



저작자표시-비영리-동일조건변경허락 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.
- 이차적 저작물을 작성할 수 있습니다.

다음과 같은 조건을 따라야 합니다:



저작자표시. 귀하는 원저작자를 표시하여야 합니다.



비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.



동일조건변경허락. 귀하가 이 저작물을 개작, 변형 또는 가공했을 경우에는, 이 저작물과 동일한 이용허락조건하에서만 배포할 수 있습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#)

**A Thesis
for the Degree of Master of Science**

**Insecticidal Activity of 28 Essential Oils and
a Commercial Product Containing
Cinnamomum cassia Bark Essential Oil against
Sitophilus zeamais Motschulsky**

어리쌀바구미(*Sitophilus zeamais*)에 대한
시나몬(*Cinnamomum cassia*) 정유를 함유한 제품과
28종 에센셜 오일의 살충 활성

**By
Yunho Yang**

**Major in Entomology
Department of Agricultural Biotechnology
Seoul National University
February 2021**

**Insecticidal Activity of 28 Essential Oils and
a Commercial Product Containing
Cinnamomum cassia Bark Essential Oil against
Sitophilus zeamais Motschulsky**

어리쌀바구미(*Sitophilus zeamais*)에 대한
시나몬(*Cinnamomum cassia*) 정유를 함유한 제품과
28종 에센셜 오일의 살충 활성

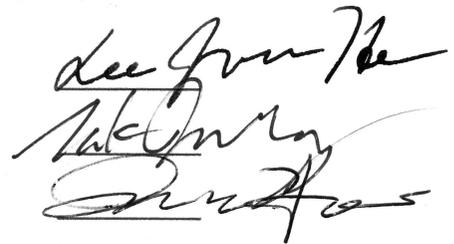
**Under the Direction of Adviser Jun-Hyung Tak
Submitted to the Faculty of the Graduate School of
Seoul National University**

**By
Yunho Yang**

**Major in Entomology
Department of Agricultural Biotechnology
Seoul National University
February 2021**

**Approved as a Qualified Thesis of Yunho Yang
for the Degree of Master of Science
By the Committee Members**

Chair	Joon-Ho Lee
Vice Chair	Jun-Hyung Tak
Examiner	Yeon Ho Je



Abstract

Insecticidal Activity of 28 Essential Oils and a Commercial Product Containing *Cinnamomum cassia* Bark Essential Oil against *Sitophilus zeamais* Motschulsky

Yunho Yang
Major in Entomology
Department of Agricultural Biotechnology
Seoul National University

The maize weevils, *Sitophilus zeamais*, are stored product pests mostly found in warm and humid regions around the globe. In the present study, acute toxicity via contact and residual bioassay and fumigant bioassay of 28 essential oils as well as their attraction-inhibitory activity against the adults of *S. zeamais* were evaluated. Chemical composition of the essential oils was analyzed by gas chromatography-mass spectrometry, and a compound elimination assay was conducted on the four most active oils (cinnamon, tea tree, ylang ylang, and marjoram oils) to identify major active constituents. Amongst the oils examined, cinnamon oil was the most active in both contact/residual and fumigant bioassays and exhibited strong

behavioral inhibitory activity. Based on the compound elimination assay and chemical analyses, *trans*-cinnamaldehyde in cinnamon oil, and terpinen-4-ol in tea tree and marjoram oils were identified as the major active components. Although cinnamon oil seemed promising in the lab-scale bioassay without rice grains, it failed to exhibit strong insecticidal activity when the container was filled with rice. When a cinnamon oil-based product was applied both in an empty glass jar and a rice-filled container, all weevils in the empty jar were killed, whereas fewer than 15% died in the rice-filled container. Further study is required to understand the absorption of active fumigants by grains, and to enhance efficacy through the formulation for household products.

Keyword: maize weevil, essential oil, fumigant toxicity, contact toxicity, attraction inhibition, formulation

Student Number: 2019-23828

Table of Contents

Abstract	i
Table of Contents	iii
List of Tables	v
List of Figures	vii
Introduction	1
Materials and Methods	5
1. Test insects.....	5
2. Test materials.....	7
3. Chemical composition of the test materials.....	9
4. Bioassays	11
4.1. Acute toxicity assay	11
4.2. Behavioral attraction–inhibitory assay.....	15
4.3. Compound elimination assay	17
4.4. Efficacy of a commercial product.....	18
5. Statistical analysis.....	19
Results	20
1. Insecticidal activity of plant essential oils	20
2. Attraction–inhibition <i>via</i> no-choice assay	23

3. Chemical composition of active essential oils.....	25
4. Comparative toxicity of the major constituents.....	27
5. Composition and efficacy of the commercial product.....	32
Discussion	34
Conclusions	43
Bibliography.....	44
Supplementary Materials.....	49
Abstract in Korean	66

List of Tables

Table 1. Plant species and essential oils tested in the this study.....	10
Table 2. Insecticidal activity of 28 essential oils against <i>Sitophilus zeamais</i> adults	22
Table 3. Chemical constituents of four most active essential oils	26
Table 4. Insecticidal activity of a commercial product on the adult of the maize weevil.....	33
Tables S1. Chemical constituents of basil essential oil.	49
Tables S2. Chemical constituents of bergamot essential oil.....	50
Tables S3. Chemical constituents of cinnamon essential oil.	50
Tables S4. Chemical constituents of citronella essential oil.....	51
Tables S5. Chemical constituents of clary sage essential oil.....	52
Tables S6. Chemical constituents of clove bud essential oil.	52
Tables S7. Chemical constituents of cypress essential oil.....	53
Tables S8. Chemical constituents of <i>Eucalyptus globulus</i> essential oil.....	53
Tables S9. Chemical constituents of <i>Eucalyptus radiata</i> essential oil.....	54
Tables S10. Chemical constituents of fennel essential oil.....	54

Tables S11. Chemical constituents of fennel sweet essential oil.	55
Tables S12. Chemical constituents of frankincense essential oil.....	55
Tables S13. Chemical constituents of geranium essential oil.....	56
Tables S14. Chemical constituents of lavender (French) essential oil.....	56
Tables S15. Chemical constituents of lavender (Bulgarian) essential oil.....	57
Tables S16. Chemical constituents of lemon essential oil.....	57
Tables S17. Chemical constituents of lemongrass essential oil.....	58
Tables S18. Chemical constituents of mandarin essential oil.....	58
Tables S19. Chemical constituents of marjoram essential oil.....	59
Tables S20. Chemical constituents of orange sweet essential oil.....	60
Tables S21. Chemical constituents of patchuli essential oil.....	61
Tables S22. Chemical constituents of peppermint essential oil.....	62
Tables S23. Chemical constituents of pine essential oil.....	62
Tables S24. Chemical constituents of rosemary essential oil.....	63
Tables S25. Chemical constituents of sandal wood essential oil.....	63
Tables S26. Chemical constituents of spearmint essential oil.....	64
Tables S27. Chemical constituents of tree essential oil.....	64
Tables S28. Chemical constituents of ylang ylang essential oil.....	65

List of Figures

Figure 1. Rearing cages of <i>S. zeamais</i>	6
Figure 2. Schematic diagram of contact and residual application assay arena.....	12
Figure 3. Schematic diagram of fumigation assay arena.....	13
Figure 4. Schematic diagram of attraction–inhibitory activity assay	15
Figure 5. Attraction–inhibition activity of essential oils	24
Figure 6. Compound elimination assay of cinnamon, tea tree and marjoram oils via contact and residual application.....	29
Figure 7. Compound elimination assay of cinnamon, tea tree and ylang ylang oils via fumigation assay	30
Figure 8. Correlation among contact/residual, fumigation, and anti-attraction activity of 28 essential oils tested.....	31

Introduction

Stored-product weevils including the maize weevil, *Sitophilus zeamais* Motschulsky, and the rice weevil, *S. oryzae* Linnaeus, both belonging to the family Curculionidae, show cosmopolitan distribution as occurring in numerous warm and humid regions worldwide (López-Castillo et al., 2018). According to the database of Invasive Species Compendium (ISC), *S. zeamais* was reported to be present in 150 regions from 107 countries worldwide (CABI, 2019). In the case of South Korea, Hong et al. (2018) examined 52 Rice processing complexes (RPCs) from 2015 to 2016 using corrugated cardboard traps and by sampling rice grains. Established colonies of *S. oryzae* were found in four out of 52 RPCs, whereas the rest of the RPCs were found to have *S. zeamais* infestation, indicating the maize weevils as the predominant species in South Korean granaries.

The maize weevils not only cause significant damage in stored grains including the reduction in nutritional quality, weight and germination rates of seeds in developing countries (Barney et al., 1991; Caneppele et al., 2003), but are also associated with human health due to allergen production and food safety in developed countries as well, since they can transmit fungi including *Aspergillus flavus* and several types of bacteria (Hubert et al., 2018). These damages and losses inflicted on the grains are closely related

to the *Sitophilus* spp.'s destructive life cycle. A female weevil drills a hole in a grain and lays eggs. After oviposition, the hole is plugged with a mucilaginous seal secreted by the female weevil. The hatched larvae grow as feeding the grain from inside and complete their developments within the grain until emergence (Devi et al., 2017). In addition, in the case of outbreaks, the increase in temperature and humidity caused by the metabolism creates a favorable environment for fungi (Sinha and Sinha, 1991).

Control of stored grain insect pests including the maize weevils has been largely relying on fumigation agents. An organobromine compound, methyl bromide, was extensively used until being phased out and banned due to environmental issues (Ristaino and Thomas, 1997). The restriction (or regulation) on the use of methyl bromide let phosphine gas to become the most frequently selected fumigant for stored product pests control. Nonetheless, heavy reliance on phosphine treatment has resulted in the global-scale development of insecticide resistance in numerous stored product insects including *Sitophilus* spp. in grain stores (Nayak et al., 2020) as well as in the food industry and flour mills (Aulicky et al., 2015). Ethyl formate are currently also being used as an eco-friendly substitute for methyl bromide to control the insect pests in dried fruits and in stored grains. Ethyl formate is a volatile compound which can be found in a variety of products including cheese and wine and in a body of ants and stingers of bees, and generally recognized as safe by the U.S. Food and Drug

Administration (21CFR184.1295). While ethyl formate left no or low residues after fumigation, insecticidal effect was largely influenced by the sorption to the grains (Damcevski and Annis, 2006). On top of it, due to the strong volatility of ethyl formate, there is a potential flammability hazard while fumigating the area or during the storage, which requires special attention (Ryan and De Lima, 2014). In order to compensate these shortcomings, Haritos et al. (2006) combined carbon dioxide (5-20%) with ethyl formate, and this formulation enhanced efficacy by contributing even distribution of fumigant between grains and lowering its flammability.

Aside from phosphine and ethyl formate treatments, there are considerable interests in screening and developing safer alternatives of them, and botanicals have been receiving great attention from many researchers. With a few exceptions such as nicotine, botanicals tend to pose little threat to human health and the environment owing to their low mammalian toxicity and minimal environmental persistence (Isman, 2006). An essential oil, which is one of the plant-derived extracts, can be extracted from various parts of plants including barks, flowers, buds, leaves, peels, and resins, mainly *via* a steam distillation method, and may contain hundreds of different monoterpenes, sesquiterpenes, and their derivatives. Essential oils are known to display various biological activities including acute and chronic toxicity, repellent activity, and inhibition of oviposition, growth, feeding and development against insect pest species (Benelli et al., 2017; Papachristos and Stamopoulos, 2002; Reddy et al., 2016). In addition,

attempts have been made to enhance the insecticidal efficacy on insect pests by incorporating essential oils to novel formulations such as chitosan nanocapsules, zein nanoparticles, micro/nanofibrous matrices, and β -cyclodextrin inclusion complexes (Ahmadi et al., 2018; de Oliveira et al., 2019; Iliou et al., 2019; Galvão et al., 2019).

The aim of this study was to evaluate the insecticidal activity of 28 essential oils and their attraction–inhibitory (i.e., deterrent) activity to rice grains using the adults of *S. zeamais* in laboratory bioassays. GC-MS analyses and compound elimination assays were performed to identify the major active constituents of the active oils, and the efficacy of a cinnamon oil-bearing commercial product was examined as well.

Materials and Methods

1. Test insects

The maize weevils used in this study was originally collected from home storages in Yongin, South Korea (37° 11' 02.2" N 127° 12' 24.8" E) in late 2018, and the colony had been maintained since then in an insectary at Seoul National University, Seoul, South Korea, without exposure to any known insecticides at 26 ± 1 °C, 50–60% RH, and a 14:10 h L:D photoperiod. The colony was kept in a 2 L plastic container containing 800 g of rice grains (*Oryza sativa* L.) (Figure 1). The rice grains in the breeding container were exchanged for new rice grains every two months or when corrupted with fungi. Unsexed (both male and female) adult weevils less than 1-month old were used in all experiments.



Figure 1. *S. zeamais* colony maintained.

