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RESEARCH ARTICLE

# Evaluation of efficacy of the essential oil from *Ostericum viridiflorum* (Turcz.) Kitagawa in control of stored product insects

Hai Ming Zhang [1,2] · Shan Shan Guo [3] · Bin Fan [1,2] · Shu Shan Du [3] · Yong Yan Wang [3] · Zhi Wei Deng [4]

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## Abstract

The natural and ecologically safe control of stored product insects has gained considerable attention in modern society. In this study of further searching for botanical pesticides from wild-growing plant, the contact toxicity and repellency towards *Tribolium castaneum* and *Liposcelis bostrychophila* were assessed for the essential oil (EO) from *Ostericum viridiflorum*. The EO was distilled from aboveground parts of *O. viridiflorum* and checked by gas chromatography-mass spectrometry. Twenty-two compounds were identified and the main components were $\beta$-caryophyllene (24.3%), $\alpha$-humulene (21.0%), apiol (10.2%), and carotol (2.5%). For bioactivity tests, results indicated that the EO and its two main compounds ($\beta$-caryophyllene and $\alpha$-humulene) all showed potent contact toxicity towards *L. bostrychophila* with $LD_{50}$ values of 44.52 μg/cm$^2$, 74.11 μg/cm$^2$, and 118.56 μg/cm$^2$, respectively. The EO and the two main compounds also exhibited comparable repellency towards *T. castaneum* and *L. bostrychophila*. The results evidenced the EO of *O. viridiflorum* aboveground parts and its major compounds could be considered for the development of eco-friendly botanical insecticides and repellents in controlling stored product insects.

**Keywords** *Ostericum viridiflorum* · Essential oil · Components · Contact toxicity · Repellency · *Tribolium castaneum* · *Liposcelis bostrychophila*

## Introduction

Pest infestation in stored products has been a major problem throughout the world. Pest insects cause substantial quantitative and qualitative losses of stored products (Karaborklu 2014). The red flour beetle, *Tribolium castaneum*, and the psocid, *Liposcelis bostrychophila*, are common and destructive stored product insects. Presently, the synthetic pesticides are widely used to control these insects. However, the widespread use of synthetic toxic pesticides has posed numerous problems including environmental pollution, possible health hazards to humans and animals, and the development of insecticide resistance due to their long persistence and accumulation in the environment and ecosystems (Tang et al. 2018; Bolognesi and Merlo 2011). Therefore, it is necessary to develop safe, eco-friendly, efficient pest control methods. In recent years, botanical pesticides, such as plant essential oil (EOs), are becoming increasingly popular as agents for control of a number of insect pests (Mukandiwa et al. 2016), vectors (Rajeswary et al. 2018), mites (Song et al. 2016), and ticks (Benelli and Pavela 2018). This is mainly attributed to EOs having multiple mechanisms of action, such as growth, development and fecundity reduction, molting and respiration disruption, acetylcholinesterase inhibition, and neurotoxic effects (Morya et al. 2010; Attia et al. 2012; Banken and Stark 1997; Dohi et al. 2009; Jankowska et al. 2018). EOs consist of diverse secondary metabolites that are biosynthesized by plants for specific functions, using different metabolic routes in plants. These secondary plant metabolites are complex mixtures of volatile molecules of low molecular weight, mainly terpenoids (monoterpenes and sesquiterpenes)

Responsible editor: Giovanni Benelli

✉ Shu Shan Du
dushushan@bnu.edu.cn

[1] Experimental Research Center, China Academy of Chinese Medical Sciences, Beijing 100700, China

[2] State Key Laboratory Breeding Base of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijng 100700, China

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

[4] Analytical and Testing Center, Beijing Normal University, Haidian District, Beijing 100875, China

