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Composition of the essential oil from *Alpinia galanga* rhizomes and its bioactivity on *Lasioderma serricorne*

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Abstract

The aim of this research was to determine the chemical constituents and bioactivity of the essential oil derived from *Alpinia galanga* (L.) Willd. rhizomes against the cigarette beetle *Lasioderma serricorne* (F.) (Coleoptera Anobiidae) adults. The essential oil of *A. galanga* obtained by hydrodistillation was investigated by gas chromatography-mass spectrometry (GC-MS). The main components of the essential oil were identified to be eucalyptol (22.63%), (1S)-(1)-β-pinene (14.36%), 1R-α-pinene (10.89%), α-terpineol (8.59%) and L(-)-borneol (8.41%) followed by (-)-camphor (4.21%) and camphene (4.14%). Eucalyptol, β-pinene, α-pinene, α-terpineol and borneol were separated and purified by silica gel column chromatography and preparative thin layer chromatography, and further identified by means of physicochemical and spectrometric analysis. The essential oil of *A. galanga* rhizomes was found to possess strong contact toxicity against *L. serricorne* adults with LD_{50} value of 12.2 μg/adult, and also showed strong fumigant toxicity against *L. serricorne* adults with LC_{50} value of 3.5 mg/L. α-Terpineol and eucalyptol showed strong contact toxicity against *L. serricorne* (LD_{50} = 13.3 and 15.6 μg/adult, respectively) and fumigant toxicity against *L. serricorne* (LC_{50} = 2.8 and 5.2 mg/L air, respectively). Moreover, the essential oil and eucalyptol also exhibited the strong repellency against *L. serricorne* adults whereas borneol exhibited weaker repellency relative to the positive control, DEET. The study revealed that the bioactivity properties of the essential oil can be attributed to the synergistic effects of its diverse major and minor components. The results indicate that the essential oil of *A. galanga* and the isolated compounds have potential to be developed into natural insecticides or repellents in controlling insects in stored grains and traditional Chinese medicinal materials.

Key words: *Alpinia galanga*, *Lasioderma serricorne*, contact toxicity, fumigant toxicity, repellency.

Introduction

Some secondary metabolites of plants play an important role in plant-insect interaction, and are commonly responsible for plant resistance to insects (Mann, 1987). Essential oils, which are aromatic plant secondary metabolites, have been shown to possess a broad spectrum of pest control properties of their larvicidal, toxic, repellent, growth inhibiting, ovicidal, antifeedant and antioviposition effects (Isman, 2000; 2006; Rajendran and Srianjini, 2008). The components of essential oil often biodegrade to nontoxic products, so they could be much safer insect control agents and more suitable for use in integrated pest management (IPM) (Dev and Koul, 1997). Ideally, botanical insecticides should be very toxic to target insects, yet should be not toxic to nontarget organisms such as plants, insects (e.g., parasites, predators and pollinators) and other animals, such as fish and birds. Above all, the risk to workers and consumers must be very low (Suthisut *et al.*, 2011). Because of the worldwide attention toward pesticide residues in agricultural products, insecticides of natural origin are very important in food safety (Ueno *et al.*, 2003). In the course of screening for significant naturally occurring insecticidal plants, *Alpinia galanga* was found to exhibit insecticidal and repellent activity against *Lasioderma serricorne* (F.).

Alpinia galanga (L.) Willd., family Zingiberaceae commonly referred to as galangal, is widely cultivated in South-east Asian countries such as Philippines, Indonesia, Thailand, India, and China (Kaushik *et al.*, 2011). It is extensively used in diets as well as in the traditional systems of medicine viz., Thai, Ayurveda, Unani and Chinese folk medicine (Chudiwal *et al.*, 2010). Galangal has been known for its use as anti-inflammatory, antipyretic, emmenagogue, carminative, abortifacient and aphrodisiac and is used in the treatment of various diseases such as renal calculus, diabetes, heart diseases, bronchitis, rheumatism, chronic enteritis and kidney disorders (Chudiwal *et al.*, 2010; Sawangjaroen *et al.*, 2005). Recent studies also show that this plant has antibacterial (Khattak *et al.*, 2005), antiplasmid (Latha *et al.*, 2009), antioxidant and anti-microbial (Wong *et al.*, 2009) properties. The essential oil and compounds therein are responsible for such activities (Raina *et al.*, 2002; Zhu *et al.*, 2009; Latha *et al.*, 2009; Barik *et al.*, 1987). The toxicity of essential oil extracted from *A. galanga* rhizomes against *Bactrocera dorsalis* (Hendel) have been studied (Sukhirun *et al.*, 2009; 2011).

The cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera Anobiidae) is widely distributed and is of considerable economic importance in tropical to temperate climates (Ashworth, 1993). This beetle is destructive primary insect pests of stored cereals, tobacco, oilseeds,

dried fruits and traditional Chinese medicinal materials (Ebadollahi *et al.*, 2010). Development and survival are affected by type of food, temperature and humidity, therefore the life cycle is different (Ashworth, 1993). Control of this pest relies on the widespread use of various synthetic chemical insecticides and fumigants which had led to a number of problems such as environmental pollution, pesticide residue in food grains, development of insecticide resistance and toxicity to non-target organisms over the years (Yusof and Ho, 1992; Lorini and Galley, 1999; Cosimi *et al.*, 2009; Sousa *et al.*, 2009).

As far as our literature survey could ascertain, no information concerning insecticidal and repellent activity of the essential oil from *A. galanga* rhizomes against *L. serricorne* has been published openly. Therefore, in the present paper, we describe the identification of the chemical components of *A. galanga* by a gas chromatography-mass spectroscopy (GC-MS) method, and evaluate the insecticidal and repellent property of the essential oil against *L. serricorne*.

Materials and methods

Insect rearing

Cultures of the cigarette beetle, *L. serricorne*, were maintained in the laboratory without exposure to any insecticide. They were reared on sterilized diet (wheat-flour/yeast, 10:1, w/w) at 29-30 °C, 70-80% RH in the dark for the last two years. The unsexed adult beetles used in all the experiments were about 1-2 weeks old. All containers housing insects used in experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon).

Plant material and essential oil extraction

The fresh rhizomes (2.0 kg) of *A. galanga* were harvested from Menghai (21°28'~22°28'N latitude and 99°56'~100°41'E longitude), Yunnan Province, China in October 2013. The plant was identified, and a voucher specimen (BNU-dushushan-2013-10-09-23) was deposited at the Herbarium (BNU) of College of Resources Science and Technology, Beijing Normal University. The harvested material was air-dried at room temperature (20-25 °C) for one week and then stored in cloth bags.

Essential oil was extracted from rhizomes (1.0 kg of dry matter) subjected to hydrodistillation during 6 h using a modified Clevenger-type apparatus. Anhydrous sodium sulphate was used to remove water after extraction. The essential oil was stored in airtight containers in a refrigerator at 4 °C for subsequent experiments.

GC-FID and GC-MS analysis

The obtained essential oil was packed in amber vial, lightless. A sample of the oil was diluted in *n*-hexane and subjected to analysis by gas chromatography coupled to flame ionization detector (GC-FID) and gas chromatography coupled to spectrometry (GC-MS) in Beijing Normal University, Beijing, China.

Analysis condition by GC-FID were: column HP-5MS (30 m × 0.25 mm × 0.25 μm). The column temperature was programmed at 50 °C for 2 min, then increased at 2 °C/min to the temperature of 150 °C and held for 2 min, and then increased at 10 °C/min until the final temperature of 250 °C was reached, where it was held for 5 min, using hydrogen and synthetic air as carrier gases. The Retention Indices (RI) were determined from the retention time of a homologous series of hydrocarbons (C_5-C_{36}), obtained by GC-FID under the same condition of analysis of essential oil.