2. Test materials

The essential oils used in the present study are listed in Table 1. Bergamot, mandarin, and orange sweet oils were obtained via a cold-pressing process, and all the remaining oils were prepared via a steam distillation method from various plant parts including barks, flowers, buds, leaves, peels, and resins. They were purchased from Absolute Aromas (Hampshire, UK), Klimtech (Dimitrovgrad, Bulgaria), Plant Therapy (Twin Falls, ID, USA), or Sun Essential Oils (Phoenix, AZ, USA).

Pure chemical compounds in the essential oils were obtained in their technical grades, which were of the highest purity available. *o*-Cymene (>99.0%) and (E)-4-methoxycinnamaldehyde (>97.0%) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), and benzyl acetate ($\geq 99\%$), benzyl benzoate ($\geq 99\%$), benzyl salicylate (98%), β -caryophyllene ($\geq 80\%$), *trans*-cinnamaldehyde (99%), cinnamyl acetate (99%), coumarin ($\geq 99\%$), eucalyptol (99%), geranyl acetate ($\geq 97\%$), linalool (97%), linalyl acetate ($\geq 97\%$), methyl benzoate (99%), 4-methylanisole (99%), (+)- α -pinene (98%), (-)-terpinen-4-ol ($\geq 95\%$), α -terpinene ($\geq 89\%$), γ -terpinene ($\geq 95\%$), α -terpineol (90%), and terpinolene ($\geq 85\%$) were from Sigma-Aldrich (St. Louis, MO, USA). Two of the positive control insecticides, deltamethrin (>97%) and ethyl formate (97%), were purchased from Tokyo Chemical Industry Co., Ltd. and Sigma-Aldrich, respectively.

To evaluate the efficacy of a commercial product containing cinnamon oil as its active ingredient, 'Rice Weevil Eradication' (manufacturer: Hub Club, Siheung, Korea) was purchased from an online retail market (Auction, <http://www.auction.co.kr/>). The product was made with its liquid contents sealed in a breathable non-woven fabric, and evaporation was initiated when the seal was removed.

3. Chemical composition of essential oils and commercial products

To identify the major constituents of the oils and test product, gas chromatography-mass spectrometry (GC-MS) analyses were performed with an ISQ gas chromatograph mass spectrometer (Thermo Scientific, Waltham, MA, USA) operating in EI mode fitted with a VF5ms column (60 m × 0.25 mm i.d., 0.25 μm thickness). Helium (99.999%) was used as a carrier gas with the constant flow of 1.0 mL/min. The injection volume was 1.0 μL, and the initial temperature for the oven was set at 50 °C for 5 min, then increased to 65, 120, 180, 210, and 325 °C with each rate of 10, 5, 5, 5, and 20 °C/min, respectively. Each stage was held for 30, 10, 0, 10, and 10 min, respectively. The data were analyzed using NIST Mass Spectral Search software (version 2.0), and the major constituents were determined by matching the spectra against the NIST/EPA/NIH Mass Spectral Libraries.

To monitor the changes in chemical composition of the commercial product during use, the seal of the product was opened, and the liquid contents were allowed to evaporate at room temperature for 0, 1, and 2 months, respectively, and GC-MS analyses were conducted. At each time of monitoring, three aliquots were analyzed for a total of nine samples.

Table 1. Plant species and essential oils tested in this study

Essential oil	Family	Scientific name	Plant parts Extracted from	Manufacturer
Basil	Lamiaceae	<i>Ocimum basilicum</i>	leaf, flower	Sun Essential Oils
Bergamot	Rutaceae	<i>Citrus bigaradia</i>	peel	Klimtech
Cinnamon	Lauraceae	<i>Cinnamomum cassia</i>	bark	Plant Therapy
Citronella	Poaceae	<i>Cymbopogon nardus</i>	leaf	Absolute Aromas
Clary sage	Lamiaceae	<i>Salvia sclarea</i>	flower	Klimtech
Clove bud	Myrtaceae	<i>Syzygium aromaticum</i>	flower bud	Absolute Aromas
Cypress	Cupressaceae	<i>Cupressus sempervirens</i>	leaf	Klimtech
<i>Eucalyptus globulus</i>	Myrtaceae	<i>Eucalyptus globulus</i>	leaf	Klimtech
<i>Eucalyptus radiata</i>	Myrtaceae	<i>Eucalyptus radiata</i>	leaf	Klimtech
Fennel	Apiaceae	<i>Foeniculum vulgare</i>	seed	Sun Essential Oils
Fennel sweet	Apiaceae	<i>Foeniculum vulgare</i>	seed	Klimtech
Frankincense	Burseraceae	<i>Boswellia carterii</i>	resin	Klimtech
Geranium	Geraniaceae	<i>Pelargonium graveolens</i>	flower	Klimtech
Lavender (French)	Lamiaceae	<i>Lavandula angustifolia</i>	flower bud	Absolute Aromas
Lavender (Bulgarian)	Lamiaceae	<i>Lavandula angustifolia</i>	flower	Klimtech
Lemon	Rutaceae	<i>Citrus limonum</i>	peel	Klimtech
Lemongrass	Poaceae	<i>Cymbopogon citratus</i>	leaf	Klimtech
Mandarin	Rutaceae	<i>Citrus reticulata</i>	peel	Klimtech
Marjoram	Lamiaceae	<i>Origanum majorana</i>	leaf	Klimtech
Orange sweet	Rutaceae	<i>Citrus aurantium</i>	peel	Klimtech
Patchouli	Lamiaceae	<i>Pogostemon cablin</i>	leaf	Klimtech
Peppermint	Lamiaceae	<i>Mentha piperita</i>	leaf	Klimtech
Pine	Pinaceae	<i>Pinus</i> spp.	needle	Sun Essential Oils
Rosemary	Lamiaceae	<i>Rosmarinus officinalis</i>	leaf	Klimtech
Sandal wood	Santalaceae	<i>Santalum album</i>	wood	Klimtech
Spearmint	Lamiaceae	<i>Mentha spicata</i>	leaf, flower	Absolute Aromas
Tea tree	Myrtaceae	<i>Melaleuca alternifolia</i>	leaf	Klimtech
Ylang ylang	Annonaceae	<i>Cananga odorata</i>	flower	Klimtech

4. Bioassays

4.1. Acute toxicity of plant essential oils

The insecticidal activity of the 28 oils was evaluated via a contact and residual application and a fumigation method described by Tak et al. (2006) with a slight modification. For the contact and residual application assay, a dose of up to 150 mg of each essential oil in 200 μ L of acetone was applied to a filter paper (Whatman No. 2, 5.5 cm in diameter) and allowed to dry for 2 min. The treated filter paper was placed into a Petri dish (Hyundai micro, Anseong, Korea, 6.0 cm in diameter), and ten adults of the same age of *S. zeamais* were released into the Petri dish then sealed with Parafilm (Figure 2). Negative control had a filter paper treated with acetone alone, and the synthetic pyrethroid insecticide, deltamethrin, was used as a positive control. The dishes were held under the same conditions as mentioned above for colony maintenance.

For the fumigation assay, ten weevil adults were placed in a 1.5 mL micro centrifuge tube aerated with 200-mesh screen on both ends of the tube (Figure 3), and the tube was placed to the bottom of a 155 mL plastic cup. Up to 100 mg of each essential oil in 200 μ L of acetone was applied to a filter paper and allowed to dry for 2 min, then placed in the cup with the lid closed. Negative control received acetone alone and ethyl formate was used as a positive control. Mortality was recorded after 24 h, with weevils considered dead if their appendages did not move when prodded with fine point forceps. All treatments were replicated at the minimum of three times

(up to nine times) using different cohorts of weevils.

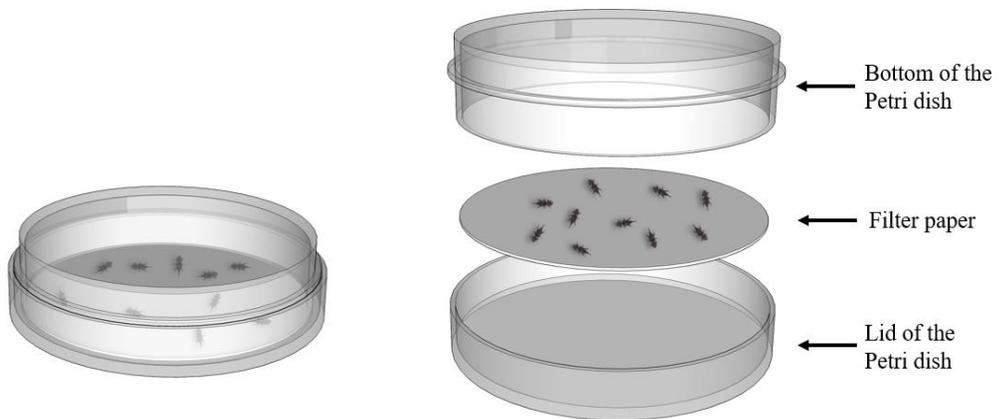


Figure 2. Schematic diagram of contact and residual application assay arena

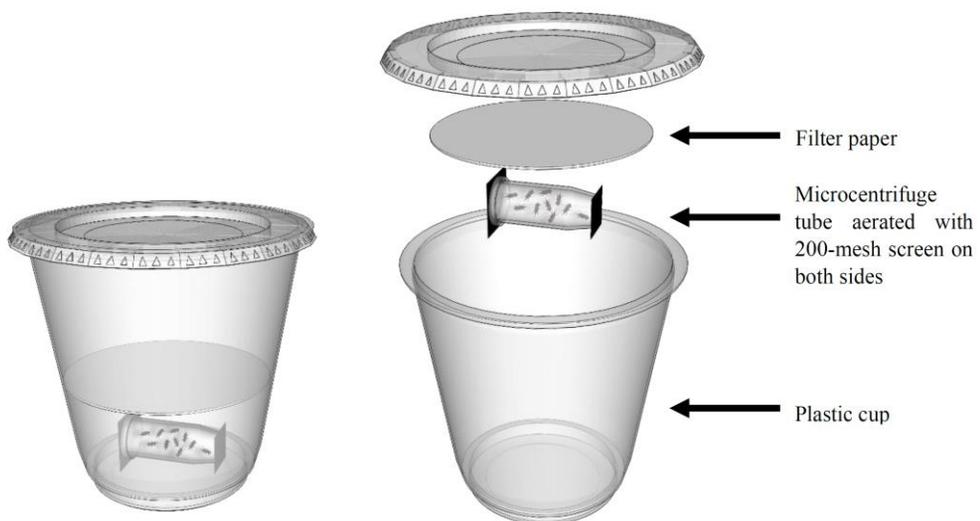


Figure 3. Schematic diagram of fumigation assay arena

4.2. Behavioral attraction–inhibitory activity to rice grains via no-choice assay

In the attraction–inhibitory activity bioassay, adult weevils were starved for 24 h prior to the test. Approximately 1.3 g of rice grains was placed in the bottom of a borosilicate glass test tube (1.1 cm i.d. × 10 cm in length) with a piece of non-woven fabric (1 × 1 cm) located on top of the grains. Since the fabric piece was too small to hold a large volume of test solution, 50 µL of undiluted crude essential oil was directly applied to it using a micropipette; then, the glass tube was promptly assembled to a 3D printout structure (port) and laid horizontally (Figure 4). Ten previously starved adult weevils were kept in the microcentrifuge tube and introduced through a hole in the port. The number of weevils that initiated attraction behavior to the glass tube containing rice grains was recorded at 1, 3, and 24 h post-treatment. The grains with non-treated fabric were used as the negative control, and the test was repeated three times. Percent inhibition (PI) for a given time point was calculated using the following formula (Yoon et al., 2007);

$$PI (\%) = (N - n)/N \times 100$$

where N is the total number of insects introduced, and n is the number of insects attracted.

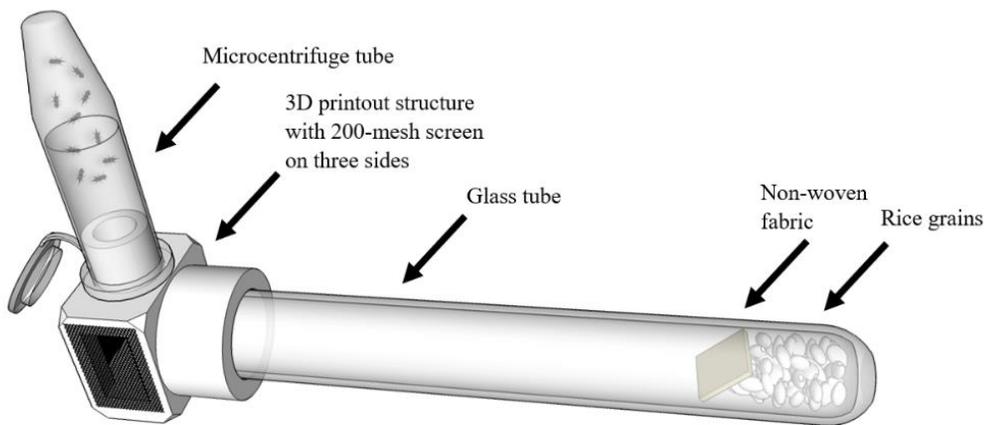


Figure 4. Schematic diagram of attraction–inhibitory activity assay

4.3. Compound elimination assay

To examine the contribution of each major constituent of the essential oils to the overall contact and/or fumigant toxicity, a compound elimination assay was conducted on the four most active oils (cinnamon, tea tree, ylang ylang, and marjoram oils). Major compounds which constitute >2% of each oil were blended according to their natural ratio to make an artificial full mixture (FM), and a series of artificial mixtures was prepared by excluding each compound from FM (Kim et al., 2013). The missing volume of excluded compound was supplemented with acetone, and the dose or concentration of each artificial mixture was prepared at the equivalent level of LD₉₅ or LC₉₅ of the original oils. The insecticidal activity of the artificial mixtures and corresponding oils was compared via either the contact or fumigation bioassay as mentioned above.

