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Mite color alteration and acaricidal activity of 3,7-dimethyl-2,6-octadienal and its structural analogues against the stored food pest mite *Tyrophagus putrescentiae*

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Abstract
Acaricidal activities of the active component isolated from *Melissa officinalis* oil and its structural analogues against *Tyrophagus putrescentiae* were evaluated using fumigant and contact bioassays. The structure of 3,7-dimethyl-2,6-octadienal purified from *M. officinalis* oil was elucidated with EI-MS, ^{1}H- and ^{13}C-NMR, ^{1}H–^{1}H COSY, and DEPT-NMR. Based on the LD_{50} values of 3,7-dimethyl-2,6-octadienal analogues in fumigant and contact bioassays, respectively, 2,4-octadienal showed the highest activity (LD_{50} = 2.09 μg/cm^3 and 11.08 μg/cm^2), followed by 3,7-dimethyl-6-octenal (3.60 μg/cm^3 and 29.34 μg/cm^2), 3,7-dimethyl-2,6-octadienal (6.18 μg/cm^3 and 36.17 μg/cm^2), 2-octenal (7.45 μg/cm^3 and 47.36 μg/cm^2) and *M. officinalis* oil (8.89 μg/cm^3 and 23.83 μg/cm^2). Comparing the acaricidal activities of the aldehyde group based on the degree of unsaturation, 2,4-octadienal containing two double bonds was more potent than 2-octenal with a single double bond. Based on the acaricidal activities of the methyl group, on the other hand, 3,7-dimethyl-6-octenal containing a single double bond was more acaricidal than 3,7-dimethyl-2,6-octadienal with two double bonds. These results indicate that 3,7-dimethyl-2,6-octadienal analogues are useful to control food mites.

Keywords Acaricidal activity · Octadienal · Mite color alteration · Structural analogues · *Tyrophagus putrescentiae*

Introduction

Stored foods are regularly infested by more than 1025 arthropod species—primarily mites, moths, and beetles—resulting in reduced quality and quantity of stored foods (Alfonzo et al. 2017; Choi et al. 2017; Hernandez-Lambraño et al. 2015). *Tyrophagus putrescentiae* is the most common pest causing serious damage to stored foods with protein and fat-rich

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content (Hughes 1976). These foods transport bacterial and fungal spores when contaminated with *T. putrescentiae* and cause enteritis, diarrhea, and allergic reactions (Franzolina et al. 1999). Commercial acaricides have been used to control the growth and emergence of stored product mites in the stored foods. However, consumers are concerned about food safety and are demanding stored foods without insecticides (Jeon et al. 2017). Therefore, plant oils are of great interest as alternatives to commercial acaricides against stored product mites.

Plant essential oils are 'generally recognized as safe' materials by the US Foods and Drugs Administration (Alfonzo et al. 2017). Due to their chemical composition, plant essential oils show a broad range of antifungal and insecticidal effects against bacteria, fungi, and stored food insects (Bae et al. 2017; Jeon et al. 2017; Kim and Lee 2016; Lee and Lee 2016; Nguefack et al. 2004; Oussalah et al. 2007; Park and Lee 2017). In this regard, *Melissa officinalis* L., widely cultivated throughout Europe, is a well-known plant (Mimica-Dukic et al. 2004). The *M. officinalis* oil was evaluated for antibacterial, antioxidant, and antifungal activity and is applied in cosmetic, food, medicine, and perfume industries (Jeon et al. 2008; Salanski et al. 1998). In our previous studies on *M. officinalis* oil, 3,7-dimethyl-2,6-octadienal was found to be toxic to *Dermatophagoides farina* and *D. pteronyssinus* and caused alteration to a golden brown color in the body (Park and Lee 2018). However, the acaricidal effect of *M. officinalis* oil against stored product mites has yet to be investigated. Therefore, this study evaluated the acaricidal effect of the essential oil extracted from *M. officinalis* leaves against *T. putrescentiae*, and its chemical constituents were isolated and identified. In addition, the acaricidal effects of active component isolated from *M. officinalis* leaves and its structural analogues were analyzed to determine the acaricidal effects based on chemical structures.

