but citral was less repellent than *L. cubeba* essential oil.

The contact insecticide have been used for control the stored-product insects such as malathion, permethrin and fenitrothion (Zetter and McDonald, 1984). Insect resistance to commercial contact insecticides could be appeared around the world. Therefore, botanical insecticide should be replaced the chemical insecticide but the risk to user and environment need to research.

The mammalian selectivity ratios were calculated with LD<sub>50</sub> or LC<sub>50</sub> of *L. serricornis* and rat acute oral LC<sub>50</sub> (Table 6). Higher mammalian selectivity ratios indicate safer products. The mammalian selectivity ratio for citral was >1.95, which is higher than unsynergized pyrethrin, but lower than the other contact insecticides. Similar results were seen for other essential oil constituents (Suthisut et al., 2011a). Therefore, further testing would be required to determine if the essential oils from *L. cubeba* are effective and safe to be used as a stored-grain protectant.

### 3.3. Fumigant toxicity

*Lasioderma serricornis* adults were more resistant to *L. cubeba* essential oil than *S. paniceum*; LC<sub>50</sub> of *L. cubeba* essential oil for *L. serricornis* and *S. paniceum* were 130.0 and 2.9 µL/L in air after 48 h. (Table 5). In addition, *L. cubeba* essential oil was also tested with the other stored-product insects; *S. zeamais* and *T. castaneum* adult by Ko et al. (2009) which was similar to this report. The LC<sub>50</sub> values of *S. zeamais* and *T. castaneum* were 549.6 and 92.5 µL/L in air after 24 h. Hence, *S. paniceum* adults were more susceptible to *L. cubeba* essential oil than *T. castaneum*, *L. serricornis* and *S. zeamais*.

Citral was toxic to *L. serricornis* and *S. paniceum* adults. The mortality of both species could be found at 12 h (Table 3). The mortality of *S. paniceum* was 100% at the concentration of

citral was 60  $\mu\text{L/L}$  in air or over after 48 h. While the mortality of *L. serricorne* was 89.0% at the highest concentration, the  $\text{LC}_{50}$  values were 21.6 and 4.4  $\mu\text{L/L}$  in air after 24 h; 20.2 and 4.2  $\mu\text{L/L}$  in air after 48 h. *Lasioderma serricorne* adults were more resistant to *L. cubeba* essential oil and citral than *S. paniceum*. Citral and *L. cubeba* essential oil had similar toxicity to *S. paniceum*. Although, citral acted as a fumigant to *L. serricorne* and *S. paniceum* adults in this study, citral did not affect *S. oryzae*,  $\text{LD}_{50} > 150 \mu\text{L/L}$  in air after 24 h (Lee, et al., 2001). However, Ukeh and Umoetok (2011) summarized that citral could be used for contact and fumigant insecticide to control *T. castaneum* and *Rhyzopertha dominica* (F.).

The CT (concentration x time) product data was explained and analyzed for commercial fumigants, essential oils and pure compounds (Suthisit et al., 2011b). The CT of methyl bromide, phosphine and sulfuryl fluoride to kill 95% of *Tribolium confusum* du Val adults were 27, 0.24 and 15  $\text{g-h/m}^3$  (Kenaga, 1957; Lindgren and Vincent, 1965, 1966). In this research, the CT of *L. cubeba* to kill 95% adults of *L. serricorne* could not be calculated while, *S. paniceum* were 3,163  $\text{g-h/m}^3$  at 24h. The CT product data of citral to both insects were 5,343 and 665  $\text{g-h/m}^3$  at 24h, respectively. It could be concluded that commercial fumigant were more effective than the essential oils and their constituents compound.

**Table 2** Mortality of *Lasioderma serricorne* and *Stegobium paniceum* adults treated with *Litsea cubeba* essential oil or citral at different concentrations and durations using filter paper contact bioassay.

