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Toxicity of Basil Oil Constituents and Related Compounds and the Efficacy of Spray Formulations to *Dermatophagoides farinae* (Acari: Pyroglyphidae)

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ABSTRACT Pyroglyphid house dust mites are the most common cause of allergic symptoms in humans. An assessment was made of the toxicity of basil, *Ocimum basilicum* L, essential oil, 11 basil oil constituents, seven structurally related compounds, and another 22 previously known basil oil constituents to adult American house dust mites, *Dermatophagoides farinae* Hughes. The efficacy of four experimental spray formulations containing basil oil (1, 2, 3, and 4% sprays) was also assessed. Results were compared with those of two conventional acaricides benzyl benzoate and *N,N*-diethyl-3-methylbenzamide. The active principles of basil oil were determined to be citral, α -terpineol, and linalool. Citral (24 h LC₅₀, 1.13 $\mu\text{g}/\text{cm}^2$) and menthol (1.69 $\mu\text{g}/\text{cm}^2$) were the most toxic compounds, followed by methyl eugenol (5.78 $\mu\text{g}/\text{cm}^2$). These compounds exhibited toxicity greater than benzyl benzoate (LC₅₀, 8.41 $\mu\text{g}/\text{cm}^2$) and *N,N*-diethyl-3-methylbenzamide (37.67 $\mu\text{g}/\text{cm}^2$). Potent toxicity was also observed with eugenol, menthone, spathulenol, α -terpineol, nerolidol, zerumbone, and nerol (LC₅₀, 12.52–21.44 $\mu\text{g}/\text{cm}^2$). Interestingly, the sesquiterpenoid α -humulene, lacking only the carbonyl group present in zerumbone, was significantly less effective than zerumbone, indicating that the α,β -unsaturated carbonyl group of zerumbone is a prerequisite component for toxicity. These compounds were consistently more toxic in closed versus open containers, indicating that their mode of delivery was largely a result of vapor action. Basil oil applied as 3 and 4% sprays provided 97 and 100% mortality against the mites, respectively, whereas permethrin (cis:trans, 25:75) 2.5 g/liter spray treatment resulted in 17% mortality. Our results indicate that practical dust mite control in indoor environments can be achieved by basil oil spray formulations (3 and 4% sprays) as potential contact-action fumigants.

KEY WORDS *Dermatophagoides farinae*, *Ocimum basilicum*, botanical acaricide, natural fumigant, spray formulation

Pyroglyphid house dust mites are the most common cause of allergic symptoms in humans, affecting 65 million to 1.2 billion people worldwide (Colloff 2009, Hammad et al. 2009). The American house dust mite, *Dermatophagoides farinae* Hughes (Acari: Pyroglyphidae), and the European house dust mite, *Dermatophagoides pteronyssinus* Trouessart, are two of the most important producers of various allergens in the domestic environment (Platts-Mills et al. 1997, Arlian 2002, Colloff 2009). Allergies to the pyroglyphid mites are increasing and are one of the most serious global public health problems. They are closely associated with changes in the living environment of humans, such as central heating, space heating, tighter win-

dows, humidity control with humidifier, poor ventilation, and fitted carpets, all of which allow the growth of the mites (Pollart et al. 1987, Institute of Medicine 2000).

Increasing public concern for the environmental effects of acaricides, human health effects, and undesirable effects on nontarget organisms intensifies when repeated applications of conventional synthetic acaricides (Pollart et al. 1987, Ahn et al. 2006, World Health Organization [WHO] 2006) become necessary. In addition, the number of approved acaricides may be reduced in the near future in the United States by the U.S. Environmental Protection Agency (U.S. EPA) as reregistration occurs under the 1996 Food Quality and Protection Act (U.S. EPA 2011). A reregistration requirement is also a concern in other regions including in the European Union, where it is under the control of the Commission Regulation (EC) No. 1048/2005 (Anonymous 2005). There is, therefore, a pressing need for the development of selective alternatives for the control of house dust mites, particularly those

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Table 1. The 40 organic pure compounds examined in this study