Materials and methods

Chemicals and plant materials

3,7-Dimethyl-2,6-octadienal (96%, cat no. W230316), 3,7-dimethyl-6-octenal (95%, cat no. 27470), 2,4-octadienal (95%, cat no. W372102), octanal (99%, cat no. O5608), and 2-octenal (95%, cat no. W321508) were purchased from Aldrich (Missouri, USA) and *N*,*N*-diethyl-*m*-toluamide (DEET) (95%, cat no. 32570) was supplied by Fluka (Buchs, Switzerland). The voucher specimen of *M. officinalis* was identified by Prof. Jeongmoon Kim (Chonbuk National University, South Korea). Samples were extracted by steam distillation. The extracted oil was concentrated to dryness by rotary evaporation at 26 °C.

Isolation and identification

Melissa officinalis oil (12 g) was isolated by silica gel column chromatography (Merck 70-230 mesh, 605 g, 550 mm i.d.×701 mm; NJ, UK) and continuously evaluated with chloroform:ethyl acetate (10:0, 9:1, and 0:10, vol/vol). Each fraction was analyzed to distinguish similar fractions using thin-layer chromatography, which yielded four fractions designated ME1–ME4. Acaricidal toxicities of all the fractions were evaluated against *T. putrescentiae* at 20 μg/cm^3. The ME4 fraction exhibited the strongest activity among all the fractions analyzed. Consequently, the active ME4 (4.61 g) was sequentially purified by HPLC (Japan Analytical Industry, Tokyo, Japan) and isolated successfully as a single peak.

The HPLC was performed using a W series column (21.5 mm i.d. × 1000 mm; Japan Analytical Industry) with methanol (100%) as a mobile phase at a flow rate of 3.0 mL/min and monitored with a UV detector (270 nm). Finally, ME41 (800 mg) was isolated.

The molecular weight of ME41 was investigated using EI-MS (model name: JEOL GSX 400 mass spectrometer; JEOL, Tokyo, Japan) spectra. The chemical structure of ME41 was determined using nuclear magnetic resonance (JNM–ECA600 spectrometer; JEOL). The ^{13}C-, ^{1}H-, and DEPT-NMR were used to confirm the number of carbons and protons at 600 and 150 MHz. Two-dimensional (2D) NMR (HMQC and ^{1}H–^{1}H COSY) was carried out to investigate the relationship between carbon and proton.

Experimental mites

The mites were reared on yeast and fry powder (1:1 by weight) located in the plastic box (15 × 13 × 8 cm). The rearing box was stored in an incubator at 25 ± 2 °C and 75% relative humidity within a plastic cage (18 × 18 × 17 cm) that contained a saturated solution of NaCl to prevent the escape of mites and to maintain relative humidity. Fry powder consisted of protein (43.8%), cellulose (4.2%), lipid (3.2%), phosphorus (1.0%), calcium (1.9%), and others (54.8%).

Acaricidal toxicity

Acaricidal toxicities of *M. officinalis* oil, 3,7-dimethyl-2,6-octadienal and its structural analogues were assessed using fumigant and contact toxicity bioassays against *T. putrescentiae* based on a modified method originally reported by Yang and Lee (2012).

To test for fumigant toxicity, various concentrations (60, 40, 20, 10, 5, 2.5, 2.0, 1.5, 1.0, 0.5, 0.25, 0.20, 0.10, and 0.05 μg/cm^3) of each sample dissolved in acetone (10 μL) were applied to a paper disk (8 mm i.d. × 1 mm thick; Advantec, Tokyo, Japan). Negative and positive control paper discs received acetone and DEET, respectively. The treated disks were dried under a hood for 10 min and later placed in the lid of a microtube (2 mL; Greiner bio-one, Frickenhausen, Germany). Thirty adult mites (both sexes, 8–11 days old) were separately inoculated in each microtube.

The contact bioassay was conducted on a filter paper (5.5 cm i.d. × 25 μm thick; Whatman, Maidstone, UK) and treated with 50 μL of a sample (Kim and Lee 2016; Lee and Lee 2016). Various concentrations (60, 40, 30, 20, 10, 5.0, 2.5, 2.0, 1.0, 0.50, 0.25, 0.20, 0.10, and 0.05 μg/cm^2) of test sample were dissolved in acetone (50 μL). Negative and positive controls were acetone and DEET, respectively. The treated papers were dried under a fume hood for 20 min and then placed at the bottom of a Petri dish (9 cm i.d. × 1.5 cm deep). Thirty randomly selected adult mites (both sexes, 7–10 days old) were added to each dish, and sealed with a lid. For the contact and fumigant methods, treatments were performed at 25 ± 2 °C and 75% relative humidity in darkness. Dead mites were confirmed 24 h after treatment by microscopic examination (× 20).