4.4. Insecticidal efficacy of a commercial product

The insecticidal activity of a commercial product, Hub Club, was evaluated in two different test settings: a 13 L plastic container, and a 500 mL Mason jar. For the 13 L container test, rice grains were filled in six of 50 mL Conical tubes aerated with mesh screen on both ends, and 50 adult maize weevils were introduced into each tube. Three of the tubes were placed in the bottom of the container, and 10 kg of rice were filled, with the remaining three tubes buried on top of the grains. Test products which were either 0, 1 or 2 months-aged after the seal of the wrapper was opened in room condition were placed on the top of the rice and the lid was covered. The container was held at room temperature, and mortality was recorded two weeks after the treatment. Negative control did not contain the product, only the rice grains.

For the 500 mL container test, two 1.5 mL aerated micro centrifuge tubes containing 10 weevils were placed in the bottom and another two on top of the mason jar, and the container was either filled with rice or remained empty, and the newly sealed-off product was introduced into the container. The mortality was observed at 24 h post-introduction of the products, and all tests were repeated three times.

5. Statistical analysis

Probit analyses were conducted to determine LD₅₀ or LC₅₀ values of the essential oils and insecticides, and mortality in the compound elimination assay and attraction–inhibition assay was subjected to analysis of variance (ANOVA) using SPSS software (version 2.5, IBM, Armonk, NY, USA).

Results

1. Insecticidal activity of plant essential oils

The insecticidal activity of 28 essential oils against the adults of the *S. zeamais* are reported in Table 2. In the contact and residual bioassay, cinnamon oil was the most active oil ($LD_{50} = 0.04 \text{ mg/cm}^2$), followed by tea tree and marjoram oils ($LD_{50} = 0.15$ and 0.18 mg/cm^2 , respectively). Seven out of 28 oils failed to produce $>50\%$ of mortality at the highest dose tested (6.3 mg/cm^2). Interestingly, several oils tested in the present study showed greater insecticidal activity than deltamethrin did, where the LD_{50} value of deltamethrin was 3.75 mg/cm^2 , indicating their strong residual effect, and presumably, complex insecticidal actions.

In the fumigation bioassay, we could evaluate LC_{50} values for only six essential oils (cinnamon, tea tree, ylang ylang, *E. radiata*, rosemary, *E. globulus* oils), since the remaining oils produced $< 50\%$ mortality at the highest concentration tested. Cinnamon oil showed the greatest fumigant toxicity among the oils tested, followed by tea tree and ylang ylang oils ($LC_{50} = 10.6, 25.1, \text{ and } 52.0 \text{ mg/L air}$, respectively). Interestingly, some essential oils, including marjoram, peppermint, and Bulgarian lavender oils, which showed strong contact toxicity ($LD_{50} < 0.31 \text{ mg/cm}^2$) failed to exhibit corresponding fumigant toxicity effect ($LC_{50} > 560.4 \text{ mg/L air}$). Several oils

moderately active in the contact bioassay ($0.36 < LD_{50} < 0.47$ mg/cm²) also failed to produce notable fumigant toxicity effect, whereas some less toxic oils in the contact assay including *E. radiata*, rosemary, and *E. globulus* oils, had greater vapor toxicity than those mentioned above (LC₅₀ values of 96.0, 121.8, and 137.9 mg/L air, respectively). Based on their LC₅₀ values, cinnamon oil (10.6 mg/L air) showed greater toxicity than the positive control, ethyl formate (16.1 mg/L air).

Table 2. Insecticidal activity of 28 essential oils against *Sitophilus zeamais* adults

Essential Oils	Contact Toxicity			Fumigation Toxicity		
	LD ₅₀ (95% CL) ^a	Slope ± SE ^b	χ ² (d.f)	LC ₅₀ (95% CL) ^c	Slope ± SE	χ ² (d.f)
Cinnamon	0.04 (0.03–0.04)	7.3 ± 1.2	5.0 (16)	14.0 (11.6–16.8)	2.4 ± 0.3	32.0 (25)
Tea Tree	0.15 (0.14–0.16)	13.8 ± 2.1	5.7 (13)	18.3 (15.5–21.8)	4.9 ± 0.6	50.5 (22)
Marjoram	0.18 (0.15–0.23)	9.2 ± 1.3	94.2 (16)	>565.8	-	
Peppermint	0.24 (0.22–0.26)	8.1 ± 1.4	13.6 (13)	>560.4	-	
Lavender (Bulgarian)	0.31 (0.24–0.39)	2.5 ± 0.4	7.8 (10)	>565.2	-	
Ylang ylang	0.32 (0.23–0.43)	4.5 ± 0.7	22.1 (10)	52.0 (45.8–58.1)	3.8 ± 0.4	26.2 (31)
Geranium	0.36 (0.28–0.49)	1.8 ± 0.2	15.2 (16)	>547.3	-	
Lemongrass	0.37 (0.25–0.50)	1.5 ± 0.2	16.9 (19)	>560.2	-	
Patchouli	0.40 (0.35–0.49)	11.8 ± 1.9	45.8 (13)	>571.6	-	
Spearmint	0.40 (0.30–0.51)	1.5 ± 0.1	52.8 (40)	>583.2	-	
Clary Sage	0.42 (0.28–0.60)	1.3 ± 0.2	22.0 (19)	>568.8	-	
Clove bud	0.47 (0.35–0.60)	1.3 ± 0.1	31.2 (37)	>654.0	-	
<i>E. radiata</i>	0.61 (0.56–0.66)	6.5 ± 0.8	20.3 (25)	96.0 (75.7–121.1)	6.3 ± 0.8	198.1 (28)
Rosemary	0.67 (0.57–0.78)	3.7 ± 0.7	18.1 (13)	121.8 (107.8–133.0)	4.9 ± 0.6	29.9 (34)
Basil	0.77 (0.49–1.16)	1.5 ± 0.2	29.0 (19)	>605.2	-	

<i>E. globulus</i>	0.89 (0.82–0.96)	9.7 ± 1.5	9.4 (16)	137.9 (121.6–181.1)	8.9 ± 1.2	117.7 (22)
Citronella	1.01 (0.73–1.38)	1.3 ± 0.2	40.1 (31)	>555.5	-	
Cypress	1.23 (1.04–1.50)	4.5 ± 0.6	28.1 (16)	>556.8	-	
Orange sweet	1.40 (1.15–1.71)	2.6 ± 0.3	17.1 (16)	>560.2	-	
Bergamot	1.70 (1.05–2.66)	1.8 ± 0.3	23.2 (13)	>551.4	-	
Sandal wood	1.87 (1.49–2.39)	2.0 ± 0.3	22.0 (22)	>555.2	-	
Fennel	>3.59	-		>551.0	-	
Frankincense	>3.53	-		>541.6	-	
Lemon	>3.53	-		>541.1	-	
Mandarin	>3.55	-		>543.7	-	
Lavender (French)	>3.62	-		>554.6	-	
Pine	>3.64	-		>558.3	-	
Fennel sweet	>4.07	-		>623.4	-	
<i>trans</i> -Cinnamaldehyde	0.02 (0.02–0.02)	8.4 ± 1.3	11.5 (19)	12.1 (9.7–15.6)	2.8 ± 0.2	117.1 (40)
Terpinen-4-ol	0.06 (0.05–0.06)	15.3 ± 2.6	29.8 (19)	11.2 (10.2–12.2)	4.2 ± 0.4	41.2 (37)
Deltamethrin	3.75 (2.24–8.37)	1.0 ± 0.1	50.6 (40)	n.t. ^d		
Ethyl formate	n.t.			16.1 (13.8–18.9)	3.9 ± 0.4	92.5 (42)

^a mg/cm²; ^b Standard Error; ^c mg/L air; ^d Not tested.

2. Attraction–inhibition via no-choice assay

The attraction–inhibitory activity of the 28 essential oils against the adults of *S. zeamais* was observed at 1, 3, and 24 h post-treatment (Figure 5). At 1 h after the release of the weevils, *E. radiata*, lemon, and cinnamon oils showed strong inhibition activity (>70%), and moderate activity (40–70% inhibition) was produced by 12 essential oils including mandarin, rosemary, patchouli, clary sage, frankincense, fennel sweet, bergamot, orang sweet, cypress, clove bud, *E. globulus*, and spearmint oils. Several oils active in the contact and residual toxicity bioassay, including tea tree, peppermint, Bulgarian lavender, and ylang ylang oils failed to generate notable attraction–inhibitory activity, showing no statistical difference to that of the control ($p > 0.05$).

Over time, the attraction–inhibition effect of the active oils diminished, possibly due to either the evaporation of the oils through the opening of the test chamber or the loss of concentration gradient in the air of test tubes. Whereas 18 and 20 oils showed significant inhibitory activity at 1 and 3 h post-treatment ($p < 0.05$), only frankincense and lemon oils displayed moderate activity (> 40%) after 24 h of application.

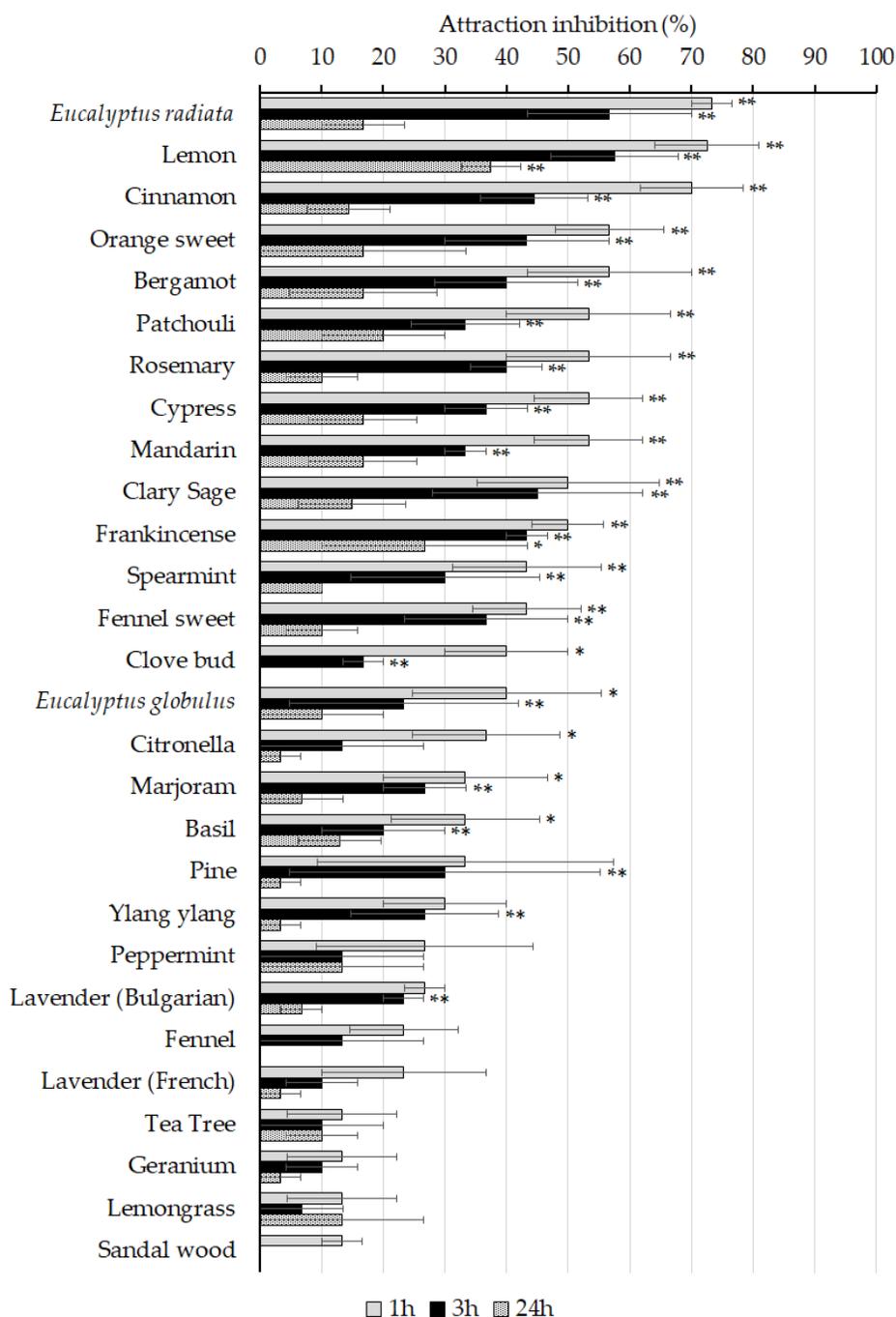


Figure 5. Attraction–inhibition activity. Control attraction–inhibition at 1, 3, and 24 h were 12.9 ± 4.3 , 2.9 ± 1.7 , and $7.1 \pm 3.2\%$, respectively. Asterisks denote significant differences between the control repellency at $p < 0.05$ (*) and $p < 0.01$ (**) in one-way ANOVA followed by the Tukey’s b test

3. Chemical composition of active essential oils

GC-MS analyses were conducted on all the essential oils tested, and the chemical compositions of the four most active oils in contact/residual and fumigation bioassays are listed in Table 3. The most abundant constituent in both the tea tree and marjoram oils was terpinen-4-ol (48.7 and 30.4%, respectively), and *trans*-cinnamaldehyde (74.6%) was the major constituent in cinnamon oil. Benzyl acetate (19.9%) was the most abundant constituent in ylang ylang oil, followed by linalool, benzyl salicylate, and 4-methylanisole, and their proportions in the oil were similar (18.0, 14.6, and 13.0% respectively). The full results of chemical analyses of 28 essential oils are available in Supplementary Information (Tables S1–S28).

