

NOTE

Composition and Insecticidal Activity of *Elsholtzia kachinensis* Prain, a Traditional Vegetable and Herbal Medicine

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Abstract: In recent years, secondary metabolites of plants have attracted researchers' interest as a substitute for synthetic insecticides with many advantages. *Elsholtzia kachinensis* is an annual herb with medicinal and edible value. In this study, the essential oil (EO) of the aerial part of *E. kachinensis* was extracted by hydrodistillation, and GC-MS analysed essential oil components. The results show that carvone and dehydroelsholtzia ketone are the main components of the essential oil, accounting for 32.298% and 31.540%, respectively. EO, carvone and dehydroelsholtzia ketone are used to determine the effects against stored-product insects *Lasioderma serricorne*, *Tribolium castaneum*, *Sitophilus oryzae* and *Liposcelis bostrychophila*. The essential oil showed the most vital contact and fumigation toxicity to *L. serricorne*, of which LD₅₀ and LC₅₀ values were 3.85 µg/adult and 7.74 mg/L air, respectively. *S. oryzae* did not show repellent activity, but the repellent rate of the other three species reached 90% under EO treatment at a concentration of 78.63 nL/cm². Therefore, the essential oil of *E. kachinensis* has an insecticidal effect and has the potential to be developed as a new eco-friendly insecticide.

Key words: *Elsholtzia kachinensis*, contact toxicity, fumigant toxicity, repellency, stored-product insects

1 Introduction

Demand for food has been increasing with the world's population continually growing. According to the Food and Agriculture Organization of the United Nations, cereals production in 2019 increased by 20% compared to 2010¹⁾. According to reports, various insects have spread worldwide, harming stored products. In 2018, about 400,000 tons of insecticides were used globally, accounting for about 10% of all pesticides used²⁾. Four typical storage insects are the cigarette beetle *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae), the red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), the rice weevil, *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae) and the booklice *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae). They damage tobacco, crops, books and other stored product by producing primary or secondary hazards^{3,4)}.

In the past few decades, synthetic insecticides have been widely used in insect control. However, the environmental concerns brought by synthetic insecticides and their effects on human health are also gradually emerging⁵⁾. Therefore, researchers have to find new biomaterials to replace synthetic insecticides to alleviate the abovementioned problems. Essential oils are the secondary metabolites secreted by aromatic plants, mainly composed of aromatic components and give a distinctive odour, flavour or scent⁶⁾. Due to the pleasant aroma and pharmacological effects of EOs, they have been favoured by consumers in recent years⁷⁾. In developing countries, farmers often use plant materials or extract to prevent pests for a long time⁸⁾, but few are used in commercial applications. Therefore, following the rapid development of EO, exploring the role of EOs in insecticides is a powerful means to promote the progress of this industry. Several studies have attempted to

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find the relationship between EOs and storage pests and described lethality and sublethal effects through modes of action^{5, 9)}. At the same time, researchers have also indicated that EOs has little impact on non-target species and the human body. Thereout, it is a consensus of scholars to consider EOs as a new environment-friendly insecticide.

Among the plants with significant insecticidal activity recorded at present, plants from Lamiaceae accounts for two sevenths, among which the genus *Elsholtzia* is distributed in East Asia, Africa, North America and Europe¹⁰⁾, often used in vegetables and herbs^{11, 12)}. In traditional Chinese medicine, *E. kachinensis*, a species in *Elsholtzia*, is used to treat fever, headache, diarrhea, edema and other diseases¹³⁾. A recent study indicated that *E. kachinensis* could be antibacterial and inhibit the activity of inflammatory factor NO¹⁴⁾. Although about 20 kinds of EO from *Elsholtzia* have been determined for their chemical composition, only a few species, like *E. ciliate*, *E. cypriani*, *E. winitiana*, or *E. stauntoni*, have been tested in insecticidal activities trials^{15–21)}. So evaluating the insecticidal activities of *E. kachinensis* may provide valuable information about EOs from *Elsholtzia* spp.

Our work aims to determine the composition of EO of *E. kachinensis* and the insecticidal activity against four species of insects mentioned above, including fumigant and contact toxicity and repellent activity.

