Toxicity and Repellent Activity of *Hedychium flavum* Rhizome Essential Oil and Major Constituents Against Stored Product Insects[1]

Jia-Zhu Wang, Jia-Wei Zhang, Yuan-Tong Qi, Yue-Shen Du, Ding Zeng[2,3], and Shu-Shan Du[3]

Beijing Key Laboratory of Traditional Chinese Medicine Protection and Utilization, Faculty of Geographical Science, Beijing Normal University, Beijing, 100875, China

J. Entomol. Sci. 60(1): 72–85 (January 2025)
DOI: 10.18474/JES23-112

Abstract An essential oil extracted from the rhizome of wild ginger, *Hedychium flavum* Roxburgh (Zingiberaceae), was evaluated for its toxic and repellent activity against adults of the stored product pests *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), and *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae). Gas chromatography–flame ionization detection and gas chromatography–mass spectrometry revealed 24 compounds that composed 90.52% of the constituents of the extracted oil. The main compounds identified were β-pinene (33.52%), linalool (15.56%), and 1,8-cineole (11.20%). These three compounds and the rhizome essential oil were further assayed for their contact toxicity, fumigant, and repellent properties against the three stored product pests. Contact toxicity bioassays determined median lethal dosages (LD_{50} values) of the essential oil as 22.3 μg/adult for *T. castaneum,* 11.3 μg/adult for *L. serricorne,* and 109.9 μg/cm^2 for *L. bostrychophila*. Analysis of the fumigant toxicity bioassay established LC_{50} values of 15.6 mg/L air for *T. castaneum* and 7.6 mg/L air for *L. serricorne.* The rhizome essential oil had higher repellent activity against the three stored product pests. Our results indicate the potential of the essential oil from rhizomes of *H. flavum* and its three main components as potential botanical insecticides for management of stored product pests.

Key Words *Hedychium flavum*, essential oil, contact toxicity, fumigant, stored product insects

Essential oils derived from plants can have pesticidal, repellent, and fumigant activity against insect and mite pests. These plant extracts have a high vapor pressure and are easily produced by steam distillation of plant materials (Rajkumar et al. 2019). The extracts are a complex mixture of volatile substances, such as monoterpenes, sesquiterpenes, phenylpropanoids, and aromatic hydrocarbons, that have pesticidal, antimicrobial, antioxidant, and medicinal properties (Pavela and Benelli 2016, Paw et al. 2020, Stevenson et al. 2017, Torres et al. 2017). Many are being evaluated as potential alternatives to chemical pesticides (Dai and Mumper 2010, Miresmailli and Isman 2014).

Members of the botanical family Zingiberaceae are distributed in tropical and subtropical regions worldwide but mainly in tropical Asia. These plants, including

[1]Received 26 December 2023; accepted for publication 6 April 2024.
[2]Department of Burns and Plastic Surgery, PLA Rocket Force Characteristic Medical Center, Beijing, 100088, China.
[3]Corresponding authors (email: dushushan@bnu.edu.cn [S.S.D.]; zengding@sohu.com [D.Z.]).

Zingiber officinale Roscoe, *Curcuma longa* (L.), and *Kaempferia galanga* L., have insecticidal and repellent properties (AlSalhi et al. 2020, Barbosa et al. 2017, Tavares et al. 2013, Ukeh et al. 2009). The essential oil of *Hedychium* acted as either an ant repellent or ant attractant depending on the plant genotype and oil concentration (Sakhanokho et al. 2013). In other studies, the essential oils from other *Hedychium* species, such as *Hedychium glabrum* S.Q. Tong, *Hedychium coronarium* Koehne, and *Hedychium yunnanense* Gagnepain, were deemed potential insecticides and repellents (Wang et al. 2024). However, comprehensive data on the bioactivity of *Hedychium flavum* Roxburgh essential oil and its components against stored product pests are not available.

Hedychium flavum grows in China, India, and Southeast Asia. It is fragrant and can be used in landscaping and as a medicine to treat alimentary maladies (Gao et al. 2008, Thanh et al. 2014). The chemical composition, cultivation, and maintenance of *H. flavum* have been studied, but research on the insecticidal activity of *H. flavum* is rare. We chose the rhizomes of *H. flavum* for this study because this part of the plant is harvested and used for medicinal and other purposes. Our objectives were to identify the chemical composition of the essential oil extracted from *H. flavum* rhizomes and to explore the contact toxicity, repellent, and fumigant activity of the essential oil and its main constituents against the important stored product insects *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), and *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae). Our overall aim was to determine the potential for development of *H. flavum* rhizome essential oil or its chemical constituents as insecticides.

Materials and Methods

Plant material. Rhizomes of *H. flavum* (2.01 kg) were collected from Kaili City (107°55′ E, 26°35′ N), Guizhou Province, China, in March 2019. The identification of the collections was verified by Dr. Q.R. Liu (College of Life Science, Beijing Normal University). Voucher specimens (BNU-dushushan-2019-03-03) were deposited in the herbarium of the College of Resources, Science, and Technology (Faculty of Geographical Science, Beijing Normal University).

Insects. *Tribolium castaneum* and *L. serricorne* were reared in 250-ml glass containers filled with flour and yeast (10:1, w/w). *Liposcelis bostrychophila* was reared in a conical flask with a mixture of flour, milk powder, and active yeast (10:1:1, w/w/w). Insects were reared in total darkness in incubators maintained at 30 $\pm$ 1°C and 70% $\pm$ 10% relative humidity (RH). All bioassays were performed on 2-wk-old adults removed from the colonies.

