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Bioactivity of essential oil of *Litsea cubeba* from China and its main compounds against two stored product insects

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ABSTRACT

During our screening program for agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *Litsea cubeba* fruits was found to possess strong contact toxicity against the cigarette beetle *Lasioderma sericorne* adults and the booklouse *Liposcelis bostrychophila*, with LD_{50} values of 27.33 μg/adult and 71.56 μg/cm^2, respectively, and also showed strong fumigant toxicity against the two stored product insects with LC_{50} values of 22.97 and 0.73 mg/L, respectively. The essential oil obtained by hydrodistillation was investigated by GC MS. The main components of the essential oil were identified to be E-citral (geranial) (27.49%), Z-citral (neral) (23.57%) and D-limonene (18.82%) followed by β-thujene (3.34%), β-pinene (2.85%), α-pinene (2.57%), 6-methyl-5-hepten-2-one (2.40%) and linalool (2.36%). Citral (Z/E-citral), D-limonene, β-pinene, α-pinene and linalool were separated and purified by silica gel column chromatography and preparative thin layer chromatography, and further identified by means of physicochemical and spectrometric analysis. Citral and linalool showed strong contact toxicity against *L. sericorne* and *L. bostrychophila* (LD_{50} = 11.76, 12.74 μg/adult and 20.15, 99.97 μg/cm^2, respectively) and fumigant toxicity against *L. sericorne* and *L. bostrychophila* (16.54, 18.04 mg/L air and 0.14, 0.71 mg/L air, respectively). Otherwise, citral, D-limonene and linalool were strongly repellent against the cigarette beetle *L. sericorne* as the essential oil whereas β-pinene and α-pinene exhibited weaker repellency against the cigarette beetle compared with the positive control, DEET. Moreover, except α-pinene and linalool, the other three compounds as well as the essential oil exhibited comparable repellency against the booklouse relative to DEET.

Introduction

The cigarette beetle *Lasioderma sericorne* (Fabricius) (Coleoptera: Anobiidae) is distributed worldwide and is of considerable economic importance in tropical to temperate climates (Ashworth, 1993). This beetle is a destructive primary insect pest of stored cereals, tobacco, oilseed, dried fruits and traditional Chinese medicinal materials (Ebadollahi et al., 2010a). Development and survival are affected by type of food, temperature and humidity, therefore the life cycle is different (Ashworth, 1993). The booklouse *Liposcelis bostrychophila* (Badonnel) (Psocoptera: Liposcelididae) is frequently found in stored product grains and traditional medicines, often in extremely high numbers, and in amylaceous products (Zhou et al., 2012). It is widely distributed in several tropical and subtropical countries in Asia, Europe, North America, South America, Africa and Australia (Turner, 1999). This insect's parthenogenic mode of reproduction coupled by its short life cycle in favorable conditions makes it particularly troublesome, as it can rapidly infest susceptible commodities (Fisher, 1985; Mills et al., 1992). In addition, they require humidity >60% and temperatures >10 °C, but <40 °C in order to survive and reproduce (Turner, 1994; Beckett and Morton, 2002).

Currently, control of stored product insect pests around the world is primarily dependent upon continued applications of liquid insecticides such as pyrethroids and gaseous insecticides such as phosphine and dichlorvos, which are still the most effective for the protection of stored food, feedstuffs and other agricultural commodities from insect infestation (Champ and Dyte, 1977; White and Leesch, 1995; Nayak et al., 2003; Hori and Kasaishi, 2005). Although effective, their repeated use for decades has disrupted natural biological control systems and led to outbreaks of stored product insect pests, development of resistance to various types of insecticides, undesirable effects on non-target organisms, and environmental and human health concerns (Champ and Dyte, 1977; Subramanyam and Hagstrum, 1995; White and Leesch, 1995).

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Moreover, the use of methyl bromide will be prohibited in the near future because of its ozone depletion potential (Anonymous, 1993). These problems have highlighted the need to develop new types of eco-friendly insect-control alternatives with fumigant action. Many plant essential oils and their components have been evaluated to possess potential to be developed as new fumigants and they may have the advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability (Isman, 2000, 2006). Most of the essential oil constituents are monoterpenoids and most monoterpenes are pleasantly aromatic. The toxicity of a large number of essential oils and their constituents has been also evaluated against a number of stored product insects (Kim and Ahn, 2001; Hori, 2003; Rajendran and Srianjini, 2008; Ebadollahi et al., 2010b; Chaubey, 2012; Jahromi et al., 2012).

