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Repellency, Fumigant and Contact Toxicities of *Litsea cubeba* (Lour.) Persoon Against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst)

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ABSTRACT

Litsea cubeba is found in many parts of Thailand and has medicinal properties. Mature fruit of *L. cubeba* was collected from Doi Ang-khang, in the Fang District of Chiang Mai Province, Thailand in June 2007 and the essential oil was extracted by a water-distillation method. The insecticidal properties of *L. cubeba* were evaluated under laboratory conditions. The results showed that the essential oil of *L. cubeba* strongly repelled *Sitophilus zeamais* and *Tribolium castaneum* even at low concentrations, but its repellency was more marked toward *T. castaneum*. Of interest was the fact that the repellency against *T. castaneum* was fairly consistent over the 5 h period of the experiment. Moreover, it showed both contact and fumigant toxicities against the tested species. Probit analysis showed that *S. zeamais* was more susceptible than *T. castaneum* in both the fumigant and contact bioassays. Hence, the essential oil of *L. cubeba* might be used as an alternative for grain protection against stored-grain insects. Further studies are needed prior to its commercial use.

Key words: contact, fumigant, *Litsea cubeba*, repellency, grain protection

INTRODUCTION

Litsea cubeba (Lour.) Persoon (Lauraceae) is found in many parts of Thailand (Ngernsaengsaruay, 2005) and is known as chakhai-ton or takhrai or takhrai-ton in the northern, southwestern and northeastern parts of Thailand, respectively. All plant parts of *L. cubeba* are used medicinally and have antiparalytic, anticephalalgic, antihysteric, carminative, spasmolytic and diuretic properties (Nor Azah and Susiarti, 1999). Moreover, *L. cubeba* oil has *in vitro* antifungal properties against several pathogens such as *Alternaria alternate* (Fr.) Keissl., *Aspergillus niger* van Tieghem, *Candida albicans* (C. P. Robin), *Fusarium* spp. and *Helminthosporium* spp. (Nor Azah and Susiarti, 1999). Liu *et al*. (2007) showed that hexane and methanol leaf extracts of *L. cubeba* had contact and fumigant toxicities and feeding deterrent effects against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst).

In agriculture, synthetic insecticides are used both to increase yields and protect stored products. However, these insecticides are often associated with residuals that are dangerous for the consumer and the environment (Lamiri *et al*., 2001). In addition, the risk of developing insect

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resistance and the high cost-benefit ratio of synthetic pesticides have pushed researchers to find alternative insecticides. The number of insect species with confirmed resistance to synthetic pesticides has continued to rise, apart from the risks associated with the use of these chemicals (Lamiri *et a*l., 2001).

Researchers in pest control have recently concentrated their efforts on the search for active natural products from plants as alternatives to conventional insecticides (Garcia *et al*., 2005). An alternative to synthetic pesticides is the use of natural compounds such as essential oils resulting from secondary metabolism in plants, with the toxicity of a large number of essential oils and their constituents having been evaluated against a number of stored-product insects (Paranagama *et al*., 2003).

Sitophilus zeamais is found throughout the warmer parts of the world, extending as far north as Japan and southern Europe (Dennis, 1983). This pest is an internal feeder causing considerable loss to cereals and affecting the quantity as well as the quality of the grain (Gupta *et al*., 1999). *Tribolium castaneum* is a common pest infesting many flour mills, warehouses and grocery stores. It has a world-wide distribution and is among the most economically-important, stored-product pests (Garcia *et al*., 2005). The purpose of the present study was to investigate the insecticidal activities of the essential oil from the mature fruits of *L. cubeba* against *S. zeamais* and *T. castaneum.*

MATERIALS AND METHODS

Insects

Sitophilus zeamais and *T. castaneum* from the Department of Agriculture, Ministry of Agriculture and Co-operatives, Thailand were used throughout this study. *Sitophilus zeamais* was reared on rice with 12–13% moisture content, while *T. castaneum* was reared on rice bran. The cultures were maintained in the laboratory at 29-32°C and 70–80% RH.

Extraction of the essential oils

Mature fruit of *L. cubeba* was collected at Doi Ang-khang (19°54’N, 99°2’E), in the Fang District of Chiang Mai Province in June 2007. The voucher specimen (#CHKU 00022) was deposited at the Bangkok Herbarium, Botanical Research Unit, Department of Agriculture, Bangkok, Thailand. The essential oil was extracted by water-distillation using a Clevenger-type apparatus for 6 h. The superior phase was collected from the condenser, dried over anhydrous sodium sulphate and stored in amber-colored vials at 10-12°C for use in further experiments.

