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Insecticidal activities of constituents of *Litsea cubeba* fruit extracts effective against the maize weevil (Coleoptera: Curculionidae)

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Abstract

In this study, we investigated the insecticidal activities, including contact toxicity, fumigant toxicity, and repellent activity, of *Litsea cubeba* fruit extracts against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). The extracts, obtained by liquid–liquid extraction in *n*-hexane, ethyl acetate, chloroform, and water were analyzed by gas chromatography–mass spectrometry. Among the different extract types, chloroform extracts exhibited the strongest repellent, contact, and fumigant activities against *S. zeamais*. The main components of the chloroform extracts were identified as laurine (21.15%) and 2,6-diisopropyl aniline (16.14%), followed by chlorobutanol (10.54%), 3-*O*-methyl-*N*-acetyl-d-glucosamine (10.03%), and 6-methyl-5-hepten-2-one (8.33%). Among the identified components of the chloroform extracts, chlorobutanol showed the strongest fumigant toxicity (LD_{50} = 21.91 mg/liter), contact toxicity (LD_{50} = 54.25 µg/adult), and repellent activity against *S. zeamais*. These results indicate that *L. cubeba* fruit extracts possess natural insecticide-like activities against *S. zeamais*.

Key words: extract, insecticidal activity, component identification, botanical pesticide

The maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), which is considered a destructive insect pest in subtropical and tropical regions (Lopez et al. 2008), attacks many types of stored products, including maize, rice, wheat, and cottonseed. *S. zeamais* is an internal feeder. The females lay eggs directly into the kernel of grains, and although more than one egg may be laid in a single grain, only one larva generally develops to maturity, as a consequence of cannibalism (Longstaff 1981). The larvae hatch and develop inside the kernel until they reach the adult stage (Hill 2002, Lopez et al. 2008). Upon reaching the adult stage, they are capable of flight, and rapidly infest stored grain (Hill 2002). Recent research on the behavior of the congeneric rice weevil *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae) has shown that male mating success and laterality are affected by geographical origin and the rearing substrate, and that *S. oryzae* males show population-level left-biased copulatory approaches to potential mates (Romano et al. 2016, Benelli et al. 2017).

S. zeamais not only attacks intact grain but also causes an increase in the temperature and moisture of stored grain (Magan et al. 2003). Currently, the control of stored insect pests is primarily dependent on synthetic insecticides such as phosphine. However, long-term, excessive, and non-standard use of phosphine has caused serious '3R' problems: resistance of the pest to phosphine; resurgence of the pest; and residual phosphine in stored grain (Nayak et al. 2003, Hori and Kasaishi 2005, Isman 2006). In an effort to solve the resistance problem, several new insecticides, including

diatomaceous earth (DE) and chlorfenapyr, have been experimentally assessed and practically applied. DEs comprise fossilized phytoplankton that can abrade and absorb the exterior protective waxy cuticle layer of insects, causing death through dehydration (Korunic 1998). Previous studies have indicated that when combined with other insecticides, such as botanicals and entomopathogenic fungi, low doses of DE can be effective against several insects (Athanassiou et al. 2007, Kavallieratos et al. 2015). Chlorfenapyr, which causes disruption of mitochondrial ATP synthesis (Hunt 1996), has been registered in the United States for use against agricultural pests and has also been evaluated with success against several stored-product insect pests (Kavallieratos et al. 2011). Other eco-friendly insecticides are botanical pesticides, which are synthesized by plants or produced from extracts of plants with insecticidal activity. These biopesticides have considerable advantages over synthetic insecticides in terms of their high selectivity, low mammalian toxicity, rapid degradation, and environmental friendliness. Moreover, botanical pesticides are produced by plants that have undergone long-term co-evolution with pests, and possess diverse insecticidal activities. Accordingly, it is more difficult for pests to develop resistance to these natural products (Isman 2000, Isman and Akhtar 2007). Not surprisingly, therefore, the prospect of using botanical pesticides as alternatives to controversial traditional pesticides has gained increasing interest (White and Leesch 1995, Nukenine et al. 2010, 2011, Huang et al. 2011, Rani et al. 2011).