Litsea cubeba (Lour.) Pers. (Lauraceae), one of the oldest herbs known and with its pleasant aroma, is distributed in southern China, Japan as well as Southeast Asian countries. Its fruits are used in pharmacy, perfumery and food. The dried fruits of *L. cubeba* are used in traditional Chinese medicine and other folk medicines, since it is considered a carminative, diuretic, expectorant, stimulant, stomachic, antiasthmatic, sedative, anti-dysenteric and antiseptic (Li et al., 1980; ChPC, 2010). The chemical composition of the essential oil of *L. cubeba* collected from various countries and areas from China has been studied (Wang and Liu, 2010; Si et al., 2012). Some variations in essential oil content and chemical composition of the essential oil were found (Wang and Liu, 2010; Yu et al., 2010). Many data indicated that the essential oil possessed antimicrobial, antibacterial, antioxidant, antiparasitic activity, acute and genetic toxicity, cytotoxicity as well as being a potential cancer prevention agent (Wang et al., 1985; Gogoi et al., 1997; Luo et al., 2005; Ho et al., 2010; Wang and Liu, 2010; Wang et al., 2012; Huang et al., 2013). The essential oil of *L. cubeba* has been demonstrated to possess insecticidal activity as well as repellency against several insects, e.g. the cabbage looper *Trichoplusia ni*, Japanese termite *Reticulitermes speratus* Kolbe, mosquito *Aedes aegypti*, maize weevil *Sitophilus zeamais*, red flour beetle *Tribolium castaneum* and nematicidal activity against pine wood nematode *Bursaphelenchus xylophilus* (Park et al., 2007; Noosidum et al., 2008; Jiang et al., 2009; Ko et al., 2009; Seo et al., 2009). However, there is no report on insecticidal activity of essential oil of *L. cubeba* fruits collected from China against the two species of stored product insects.

This research was carried out to assess the contact, fumigant toxicity and repellency of *L. cubeba* essential oil and five main components derived from it against *L. serricorne* and *L. bostrychophila*.

Materials and methods

General

^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DRX 500 instruments using $CDCl_3$ as solvent with TMS as internal standard. EIMS were determined on a ThermoQuest Trace 2000 mass spectrometer at 70 eV (probe). Silica gel (160–200 mesh) and pre-coated G plates were purchased from Qingdao Marine Chemical Plant (Shandong Province, China). Fluon was purchased from Beijing Sino-Rich Co. (China). C_8–C_{24} *n*-alkanes were purchased from Sigma-Aldrich (USA). All other unlabeled chemicals and reagents were of analytical grade. The positive control, pyrethrins (pyrethrin 1: 24%; pyrethrin 2: 13%; cinnerin 1: 2%; cinnerin 2: 2%; jasmolin 1: 1%; jasmolin 2: 1%) and DEET (*N*, *N*-diethyl-3-methylbenzamide) were purchased from Dr. Ehrenstorfer, Germany.

Plant material

Fruits (1 kg) of *L. cubeba* were purchased from Guangxi Zhuang Autonomous, (east longitude: 104°26′–112°04′; north latitude: 20°54′–26°24′), China. The fruits were air-dried for one week and ground to a powder. The voucher specimens (BNU-Dushushan-bichengqie-2011-06-05) were deposited at the Herbarium (BNU) of the College of Resources Science and Technology, Beijing Normal University.

Insects

The cigarette beetles (*L. serricorne*) and the booklice (*L. bostrychophila*) were obtained from laboratory cultures maintained in the dark in incubators at 29–30 °C and 70–80% r.h. The cigarette beetles were reared on wheat flour mixed with yeast (10:1, w/w) at 12–13% moisture content and the booklice were reared on a 1:1:1 mixture, by mass, of milk powder, active yeast and flour. Unsexed adult beetles and booklice used in all the experiments were about 1 weeks old. All containers housing insects and the Petri dishes used in the experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon).

Essential oil distillation

The ground powder of *L. cubeba* fruits was subjected to hydrodistillation using a modified Clevenger-type apparatus for 10 h and extracted with *n*-hexane. Anhydrous sodium sulfate was used to remove water after extraction. The essential oil was stored in airtight containers in a refrigerator at 4 °C. The oil yield was 2.3% v/w.

Gas chromatography and mass spectrometry

Components of the essential oil were separated and identified by gas chromatography-flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC–MS) on an Agilent 6890N gas chromatograph hooked to an Agilent 5973N mass selective detector. The same column and analysis conditions were used for both GC-FID and GC–MS. They were equipped with a flame ionization detector and a capillary column with HP-5MS (30 m × 0.25 mm × 0.25 μm). The GC settings were as follows: The initial oven temperature was held at 60 °C for 1 min and ramped at 10 °C/min to 180 °C for 1 min, and then ramped at 20 °C/min to 280 °C for 15 min. The injector temperature was maintained at 270 °C. The samples (1 μL, dilute to 1% with hexane) were injected neat, with a split ratio of 1:10. The carrier gas was helium at flow rate of 1.0 mL/min. Spectra were scanned from 20 to 550 m/z at 2 scans per second. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8–C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 (Standard Reference Data, Gaithersburg, MD, USA) and Wiley 275 libraries (Wiley, New York, NY, USA) or with mass spectra from the literature (Adams, 2001; Laribi et al., 2010; Samojlik et al., 2010). Relative percentages of the individual components of the essential oil were obtained by averaging the GC-FID peak area% reports.