Essential oil extraction. Rhizomes of *H. flavum* were crushed and subjected to hydrodistillation for 6 h in a Clevenger-type apparatus to obtain the essential oil. The volume was determined by dehydration with anhydrous sodium sulfate after extraction. The essential oil was stored in an airtight glass vial at 4°C until used.

Gas chromatography and mass spectrometry. Gas chromatography–mass spectrometry (GC-MS) analysis was performed on a Thermo Finnigan Trace DSQ instrument equipped with a flame ionization detector (GC-FID) and a capillary column of DB-5MS (30 m $\times$ 0.25 mm $\times$ 0.25 μm). The injector temperature was maintained at 250°C. The injected volume was 1 μL of 1% solution (diluted in *n*-hexane). The carrier gas was helium, and the flow rate was 1.0 mL/min. The oven temperature was

held at 50°C for 3 min then increased to 290°C at 10°C/min. The vaporizer temperature was 250°C, and the spectral scanning range was 45–650 *m/z* in full scan mode. GC-MS and GC-FID were performed under the same operating conditions, and the retention indices were calculated with a homologous series of *n*-alkanes (C_5–C_{36}). The relative percentages of individual compounds were determined by the GC-FID peak area percentage. The mass spectrum and retention index were compared with the mass spectra in NIST 05 (Standard Reference Data, Gaithersburg, MD).

Fumigant bioassay. The fumigant toxicity bioassay with *T. castaneum* and *L. serricorne* were conducted following the methods of Liu and Ho (1999), and the methods of Zhao et al. (2012) were used for *L. bostrychophila.* Preliminary testing established the range of concentrations used against each of the pest insects: 1.93, 2.89, 4.33, 6.5, and 10% for *T. castaneum* and 1.28, 1.93, 2.89, 4.33, and 6.5% for *L. serricorne.* Each concentration experiment was replicated five times with *n*-hexane as the solvent and negative control.

For the bioassays with *T. castaneum* and *L. serricorne,* 10 μL of the solution was dropped on a filter paper disk (Whatman, 2 cm in diameter) and allowed to evaporate for 20 s. The treated filter paper was attached to the inside of the screw cap of a 25-ml glass vial (diameter 2.5 cm, height 5.5 cm). Ten adult insects were placed in each glass vial. The screw cap was attached to the vial and sealed with Parafilm® (Amcor, Zurich, Switzerland). The vials were maintained in darkness at 30 ± 1°C and 70 ± 10% RH, and mortality of the target insect was recorded 24 h later.

For *L. bostrychophila,* 10 adults were placed in a glass vial (8 mL), and the vial was placed in a glass jar (250 mL). The filter paper strip (3.5 cm × 1.5 cm) was attached to the cap of the jar, and 10 μL of the test solution was applied to it. The lid of the jar was tightly screwed down and sealed with Parafilm. Vials and jars were kept at room temperature, and mortality was recorded after 24 h.

Contact toxicity bioassay. The bioassay methods used with *T. castaneum* and *L. serricorne* were similar to those of Liu and Ho (1999). Based on preliminary testing, a range of sequential dilutions of bioassay concentrations was established for *T. castaneum* (1.28, 1.93, 2.98, 4.33, and 6.5%) and *L. serricorne* (0.89, 1.33, 2.0, 3.0, and 4.5%) using *n*-hexane as the diluent. For each concentration, a 0.5-μL droplet of solution was placed on the dorsum of the thorax of each of the 10 insects treated with each concentration. Treated insects were transferred individually to glass vials.

The contact toxicity bioassay with *L. bostrychophila* was conducted as described by Zhao et al. (2012). Preliminary testing established the range of concentrations appropriate for the assay: 0.8, 0.9, 1.56, 2.33, and 3.5%. For each concentration, 300 μL of the solution was applied to a filter paper disk (5.5 cm diameter) and allowed to evaporate for 20 s at room temperature. The filter paper disk was then glued to the bottom of a 5.5-cm petri dish, and 10 *L. bostrychophila* adults from the laboratory colony were placed inside the dish. To prevent *L. bostrychophila* from escaping, the inner wall of the dish was coated with Fluon® (AGC Chemicals Co., Tokyo, Japan), and the dish was covered with a glass lid.

For each bioassay, *n*-hexane was used as the solvent and negative control. Treated insects were maintained in darkness at 30 ± 1°C and 70 ± 10% RH, and mortality was recorded after 24 h of exposure. Each bioassay was conducted five times.

Repellent bioassay. The area preference method of Liu and Ho (1999) was used for the repellent bioassays. For *T. castaneum* and *L. serricorne,* the test concentrations of 0.13, 0.63, 3.15, 15.73, and 78.63 nL/cm^2 were prepared by serial dilutions in *n*-hexane. Filter paper disks (9 cm in diameter) were cut into two equal halves. One half was treated with 500 μL of the test solution, and the other half was treated with 500 μL of *n*-hexane as a control. After air drying for 30 s, the treated and control filter paper halves were placed side by side on the bottom of a 9-cm-diameter petri dish. Twenty adult insects were placed in the middle of the dish, which was then covered with its top. The number of insects on each half of the filter paper in each dish was counted at 2 and 4 h after initial exposure.

The same method was used for bioassays with *L. bostrychophila*; however, some adjustments to the protocol were made. A smaller filter paper disk (5.5 cm in diameter) was used, and the concentrations of the test solutions were 0.10, 0.51, 2.53, 12.63, and 63.17 nL/cm^2. The filter paper disks were halved, treated with 150 μL of the test solution or *n*-hexane (control), allowed to air dry for 30 s, and placed in the bottom of petri dishes as previously described. Twenty adult insects were added to the center of the dish, which was then covered. The number of insects on each half of the filter paper was counted after 2 and 4 h. Treatments were conducted five times.