Table 3. Chemical constituents of four most active essential oils

RT (min)	Compounds	Composition (%)			
		Tea tree	Marjoram	Cinnamon	Ylang ylang
27.22	α -Pinene	4.6	1.4	-	-
34.38	Sabinene	-	2.6	-	-
35.48	β -Pinene	-	1.0	-	-
40.73	3-Carene	-	2.7	-	-
41.08	4-Methylanisole	-	-	-	13.0
41.71	α -Terpinene	3.7	1.8	-	-
42.62	o-Cymene	5.6	9.0	-	-
43.03	Limonene	1.2	-	-	-
43.35	Eucalyptol	4.8	-	-	-
45.61	γ -Terpinene	15.8	6.1	-	-
47.54	Methyl benzoate	-	-	-	5.8
47.57	Terpinolene	6.8	2.2	-	-
48.52	Linalool	-	6.9	-	18.0
52.35	Benzyl acetate	-	-	-	19.9
54.84	Terpinen-4-ol	48.7	30.4	-	-
55.96	α -Terpineol	-	4.9	-	-
60.09	Linalyl acetate	-	11.2	-	-
60.88	Piperitone	-	1.3	-	-
62.51	<i>trans</i> -Cinnamaldehyde	-	-	74.6	-
66.78	Geranyl acetate	-	-	-	6.2
68.96	Methyl undecanoate	1.7	1.9	1.8	3.0
69.36	β -Caryophyllene	-	8.5	-	4.8
69.58	Cinnamyl acetate	-	-	-	3.7
70.06	<i>trans</i> -Cinnamyl acetate	-	-	2.8	-
70.17	Coumarin	-	-	1.9	-
70.29	α -Caryophyllene	-	-	-	1.6
73.06	3-Methoxycinnamaldehyde	-	-	9.5	-
79.60	Benzyl benzoate	-	-	-	3.8
83.34	Benzyl salicylate	-	-	-	14.6
Total identified		92.9	91.8	90.6	94.4

4. Comparative toxicity of the major constituents

In the compound elimination assay using marjoram and tea tree oils via the contact and residual application method, the artificial mixtures failed to cause any mortality to the adult weevils when terpinen-4-ol was excluded from the full mixtures (Figure 6a, b). On the other hand, the mortality of all the remaining combinations containing terpinen-4-ol including the full mixture showed no statistical difference when compared to those of the corresponding natural essential oils ($p > 0.05$), implicating terpinen-4-ol as the main constituent responsible for the insecticidal activity of those two oils (Figure 6b, c). Likewise, terpinen-4-ol was identified as the main active fumigant in tea tree oil, producing no mortality when removed from the full mixture (Figure 7b). Among the constituents of cinnamon oil, *trans*-cinnamaldehyde was shown to be the sole active compound in both the contact/residual and fumigant bioassays against the maize weevil, since no other compounds showed statistical difference when excluded from the full mixture ($p > 0.05$, Figures 6a and 7a). Ylang ylang oil, although the artificial mixture lacking benzyl acetate, the most abundant compound, caused low mortality (< 40%) that was statistically different ($p < 0.05$), it failed to completely nullify the toxicity unlike the other oils. Nonetheless, the other artificial mixtures showed no statistical difference in mortality when compared to that of the natural ylang ylang oil ($p > 0.05$, Figure 7c).

In In the comparison between the values of LD₅₀ in the contact and

residual bioassay and LC_{50} in fumigant assay, six essential oils with strong contact toxicity were found to possess the equivalent level of fumigant toxicity, displaying high correlation between the two groups ($R^2 = 0.9842$), whereas the other fifteen essential oils which showed contact toxicity ($LD_{50} < 1.9 \text{ mg/cm}^2$) failed to show corresponding fumigant toxicity (Figure 8). In the meantime, no direct correlation was found between attraction–inhibitory activity and either contact/residual or fumigant toxicity, with low R^2 values of 0.011 and 0.031, respectively.

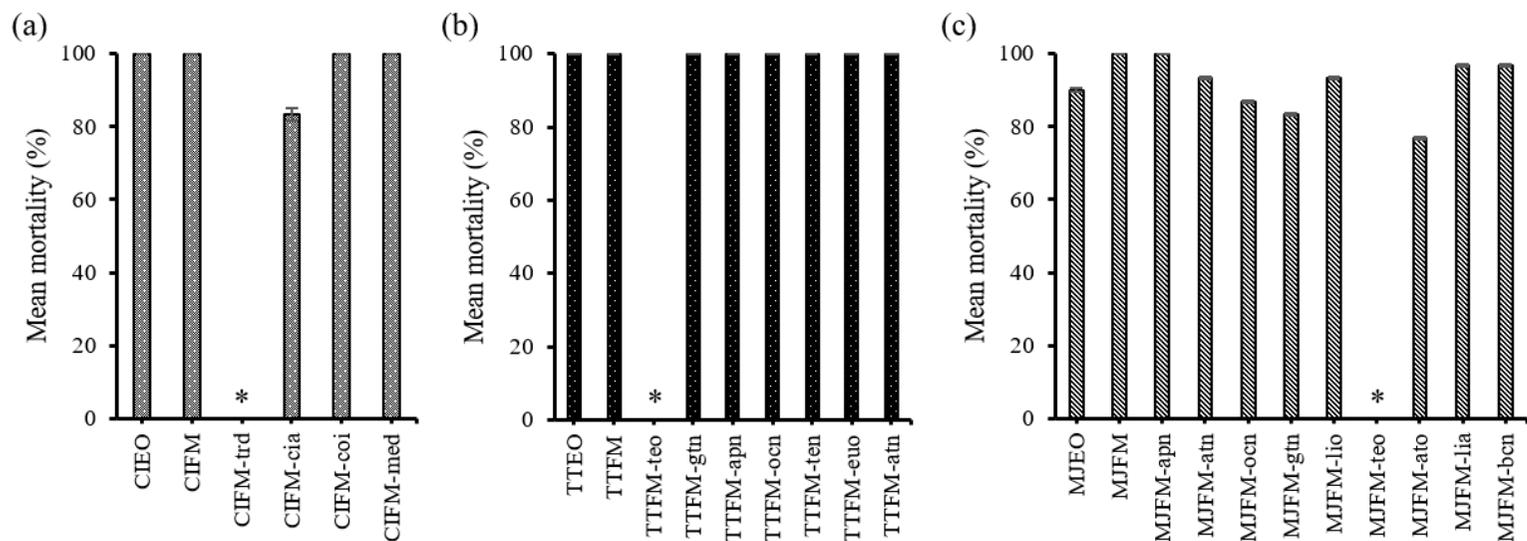


Figure 6. Compound elimination assay via contact and residual application: (a) cinnamon oil at LD₉₅ of 0.22 mg/cm²; (b) tea tree oil at LD₉₅ of 0.37 mg/cm²; (c) marjoram oil at LD₉₅ of 0.37 mg/cm² against *S. zeamais* adults. Asterisks denote significant differences at $p = 0.05$. (apn: (+)- α -pinene, atn: α -terpinene, ato: α -terpineol, bcn: β -caryophyllene, cia: cinnamyl acetate, coi: coumarin, euo: eucalyptol, gtn: γ -terpinene, lia: linalyl acetate, lia: linalyl acetate, lio: linalool, med: (E)-4-methoxycinnamaldehyde, ocn: o-cymene, ten: terpinolene, teo: (-)-terpinen-4-ol, and trd: *trans*-cinnamaldehyde)

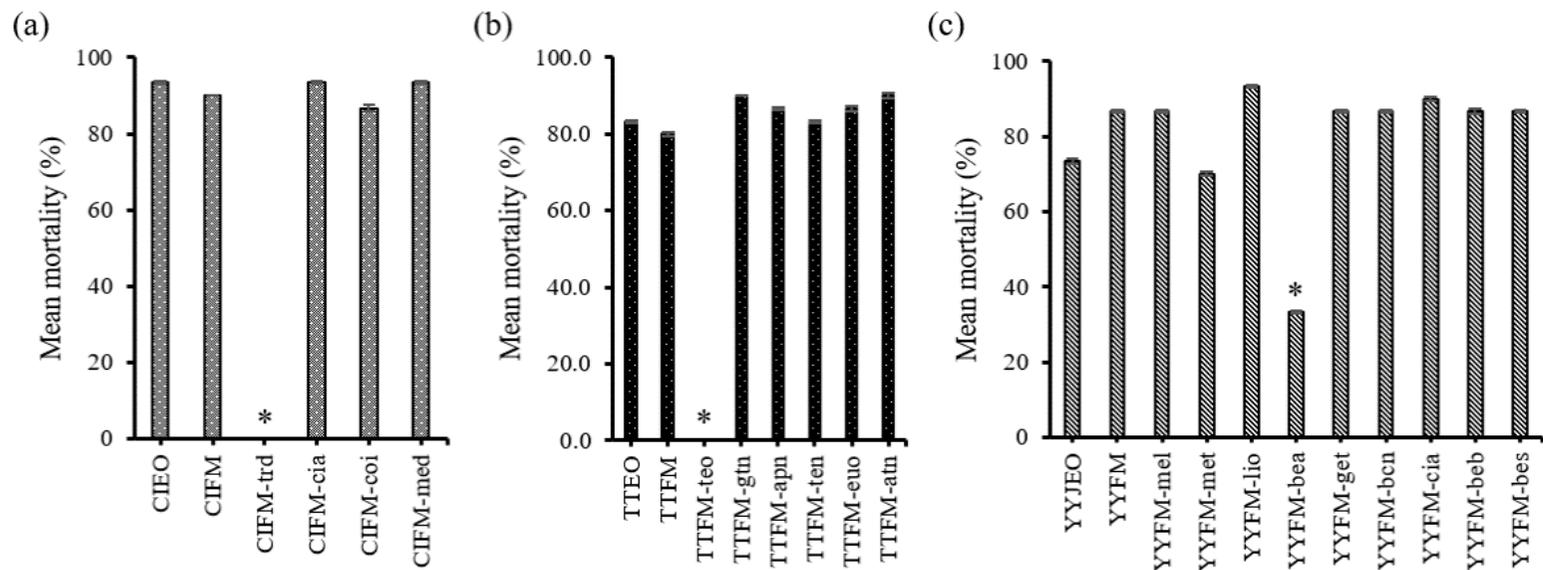


Figure 7. Compound elimination assay via fumigation assay: (a) cinnamon oil at LC_{95} of 238.6 mg/L air; (b) tea tree oil at LC_{95} of 114.5 mg/L air; (c) ylang ylang oil at LC_{95} of 142.0 mg/L air against *S. zeamais* adults. Asterisks denote significant differences at $p = 0.05$. (apn: (+)- α -pinene, atn: α -terpinene, bcn: β -caryophyllene, bea: benzyl acetate, beb: benzyl benzoate, bes: benzyl salicylate, cia: cinnamyl acetate, coi: coumarin, euo: eucalyptol, get: geranyl acetate, gtn: γ -terpinene, lio: linalool, med: (E)-4-methoxycinnamaldehyde, mel: 4-methylanisole, met: methyl benzoate, ten: terpinolene, teo: (-)-terpinen-4-ol, trd: *trans*-cinnamaldehyde)

