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Insecticidal Activities of Essential Oils from Fruits of *Litsea salicifolia* Roxb. ex Wall. Against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst)

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Abstract.- The insecticidal activities of essential oil extracted from *Litsea salicifolia* to *Sitophilus zeamais* and *Tribolium castaneum* were investigated under laboratory conditions. Essential oil was extracted by hydro-distillation method and then analyzed by GC/MS. The major components of *L. salicifolia* were (E)-citral and (Z)-citral. *Litsea salicifolia* had repellency effect on both insect species even at the lowest application rate (0.16 µg/cm^2). In addition, it exhibited fumigant toxicity to *S. zeamais*, contact toxicity to both species and high antifeedant toxicity to *T. castaneum* compared to *S. zeamais.* Hence, *L. salicifolia* might be considered as a grain protectant to major stored product insects.

Key words: Essential oil, repellency, fumigant, contact toxicity, *Litsea salicifolia*, grain protectant.

INTRODUCTION

Chemicals derived from plants are an important source of insecticides as various plant extracts have been used by humans for control of insects since the time of ancient Romans. During the 20th century, a few natural compounds such as nicotine, rotenone and pyrethrin have been used commercially as insecticides (Arnason *et al*., 1981). In many areas of Africa and Asia, locally available plants are widely used as an alternative to synthetic pesticides to protect stored products from insect infestation (Golob and Webley, 1980; Su *et al*., 1982; Zehrer, 1984; Ahmed and Koppel, 1985; Khalique *et al*., 1988). Hence, the use of plants for pest control on stored grains seems to offer desirable solutions, especially in the developing tropical countries where plants are found in abundance everywhere throughout the year. Moreover, there has been growing interest in the use of both plant extracts and their essential oils since they exhibit low mammalian toxicity and low persistence in the environment (Raja *et al*., 2001; Papachristos and Stamopoulos, 2002).

The efficient and effective control of storage insects like *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) are mainly dependent on the use of synthetic insecticides (Isikber *et al*., 2006; Mondal and Khalequzzaman, 2006). Pest control in many storage systems depends on fumigation with either methyl bromide or phosphine. However, under the Clean Air Act and Montreal Protocol, the use of methyl bromide has been prohibited in the developed countries since 2005 and will soon be restricted in the developing countries in 2010 because of its potential to damage the atmospheric ozone layer (TEAP, 2000). Moreover, some stored-product insects are found to develop resistance to phosphine in many countries, thus the further use of phosphine could be threatened by further development of resistant strains (Bell and Wilson, 1995; Collins *et al*., 2001). Therefore, this wide role of methyl bromide and phosphine is likely to be more restricted in the future. Increased public concern over the residual toxicity of insecticides applied to stored insect strains and the necessary precaution to work with traditional chemical insecticides calls for new approaches in controlling stored product insect pests (Yilsrim *et al.,* 2001).

Litsea salicifolia Roxb. ex Wall. is one of the many plants used as phytopesticide, traditionally by various tribes of Assam (Phukan and Kalita, 2005). The hexane extract (2000 ppm) of *L. salicifolia*

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exhibited 70% repellent activity for 3 h against *Aedes aegypti* (L.) and 46% activity for 3 h against *Culex quinquefasciatus* where LC_{50} value of *L. salicifolia* against *A. aegypti* was 0.72% (Phukan and Kalita, 2004). In addition, Noosidum *et al*. (2008) found that fruit oil of *L. salicifolia* produced vigorous contact irritancy and non-contact repellency at low concentration (0.5%) against *A. aegypti* . At present, there has been no information concerning the insecticidal activities of *L. salicifolia* against stored-product insects. Hence, the study was aimed to investigate their repellency, fumigant, contact and antifeedant activities under laboratory conditions.

MATERIALS AND METHODS

Insects

Sitophilus zeamais and *T. castaneum* provided from Department of Agriculture, Ministry of Agriculture and Co-operatives, Thailand were used throughout this study. *Sitophilus zeamais* was reared on rice of 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% relative humidity (RH).