Purification and identification of constituent compounds

The crude essential oil (20 mL) of *L. cubeba* was chromatographed on a silica gel column by gradient elution with *n*-hexane first, then with *n*-hexane-ethyl acetate, and last with acetone to obtain 25 fractions. Based on the contact/fumigant toxicity, fractions (2, 3, 8, 10 and 16) were further separated by preparative silica gel column chromatography (PTLC) to obtain five pure compounds for determining structure. The spectral data of citral (1.9 g) matched with the previous report (Elgendy and Khayyat, 2008). The data of D-limonene (1.6 g) matched with the previous reports (Pouchert and Behnke, 1993; Liu et al., 2012a). The spectral data were identical to the published data of β-pinene (0.8 g) (Badiah-Hadj-Ahmed et al., 1992). The spectral data of α-pinene (1.2 g) matched with the previous report (Badiah-

Hadj-Ahmed et al., 1992) and the data of linalool (1.0 g) matched with the previous reports (Bohlmann et al., 1975; Phutdhawong et al., 2007).

Contact toxicity

The contact toxicity of the essential oil/pure 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) was prepared in *n*-hexane. Aliquots of 0.5 μL of the dilutions were applied topically to the dorsal thorax of the insects. Controls were determined using *n*-hexane. Five replicates were carried out for all treatments and controls. Both treated and control insects were then transferred to glass vials (diameter 2.5 cm, height 5.5 cm, volume 25 mL) (10 insects/vial) with culture media and kept in incubators (29–30 °C and 70–80% r.h). Mortalities of insects were observed after 24 h. The observed mortality data were corrected for control mortality using Abbott's formula. The LD_{50} values were calculated by using Probit analysis (IBM SPSS V20.0) (Sakuma, 1998).

The contact toxicity of the essential oil/pure compounds against *L. bostrychophila* was tested as described by Liu (Zhou et al., 2012). A 5.5 cm diameter filter paper was treated with 300 μL of the solution of the essential oil/compounds. The filter paper after being treated with solid glue (used to paste the filter paper and Petri dish together) was placed in a 5.5 cm diameter Petri dish and 10 booklice were put on the filter paper. A cover was put and all the Petri dishes were kept in incubators. *n*-Hexane was used as a negative control. Five concentrations (in *n*-hexane) and five replicates of each concentration were used. Mortalities of insects were observed after 24 h. The observed mortality data were corrected for control mortality using Abbott's formula. The LD_{50} values were calculated by using Probit analysis (IBM SPSS V20.0) (Sakuma, 1998).

Fumigant toxicity

The fumigant activity of the essential oil and the pure 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) 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 20 s before the cap was placed tightly on the glass vial to form a sealed chamber, each of which contained 10 insects. Preliminary experiments demonstrated that 20 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 for 24 h (29–30 °C and 70–80% r.h.). The insects were then transferred to clean vials with some culture media and returned to the incubator for 24 h. Mortalities of insects were observed and results from all replicates were calculated by using Probit analysis to determine LC_{50} values (IBM SPSS V20.0) (Sakuma, 1998).

The fumigant activity of the essential oil and the pure compounds against *L. bostrychophila* adults was tested as described (Zhou et al., 2012). A filter paper strip (3.5 × 1.5 cm) was treated with 10 μL of an appropriate concentration of the test essential oil/compounds. The impregnated filter paper was then placed in the bottom cover of a 250 mL volume of glass bottle. Ten unsexed adult booklice in a small glass bottle (8 mL) were transferred to be observed conveniently into another glass bottle (250 mL) and exposed for 24 h. Five concentrations of the oil/compounds were used in the experiments and each concentration had five replicates. *n*-Hexane was used as a negative control. The observed mortality data were corrected for control mortality using Abbott's formula. The LC_{50} values were calculated by using Probit analysis (IBM SPSS V20.0) (Sakuma, 1998).

Repellency tests

The repellent effects of the essential oil and some of their individual components against *L. serricorne* and *L. bostrychophila* were assessed by using assays on Petri dishes (Chaubey, 2007). Petri dishes 9 cm in diameter were used to confine beetles during the experiment. The essential oil of *L. cubeba* and the isolated compounds were prepared in *n*-hexane (78.63, 15.73, 3.15, 0.63 and 0.13 nL/cm^2), and absolute *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 one half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 500 μL of absolute *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. Each reassembled filter paper after treatment with solid glue was placed in a Petri dish with the seam oriented in one of four different randomly selected 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

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

where N_c is the number of insects present in the negative control half and N_t is the number of insects present in the treated half.

As for the booklice, Petri dishes and filter papers were changed to 6 cm in diameter and the concentration of the oil and isolated constituents used in the experiments were 31.58, 6.32, 1.26, 0.25, 0.05 nL/cm^2. The half filter paper was treated with 150 μL of the solution. As a positive control, a commercial repellent DEET (*N*, *N*-diethyl-3-methylbenzamide), was used under the same conditions as the oil. Repellency against the insects was determined and transformed to arcsine square root values for analysis by using a general linear model (GLM) with three factors (compound, concentration and time) with interactions. Significant differences in repellence rates were detected by using LSD test (IBM SPSS V20.0, GLM).