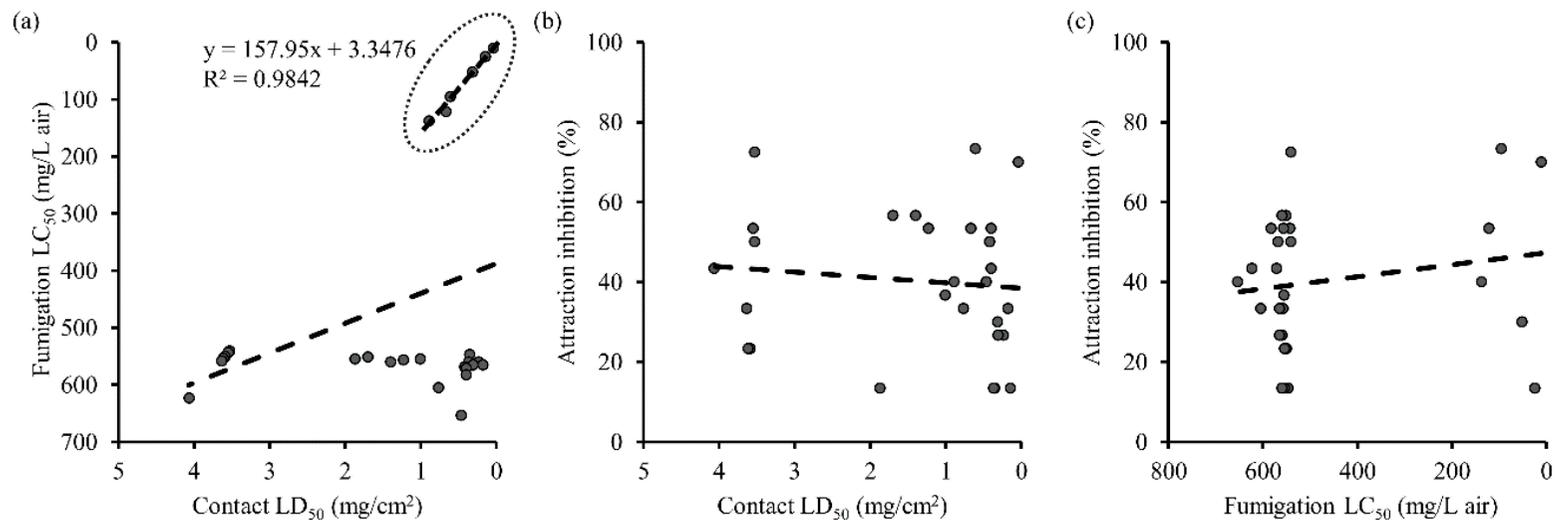


Figure 8. Correlation among contact/residual, fumigation, and anti-attraction activity of 28 essential oils tested: (a) contact/residual and fumigation (df = 1, 26; F = 3.60; $p = 0.069$); (b) contact/residual and 1 h attraction inhibition (df = 1, 26; F = 0.28; $p = 0.600$); (c) fumigation and 1 h attraction inhibition (df = 1, 26; F = 0.84; $p = 0.367$)

5. Chemical composition and efficacy of the commercial product

The label of the commercial product indicated cinnamon oil as its active ingredient, and GC-MS result confirmed the presence of *trans*-cinnamaldehyde in the product. While the newly opened product had 12.0% of this compound in its liquid contents, the concentration of the compound in the liquid increased to $53.1 \pm 4.7\%$ when the product remained open for two months, indicating its slower evaporation rate compared with other chemical constituents. Surprisingly, in terms of the efficacy of the product, it produced limited mortality for two weeks' observation, with the greatest mortality at only $12.0 \pm 6.7\%$ in the one-month-old product (Table 4). It is notable that in the absence of rice, it showed complete mortality ($100.0 \pm 0.0\%$) within 24 h, whereas it failed to show any insecticidal activity when rice grains were present in the container, suggesting that rice grains counteract the efficacy of the oil or the product.

Table 4. Insecticidal activity of a commercial product on the adult of the maize weevil.

Months evaporated	Mortality (% ± SE)				<i>trans</i> -Cinnamaldehyde content (% ± SE)
	without	with	with	with	
0	1.3 ± 0.8	2.1 ± 0.9	0.0 ± 0.0	100.0 ± 0.0 *	12.2 ± 0.2
1	1.0 ± 0.7	12.0 ± 6.7 *	-	-	23.4 ± 1.6
2	1.3 ± 0.7	1.8 ± 0.2	-	-	53.1 ± 4.7
Product	without	with	with	with	
Container volume	13 L	13 L	500 mL	500 mL	
Rice grain	with	with	with	without	

* Asterisks denote significant differences at $p = 0.05$

Discussion

Insecticide fumigation is one of the most widely adopted control methods for the protection of stored products from insect infestations. Plant-derived natural products are known to have relatively low mammalian toxicity, and they tend to be rapidly degraded in the environment, making them potential alternatives to conventional fumigants (Rajendran and Sriranjini, 2008). The insecticidal and repellent effect of plant extracts and essential oils against various stored product pests have been explored in many previous studies (Nattudurai et al.,2017; Aydin et al., 2017; Franz et al.,2011; Khani et al.,2017; Negahban et al., 2007). In this study, acute toxicity and attraction–inhibitory activity of 28 commercially obtained essential oils and their major constituents were examined against the adults of *S. zeamais*. Cinnamon oil showed the greatest contact and fumigant toxicity amongst the tested essential oils (Table 2). Cinnamon oil and *trans*-cinnamaldehyde, the most abundant constituent of the oil, are known to have insecticidal activity against several other coleopteran stored product insects including the rice weevil, *S. oryzae* L., Chinese bruchid, *Callosobruchus chinensis* L. (Kim et al.,2003a), the red flour beetle, *Tribolium castaneum* Herbst (Huang et al.,1998), and the cigarette beetle, *Lasioderma serricorne* Fabricius (Kim et al.,2003b). The content of *trans*-cinnamaldehyde in the

cinnamon oil in the present study was 74.6%, which was similar to that in *C. cassia* bark essential oil (66.3–77.2%) as reported by Li et al. (Li et al.,2013). According to Liu et al. (Liu et al., 2014), *trans*-cinnamaldehyde was identified as the major toxicant in *C. cassia* essential oil against the booklouse, *Liposcelis bostrychophila* Badonnel, and *trans*-cinnamaldehyde in *C. osmophloeum* essential oil also showed notable larvicidal activity on three species of mosquito larvae including the Asian tiger mosquito, *Aedes albopictus* Skuse, southern house mosquito, *Culex quinquefasciatus* Say, and *Armigeres subalbatus* Coquillett (Cheng et al.,2009). Our results from the compound elimination assay (Figures 6 and 7) also revealed that *trans*-cinnamaldehyde acts as the major active compound for contact/residual and fumigation toxicity against the maize weevil. Besides cinnamon oil, marjoram and tea tree oils also showed highly effective contact toxicity, and terpinen-4-ol was the most abundant component in both oils. Terpinen-4-ol content in marjoram oil (30.4%) was comparable to the oils from other *Majorana hortensis* (*O. majorana*) plants in Egypt (30.0%) (Abbassy et al., 2009), whereas in our tea tree oil (48.7%) it was lower than oils of *M. alternifolia* plants in Brazil (53.7%) (Silva et al., 2007). The difference in the proportion of the major constituents and the composition of minor constituents may vary depending on environmental (Novak et al., 2008) or nutritional factors (Baatour et al., 2010). Abbassy et al. (2009) suggested that terpinen-4-ol is one of the main toxic constituents of marjoram oil against the black bean aphid, *Aphis fabae* Scop., and the Egyptian cotton leafworm, *Spodoptera littoralis* Boisduval. Likewise, compound elimination

test results for marjoram and tea tree oils to *S. zeamais* in the present study indicated that terpinen-4-ol as the major contributing component. Seven other oils, including fennel, frankincense, lemon, mandarin, French lavender, pine and fennel sweet, did not appear to have any acute contact toxicity after 24 h of application (Table 2). As reported by Kim et al. (2003a), some methanol extracts from aromatic medicinal plant species that lacked acute toxicity against *S. oryzae* and *C. chinensis*, produced >90% mortality at 3 or 4 days post-treatment. Therefore, the possibility of prolonged insecticidal activity should not be ignored for plant extracts and/or essential oils even if their acute toxicity is unapparent.

In contrast to this study, Pavela et al. (2016) reported that *F. vulgare* essential oil has an acute toxic effect on the larvae of *S. littoralis* and *C. quinquefasciatus* and the adults of *Musca domestica*, while this oil failed to show effective toxicity in the present study. Interspecific differences in biological activity, especially for insecticidal activity, are common and well-known in many insect pests. In earlier studies against four insect species (*S. littoralis* Fabricius, *Drosophila melanogaster* Meigen, *M. domestica*, *Diabrotica virgifera* LeConte) and the two-spotted spider mite, *Tetranychus urticae* Koch, toxicity of monoterpene compounds from essential oils including eugenol, carvacrol, α -terpineol, and terpinen-4-ol showed wide variety in their LD₅₀ or LC₅₀ values (Isman, 2000). These types of discrepancy in toxicity can be intriguing in many fields of research, including biochemical research on detoxification, physicochemical study on

cuticular penetration, electrophysiological studies of antennal perception of airborne particles, and physiological work on modes-of-action to elucidate the underlying mechanisms for interspecific difference in toxicity and repellent activity. In terms of different modes-of-action, these active essential oils seemed to possess different modes-of-action than deltamethrin. While deltamethrin displayed distinctive knock-down activity at relatively low dosages applied while many essential oils failed to exhibit any acute toxic responses, its LD₅₀ value was greater than those of some active oils in the present study (Table 2). In the previous study of Fouad and da Camara (Fouad and Camara, 2017), the LD₅₀ for deltamethrin was 2.53 µL/mL against *S. zeamais* adults, which is equivalent to 0.03 mg/cm², which is 140-fold more active than in our contact bioassay. One possible explanation for the significant difference in toxicity of the compound might be the different judgement standard of mortality, since I considered the weevils dead when they completely stopped moving when probed, regardless of their knock-down activity. Another possible reason is the difference in test methods, since I applied the compound onto filter papers whereas the previous study applied the insecticide directly on the glass surface of the Petri dish. Another previous study showed wide differences in the toxicity at the same dose of the same compounds but on different test surfaces (Tak and Isman, 2017).

Ylang ylang oil showed effective contact and fumigation toxicity, and GC-MS analysis data showed that 4-methylanisole, linalool, benzyl acetate, and benzyl salicylate were the major constituents comprising 13.0,

18.0, 19.9, and 14.6% of the oil, respectively. These monoterpenes and phenylpropanoid compounds are commonly found in the essential oils extracted from flowers of ylang ylang (Tan et al., 2015). The compound elimination assay with ylang ylang oil demonstrated that the artificial full mixture excluding benzyl acetate (YYFM-bea in Figure 7c) showed lower mortality than the other combinations. Although the elimination of benzyl acetate from the full mixture resulted in a significant decrease in fumigant toxicity ($p < 0.05$), combination of the remaining constituents still exhibited modest insecticidal activity (33.3%), indicating that bioactivity of ylang ylang oil is cannot be solely attributed to benzyl acetate, but possibly in association with the remaining compounds, through either additive or synergistic interactions. Previous studies show complex interactions among the major constituents of essential oils against various insect and arthropod pests (de Assis et al., 2011; Miresmailli et al., 2006; Shimizu and Hori, 2009; Tak et al., 2016).

The attraction–inhibitory effect against adult *S. zeamais* decreased over time, as most of the oils showed no statistical difference in their 24-h activity when compared to the control except for frankincense and lemon oils (Figure 5). The inhibitory activity was most evident at 1 h post-treatment, with the average inhibition of 42.7%, and the most active treatment was *E. radiata* oil followed by lemon and cinnamon oils. Most constituents of plant essential oils are highly volatile due to their low molecular weight (Regnault-Roger et al., 2012), and the volatility of

essential oils can be affected by the types and structure of a test surface and formulation. For example, *E. radiata* oil was reported to have a repellent effect against *C. quinquefasciatus* for 8 h when applied on the skin of human volunteers (Amer & Mehlhorn, 2006). Likewise, the repellent activity of 20 monoterpene compounds frequently found in many essential oils showed significant differences against two-spotted spider mites when applied to the leaves of bean and cabbage (Tak and Isman, 2017).