2 Materials and Methods

2.1 Plant materials and chemicals

Fresh aerial parts from *E. kachinensis* weighing 3 kg were collected from Yunnan Province, China, in November 2020. The species was identified by Dr. Q.R., Liu (College of Life Sciences, Beijing Normal University, Beijing, China), and a voucher specimen (BNU-dushushan-2020-11-14-001) was deposited in the Herbarium of the College of Resources Science and Technology, Faculty of Geographical Science, Beijing Normal University (Beijing, China).

Carvone was bought from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. Dehydroelsholtzia ketone was obtained from EO of *E. rugulosa*, which contained 95.88% of dehydroelsholtzia ketone. The extraction method of EO was presented in 2.2.

2.2 Extraction of essential oil

After air-drying in shade at room temperature, the plant material was grounded into powder and extracted by hydrodistillation for essential oil. The dehydration was done by anhydrous sodium sulfate. 2.2 mL essential oil was finally obtained, and it was stored in a sealed glass container at 4°C in the refrigerator.

2.3 GC-MS and GC-FID analysis

Gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionisation detection (GC-FID) were performed on an Agilent 6890N gas chromatograph coupled to an Agilent 5973N mass selective detector (70 eV). DB-5MS column (30 m × 0.25 mm × 0.25 µm) took responsibility to distinguish the compounds. The oven temperature programming was 50°C for 2 min, then 150°C by 2°C/min, finally reached 250°C by 10°C/min and holding for 5 min. EO was diluted in *n*-hexane with a solution of 1% and carried by helium. The carrier gas was maintained a constant flow rate at 1.0 mL/min, and the injector temperature was 250°C. By comparing to a homologous series of *n*-alkanes (C₆-C₄₀), the retention indices (RI) were calculated. The components of EO were identified by comparing their mass spectra with those stored in NIST 05 (Standard Reference Data, Gaithersburg, MD, USA), Wiley 275 libraries (Wiley, New York, NY, USA) and pure standards²²⁾. The capillary column and temperature program during GC-FID separation were the same as GC-MS. GC-FID peak area% was used to determine the relative percentage of each component.

2.4 Insects

L. serricorne, *T. castaneum*, *S. oryzae* and *L. bostrychophila* were obtained from a laboratory colony cultured in dark incubators at the temperature of 28–30°C and relative humidity of 70–80%. *L. serricorne*, *T. castaneum* were bred on the mixture of yeast and wheat flour (1:10, w/w) at 12–13% moisture content, *L. bostrychophila* was reared with a mixture of milk powder, yeast and wheat power (1:1:10, w/w), while *S. oryzae* were fed with whole wheat. The mixed-sex adults about ten days old will be used for bioassays. The containers in the bioassay for insects were smeared with polytetrafluoroethylene (Beijing Sino-Rich Material Science Co., Ltd., Beijing, China) to prevent insects from escaping and contacting the solutions.

2.5 Contact toxicity

A group of preliminary experiments (concentrations of 50%, 10% and 2%) were performed before the formal assays to find an appropriate concentration range.

The method of contact toxicity of EO against *L. serricorne*, *T. castaneum*, *S. oryzae* is based on the description by Liu and Ho²³⁾. Dilute EO into five concentrations based on the results of the preliminary experiment with *n*-hexane. Each insect was treated with a 0.5 µL solution or *n*-hexane (as control) to the dorsal thorax. Ten insects were transferred into a glass bottle and five bottles for each concentration as a repeat.

For *L. bostrychophila*, a method carried by Zhao has been adapted as follows²⁴⁾. Filter papers (diameter 5.5 cm) treated with 300 µL solutions were stuck to the Petri dish (diameter 5.5 cm) to load solutions. Ten *L. bostrychophila*

were released in the centre of the Petri dish, and the dishes were covered with lids. Repeat five times at each concentration.

Treated insects were kept under the same condition as the insect rearing. The mortality was recorded after 24 h to calculate the LD₅₀ value. The test methods of individual compounds were set as above.