and phenylpropanoids. The EOs generally are obtained by hydrodistillation, steam distillation, dry distillation, supercritical fluid extraction, and microwave-assisted process methods (Aziz et al. 2018). EOs have the advantage of low mammalian toxicity, rapid biodegradation with broad-spectrum activity, short environmental persistence, and minimal residual activity (Regnault-Roger et al. 2012). Many studies have demonstrated that EOs offer protection against insect pests through various activities, including contact and fumigant toxicities, larvicidal, ovicidal, adulticidal, and repellent activities, antifeedant, and oviposition deterrence effects (Sowndhararajan et al. 2017; Soonwera et al. 2018; Darabi and Khajehali 2017; Zahran et al. 2017). In addition to the bioactivities of EOs, there are some considerations and challenges for EOs (e.g., low stability and short persistence) that need to be solved (Pavela and Benelli 2016). During our ongoing search for new insecticides of plant origin, we have reported insecticidal activities of the EOs from two species (*Ostericum sieboldii* and *O. grosseserratum*) of genus *Ostericum* (Liu et al. 2011; Chu et al. 2013). In order to further search for botanical pesticides from genus *Ostericum*, this study continues to evaluate the bioactivities of EO from other species of this genus.

*Ostericum* (Family: Umbelliferae) is a separate subgenus from the genus *Angelica* on the basis of new phytochemical and molecular data, together with the serological data of *Ostericum* (Harborne et al. 1986; Shneyer et al. 2003; Suk et al. 1974). This genus comprises ten species distributed in North Korea, Japan, and the Far East, of which seven species are present in China (Pan et al. 1997). Among them, *Ostericum viridiflorum* (Turcz.) Kitagawa is a perennial herb with a yellowish brown conic root, purplish green and acute-angled stem, acute-triangular petioles, and triangular-ovate leaf blade. The inflorescence of compound umbel is 4–9 cm in diameter. Petals are green or greenish white. It is mainly distributed in northeast China and Inner Mongolia, and there are also distributions in eastern Siberia and the Far East. It grows in damp meadows, riversides, and stream banks (Gorovoi 1966; Kitagawa 1979; Fu and Hong 2001; Ohwi 1965; Park 2007). This plant is rich in valuable EOs, and has the ability of expelling wind, removing dampness, dispersing cold, and relieving pain. The young leaves of this plant are eaten as a spring vegetable and the roots are used to treat diseases like rheumatic pain, waist and knee pain, cold headache, and carbuncle swollen (Editorial Board of Zhong Hua Ben Cao, State Administration of Traditional Chinese Medicine 2004). Hence, in this study, we assess the contact toxicity and repellency of the EO from *O. viridiflorum* aboveground parts towards the red flour beetles and psocids for the first time. The first section of this paper analyzed the chemical composition of the EO from *O. viridiflorum* aboveground parts and then evaluated the contact toxicity and repellency towards the two stored product insects for the EO and its two major sesquiterpenes ($\alpha$-humulene and $\beta$-caryophyllene).

## Materials and methods

### Essential oil extraction

Aboveground parts of *O. viridiflorum* were collected from Qianshan Mountain Scenic Spot, Anshan City, Liaoning Province, China (41.07° N latitude and 122.97° E longitude), in September 2016. The plant specimen was identified and authenticated by Prof. B. Fan from the Experimental Research Center, China Academy of Chinese Medical Sciences. The specimen (no. ERC-20160917) was deposited in the Herbarium (ERC) of Experimental Research Center. The sample was shade-dried at room temperature and grounded to powders. The ground powders were extracted by steam distillation for 8 h. The obtained EO sample was kept in hermetic brown bottle at 4 °C for further analysis.

### Analysis of essential oil

GC-MS detection of the EO was conducted on Agilent 7890A GC-FID and Agilent 7890A GC-5975C MSD. The two systems were performed using the same condition. Analytical conditions were as follows: capillary column used HP-5MS (30 m × 0.25 mm × 0.25 μm) and temperature was set initially at 50 °C, kept for 1 min, and increased to 150 °C at 2 °C/min for 2 min, finally ramped to 240 °C at 10 °C/min. The injector was programmed at 250 °C. Dry helium was the carrier gas (1.0 mL/min). Volume of injected was 1 μL. Mass range was m/z 50 to 550. Identification of constituents was carried out by retention indices, compared to reported compounds in the literatures and authentic chemicals standards in authors' laboratory. Under the same experimental conditions, the retention index for each compound was calculated with a standard *n*-alkanes series ($C_5$–$C_{36}$). Composition quantification was carried out using area normalization of the FID chromatograms.