Litsea cubeba (Lour.) Pers., a member of the Lauraceae plant family, is mainly distributed in the south of China and Southwestern Asia (ChPC 2010, Zhang et al. 2014) and is one of the most popular herbs because of its wide-ranging properties, including stomachic, decongestant, antimicrobial, carminative, and antiseptic activities (Gogoi et al. 1997, Ho et al. 2010, Huang et al. 2013). The fruits of *L. cubeba* are variously used in food, traditional Chinese medicine, and perfumery. Many studies have shown that the essential oil of *L. cubeba* fruit not only possesses antioxidant, antimicrobial, and cytotoxic properties, but also shows repellency and contact toxicity against certain pest insects, including *S. zeamais* (Ko et al. 2009), red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Ko et al. 2009), cabbage looper *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) (Jiang et al. 2009), pine wood nematode *Bursaphelenchus xylophilus* (Park et al. 2007), cigarette beetle *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) (Yang et al. 2014), and booklouse *Liposcelis bostrychophila* (Yang et al. 2014).

Most previous studies on the pesticide potential of *L. cubeba* have focused on the activity of its essential oils, and there has been relatively little attention devoted to the efficacy of other extracts of this plant. Accordingly, in the present study, we aimed to investigate the insecticidal activity of different fractions of 95% ethanol extracts of *L. cubeba* fruit separated by liquid–liquid extraction. We examined the bioactivity of extracts in *n*-hexane, ethyl acetate, chloroform, and water against *S. zeamais*, and also assessed the repellent, fumigant, and contact toxicities of the four main components derived from chloroform extracts against this insect pest.

Materials and Methods

General Considerations

For the analysis of *L. cubeba* extracts, we used a capillary gas chromatography–mass spectrometry (GC–MS) instrument (HP6890/5973MSD), Ethanol (95%), *n*-hexane, ethyl acetate, chloroform, laurine, 2,6-diisopropyl aniline, chlorobutanol, and 6-methyl-5-hepten-2-one. Pyrethrins were purchased from Dr Ehrenstorfer, Germany. All chemicals and reagents used were of analytical grade.

Plant Material

L. cubeba fruits from Guangxi Zhuang Autonomous Region, China, were purchased from a supermarket in Hainan, China. The *L. cubeba* fruits were air-dried for 2 wk, ground to a powder, and sifted through a 40-mesh sieve.

Insects

Adults of *S. zeamais* were originally captured from the Hainan Dalu Grain Store (China), and identified as described by Boudreaux (1969) and Halstead (1963). The insects have been maintained in the insectarium of Hainan Institute of Grain and Oil Science for 4 yr, and were reared on wheat (12–14% moisture content) in incubators at 27–29°C and 70–80% relative humidity (RH) and in continuous darkness. The adults were cultured for oviposition and then sieved out after 7 d. Second-generation unsexed adults (<2 wk old) were used for research (Li et al. 2013).

Crude Extract Preparation

The ground powder of *L. cubeba* fruits was extracted with 95% ethanol in a ratio of 1:5 (w/v) for 48 h at room temperature and then filtered. The filtrate was dried using a vacuum rotary evaporator at

80°C. The crude extract was stored in air-tight brown glass bottles at 4°C.

Separation of Crude Extracts

Aliquots (10.00 g) of crude extract were weighted accurately. To these, distilled water was added in a ratio of 1:10 (w/v), followed by ultrasonication for 10 min under 99% power at 30°C to promote dissolution. The resultant mixture was then filtered and the filter residue (RE) was used as the sample. The filtrate (aqueous phase) was extracted three times with 100 ml *n*-hexane (polarity, 0.06; non-polar) in a separating funnel, shaking for 3 min, and then left to stand for 10 min. The *n*-hexane phase was subsequently separated from the aqueous phase and then dried using a vacuum rotary evaporator at 80°C to yield the *n*-hexane extract (NHE). Using the same procedure as described earlier, the aqueous phase was sequentially extracted with chloroform (CHE: polarity, 4.4; weakly polar) and then ethyl acetate (EAE: polarity, 4.3; weakly polar). The remaining aqueous phase sample was then evaporated in a vacuum rotary evaporator at 95°C to obtain the water extract (WAE).