2.6 Fumigant toxicity

The pre-experiment was the same as the contact toxicity test. The fumigant activity of the essential oil against *L. serricorne*, *T. castaneum*, *S. oryzae* adults was tested as described by Liu and Ho²³⁾. A serial dilution of five concentrations of EO was dissolved in *n*-hexane. Each filter paper (diameter 2.0 cm) was applied with 10 µL dilution and fixed in the underside of the screw cap of a glass bottle (diameter 2.5 cm, height 5.5 cm, volume 25 mL). Before screwing the cap, the solvent was allowed to evaporate for 20 s to prevent it from affecting insects; each bottle contained 10 insects and bottles were sealed with sealing films.

As for *L. bostrychophila*, ten adults were introduced into a vial (8 mL) without a cap placed in a glass wide-mouth bottle. A paper strip (3.5 cm × 1.5 cm) was stuck on the bottle's lid. Lids were quickly closed and sealed with sealing films after being treated a 10 µL solution on the paper strip.

An equal volume of *n*-hexane was used as a control. Five replicates were carried out for each treatment, and they were incubated under the same conditions as rearing. The number of dead insects was recorded after 24 h for calculating LC₅₀ value. The test methods of individual compounds were set as above.

2.7 Repellent activity

The repellent activity of EO and its major compounds against insects was evaluated by the method described in the literature²⁵⁾. Samples were diluted with *n*-hexane to five concentrations (78.63, 15.73, 3.15, 0.63 and 0.13 nL/cm² for *L. serricorne*, *T. castaneum*, *S. oryzae*, and 63.17, 12.63, 2.53, 0.51, and 0.10 nL/cm² for *L. bostrychophila*). Cutting the filter paper disk (9 cm diameter for *L. serricorne*, *T. castaneum*, *S. oryzae*, and 5.5 cm diameter for *L. bostrychophila*) into semicircles, and treated halves of the disks with solutions (500 µL diameter for *L. serricorne*, *T. castaneum*, *S. oryzae*, and 150 µL diameter for *L. bostrychophila*) and the other with *n*-hexane of the same volume as the solution as a negative control. The two halves were then reassembled and pasted on the bottom of the Petri dishes. Twenty insects were released in the centre of the dish, and then the dish was covered with a lid. The numbers of insects settled on the negative control (Nc), and the testing (Nt) halves were counted after 2 h and 4 h, respectively.

2.8 Statistical analysis

Probit analysis was used to deal with the number of dead insects to calculate LD₅₀ and LC₅₀ values in contact and fumigant assays. The confidence intervals, related parameters, and chi-square values of LD₅₀ and LC₅₀ value were presented.

In repellent assays, the value of percent repellency (PR) was estimated as follows: $PR(\%) = [(Nc - Nt) / (Nc + Nt)] \times 100$. PR values were transformed into arcsine, and square root values for one-way ANOVA followed by Tukey's test. Differences between means were considered significant when $p < 0.05$. All data were analysed by SPSS v20.0 (IBM).

3 Results

3.1 Chemical constitutions

Table 1 indicated the chemical constitutions of the EO of *E. kachinensis*, and the GC chromatograph was shown in Fig. 1. Thirty-six components were identified in the EO, accounting for 98.767% of the total oil. The principal compounds of the EO were carvone and dehydroelsholtzia ketone, which together constituted more than 60% of the essential oil components, and the contents are 32.298% and 31.540%, respectively. They were followed by (*E*)- β -farnesene with content of 10.096%. Sesquiterpenes and oxygenated sesquiterpenes account for more than a quarter of all chemical constituents.

3.2 Contact activity

Table 2 showed the contact activities of *E. kachinensis* EO and individual compounds against four insect species. For EO, *L. serricorne* was the most susceptible in *L. serricorne*, *T. castaneum*, *S. oryzae* with the LD₅₀ value of 3.85 µg/adult, whilst the LD₅₀ value of *L. bostrychophila* was 65.38 µg/cm². *T. castaneum*, *S. oryzae* and *L. bostrychophila* were more sensitive against individual compounds than EO, but EO performed lower lethal concentrations than compounds for *L. serricorne*.

3.3 Fumigant activity

In the fumigant activity test, *L. bostrychophila* performed the lowest value of LC₅₀, which was 0.30 mg/L air, but *T. castaneum* was duller compared with *S. oryzae* in this test, which is different from contact activity. Unlike the fumigation result, carvone presented higher lethality than dehydroelsholtzia ketone and EO for *S. oryzae* and *L. bostrychophila* (Table 3).