### Insect rearing

All tested insects used for the study were obtained from our laboratory cultures (Faculty of Geographical Science, Beijing Normal University) and were maintained for the last 2 years. The rearing was conducted under the dark in incubators at temperature of 28 to 30 °C and 60 to 70% r.h. *Tribolium castaneum* was fed on wheat flour mixed with yeast (10:1, *w*/*w*). The psocid was fed on an artificial diet composed of skim milk, dried yeast, as well as wholemeal (the proportion was 1:1:10). Adult unsexed beetles/booklice (1–2 weeks old) were employed in the further studies. All the insect containers and petri dishes were coated with polytetrafluoroethylene so that insects could not escape.

### Contact toxicity

The contact toxicity towards *T. castaneum* for the EO and major constituents referenced the topical application method (Liu and Ho 1999). Range-finding studies were run to determine the appropriate testing concentrations. The EO and the two compounds were dissolved in *n*-hexane to obtain five series concentrations using serial dilution method. A volume of 0.5 μL of each dilution was dropped on the dorsal thorax of each insect. Both treatments and controls (using *n*-hexane) were put into glass bottles and then placed in incubators. The control and each test concentration were repeated five times with ten adult insects in each replication. Mortalities were recorded after 24 h. The bioassay of psocids was measured as residual film method (Zhao et al. 2012). *n*-Hexane was used to dilute five different concentrations of EO and compounds. A filter paper (5.5 cm diameter) was impregnated with 300 μL dilutions of each sample. Then the filter paper was posted in a Petri dish. Ten psocids were put in a Petri dish placed in an incubator and were exposed for 24 h after the lids were closed. Control group (using *n*-hexane) and each dose group were repeated five times.

### Repellency tests

Repellency towards the red flour beetles for the EO and major constituents was measured following the method of Zhang et al. (2011). Five concentrations (0.13–78.63 nL/cm$^2$) for each sample were designed together with the positive control DEET using *n*-hexane as solvent. Each treatment had a glass Petri dish (9 cm diameter) covered with the same size filter paper. This filter paper was split into two pieces, one half was added with 0.5 mL of each solution and the rest was considered as the control (0.5 mL of *n*-hexane). Both the two pieces were naturally dried leaving the residual *n*-hexane on them. As for psocids, the method used was similar to that described above and the only differences were the diameter of Petri dishes and filter papers (5.5 cm), five series concentration for each sample (0.10–63.17 nL/cm$^2$), and the treatment dose (150 μL). Twenty beetles/booklice were located in the middle of filter paper for each assay, and the Petri dishes were transferred to incubators after the lids of Petri dish were closed. The experiment in each group was repeated five times and the percent repellency (PR) of each sample was counted at 2 and 4 h as follows: PR (%) = [(Nc-Nt)/(Nc + Nt)] × 100, N = number of insects; c = negative control half; t = treated half.

### Data analysis

The mortality data generated in contact toxicity assay were calculated by Abbott's formula to obtain natural mortality (Fleming and Retnakaran 1985). The $LD_{50}$ values, their 95% confidence intervals, slope, and chi-square values were estimated by Probit analysis. In repellency tests, the percentages were normalized using square arcsine transformation before ANOVA. Mean (±SE) values were compared and differed using Tukey's test (SPSS version 19.0, Windows XP) (Jia 2006).

## Results and discussion

### Chemical constituents of essential oil

The aboveground parts of *O. viridiflorum* yielded 0.025% EO. Totally, 22 compounds, accounting for 70.2% of the EO of *O. viridiflorum* aboveground parts, were detected (Table 1). The main constituents of EO from *O. viridiflorum* aboveground parts were β-caryophyllene (24.3%), α-humulene (21.0%), apiol (10.2%), and carotol (2.5%). The results were not in accordance with the only one published report on the components of the EO of *O. viridiflorum* aboveground parts from Shkotovo,