The extraction rate (ER, in %) was calculated from the quality of the separated extracts.

$$ER = \frac{EQ}{CE} \times 100\%,$$

where EQ is the quality of extracts and CE is the quality of the crude extract.

Contact Toxicity

The contact toxicity of *L. cubeba* fruit extracts against *S. zeamais* was assessed as described by Liu and Ho (1999) with some modifications. All extracts were diluted to 0.5 g/ml with acetone. *S. zeamais* adults were immobilized using a homemade negative pressure pipette. Aliquots (0.5 µl) of the dilutions were applied to the dorsal thorax of *S. zeamais* individuals, using a BS00279 Burkard microapplicator (syringe volume 1 ml, needle no. 3/10 × 25 mm). Controls were treated similarly using acetone. Thirty insects were treated with each of the extracts (or the control), and the experiment was replicated four times. The treated and control insects were placed in glass vials (volume 25 ml; 10 insects/vial) containing culture medium and maintained in incubators at 27–29°C and 70–80% RH. *S. zeamais* mortality was recorded after 24 h. Test insects were considered dead if their appendages did not move when prodded with a pin. The observed mortality data were corrected for control mortality using Abbott's formula.

Following the same procedure, serial dilutions of pyrethrin and the pure compounds derived from extracts (at five concentrations: 30, 60, 120, 240, and 480 mg/ml) were prepared in acetone. The concentrations of the pure compounds corresponded to doses of 15, 30, 60, 120, and 240 µg/adult, respectively.

Fumigant Toxicity

The fumigant toxicity of extracts of *L. cubeba* fruits against *S. zeamais* was assessed according to Liu and Ho (1999), with some modifications. All extracts were diluted to 0.25 g/ml with acetone. Aliquots (10 µl) of the dilutions were applied to filter papers (diameter 2 cm), which were then placed on the undersides of the screw caps of glass vials (diameter 2.5 cm, height 5.5 cm, volume 25 ml). The solvent was allowed to evaporate for 20 s, after which 10 insects were placed into the glass vial and the cap screwed tightly. The insects were incubated for 24 h (27–29°C and 70–80% RH), and

then transferred to clean vials containing the same culture medium for 24 h. Acetone was used as a control. Four replicates were carried out for all treatments and controls. Test insects were considered dead if their appendages did not move when prodded with a pin. Insect mortalities were recorded and the observed mortality data were corrected for control mortality using Abbott's formula.

Following the same procedure, serial dilutions of the pure compounds derived from extracts (at five concentrations: 25, 62.5, 100, 137.5, and 175 mg/mL) were prepared in acetone. The concentrations of the pure compounds corresponded to doses of 10, 25, 40, 55, 70 mg/liter, respectively. The toxicity of phosphine against *S. zeamais* was assessed according to Anon (1975).

Repellency Test

The repellency test of *L. cubeba* fruit extracts against *S. zeamais* was performed according to Chabey (2007), with some modifications. All extracts were diluted to 0.5 g/ml with acetone. Petri dishes (diameter 9.0 cm) were washed and dried. Filter paper disks (diameter 9 cm) were cut in half, and to one half, 0.5-ml aliquots of extract dilutions were applied, equivalent to 7.86 mg/cm^2. The other half of the filter paper (control) was treated with 0.5 ml of acetone. Solvents in the filter paper were allowed to evaporate for 20 s, after which a full filter paper was remade by attaching the control half to the treatment half with adhesive tape and then placed in a Petri dish (diameter 9.0 cm) with the same orientation in replicates in order to avoid the effects of any external directional stimulus. Twenty insects were released on the center of each filter paper disk, and a cover was placed over the Petri dishes, which were then maintained under darkness. Treatment with each extract was replicated four times. After 4 h, the number of insects present on each half of the filter paper disk was counted in mild light, and the percentage repellency (PR, in %) of each extract was calculated using the following formula:

$$PR = \frac{N_c - N_t}{N_C + N_t} \times 100$$

where N_c is the number of insects present on the control half of the disk and N_t is the number of insects present on the treated half.