3.4 Repellent activity

A-F in Fig. 2 represented the PR value of *L. serricorne*, *T. castaneum*, *S. oryzae* and *L. bostrychophila* in 2 h and 4 h, respectively. In most cases, the value is positively cor-

Table 1 Chemical constituents identified in the essential oils from aerial parts of *E. kachinensis*.

No.	Compound Name	Molecular formula	RT ^a	RI ^b	Content (%)
1	<i>meso</i> -2,5-Dimethyl-3,4-hexanediol	C ₈ H ₁₈ O ₂	8.035	940	0.189
2	Benzaldehyde	C ₇ H ₆ O	8.953	984	0.652
3	1,1,2,2-Tetra- <i>t</i> -butylethane	C ₁₈ H ₃₈	9.433	1009	0.102
4	<i>m</i> -Cymene	C ₁₀ H ₁₄	10.445	1069	0.152
5	Limonene	C ₁₀ H ₁₆	10.549	1075	1.339
6	<i>trans</i> - β -Ocimene	C ₁₀ H ₁₆	10.680	1083	0.146
7	β -Ocimene	C ₁₀ H ₁₆	10.905	1096	1.231
8	γ -Terpinene	C ₁₀ H ₁₆	11.160	1114	1.064
9	α,α -Dihydroxyacetophenone	C ₈ H ₈ O ₃	11.285	1122	0.662
10	Rosefuran	C ₁₀ H ₁₄ O	11.814	1160	0.389
11	Linalool	C ₁₀ H ₁₈ O	11.970	1170	0.141
12	1-Octen-3-yl-acetate	C ₁₀ H ₁₈ O ₂	12.098	1179	4.123
13	Terpinen-4-ol	C ₁₀ H ₁₈ O	13.504	1287	0.111
14	Terpineol	C ₁₀ H ₁₈ O	13.747	1307	0.237
15	<i>trans</i> -8a-Methylperhydroazulen-4(1 <i>H</i>)-one	C ₁₁ H ₁₈ O	13.799	1311	1.898
16	Thymol methyl ether	C ₁₁ H ₁₆ O	14.193	1345	0.468
17	Carvone	C ₁₀ H ₁₄ O	14.587	1379	32.298
18	2-Cyclohexen-1-one, 3,5-dimethyl	C ₈ H ₁₂ O	14.978	1413	0.190
19	Dehydroelsholtzia ketone	C ₁₀ H ₁₂ O ₂	15.455	1454	31.540
20	Elemene isomer	C ₁₅ H ₂₄	15.988	1506	0.151
21	Bi-2-cyclohexen-1-yl	C ₁₂ H ₁₈	16.802	1585	0.642
22	2,4,6-Trimethoxystyrene	C ₁₁ H ₁₄ O ₃	17.064	1611	0.433
23	(<i>E</i>)- β -Farnesene	C ₁₅ H ₂₄	17.642	1671	10.096
24	Humulene	C ₁₅ H ₂₄	17.842	1692	0.674
25	α -Curcumene	C ₁₅ H ₂₂	18.085	1717	0.077
26	Germacrene D	C ₁₅ H ₂₄	18.186	1728	0.877
27	7- <i>epi</i> -Sesquithujene	C ₁₅ H ₂₄	18.268	1737	0.210
28	Bicyclogermacrene	C ₁₅ H ₂₄	18.379	1749	0.976
29	β -Curcumene	C ₁₅ H ₂₄	18.465	1759	0.406
30	δ -Amorphene	C ₁₅ H ₂₄	18.628	1776	0.159
31	(<i>E</i>)- γ -Bisabolene	C ₁₅ H ₂₄	18.718	1786	0.063
32	3,7,11-Trimethyl-3-hydroxy-6,10-dodecadien-1-yl acetate	C ₁₇ H ₃₀ O ₃	19.103	1829	0.314
33	[2,2-dimethyl-4-(3-methylbut-2-enyl)-6-methylidenecyclohexyl] methanol	C ₁₅ H ₂₆ O	19.453	1869	4.781
34	α -Cadinol	C ₁₅ H ₂₆ O	20.422	1983	0.351
35	3,4-Nonadien-6-yne, 5-ethyl-3-methyl	C ₁₂ H ₁₈	21.648	2138	1.625
	Monoterpenes				3.780
	Oxygenated monoterpenes				32.687
	Sesquiterpenes				13.689
	Oxygenated sesquiterpenes				5.132
	Aromatics				2.444
	Others				41.035
	Total				98.767