According to Obeng-Ofori et al. (1997), the area preference test using 1,8-cineole, which is a major component of *Ocimum kenyense* oil, demonstrated strong repellent activity against *S. granarius* and *S. zeamais*, and the GC-MS result in this study indicated that the major component of *E. radiata* oil was eucalyptol (=1,8-cineole, 65.12%). Therefore, eucalyptol may have a major influence on the anti-attraction effect of *E. radiata* oil. The oils extracted from the fruit peels of plants belonging to the genus *Citrus* (lemon, orange sweet, bergamot, and mandarin) exhibited moderate attraction–inhibition against *S. zeamais* maintaining more than 50% activity at 1 h post-treatment. Peel oils of the genus *Citrus* are known to be rich in limonene (Ladaniya , 2008), and our GC-MS analysis results (Tables S2, S16, S18 and S20) confirmed that limonene was the major component in bergamot (46.0%), lemon (75.7%), mandarin (71.9%), and sweet orange (83.8%) oils. A previous study reported the repellent and insecticidal activity of limonene (Fouad and Camara, 2017), and the attraction–inhibition activity of those oils observed with *S. zeamais* could be attributed to

limonene. Nonetheless, not all the acutely toxic essential oils induced strong inhibition on attraction to the rice grains. For instance, essential oils including tea tree and ylang ylang produced somewhat notable toxicity, but their inhibitory effect at 1 h post-treatment was not proportionate. In an earlier study, similar results were obtained by Tak and Isman (2017) in that camphor, geranic acid, menthone, and α -pinene showed relatively strong or moderate toxicity against *Tetranychus urticae* but did not show a corresponding repellent effect. This indicates that toxicity cannot be directly related to the repellent or attraction–inhibitory effect, and complex and various mechanisms of action may be involved.

As the use of methyl bromide was being phased-out in the stored product pests control programs, phosphine fumigation became the most popular control method around the globe. Compared to other potential alternatives such as sulfuryl fluoride, carbonyl sulfide, propylene oxide, ethyl formate, and hydrogen cyanide, phosphine has unique benefits including lowest costs, various formulations that are easy to apply, rapid dispersion into the treated areas due to its similar density to air, and fast break down after fumigation (Nayak et al., 2020). However, the lack of compatible alternatives and repeated use of phosphine treatment in industrial storages as well as in flour mills has resulted in the development of resistance in various stored product insect pests, which emphasizes the need for additional pest management products. The current situation of phosphine resistance is well documented (Nayak et al., 2020).

In household environments, on the other hand, the control of grain pests should not rely on synthetic chemical control due to safety concerns, since the grains are readily eaten by consumers in their homes. Botanical sources are frequently adopted as good alternatives in this particular situation, and many commercial products are available, especially in Asian countries including South Korea and Japan. Many of the products tend to be made from strong scented plants such as pepper, wasabi, and horseradish. The product tested in the present study used cinnamon oil as the active ingredient and, as shown in Table 2, the oil itself seems promising to control maize weevils. However, our experiment with the formulated product produced a result opposite to that expected when it was deployed with rice grains. In this case, the insecticidal activity was almost nullified by the grains. Possible explanations include the rapid absorption to the surface of rice grains, degradation of active compounds by the metabolic process of grains, or limited (or blocked) evaporation of active constituents. In a previous study, Lee et al. (2004) observed a similar result in that fumigant toxicity of six essential oils against *S. oryzae* was three to nine times lower with a 50% filling ratio of wheat, compared to vessels lacking wheat. Likewise, the presence or absence of grain also significantly affected the fumigant toxicity of ethyl formate to *S. oryzae* as well (Damcevski and Annis, 2006). Further studies should focus on understanding the absorption nature of essential oils and their active principles, and/or formulation approaches to control or decrease the absorption or attachment to the surface of grains to enhance the efficacy of control agents. Finally, organoleptic

evaluation of the treated grains (e.g., for color, flavor, odor, taste, and texture) must be considered.

The label of the commercial product indicated cinnamon oil as its active ingredient, and GC-MS result confirmed the presence of *trans*-cinnamaldehyde in the product. While the newly opened product had 12.0% of this compound in its liquid contents, the concentration

Conclusions

The present study evaluated the susceptibility of *S. zeamais* adults to the acute contact and vapor phase toxicity of 28 essential oils and their major compounds. Amongst 28 oils tested, cinnamon oil exhibited the greatest toxicity and attraction–inhibitory (= deterrent) activity to rice. Toxicity and attraction–inhibition activity were not correlated in most cases. *E. radiata* and cinnamon essential oils were the most active attraction inhibitors at 1 h, and several limonene-containing essential oils showed moderate activity in this bioassay. Although cinnamon oil was the most active in all three laboratory bioassays, a cinnamon oil-bearing commercial product showed limited efficacy with a rice-filled container. Further study is required to understand the absorption of active fumigants by grains, and to enhance efficacy through the formulation for household products.

Bibliography

- Abbassy, M.A.; Abdelgaleil, S.A.; Rabie, R.Y. Insecticidal and synergistic effects of *Majorana hortensis* essential oil and some of its major constituents. *Entomol. Exp. Appl.* **2009**, 131, 225–232.
- Ahmadi, Z., Saber, M., Bagheri, M., & Mahdavinia, G. R. *Achillea millefolium* essential oil and chitosan nanocapsules with enhanced activity against *Tetranychus urticae*. *J. Pest. Sci.* **2018**, 91, 837-848.
- Amer, A.; Mehlhorn, H. Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). *Parasitol. Res.* **2006**, 99, 466–472.
- Aulicky, R.; Stejskal, V.; Frydova, B.; Athanassiou, C.G. Susceptibility of two strains of the confused flour beetle (Coleoptera: Tenebrionidae) following phosphine structural mill fumigation: Effects of concentration, temperature, and flour deposits. *J. Econ. Entomol.* **2015**, 108, 2823–2830.
- Aydin, T.; Bayrak, N.; Baran, E.; Cakir, A. Insecticidal effects of extracts of *Humulus lupulus* (hops) L. cones and its principal component, xanthohumol. *Bull. Entomol. Res.* **2017**, 107, 543–549.
- Baatour, O.; Kaddour, R.; Wannas, W.A.; Lachaâl, M.; Marzouk, B. Salt effects on the growth, mineral nutrition, essential oil yield and composition of marjoram (*Origanum majorana*). *Acta Physiol. Plant.* **2010**, 32, 45.
- Barney, R.J.; Sedlacek, J.D.; Siddiqui, M.; Price, B.D. Quality of stored corn (maize) as influenced by *Sitophilus zeamais* Motsch. and several management practices. *J. Stored Prod. Res.* **1991**, 27, 225–237.
- Benelli, G.; Pavela, R.; Canale, A.; Cianfaglione, K.; Ciaschetti, G.; Conti, F.; Nicoletti, M.; Senthil-Nathan, S.; Mehlhorn, H.; Maggi, F. Acute larvicidal toxicity of five essential oils (*Pinus nigra*, *Hyssopus officinalis*, *Satureja montana*, *Aloysia citrodora* and *Pelargonium graveolens*) against the filariasis vector *Culex quinquefasciatus*: Synergistic and antagonistic effects. *Parasitol. Int.* **2017**, 66, 166–171.
- CABI, *Sitophilus zeamais*, Invasive Species Compendium, CAB International, Wallingford, UK (2017), www.cabi.org/isc
- Caneppele, M.A.B.; Caneppele, C.; Lázari, F.A.; Lázari, S.M.N. Correlation between the infestation level of *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera, Curculionidae) and the quality factors of stored corn, *Zea mays* L.(Poaceae). *Rev. Bras. Entomol.* **2003**, 47, 625–630.

- Cheng, S.-S.; Liu, J.-Y.; Huang, C.-G.; Hsui, Y.-R.; Chen, W.-J.; Chang, S.-T. Insecticidal activities of leaf essential oils from *Cinnamomum osmophloeum* against three mosquito species. *Bioresour. Technol.* **2009**, *100*, 457–464.
- Damcevski, K.A.; Annis, P.C. Influence of grain and relative humidity on the mortality of *Sitophilus oryzae* (L.) adults exposed to ethyl formate vapour. *J. Stored Prod. Res.* **2006**, *42*, 61–74.
- de Assis, C.P.; Gondim, M.G., Jr.; de Siqueira, H.A.; da Câmara, C.A. Toxicity of essential oils from plants towards *Tyrophagus putrescentiae* (Schrank) and *Suidasia pontifica* Oudemans (Acari: Astigmata). *J. Stored Prod. Res.* **2011**, *47*, 311–315.
- de Oliveira, J. L., Campos, E. V., Germano-Costa, T., Lima, R., Vechia, J. F. D., Soares, S. T., ... & Fraceto, L. F. Association of zein nanoparticles with botanical compounds for effective pest control systems. *Pest Manag. Sci.* **2019**, *75*, 1855-1865.
- Devi, S. R., Thomas, A., Rebijith, K. B., & Ramamurthy, V. V. Biology, morphology and molecular characterization of *Sitophilus oryzae* and *S. zeamais* (Coleoptera: Curculionidae). *J. Stored Prod. Res.* **2017**, *73*, 135-141.
- Fouad, H.A.; da Camara, C.A. Chemical composition and bioactivity of peel oils from *Citrus aurantiifolia* and *Citrus reticulata* and enantiomers of their major constituent against *Sitophilus zeamais* (Coleoptera: Curculionidae). *J. Stored Prod. Res.* **2017**, *73*, 30–36.
- Franz, A.R.; Knaak, N.; Fiuza, L.M. Toxic effects of essential plant oils in adult *Sitophilus oryzae* (Linnaeus)(Coleoptera, Curculionidae). *Rev. Bras. Entomol.* **2011**, *55*, 116–120.
- Galvão, J. G., Cerpe, P., Santos, D. A., Gonsalves, J. K., Santos, A. J., Nunes, R. K., ... & Silva, G. F. *Lippia gracilis* essential oil in β -cyclodextrin inclusion complexes: an environmentally safe formulation to control *Aedes aegypti* larvae. *Pest Manag. Sci.* **2019**, *75*, 452-459.
- Haritos, V. S., Damcevski, K. A., & Dojchinov, G. Improved efficacy of ethyl formate against stored grain insects by combination with carbon dioxide in a ‘dynamic’ application. *Pest Manag. Sci.* **2006**, *62*, 325-333.
- Hong, K. J., Lee, W., Park, Y. J., & Yang, J. O. First confirmation of the distribution of rice weevil, *Sitophilus oryzae*, in South Korea. *J. Asia Pac. Biodivers.* **2018**, *11*, 69-75.
- Huang, Y.; Ho, S. Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *J. Stored Prod. Res.* **1998**, *34*, 11–17.
- Hubert, J.; Stejskal, V.; Athanassiou, C.G.; Throne, J.E. Health hazards associated with arthropod infestation of stored products. *Annu. Rev. Entomol.* **2018**, *63*, 553–573.
- Iliou, K., Kikionis, S., Petrakis, P. V., Ioannou, E., & Roussis, V. Citronella oil-loaded electrospun micro/nanofibrous matrices as sustained repellency systems for the Asian tiger mosquito *Aedes albopictus*. *Pest Manag. Sci.* **2019**, *75*, 2142-2147.

- Isman, M.B. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* **2006**, 51, 45–66.
- Isman, M.B. Plant essential oils for pest and disease management. *Crop. Prot.* **2000**, 19, 603–608.
- Khani, M.; Marouf, A.; Amini, S.; Yazdani, D.; Farashiani, M.E.; Ahvazi, M.; Khalighi-Sigaroodi, F.; Hosseini-Gharalari, A. Efficacy of three herbal essential oils against rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae). *J. Essent. Oil Bear. Plants* **2017**, 20, 937–950.
- Kim, S.-I.; Park, C.; Ohh, M.-H.; Cho, H.-C.; Ahn, Y.-J. Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricornis* (Coleoptera: Anobiidae). *J. Stored Prod. Res.* **2003b**, 39, 11–19.
- Kim, S.-I.; Roh, J.-Y.; Kim, D.-H.; Lee, H.-S.; Ahn, Y.-J. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. *J. Stored Prod. Res.* **2003a**, 39, 293–303.
- Kim, S.-W.; Kang, J.; Park, I.-K. Fumigant toxicity of Apiaceae essential oils and their constituents against *Sitophilus oryzae* and their acetylcholinesterase inhibitory activity. *J. Asia Pac. Entomol.* **2013**, 16, 443–448.
- Ladaniya, M. Citrus Fruit: Biology, Technology and Evaluation, 1st ed.; Academic Press: Cambridge, MA, USA, **2008**, pp. 173–176.
- Lee, B.H.; Annis, P.C.; Tumaalii, F.; Choi, W.S. Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects. *J. Stored Prod. Res.* **2004**, 40, 553–564.
- Li, Y.-q.; Kong, D.-x.; Wu, H. Analysis and evaluation of essential oil components of cinnamon barks using GC–MS and FTIR spectroscopy. *Ind. Crops Prod.* **2013**, 41, 269–278.
- Liu, X.C.; Cheng, J.; Zhao, N.N.; Liu, Z.L. Insecticidal activity of essential oil of *Cinnamomum cassia* and its main constituent, *trans*-cinnamaldehyde, against the booklice, *Liposcelis bostrychophila*. *Trop. J. Pharm. Res.* **2014**, 13, 1697–1702.
- López-Castillo, L.M.; Silva-Fernández, S.E.; Winkler, R.; Bergvinson, D.J.; Arnason, J.T.; García-Lara, S. Postharvest insect resistance in maize. *J. Stored Prod. Res.* **2018**, 77, 66–76.
- Miresmailli, S.; Bradbury, R.; Isman, M.B. Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. *Pest. Manag. Sci.* **2006**, 62, 366–371.
- Nattudurai, G.; Baskar, K.; Paulraj, M.G.; Islam, V.I.H.; Ignacimuthu, S.; Durairandiyar, V. Toxic effect of *Atalantia monophylla* essential oil on *Callosobruchus maculatus* and *Sitophilus oryzae*. *Environ. Sci. Pollut. Res. Int.* **2017**, 24, 1619–1629.