All experiments were performed in triplicate and the 50% lethal dose (LD_{50}) values were calculated by probit analysis (SAS Institute 1990). Relative toxicity (RT) was expressed based on the ratio of DEET LD_{50} to each chemical LD_{50}, as described previously (Bae et al. 2017; Lee and Lee 2016). The altered color of food mites was determined using a fumigant. In addition, color alterations of stored product mites exposed to 3,7-dimethyl-2,6-octadienal, 2,4-octadienal and DEET were visualized via optical microscopy (× 100; Olympus, Japan).

Results and discussion

Based on the LD_{50} values in the fumigant bioassay, the essential oil (LD_{50} = 8.89 μg/cm^3) of *M. officinalis* oil was about 3.5-fold more active than DEET (LD_{50} = 31.47 μg/cm^3) against *T. putrescentiae* (Table 1). In case of the contact bioassay, *M. officinalis* oil (LD_{50} = 23.83 μg/cm^2) was about 0.7-fold more effective than DEET (LD_{50} = 16.26 μg/cm^2) against *T. putrescentiae* (Table 1). The negative control, applied to acetone, failed to show any acaricidal activity against *T. putrescentiae* in the fumigant and contact bioassays.

In order to isolate the active components of *M. officinalis* oil, silica gel chromatography and HPLC were conducted using organic solvents. ME41 was successfully isolated. The active component was purified using spectroscopic methods, including ^{1}H-NMR, ^{13}C-NMR, EI-MS, DEPT-NMR, ^{1}H-^{1}H COSY and HMQC. The spectroscopic analyses resulted in the identification of 3,7-dimethyl-2,6-octadienal based on the following evidence: 3,7-dimethyl-2,6-octadienal ($C_{10}H_{16}O$); EI-MS (70 eV) m/z (% relative intensity) M^+ 152.23; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ = 5.835–5.874 (1H, t), 5.089–5.228 (2H, d), 3.974–4.094 (1H, d), 3.974–4.094 (2H, t), 1.815 (1H, s), 1.295–1.306 (2H, d), and 1.318–1.323 ppm (2H, d); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ = 141.430, 114.573, 73.334, 37.065, 31.828, 25.077, 22.374, and 14.095 ppm. These results were consistent with previously reported data (Salanski et al. 1998).

In the fumigant bioassay, 3,7-dimethyl-2,6-octadienal (LD_{50} = 6.18 μg/cm^3) was approximately 5.1-fold more effective than DEET (LD_{50} = 31.47 μg/cm^3) (Table 2). In the contact bioassay, 3,7-dimethyl-2,6-octadienal (LD_{50} = 36.17 μg/cm^3) was less effective than DEET (LD_{50} = 17.26 μg/cm^3). The negative control (only solvent) did not kill *T. putrescentiae*. These results indicate that the acaricidal activity of 3,7-dimethyl-2,6-octadienal is mainly mediated via fumigant action. Early reports analyzing the acaricidal activity of 3,7-dimethyl-2,6-octadienal against *Dermatophagoides farinae* showed that this was largely a result of fumigant action (Lee et al. 2013), consistent with our present study results. Plant-derived oils containing high levels of 3,7-dimethyl-2,6-octadienal also showed potent fumigant toxicity against the insect pest *Tribolium castaneum* (Olivero-Verbel et al. 2010).

To evaluate the structure–activity relationships (SARs) of 3,7-dimethyl-2,6-octadienal and its structural analogues, we selected octanal, 2-octenal, 2,4-octadienal, and 3,7-dimethyl-6-octenal (Table 2). In the fumigant bioassay, 2,4-octadienal was the most active compound (LD_{50} = 2.09 μg/cm^3), which was approximately 15.0 × as effective as DEET (LD_{50} = 31.47 μg/cm^3), followed by 3,7-dimethyl-6-octenal (3.60 μg/cm^3), 3,7-dimethyl-2,6-octadienal (6.18 μg/cm^3), and 2-octenal (7.45 μg/cm^3). In the contact bioassay, 2,4-octadienal was the most active compound (LD_{50} = 11.08 μg/cm^2), approximately 1.6 × as effective as DEET (17.26 μg/cm^2), followed by 3,7-dimethyl-6-octenal (29.34 μg/cm^2), 3,7-dimethyl-2,6-octadienal (36.17 μg/cm^2) and 2-octenal (47.36 μg/cm^2), whereas octanal did not show any acaricidal activity.