Statistical analysis. In the fumigation toxicity and contact toxicity bioassays, probit analysis was used with the mortality data to calculate median lethal concentration (LC_{50}) (fumigation and contact), median lethal dose (LD_{50}) (contact), the 95% confidence intervals (CIs) for each, and other related parameters. In the repellency bioassays, percent repellency (PR) was calculated with the numbers of adult insects on either the treated or control filter paper using the formula $PR = [(Nc - Nt)/(Nc + Nt)] \times 100$, where Nc is the number of insects in the control group and Nt is the number of insects in the treatment group. PR values were normalized by converting to arcsine and square root values before being subjected to a one-way analysis of variance. Treatment means were subjected to Tukey's honestly significant difference test, with statistical significance set at $P < 0.05$. All data were processed by SPSS 20.0 (IBM, Armonk, NY).

Results and Discussion

Chemical composition of essential oil. The yield of essential oil obtained from *H. flavum* rhizomes by hydrodistillation was 0.17% (v/w) each. The oil was pale yellow with a density of 0.89 g/mL. Twenty-four compounds were identified by GC-MS, accounting for 90.52% of the essential oil extracted. These compounds were grouped into five classes: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and others (Fig. 1). The major components were β-pinene (33.52%), linalool (15.56%), and 1,8-cineole (11.20%) (Table 1). Terpenes were the most abundant compounds, among which monoterpenes (43.41%) and sesquiterpenes (37.25%) accounted for a large proportion and had a strong and unique aroma.

These results are similar to those of Fan et al. (2017) in their analysis of the chemical composition of four *Hedychium* species collected from Guangdong Province, China. Their analysis revealed that the major components of the essential oil from *H. flavum* flower were linalool, 1,8-cineole, (E)-ocimenone, and α-farnesene, in agreement with our results. Fan et al. (2017) further speculated that based on the

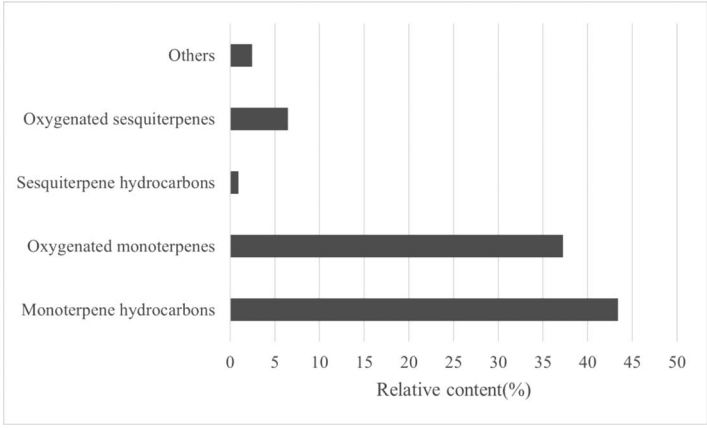

Fig. 1. Chemical classes of components of *H. flavum* rhizome essential oil.

compounds in the essential oils and the intensity of their odor, the aromatic intensity of *Hedychium* species may be closely tied to volatile terpene compounds. The aroma intensity of the essential oil of *H. flavum* rhizomes may be associated with linalool and 1,8-cineole.

In contrast to those findings, Tian et al. (2020) identified 77 components collected from *H. flavum* in Guangxi Province, China. Of those compounds, the dominant ones were coronation E (20.3%), β-pinene (16.8%), (E)-nerolidol (11.8%), and linalool (8.5%); coronation E was identified for the first time. Tian et al. (2023) found 55 volatile compounds in an essential oil extracted from *H. flavum* flowers collected from Nayong, Bijie, Guizhou Province, China. The predominant constituents were β-pinene (20.2%), α-pinene (9.3%), and α-phellandrene (8.3%). *Hedychium flavum* collected from Vietnam yielded 49 volatile compounds, which accounted for 99.3% of the total constituents of the essential oil. The predominant constituent was α-pinene (21.8%) (Thanh et al. 2014).

The number of compounds we identified from *H. flavum* rhizomes was lower than the numbers reported by Tian et al. (2020, 2023) and Thanh et al. (2014), and the primary compounds differed among these studies. These differences may be linked to climate and environmental conditions at the collection sites (Figueiredo et al. 2008). Rawat et al. (2020) also found that the percentages of β-pinene and 1,8-cineole were negatively correlated with elevation and, thus, another possible contributing factor in the observed differences. The percentage of 1,8-cineole during the rainy season and the summer was higher than that in winter (Bhardwaj et al. 2019). Prinsloo and Nogemane (2018) also noted that chemical composition and physical and chemical properties of the essential oil may differ among the seasons. Given that the chemical composition of the essential oils changes with environmental conditions, geographic location, habitats, plant growth stage, growing season, and extraction methods, resulting changes could impact bioactivity (Figueiredo et al. 2008). The composition of *H. flavum* essential oils is especially variable, and choice of optimal planting time, geographic location, and harvest time can significantly improve the yield of bioactive essential oils, thus enhancing the potential for the development of related industries.

Table 1. Chemical composition of the essential oil from *H. flavum* rhizome.