Following the same procedure, serial dilutions of the pure compounds derived from extracts (at four concentrations: 500.0, 166.6, 55.3, and 18.4 mg/ml) were prepared in acetone. The concentrations of the pure compounds corresponded to doses of 7.86, 2.62, 0.87, and 0.29 mg/cm^2, respectively. After completion of the above processing, the insects that settled on each half of the filter paper disk were counted and the PR of each pure compound was calculated.

Gas Chromatography and Mass Spectrometry

A certain amount of the CHE was diluted with chloroform, and directly analyzed by GC–MS. The gas chromatograph was equipped with an HP-INNOWax fused-silica capillary column (30 m × 0.25 mm; 0.25 µm). The oven temperature was held at 80°C, ramped at 10°C min^{-1} to 150°C, and then programmed at 6°C min^{-1} to 250°C for 10 min. The carrier gas was helium (1.0 ml/min, split ratio

1:10), and the detector and injector temperatures were both 280°C. One-microliter sample was injected rapidly and evenly into the chromatograph. Mass spectrometry was performed at 70 eV, and the mass range was *m/z* 10–500 Aum. The ion source temperature was held at 230°C. The compounds were identified by their mass spectra based on reference to spectra stored in an MS database (NIST2005).

Statistical Analyses

The contact, fumigant, and repellent activities and extraction rates for crude extracts were analyzed using ANOVA procedures, and means were compared using Tukey's test (SPSS 17.0), at a significance level of α = 0.05. LD$_{50}$ values of the pure compounds were calculated using Probit analysis (IBM SPSS 17.0).

Results and Discussion

The Separation of Crude Extracts

The extraction rates of the crude extracts in different solvents are shown in Table 1. Significant differences in extraction rate were detected as a function of solvent ($F_{(4,15)}$ = 338.810, df = 4, $P < 0.001$). Among the various extracts we prepared, the extraction rate was highest for RE at 46.94%, followed by that for WAE and CHE at 28.5 and 19.85%, respectively. The extraction rates for both NHE and EAE were considerably lower (below 3%).

The Bioactivity of Extracts in Different Solvents

On testing the bioactivity of extracts in different solvents against *S. zeamais* (Table 2), we detected significant differences in control-corrected mean contact mortality (*F*-test: $F_{(4,15)}$ = 238.618, df = 4, $P < 0.001$), PR ($F_{(4,15)}$ = 89.286, df = 4, $P < 0.001$), and control-corrected mean fumigant mortality ($F_{(4,15)}$ = 56.791, df = 4, $P < 0.001$), as a function of extract. The control-corrected mean contact mortality caused by CHE against *S. zeamais* was 75.98%, which was significantly higher than that of extracts in the other solvents. CHE also had the highest PR at 81.26%, followed by RE at only 28.85%. On the basis of differences in PR, the repellency of extracts was divided into six grades (Malik 1984). The repellency of CHE reached the highest level (level V), whereas the repellency of RE was level II, and that of the others was only level I. The fumigant toxicity of the five extracts was ordered as follows: CHE > RE > EAE > WAE > NHE. The fumigant toxicity of CHE was significantly higher than that of the others. The polarities of *n*-hexane, chloroform, ethyl acetate, and water were 0.06, 4.4, 4.3, and 10.2, respectively. According to the principle of similar compatibility, it can be concluded that the insecticidal components of these extracts were mostly weakly polar, which is consistent with the findings of Li et al. (2013) and Sun et al. (2007). Although chloroform and ethyl acetate are both weakly polar, the bioactivity of CHE was notably stronger than that of EAE. This difference might be explained by the fact that CHE enters the tissues and organs of trial insects more readily and affects the activities of various detoxifying enzymes (Matthews et al. 2010, Xiong et al. 2010).