The essential oil was analyzed by GC/MS (Shimadzu QP 5050A) equipped with a DB-5 capillary column (60 m, 0.25 mm, 0.25 μm film thickness) (J&W Scientific). The column temperature was programmed at 60°C for 5 min then increased at 1°C/min to 80°C and then finally 4°C/min to 200°C, where it was held for 10 min. The injector and detector temperatures were 250°C, using helium as the carrier gas, at a flow rate of 1.2 mL/min. The injection volume was 1 μL with a split ratio of 1:7. The essential oil components were identified by comparing their GC retention times and their mass spectra with those presented in the MS library.

Repellent activity

Petri dishes 9 cm in diameter were used to confine insects during the experiment. The essential oil of *L. cubeba* was diluted in ethanol to different concentrations (0.5, 1, 1.5 and 2% or 0.16, 0.31, 0.47 and 0.63 $\mu g/cm^2$) and absolute ethanol was used as the control. Filter paper with a 9 cm diameter, was cut in half and 1 ml of each concentration was applied separately to one half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 1 ml of absolute ethanol. Both the treated half and the control half were then air dried to evaporate the solvent completely. A full disc was carefully remade by attaching the tested half to the control half with tape. Care was taken so that the attachment did not prevent free movement of insects from the one half to another, but the distance between the filter-paper halves remained sufficient to prevent seepage of test samples from one half to another. Each remade filter paper was placed in a petri dish with the seam oriented in one of four randomly selected different directions to avoid any insecticidal stimuli affecting the distribution of insects. Ten insects were released in the center of each filter-paper disc and a cover was placed over the petri dish. Five replicates were used and the experiment was repeated once. Counts of the insects present on each strip were made after 1 h and at hourly intervals up to the fifth hour. The percent repellency of each volatile oil was then calculated using the formula:

$$PR\ (\%) = [(N_c - N_t)/(N_c + N_t)] \times 100$$

where: N_c was the number of insects present in the control half,

N_t was the number of insects present in the treated half.

Fumigant toxicity

To determine the fumigant toxicity, filter paper (Whatman No. 1, cut into 2-cm diameter pieces) was impregnated with oil at doses calculated to give equivalent fumigant concentrations of 37, 56, 94, 130, 185, 296, 370, 444 and 556 μL/L air. The impregnated filter paper was then attached to the undersurface of the screw cap of a glass vial (27 mL). The caps were screwed tightly onto a vial containing 10 adults (1-7 days old) of either *S. zeamais* or *T. castaneum*. Each concentration and the control was replicated five times. Mortality was determined after 3, 6, 9, 12 and 24 h from the commencement of exposure. When no leg or antennal movements were observed, insects were considered dead. The percentage insect mortality was calculated using Abbott's formula for natural mortality in untreated controls (Abbott, 1925). Probit analysis was used to estimate LC_{50} and LC_{95} values. The experiment was arranged by randomized complete block design and ANOVA was computed using the SPSS version 16.0 software package.

Contact toxicity

Aliquots of 0.5 μL of the dilutions (10, 20, 30 and 40%) of the essential oil samples were applied topically to the thorax of the *S. zeamais* and *T. castaneum* using a Burkard Arnold microapplicator (Burkard Manufacturing Company Ltd., England) and ethanol was used as the control. Both treated and control insects were then transferred to glass vials (10 insects/vial) (2 cm diam and 5.5 cm height, plastic cap) and kept in incubators set at 27.7-28°C and 58-62% RH. Culture media were added to each treatment after 24 h. The mortality of insects was observed daily until end-point mortality was reached 1 week after treatment.

RESULTS AND DISCUSSION

The essential oil of *L. cubeba* strongly repelled *S. zeamais* and *T. castaneum* even at the lowest concentration and the repellency was more marked toward *T. castaneum* (Table 1). Generally, repellency increased when the concentrations were increased. However, repellency was almost persistent for *T. castaneum* where 80-96% repellency was detected 1 h after application at concentrations from 0.16 to 0.63 μg/cm^2. Complete 100% repellency only occurred with *S. zeamais* when the highest concentration (0.63 μg/cm^2) was applied for 4 h. In addition, the 100% repellency in *T. castaneum* was observed from the lowest to highest concentrations starting from 2-3 h onwards after application.

The essential oil derived from mature fruits of *L. cubeba* demonstrated fumigant toxicity to *S. zeamais* and *T. castaneum* (Table 2). However, *S. zeamais* (LC_{50} = 92.46 μL/L) was considerably more susceptible than *T. castaneum* (LC_{50} = 549.57 μL/L).