GC-MS analysis was performed on a Thermo Finnigan Trace DSQ instrument equipped with a flame ionization detector and a HP-5MS (30 m × 0.25 mm × 0.25 μm) capillary column. The column temperature was programmed at 50 °C for 2 min, then increased at 2 °C/min to the temperature of 150 °C and held for 2 min, and then increased at 10 °C/min until the final temperature of 250 °C was reached, where it was held for 5 min. The injector temperature was maintained at 250 °C and the volume injected was 0.1 mL of 1% solution (diluted in *n*-hexane). The carrier gas was helium at flow rate of 1.0 mL/min. Spectra were scanned from 50 to 550 m/z. Most constituents were identified by comparison of their retention indices with those reported in the literatures. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_5-C_{36}) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature (Adams, 2001). Relative percentages of the individual components of the essential oil were obtained by averaging the GC peak area % reports.

Purification and identification of five constituent compounds

The crude essential oil (1.0 mL) was chromatographed on a silica gel (Qingdao Marine Chemical Plant, Shandong province, China) column (45 mm i.d., 500 mm length) by gradient elution with *n*-hexane first, then with *n*-hexane-ethyl acetate, and last with ethyl acetate. Fractions (120 mL) were collected and concentrated at 35 °C, and similar fractions according to thin layer chromatography (TLC) profiles were combined to yield 35 fractions. Of these, fraction 3, 9, 13, 20 and 25 were further separated on repeated silica gel columns and PTLC to afford five pure compounds for determining structure. The purified subfractions were subjected to nuclear magnetic resonance (NMR) analysis. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker AMX500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] using $CDCl_3$ as the solvent with TMS as internal standard. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. The spectral data of eucalyptol (1.9 g) matched with the previous report (Ashnagar *et al.*, 2009). The spectral data were identical to the published data of β-pinene (0.9 g) (Badiah-Hadj-Ahmed *et al.*, 1992). The spectral data of α-pinene (1.0 g) matched with the previous report (Badiah-Hadj-Ahmed *et al.*, 1992). The data of α-terpineol (0.6 g) matched with the previous reports (Krings *et al.*, 2006) and the data of borneol (0.5 g) matched with the previous reports (Matos and Andrade, 2008; Gerdov *et al.*, 2005).

Contact toxicity bioassays

The contact toxicity of the crude essential oil and isolated compounds against *L. serricorne* adults was measured as described by Liu and Ho (1999). Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil/compounds (five concentrations for each: 1.97%-10.00% for oil, eucalyptol and α-terpineol; 3.95%-20.00% for β-pinene; 5.93%-30.00% for α-pinene; 2.96%-15.00% for borneol) was prepared in *n*-hexane. Aliquots of 0.5 μL of the dilutions were applied topically to the dorsal thorax of the insects with a range of 2.5 microliter pipette. Controls were determined using *n*-hexane. Ten insects were used for each concentration and control, and the experiment was replicated five times. Both treated and control insects were then transferred to glass vials with culture media and kept in incubators. Mortality was recorded after 24 h by direct observation of the insects which didn't move after five minutes because of the suspended animation. And the LD_{50} values (short for median lethal dose which means capable of causing the death of half of the test animals dose, usually expressed by the logarithm of a lethal dose of the drug) were calculated using Probit analysis (IBM SPSS V20.0) (Sakuma, 1998).

Fumigant toxicity bioassays

The fumigant activity of the crude essential oil and isolated compounds against *L. serricorne* adults was tested as described by Liu and Ho (1999). Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil/compounds (five concentrations for each: 0.40%-2.00% for oil; 0.99%-5.00% for eucalyptol and borneol; 3.95%-20.00% for β-pinene and α-pinene; 0.63%-1.33% for α-terpineol) was prepared in *n*-hexane. A Whatman filter paper (diameter 2.0 cm) was impregnated with 10 μL dilution and then placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 25 mL). The solvent was allowed to evaporate for 10 s before the cap was placed tightly on the glass vial, each of which contained 10 insects inside to form a sealed chamber. Fluon (ICI America Inc.) was used inside the glass vial to prevent insects from contacting the treated filter paper. Preliminary experiments demonstrated that 10 s was sufficient for the evaporation of solvents. *n*-Hexane was used as a control. Five replicates were carried out for all treatments and controls, and they were incubated under the same conditions as rearing. Mortality was determined after 24 h of treatment, and the LC_{50} values were calculated using Probit analysis (IBM SPSS V20.0) (Sakuma, 1998).