Extraction of essential oils

Mature fruits of *L. salicifolia* were collected at Doi Ang-khang (19º54'N, 99º2'E), Fang District, Chiang Mai Province in June 2007. The voucher specimens (#CHKU 00023) was deposited at the Bangkok Herbarium, Botanical Research Unit, Department of Agriculture, Bangkok, Thailand. Essential oil was extracted by hydro-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 the amber-colored vials at 10-12ºC for further experiments. 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, finally 4°C/min to 200°C, held for 10 min. The injector and detector temperatures were 250°C. Helium was the carrier gas, at a flow rate of 1.2 mL/min. The injection volume was 1 μL with split ratio 1:7. 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 of 9 cm in diameter were used to confine insects during the experiment. Essential oil of *L. salicifolia* was diluted in ethanol to different concentrations (0.5%, 1%, 1.5% and 2% or 0.16, 0.31, 0.47 and 0.63 μg/cm^2, respectively) while absolute ethanol was used as the control. Filter papers (9 cm diameter) were cut in half. One mL of 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 sides 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 sellotape. 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 on each Petri dish. Five replicates were performed and the experiment was repeated once. Counts of insects present in each strip were made after 1 h and at hourly interval 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 presented in the control half and N_t was the number of insects presented in the treated half.

Fumigant toxicity

To determine the fumigant toxicity, filter papers (Whatman No. 1, cut into 2-cm diameter pieces) were impregnated with oil at doses calculated to give equivalent fumigant concentrations of 37, 56, 94, 130, 185, 296, 370, 444 and 556 μL/L in air. Then the impregnated filter paper was attached to the under-surface of the screw cap of a glass vial (27 mL). The caps were screwed tightly on the vial containing 10 adults (1-7 days old) of either *S. zeamais* or *T. castaneum*. Each concentration and control was replicated five

times. Mortality was determined after 3, 6, 9, 12 and 24 h from commencement of exposure. When no leg or antennal movements were observed, insects were considered dead. Percentage of insect mortality was calculated using the 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 completely randomized block design and ANOVA was computed using SPSS version16.0 software package.

Contact toxicity

Aliquots of 0.5 µL of the dilutions (10%, 20%, 30% and 40%) of the essential oil were applied topically to the thorax of *S. zeamais* and *T. castaneum* using a Burkard Arnold microapplicator (Burkard Manufacturing Company Ltd. England). The control was prepared using ethanol. Both the treated and control insects were then transferred to glass vials (10 insects/vial) (2 cm diam and 5.5 cm height with plastic cap) and kept in the incubator set at 27-28˚C and 58-62% RH. Culture media were added to each treatment after 24 h. Mortality of insects was observed daily until end-point mortality (when the number of dead insects no longer increased with time) was reached 1 week after treatment.

Antifeedant test

Aliquots of 200 µL of a suspension of wheat flour in water (10 g in 50 mL) were dropped onto a clean plastic placed in a tray. The discs were left to dry in the fume hood for 24 h, after which they were placed in the hot air oven at 60˚C for 1 h. Each essential oil was diluted in ethanol to get different concentrations (4% 6%, 8% and 10%) and absolute ethanol and non-treated discs were used as the controls. After evaporation of solvent, 2 discs for *S. zeamais* or 1 disc for *T. castaneum* were placed in glass vials (2.5 cm diameter, 3 cm height). All insects were starved for 24 h before use. Then, ten group-weighed, unsexed adults were added to each preweighed vial containing the disc. Glass vials containing flour discs but without insects were also prepared to determine if any decrease in weight occurred due to evaporation. A 5 µL of different concentrations was dropped onto each flour disc and ethanol was used as the control. Five replicates were prepared for this experiment.

After three days, the glass vials with flour discs were weighed again and mortality of insect, if any, was recorded. For antifeedant action, the formula described by Huang *et al.* (2000) was modified in calculating the feeding deterrence index FDI (%) = [(C-T)/C] x 100, where C = the consumption of control discs and T = the consumption of treated discs. The following criteria were adopted to categorize the essential oils:

FDI% < 20%	No feeding deterrence
50% > FDI% ≥ 20%	Weak feeding deterrence
70% > FDI% ≥ 50%	Moderate feeding deterrence
FDI% ≥ 70%	Strong feeding deterrence