Results and discussion

Chemical composition of the essential oil

The chemical compositions of the essential oil derived from *L. cubeba* fruits collected from Guangxi, China are shown in Table 1. The main constituents of *L. cubeba* essential oil are E-citral (geranial) (27.49%), Z-citral (neral) (23.57%) and D-limonene (18.82%) followed by β-thujene (3.34%), β-pinene (2.85%), α-pinene (2.57%), 6-methyl-5-hepten-2-one (2.40%) and linalool (2.36%). A total of 33 components were identified in the essential oil of *L. cubeba*, accounting for 98.01% of the total oil (Table 1).

The results were different from 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, fruit essential oil collected from Fujian, Jiangxi, Guizhou, Hunan, Yunnan and Sichuan Provinces contained geranial (44.4–50.0%), neral (34.2–37.4%) as its main constituents whereas D-limonene was only a minor constituent (0.7–5.3%) (Si et al., 2012). Wang and Liu (2010) reported that neral (63.75%) and limonene (7.38%) were the two main constituents followed by methyl heptenone (3.54%), camphene (3.12%), α-pinene (2.87%), and p-cymene (2.14%) in the essential oil of *L. cubeba* from southern areas of China. The essential oil collected from Nanchang, Jiangxi Province, China by Wang et al. (2009) contained limonene (26.25%), geranial (25.97%), neral (21.90%), β-pinene (6.20%), β-

Table 1
Chemical composition of the essential oil of *Litsea cubeba* fruits.

Compounds	RI[a]	Relative content (%)
α-Thujene	927	0.22
α-Pinene	931	2.57
Camphene	941	0.68
β-Thujene	967	3.34
β-Pinene	981	2.85
6-Methyl-5-hepten-2-one	996	2.40
2,3-Dehydro-1,8-cineole	1004	0.19
o-Cymene	1012	0.51
D-Limonene	1014	18.82
Eucalyptol	1017	2.07
γ-Terpinene	1047	0.24
cis-β-Terpineol	1060	0.36
Linalool Oxide	1078	0.33
Linalool	1094	2.36
trans-p-Mentha-2,8-dienol	1126	0.25
trans-Limonene oxide	1132	0.59
cis-Verbenol	1143	1.78
Citronellal	1152	0.67
Methyl-2-methyl-3-methylenecyclopentanecarboxylate	1162	0.16
4-Terpineol	1174	1.99
α-Terpineol	1181	0.67
cis-Carveol	1222	0.27
2-Isopropenyl-5-methylhex-4-enal	1231	0.97
Z-Citral	1240	23.57
(+)-Carvone	1242	0.18
trans-Geraniol	1252	0.35
Piperitone	1520	0.07
E-Citral	1560	27.49
Methyl 10,11-tetradecadienoate	1562	0.21
Geranic acid	1570	0.64
8-Hydroxycarvotanacetone	1574	0.41
Spathulenol	1578	0.17
Caryophyllene oxide	1583	0.63
Total		98.01

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

phellandrene (4.51%) and α-pinene (3.80%) as the main components of the fruit oil. However, the main components of the fruit oil from Nantou, Taiwan were found to be geranial (37.16%), neral (28.29%), D-limonene (22.90%) and β-myrcene (2.06%) (Chen et al., 2012). On the other hand, the essential oil of *L. cubeba* fruit collected in Bomi County, Tibet, China possessed limonol (44.2%), β-linalool (8.8%), 1,8-cineole (5.4%) as its main components followed by elemicin (3.9%), methyleugenol (3.8%), esteragenol (2.8%), deoxygeraniol (2.6%) (Yu et al., 2010), while neral (41.31%), geranial (30.08%), methylheptenone (5.56%) were the main compounds followed by 1,8-cineole (3.19%), limonene (2.75%), camphor (2.18%) and linalool (2.16%) in the essential oil of *L. cubeba* fruit oil from Doi Angkang, Chiang Mai Province, Thailand (Ko et al., 2009). The above findings suggest that further studies on plant cultivation and essential oil standardization are needed because chemical composition and content of constituents of the essential oil varies greatly with the plant population.

Contact and fumigant toxicity

The essential oil of *L. cubeba* showed contact toxicity against *L. serricorne* adults with a LD_{50} value of 27.33 μg/adult (Table 2). Compared with the famous botanical insecticide, pyrethrins, the essential oil was 114 times less active against *L. serricorne* adults because pyrethrins displayed a LD_{50} value of 0.24 μg/adult (Table 2). *L. cubeba* essential oil also possessed strong contact toxicity (LD_{50} = 71.56 μg/cm^2) against *L. bostrychophila* adults (Table 3). When compared with the positive control, pyrethrins, the essential oil was 4 times less active against the booklice because pyrethrins displayed a LD_{50} value of 18.72 μg/cm^2. It showed that *L. bostrychophila* was more susceptible than *L. serricorne* to the contact toxicity of the essential oil of *L. cubeba*. However, compared with the other essential oils in the literature, the essential oil of *L. cubeba* possessed stronger contact toxicity against booklice, for example, essential oil of *Curcuma wenyujin* (Zingiberaceae) (LD_{50} = 208.85 μg/cm^2), *Acorus calamus* (Araceae) (LD_{50} = 100.21 μg/cm^2), *Foeniculum vulgare* (Umbelliferae) (LD_{50} = 90.36 μg/cm^2) (Liu et al., 2012b; Zhao et a., 2012; Liu et al., 2013). Nevertheless, the essential oil showed weaker contact toxicity against booklice than the essential oil of *Lonicera japonica* (Caprifoliaceae) (LD_{50} = 64.04 μg/cm^2) (Zhou et al., 2012).