- Nayak, M.K.; Daghli, G.J.; Phillips, T.W.; Ebert, P.R. Resistance to the fumigant phosphine and its management in insect pests of stored products: A global perspective. *Annu. Rev. Entomol.* **2020**, *65*, 333–350.
- Negahban, M.; Moharramipour, S.; Sefidkon, F. Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-product insects. *J. Stored Prod. Res.* **2007**, *43*, 123–128.
- Novak, J.; Lukas, B.; Franz, C.M. The essential oil composition of wild growing sweet marjoram (*Origanum majorana* L., Lamiaceae) from Cyprus—Three chemotypes. *J. Essent. Oil Res.* **2008**, *20*, 339–341.
- Obeng-Ofori, D.; Reichmuth, C.; Bekele, J.; Hassanali, A. Biological activity of 1, 8 cineole, a major component of essential oil of *Ocimum kenyense* (Ayobangira) against stored product beetles. *J. Appl. Entomol.* **1997**, *121*, 237–243.
- Papachristos, D.; Stamopoulos, D. Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say)(Coleoptera: Bruchidae). *J. Stored Prod. Res.* **2002**, *38*, 117–128.
- Pavela, R.; Žabka, M.; Bednář, J.; Tříška, J.; Vrchotová, N. New knowledge for yield, composition and insecticidal activity of essential oils obtained from the aerial parts or seeds of fennel (*Foeniculum vulgare* Mill.). *Ind. Crops Prod.* **2016**, *83*, 275–282.
- Rajendran, S.; Sriranjini, V. Plant products as fumigants for stored-product insect control. *J. Stored Prod. Res.* **2008**, *44*, 126–135.
- Reddy, S.E.; Kirti Dolma, S.; Koundal, R.; Singh, B. Chemical composition and insecticidal activities of essential oils against diamondback moth, *Plutella xylostella* (L.)(Lepidoptera: Yponomeutidae). *Nat. Prod. Res.* **2016**, *30*, 1834–1838.
- Regnault-Roger, C.; Vincent, C.; Arnason, J.T. Essential oils in insect control: Low-risk products in a high-stakes world. *Annu. Rev. Entomol.* **2012**, *57*, 405–424.
- Ristaino, J. B., & Thomas, W. Agriculture, methyl bromide, and the ozone hole: can we fill the gaps? *Plant Dis.* **1997**, *81*, 964-977.
- Ryan, R. F., & De Lima, C. P. F. Ethyl formate fumigation an overview update. In Proceedings 11th international working conference on stored product protection **2014**, pp. 24-28.
- Shimizu, C.; Hori, M. Repellency and toxicity of troponoid compounds against the adzuki bean beetle, *Callosobruchus chinensis* (L.)(Coleoptera: Bruchidae). *J. Stored Prod. Res.* **2009**, *45*, 49–53.
- Silva, C.J.; Barbosa, L.C.; Maltha, C.R.; Pinheiro, A.L.; Ismail, F.M. Comparative study of the essential oils of seven *Melaleuca* (Myrtaceae) species grown in Brazil. *Flavour Fragr. J.* **2007**, *22*, 474–478.
- Sinha, K. K., & Sinha, A. K. Effect of *Sitophilus oryzae* infestation on *Aspergillus flavus* infection and aflatoxin contamination in stored wheat. *J. Stored Prod. Res.* **1991**, *27*,

65-68.

- Tak, J.H.; Isman, M.B. Acaricidal and repellent activity of plant essential oil-derived terpenes and the effect of binary mixtures against *Tetranychus urticae* Koch (Acari: Tetranychidae). *Ind. Crops Prod.* **2017**, 108, 786–792.
- Tak, J.H.; Jovel, E.; Isman, M.B. Comparative and synergistic activity of *Rosmarinus officinalis* L. essential oil constituents against the larvae and an ovarian cell line of the cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). *Pest. Manag. Sci.* **2016**, 72, 474–480.
- Tak, J.H.; Kim, H.K.; Lee, S.H.; Ahn, Y.J. Acaricidal activities of paeonol and benzoic acid from *Paeonia suffruticosa* root bark and monoterpenoids against *Tyrophagus putrescentiae* (Acari: Acaridae). *Pest. Manag. Sci.* **2006**, 62, 551–557.
- Tan, L.T.H.; Lee, L.H.; Yin, W.F.; Chan, C.K.; Abdul Kadir, H.; Chan, K.G.; Goh, B.H. Traditional uses, phytochemistry, and bioactivities of *Cananga odorata* (Ylang-Ylang). *Evid. Based Complement. Alternat Med.* **2015**, doi:1155/2015/896314.
- Yoon, C.; Kang, S.-H.; Jang, S.-A.; Kim, Y.-J.; Kim, G.-H. Repellent efficacy of caraway and grapefruit oils for *Sitophilus oryzae* (Coleoptera: Curculionidae). *J. Asia Pac. Entomol.* **2007**, 10, 263–267.

Supplementary Materials

Table S1. Chemical constituents of basil essential oil

retention time (min)	constituent	area %
48.34	Linalool	19.7
54.08	Levomenthol	0.5
55.83	Estragole	70.3
59.18	Z-Citral	0.4
61.29	E-Citral	0.6
68.98	Methyl undecanoate	1.7
69.27	β -Caryophyllene	0.5
69.61	<i>trans</i> - α -Bergamotene	0.7
71.50	Germacrene D	0.4
73.17	<i>cis</i> - α -Bisabolene	1.8
total		96.6

Table S2. Chemical constituents of bergamot essential oil

retention time (min)	constituent	area %
25.83	α -Thujene	0.5
27.00	α -Pinene	24.5
29.64	Camphene	0.7
34.88	β -Pinene	0.8
37.50	α -Myrcene	0.5
40.42	3-Carene	5.0
41.19	Isocineole	2.0
42.27	o-Cymene	0.4
42.80	D-Limonene	46.0
43.05	Eucalyptol	1.5
45.41	γ -Terpinene	0.4
47.43	α -Terpinolene	3.7
48.34	Linalool	3.3
55.54	α -Terpineol	0.7
60.04	Linalyl acetate	3.2
65.26	Triacetin	0.7
65.83	alpha-Terpinyl acetate	0.7
68.98	Methyl undecanoate	2.1
total		96.6

Table S3. Chemical constituents of cinnamon essential oil

retention time (min)	constituent	area %
49.53	Phenylethyl Alcohol	0.3
59.80	2,3-Dihydro-benzofuran-3-ol	0.4
62.51	<i>trans</i> -Cinnamaldehyde	74.6
68.95	Methyl undecanoate	1.8
70.06	<i>trans</i> -Cinnamyl acetate	2.8
70.17	Coumarin	1.9
73.06	3-Methoxycinnamaldehyde	9.5
76.69	1,5-Dihydroxy-1,2,3,4-tetrahydronaphthalene	0.3
total		91.7

Table S4. Chemical constituents of citronella essential oil

retention time (min)	constituent	area %
42.55	Limonene	3.8
48.20	Linalool	0.8
51.67	Isopulegol	1.2
51.98	Citronellal	35.7
52.47	Isopulegol	0.6
58.03	α -Citronellol	13.4
59.99	Geraniol	19.6
61.14	E-Citral	0.5
65.68	Citronellyl acetate	4.2
65.94	Eugenol	0.6
67.00	Geranyl acetate	2.6
67.78	α -elemene	1.7
68.87	Methyl undecanoate	2.9
71.40	Germacrene D	1.2
71.89	α -Muurolene	0.7
72.42	γ -Muurolene	0.6
72.53	δ -Cadinene	2.2
73.45	Elemol	2.2
74.40	Cubenol	0.5
76.24	δ -Muurolol	0.4
76.57	α -Cadinol	0.5
76.67	α -Eudesmol	0.6
total		96.5

Table S5. Chemical constituents of clary sage essential oil

retention time (min)	constituent	area %
48.37	Linalool	32.4
55.54	α -Terpineol	3.9
55.97	2-Carene	0.4
58.06	Geraniol	1.4
60.07	Linalyl acetate	45.2
61.33	dihydro linalool	0.5
65.83	α -Terpinyl acetate	3.5
65.93	Ocimenyl acetate	0.3
66.24	Neryl acetate	3.1
67.12	Geranyl acetate	4.4
68.98	Methyl undecanoate	2.0
total		97.2

Table S6. Chemical constituents of clove bud essential oil

retention time (min)	constituent	area %
65.91	Eugenol	94.4
68.69	Methyl undecanoate	1.3
68.96	β -Caryophyllene	1.5
72.04	Phenol, 2-methoxy-4-(2-propenyl)-,acetate	2.1
total		99.3

Table S7. Chemical constituents of cypress essential oil

retention time (min)	constituent	area %
26.96	α -Pinene	1.7
29.62	Camphene	0.5
33.90	Sabinene	0.6
34.91	β -Pinene	48.8
37.47	α -Myrcene	2.7
40.41	3-carene	20.9
42.26	o-Cymene	1.0
42.76	Limonene	3.9
42.88	α -Phellandrene	0.4
47.42	α -Terpinolene	3.5
47.69	o-Isopropenyltoluene	0.4
48.33	Linalool	0.5
51.32	Isopinocarveol	0.4
54.37	Terpinen-4-ol	3.7
54.80	p-Cymen-8-ol	0.4
55.70	Myrtenol	0.4
65.83	α -Terpinyl acetate	3.9
65.94	Bicyclo[3.1.0]hexene, 6-isopropyl	0.4
68.98	Methyl undecanoate	2.0
total		95.8

Table S8. Chemical constituents of *Eucalyptus globulus* essential oil

retention time (min)	constituent	area %
26.95	α -Pinene	2.3
40.39	3-Carene	1.9
42.25	o-Cymene	2.4
42.76	D-Limonene	5.7
43.06	Eucalyptol	75.6
45.39	γ -Terpinene	2.8
47.41	α -Terpinene	1.3
54.37	Terpinen-4-ol	0.3
67.36	α -Copaene	0.4
68.97	Methyl undecanoate	1.8
69.27	β -Caryophyllene	0.8
total		95.3

Table S9. Chemical constituents of *Eucalyptus radiata* essential oil

retention time (min)	constituent	area %
26.97	α -Pinene	2.4
34.88	β -Pinene	0.5
37.49	α -Myrcene	0.3
40.41	3-carene	1.9
42.26	o-Cymene	2.8
42.77	D-Limonene	6.2
42.90	α -Phellandrene	0.4
43.07	Eucalyptol	65.1
45.40	γ -Terpinene	2.7
47.42	α -Terpinolene	1.3
54.37	Terpinen-4-ol	0.4
55.53	α -Terpineol	7.3
55.97	γ -Terpineol	1.1
67.36	α -Copaene	0.4
68.98	Methyl undecanoate	1.8
69.27	β -Caryophyllene	0.9
total		95.5

Table S10. Chemical constituents of fennel essential oil

retention time (min)	constituent	area %
26.09	α -Pinene	3.2
36.32	α -Myrcene	0.7
39.22	l-Phellandrene	1.9
42.14	D-Limonene	2.6
47.17	Fenchone	1.2
62.12	Anethole	44.6
68.66	Methyl undecanoate	21.2
69.09	Undecanoic acid, 2-methyl-	1.3
total		76.7

Table S11. Chemical constituents of fennel sweet essential oil

retention time (min)	constituent	area %
26.08	α -Pinene	0.8
41.60	o-Cymene	1.0
42.14	D-Limonene	4.0
47.15	L-Fenchone	1.5
55.19	Estragole	3.6
59.95	Anisaldehyde	2.1
62.28	Anethole	79.6
68.65	Methyl undecanoate	3.2
total		95.8

Table S12. Chemical constituents of frankincense essential oil

retention time (min)	constituent	area %
25.08	2-Thujene	15.3
26.27	α -Pinene	44.4
32.82	Sabinene	7.5
33.71	β -Pinene	2.7
36.33	α -Myrcene	1.4
39.64	3-Carene	1.0
41.66	o-Cymene	6.1
42.22	Limonene	14.9
68.67	Methyl undecanoate	2.8
total		96.3