No.	Compound	RI*	Identification method†	Relative content (%)‡
1	Sabinene	920	MS	1.50
2	α-Pinene	940	RI, MS	4.32
3	Camphene	958	RI, MS	1.16
4	β-Pinene	981	RI, MS, Co	33.52
5	β-Phellandrene	1,010	RI, MS	0.66
6	*o*-Cymene	1,019	RI, MS	0.64
7	Cyclohexene, 1-methyl-5-(1-methylethenyl)-	1,030	MS	2.12
8	1,8-Cineole	1,038	RI, MS, Co	11.20
9	Terpinolene	1,081	RI, MS	0.14
10	Linalool	1,103	RI, MS, Co	15.56
11	Camphor	1,144	RI, MS	0.51
12	Pinocarvone	1,165	RI, MS	0.17
13	Borneol	1,173	RI, MS	0.87
14	Terpinen-4-ol	1,178	RI, MS	1.75
15	α-Terpineol	1,200	RI, MS	7.00
16	Bornyl acetate	1,281	RI, MS	0.18
17	Caryophyllene	1,419	RI, MS	0.59
18	Germacrene D	1,469	RI, MS	0.06
19	Curcumene	1,479	RI, MS	0.40
20	α-Bergamotene	1,552	RI, MS	0.28
21	Myristicin	1,566	RI, MS	1.15
22	Nerolidol	1,571	RI, MS	6.00
23	Caryophyllene oxide	1,595	RI, MS	0.46
24	α-Springene	1,970	RI, MS	0.29
	Monoterpene hydrocarbons			43.41
	Oxygenated monoterpenes			37.25
	Sesquiterpene hydrocarbons			0.93
	Oxygenated sesquiterpenes			6.47
	Others			2.48
	Total			90.52

* RI, retention index as determined on DB-5MS column using the homologous series (C_5–C_{36}) of *n*-hydrocarbons.
† MS, based on comparison of mass spectra with those listed in the NIST 05; Co, coinjection with standard compound.
‡ Relative area (peak area relative to the total peak area).

Table 2. Fumigant toxicity of essential oil from *H. flavum* (EOHFR) and its major constituents against *T. castaneum* (TC), *L. serricorne* (LS), and *L. bostrychophila* (LB) at 24 h.

Insect	Sample	LC_{50} (95% CI)* (mg/L air)	Slope (Mean ± SE)	χ^2	*P* value
TC	EOHFR	15.6 (13.7–17.8)	3.7 ± 0.5	11.5	0.871
	β-Pinene	13.2 (12.0–14.4)	4.9 ± 0.5	14.4	0.916
	Linalool	10.4 (8.9–12.0)	2.7 ± 0.4	5.1	1.000
	1,8-Cineole	5.7 (5.0–6.4)	3.8 ± 0.5	16.8	0.817
LS	EOHFR	7.6 (6.0–9.0)	2.2 ± 0.4	13.1	0.950
	β-Pinene	24.9 (22.3–27.5)	4.4 ± 0.5	8.4	0.998
	Linalool	5.5 (4.9–6.2)	4.0 ± 0.5	8.2	0.998
	1,8-Cineole	3.2 (2.8–3.7)	3.0 ± 0.4	14.0	0.928
LB	EOHFR	>50.0 (mortality 23% ± 1.5%)			
	β-Pinene	>50.0 (mortality 37% ± 1.5%)			
	Linalool	77.1 (66.5–89.2)	2.7 ± 0.4	5.0	1.000
	1,8-Cineole	12.8 (11.9–13.7)	5.7 ± 0.8	18.6	0.724

* CI, confidence interval.

Fumigant toxicity. The fumigant toxicity of the essential oil from *H. flavum* rhizomes and its three primary chemical constituents against *T. castaneum, L. serricorne,* and *L. bostrychophila* was determined by concentration–mortality response bioassays. By using nonoverlapping 95% CIs as indicators of statistical significance, we determined that the fumigant activity of 1,8-cineole was significantly superior to that of the other three solutions against *T. castaneum* (Table 2). Linalool was superior to β-pinene and the *H. flavum* essential oil, and these were statistically equal in their fumigant toxicity. Liu and Ho (1999) reported an LC_{50} of 1.8 mg/L air for methyl bromide against *T. castaneum*.

For *L. serricorne,* the fumigant toxicity of the four test solutions was 1,8-cineole > linalool = *H. flavum* essential oil > β-pinene (Table 2). Phosphine had an LC_{50} of 9.2×10^{-3} mg/L air for *L. serricorne* (Yang et al. 2014). We also determined that 1,8-cineole was superior to linalool as a fumigant against *L. bostrychophila*; however, we were unable to calculate the LC_{50} values of β-pinene or the *H. flavum* essential oil because 50% concentrations of each did not cause 100% mortality of *L. bostrychophila* (Table 2). Liu et al. (2013) obtained an LC_{50} of 1.4×10^{-3} mg/L air for dichlorvos against *L. bostrychophila*.

Although we did not find significant differences among the three insect species in their responses to the chemicals, 1,8-cineole, the most abundant compound in *H. flavum* essential oil, had effective fumigant activity against the three insect pests (Table 2). Our finding support those of similar studies; 1,8-cineole, as a major

component of a variety of plants such as *Stachys riederi* Chamisso, *Eucalyptus globulus* Labill, and *Zanthoxylum armatum* de Candolle, exhibited excellent fumigant toxicity against *L. bostrychophila, Oryzaephilus surinamensis* L., and *T. castaneum.* The toxic effect of 1,8-cineole also was comparable to that of synthetic insecticides (Lee et al. 2000, Quan et al. 2018, Wang et al. 2015). Therefore, this compound might play a critical role in the fumigant bioactivity of the essential oil.