Table 2
Contact toxicity of essential oil of *Litsea cubeba* and its components against *Lasioderma serricorne* adults.

Compounds	LD_{50}[a]	95% FL[a]	Slope ± SE	χ^2 (*df*)	*P*-value
Essential oil	27.33	23.56–31.70	3.37 ± 0.49	15.87 (23)	0.861
Citral	11.76	8.34–14.50	3.61 ± 0.66	12.19 (23)	0.967
D-Limonene	13.66	11.63–16.18	3.21 ± 0.54	11.04 (23)	0.983
β-Pinene	65.55	58.13–76.09	3.75 ± 0.46	21.62 (23)	0.543
α-Pinene	76.82	68.69–86.82	5.41 ± 0.58	17.48 (23)	0.785
Linalol	12.74	11.25–14.16	4.87 ± 0.61	13.11 (23)	0.950
Pyrethrins	0.24	0.16–0.35	1.31 ± 0.20	17.36 (23)	0.791

[a] Dose (μg/adult); mean mortality of the control with *n*-hexane ≤2%.

The essential oil of *L. cubeba* fruits also possessed strong fumigant activity against *L. serricorne* with a LC_{50} value of 22.97 mg/L air (Table 4). Compared with the other essential oils in the previous studies, the essential oil of *L. cubeba* exhibited the same level of fumigant toxicity against cigarette beetles, e.g. essential oil of *Agastache foeniculum* (Labiatae) (LC_{50} = 21.565 μL/L) (Ebadollahi et al., 2010a), but less than that of the essential oil of *Ailanthus altissima* (Simaroubaceae) (LC_{50} = 3.1 μL/L) (Lv and Shi, 2010). At the same time, the essential oil of *L. cubeba* also exhibited fumigant toxicity against *L. bostrychophila* with a LC_{50} value of 0.73 mg/L air (Table 5). Compared with the positive control, dichlorvos (LC_{50} = 1.35 μg/L) from Liu et al. (2013), the essential oil showed 540 times less fumigant toxicity against booklice (Table 4). Based on the other essential oils in the literature, the essential oil of *L. cubeba* exhibited less fumigant toxicity against *L. bostrychophila*, e.g. essential oils of *A. calamus* (LC_{50} = 0.39 mg/L), *L. japonica* (LC_{50} = 0.20 mg/L), *F. vulgare* (LC_{50} = 0.03 mg/L) (Zhao et al., 2012; Zhou et al., 2012; Liu et al., 2013).

The isolated compounds, citral, D-limonene and linalool showed stronger contact toxicity against *L. serricorne* adults (LD_{50} = 11.76, 13.66 and 12.74 μg/adult, respectively) than the crude essential oil (LD_{50} = 27.33 μg/adult) (Table 2), while β-pinene and α-pinene exhibited weaker contact toxicity against *L. serricorne* adults (LD_{50} = 65.55 and 76.82 μg/adult, respectively) (Table 2). However, only citral possessed stronger contact toxicity against booklice (LD_{50} = 20.15 μg/cm^2, the same level of contact toxicity as the positive control, LD_{50} = 18.72 μg/cm^2), linalool exhibited the same level of contact toxicity against booklice (LD_{50} = 99.97 μg/cm^2) as the essential oil, while the other three isolated compounds showed weaker contact toxicity than did the essential oil of *L. cubeba* (Table 3).

Citral, D-limonene and linalool also possessed fumigant toxicity against cigarette beetles *L. serricorne* (16.54, 14.07 and 18.04 mg/L air, respectively), while the crude essential oil showed a LC_{50} value of

Table 3
Contact toxicity of essential oil of *Litsea cubeba* and its components against *Liposcelis bostrychophila*.

Compounds	LD_{50}[a]	95% FL[a]	Slope ± SE	χ^2 (*df*)	*P*-value
Essential oil	71.56	68.00–74.87	9.92 ± 1.12	17.02 (23)	0.808
Citral	20.15	18.37–22.01	5.41 ± 0.58	15.18 (23)	0.888
D-Limonene	259.62	238.13–283.68	5.56 ± 0.57	16.10 (23)	0.851
β-Pinene	397.92	353.60–459.21	3.71 ± 0.46	12.88 (23)	0.955
α-Pinene	873.73	830.73–921.29	8.27 ± 0.93	18.63 (23)	0.722
Linalol	99.97	93.92–105.89	7.92 ± 0.93	25.07 (23)	0.347
Pyrethrins	18.72	17.60–19.92	2.98 ± 0.40	10.56 (23)	0.987

[a] Concentration (μg/cm^2); mean mortality of the control with *n*-hexane ≤1%.