Compound	Source ^a	Purity (%)	Compound	Source ^a	Purity (%)
(E)-anethole ^b	S-A	99	Limonene ^{b,c,d,e,f}	WK	>95.0
(+)-aromadendrene ^c	S-A	≥97.0	Linalool ^{b,c,d,e,f}	WK	>98.0
Bisabolene ^{c,f}	TCI	–	Linalool oxide ^{c,e,f}	S-A	≥97.0
Camphene ^{d,e,f}	S-A	95	Menthol ^g	S-A	≥98.0
Camphor ^{b,d,e,f}	S-A	96	Menthone ^g	S-A	≥97.0
β-caryophyllene ^{b,c,d,e,f}	TCI	>90.0	(E)-p-methoxycinnamaldehyde ^c	S-A	≥98.0
Caryophyllene oxide ^c	S-A	95	Methyl chavicol ^{b,c,d,e,f}	S-A	98
(–)-α-cedrene ^f	S-A	≥99.0	Methyl eugenol ^{b,e,f}	S-A	≥98.0
1,8-cineole ^{b,d,e,f}	S-A	99	Myrcene ^{b,d,e,f}	S-A	≥95.0
Citral ^c	S-A	95	(+)-terpinen-4-ol ^{b,d,e,f}	S-A	≥95.0
(S)-(-)-β-citronellol ^g	S-A	99	Nerol ^g	TCI	>98.0
p-cymene ^{b,d,e}	TCI	>95	Nerolidol ^{c,f}	S-A	98
Eugenol ^{d,e,f}	WK	>95	α-pinene ^{b,d,e,f}	TCI	95.0
(E)-β-farnesene ^{c,e,f}	S-A	≥90.0	β-pinene ^{b,c,d,e,f}	TCI	>94
(+)-fenchone ^c	S-A	≥98.0	Spathulenol ^c	BBP	>97.0
(1R)-(-)-fenchone ^c	S-A	≥98.0	γ-terpinene ^{b,d,e,f}	S-A	≥97.0
Geraniol ^{c,f}	S-A	98	α-terpineol ^{c,d,e}	S-A	≥96
α-humulene ^{b,c,d,f}	S-A	≥96.0	Terpinyl acetate ^c	WK	90
Isoeugenol ^g	S-A	98	(–)-α-thujone ^g	S-A	≥96.0
(–)-isolongifolene ^f	S-A	≥98.0	Zerumbone ^g	S-A	≥98

^a SA, Sigma-Aldrich (St. Louis, MO); TCI, Tokyo Chemical Industry (Tokyo, Japan); WK, Wako (Osaka, Japan); BBP, BioBioPha (Kunming, Yunnan, China).

^b Constituents of basil oil reported by Koba et al. (2009).

^c Constituents identified in this study.

^d Constituents of basil oil reported by Marotti et al. (1996).

^e Constituents of basil oil reported by Viña and Murillo (2003).

^f Constituents of basil oil reported by Pripdeevecch et al. (2010).

^g Compounds structurally related to basil oils constituents.

with fumigant action, that allow effective application in indoor environments that reach deep harborages missed by many commercially available acaricides.

Bioacaricides derived from plant essential oils have been suggested as potential alternatives to conventional acaricides largely because essential oils constitute a potential source of bioactive secondary metabolites that pose fewer risks to the environment, with minimal impacts on human and animal health (Ahn et al. 2006, Isman 2006). They can act at multiple and novel target sites (Kostyukovsky et al. 2002, Priestley et al. 2003), thereby reducing the potential for resistance (Wang et al. 2012). Many essential oils are commonly used as fragrances and flavoring agents for foods, beverages, and cosmetics (Lawless 2002), and are perceived by the general public as relatively safe (Isman 2006). Much effort has been focused on them as potential sources of commercial acaricides, in part, because certain essential oil preparations meet the criteria as minimum-risk pesticides (Isman 2008). In the screening of essential oils for acaricidal activity, the essential oil from basil, *Ocimum basilicum* L. (Lamiaceae), was shown to have good biocidal activity against adult American house dust mite. No information, however, is available concerning the potential use of the essential oil and its constituents for the control of American house dust mite, despite a long history of use in fragrances and flavorings in the perfume, cosmetic, and food industries (Lawless 2002).

In the current study, an assessment was made of basil oil, 11 basil oil constituents identified, seven structurally related compounds, and another 22 previously known basil oil constituents against adult American house dust mite. The toxicities of these

materials were assessed by fabric-circle contact + fumigant and vapor-phase mortality bioassays and compared with those of two conventional acaricides benzyl benzoate and N,N-diethyl-3-methylbenzamide (DEET). The efficacy of four experimental spray formulations containing basil oil (1, 2, 3, and 4% sprays) against adult American house dust mite was evaluated using a spray bioassay to determine the most effective formulations and compared with that of commercial permethrin (cis:trans, 25:75) 2.5 g/liter spray for use as future commercial acaricides. The commercial product is registered as an acaricide for the control of dust mites in South Korea (Anonymous 2013). The mode of acaricidal action of the test compounds also is discussed.