Mortality responses to the other three compounds varied among the three pest species. In other studies, fumigant toxicity responses to essential oils also were highly variable among coleopteran stored product pests (Feng et al. 2023, Li et al. 2023). Our results are similar to those of Cao et al. (2018), who found that linalool and β-pinene were toxic for insects, but the same concentration of essential oil did not have insecticidal activity. One postulation is that antagonistic interactions occur among the components of essential oils, thus neutralizing at least a portion of the fumigant toxicity (Ukeh and Urnoetok 2011). Monoterpenes are volatile compounds with the potential to be used against insects in fumigants products, so the total monoterpene concentrations of essential oils can affect their insecticidal activities (Lee et al. 2003).

We also observed that dead insects fumigated with the essential oil often appeared black and had their wings spread. This response may be due to the action of small monoterpene molecules, indicating that the essential oil and its components may have neurotoxic effects on target insects. Subsequent studies could include assessment of the bioactivity of combinations of components against target insects to explore optimal usage.

Contact toxicity. The contact toxicity of the four test substances was determined by the dose–mortality responses of *T. castaneum* and *L. serricorne* and the concentration–mortality responses of *L. bostrychophila.* The significance of differences among the LD_{50} or LC_{50} values of the four substances for each of the three species was determined by nonoverlap of the 95% CIs. The relative contact toxicity of the substances against *T. castaneum* was 1,8-cineole = β-pinene = *H. flavum* essential oil > linalool (Table 3). No significant differences were detected among the LD_{50} values of the four substances against *L. serricorne.* Yang et al. (2014) determined the contact toxicity of pyrethrins against *T. castaneum* and *L. serricorne* adults, with LD_{50} values of 0.3 and 0.2 μg/insect, respectively.

The LC_{50} values of the four substances against L. bostrychophila indicated that the *H. flavum* essential oil was more effective than the three main constituents of the essential oil alone, with relative responses of *H. flavum* essential oil > linalool = 1,8-cineole > β-pinene (Table 3). Yang et al. (2014) also reported an LC_{50} of 18.7 μg/cm^2 for pyrethrins against *L. bostrychophila* adults.

Although the toxicity of the essential oil extracted from the rhizomes of *H. flavum* was lower than that of the positive controls, this essential oil appears to have greater contact toxicity against *T. castaneum* and *L. serricorne* than do essential oils extracted from other plants in the family Zingiberaceae. Chen et al. (2018) reported that the essential oil from *Alpinia katsumadai* Hayata seed had contact toxicity against *T. castaneum* and *L. serricorne* with LD_{50} values of 52.6 and 17.4 μg/adult, respectively. *Zingiber zerumbet* Smith essential oil had contact toxicity against *L. serricorne* with an LD_{50} value of 48.3 μg/adult (Wu et al. 2017). Our results clearly indicate that the toxicity response of *L. bostrychophila* to the combination of the components

Table 3. Contact toxicity of essential oil from *H. flavum* (EOHFR) and its major constituents against *T. castaneum* (TC), *L. serricorne* (LS), and *L. bostrychophila* (LB) at 24 h.

Insect	Sample	LC_{50} (95% Fiducial Limits)		Slope (Mean ± SE)	χ^2	*P* value
		µg/adult	µg/cm^2			
TC	EOHFR	22.3 (18.0–32.1)		2.5 ± 0.5	5.0	0.975
	β-Pinene	19.3 (14.1–23.5)		2.5 ± 0.4	4.4	0.960
	Linalool	44.8 (38.6–50.6)		3.5 ± 0.4	18.6	0.727
	1,8-Cineole	19.3 (17.6–21.2)		4.9 ± 0.5	16.5	0.834
LS	EOHFR	11.3 (9.5–14.0)		2.5 ± 0.4	7.1	0.989
	β-Pinene	7.6 (5.2–9.7)		2.0 ± 0.3	10.9	0.984
	Linalool	7.6 (5.2–9.9)		1.8 ± 0.2	14.3	0.919
	1,8-Cineole	9.8 (8.4–11.4)		2.9 ± 0.3	15.6	0.872
LB	EOHFR		109.9 (103.8–116.2)	8.3 ± 1.1	17.0	0.809
	β-Pinene		361.2 (340.0–382.0)	7.2 ± 0.9	9.9	0.992
	Linalool		222.4 (170.0–275.3)	2.2 ± 0.3	16.5	0.834
	1,8-Cineole		273.4 (243.2–293.0)	9.2 ± 1.6	14.2	0.921