^a RT: Retention time.^b RI: Retention index as determined on a HP-5MS column using the homologous series of *n*-alkanes.

related with the concentration. For *L. serricorne* and *L. bostrychophila*, the PR value of EO in both 2 h and 4 h was above 50% at the concentration of 15.73 nL/cm², and the PR value of *T. castaneum* was still greater than 50%

at the concentration of 3.15 nL/cm². Carvone and dehydroelsholtzia ketone mostly performed significantly poor PR values than EO against *L. serricorne* and *L. bostrychophila* at concentrations above 15.73% and 12.63%, respec-

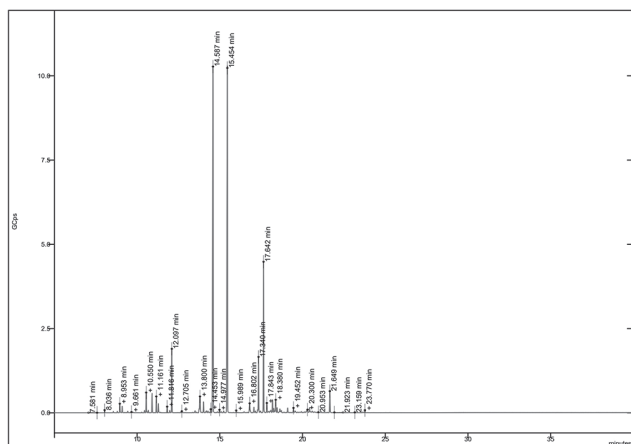


Fig. 1 GC-MS chromatogram of the essential oil of *E. kachinensis*.

tively. However, *S. oryzae* did not show repellent activity in our test.

4 Discussion

Other studies have measured the composition of *E. kachinensis*. The results of this study are different from the

results of the other two studies that obtained plant materials in Yunnan province, where we collected our material. In the study of Zhang *et al.*, *D*-(+)-carvone and 1-(3-methyl-2-furanyl)-methyl-2-butenyl-1-one were the main components of EO, accounting for 46.53% and 33.13%, respectively¹⁴. While in the study of Kang, β -dehydroelsholtzia ketone and 1-octen-3-yl-acetate are the main components, accounting for 78.33% and 5.43%, respectively²⁹. EO of *E. kachinensis* collected from Guizhou Province, China, was analysed to have 56 compounds, most in literature records. The highest percentage was 16.66% benzenemethanol, 4-(1-methylethyl)- and 15.82% 1-octen-3-yl-acetate¹³. According to the current results, EOs from *E. kachinensis* have diverse chemical compositions, so it was difficult to characterise the typical chemotype of EO. This condition may be influenced by the growth conditions and the harvest season. Lesueur *et al.* measured the composition of EO from *E. blanda* and *E. winitiana* collected from August and November³⁰. The results show that the compounds of two EO plants collected at different seasons are very different. EOs from *E. blanda* collected in August and November contained 75.2% and 56.8% of linalool, respectively. Twenty-eight percent of 1,8-cineole was detected in EO from November, which was not in EO from August. For *E. winitiana*, the main component collected in August

Table 2 Contact toxicity of the essential oil from *E. kachinensis* against four kinds of insects.