Compound	Insect	Concentration ( $\mu\text{L/cm}^2$ )	Mortality (%) at different durations (Mean $\pm$ SE)				
			1 h	3 h	6 h	24 h	
<i>L. cubeba</i> essential oil	<i>L. serricorne</i>	0	0	0 $\pm$ 0b	3.0 $\pm$ 1.2 c	4.0 $\pm$ 1.9 c	
		0.16	0	2.0 $\pm$ 2.0b	15.0 $\pm$ 5.7 bc	16.0 $\pm$ 6.6 bc	
		0.32	2.0	5.0 $\pm$ 1.6ab	16.0 $\pm$ 5.1 bc	23.0 $\pm$ 9.0 bc	
		0.64	2.0	9.0 $\pm$ 3.3 ab	15.0 $\pm$ 5.7 bc	27.0 $\pm$ 8.5 bc	
		1.27	3.0	6.0 $\pm$ 1.9 ab	35.0 $\pm$ 5.7 b	36.0 $\pm$ 5.1 b	
		2.54	1.0	19.0 $\pm$ 8.0 a	67.0 $\pm$ 11.1 a	73.0 $\pm$ 9.2 a	
		<i>S. paniceum</i>	0	0 $\pm$ 0 c	0 $\pm$ 0 b	0 $\pm$ 0 b	0 $\pm$ 0 b
	0.16		5.0 $\pm$ 2.7 bc	7.0 $\pm$ 2.5 b	8.2 $\pm$ 1.9 b	8.9 $\pm$ 3.8 b	
	0.32		8.0 $\pm$ 4.3 bc	11.0 $\pm$ 4.0 b	8.2 $\pm$ 4.1 b	11.1 $\pm$ 5.8b	
	0.64		10.0 $\pm$ 2.7 b	55.0 $\pm$ 5.2 a	58.8 $\pm$ 6.5 a	56.7 $\pm$ 6.4 b	
	1.27		13.0 $\pm$ 2.9 b	47.0 $\pm$ 2.5 a	48.4 $\pm$ 3.3 a	58.9 $\pm$ 6.2 b	
	2.54		20.0 $\pm$ 4.6 a	53.0 $\pm$ 5.2 a	63.9 $\pm$ 4.3 a	83.3 $\pm$ 4.7 a	
	Citral		<i>L. serricorne</i>	0	0	0	0
		0.16		0	0	0	0
0.32		0		0	1.0	1.0	
0.64		0		0	2.0	2.0	
1.27		0		0	0.0	0.0	
2.54		0		0	2.0	5.0	
<i>S. paniceum</i>		0		0 $\pm$ 0 b	0 $\pm$ b	0 $\pm$ 0 b	0 $\pm$ 0 b

0.16	0±0 b	0± b	0±0 b	2.0±1.2 b
0.32	1.0±1.0 b	2.0±2.0 b	3.0±2.0 b	7.0±2.2 b
0.64	2.7±1.2 b	2.0±1.2 b	2.0±1.2 b	3.0±2.0 b
1.27	5.0±2.2 b	5.0±2.2 b	5.0±2.2 b	7.0±2.2 b
2.54	26.8±12.0 a	57.0±14.5 a	92.0±5.2 a	94.0±3.7 a

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\*Five replicates of 20 insects in each replication, means in same column followed by different letters are significantly different ( $p < 0.05$ ) Tukey's multiple range test.

**Table 3** Mortality of *Lasioderma serricorne* and *Stegobium paniceum* adults were fumigated with *Litsea cubeba* essential oil or citral at 12, 24 and 48 h after exposure.