RESULTS AND DISCUSSION

The repellency effects of *L. salicifolia* are shown in Table I. It could be seen that the essential oil of *L. salicifolia* strongly repelled both *S. zeamais* and *T. castaneum* even at a very low concentration. Generally, the repellency of all application rates to *S. zeamais* was 72-100% while that for *T. castaneum* was 76-100% during 5 h periods. The 100% repellency was detected at the lowest application rates for 2 h duration. In addition, 100% repellency was also observed in the second lowest application rate for 1 h and 2 h. At the highest application rate, 100% repellency was found at 2 h period for *S. zeamais* and *T. castaneum*. When the repellency effect of all application rates during 5 h periods were compared, no statistical difference was found (Table I).

Meanwhile, 100% repellency to *T. castaneum* occurred at the lowest and second lowest applications at 2 h period. In addition, 100% repellency was also found in the second highest application rate at 1, 3 and 4 h. At the highest application rate, 100% repellency could be detected at all periods except for 1 h. Generally speaking, *L. salicifolia* seemed to demonstrate higher repellent effect to *T. castaneum* than *S. zeamais*.

Fumigant toxicities of *L. salicifolia* against *S. zeamais* and *T. castaneum* is demonstrated in Table II. *L. salicifolia* oil evoked more tremendous fumigant effect on *S. zeamais* than *T. castaneum*

Table I.- Percent repellency (PR) of *Litsea salicifolia* fruit essential oil to *Sitopilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) using treated filter paper test.*

Insect	Oil (µg/cm^2)	PR (Mean% ± *SD*)** hours after insect release***					PR (Mean %)
		1	2	3	4	5	
S. zeamais	0.16	92 ± 11a	100 ± 0a	72 ± 33a	88 ± 18a	88 ± 18a	88.0
	0.31	100 ± 0a	100 ± 0a	92 ± 11a	96 ± 9a	80 ± 28a	93.6
	0.47	92 ± 11a	96 ± 9a	80 ± 14a	88 ± 11a	92 ± 11a	89.6
	0.63	84 ± 17a	100 ± 0a	92 ± 18a	88 ± 18a	88 ± 18a	90.4
$F_{(3, 16)}$		0.917	1.000	1.636	0.381	2.794	
P		0.455	0.418	0.221	0.768	0.074	
T. castaneum	0.16	92 ± 11a	100 ± 0a	88 ± 18a	100 ± 0a	76 ± 26a	91.2
	0.31	92 ± 11a	100 ± 0a	90 ± 11a	100 ± 0a	96 ± 8a	95.6
	0.47	100 ± 0a	96 ± 9a	100 ± 0a	100 ± 0a	96 ± 9a	98.4
	0.63	96 ± 9a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 6a	99.2
$F_{(3, 16)}$		0.917	7.111	1.636	-	2.794	
P		0.455	0.418	0.221	-	0.074	

* Values were based on 4 levels of content (0.16, 0.31, 0.47 and 0.63 µg/cm^2), five replicates of 10 insects in each replication.
** Values were means of 4 levels of content (0.16, 0.31, 0.47 and 0.63 µg/cm^2) over the 5 h duration (at 1, 2, 3, 4, 5 hours 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.

Table II.- Fumigant toxicities of *Litsea salicifolia* fruit essential oil against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* Herbst.

Insect species	LC_{50} *, **	LC_{95} a, b	Slope ± SE	Degrees of freedom	Chi-square (x^2)
S. zeamais	4.435 (–)	174.63 (–)	0.10 ± 0.001	8	390.511
T. castaneum	845.16 (729.81-1052.01)	1345.00 (1118.69-1760.79)	0.003 ± 0.01	8	4.8

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

Table III.- Contact toxicities of *Litsea salicifolia* fruit essential oil applied topically to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst).

Insect	LD_{50} (95% fiducial limit) (µL/insect)	LD_{95} (95% fiducial limit) (µL/insect)	Slope ± S. E	Y-intercept ± S. E
S. zeamais	0.079 (-0.003 - 0.150)	0.144 (0.102 – 0.581)	25.069 ± 1.931	- 1.973 ± 1.931
T. castaneum	0.111 (-)	0.304 (–)	8.556 ± 0.903	-0.952 ± 0.903

where the LC_{50} values of *L. salicifolia* oil on *S. zeamais* was 211 folds greater than on *T. castaneum*.