Insects ^a	Samples	LD ₅₀ ^b	95% LCL-UCL ^c	Slope ± SE	χ ²	P
LS	EO	3.85	3.30-4.42	4.77 ± 0.67	9.78	0.99
	Carvone	4.80	4.23-5.40	1.47 ± 0.18	14.36	0.92
	Dehydroelshotzia ketone	4.77	4.13-5.43	1.28 ± 0.17	10.29	0.99
	Pyrethrins ^d	0.24	0.16-0.35	1.31 ± 0.20	17.36	0.79
TC	EO	26.00	23.27-29.13	9.00 ± 1.17	19.59	0.67
	Carvone	8.88	7.29-10.28	0.61 ± 0.08	7.94	1.00
	Dehydroelshotzia ketone	16.30	14.23-19.06	0.39 ± 0.06	6.22	1.00
	Pyrethrins ^e	0.36	0.22-0.30	3.34 ± 0.32	13.11	0.95
SO	EO	29.62	27.12-32.00	13.32 ± 2.02	7.726	1.00
	Carvone	22.35	20.37-24.51	0.48 ± 0.06	10.84	0.99
	Dehydroelshotzia ketone	28.82	26.14-31.88	0.32 ± 0.04	17.35	0.79
	Deltamethrin	0.39	0.00-0.63	1.03 ± 0.24	1.90	1.00
LB	EO	65.38	61.42-69.85	20.50 ± 2.92	16.55	0.83
	Carvone	37.75	35.09-40.65	12.11 ± 1.70	12.68	0.96
	Dehydroelshotzia ketone	42.98	39.87-46.58	8.77 ± 1.12	10.17	0.99
	Pyrethrins ^d	18.72	17.60-19.92	2.98 ± 0.40	10.56	0.99

^a LS: *L. serricorne*; TC: *T. castaneum*; SO: *S. oryzae*; LB: *L. bostrychophila*.

^b LD₅₀: µg/adult (*L. serricorne*, *T. castaneum* and *S. oryzae*); µg/cm² (*L. bostrychophila*).

^c LCL-UCL: lower confidence limit - upper confidence limit.

^d Data from Yang *et al.*²⁶⁾.

^c Data from Wang *et al.*²⁷⁾.

Table 3 Fumigant toxicity of the essential oil from *E. kachinensis* against four kinds of insects.

Insects ^a	Samples	LD ₅₀ ^b	95% LCL-UCL ^c	Slope ± SE	χ ²	P
LS	EO	7.74	7.06-8.46	9.62 ± 1.13	18.60	0.72
	Carvone	14.88	13.49-16.31	0.58 ± 0.07	11.55	0.98
	Dehydroelsholtzia ketone	20.55	18.90-22.38	0.46 ± 0.05	19.91	0.65
	Phosphine ^d	9.23 × 10 ⁻³	7.13 × 10 ⁻³ - 11.37 × 10 ⁻³	2.12 ± 0.27	11.96	0.97
TC	EO	20.32	18.17-22.80	8.79 ± 1.13	20.65	0.60
	Carvone	4.04	3.75-4.38	2.81 ± 0.32	12.85	0.96
	Dehydroelsholtzia ketone	10.05	9.31-10.89	1.11 ± 0.12	10.21	0.99
	MeBr ^e	1.80	—	—	—	—
SO	EO	12.36	11.46-13.35	13.46 ± 1.74	14.03	0.93
	Carvone	9.73	8.91-10.71	1.20 ± 0.16	6.44	1.00
	Dehydroelsholtzia ketone	23.45	21.65-25.49	0.43 ± 0.04	15.95	0.86
	DDVP ^f	0.05	0.05-0.06	153.68 ± 18.72	14.63	0.91
LB	EO	0.30	0.27-0.33	8.84 ± 1.19	7.98	1.00
	Carvone	0.21	0.20-0.24	5.61 ± 0.72	7.97	1.00
	Dehydroelsholtzia ketone	0.68	0.63-0.73	1.85 ± 0.24	10.04	0.99
	Pyrethrins ^g	1.35 × 10 ⁻³	1.25 × 10 ⁻³ - 1.47 × 10 ⁻³	6.87 ± 0.77	—	—

^a LS: *L. serricorne*; TC: *T. castaneum*; SO: *S. oryzae*; LB: *L. bostrychophila*.^b LC₅₀: mg/L air.^c LCL-UCL: lower confidence limit - upper confidence limit.^d Data from Yang *et al.*²⁶⁾.^e Data from Liu and Ho²³⁾.^f Data from Zheng *et al.* (unpublished).^g Data from Liu *et al.*²⁸⁾.

were rosefuran and dehydroelsholtzia ketone, while in November, it was elsholtzia ketone.