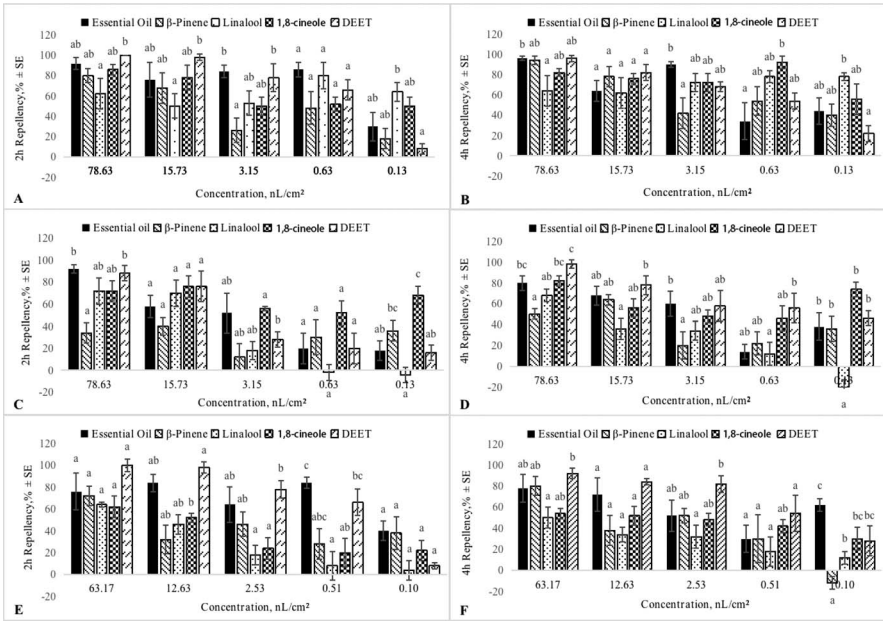

Fig. 2. Percent repellency of *H. flavum* rhizome essential oil and its three major components against *T. castaneum*, *L. serricorne*, and *L. bostrychophila* adults, respectively, after 2 h of exposure (A, C, E) and 4 h of exposure (B, D, F).

exceeded that of the components used individually. The synergistic or additive toxicity of monoterpene mixtures to insects was manifested in increased epidermal permeability, and essential oils may have a more significant neurosuppressive effect than do synthetic insecticides. Therefore, the essential oil of *H. flavum* may be useful as a plant insecticide.

López and Pascual-Villalobos (2010) also noted that monoterpenes, especially linalool that we identified in the *H. flavum* essential oil, have antiacetylcholinesterase properties, accounting for the high mortality in treated stored product insects. In our contact toxicity assay, we also observed insect behaviors, such as tremors, agitation, and the spreading of wings, that are indicative of exposure to neurotoxins. These behaviors were also reported by Kostromytska et al. (2011). We speculate that the mode of action of certain essential oils against insects may be neurotoxic. The synergistic or additive effects of multiple plant secondary metabolites are undoubtedly one of the most effective defense systems against stored product insects.

Repellent activity. Various concentration-dependent and time-dependent responses were observed in the repellency of the four test substances against the three stored product insect pests (Fig. 2). As concentration decreased, the repellent effect also decreased and reached the lowest activity at the lower concentrations tested. β-Pinene, linalool, and 1,8-cineole, as the major components of *H. flavum* essential oil, had some repellent effects on the three stored product insects, similar to previous reports (Cao et al. 2018, You et al. 2015, Zhang et al. 2015). Of the

substances tested, *H. flavum* essential oil was most consistent in repelling the three insect species. This effect may be related to the synergistic or additive effect of the compounds in the oil. For example, Chen et al. (2021) confirmed the synergistic repellent effect of four individual components: α-thujone, (+)-camphor, 1,8-cineole, and α-caryophyllene. This synergism can enhance the bioactivity of mixtures of chemicals, such as seen with essential oils. Synergism has also been reported for the repellency of mixtures of essential oils. Liu et al. (2006) reported synergism in the repellency of the mixture of essential oils from *Artemisia princeps* Pampanini and *Cinnamomum camphora* (L.) against *Sitophillus oryzae* L. and *Bruchus rugimanus* Boheman, which was significantly higher than the repellency of the individual oils.

Essential oils and their volatile components commonly act in a vapor phase, which imparts a repellent action and drives pests away from the odorous source. However, this repellent barrier diminishes relatively quickly, thus limiting the effectiveness of these repellents. The repellent activity of essential oils, especially its duration, is highly dependent on the chemical content of the essential oil (Zhu et al. 2001). More research is needed to understand the mechanism underlying the interactions among the components of an essential oil and how they enhance its repellent properties. Although the positive control (*N,N*-diethyl-meta-toluamide) in this assay was more repellent against these three species of stored product insects, concerns about environmental contamination and human safety regarding commercial pesticides may serve as impetus for continued development of essential oils for the management of insect pests.

Concluding remarks. The essential oil extracted from the rhizomes of *H. flavum* and the three major components of this oil (β-pinene, linalool, and 1,8-cineole) had significant fumigant, contact toxicity, and repellent activity against *T. castaneum, L. serricorne,* and *L. bostrychophila.* The lethal toxicity of this essential oil and its three main components suggests that they have potential for development in the management of stored product insect pests. Although the essential oil and its terpenoids individually had relatively effective fumigant, contact toxicity, and repellent activity against the three insects, the modes of action should be studied to determine the best mechanisms to enhance the effectiveness of the essential oil, including exploring possible synergistic mixtures.

Acknowledgments

This work was supported by the Second Tibetan Plateau Scientific Expedition and Research Program (2019QZKK0608). The authors have no conflicts of interest to declare that are relevant to the content of this article. J.Z.W. performed the experiments, analyzed data, and wrote the manuscript. Y.S.D. performed the experiments and analyzed data. Y.T.Q. J.W.Z., D.Z., and S.S.D. revised the manuscript together. S.S.D. provided materials.

References Cited

AlSalhi, M.S., K. Elumalai, S. Devanesan, M. Govindarajan, K. Krishnappa and F. Maggi. 2020. The aromatic ginger *Kaempferia galanga* L. (Zingiberaceae) essential oil and its main compounds are effective larvicidal agents against *Aedes vittatus* and *Anopheles maculatus* without toxicity on the non-target aquatic fauna. Ind. Crops Prod. 158: 113012.

Barbosa, G.B., N.S. Jayasinghe, S.H.A. Natera, E.D. Inutan, N.P. Peteros and U. Roessner. 2017. From common to rare Zingiberaceae plants—A metabolomics study using GC-MS. Phytochemistry 140: 141–150.