Table 1. The ERs for crude extracts in different solvents

Types of extract	RE	NHE	CHE	EAE	WAE
ER (%)	46.94 ± 2.22a	2.85 ± 0.14d	19.85 ± 0.71c	2.64 ± 0.16d	28.50 ± 1.56b

Means ± SE followed by a different letter differ significantly, based on Tukey's test at the 5% level of significance. $F_{(4,15)}$ = 338.810, df = 4, $P < 0.001$.
RE (crude extract residue).

Table 2. The bioactivity of extracts in different solvents against *S. zeamais*

Types of bioactivity	Types of extract					$F_{(4,15)}$
	RE	NHE	CHE	EAE	WAE	
CMC (%)	24.53 ± 0.91b	10.65 ± 1.02d	75.98 ± 2.37a	12.9 ± 2.54cd	19.3 ± 2.59bc	238.618***
PR (%)	28.85 ± 1.95b	8.47 ± 2.67d	81.26 ± 1.49a	12.0 ± 0.72cd	15.20 ± 1.96c	89.286***
CMF (%)	35.78 ± 1.23b	14.40 ± 1.27d	58.48 ± 2.12a	20.02 ± 0.48c	17.3 ± 2.30cd	56.971***

CMC, PR, and CMF represent control-corrected mean contact mortality, percentage repellency, and control-corrected mean fumigant mortality, respectively.
Means ± SE followed by a different letter in the same row differ significantly, based on Tukey's test at the 5% level of significance.
***P < 0.001.

Table 3. Chemical composition of the CHE

Compounds	RT (min)	Composition (%)
Epoxy-linalool oxide	11.35	1.16
3-Hexanone,2,5-dimethyl-4-nitro	12.34	1.10
Phenol, 3-methyl-	12.87	1.14
Cyclobutaneethanol,1-methyl-2-(1-methylethenyl)-, (1*R-cis*)-	13.75	2.75
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy	14.73	3.56
2(1H)-Pyrimidinone, 4-amino-	15.47	1.88
6-Methyl-5-hepten-2-one	18.22	8.33
Laurine	18.83	21.15
Cyclohexanone, 2-ethyl-	19.79	1.65
Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester	20.17	2.88
2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol	20.52	1.00
3-*O*-Methyl-*N*-acetyl-d-glucosamine	21.40	10.03
Chlorobutanol	21.78	10.54
3,4-Dimethyl-3-pyrrolin-2-one	22.11	1.35
1H-Pyrazole, 1-ethyl-3,5-dimethyl-	23.28	0.66
2-Hexadecanol	24.04	3.31
2-Pyrazoline, 1,3,4-trimethyl-	24.56	5.93
2,6-Diisopropylaniline	26.95	16.14
Oleic acid	27.83	3.55
9,12-Octadecadienoic acid (*Z,Z*)-	28.75	1.86
Total		99.97

RT (retention time).

In conclusion, among the different solvent extracts examined in the present study, CHE showed the strongest bioactivity against *S. zeamais*.

Chemical Composition of CHE

Twenty chemical components were identified in CHE, most of which were ketones, aldehydes, and alcohols (Table 3). The main components in CHE were laurine (21.5%), 2,6-diisopropylaniline (16.14%), chlorobutanol (10.54%), 3-O-methyl-*N*-acetyl-d-glucosamine (10.03%), 6-methyl-5-hepten-2-one (8.33%), and 1,3,4-trimethyl-2-pyrazoline (5.93%). These percentages differ considerably from those reported previously for *L. cubeba* extracts, which might be attributable to different methods of extraction. For example, Kai Yang (2014), using hydrodistillation, reported that the main components of *L. cubeba* fruits were *E*-citral (27.49%), *Z*-citral (23.57%), and d-limonene (18.82%), whereas Si et al. (2012) reported that fruit extract contained mainly geranial (44.4–50.0%) and neral (34.2–37.4%).