Table 1 Percent repellency (PR) of essential oil from the fruit of *Litsea cubeba* against *Sitopilus zeamais* and *Tribolium castaneum* using a treated filter paper test.*

Insect	Oil	PR (Mean% ± *SD*)[a] hours after insect release*					PR
	(µg/cm²)	1	2	3	4	5	(Mean %)[b]
S. zeamais	0.16	76 ± 13.2a	60 ± 11a	48 ± 13b	96 ± 3.5a	88 ± 4a	75.0
	0.31	68 ± 13.2a	92 ± 11a	96 ± 13a	96 ± 3.5a	96 ± 4a	90.0
	0.47	100 ± 13.2a	96 ± 11a	84 ± 13ab	96 ± 3.5a	92 ± 4a	94.0
	0.63	84 ± 13.2a	100 ± 11a	92 ± 13a	100 ± 0.08a	100 ± 4a	95.2
$F_{(3, 16)}$		1.073	2.789	2.824	0.333	1.667	
P		0.388	0.074	0.072	0.801	0.214	
T. castaneum	0.16	96 ± 9a	96 ± 9a	100 ± 0a	92 ± 11a	100 ± 0a	96.8
	0.31	92 ± 11a	96 ± 9a	100 ± 0a	100 ± 0a	100 ± 0a	97.6
	0.47	80 ± 45a	100 ± 0a	100 ± 0a	96 ± 9a	100 ± 0a	95.2
	0.63	96 ± 9a	96 ± 9a	100 ± 0a	100 ± 0a	100 ± 0a	98.4
$F_{(3, 16)}$		0.503	0.333	-	1.467	-	
P		0.686	0.801	-	0.261	-	

[a] Values were based on 4 levels of content (0.16,0.31, 0.47 and 0.63 µg/cm²), five replicates of 10 insects in each replication.

[b] Values were means of 4 levels of content (0.16,0.31, 0.47 and 0.63 µg/cm²) over the 5 h duration (at 1, 2, 3, 4, 5 h after insects were released).

* For each insect species, means in the same column followed by the same letters do not differ significantly ($P > 0.05$) as determined by Lsd test.

The contact toxicity of *L. cubeba* was very obvious when applied topically to the dorsal surface of *S. zeamais* and *T. castaneum* (Table 3). On the basis of the LD_{50} and LD_{95} values, *S. zeamais* adults were more susceptible to the essential oil of *L. cubeba* than *T. castaneum* (Table 3).

In this study, the essential oil of *L. cubeba* was shown to possess repellent, fumigant and contact activities against *S. zeamais* and *T. castaneum* which was similar to the results of Liu *et al*. (2007) that showed hexane and methanol leaf extracts of *L. cubeba* had fumigant, contact and antifeedant toxicities against these two species. Therefore, it could be concluded that not only crude leaf extracts, but also the fruit essential oil of *L. cubeba* exhibited insecticidal activities against *S. zeamais* and *T. castaneum*. In 1999, Liu and Ho demonstrated that the essential oils from *Evodia rutaecarpa* Hook f. et Thomas had contact, repellency and antifeedant toxicities against *S. zeamais* and *T. castaneum*. Moreover, Chiam *et al*. (1999) showed that an allyl disulfide compound from garlic contributed to the contact and antifeedant effects on these two species. Huang *et al*. (2002) also showed contact toxicity of eugenol, isoeugenol and methyleugenol on these two pest species.

Rajendran and Sriranjini (2008) mentioned that essential oils of plants (mainly belonging to Apiaceae, Lamiaceae, Lauraceae and Myrtaceae) and their components (monoterpenoids and others) had been tested for fumigant toxicity, with many of them showing positive results against stored insect pests including *S. zeamais* and *T. castaneum*. According to Liu and Ho (1999), *E. rutaecarpa* showed fumigant toxicity to *S. zeamais* and *T. castaneum* with LC_{50} equal to 41 and 11.7 41 µL/L air, respectively. This result showed that the essential oil of *L. cubeba* demonstrated fumigant toxicity to *S. zeamais* and *T. castaneum* whose LC_{50} values

Table 2 Fumigant toxicity of essential oil from the fruit of *Litsea cubeba* against *Sitophilus zeamais* and *Tribolium castaneum*.