Compound	Insect	concentration ( $\mu\text{L/L}$ )	Mortality (%) at different durations (Mean $\pm$ SE)		
			12 h	24 h	48 h
<i>L. cubeba</i> essential oil	<i>L. serricorne</i>	0	0	0 $\pm$ 0 b	1.0 $\pm$ 1.0 c
		3	0	1.0 $\pm$ 1.0 b	1.1 $\pm$ 1.1 c
		15	0	1.0 $\pm$ 1.0 b	7.7 $\pm$ 5.3 bc
		30	0	6.0 $\pm$ 3.0 ab	18.0 $\pm$ 6.0 bc
		45	0	5.0 $\pm$ 2.2 b	27.0 $\pm$ 4.0 b
		60	0	9.9 $\pm$ 3.5 ab	52.9 $\pm$ 3.1 a
		121	0	8.9 $\pm$ 2.9 ab	50.4 $\pm$ 9.0 a
		242	0	15.9 $\pm$ 2.4 a	53.3 $\pm$ 5.8 a
		<i>S. paniceum</i>	0	0 $\pm$ 0 c	0 $\pm$ 0 c
	3		42.2 $\pm$ 7.6 b	44.0 $\pm$ 5.0 b	48.2 $\pm$ 4.4b
	15		78.6 $\pm$ 3.3 a	79.8 $\pm$ 4.5 a	85.2 $\pm$ 4.8 a
	30		82.8 $\pm$ 2.5 a	82.1 $\pm$ 2.6 a	86.2 $\pm$ 2.8 a
	45		91.8 $\pm$ 6.0 a	91.4 $\pm$ 6.3 a	91.7 $\pm$ 4.0 a
	60		91.8 $\pm$ 3.1 a	91.4 $\pm$ 3.2 a	91.7 $\pm$ 3.1 a
	121		87.7 $\pm$ 5.8 a	87.1 $\pm$ 6.0 a	91.0 $\pm$ 4.7 a
242	92.9 $\pm$ 1.3 a		97.8 $\pm$ 1.3 a	98.9 $\pm$ 1.1 a	
Citral	<i>L. serricorne</i>	0	0 $\pm$ 0 b	0 $\pm$ 0 c	0 $\pm$ 0c
		3	2.0 $\pm$ 1.2 b	2.0 $\pm$ 1.2 c	2.0 $\pm$ 1.2 c
		15	2.1 $\pm$ 9.4 b	32.3 $\pm$ 12.5 b	33.3 $\pm$ 12.1 b
		30	51.4 $\pm$ 4.4 a	75.2 $\pm$ 5.7 a	76.2 $\pm$ 4.8 a
		45	65.3 $\pm$ 3.4 a	82.0 $\pm$ 5.2 a	85.0 $\pm$ 5.5 a
		60	72.0 $\pm$ 4.1 a	84.0 $\pm$ 4.9 a	85.0 $\pm$ 4.7 a
		121	64.0 $\pm$ 4.3 a	78.0 $\pm$ 5.2 a	83.0 $\pm$ 4.9 a
		242	71.0 $\pm$ 5.8 a	87.0 $\pm$ 6.0 a	89.0 $\pm$ 4.9 a
		<i>S. paniceum</i>	0	0 $\pm$ 0 d	0 $\pm$ 0 d
	3		40.8 $\pm$ 5.0 c	40.1 $\pm$ 5.6 c	40.6 $\pm$ 6.0 c
	15		78.6 $\pm$ 6.3 b	76.9 $\pm$ 6.8 b	76.9 $\pm$ 7.0 b
	30		95.9 $\pm$ 1.9 a	96.7 $\pm$ 1.3 a	96.7 $\pm$ 1.4 a
	45		95.9 $\pm$ 1.0 a	96.7 $\pm$ 1.3 a	96.7 $\pm$ 1.4 a
	60		98.8 $\pm$ 1.0 a	100.0 $\pm$ 0 a	100.0 $\pm$ 0 a
	121		100.0 $\pm$ 0 a	100.0 $\pm$ 0 a	100.0 $\pm$ 0 a
242	96.9 $\pm$ 2.0 a		100.0 $\pm$ 0 a	100.0 $\pm$ 0 a	

\*Five replicates of 20 insects in each replication, mean in same column followed by different letters are significantly different ( $p < 0.05$ ) Tukey's multiple range test.

**Table 4** Contact toxicity of extracted essential oils from *Litsea cubeba* mature fruits or citral against adults of *Lasioderma serricorne* and *Stegobium paniceum*, 6 and 24 h after exposure.