**Table 1** Volatile composition of essential oil from *O. viridiflorum* aboveground parts

| Number | Retention index [a] | Compounds | Relative content (%) |
|---|---|---|---|
| | Monoterpenoids | | 0.6 |
| 1 | 1030 | Limonene | 0.6 |
| | Sesquiterpenoids | | 57.4 |
| 2 | 1376 | Farnesane | 0.2 |
| 3 | 1378 | Copaene | 1.2 |
| 4 | 1386 | α-Bourbonene | 0.1 |
| 5 | 1391 | β-Elemen | 1.1 |
| 6 | 1408 | Isocaryophillene | 0.1 |
| 7 | 1412 | Isoledene | 0.7 |
| 8 | 1423 | β-Caryophyllene | 24.3 |
| 9 | 1456 | α-Humulene | 21.0 |
| 10 | 1458 | Elixene | 0.5 |
| 11 | 1485 | Germacrene D | 0.1 |
| 12 | 1491 | α-Farnesene | 1.2 |
| 13 | 1502 | Cuparene | 0.1 |
| 14 | 1528 | Cadinene | 1.1 |
| 15 | 1582 | Spathulenol | 0.4 |
| 16 | 1589 | β-Caryophyllene oxide | 1.5 |
| 17 | 1606 | Humulene oxide II | 1.2 |
| 18 | 1608 | Dihydro-cis-α-copaene-8-ol | 0.1 |
| 19 | 1574 | Carotol | 2.5 |
| | Others | | 12.2 |
| 20 | 1689 | Apiol | 10.2 |
| 21 | 1716 | Pentadecanal | 1.5 |
| 22 | 1855 | Perhydrofarnesyl acetone | 0.5 |
| | Total identified | | 70.2 |

[a] Retention index in this experiment calculated with a standard *n*-alkanes series in a capillary column HP-5 MS

Shkotovskii District; the major compounds of the EO were caryophyllene oxide (61.7%), 3,4-dimethyl-3-cyclohexan-1-carboxaldehdye (5.8%), isobutyl-2-methylpent-3-yl ester of phthalic acid (5.5%), and vulgarol (4.0%) (Suleimen et al. 2014). Though there was only one report on the constituents of the EO of *O. viridiflorum*, there were a few reports on the components of EOs from other species of *Ostericum*. For example, Song et al. (2015) found that β-phellandrene (38.2%), α-bisabolol (9.4%), *m*-cresol (6.7%), terpinolen (5.5%), and 1-acetoxy-1,2-epoxycyclohexane (5.0%) were the principal constituents of the EO of *O.koreanum* rhizome collected from Jeonju, Korea. Moreover, α-pinene (41.1%), ρ-cresol (18.0%), 4-methylacetophenone (7.9%), sabinene (7.6%), and α-bisabolol (2.1%) were detected in the EO of *O. koreanum* leaves from Chungcheongbuk-do, Jecheon, South Korea (Shin 2005). The differences in EO composition and content from the same species might be arise from various external or internal factors such as the geographical location, collection parts, harvest time, climate, storage duration, as well as plant parts (roots, leaves, flowers, and stems), which may affect different biological activities (Salamon 2007). Therefore, further studies regarding EO quality control and standardization are required.

## Contact toxicity

The EO showed potent contact toxicity towards psocids as it exhibited a $LD_{50}$ value of 44.52 μg/cm$^2$, but displayed weak toxicity against *T. castaneum* within measurement range (Table 2). The EO possessed only 2.4 times less toxicity than pyrethrins (positive control, $LD_{50}$ = 18.72 μg/cm$^2$) against psocids. Meanwhile, the EO showed higher contact toxicity against psocids than the two main compounds, which had almost 2.7 and 1.7 times more toxicity than α-humulene ($LD_{50}$ = 118.56 μg/cm$^2$) and β-caryophyllene ($LD_{50}$ = 74.11 μg/cm$^2$) against psocids. The high toxicity of the EO against psocids could be due to the interaction between major compounds and others. The other ingredients in the oil often play a key part in contact toxicity. Such as β-caryophyllene oxide (1.5%), it exhibited strong contact toxicity against psocids with $LD_{50}$ values of 35.40 μg/cm$^2$ and 27.20 μg/cm$^2$ respectively in the previous reports (Cao et al. 2018a, b). From the above, β-caryophyllene oxide and structural analogues (α-humulene and β-caryophyllene) all demonstrated potent contact toxicity against psocids. By comparing the chemical structures with their bioactivity, these three sesquiterpenes had the same sesquiterpene structure (an 11-membered macrocycle). However, as for the red flour beetles, α-humulene ($LD_{50}$ = 14.23 μg/cm$^2$), β-caryophyllene ($LD_{50}$ = 41.72 μg/cm$^2$), and β-caryophyllene oxide ($LD_{50}$ = 45.56 μg/cm$^2$) (Liu et al. 2012) exhibited less toxicity (no overlap in 95% fiducial limit) than the positive control, pyrethrins ($LD_{50}$ = 0.26 μg/cm$^2$), suggesting that the sesquiterpene structure may have more effective toxic properties against the psocids than against the red flour beetles. It should be added that further research is needed to explore the insecticidal mechanism of above sesquiterpene hydrocarbons.