Materials and Methods

Materials. Basil oil was purchased from Berjé (Carteret, NJ). The 11 commercially available organic pure compounds identified in the basil oil, seven structurally related compounds, and another 22 previously known basil oil constituents (Marotti et al. 1996, Viña and Murillo 2003, Koba et al. 2009, Pripdeevecch et al. 2010) are listed in Table 1, along with their sources. α-Humulene, a zerumbone analog lacking the α,β-unsaturated carbonyl group, was also used in this study for structure–activity relationship (Fig. 1). Benzyl benzoate (≥99.0% purity) and DEET (97% purity) were purchased from Sigma-Aldrich (St. Louis, MO). A commercially available acaricide examined in this study was permethrin (cis:trans, 25:75) 2.5 g/liter spray (Dongsung Pharmaceuticals, Asan, Chungnam, South Korea). Polyoxyethylene + polyoxypropylene

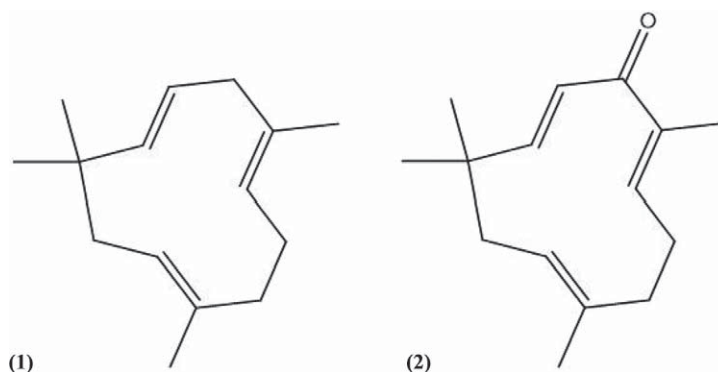


Fig. 1. Structures of α -humulene (1) and zerumbone (2).

(9:1) styrenated phenyl ether (Koremul-SP-1008R), a surfactant, was supplied by Hannong Chemical (Anyang, Gyeonggi, South Korea). All of the other chemicals used in this study were of reagent-grade quality and available commercially.

House Dust Mite. The stock cultures of American house dust mite (Kim et al. 2007) have been maintained in a temperature-controlled incubator without exposure to any known acaricide. Mites were reared in plastic containers (12.5 by 10.5 by 5.0 cm) containing 40 g of sterilized diet (fry feed no. 1: dried yeast, 1:1 by weight). The containers were incubated at $25 \pm 1^\circ\text{C}$ and 70–80% relative humidity in darkness. Fry feed and dried yeast were purchased from Korea Special Feed Meal (Inchon, South Korea) and Daeheung Pharmaceutical (Seoul, South Korea), respectively.

Experimental Spray Formulations. Four experimental spray formulations containing basil oil in 5-ml glass containers with a Homewell polypropylene pump spray nozzle (Seoul) were prepared to determine the effective acaricide products. The 1, 2, 3, and 4% spray formulations were, respectively, composed of 1, 2, 3, and 4% of the corresponding oils, 3% surfactant, 5% ethanol, and sterile distilled water at 91, 90, 89, and 88%. Single spray applications of 1, 2, 3, and 4% concentrations of the oil preparations delivered ≈ 17.94 , 35.87, 71.75, and $107.62 \mu\text{g}/\text{cm}^2$ of total material to a 5-cm-diameter black cotton-fabric circle, respectively.

Chromatographic Analysis. An Agilent 7890A GC System (Palo Alto, CA), equipped with a splitless injector and a flame ionization detection system, was used to separate and detect the constituents of basil oil. Constituents were separated with a 30 m by 0.32 mm i.d. ($d_f = 0.25 \mu\text{m}$) HP-5 capillary column (Agilent J&W Scientific, Folsom, CA). Flow rate of the nitrogen carrier gas was 1.0 ml/min. The oven temperature was kept at 50°C (5 min isothermal) and programmed to 280°C at a rate of $5^\circ\text{C}/\text{min}$, then isothermal at 280°C for 10 min. The injector temperature was 280°C . Chemical constituents were identified by coelution of authenticated samples after coinjection.