Based on the structure of 3,7-dimethyl-2,6-octadienal and its structural analogues, acaricidal activities were determined by the degrees of unsaturation and functional groups (aldehyde and methyl groups) (Table 3). Comparing the acaricidal activities of aldehyde groups with regard to degrees of unsaturation, 2,4-octadienal with two double bonds was more potent than 2-octenal containing a single double bond. However, 2-octenal with one double bond had more acaricidal activity than octanal. Based on methyl groups, on the other hand, 3,7-dimethyl-6-octenal containing one double bond was more acaricidal than 3,7-dimethyl-2,6-octadienal with two double bonds. With regard to structure, the fumigant toxicity of 22 monoterpenoids against the house fly *Musca domestica* was affected

Table 1 Acaricidal toxicities of *Melissa officinalis* oil and commercial acaricide against *Tyrophagus putrescentiae*, using fumigant and contact bioassays with 24-h exposure

Samples	Bioassay	LD_{50} (95% CI)[a]	LD_{90} (95% CI)	Slope ± SE	χ^2 (df, p)[b]	RT[c]
M. officinalis oil	Fumigant (μg/cm^3)	8.89 (7.17–10.85)	28.82 (21.71–44.57)	2.51 ± 0.33	3.42 (8, 0.91)	3.54
	Contact (μg/cm^2)	23.83 (20.72–27.22)	52.65 (42.82–74.50)	3.72 ± 0.55	8.15 (8, 0.42)	0.72
DEET	Fumigant (μg/cm^3)	31.47 (26.36–37.59)	86.74 (66.67–131.59)	2.91 ± 0.39	10.40 (8, 0.24)	1.00
	Contact (μg/cm^2)	17.26 (14.17–20.90)	48.78 (36.93–76.97)	2.84 ± 0.41	8.06 (7, 0.33)	1.00

[a]Activity is considered significantly different if the 95% confidence intervals do not overlap

[b]Pearson's χ^2 goodness of fit test

[c]Relative toxicity: LD_{50} value of DEET/LD_{50} value of *M. officinalis* oil

Table 2 Acaricidal toxicities of 3,7-dimethyl-2,6-octadienal analogues and commercial acaricide against *Tyrophagus putrescentiae*, based on fumigant and contact bioassays with 24-h exposure

Samples	Bioassay	LD_{50} (95% CI)[a]	LD_{90} (95% CI)	Slope ± SE	χ^2 (df, p)[b]	RT[c]
Octanal	Fumigant (μg/cm^3)	–[d]	–	–	–	–
	Contact (μg/cm^2)	–	–	–	–	–
2-Octenal	Fumigant (μg/cm^3)	7.45 (5.76–9.63)	29.76 (21.13–48.72)	2.13 ± 0.25	3.04 (8, 0.93)	4.23
	Contact (μg/cm^2)	47.36 (41.64–53.84)	102.21 (84.06–139.54)	3.84 ± 0.52	4.20 (8, 0.84)	0.36
2,4-Octadienal	Fumigant (μg/cm^3)	2.09 (1.55–2.83)	9.06 (6.07–16.47)	2.01 ± 0.25	2.60 (7, 0.92)	15.03
	Contact (μg/cm^2)	11.08 (9.46–12.99)	24.45 (19.86–33.11)	3.73 ± 0.45	3.42 (7, 0.84)	1.56
3,7-Dimethyl-6-octenal	Fumigant (μg/cm^3)	3.60 (2.94–4.35)	9.90 (7.76–14.06)	2.92 ± 0.35	5.14 (7, 0.64)	8.74
	Contact (μg/cm^2)	29.34 (25.15–34.34)	68.94 (54.92–98.52)	3.45 ± 0.46	3.00 (8, 0.935)	0.59
3,7-Dimethyl-2,6-octadienal	Fumigant (μg/cm^3)	6.18 (4.81–7.88)	22.78 (16.54–36.12)	2.26 ± 0.27	4.38 (8, 0.82)	5.10
	Contact (μg/cm^2)	36.17 (31.38–41.60)	77.17 (63.07–106.79)	3.89 ± 0.55	6.10 (7, 0.53)	0.48
DEET	Fumigant (μg/cm^3)	31.47 (26.36–37.59)	86.74 (66.67–131.59)	2.91 ± 0.39	10.40 (8, 0.24)	1.00
	Contact (μg/cm^2)	17.26 (14.17–20.90)	48.78 (36.93–76.97)	2.84 ± 0.41	8.06 (7, 0.33)	1.00