Repellency tests

The repellent activity to *L. serricorne* adults was tested using the area preference method (Zhang *et al.*, 2011). Petri dishes (9 cm in diameter) were used to confine cigarette beetles during the experiment. The crude essential oil and the isolated compounds were diluted in *n*-hexane to five concentrations (39.32, 7.86, 1.57, 0.31 and 0.06 nL/cm^2), and *n*-hexane was used as the control. Filter paper (9 cm in diameter) was cut in half and 500 μL of each concentration was applied separately to half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 500 μL of *n*-hexane. Both the treated half and the control half were then air-dried to evaporate the solvent completely (30 s). A full disk was carefully remade by attaching the tested half to the negative control half with tape. Care was taken so that the attachment did not prevent free movement of insects from the one half to the other, but the distance between the filter paper halves remained sufficient to prevent seepage of test samples from one half to the other. Each remade filter paper after treatment with solid glue 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. Twenty insects were released in the center of each filter paper disk, and a cover was placed over the Petri dish. Five replicates were used and the experiment was repeated three times. Counts of the insects present on each strip were made after 2 and 4 h. The percent repellency (PR) of each volatile oil/compound was then calculated using the formula:

$$PR\ (\%) = [(Nc - Nt) / (Nc + Nt)] \times 100$$

Nc is the number of insects present in the negative control half, while Nt is the number of insects present in the treated half. Analysis of variance (One-Way ANOVA and GLM Univariate) and Tukey's test were conducted by using SPSS 20.0 for Windows 2007. Percentage was subjected to an arcsine √ transformation before variance and Tukey's tests. The averages were then assigned to different classes (Class 0, PR is > 0.01 to < 0.1; Class I, PR is 0.1-20; Class II, PR is 20.1-40; Class III, PR is 40.1-60; Class IV, PR is 60.1-80; Class V, PR is 80.1-100) (Nerio *et al.*, 2010). Significant differences in repellence rates were detected by using LSD test (IBM SPSS V20.0, GLM). A commercial repellent, DEET (N,N-diethyl-3-methyl-benzamide), was purchased from the National Center of Pesticide Standards (8 Shenliao West Road, Tiexi District, Shenyang 110021, China) and used as a positive control.

Results and discussion

Chemical constituents of the essential oil

The chemical compositions of the essential oil derived from *A. galanga* rhizomes collected from Yunnan, China are shown in table 1. The essential oil was obtained by hydrodistillation from the dried rhizomes of *A. galanga* with the yield of 0.30×10^{-2} L/kg (v/w) and the density of 0.87 g/mL. A total of 51 components were identified in the essential oil of *A. galanga* accounting for 96.06% of the total oil. The main constituents of *A. galanga* essential oil were eucalyptol (22.63%), (1S)-(1)-β-pinene (14.36%), 1R-α-pinene (10.89%), α-terpineol (8.59%) and L(-)-borneol (8.41%) followed by (-)-camphor (4.21%) and camphene (4.14%).

It is noteworthy that the chemical constituents of the essential oil are in partial agreement with the previous reports. These differences might have been due to harvest time and local, climatic and seasonal factors as well

as storage duration of medicinal herbs. For example, the oil from Indonesia was made up predominantly of monoterpenoids with pinenes (18.6%) and 1,8-cineole (47.3%), while the Malaysian oil was characterized by sesquiterpenoids with (*E*)-β-farnesene (18.2%) and β-bisabolene (16.2%) as the major components (Scheffer

Table 1. Chemical constituents of the essential oil derived from *A. galanga* rhizome parts.

