Ecotoxicology and Environmental Safety 160 (2018) 342–348

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Toxicity and repellency of essential oil from *Evodia lenticellata* Huang fruits and its major monoterpenes against three stored-product insects

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ARTICLE INFO

Keywords:
Evodia lenticellata
Fumigant toxicity
Contact toxicity
Repellency
Monoterpenes
Stored-product insects

ABSTRACT

In this work, the essential oil (EO) was extracted from the fruits of *Evodia lenticellata*, and the fumigant toxicity, contact toxicity and repellency against three stored-product insect species were evaluated for the obtained EO and several of its chemical components. The target insects were the adults of *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Lasioderma serricorne* (Coleoptera: Anobiidae) and *Liposcelis bostrychophila* (Psocoptera: Liposcelididae). The EO was obtained with hydrodistillation and its chemical components were analyzed with the gas chromatography-mass spectrometry (GC-MS). Twenty-seven compounds, accounting for 83.1% of the total amount of the oil, were identified from the EO sample. The main compounds included linalool (12.0%), *β*-pinene (11.5%), 3-carene (9.6%), caryophyllene oxide (8.7%) and *β*-caryophyllene (7.9%). Among them, the amounts of monoterpenes and sesquiterpenes were as high as 52.7% and 22.7% to the total amount of EO respectively. The results of bioactivity test showed that the EO and its testing compounds had interspecific toxicity and repellent activity. So that, it might be expected that the EO extracted from the fruits of *E. lenticellata* could be developed to a new type of eco-friendly natural insecticide or repellent for the control of stored-product insects.

1. Introduction

The damage caused by stored-product insects includes weight loss, nutrient loss, contamination and health risks to consumers (Hagstrum and Subramanyam, 2006). In this work, *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Lasioderma serricorne* (Coleoptera: Anobiidae) and *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) were selected as the target insects. *T. castaneum* is an important cosmopolitan pest with short life cycle and high fecundity. It feeds and reproduces on a wide range of grains and value-added food products across the storage system and supply chain (Richards et al., 2008). *L. serricorne* is one of the most varied taste storage insect. It breeds on stored cereals, tobacco, seeds, dried fruits and even animal matter (Howe, 1957). *L. bostrychophila* is a worldwide distributed insect pest as reported. This specie has a remarkable reproductive capacity owing to its parthenogenetic charact eristic (Bryan, 1994). Synthetic insecticides have been used as one of the popular control method for stored-product insects. However, they have long been considered to be replaced due to their unexpected toxicity on non-target organisms, endangerment on human health and the environment safety (Arthur, 1996; Isman, 2000).

Botanical insecticides have been considered as suitable alternatives to conventional insecticides for pest control owing to insecticidal potential of their secondary chemicals inspired by plant-insect chemical interactions (Miresmailli and Isman, 2014). Meanwhile, they are relatively low toxic to environment and human health contrasted to synthetic chemical insecticides (Isman, 2006). Essential oils are one of the major types of botanical products which have been used for insect control, owing to their low risk to the environment and non-target insects. This is because their volatile nature leads minimal residual activity (Isman, 2006). Another benefit of the EOs would be the slowly developed resistance owing to the complex mixtures of the compounds (Koul et al., 2008). Essential oils are usually mixtures of monoterpenes, sesquiterpenes, aromatic and aliphatic compounds with small-molecular-weight and high volatility (Bassolé and Juliani, 2012). Most of the compounds in essential oils are nontoxic to mammals, birds, and fishes (Stroh et al., 1998). Monoterpenes are usually cited as one of the

Abbreviations: E. lenticellata, *Evodia lenticellata*; EO, essential oil; *T. castaneum*, *Tribolium castaneum*; *L. serricorne*, *Lasioderma serricorne*; *L. bostrychophila*, *Liposcelis bostrychophila*; RI, Retention Index; MS, mass spectrum; DEET, N, N-diethyl-3-methyl-benzamide

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https://doi.org/10.1016/j.ecoenv.2018.05.054
Received 28 December 2017; Received in revised form 6 April 2018; Accepted 22 May 2018

dominant factors responsible for biological activities of EOs (Habtemariam, 2017). They are used as contact toxicants, fumigants, attractants or repellents to control insect pests (Miresmailli and Isman, 2014).

Evodia of family Rutaceae were reported to possess insecticidal and repellent activity against stored-product insects. e.g., Essential oil of *Evodia rutaecarpa* unripe fruits showed fumigant toxicity against *Sitophilus zeamais* and *T. castaneum* adults with LC_{50} values of 36.89 and 24.57 mg/L air (Liu and Du, 2011). *E. rutaecarpa* unripe fruits is officially listed in the *Chinese Pharmacopoeia* and used as an analgesic, antiemetic, anti-inflammatory, astringent agents and treatment of hypertension (Tang and Eisenbrand, 1992). The major compounds isolated from *E. rutaecarpa* were identified as dehydroevodiamine, evodiamine and rutaecarpine, displaying biological activities related to inflammatory (Tang and Eisenbrand, 1992). *Evodia lenticellata*, mainly distributed in Southwest Shannxi province of China, belonging to family Rutaceae, has similar morphology and medicinal effects as the fruit of *Evodia rutaecarpa* (Ma, 2016). Evodiamine and rutaecarpine have also been isolated from *E. lenticellata* fruits as major active constitutes which exhibit similar activities as *E. rutaecarpa* fruits (Ma, 2016). Besides, EOs from *Evodia calcicola* and *Evodia trichotoma* leaves were also reported exhibit strong repellency to *T. castaneum*, *L. serricorne* and *L. bostrychophila* (Yang et al., 2014b). These prompt us to conduct a systematic investigation on bioactivity of this genus against stored-product insects. Hence, in this research, we focus our attention on this overlooked local traditional medicine herbs, *E. lenticellata*, which is selected as a source of compounds showing insecticidal activity against three target stored-product insect adults, *T. castaneum*, *L. serricorne* and *L. bostrychophila*. Meanwhile, no published studies so far have evaluated the insecticidal activity of *E. lenticellata* fruit EO on stored-product insects. Thus, it is reasonable to hypothesize that EO of *E. lenticellata* fruit may also exhibit insecticidal and repellent activity against stored-product insects.