Bhardwaj, S., Rashmi and V. Parcha. 2019. Effect of seasonal variation on chemical composition and physicochemical properties of *Hedychium spicatum* rhizomes essential oil. J. Essent. Oil Bear. Plants 22: 1593–1600.

Cao, J.Q., S.S. Guo, Y. Wang, X. Pang, Z.F. Geng and S.S. Du. 2018. Toxicity and repellency of essential oil from *Evodia lenticellata* Huang fruits and its major monoterpenes against three stored-product insects. Ecotoxicol. Environ. Saf. 160: 342–348.

Chen, Y.J., J.X. Luo, N. Zhang, W.J. Yu, J.X. Jiang and G.H. Dai. 2021. Insecticidal activities of *Salvia hispanica* L. essential oil and combinations of their main compounds against the beet armyworm *Spodoptera exigua*. Ind. Crops Prod. 162: 113271.

Chen, Z.Y., S.S. Guo, J.Q. Cao, X. Pang, Z.F. Geng, Y. Wang, Z. Zhang and S.S. Du. 2018. Insecticidal and repellent activity of essential oil from *Amomum villosum* Lour. and its main compounds against two stored-product insects. Int. J. Food Prop. 21: 2265–2275.

Dai, J. and R.J. Mumper. 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules 15: 7313–7352.

Fan, Y.P., X.R. Wang, R.C. Yu and P. Yang. 2017. Analysis on the aroma components in several species of *Hedychium*. J. Hortic. 34: 231–234.

Feng, Y.X., X.X. Lu, Y.S. Du, J.W. Zhang, B. Almaz, D. Zeng and S.S. Du. 2023. Synergized potential, insecticidal and repellent activity of essential oils from two *Rhododendron* species against three stored product insects. J. Essent. Oil Res. 35: 91–101.

Figueiredo, A.C., J.G. Barroso, L.G. Pedro and J.J.C. Scheffer. 2008. Factors affecting secondary metabolite production in plants: Volatile components and essential oils. Flavour Frag. J. 23: 213–226.

Gao, L.X., N.A. Liu, B.H. Huang and X. Hu. 2008. Phylogenetic analysis and genetic mapping of Chinese *Hedychium* using SRAP markers. Sci. Hortic. 117: 369–377.

Kostromytska, O.S., E.A. Buss and M.E. Scharf. 2011. Toxicity and neurophysiological effects of selected insecticides on the mole cricket, *Scapteriscus vicinus* (Orthoptera: Gryllotalpidae). Pestic. Biochem. Phys. 100: 27–34.

Lee, S., C.J. Peterson and J.R. Coats. 2003. Fumigation toxicity of monoterpenoids to several stored product insects. J. Stored Prod. Res. 39: 77–85.

Lee, S.E., W.S. Choi, H.S. Lee and B.S. Park. 2000. Cross-resistance of a chlorpyrifos-methyl resistant strain of *Oryzaephilus surinamensis* (Coleoptera: Cucujidae) to fumigant toxicity of essential oil extracted from *Eucalyptus globulus* and its major monoterpene, 1,8-cineole. J. Stored Prod. Res. 36: 383–389.

Li, B.Y., J.W. Zhang, Y. Zheng, D. Wang, C.F. Wan and S.S. Du. 2023. Insecticidal and repellent effects of the essential oils extract from *Zanthoxylum myriacanthum* against three storage pests. Chem. Biodivers. 20(2): e202200493.

Liu, C.H., A.K. Mishra, R.X. Tan, C. Tang, H. Yang and Y.F. Shen. 2006. Repellent and insecticidal activities of essential oils from *Artemisia princeps* and *Cinnamomum camphora* and their effect on seed germination of wheat and broad bean. Bioresour. Technol. 97: 1969–1973.

Liu, X.C., L.G. Zhou, Z.L. Liu and S.S. Du. 2013. Identification of insecticidal constituents of the essential oil of *Acorus calamus* rhizomes against *Liposcelis bostrychophila* Badonnel. Molecules 18: 5684–5696.

Liu, Z.L. and S.H. Ho. 1999. Bioactivity of the essential oil extracted from *Evodia rutaecarpa* Hook f. et Thomas against the grain storage insects, *Sitophilus zeamais* Motsch and *Tribolium castaneum* (Herbst). J. Stored Prod. Res. 35: 317–328.

López, M.D. and M.J. Pascual-Villalobos. 2010. Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Ind. Crop. Prod. 31: 284–288.

Miresmailli, S. and M.B. Isman. 2014. Botanical insecticides inspired by plant–herbivore chemical interactions. Trends Plant Sci. 19: 29–35.

Pavela, R. and G. Benelli. 2016. Essential oils as ecofriendly biopesticides? Challenges and constraints. Trends Plant Sci. 21: 1000–1007.

Paw, M., T. Begum, R. Gogoi, S.K. Pandey and M. Lal. 2020. Chemical composition of *Citrus limon* L. Burmf peel essential oil from north east India. J. Essent. Oil Bear. Plants 23: 337–344.

Prinsloo, G. and N. Nogemane. 2018. The effects of season and water availability on chemical composition, secondary metabolites and biological activity in plants. Phytochem. Rev. 17: 889–902.

Quan, M.R., Q.Z. Liu and Z.L. Liu. 2018. Identification of insecticidal constituents from the essential oil from the aerial parts *Stachys riederi* var. *japonica*. Molecules 23: 1200.

Rajkumar, V., C. Gunasekaran, I.K. Christy, J. Dharmaraj, P. Chinnaraj and C.A. Paul. 2019. Toxicity, antifeedant and biochemical efficacy of *Mentha piperita* L. essential oil and their major constituents against stored grain pest. Pest. Biochem. Physiol. 156: 138–144.