Secondary metabolites affect the growth and metabolism of insects by changing the process of physiological and enzymatic activities³¹⁾. The activity of EOs against insects depends on penetration ability or the sensitivity of the insect respiratory, dermal or nervous system³²⁻³⁴⁾. *T. castaneum* and *L. bostrychophila* were sensitive to EO of *E. ciliata* in contact toxicity^{15, 16)}. EOs and the compositions differ a lot from kinds of plants. Different major components and the ratio of components both determine the insecticidal effect of EOs^{35, 36)}. Besides, LD₅₀ value depended on the duration of exposure, the concentration and the body part where the medicine was applied⁵⁾.

In the fumigant toxicity test, the higher value of LC₅₀ of *E. stauntonii* after 48 h exposure was reported by Lü *et al.*, which value was 10.99 µL/L air. EO from *E. ciliata* exhibited an LC₅₀ value of 11.61 mg/L air and 0.48 mg/L air against *L. serricorne* and *L. bostrychophila*, respectively, showed that these two insects are more sensitive to EO from *E. kachinensis*^{15, 16, 20)}. Several advantages of fumiga-

tion are apparent: the ability to maintain an effective concentration inside a closed space, the possibility to distribute the substance even in hard-to-reach places, and what's more, the application method of fumigation is easy to use⁵⁾. Evaporation of EO does not cause any change in the colour or smell of the grain, and it has little or no cons to the germination ability. Therefore, the market value of the grain will not be affected³⁷⁾. EO was more effective in controlling *L. serricorne* than carvone and dehydroelsholtzia ketone in the three assays. It may be due to the synergy of these two main ingredients. For *L. bostrychophila*, however, antagonistic action may act as a dominant factor in the contact test.

In addition to fumigation, insects can be controlled using gaseous substances when they do not contaminate the grain. We tested the repellent effect of EO from *E. kachinensis* on four species of insects. Except for *S. oryzae*, nearly 100% PR was observed at the concentration of 78.63 nL/cm² for *L. serricorne*, *T. castaneum*, and 63.17 nL/cm² for *L. bostrychophila*. Furthermore, we observed that 4 h PR value is higher than what at 2 h, it may be