Insect species	LC_{50} [a, b]	LC_{95} [a, b]	Slope ± SE	Degrees of freedom	Chi-square (x^2)
S. zeamais	92.46 (72.49 – 113.16)	224.53 (188.09 – 290.22)	0.012 ± 0.001	8	27.509
T. castaneum	549.57 (457.43 – 720.31)	1.173E3 (935.99 – 1673.11)	0.003 ± 0	8	18.96

[a] Units LC_{50} and LC_{95} = µL/L air, applied for 24 h at 27°C.
[b] 95% lower and upper fiducial limits are shown in parenthesis

Table 3 Contact toxicity of essential oil from the fruit of *Litsea cubeba* applied topically to *Sitophilus zeamais* and *Tribolium castaneum*.

Insect	LD_{50} (95% fiducial limit) (µL/insect)	LD_{95} (95% fiducial limit) (µL/insect)	Slope ± S. E	Y-intercept ± S. E
S. zeamais	0.107 (0.101 - 0.114)	0.17 (0.159 – 0.183)	26.405 ± 2.064	- 2.832 ± 2.064
T. castaneum	0.212 (0.145 – 5.009)	0.426 (0.269 – 16.746)	7.672 ± 1.022	-1.623 ± 1.022

of 92.46 and 549.57 μL/L air were much higher than the values reported by Liu and Ho (1999). Moreover, Negahban *et al*. (2007) found that *Artemisia sieberi* Besser showed fumigant toxicity to *T. castaneum* with LC_{50} value of only 16.76 μL/L air and lime peel oil exhibited fumigant toxicity against *S. zeamais* with an LC_{50} value of 11.75 μL/L (Don-Pedro, 1996). Hence, the fumigant toxicity of *L. cubeba* essential oil in this study was not as good as their findings.

The major constituents of the essential oil extracted from the mature fruits of *L. cubeba* were (E)-citral and (Z)-citral and other components were limonene, terpinen-4-ol, 1, 8-cineole (eucalyptol), α-pinene, β-pinene, camphor and linalool (Table 4). These constituents had insecticidal activities against several stored-product insect pests. For example, terpinen-4-ol was reported as toxic and also showed fumigant toxicity on some stored-product insects (Lee *et al*., 2001; Papachristos *et al*., 2004; Erler, 2005; Stamopoulos *et al*., 2007). The 1, 8-cineol had toxicity effects and was highly repellent to *S. zeamais* and other stored-product insects (Obeng-Ofori *et al*., 1997; Tripathi *et al*., 2001; Lee, 2003; Papachristos *et al*., 2004; Stamopoulos *et al*., 2007). It also showed fumigant (Tripathi *et al*., 2001; Lee *et al*., 2004a, b; Rozman *et al*., 2007) and antifeedant toxicities on major stored-product pests including *T. castaneum* (Tripathi *et al*., 2001). Papachristos *et al*. (2004) and Stamopoulos *et al*. (2007) indicated that linalool had toxicity against some stored-product insects whereas fumigant toxicity was reported by Rozman *et al*. (2007). In addition, limonene, α-pinene, β-pinene and camphor were also known to induce insecticidal activities against stored-product insects (Prates *et al*., 1998; Papachristos *et al*., 2004; Garcia *et al*., 2005; Rozman *et al*., 2007). These findings clearly supported the results of this study.

Table 4 Chemical constituents of essential oil from the fruit of *Litsea cubeba* collected from Doi Ang-kang, Chiang Mai Province, Thailand.

Compound	Retention Time (RT)	% Composition
β - Myrcene	18.357	1.47
Limonene	21.758	2.75
Terpinol-4-ol	33.718	0.31
β– Pinene	16.934	1.51
Methylheptenone	18.058	5.56
1, 8- Cineole	22.052	3.19
Fenchone	27.479	1.80
Linalool	28.589	2.16
Camphor	31.617	2.18
Citronella	32.217	1.78
Unknown	32.937	1.02
Unknown	33.998	1.88
(Z)- Geraniol	36.526	0.92
(Z)- Citral	37.150	30.08
(E)- Geraniol	37.777	1.37
(E)- Citral	38.543	41.31
Linalool acetate	42.944	0.61

CONCLUSIONS

The essential oil extracted from the mature fruit of *L. cubeba* showed insecticidal activities on *S. zeamais* and *T. castaneum* adults and might have a potential role as an alternative pest control partly because of the content of limonene, terpinen-4-ol, 1,8-cineole (eucalyptol), α-pinene, β-pinene, camphor and linalool. Since there is no record of any earlier report on the efficacy of *L. cubeba* essential oil against stored-product insects, this study represented the first record of its insecticidal activities against *S. zeamais* and *T. castaneum*. However, it is necessary to further define the repellent, fumigant and contact toxicity effects from *L. cubeba* essential oil on major storage insects.

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