Rawat, S., I.D. Bhatt and R.S. Rawal. 2020. Variation in essential oil composition in rhizomes of natural populations of *Hedychium spicatum* in different environmental condition and habitats. J. Essent. Oil Res. 32: 348–360.

Sakhanokho, H.F., B.J. Sampson, N. Tabanca, D.E. Wedge, B. Demirci, K.H.C. Baser, U.R. Bernier, M. Tsikolia, N.M. Agramonte, J.J. Becnel, J. Chen, K. Rajasekaran and J.M. Spiers. 2013. Chemical composition, antifungal and insecticidal activities of *Hedychium* essential oils. Molecules 18: 4308–4327.

Stevenson, P.C., M.B. Isman and S.R. Belmain. 2017. Pesticidal plants in Africa: A global vision of new biological control products from local uses. Ind. Crop. Prod. 110: 2–9.

Tavares, W.D., S.D. Freitas, G.H. Grazziotti, L.M.L. Parente, L.M. Liao and J.C. Zanuncio. 2013. *Ar*-turmerone from *Curcuma longa* (Zingiberaceae) rhizomes and effects on *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Ind. Crop. Prod. 46: 158–164.

Thanh, B.V., D.N. Dai, T.D. Thang, N.Q. Binh, L.D.N. Anh and I.A. Ogunwande. 2014. Composition of essential oils of four *Hedychium* species from Vietnam. Chem. Cent. J. 8: 54.

Tian, M.Y., X.H. Wu, T.Y. Lu, X.G. Zhao, F. Wei, G.D. Deng and Y. Zhou. 2020. Phytochemical analysis, antioxidant, antibacterial, cytotoxic, and enzyme inhibitory activities of *Hedychium flavum* rhizome. Front. Pharmacol. 11: 572659.

Tian, M.Y., D. Xie, Y. Yang, Y.F. Tian, X.Y. Jia, Q.Q. Wang, G.D. Deng and Y. Zhou. 2023. *Hedychium flavum* flower essential oil: Chemical composition, anti-inflammatory activities and related mechanisms *in vitro* and *in vivo*. J. Ethnopharmacol. 301: 115846.

Torres, J.E.D., F. Gassara, A.P. Kouassi, S.K. Brar and K. Belkacemi. 2017. Spice use in food: Properties and benefits. Crit. Rev. Food Sci. 57: 1078–1088.

Ukeh, D.A., M.A. Birkett, J.A. Pickett, A.S. Bowman and A.J.M. Luntz. 2009. Repellent activity of alligator pepper, *Aframomum melegueta,* and ginger, *Zingiber officinale,* against the maize weevil, *Sitophilus zeamais*. Phytochemistry 70: 751–758.

Ukeh, D.A. and S.B.A. Urnoetok. 2011. Repellent effects of five monoterpenoid odours against *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) in Calabar, Nigeria. Crop Prot. 30: 1351–1355.

Wang, C.F., W.J. Zhang, C.X. You, S.S. Guo, Z.F. Geng, L. Fan, S.S. Du, Z.W. Deng and Y.Y. Wang. 2015. Insecticidal constituents of essential oil derived from *Zanthoxylum armatum* against two stored-product insects. J. Oleo Sci. 64: 861–868.

Wang, J.Z., S.S. Du, Y.S. Du, Y.T. Qi, C. Zhang, Z.R. Zhang and H.M. Zhang. 2024. Chemical composition, toxicity, and repellency of essential oils from three *Hedychium* species against stored-product insects. Chem. Biodivers. 21(4): e202301711.

Wu, Y., S.S. Guo, D.Y. Huang, C.F. Wang, J.Y. Wei, Z.H. Li, J.S. Sun, J.F. Bai, Z.F. Tian, P.J. Wang and S.S. Du. 2017. Contact and repellant activities of zerumbone and its analogues from the essential oil of *Zingiber zerumbet* (L.) Smith against *Lasioderma serricorne*. J. Oleo Sci. 66: 399–405.

Yang, K., C.F. Wang, C.X. You, Z.F. Geng, R.Q. Sun, S.S. Guo, S.S. Du, Z.L. Liu and Z.W. Deng. 2014. Bioactivity of essential oil of *Litsea cubeba* from China and its main compounds against two stored product insects. J. Asia Pac. Entomol. 17: 459–466.

You, C.X., S.S. Guo, W.J. Zhang, K. Yang, Z.F. Geng, S.S. Du, C.F. Wang and Z.W. Deng. 2015. Identification of repellent and insecticidal constituents from *Artemisia mongolica* essential oil against *Lasioderma serricorne*. J. Chem. 2015: 549057.

Zhang, J.W., K. Yang, C.X. You, Y. Wang, C.F. Wang, Y. Wu, Z.F. Geng, Y. Su, S.S. Dui and Z.W. Deng. 2015. Bioactivity of essential oil from *Artemisia stolonifera* (Maxim.) Komar. and its main compounds against two stored-product insects. J. Oleo Sci. 64: 299–307.

Zhao, N.N., L.G. Zhou, Z.L. Liu, S.S. Du and Z.W. Deng. 2012. Evaluation of the toxicity of the essential oils of some common Chinese spices against *Liposcelis bostrychophila*. Food Control 26: 486–490.

Zhu, B.C.R., G. Henderson, F. Chen, H.X. Fei and R.A. Laine. 2001. Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite. J. Chem. Ecol. 27: 1617–1625.