Table 4
Fumigant toxicity of essential oil of *Litsea cubeba* and its components against *Lasioderma serricorne*.

Compounds	LC_{50}[a]	95% FL[a]	Slope ± SE	$\chi 2$ (*df*)	*P*-value
Essential oil	22.97	18.40–27.56	2.74 ± 0.43	12.65 (23)	0.959
Citral	16.54	13.35–19.56	3.32 ± 0.53	16.10 (23)	0.851
D-Limonene	14.07	12.41–15.76	4.26 ± 0.51	22.08 (23)	0.515
β-Pinene	29.03	26.38–31.79	5.41 ± 0.58	17.48 (23)	0.785
α-Pinene	38.07	34.49–41.72	5.83 ± 0.72	20.01 (23)	0.641
Linalol	18.04	12.28–22.72	1.83 ± 0.36	16.33 (23)	0.841
Phosphine	9.23×10^{-3}	7.13×10^{-3}-11.37×10^{-3}	2.12 ± 0.27	11.96 (23)	0.971

[a] Concentration (mg/L air); mean mortality of the control with *n*-hexane ≤1%.

22.97 mg/L air (Table 4). However, β-pinene and α-pinene exhibited less toxicity than the crude essential oil against adults (Table 4). At the same time, only citral and linalool exhibited stronger fumigant toxicity against booklice *L. bostrychophila* (LC_{50} = 0.14 and 0.71 mg/L air, respectively) than the crude essential oil, while the other three isolated compounds showed weaker fumigant toxicity than the crude essential oil (Table 4). The results from our experiment compared with the result of the positive control, dichlorvos (LC_{50} = 1.35 μg/L air) from Liu et al. (2013) indicate that citral and linalool exhibited almost 100 and 526 times less toxicity to *L. bostrychophila*, respectively.

In previous reports, the five components had been demonstrated to possess insecticidal activities against several stored product insects such as lesser grain borer *Rhyzopertha dominica* (Coleoptera: Bostrychidae), maize weevil *Sitophilus oryzae* (Coleoptera: Curculionidae), red flour beetle *T. castaneum* (Coleoptera: Tenebrionidae), flat grain beetle *Cryptolestes pusillus* (Coleoptera: Laemophloeidae) (Arun et al., 2003; Lopez et al., 2008; Suthisut et al., 2011) as well as against German cockroach *Blattella germanica* (Blattaria) (Jang et al., 2005). The high volatility of these toxic mono- and sesquiterpene compounds likely delivered fumigant toxicity by vapor action via the respiratory system, but further work is needed to confirm their extract mode of action. Among the five compounds, linalool and D-limonene have been demonstrated to be potent inhibitors of acetylcholinesterase (AChE) activity from several stored product insects (Abdelgaleil et al., 2009; Lopez and Pascual-Villalobos, 2010).

Considering that the currently used fumigants are synthetic insecticides, fumigant activities of the crude essential oil and citral are quite promising and they show potential for development as possible natural fumigants for the control of stored product insects. However, for the practical application of the essential oil/compounds as novel fumigants, further studies on the safety of the essential oil/compounds to humans and on development of formulation are necessary to improve the efficacy and stability and to reduce cost.

Repellent activity of the oil and isolated constituents

The repellent activities of the essential oil of *L. cubeba* and isolated constituents to *L. serricorne* and *L. bostrychophila* adults were tested using the area preference method 2 h and 4 h after treatment (Tables 6 and 7). Moreover, significant differences in repellence rates were detected, as a function of compound (F = 8.768, df = 6, P = 0.000), concentration (F = 113.428, df = 4, P = 0.000), but no significant differences in repellence rates were detected as a function of time (F = 1.732, df = 1, P = 0.189) and their interaction (F = 0.610, df = 24, P = 0.925). At 78.63 nL/cm^2, the *L. cubeba* essential oil showed 78% and 82% repellency against *L. serricorne* adults. Its activity was decreased gradually with the decreasing of the sample concentration. Among the five constituents of the *L. cubeba* oil, citral which had the strongest insecticidal activity, produced strong repellency 2 h (84% and 94%, respectively, at 78.63 and 15.73 nL/cm^2) (Table 6) and 4 h (72% and 84%, respectively, at 78.63 and 15.73 nL/cm^2) after treatment (Table 7). At 0.63 nL/cm^2, the repellent response of *L. serricorne* adults to citral decreased significantly compared to the high concentration treatment. Data showed that at all the assayed concentrations, D-limonene and linalool showed the same level of repellency against cigarette beetles compared with the positive control, DEET. However, β-pinene and α-pinene exhibited lower level of repellency against cigarette beetles compared with the positive control, DEET. At the highest concentration (78.63 nL/cm^2), the compounds exhibited only 50 and 52%, respectively, repellency against cigarette beetles at 4 h after exposure (Table 7).