Table S13. Chemical constituents of geranium essential oil

retention time (min)	constituent	area %
24.73	Hexylene glycol	0.4
48.36	Linalool	11.3
52.44	l-Menthone	0.9
53.16	p-Menthone	5.2
58.22	α -Citronellol	39.6
60.09	Geraniol	15.3
61.65	Citronellyl formate	11.0
63.19	Geraniol formate	4.7
65.82	Citronellyl acetate	0.8
67.13	Geranyl acetate	2.2
68.39	Diphenyl ether	0.6
68.78	α -Gurjunene	0.5
69.00	Methyl undecanoate	2.2
69.59	Diphenylmethane	0.3
total		95.2

Table S14. Chemical constituents of lavender (French) essential oil

retention time (min)	constituent	area %
35.23	3-Octanone	0.8
42.94	<i>trans</i> - α -Ocimene	0.9
43.93	β -Ocimene	0.6
48.05	Linalool	33.6
48.48	1-Octen-3-yl-acetate	0.8
51.27	(-)-Camphor	0.5
59.64	Linalyl acetate	47.5
68.67	Methyl undecanoate	3.7
68.77	α -Santalene	0.7
68.94	β -Caryophyllene	3.3
69.86	α -Farnesene	1.7
74.48	Caryophyllene oxide	0.6
total		94.7

Table S15. Chemical constituents of lavender (Bulgarian) essential oil

retention time (min)	constituent	area %
27.03	α -Pinene	0.3
42.31	o-Cymene	3.2
42.80	Limonene	2.2
43.07	Eucalyptol	3.5
48.39	Linalool	33.3
53.52	3,5,5-Trimethylhexyl acetate	0.4
54.41	Terpinen-4-ol	3.7
55.58	α -Terpineol	0.7
60.09	Linalyl acetate	35.5
61.36	Dihydro linalool	0.4
65.85	α -Terpinyl acetate	0.6
66.26	Neryl acetate	1.1
67.14	Geranyl acetate	3.4
69.00	Methyl undecanoate	1.9
69.30	β -Caryophyllene	4.7
74.78	Caryophyllene oxide	1.2
total		96.1

Table S16. Chemical constituents of lemon essential oil

retention time (min)	constituent	area %
26.32	α -Pinene	3.1
34.01	β -Pinene	9.2
36.60	α -Myrcene	0.8
39.82	3-Carene	2.1
41.81	o-Cymene	1.2
42.53	D-Limonene	75.7
45.06	γ -Terpinene	4.5
47.11	α -Terpinolene	0.4
68.75	Methyl undecanoate	0.8
total		97.7

Table S17. Chemical constituents of lemongrass essential oil

retention time (min)	constituent	area %
27.02	α -Pinene	1.1
42.80	Limonene	6.6
43.07	Eucalyptol	0.9
48.36	Linalool	3.5
54.23	Verbenol	0.4
58.20	α -Citronellol	12.5
59.24	Z-Citral	23.0
60.09	Geraniol	9.4
61.34	E-Citral	28.6
66.26	Neryl acetate	1.1
67.14	Geranyl acetate	3.7
69.00	Methyl undecanoate	2.1
69.30	β -Caryophyllene	2.8
total		95.5

Table S18. Chemical constituents of mandarin essential oil

retention time (min)	constituent	area %
26.12	α -Pinene	2.7
33.71	β -Pinene	7.0
36.33	α -Myrcene	1.1
41.65	o-Cymene	2.6
42.34	D-Limonene	71.9
44.94	γ -Terpinene	6.2
68.66	Methyl undecanoate	3.4
total		94.9

Table S19. Chemical constituents of marjoram essential oil

retention time (min)	constituent	area %
27.22	α -Pinene	1.4
34.38	Sabinene	2.6
35.48	β -Pinene	1.0
40.73	3-Carene	2.7
41.71	α -Terpinene	1.8
42.62	o-Cymene	9.0
43.03	Limonene	1.0
43.20	α -Phellandrene	0.5
45.61	γ -Terpinene	6.1
47.57	Terpinolene	2.2
48.52	Linalool	6.9
54.84	Terpinen-4-ol	30.4
54.99	α -Thujone	0.7
55.96	α -Terpineol	4.9
56.34	γ -Terpineol	0.5
60.09	Linalyl acetate	11.2
60.88	Piperitone	1.3
68.96	Methyl undecanoate	1.9
69.36	β -Caryophyllene	8.5
total		94.6

Table S20. Chemical constituents of orange sweet essential oil

retention time (min)	constituent	area %
26.99	α -Pinene	0.4
37.52	α -Myrcene	0.4
42.83	Limonene	83.8
48.35	Linalool	0.3
49.89	<i>trans-p</i> -Mentha-2,8-dienol	0.6
50.71	Limonene oxide	0.4
50.94	<i>cis-p</i> -Mentha-2,8-dien-1-ol	0.6
51.02	<i>trans</i> -Limonene oxide	0.7
56.02	Perilla alcohol	0.5
57.65	<i>trans</i> -Carveol	1.1
58.78	<i>cis</i> -Carveol	0.5
59.20	Z-Citral	0.6
59.74	Carvone	1.3
61.31	E-Citral	0.8
65.69	7-Oxabicyclo[4.1.0]heptane	1.8
68.42	<i>cis</i> -Limonene oxide	0.5
68.99	Methyl undecanoate	1.9
80.16	Cedrene	0.4
87.15	Phenylethyl salicylate	0.4
total		96.7

Table S21. Chemical constituents of patchuli essential oil

retention time (min)	constituent	area %
42.29	o-Cymene	11.1
43.04	Benzyl alcohol	2.8
48.35	Linalool	9.2
67.87	α -Patchoulene	0.9
68.66	α -Gurjunene	0.4
68.77	Isoledene	7.1
68.99	Methyl undecanoate	2.0
69.18	Di-epi- α -cedrene	2.8
69.29	β -Caryophyllene	24.6
69.52	Cedrene	0.7
69.78	α -Guaiene	3.3
69.90	Thujopsene	3.7
70.50	Seychellene	1.9
70.81	Aromadendrene	3.2
70.93	α -Patchoulene	1.5
71.04	γ -Gurjunene	0.4
71.20	Azulene	1.3
71.80	Ledene	0.3
71.96	Azulene	0.7
72.17	α -Bulnesene	4.0
72.42	Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)-	1.3
75.47	Widdrol	0.5
75.58	Cedrol	3.2
75.89	Isoaromadendrene epoxide	0.3
76.79	Veridiflorol	0.5
77.46	Patchouli alcohol	8.0
total		95.7

Table S22. Chemical constituents of peppermint essential oil

retention time (min)	constituent	area %
27.04	α -Pinene	0.4
34.97	β -Pinene	0.7
42.31	o-Cymene	6.0
42.81	D-Limonene	6.8
48.37	Linalool	11.0
51.88	Isopulegol	0.8
52.47	Isomenthone	28.1
53.17	p-Menthone	11.2
53.52	Menthol	2.2
54.14	Levomenthol	18.4
54.32	Isopulegol	0.5
60.53	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	0.4
62.81	Menthyl acetate	1.6
69.00	Methyl undecanoate	1.9
69.30	β -Caryophyllene	5.2
total		95.1

Table S23. Chemical constituents of pine essential oil

retention time (min)	constituent	area %
25.90	α -Pinene	43.9
28.40	Camphene	1.0
33.36	β -Pinene	7.6
36.00	α -Myrcene	2.2
39.40	3-Carene	8.0
41.45	o-Cymene	1.0
41.98	D-Limonene	5.9
42.10	α -Phellandrene	1.4
61.94	(-)-Bornyl acetate	0.6
68.48	Longifolene	0.4
68.60	Methyl undecanoate	16.2
68.83	β -Caryophyllene	2.5
69.05	Undecanoic acid, 2-methyl	2.6
total		93.3

Table S24. Chemical constituents of rosemary essential oil

retention time (min)	constituent	area %
26.98	α -Pinene	15.9
29.63	Camphene	0.4
34.89	β -Pinene	9.0
40.41	3-Carene	1.8
42.27	o-Cymene	6.8
42.77	D-Limonene	7.9
43.05	Eucalyptol	30.3
45.40	γ -Terpinene	0.8
47.42	α -Terpinolene	1.1
51.78	Camphor	19.9
54.37	Terpinen-4-ol	0.3
68.98	Methyl undecanoate	1.8
total		96.0

Table S25. Chemical constituents of sandal wood essential oil

retention time (min)	constituent	area %
42.96	Benzyl alcohol	14.1
68.87	Methyl undecanoate	2.9
77.08	Norbornane	14.5
78.28	α -Santalol	5.8
80.71	Geranylgeraniol	3.9
81.02	Corymbolone	3.8
81.15	Eudesma-3,11-dien-2-one	11.1
81.27	2,6,11-Tridecatrien-10-ol, 2,6,10-trimethyl-	3.5
81.88	Longipinocarvone	4.4
82.21	Geranylgeraniol	3.4
82.43	1-Cyclohexene-1-butanal, α ,2,6,6-tetramethyl-	5.0
82.61	Acetic acid, 1-[2-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-ethyl]-vinyl ester	8.9
82.88	9,17-Octadecadienal, (Z)-	2.1
total		83.4

Table S26. Chemical constituents of spearmint essential oil

retention time (min)	constituent	area %
26.65	α -Pinene	0.7
33.48	Sabinene	0.5
34.41	β -Pinene	0.9
37.08	Myrcene	1.6
38.34	3-Octanol	0.3
42.61	D-Limonene	21.2
42.82	Eucalyptol	2.1
53.89	Levomenthol	0.6
54.16	Terpinen-4-ol	0.6
55.60	Dihydrocarvone	1.8
59.75	(-)-Carvone	61.4
67.64	α -Bourbonene	0.8
68.87	Methyl undecanoate	2.4
69.15	β -Caryophyllene	0.6
70.06	<i>trans</i> - β -Farnesene	0.4
71.39	Germacrene D	0.4
total		96.1

Table S27. Chemical constituents of tea tree essential oil

retention time (min)	constituent	area %
27.25	α -Pinene	4.6
41.72	α -Terpinene	3.7
42.60	o-Cymene	5.6
43.03	Limonene	1.2
43.35	Eucalyptol	4.8
45.64	γ -Terpinene	15.8
47.59	Terpinolene	6.8
54.91	Terpinen-4-ol	48.7
55.92	α -Terpineol	0.4
60.87	Piperitone	0.5
68.95	Methyl undecanoate	1.7
total		93.9

Table S28. Chemical constituents of ylang ylang essential oil

retention time (min)	constituent	area %
41.08	4-Methylanisole	13.0
47.54	Methyl benzoate	5.8
47.98	Linalool	18.0
52.35	Benzyl acetate	19.9
54.74	Methyl salicylate	0.5
62.11	Anethole	0.4
66.78	Geranyl acetate	6.2
68.66	Methyl undecanoate	3.0
68.93	β -Caryophyllene	4.8
69.58	Cinnamyl acetate	3.7
70.29	α -Caryophyllene	1.6
72.89	cis- α -Bisabolene	0.4
73.45	Nerolidol	0.4
79.60	Benzyl benzoate	3.8
83.34	Benzyl salicylate	14.6
total		95.9

Abstract in Korean

어리쌀바구미(*Sitophilus zeamais*)에 대한 시나몬(*Cinnamomum cassia*) 정유를 함유한 제품과 28종 에센셜 오일의 살충 활성

양 윤 호

농생명공학부 곤충학 전공

서울대학교 대학원

초록

어리쌀바구미(*Sitophilus zeamais*)는 따뜻하고 습한 지역에서 발견되는 대표적인 저장곡물 해충의 하나이다. 본 연구에서는 어리쌀바구미에 대한 28종 에센셜 오일의 접촉 및 훈증 독성 그리고 유인 저해 효과를 평가하였다. 가스 크로마토그래프 질량분석법(GC-MS)을 이용해 에센셜 오일의 주요 성분들이 확인하였다. 또한 주성분 소거 평가(compound elimination assay)를 진행하여 독성 평가에서 효과가 좋았던 시나몬, 티트리, 일랑일랑, 마조람 에센셜 오일의 주요 활성 성분을 파악하였다. 평가된

에센셜 오일들 중에서 시나몬 오일이 독성평가와 유인 저해 평가에서 가장 뛰어난 효과를 보였다. GC-MS 분석과 주성분 소거 평가 결과를 토대로 시나몬 오일의 *trans*-cinnamaldehyde와 티트리, 마조람 오일의 terpinen-4-ol 이 독성에 관여하는 주요 성분이라는 것을 확인할 수 있었다. 시나몬 오일은 쌀을 넣지 않았던 용기 평가에서는 100% 살충률을 보여주었으나, 쌀이 채워진 용기 조건에서는 15% 이하의 낮은 살충력을 보이며 독성 효과가 떨어졌다. 곡물에 의한 에센셜 오일의 활성성분 흡수 양상에 대한 이해와 효과를 높일 수 있는 적절한 제형 개발이 차후 연구 과제로 남을 것으로 보인다.

검색어: 어리쌀바구미, 에센셜 오일, 훈증독성, 접촉독성, 유인 저해, 제형

학번: 2019-23828