Peak no.	Components	RI[a]	Peak area (%)
1	β-Terpinen	1151	0.09
2	1R-α-Pinene	1182	10.89
3	Camphene	1206	4.14
4	2,4(8)-*p*-Menthadiene	1214	2.14
5	(1S)-(1)-β-Pinene	1229	14.36
6	α-Phellandrene	1255	0.88
7	2-Isopropyltoluene	1274	1.74
8	Eucalyptol	1280	22.63
9	1-Methyl-3-(1'-methylcyclopropyl)cyclopentene	1285	0.07
10	2,2-Dimethylheptane	1404	0.08
11	γ-Terpinene	1409	1.15
12	Undecane	1416	0.22
13	Linalool	1456	0.76
14	2-Methyloctane	1460	0.10
15	Fenchol	1467	1.72
16	Fenchene	1477	0.16
17	(-)-Camphor	1602	4.21
18	Camphene hydrate	1612	1.07
19	L(-)-Borneol	1662	8.41
20	4-Carvomenthenol	1695	3.76
21	α-Terpineol	1736	8.59
22	Myrtenol	1751	0.10
23	(+)-Sabinol	1765	0.10
24	Fenchyl acetate	1912	0.08
25	Citronellyl formate	1930	0.09
26	Benzylacetone	1949	0.65
27	*p*-Menth-1-en-3-one	1964	0.05
28	(*Z*)-Geraniol	1971	2.13
29	l-Bornyl acetate	2105	1.29
30	Thymyl acetate	2114	0.11
31	Benzalacetone	2139	0.11
32	Nerol acetate	2155	0.14
33	Caryophyllene	2168	0.39
34	α-Caryophyllene	2184	0.73
35	Methyl isoeugenol	2189	0.05
36	Dihydro-cis-α-copaene-8-ol	2199	0.13
37	Aciphyllene	2607	0.05
38	2-methyl-Decane	2615	0.08
39	3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate	2685	0.09
40	1-Methylene-2*b*-hydroxymethyl-3,3-dimethyl-4*b*-(3-methylbut-2-enyl)-cyclohexane	2697	0.06
41	2-(1,1-Dimethylethyl)-6-(1-methylethyl)phenol	2716	0.52
42	1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane	2723	0.21
43	Methyltrans-2-phenyl-1-cyclopropanecarboxylate	2752	0.67
44	Bicyclo[6.1.0]non-1-ene	2782	0.05
45	3,5-Dimethyl-4-octanone	2830	0.56
46	2-Methyl-3-(3-methylbut-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	2837	0.07
47	Bornylane	2891	0.12
48	Scytalone	2900	0.06
49	3-Ethyl-3-methyl-decane	2916	0.05
50	9-Oxononanoic acid	2926	0.09
51	2-(Fench-2-yl)fenchane	2932	0.06
	Total		96.06

[a]RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons as reference.

Table 2. Contact toxicity of essential oil from *A. galanga* rhizome parts and its main components against *L. sericorne* adults.

Treatment	LD_{50} (μg/adult)	95% FL[a]	Slope ± SE	N
Alpinia galanga	12.2	10.4-14.0	2.38 ± 0.3	250
Eucalyptol	15.6	12.9-18.0	3.87 ± 0.6	250
β-Pinene	65.6	58.1-76.1	3.75 ± 0.5	250
α-Pinene	76.8	68.7-86.8	5.41 ± 0.6	250
α-Terpineol	13.3	11.9-14.7	3.61 ± 0.5	250
Borneol[b]	-	-	-	250
Pyrethrins[c]	0.2	0.1-0.4	1.31 ± 0.2	250

[a]Fiducial limits; mean mortality of the control with *n*-hexane ≤ 2%;
[b]Borneol did not show contact toxicity at the tested concentrations;
[c]Data from Yang *et al.* (2014).

Table 3. Fumigant toxicity of essential oil from *A. galanga* rhizome parts and its main components against *L. sericorne* adults.

Treatment	LC_{50} (mg/L air)	95% FL[a]	Slope ± SE	N
Alpinia galanga	3.5	2.8-5.0	1.35 ± 0.3	250
Eucalyptol	5.2	4.6-5.7	4.84 ± 0.6	250
β-Pinene	29.0	26.4-31.8	5.41 ± 0.6	250
α-Pinene	38.1	34.5-41.7	5.83 ± 0.7	250
α-Terpineol	2.8	2.7-2.9	11.25 ± 1.4	250
Borneol[b]	-	-	-	250
Phosphine[c]	9.2×10^{-3}	7.1×10^{-3}-11.4×10^{-3}	2.12 ± 0.3	250