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using a PerkinElmer Clarus 680T gas chromatography-mass spectrometer (PerkinElmer,

Fort Belvoir, VA). An Agilent 30 m by 0.25 mm i.d. ($d_f = 0.5 \mu\text{m}$) DB-5MS capillary column (Agilent J&W Scientific, Folsom, CA) was used. The oven temperature was kept at 50°C (5 min isothermal) and programmed to 280°C at a rate of $4^\circ\text{C}/\text{min}$, then isothermal at 280°C for 10 min. The flow velocity of the helium carrier gas was 1.0 ml/min. The ion source temperature was 250°C . The interface temperature was kept at 260°C , and mass spectra were obtained at 70 eV. The sector mass analyzer was set to scan from 35 to 550 atomic mass units every 0.2 s. Chemical constituents were identified by comparison of mass spectra of each peak with those of authentic samples in a mass spectrum library (Anonymous 2008).

Contact + Fumigant Mortality Bioassay. A fabric-circle contact + fumigant mortality bioassay (Yun et al. 2012) was used to evaluate the toxicity of basil oil and all compounds to adult American house dust mite. Based on the preliminary test results, 4–5 concentrations of the test materials, each in 100 μl of ethanol, were applied to 5-cm-diameter black cotton-fabric circles. After drying in a fume hood for 90 s, each fabric circle was placed onto the bottom section of a disposable petri dish (5 cm in diameter $\times$ 1 cm). Groups of 40–50 adult mites (including both sexes, 7–10 d old) were separately placed onto the treated fabric circles. Each petri dish was then sealed with the original tight-fitting lid and wrapped with Bemis Parafilm M (Neenah, WI). Benzyl benzoate and DEET served as positive controls and were similarly formulated. Negative controls (i.e., no test material or acaricide) consisted of 100 μl of ethanol only.

Treated and control (ethanol only) mites were kept under the same conditions as those reared for colony maintenance for 24 h. Mortalities were determined under a dissecting microscope ($\times 20$). A mite was considered dead if its body and appendages did not move when prodded with a fine wooden dowel (Yun et al. 2012). Because not all bioassays could be conducted at the same time, treatments were blocked over time with a separate control treatment included in each block (Robertson and Preisler 1992). Freshly prepared solutions were used for each block of bioassays. All treatments were replicated three times using 40–50 adult mites per replicate.

Vapor-Phase Mortality Bioassay. The closed and open container treatment described by Kim et al. (2007) was used to determine whether the lethality of 11 selected compounds against adult American house dust mite was attributable to contact or fumigant action. Groups of 40–50 adult mites (both sexes, 7–10 d old) were placed separately onto the untreated cotton-fabric circles on the bottom section of polystyrene containers (5 cm in diameter $\times$ 2 cm). Each container was sealed with the original tight-fitting lid which had a fine wire screen covering a 3-cm-diameter central hole. Approximately twofold quantities of the contact + fumigant LC₅₀ values of each test compound were applied to 5-cm-diameter filter papers, as stated previously. Each treated filter paper was then placed on top of the wire screen, which prevented direct contact of adult mites with the test compound. Each container was sealed with either another solid lid (closed container treatment method) or sealed with another solid lid with a 4-cm-diameter central hole (open container treatment method) to investigate the potential vapor-phase toxicity of the test compounds. Control filter papers received 100 μ l of ethanol only. Mortalities were determined 24 h posttreatment, as stated previously. All bioassays were replicated three times using 40–50 adult mites per replicate.

Spray Bioassay. A spray bioassay (Lee et al. 2013) was used to evaluate the efficacy of the four experimental spray formulations against adult American house dust mite. Each test solution was sprayed two times successively at 10 cm upwards onto the 5-cm-diameter black cotton-fabric circle. After drying for 5 min, each fabric circle was placed onto the bottom section of a disposable petri dish (5 cm in diameter $\times$ 1 cm). Groups of 40–50 adult mites (both sexes, 7–10 d old) were placed separately onto the treated fabric circles, and each dish was sealed with the original tight-fitting lid. Permethrin (cis:trans, 25:75) 2.5 g/liter spray served as positive controls. Negative controls consisted of the ethanol-polyoxyethylene + polyoxypropylene (9:1) styrenated phenyl ether solution in distilled water or water. Mortalities were recorded, as stated previously. Each treatment was replicated three times using 40–50 adults per replicate.