2. Materials and methods

2.1. Essential oil extraction

The *E. lenticellata* ripe fruits were collected from Hanzhong, Shannxi Province, China (Latitude 32°08'–33°53' N, Longitude 105°30'–108°16' E) in November 2017. The sample was identified by Dr. Liu, Q.R. and the voucher specimen (BNU-dushushan-20171208) was deposited at the Herbarium (BNU) of College of Resources Science and Technology, Beijing Normal University. The fruits were air-dried. The sample was weighted and ground to powder, then transferred into a modified *Clevenger-type* apparatus for 6 h. The extracted essential oil was dehydrated with anhydrous sodium sulfate and stored in airtight containers at 4 °C.

2.2. Gas chromatography and mass spectrometry

GC-MS analysis was performed on a Thermo Finnigan Trace DSQ instrument coupled with a flame ionization detector (FID) and a capillary column of HP-5MS (30 m × 0.25 mm × 0.25 μm). The GC-MS settings were programmed as follows: initial oven temperature was held at 50 °C for 2 min, rising to 150 °C at 2 °C/min and increased to 250 °C at 10 °C/min, then kept for 5 min. Injector temperature was maintained at 250 °C and the volume injected was 1 μL of 1% solution (diluted in n-hexane). Carrier gas used was helium at a flow rate of 1.0 mL/min. Spectra were scanned from 50 to 550 *m/z*. Under the same operating conditions, the retention indices were determined in relation to a homologous series of n-alkanes (C_5-C_{36}). Further identification was made by comparing their mass spectra with those stored in NIST 05 (Standard Reference Data, Gaithersburg, MD) and Wiley 275 libraries (Wiley, New York, NY) or with mass spectra from literature (Adams, 2001). Relative percentages of the individual components of the essential oil were obtained by averaging the GC-FID peak area% reports.

2.3. Insects culture

The insects are reared in glass containers (0.5 L) with incubators (29 ± 1 °C and 65% ± 5% relative humidity) that are kept in permanent darkness. The red flour beetles (*T. castaneum*) and cigarette beetles (L. *serricorne*) are reared on wheat flour mixed with additional 10% yeast. L. *bostrychophila* was reared with the mixture of flour, milk powder and yeast (the mass ratio is 10:1:1). The unsexed adults of all species used in the tests were two weeks post-emergence.

2.4. Bioactivity

2.4.1. Fumigant toxicity

The five concentrations of essential oil and individual compounds were determined by pre-experiments (maximum concentration, 50%, V/V). The testing samples were dissolved separately in acetone to prepare serials of testing solutions. The treatments were experimented as the method described by Liu and Ho (1999). A Whatman filter paper (diameter 2.0 cm) was laid at the bottom of the screw cap and impregnated with 10 μL dilution. The solvent was allowed to evaporate for 20 s before the cap was placed tightly on the glass vial (diameter 2.5 cm, height 5.5 cm, volume 25 mL), each of which contained 10 insects inside. The negative control was acetone. Five replications were performed for each treatment. After 24 h, record the number of dead insects.

2.4.2. Contact toxicity

Contact toxicity test method of essential oil and compounds against *T. castaneum* and L. *serricorne* are similar to that described by Liu and Ho (1999). The samples were serially diluted with acetone to a suit of concentrations according to the pre-experiments (maximum concentration, 50%, V/V). Volume of 0.5 μL prepared solution was applied to dorsal thorax of each insect. The control group was treated only with acetone. The treated insects were then transferred to clean glass vial and kept in the aforementioned incubator. Each treatment was replicated five times. After 24 h, the mortality was recorded.

The test of L. *bostrychophila* was run as described (Zhao et al., 2012). A serial dilution of the EO and compounds (five concentrations) were prepared in acetone (the highest concentration of pre-experiment is 5%, V/V). The filter paper disks (5.5 cm in diameter) were treated with 300 μL of the diluted solution and then adhered to the bottom of the same size Petri dish. Twenty booklice were introduced at the center of the filter paper by using a hair brush then the Petri dish was covered with lid. Controls received only acetone. Five replications were run for each concentration. Mortality of insects was observed after 24 h.

2.4.3. Repellency tests

The area preference method (Zhang et al., 2011) was used to evaluate repellency of the EO and compounds against *T. castaneum* and L. *serricorne*. The filter paper disk (9 cm in diameter) was cut into semicircles. One half was treated with 500 μL of acetone solutions of the samples (0.13–78.63 nL/cm^2) and the other half was treated with acetone as control. Reassemble the two halves into disk again after the solvent was evaporated (85 s), and then attach them at the bottom of the same size Petri dish. Twenty adults were introduced at the center of the filter paper then the Petri dish was covered with a lid. As for L. *bostrychophila*, the same method was used. The differences are the diameter of filter paper disk (5.5 cm in diameter), concentrations of the samples (0.10–63.17 nL/cm^2) and the volume of the solution used to treat the semicircles (150 μL). Each treatment was replicated five times and numbers of insects presented on the control (Nc) and the testing (Nt) halves were recorded after 2 and 4 h, respectively. A commercial repellent, N, N-diethyl-3-methylbenzamide (DEET), was used as a