## Repellent activity

The repellent effect produced by the EO and its major sesquiterpenes towards red flour beetles and psocids was observed in Tables 3 and 4. It was found that this EO demonstrated high repellency against both *T. castaneum* and *L. bostrychophila*, especially towards *T. castaneum*. The EO and DEET exhibited the same level of repellent activity against red flour beetles at high concentrations (78.63 and 15.73 nL/cm$^2$) after 2- and 4-h treatment. Moreover, at low concentrations, EO even exhibited higher repellency than DEET against red flour beetles. For example, at three low concentrations (3.15, 0.63, and 0.13 nL/cm$^2$), EO (80, 72, and 52%, respectively) exhibited significantly stronger repellency than DEET (68, 54, and 22%, respectively) against *T. castaneum* at 4 h. Unlike the repellency

**Table 2** Results of contact toxicity towards *T. castaneum* (TC) and *L. bostrychophila* (LB) adults for the essential oil and major compounds

| Insects | Treatment | Doses (%) | Mortality insects/total insects | $LD_{50}$ (μg/adult) (μg/cm$^2$) | 95% FL | Slope ± SE | Chi-square ($\chi^2$) | *P* value |
|---|---|---|---|---|---|---|---|---|
| TC | *O. viridiflorum* | 0–50.00 | 34/90 | – | – | – | – | – |
| | α-Humulene | 1.98–10.00 | 127/250 | 14.23 | 11.73–16.58 | 2.60 ± 0.37 | 7.67 | 0.999 |
| | β-Caryophyllene | 3.95–20.00 | 99/250 | 41.72 | 37.94–45.52 | 5.28 ± 0.54 | 18.58 | 0.725 |
| | Pyrethrins [a] | – | – | 0.26 | 0.22–0.30 | 3.34 ± 0.32 | 13.11 | 0.950 |
| LB | *O. viridiflorum* | 0.49–1.00 | 203/250 | 44.52 | 40.60–48.71 | 5.49 ± 0.36 | 25.40 | 0.568 |
| | α-Humulene | 0.40–2.00 | 107/250 | 118.56 | 108.01–130.69 | 4.90 ± 0.52 | 10.04 | 0.886 |
| | β-Caryophyllene | 0.49–1.00 | 122/250 | 74.11 | 71.26–77.75 | 10.40 ± 1.08 | 20.11 | 0.635 |
| | Pyrethrins [b] | – | – | 18.72 | 17.60–19.92 | 2.98 ± 0.40 | 10.56 | 0.987 |

[a] Data from You et al. (2014)

[b] Data from Yang et al. (2014)

**Table 3** Repellency towards *T. castaneum* adults for the essential oil and major compounds after 2- and 4 h treatment

| Treatment | 2 h/(nL/cm$^2$) | | | | | 4 h/(nL/cm$^2$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 78.63 | 15.73 | 3.15 | 0.63 | 0.13 | 78.63 | 15.73 | 3.15 | 0.63 | 0.13 |
| Essential oil | 96 ± 3ab | 98 ± 2b | 94 ± 6b | 82 ± 12b | -8 ± 14a | 94 ± 6a | 82 ± 8b | 80 ± 10b | 72 ± 5c | 52 ± 14b |
| $\alpha$-Humulene | 94 ± 6ab | 90 ± 6b | 46 ± 7a | 6 ± 6a | 2 ± 7ab | 92 ± 7a | 72 ± 5b | 32 ± 7a | − 4 ± 7a | − 30 ± 6ab |
| $\beta$-Caryophyllene | 82 ± 8a | 38 ± 7a | 30 ± 4a | 16 ± 7a | 22 ± 5b | 98 ± 3a | 32 ± 8a | 28 ± 7a | 30 ± 6b | 16 ± 6a |
| DEET | 100 ± 0b | 98 ± 3b | 78 ± 14b | 66 ± 10b | 8 ± 5ab | 96 ± 3b | 82 ± 8b | 68 ± 5b | 54 ± 8bc | 22 ± 8a |