Data Analysis. Data were corrected for control mortality using Abbott's (1925) formula. Mortality percentages were transformed to arcsine square root values for analysis of variance. The Bonferroni multiple-comparison method was used to test for significant differences among the treatments (SAS Institute 2004, Cary, NC). Student's *t*-test was used to test for significant differences between two treatment methods (SAS Institute 2004). Means ($\pm$ SE) of untransformed data are reported. Concentration-mortality data were subjected to probit analysis (SAS Institute 2004). The LC₅₀ values of the treatments were considered to be significantly different from one another when 95% CL failed to overlap. A compound having LC₅₀ > 500 μ g/cm² was considered ineffective.

Table 2. Chemical composition of basil oil

Compound	RT ^a (min)	% area in GC-FID ^b
β -pinene ^c	13.55	1.03
Limonene ^c	14.79	0.39
Unknown	15.39	0.66
Linalool oxide ^c	16.68	0.19
Linalool ^c	17.02	16.41
Unknown	17.90	0.27
Unknown	19.23	0.34
α -terpineol ^c	19.77	0.13
Methyl chavicol ^c	20.06	76.31
Unknown	21.15	0.19
β -citral ^c	21.48	0.14
α -citral ^c	21.95	0.31
Unknown	22.40	0.26
Unknown	24.16	0.11
Unknown	24.69	0.16
Caryophyllene ^c	25.98	0.32
Unknown	26.28	0.44
Unknown	26.70	0.19
Humulene ^c	26.82	0.17
Unknown	28.01	0.16
(Z)- β -farnesene ^c	28.58	0.21
(E)- β -farnesene ^c	28.79	1.27
(E)- <i>p</i> -methoxycinnamaldehyde ^c	29.40	0.22
Unknown	36.71	0.11

^a Retention time.

^b Gas chromatography-flame ionization detection.

^c Identified by coinjection and mass spectrometry.

Results

Chemical Composition of Basil Oil. Basil oil was composed of 2 major ($\geq 3.0\%$) and 22 minor constituents by comparison of mass spectral data and coelution of authenticated samples after coinjection (Table 2). The two major constituents of basil oil were methyl chavicol and linalool, and comprised 76.3 and 16.4% of the oil, respectively.

Toxicity to American House Dust Mites. Based on the 24-h LC₅₀ values from fabric-circle contact + fumigant mortality bioassays, basil oil was 15.1 and 3.4 times less toxic than benzyl benzoate (8.41 μ g/cm²) and DEET (37.67 μ g/cm²) against adult American house dust mite, respectively (Table 3). Mortality in the ethanol-polyoxyethylene + polyoxypropylene (9:1)-water-treated controls was <3%.

The toxic effects of 40 organic pure compounds on adult American house dust mite was likewise compared (Table 3). As judged by the 24-h LC₅₀ values, citral and menthol were the most toxic compounds, and these compounds were 7.4 and 5 times more toxic than benzyl benzoate. Methyl eugenol and benzyl benzoate did not differ significantly in toxicity from each other. Potent toxicity was also observed with eugenol, menthone, spathulenol, α -terpineol, nerolidol, zerbombone, and nerol (LC₅₀, 12.52–21.44 μ g/cm²), and these compounds were 1.8–3.0 times more toxic than DEET. The toxicity of (+)-terpinen-4-ol, (–)- α -thujone, caryophyllene oxide, isoeugenol, camphor, terpinyl acetate, linalool, and geraniol (LC₅₀, 29.55–54.61 μ g/cm²) did not differ significantly from that of DEET. Low (LC₅₀, 91.25–252.88 μ g/cm²) or no toxicity (LC₅₀, >500 μ g/cm²) was produced by the other 16 and 6 compounds, respectively.

Table 3. Toxicity of basil oil, 40 organic pure compounds, and 2 acaricides to adult *D. farinae* using a contact + fumigant mortality bioassay during a 24-h exposure