Contact Toxicity, Fumigant Toxicity, and Repellent Activity of CHE Components

The components of CHE showed bioactivity against *S. zeamais* adults (Tables 4–6). The contact toxicity regression equations of the pure compounds are shown in Table 4. The chi-square test results (df = 18; χ^2 = 7.75–10.74; P = 0.905–0.981) indicated that the contact toxicity regression equations of the pure compounds fitted well. Chlorobutanol showed the strongest contact toxicity against *S. zeamais* adults, with an LD_{50} value of 54.25 µg/adult, whereas 2,6-diisopropylaniline, laurine, and 6-methyl-5-hepten-2-one had LD_{50} values of 107.55, 219.12, and 224.69 µg/adult, respectively. When compared with other extracts reported in the literature [e.g., extracts of *Artemisia lavandulaefolia* (LD_{50} = 55.2 µg/adult), *Illicium simonsii* fruits (LD_{50} = 112.7 µg/adult), and *Artemisia sieversiana* (LD_{50} = 113.0 µg/adult) (Chu et al. 2010, Liu et al. 2010)], chlorobutanol also exhibited greater contact toxicity against *S. zeamais*. However, the activity of chlorobutanol was 2.47 times lower when compared with commercial pyrethrin insecticides (LD_{50} = 21.91 µg/adult).

The fumigant toxicity regression equations of the pure compounds are shown in Table 5. The chi-square test results (df = 18; χ^2 = 7.75–8.23; P = 0.970–0.980) indicated that the fumigant toxicity regression equations of the pure compounds fitted well. The compounds in CHE exhibiting fumigant toxicity against *S. zeamais* adults were as follows: chlorobutanol (LD_{50} = 21.91 mg/liter), 2,6-diisopropylaniline (LD_{50} = 32.26 mg/liter), laurine (LD_{50} = 43.83 mg/liter), and 6-methyl-5-hepten-2-one (LD_{50} = 57.57 mg/liter). However, when compared with other extracts previously reported in the literature [e.g., extracts of *A. sieversiana* (LD_{50} = 15.00 mg/liter) (Liu et al. 2010), *Schizonepeta multifida* (LD_{50} = 8.89 mg/liter) (Liu et al. 2011), *Kadsura heteroclita* (LD_{50} = 14.04 mg/liter) (Li et al. 2011), and *Murraya exotica* (LD_{50} = 8.29 mg/liter) (Li et al. 2010)], chlorobutanol exhibited less fumigant toxicity against *S. zeamais*. The reason why chlorobutanol exhibited less fumigant toxicity than that in the above-mentioned extracts might be attributable to the resistance of the trial *S. zeamais*. In our research, phosphine exhibited fumigant toxicity against the trial *S. zeamais* adults with an LD_{50} = 0.017 mg/liter, whereas Liu et al. 2010 reported that phosphine exhibited fumigant toxicity against trial *S. zeamais* adults with an LD_{50} = 0.010 mg/liter.

The results of the repellency test against *S. zeamais* adults using the components of CHE are presented in Table 6. All concentrations of the components in CHE generally caused a significant increase in the PR against *S. zeamais*, which was dose dependent (df = 3; $F_{(3,12)}$ = 8.075–31.427; P < 0.01). When the concentration was >0.29 mg/cm², significant differences in PR were detected as a function of compound (df = 3; $F_{(3,12)}$ = 6.048–7.982; P < 0.01). Among the components examined, the repellent toxicities of chlorobutanol, and 2,6-diisopropylaniline (with percentage repellencies of 84.77 and 81.87%, respectively) were higher than those of the other compounds at 7.86 mg/cm². At 0.29 mg/cm², all the pure compounds