The repellent activities of the essential oil of *L. cubeba* and isolated constituents to *L. bostrychophila* adults were tested using the area preference method 2 h and 4 h after treatment (Tables 8 and 9). Moreover, significant differences in repellence rates were detected, as a function of compound (F = 44.699, df = 6, P = 0.000), concentration (F = 82.434, df = 4, P = 0.000), but no significant differences in repellence rates were detected as a function of time (F = 1.958, df = 1, P = 0.163) and their interaction (F = 1.175, df = 24, P = 0.264). At 31.58 nL/cm^2, the *L. cubeba* essential oil also showed strong repellency (84% and 78%, respectively, at 2 h and 4 h) against *L. bostrychophila* adults. Its activity was decreased gradually with the decreasing of the sample concentration. However, at 0.25 nL/cm^2, the repellent response of *L. bostrychophila* adults to *L. cubeba* essential oil decreased significantly compared to the high concentration treatment at 2 h. Among the five constituents of the *L. cubeba* oil, at all the assayed concentrations, citral exhibited the same level repellency against booklice, *L. bostrychophila*, compared with the positive control, DEET (Table 8). At 31.58 nL/cm^2, β-pinene, D-limonene and α-pinene also produced strong repellency 2 h (88%, 84% and 82%, respectively) (Table 8) and 4 h (94%, 78% and 72%, respectively) after treatment (Table 9). With the decreasing of the sample concentration, the activities of β-pinene and D-limonene were decreased gradually while α-pinene decreased rapidly at 6.32 nL/cm^2. Moreover, more adults were found on areas treated by α-pinene at the concentrations of 1.26, 0.25 and 0.05 nL/cm^2 treatment than on the control, indicating that α-pinene may attract *L. bostrychophila* adults (Tables 8 and 9). However linalool exhibited lower repellency against booklice when compared with the positive control. At the highest concentration (31.58 nL/cm^2), linalool only showed 64% and 34% at 2 h and 4 h after exposure. While with the decreasing of the concentration, the activity of linalool was decreased

Table 5
Fumigant toxicity of essential oil of *Litsea cubeba* and its components against *Liposcelis bostrychophila* adults.

Compounds	LC_{50}[a]	95% FL[a]	Slope ± SE	$\chi 2$ (*df*)	*P*-value
Essential oil	0.73	0.66–0.81	4.58 ± 0.48	19.09 (23)	0.696
Citral	0.14	0.12–0.15	5.62 ± 0.59	10.58 (23)	0.987
D-Limonene	>16.75	–	–	–	–
β-Pinene	1.36	1.30–1.41	12.14 ± 1.25	20.93 (23)	0.585
α-Pinene	1.43	1.37–1.51	9.31 ± 1.03	14.49 (23)	0.912
Linalol	0.71	0.65–0.78	5.11 ± 0.52	21.85 (23)	0.529
Dichlorvos[b]	1.35×10^{-3}	–	–	–	–

[a] Concentration (mg/L air).
[b] Data from Liu et al. (2013); mean mortality of the control with *n*-hexane ≤2%.

Table 6
Percentage repellency (PR) after 2 h for the essential oil and isolated constituents against *Lasioderma serricorne*[a,A].

Treatment	Percentage repellency ± SE (%)				
	Concentration nL/cm^2				
	78.63	15.73	3.15	0.63	0.13
Oil	76. ± 5aA	56 ± 9acAB	24 ± 12aBC	8 ± 13aCD	−2 ± 16aD
Citral	84 ± 14aAB	94 ± 7bA	56 ± 17bB	6 ± 9aC	8 ± 19abC
D-Limonene	78 ± 17aA	72 ± 15adAB	46 ± 12bcBC	32 ± 19bC	30 ± 10bcC
β-Pinene	50 ± 13bA	40 ± 14cAB	38 ± 12abAB	30 ± 8bB	40 ± 13cAB
α-Pinene	78 ± 4aA	70 ± 19adAB	42 ± 11abABC	36 ± 5bBC	22 ± 16acC
Linalol	72 ± 7abAB	78 ± 9dA	48 ± 9bcAB	38 ± 12bBC	16 ± 20acC
DEET	88 ± 7aA	76 ± 14adA	28 ± 7acB	20 ± 14abB	16 ± 7acB

a in the same column followed by different letters differ significantly ($P < 0.05$, GLM, LSD test); A in the same row followed by different letters differ significantly ($P < 0.05$, GLM, LSD test). PR was subjected to an arcsine square-root transformation before univariate analysis (GLM).

gradually. Many essential oils and their constituents have been evaluated for repellency against insects (Nerio et al., 2010; Caballero-Gallardo et al., 2011). Based on the previous reports, the essential oil of *L. cubeba* fruits have also been found to be repellent against some insects, e.g. mosquitoes *A. aegypti*, red flour beetles *T. castaneum*, maize weevils *S. zeamais* (Noosidum et al., 2008; Ko et al., 2009). In this paper, we report the repellency of the essential oil and the five components of *L. cubeba* collected from Guangxi, China against *L. serricorne* and *L. bostrychophila* specifically for the first time. These findings, considered together, suggest that the essential oil and the five compounds show potential for development as a natural repellent for stored products.