Compound	Insect	Duration times (h)	LC <sub>50</sub> (µL/cm <sup>2</sup> )	95% confidence interval (µL/cm <sup>2</sup> )	LC <sub>90</sub> (µL/cm <sup>2</sup> )	95% confidence interval (µL/cm <sup>2</sup> )	Chi-square
<i>L. cubeba</i> essential oil	<i>L. serricorne</i>	6	1.9	1.3-3.4	17.4	7.3-139.7	291.3
		24	1.6	1.0-3.2	13.0	5.3-229.2	436.9
	<i>S. paniceum</i>	6	1.2	0.9-1.6	7.9	4.6-19.8	273.9
		24	0.8	0.7-1.0	3.6	2.6-5.8	262.0
Citral	<i>L. serricorne</i>	6	>2.54	-	-	-	-
		24	>2.54	-	-	-	-
	<i>S. paniceum</i>	6	1.6	1.3-2.5	3.6	2.4-9.0	246.6
		24	1.2	1.0-1.5	3.6	2.7-5.7	329.1

**Table 5** Fumigant toxicity of extracted essential oils from *Litsea cubeba* mature fruits or citral against adults of *Lasioderma serricorne* and *Stegobium paniceum*, 24 and 48 h after exposure.

Compound	Insect	Duration times(h)	LC <sub>50</sub> (µL/L)	95% confidence Interval (µL/L)	LC <sub>90</sub> (µL/L)	95% confidence Interval (µL/L)	Chi-square
<i>L. cubeba</i> essential oil	<i>L. serricorne</i>	24	>242	-	-	-	-
		48	130.0	99-187	1277	676-3565	350.6
	<i>S. paniceum</i>	24	3.3	1.5-5.5	63	43-106	355.4
		48	2.9	1.6-4.3	37	28 -53	246.4
Citral	<i>L. serricorne</i>	24	21.6	15-29	145	100-254	672.6
		48	20.2	14-26	118	85-191	641.7
	<i>S. paniceum</i>	24	4.4	3.5-5.3	20	17-25	156.5
		48	4.2	3.3-5.2	20.9	17.4-25.9	175.8

**Table 6** The mammalian selectivity ratios of contact insecticide and *Litsea cubeba* essential oil and citral.

Insecticide / Essential oils	Chemical or Plant Family	LD <sub>50</sub> or LC <sub>50</sub> of adults (µL/cm <sup>2</sup> )		References for LD <sub>50</sub>	Rat acute oral LD <sub>50</sub> (mg/kg)	Mammalian selectivity ratio (rat LC <sub>50</sub> / <i>L. serricornes</i> ) LD <sub>50</sub> / x 10 <sup>-3</sup> )	References for rat LC <sub>50</sub>
		<i>L. serricornes</i>	<i>S. paniceum</i>				
malathion	Organophosphate	0.09 <sup>a</sup>	-	Zettler and McDonald (1984)	3,438	38.2	Tomlin (2003)
fenitrothion	Organophosphate	0.01 <sup>a</sup>	-	Zettler and McDonald (1984)	800	80	Zettler and McDonald (1984)
permithrin	Pyrethroid	0.08 <sup>a</sup>	-	Zettler and McDonald (1984)	2,000	25	Zettler and McDonald (1984)
pyrethrin	Asteraceae	26 <sup>a</sup>	19 <sup>a</sup>	Carter et al (1975)	200	0.008	Anonymous (2015b)
<i>L. cubeba</i>	Lauraceae	1.6	0.8	This study	-	-	
Citral	-	>2.54	1.2	This study	4960	>1.95	Anonymous (2015a)

<sup>a</sup> unit is µg/mg



#### 4. Conclusions

There were 10 compounds in the mature fruit of *L. cubeba* essential oil and the major compounds were E-citral and Z-citral. *Litsea cubeba* essential oil could control adult of *L. serricornis* and *S. paniceum* by contact toxicity, but the commercial citral could only control *S. paniceum*. Commercial citral could control both insects as a fumigant and was more effective than *L. cubeba* oil. For each natural compound, each insect was differently controlled. Different natural compounds could effect on different insects. Therefore, the study on various applications would be necessary to the proper utilization and the effectiveness. Effects on non-target organisms, workers, consumers and end use quality would need to be completed before this could be used in commercial grain stores. The mammalian selectivity was estimated for citral as a contact insecticide, but the data on toxicity to mammals is not known for the *L. cubeba* oil, nor is the inhalation toxicity of citral or *L. cubeba* oil known. Therefore, further testing would be required to determine if the essential oils from *L. cubeba* are effective and safe to be used as a stored-grain protectant.

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