Values are mean (% ± SE)

The difference among values in the same column with different letters is significant ($P < 0.05$) in ANOVA and *Tukey*'s tests. Values were normalized using square arcsine transformation before analysis

on *T. castaneum*, data in Table 4 explained that the EO had high repellency against psocids only at the high concentrations (≥ 12.63 nL/cm$^2$). At high concentrations (63.17 and 12.63 nL/cm$^2$), compared with DEET, the EO showed higher repellency against psocids after 2- and 4-h treatment. Especially at the highest dose (63.17 nL/cm$^2$), the EO both showed 100% repellency against psocids at 2 and 4 h when compared with DEET (94% and 92%). The repellency of the EO tended to reduce with decreasing concentration of sample. At the lowest concentration (0.10 nL/cm$^2$), compared with DEET (56% and 28%), the EO (10% and 8%) showed weaker repellency against psocids at 2 and 4 h after exposure. The two main compounds $\alpha$-humulene and $\beta$-caryophyllene showed different levels of repellency against the two insects. As for *T. castaneum*, at testing doses of 78.63, 15.73, and 3.15 nL/cm$^2$, $\alpha$-humulene demonstrated stronger repellent activity than $\beta$-caryophyllene at 2 and 4 h, while $\beta$-caryophyllene showed stronger repellent activity than $\alpha$-humulene at testing doses of 0.63 and 0.13 nL/cm$^2$ at both 2 and 4 h. Further, at the dose of 0.13 nL/cm$^2$, $\beta$-caryophyllene (22%) showed much stronger repellency at 2 h relative to DEET (8%). However, as for *L. bostrychophila*, at five tested concentrations, $\beta$-caryophyllene exhibited higher repellency than $\alpha$-humulene at both 2 and 4 h, except for the concentration of 0.10 nL/cm$^2$ at 4 h. Furthermore, at testing dose of 63.17 and 12.63 nL/cm$^2$, the PR of $\beta$-caryophyllene both reached 100% at 2 h when compared with DEET (94% and 82%), suggesting that $\beta$-caryophyllene was strongly repellent towards *L. bostrychophila*. The repellent activity of $\alpha$-humulene and $\beta$-caryophyllene has also been observed towards other insects. For example, previous research in the literature reported that $\alpha$-humulene and $\beta$-caryophyllene both showed strong repellency on *Ixodes ricinus* (Ashitani et al. 2015). Moreover, $\alpha$-humulene and $\beta$-caryophyllene achieved various degrees of repellency against *Tetranychus urticae* (Araújo et al. 2012). However, as for *Aedes aegypti*, $\alpha$-humulene showed weak repellency, $\beta$-caryophyllene was not active repellent even at the maximum test dose (Zhai et al. 2017).

$\alpha$-Humulene and $\beta$-caryophyllene were similar in chemical structure and were often found together as a mixture in many aromatic plants, but their bioactivities were different in this study. It is interesting to note that $\alpha$-humulene possessed higher contact toxicity than $\beta$-caryophyllene against *T. castaneum*; however, $\alpha$-humulene had less toxicity than $\beta$-caryophyllene against booklice. In repellency assay, $\beta$-caryophyllene demonstrated higher repellency than $\alpha$-humulene against red flour beetles only at the low concentrations (0.63 and 0.13 nL/cm$^2$), while $\beta$-caryophyllene possessed comparatively higher repellency than $\alpha$-humulene against booklice almost at five tested concentrations. $\alpha$-Humulene is a natural cyclic sesquiterpene with an 11-membered ring containing three isoprene units (Neuenschwander et al. 2012). $\beta$-