Conclusions

Based on mass screening, essential oil of *L. cubeba* fruits was examined for their insecticidal activities against cigarette beetles and booklice. The essential oil possessed strong contact and fumigant toxicity against cigarette beetles and booklice. The five isolated compounds also exhibited strong fumigant toxicities against *L. serricorne* and citral, linalool showed strong fumigant toxicities against *L. bostrychophila* as well. The essential oil and the two compounds also possessed contact toxicities against the two stored product insects. Furthermore, the essential oil as well as the five components also strongly exhibited repellence against the two stored product insects. These findings suggest that the essential oil and the five compounds show potential for development as natural fumigants and repellents for stored products.

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Table 7
Percentage repellency (PR) after 4 h for the essential oil and isolated constituents against *Lasioderma serricorne*[a,A].

Treatment	Percentage repellency ± SE (%)				
	Concentration nL/cm^2				
	78.63	15.73	3.15	0.63	0.13
Oil	82 ± 9aA	64 ± 12adAB	44 ± 12aB	16 ± 7aC	0 ± 14aC
Citral	72 ± 13acA	84 ± 13bcA	58 ± 16aA	−2 ± 20bB	4 ± 9aB
D-Limonene	74 ± 11aA	70 ± 16acdA	50 ± 13aB	36 ± 9acB	28 ± 12bcB
β-Pinene	50 ± 10bAC	64 ± 9adA	42 ± 9aAC	24 ± 12adB	34 ± 15bBC
α-Pinene	52 ± 19bcA	48 ± 17dA	48 ± 9aA	30 ± 16acAB	10 ± 13acB
Linalol	70 ± 6abA	62 ± 14adA	52 ± 5aA	48 ± 16cdAB	26 ± 15bcB
DEET	98 ± 4dA	78 ± 9acB	58 ± 15aC	56 ± 14cC	46 ± 7bC

a in the same column followed by different letters differ significantly ($P < 0.05$, GLM, LSD test); A in the same row followed by different letters differ significantly ($P < 0.05$, GLM, LSD test). PR was subjected to an arcsine square-root transformation before univariate analysis (GLM).

Table 8
Percentage repellency (PR) after 2 h for the essential oil and isolated constituents against *Liposcelis bostrychophila*[a,A].

Treatment	Percentage repellency ± SE (%)				
	Concentration nL/cm^2				
	31.58	6.32	1.26	0.25	0.05
Oil	84 ± 7adeA	64 ± 7adA	60 ± 12abeA	4 ± 13acB	4 ± 17abB
Citral	98 ± 3bA	90 ± 8bA	82 ± 8aeA	50 ± 4bdB	12 ± 14aC
D-Limonene	84 ± 7adeA	70 ± 6abdA	30 ± 10bdB	16 ± 10acB	12 ± 12aB
β-Pinene	88 ± 8abdA	60 ± 14adBC	56 ± 16abCD	30 ± 15abDE	26 ± 11acE
α-Pinene	82 ± 5acdA	10 ± 16cB	−6 ± 10cBC	−6 ± 17cBC	−20 ± 13bC
Linalol	64 ± 3cA	46 ± 13aAB	24 ± 19dBC	14 ± 14acBC	10 ± 19aC
DEET	94 ± 6beA	82 ± 5bdAB	86 ± 8eAB	70 ± 12dBC	56 ± 3cC

a in the same column followed by different letters differ significantly ($P < 0.05$, GLM, LSD test); A in the same row followed by different letters differ significantly ($P < 0.05$, GLM, LSD test). PR was subjected to an arcsine square-root transformation before univariate analysis (GLM).

Table 9
Percentage repellency (PR) after 4 h for the essential oil and isolated constituents against *Liposcelis bostrychophila*[a,A].

Treatment	Percentage repellency ± SE (%)				
	Concentration nL/cm^2				
	31.58	6.32	1.26	0.25	0.05
Oil	78 ± 3aA	72 ± 12aA	50 ± 12abAB	28 ± 8abcB	26 ± 15aB
Citral	96 ± 6bA	82 ± 9aAB	76 ± 7beB	54 ± 7bcB	22 ± 16aC
D-Limonene	78 ± 11aA	64 ± 7aA	14 ± 10cB	22 ± 8abcB	18 ± 12aB
β-Pinene	94 ± 8abA	56 ± 6aAB	32 ± 12acBC	18 ± 15aCD	12 ± 10aD
α-Pinene	72 ± 14aA	8 ± 10bB	−22 ± 14dC	10 ± 6aB	−30 ± 12bC
Linalol	34 ± 14cA	20 ± 14bA	42 ± 18aA	24 ± 14aA	12 ± 8aA
DEET	92 ± 5abA	84 ± 3aA	82 ± 8eA	54 ± 17cB	28 ± 14aC

a in the same column followed by different letters differ significantly ($P < 0.05$, GLM, LSD test); A in the same row followed by different letters differ significantly ($P < 0.05$, GLM, LSD test). PR was subjected to an arcsine square-root transformation before univariate analysis (GLM).

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