[a]Fiducial limits; mean mortality of the control with *n*-hexane ≤ 1%;
[b]Borneol did not show fumigant toxicity at the tested concentrations;
[c]Data from Yang *et al.* (2014).

as storage duration of medicinal herbs. For example, the oil from Indonesia was made up predominantly of monoterpenoids with pinenes (18.6%) and 1,8-cineole (47.3%), while the Malaysian oil was characterized by sesquiterpenoids with (*E*)-β-farnesene (18.2%) and β-bisabolene (16.2%) as the major components (Scheffer *et al.*, 1981; Mori *et al.*, 1995; Jantan *et al.*, 2004). Studies showed that *A. galanga* from Southern India contained 1,8-cineole (33.0-30.2%), camphor (5.0-14.0%), α-terpineol (2.3-9.3%), α-fenchyl acetate (1.1-12.7%), and (*E*)-methyl cinnamate (2.6-5.3%) as the main component in its rhizome oil (Mallavarapu *et al.*, 2002). *A. galanga* from North East India showed 1,8-cineole as the major compound (67.5%) and other compounds were β-sesquiphellandrene (9.4%), β-pinene (2.3%), and terpinen-4-ol (2.1%) (Dutta and Nath, 2003). The essential oil composition of *A. galanga* was reported to possess compositional differences, suggesting the existence of chemotypes in this species. The above results suggest further studies on plant cultivation and essential oil standardization are needed.

Insecticidal activities

The essential oil of *A. galanga* showed contact toxicity against *L. sericorne* adults with a LD_{50} value of 12.2 μg/adult (table 2). Compared with pyrethrins, the essential oil was 61 times less active against *L. sericorne* adults because pyrethrins displayed a LD_{50} value of 0.2 μg/adult. Compared with the other essential oils in the literature, the essential oil of *A. galanga* possessed stronger contact toxicity against *L. sericorne* than the essential oil of *Litsea cubeba* (Lour.) Pers. (LD_{50} = 27.33 μg/adult) (Yang *et al.*, 2014).

The essential oil of *A. galanga* rhizomes also possessed strong fumigant activity against *L. sericorne* with a LC_{50} value of 3.5 mg/L air (table 3). However, the currently used grain fumigant, phosphine was reported to have fumigant activity against *L. sericorne* adults with a LC_{50} value of 9.2×10^{-3} mg/L air (Yang *et al.*, 2014). Compared with the other essential oils in the previous studies, the essential oil of *A. galanga* rhizomes exhibited stronger fumigant toxicity against the cigarette beetles, e.g. essential oils of *Pistacia lentiscus* L. (LC_{50} = 8.44 mg/L air), *Elsholtzia stauntonii* Benth. (LC_{50} = 10.99 mg/L air) and *Agastache foeniculum* (Pursh) Kuntze (Lamiaceae) (LC_{50} = 21.57 mg/L air) (Bachrouch *et al.*, 2010; Lv *et al.*, 2012; Ebadollahi *et al.*, 2010).

The isolated compounds, α-terpineol and eucalyptol exhibited the same level of contact and fumigant toxicity against *L. sericorne* adults (LD_{50} = 13.3 and 15.6 μg/adult; LC_{50} = 2.8 and 5.2 mg/L air, respectively) as the essential oil, while β-pinene and α-pinene exhibited weaker contact and fumigant toxicity against *L. sericorne* adults (LD_{50} = 65.6 and 76.8 μg/adult, LC_{50} = 29.0 and 38.1 mg/L air, respectively) (tables 2 and 3). However, borneol did not show contact and fumigant toxicity at the tested concentrations. In this work, the results suggest that among five main compounds, α-terpineol and eucalyptol showed the stronger contact and fumigant

Table 4. Percentage repellency after two exposure times for the essential oil and isolated constituents against *L. serricorne* adults.