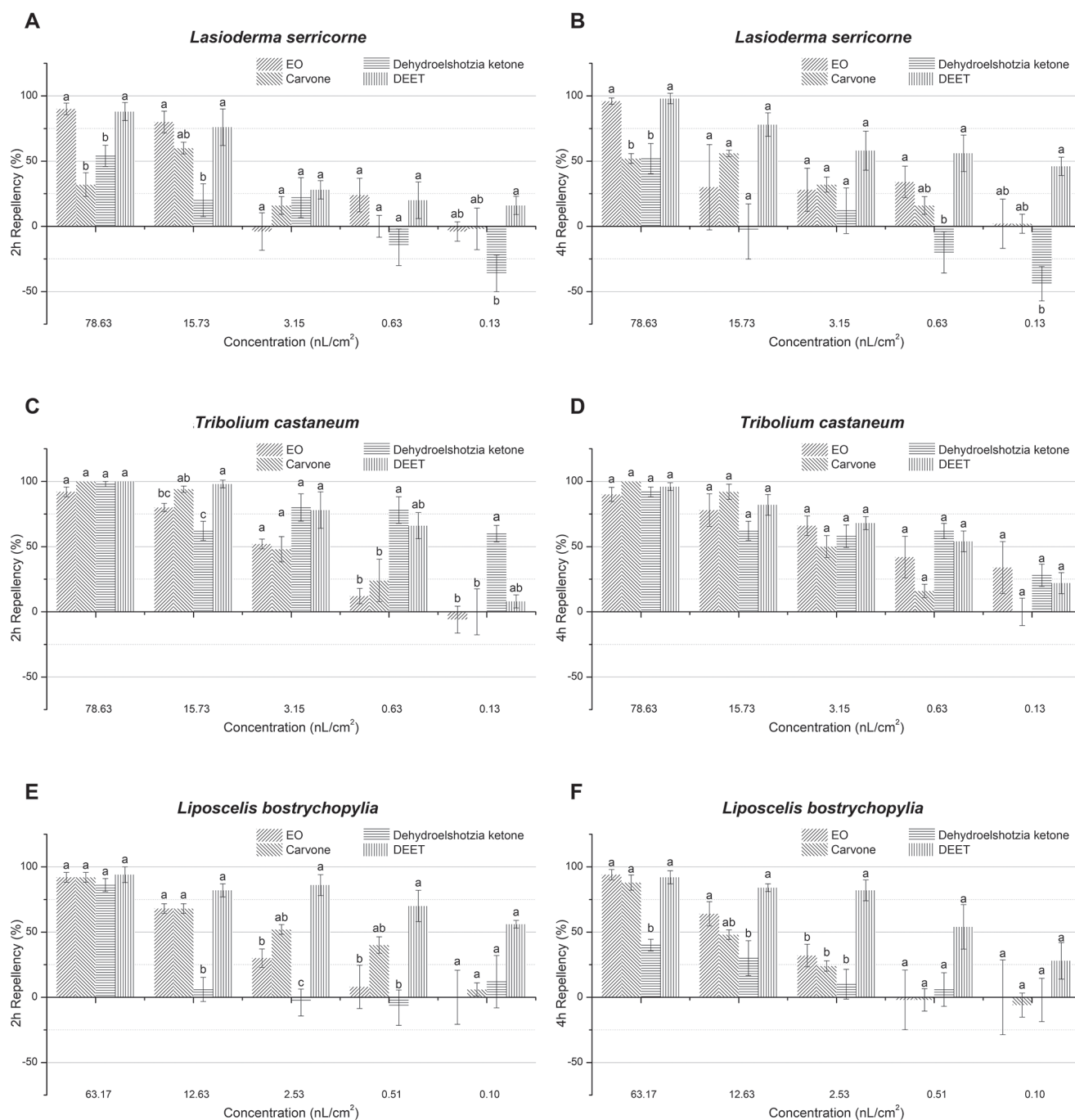


Fig. 2 Percentage repellency (PR) of the essential oils and the compounds against *L. serricorne* (A and B), *T. castaneum* (C and D) and *L. bostrychophyla* (E and F) at 2 h (A, C and E) and 4 h (B, D and F) after exposure. Means in the same column followed by the same letters do not have significant differences ($p > 0.05$) in ANOVA and Tukey's test.

caused by the accumulation of volatilisation of EO in the container with the increase of exposure time, but a record of Eljazi reported that the PR value would decrease with the further extension of time^{38, 39}. In a higher concentration, EO enters the insect body through the respiratory system, affecting the respiration rate, and then enters the insect body by midgut epithelium⁴⁰. Low concentrations of

EO may affect the functioning of olfactory neurons but not cause death⁴¹. Therefore, *T. castaneum*'s more sensitive olfactory system may be the reason for its high PR value. Besides acute toxicities or behavioural effects on the adult insect, many EOs have reproductive effects, including inhibiting reproductive behaviour, fecundity, and larval growth and development^{42, 43}. The sublethal effects limit

the extent of crop infestation and slow down crop losses, which was thought to be as crucial as the lethal effect.

Carvone and dehydroelsholtzia ketone were tested against insects according to previous studies. Zhao *et al.* reported that these two compounds have lethal effects against *L. serricorne* and *T. castaneum* in contact and fumigant tests¹⁶⁾. As a typical member of terpenoids, carvone was also tested against *S. oryzae*, *S. zeamais*, *S. granarius* and *Rhyzopertha dominica* with good results^{15, 44–46)}. So it was valuable to continue developing *E. kachinensis* and other genera from *Elsholtzia*.

5 Conclusion

Our study shows that carvone and dehydroelsholtzia ketone are the main compounds of EO from *E. kachinensis*. EO performed a well insecticidal effect against *L. serricorne*, *T. castaneum*, *S. oryzae* and *L. bostrychophila*. Among them, *L. serricorne* was the most sensitive species in contact and fumigant test. As a traditional vegetable and herbal plant source, *E. kachinensis* has no significant impact on human health, so it has the potential to become a safe and effective bio-insecticide. However, further studies are needed to determine the internal relations between the major components of EO to express the synergistic or antagonistic relationships quantitatively.

Author Contributions

Thank all co-authors for completing this research. Jia-Wei Zhang designed and performed this research, analysed data, and wrote the manuscript. Yi-Xi Feng, Yue-Shen Du, Xin-Xin Lu, Yu Zheng, and Dan Wang checked this manuscript together. Shu-Shan Du gave supporting of the research and provided the materials.

Conflicts of Interest

The authors declare there are no conflicts of interest.

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