Contact toxicities of *L. salicifolia* against *S. zeamais* and *T.castaneum* are presented in Table III. Based on the LD_{50} values, *L. salicifolia* showed more contact toxicity against *S. zeamais* than *T. castaneum*.

Antifeedant toxicities of *L. salicifolia* against *S. zeamais* and *T. castaneum* are shown in Table IV. *L. salicifolia* showed more antifeedant effect on *T.*

castaneum. The feeding deterrence index (FDI) of all concentration rates to *S. zeamais* was lower than those of *T. castaneum*. Only the highest concentration rate had weak antifeedant deterrence to *S. zeamais,* whereas the lowest and second lowest concentration rates showed moderate feeding deterrence and the highest concentration rate showed strong feeding deterrence on *T. castaneum*.

Table IV.- Comparison of Antifeedant toxicities of *Litsea salicifolia* fruit essential oil against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst).

Concentration (%)	Feeding deterrent index (FDI) %*	
	S. zeamais	*T. castaneum*
0	0 c	0 c
4	6.17 c	53.85 b
6	7.41 c	69.23 ab
8	19.75 b	76.92 ab
10	29.63 a	84.62 a

*For each species, means in the same column with the same letters do not differ significantly ($P > 0.05$) as determined by independent sample t test

In this study, *L. salicifolia* showed repellency effect on *S. zeamais* and *T. castaneum.* Liu and Ho (1999) demonstrated that the essential oils from *Evodia rutaecarpa* (Juss.) Benth. had contact, repellency and antifeedant toxicities against *S. zeamais* and *T. castaneum*. Huang *et al*. (1997) also showed that the essential oil extracted from nutmeg seeds had contact, fumigant and antifeedant toxicities against these two insects. Moreover, Huang and Ho (1998) proved that methylene chloride extract of cinnamon had contact, fumigant and antifeedant effects to *T. castaneum* and *S. zeamais*.

The major chemical constituents contained in *L. salicifolia* were (E)-citral and (Z)-citral where limonene, linalool, terpinen-4-ol and α-terpineol were minor components (Table V). Prates *et al*. (1998) reported that limonene had insecticidal activity against *T. castaneum*. In addition, the most repellent compound in *Baccharis salicifolia* (Ruiz & Pav.) Pers. essential oil against *T. castaneum* was α-terpineol (Garcia *et al*., 2005). Stamapoulos *et al*. (2007) revealed that terpinen-4-ol and linalool exhibited insecticidal activities against *T. confusum*. Moreover, Ojimelukwe and Adler (1999) reported that α-pinene showed potent repellent and toxic effects on *T. confusum*. Hence, essential oils of *L. salicifolia* had insecticidal activities to *S. zeamais* and *T. castaneum* partly because of the presence of these chemical constituents.

Table V.- Some chemical constituents of *Litsea salicifolia* fruit essential oil collected from Doi Angkang, Chiang Mai Province, Thailand.

Compounds	Retention Time (RT)	% Composition
α - pinene	13.968	0.29
methylheptenone	17.995	11.20
β – myrcene	18.286	1.74
limonene	21.686	5.59
citronellal	32.159	2.26
terpinen-4-ol	33.672	0.32
linalool	28.589	2.16
α - terpineol	34.462	0.19
(Z) – geraniol	36.478	2.01
(Z) – citral	37.099	29.10
(E) – geraniol	37.731	2.19
(E)- citral	38.491	40.88
(Z)-β-farnesene	44.455	0.19

This research provides a scientific basis for extracting and applying phytochemicals from the tested plant species against stored-product pests. *L. salicifolia* fruit oil is more effective as fumigant and contact activities against *S. zeamais* as compared to *T. castaneum*. Furthermore, high concentration (10%) of this particular oil can be used as an antifeedant against *T. castaneum* where lowest concentration (0.16 μg/cm^2) demonstrated repellent effect on both insects.

Further studies on the bioactivity of *L. salicifolia* fruit oil and their constituents against stored-product insects on a small scale basis is needed to be investigated before the commercial implementation can be considered.

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