Treatment	Repellency (%)									
	2 h					4 h				
	39.32 nL/cm^2	7.86 nL/cm^2	1.57 nL/cm^2	0.31 nL/cm^2	0.06 nL/cm^2	39.32 nL/cm^2	7.86 nL/cm^2	1.57 nL/cm^2	0.31 nL/cm^2	0.06 nL/cm^2
DEET	88±7c	76±14ac	28±7bc	20±14cd	16±7c	98±4a	78±9a	58±16ab	56±14a	46±7bc
Oil	98±4a	52±15cd	16±20cd	28±15d	76±4a	96±4a	70±13a	12±27cd	8±16b	62±16ab
Eucalyptol	60±12bd	80±13ab	28±11bc	62±15a	46±12b	82±9b	60±12ab	36±12abd	50±10a	76±9a
β-Pinene	50±13d	40±14d	38±12d	30±8d	40±13bc	50±10c	64±9ab	42±9bc	24±12ab	34±15d
α-Pinene	78±4bc	70±19ac	42±11b	36±5bd	22±16c	52±19c	48±17b	48±9ab	30±16ab	10±13de
α-Terpineol	78±13bc	82±18a	72±15a	50±12ab	22±12c	80±15b	76±12a	66±16a	52±19a	30±13ce
Borneol	86±12c	58±11bcd	2±18cd	10±16c	18±16c	92±4ab	60±17ab	2±20c	-2±23b	0±24cd

Means in the same column followed by the same letters do not differ significantly ($P > 0.05$) in ANOVA and Tukey's tests. Percentage repellency was subjected to an arcsine √ transformation before ANOVA and Tukey's tests.

toxicity against *L. serricorne*, the insecticidal properties of the essential oil can be attributed to the synergistic effects of its diverse major and minor active components. In previous reports, the two active components have been demonstrated to possess insecticidal activities against several stored product insects such as maize weevils *Sitophilus zeamais* (Motschulsky), the red flour beetle *Tribolium castaneum* (Herbst), colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera Chrysomelidae), and granary weevil *Sitophilus granarius* (L.) (Coleoptera Curculinonidae) (Chu *et al.*, 2013; Liu *et al.*, 2013; Kordali *et al.*, 2007; 2006). The high volatility of these toxic compounds likely delivered fumigant toxicity by vapour action via the respiratory system, but further work is needed to confirm their extract mode of action.

Repellent activity

The repellent activities of the essential oil of *A. galanga* and isolated constituents to *L. serricorne* adults were tested using the area preference method 2 h and 4 h after treatment (table 4). Data showed that at tested concentration of 39.32 nL/cm^2, the crude essential oil showed strongest (class V) repellency against *L. serricorne* adults at 2 h and 4 h after exposure. At the lowest assayed concentration (0.06 nL/cm^2), eucalyptol showed much stronger (class IV) repellency (76%) than the positive control, DEET (class III, PR = 46%) at 4 h after exposure against *L. serricorne* adults. Among five isolated constituents, borneol showed weakest repellency against *L. serricorne* adults at 2 h and 4 h after exposure. The other three components exhibited the same level repellency against *L. serricorne* adults at 2 h and 4 h after exposure at some concentration than the positive control, DEET. In China, the essential oils derived from Chinese medicinal herbs were also evaluated for repellent activity against insects (Liu *et al.*, 2011; Liang *et al.*, 2013; Zhang *et al.*, 2011). However, only few of these reports are on repellent activity to *L. serricorne* (Yang *et al.*, 2014). In this paper, we report the repellency of the essential oil and the five components of *A. galanga* collected from Yunnan, China against *L. serricorne* specifically for the first time.

Conclusions

These findings, considered together, suggest that the essential oil of *A. galanga* and the isolated constituents show potential to be developed as natural insecticides/repellents for control of stored product insects. The oxygenated monoterpene α-terpineol and monocyclic monoterpenes eucalyptol have been considered economically important in this context. Since the natural resources of *A. galanga* are abundant, further investigations that focus on more detailed insecticidal activity studies should be conducted to elucidate the insecticidal mechanism of tested essential oils for various applications. We have no data on the cost of the essential oils, but small farmers have extensively used botanical insecticides to protect their harvest when chemical insecticides are not available or are too expensive. The effects on end-use quality, lingering off-odours or taste and risk to humans would need to be determined before commercialization.

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