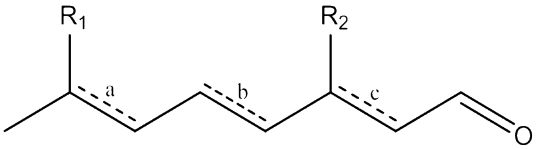
[a]Activity is considered significantly different if the 95% confidence intervals do not overlap

[b]Pearson's χ^2 goodness of fit test

[c]Relative toxicity: LD_{50} value of DEET/LD_{50} value of *M. officinalis* oil

[d]–, no activity

Table 3 Structure-activity relationships of 3,7-dimethyl-2,6-octadienal analogues



Compounds	R_1	R_2	a	b	c	LD_{50}	
						Fumigant ($\mu g/cm^3$)	Contact ($\mu g/cm^2$)
Octanal	H	H				–	–
2-Octenal	H	H			•	7.45	47.36
2,4-Octadienal	H	H		•	•	2.09	11.08
3,7-Dimethyl-6-octenal	CH_3	CH_3	•			3.60	29.34
3,7-Dimethyl-2,6-octadienal	CH_3	CH_3	•		•	6.18	36.17

–, no activity

by carbon skeleton, functional groups, degrees of unsaturation, and volatility of the test monoterpenoids (Rice and Coats 1994). The monocyclic ketone carvone with two double bonds exhibited greater fumigant activity than the saturated monocyclic ketone menthone and the monocyclic ketone pulegone with one double bond (Tak et al. 2006).

The color alterations of 3,7-dimethyl-2,6-octadienal analogues were used to identify mites microscopically following a fumigant toxicity bioassay (Fig. 1). Mites treated with 2,4-octadienal (Fig. 1a) and 3,7-dimethyl-2,6-octadienal (Fig. 1b) displayed change in body color to golden brown. In contrast, *T. putrescentiae* failed to show any color alteration by DEET (Fig. 1c). *Tyrophagus putrescentiae* was an important inductor of fungal and bacterial spores, and allergens (Lee et al. 2009). The color change in mites treated with 2,4-octadienal and 3,7-dimethyl-2,6-octadienal can be used to distinguish them with the naked eye. This discoloration is likely to be associated with enzymatic browning, which may be induced by polyphenol oxidase and tyrosinase; polyphenol oxidase exists as propolyphenol oxidase in both insects and mites, and mediates immunity and self-recognition (Lee and Lee 2008). Resistance to disease was attributed to the existence of polyphenol oxidase (Lee

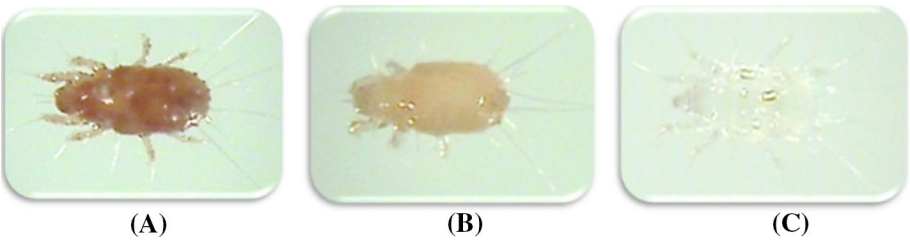


Fig. 1 Color alteration of *Tyrophagus putrescentiae* treated with **a** 2,4-octadienal, **b** 3,7-dimethyl-2,6-octadienal, and **c** DEET. (Color figure online)

2002). Furthermore, tyrosinase is an oxidase that controls melanin production in plants and animals (Lee and Lee 2008).

According to the material safety data sheet (Sigma-Aldrich 2016), acute oral toxicity of 3,7-dimethyl-2,6-octadienal in rats occurred at 2420 mg/kg. In this regard, 3,7-dimethyl-2,6-octadienal and its analogues facilitate the control of mites in stored foods and ensure food safety industrially. Further investigations are needed to evaluate the potential safety of 3,7-dimethyl-2,6-octadienal and its analogues, especially its mode of action and formulations with enhanced potency.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent was obtained from all individual participants included in the study.

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