Table 4. Contact toxicity of CHE components against *S. zeamais* adults

Pure compounds	LD_{50}[a]	Slope ± SE	95% FL[a]	χ^2 (df)	*P*-value
2,6-Diisopropylaniline	107.55	3.86 ± 0.80	95.98–125.76	7.75 (18)	0.970
Laurine	219.12	8.447 ± 1.75	208.00–235.46	7.96 (18)	0.967
Chlorobutanol	54.25	3.76 ± 0.76	48.41–63.03	7.92 (18)	0.980
6-Methyl-5-hepten-2-one	224.69	7.65 ± 1.66	212.49–244.40	10.74 (18)	0.905
Pyrethrins	21.91	2.92 ± 0.59	18.92–26.58	7.85 (18)	0.981

The number of treated insects per compound was 720. LD_{50} values of the pure compounds were calculated using Probit analysis.
[a]Dose (µg/adult); mean mortality of the control with acetone <1.5%.

Table 5. Fumigant toxicity of components of CHE against *S. zeamais* adults

Pure compounds	LD_{50}[a]	Slope ± SE	95% FL[a]	χ^2 (df)	*P*-value
2,6-Diisopropylaniline	32.26	4.58 ± 0.31	29.39–36.50	7.75 (18)	0.97
Laurine	43.83	2.92 ± 0.59	37.84–53.16	7.84 (18)	0.981
Chlorobutanol	21.91	3.76 ± 0.76	18.92–26.58	7.84 (18)	0.981
6-Methyl-5-hepten-2-one	57.57	8.55 ± 1.74	54.76–61.52	8.23 (18)	0.975
Phosphine	1.7×10^{-2}	2.00 ± 0.41	1.3×10^{-2}–2.2×10^{-2}	7.88 (18)	0.980

The number of treated insects per compound was 240. LD_{50} values of the pure compounds were calculated using Probit analysis.
[a]Concentration (mg/liter air); mean mortality of the control with acetone <1.5%.

Table 6. PR of components of CHE against *S. zeamais* adults

Pure compounds	Percentage repellency ± SE (%)				$F_{(3,12)}$
	Concentration mg/cm²				
	7.86	2.62	0.87	0.29	
2,6-Diisopropy-laniline	81.87 ± 7.58aA	62.10 ± 12.11bA	37.37 ± 11.14cAB	16.96 ± 8.89dA	23.684[***]
Laurine	67.87 ± 8.46aAB	63.13 ± 8.66aA	25.63 ± 5.52bBC	11.38 ± 10.89bA	31.427[***]
Chlorobutanol	84.77 ± 10.43aA	66.19 ± 3.90bA	42.32 ± 5.87cA	26.97 ± 13.51cA	22.973[***]
6-Methyl-5-hepten-2-one	49.25 ± 14.11aB	33.50 ± 10.62abB	15.89 ± 9.56bcC	2.95 ± 14.34dA	8.075[**]
$F_{(3,12)}$	7.234*	7.982*	6.048*	2.080[ns]	

Values in the same row followed by different lower-case letters differ significantly, based on Tukey's test at the 5% level of significance; values in the same column followed by different upper-case letters differ significantly, based on Tukey's test at the 5% level of significance.
[ns]*P* > 0.05; *P < 0.05; [**]*P* < 0.01; [***]*P* < 0.001.

exhibited lower PR compared with treatments using other concentrations, although no significant differences were detected as a function of compound (df = 3; $F_{(3,12)}$ = 2.080; *P* > 0.05).

Conclusion

The main components in 95% ethanol extracts of *L. cubeba* fruits were water insoluble, water extractable, and CHE extractable, and to a lesser extent ethyl acetate, and *n*-hexane extractable. Among these various extracts, CHE showed the highest bioactivity against *S. zeamais*. Twenty components were identified in CHE, the main ones of which were laurine, 2,6-diisopropylaniline, chlorobutanol, and 6-methyl-5-hepten-2-one. All these compounds exhibited contact toxicity, fumigant toxicity, and repellent activity against *S. zeamais*, with chlorobutanol showing the overall strongest bioactivity. All these four compounds have potential for development as natural insecticides.

Acknowledgments

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