**Table 4** Repellency towards *L. bostrychophila* adults for the essential oil and major compounds after 2- and 4 h treatment

| Treatment | 2 h/(nL/cm$^2$) | | | | | 4 h/(nL/cm$^2$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 63.17 | 12.63 | 2.53 | 0.51 | 0.10 | 63.17 | 12.63 | 2.53 | 0.51 | 0.10 |
| Essential oil | 100 ± 0a | 82 ± 8a | 8 ± 7a | 2 ± 15a | 10 ± 9a | 100 ± 0a | 94 ± 3b | 24 ± 13a | 8 ± 8a | 8 ± 13ab |
| $\alpha$-Humulene | 84 ± 10a | 60 ± 13a | 46 ± 3b | 20 ± 6ab | 16 ± 7a | 82 ± 11a | 52 ± 12a | 30 ± 13a | 18 ± 5a | 10 ± 9ab |
| $\beta$-Caryophyllene | 100 ± 0a | 100 ± 0b | 82 ± 8c | 62 ± 5bc | 20 ± 10a | 100 ± 0a | 94 ± 8b | 74 ± 8b | 18 ± 5a | − 10 ± 10a |
| DEET | 94 ± 8a | 82 ± 5a | 86 ± 8c | 70 ± 12c | 56 ± 3a | 92 ± 5a | 84 ± 3b | 82 ± 8b | 54 ± 7b | 28 ± 14b |

Values are mean (% ± SE)

The difference among values in the same column with different letters is significant ($P < 0.05$) in ANOVA and *Tukey*'s tests. Values were normalized using square arcsine transformation before analysis

Caryophyllene is an isomer of humulene and has a humulene macrocycle structure with a bicyclic 4/9 ring system, in which a trans double bond in the nine-membered ring (Tanaka et al. 1996). The molecular structural differences of the two compounds and the mechanism of insect species may account for the significant difference in bioactivities.

On the other hand, $\alpha$-humulene and $\beta$-caryophyllene were natural isomeric sesquiterpenes, widely existing in the EOs of many medicinal and spice plants. $\beta$-Caryophyllene has been shown to have various biological activities, such as antimicrobial, phage repellent, local anesthetic, anti-inflammatory, antitumoral, anti-allergic, as well useful as a flavoring agent (Tambe et al. 1996; Budavari et al. 1996; Sköld et al. 2006). Moreover, $\beta$-caryophyllene exhibited good activities against a wide range of insects, e.g., the larvae of mosquito vectors (*Aedes albopictus*, *Anopheles subpictus*, and *Culex tritaeniorhynchus*), *Spodoptera frugiperda*, *T. castaneum*, *Lasioderma serricorne*, and *Cacopsylla chinensis* (Govindarajan et al. 2016; Cárdenas-Ortega et al. 2015; Chaubey 2012; Pandey and Singh 2017; Tian et al. 2015). In addition, $\alpha$-humulene was also associated with many bioactivities (such as anti-tumor, anti-fungal, anti-asthma, anti-allergies, anti-inflammatory, and inhibition of drug-metabolizing enzymes) and was often used as an inevitable ingredient during the brewing process (Legault et al. 2003; Sabulal et al. 2006; Rogerio et al. 2010; Passos et al. 2007; Nguyen et al. 2017; Connolly and Hill 1991). In recent studies, $\alpha$-humulene was reported to have bioactivities in control of insects, e.g., *L. serricorne*, *L. bostrychophila*, larvae of *A. subpictus*, *A. albopictus*, and *C. tritaeniorhynchus*, and *Helicoverpa armigera* (Guo et al. 2017; Govindarajan and Benelli 2016; Benelli et al. 2018).

In conclusion, the above results indicated that the EO and its two main constituents would be the exploration direction of botanical insecticides and repellents in controlling stored product insects.

## Conclusion

The present study analyzed the composition of the EO of *O. viridiflorum* aboveground parts and reported for the first time the toxicity and repellent activity of the EO against the two storage pests. The results of this study demonstrate that the EO and the two selected constituents could play a vital role in the integrated pest management of stored products and safeguard the environment and health of the user from the risk of the use of synthetic insecticides. Moreover, the EOs from three species (*O. viridiflorum*, *O. sieboldii*, and *O. grosseserratum*) of *Ostericum* all showed bioactivities against stored product insects in our series of studies. This work would not only provide a scientific foundation for further development and utilization of *O. viridiflorum* resource in control of stored product insects but also establish a very good perspective for comprehensive utilization of plant resources of *Ostericum* genus. However, there are also a number of challenges (such as stability, standardization of chemical composition, quality control, non-target toxicity, and micro-encapsulation of the most effective molecules) for EOs to face, which deserve further study and discussion.

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