Material	LC ₅₀ (μg/cm ²) (95% CL ^a)	Slope ± SE	χ ^{2b}	P value
Basil oil	127.20 (109.33–145.39)	2.3 ± 0.30	4.78	0.905
Citral ^c	1.27 (0.80–1.68)	1.7 ± 0.27	1.41	0.999
Menthol ^d	1.69 (1.37–2.03)	1.4 ± 0.12	4.58	0.999
Methyl eugenol ^{e,f,g,h}	5.78 (4.87–6.86)	2.4 ± 0.29	3.99	0.947
Eugenol ^{e,g,h}	12.52 (11.14–13.88)	3.4 ± 0.33	1.32	0.987
Menthone ^d	17.28 (15.67–18.89)	4.2 ± 0.45	4.90	0.977
Spathulenol ^e	17.98 (16.27–19.76)	3.8 ± 0.45	3.81	0.993
α-terpineol ^{c,e,f,h}	18.74 (17.83–19.71)	6.5 ± 0.58	2.82	0.985
Nerolidol ^{e,g}	18.95 (17.18–20.84)	3.8 ± 0.45	3.74	0.993
Zerumbone ^d	19.42 (16.48–23.12)	2.5 ± 0.29	1.52	0.998
Nerol ^d	21.44 (20.09–22.82)	6.6 ± 0.81	1.79	0.997
(+)-terpinen-4-ol ^{c,f,g,h}	29.55 (26.77–33.05)	3.1 ± 0.40	2.39	0.992
(-)-α-thujone ^d	33.75 (28.46–40.28)	2.3 ± 0.35	4.70	0.910
Caryophyllene oxide ^e	36.07 (28.93–51.79)	2.1 ± 0.31	2.60	0.990
Isoeugenol ^d	36.10 (21.49–42.39)	2.4 ± 0.73	1.17	0.991
Camphor ^{e,f,g,h}	40.56 (37.40–43.67)	4.3 ± 0.46	3.52	0.966
Terpinyl acetate ^e	46.83 (37.69–55.02)	2.0 ± 0.29	4.78	0.905
Linalool ^{c,e,f,g,h}	47.76 (44.48–52.60)	5.4 ± 0.65	4.02	0.946
Geraniol ^{e,g}	54.61 (45.55–68.06)	1.7 ± 0.21	3.08	0.979
Methyl chavicol ^{c,e,f,g,h}	91.25 (77.80–106.79)	1.6 ± 0.22	2.28	0.993
α-humulene ^{c,f,g,h}	96.45 (80.58–114.03)	2.1 ± 0.25	1.92	0.996
(-)-isolongifolene ^g	104.60 (92.58–117.73)	2.3 ± 0.19	3.55	0.995
(+)-aromadendrene ^e	106.60 (89.76–125.28)	1.7 ± 0.18	4.05	0.945
(S)-(-)-β-citronellol ^d	116.72 (103.76–129.42)	3.2 ± 0.30	4.12	0.941
Linalool oxide ^{c,e,g}	119.86 (103.10–140.13)	2.1 ± 0.22	3.48	0.995
(E)-anethole ^d	120.26 (105.12–135.15)	2.7 ± 0.31	3.93	0.950
(E)-β-farnesene ^{c,e,g}	135.45 (117.61–154.21)	2.4 ± 0.28	1.99	0.996
β-caryophyllene ^{c,e,f,g,h}	156.24 (113.97–213.66)	1.2 ± 0.15	4.09	0.943
(+)-fenchone ^e	158.53 (140.90–179.43)	3.2 ± 0.37	3.18	0.976
1,8-cineole ^{e,f,g,h}	164.80 (142.15–190.26)	2.1 ± 0.21	4.66	0.912
(1R)-(-)-fenchone ^e	169.14 (150.03–192.83)	3.1 ± 0.37	3.43	0.969
Bisabolene ^{e,g}	176.18 (156.51–197.42)	2.1 ± 0.16	6.72	0.915
(-)-α-cedrene ^g	200.30 (161.06–244.97)	1.4 ± 0.15	1.04	0.999
γ-terpinene ^{e,f,g,h}	216.16 (177.90–256.37)	1.7 ± 0.21	2.35	0.992
(E)-p-methoxycinnamaldehyde ^e	252.88 (213.14–329.84)	2.4 ± 0.39	2.63	0.988
Camphene ^{e,g,h}	>500			
p-cymene ^{e,f,h}	>500			
Limonene ^{c,e,f,g,h}	>500			
Myrcene ^{e,f,g,h}	>500			
α-pinene ^{c,f,g,h}	>500			
β-pinene ^{c,e,g,h}	>500			
Benzyl benzoate	8.41 (6.84–10.06)	1.5 ± 0.54	8.96	0.914
DEET	37.67 (30.11–52.92)	1.3 ± 0.29	4.51	0.921

^a CL denotes confidence limit.^b Pearson's chi-square goodness-of-fit test.^c Constituents identified in this study.^d Compounds structurally related to basil oils constituents.^e Constituents of basil oil reported by Viña and Murillo (2003).^f Constituents of basil oil reported by Koba et al. (2009).^g Constituents of basil oil reported by Pripdeevec et al. (2010).^h Constituents of basil oil reported by Marotti et al. (1996).

Route of Acaricidal Action. Because of their good acaricidal activity, the fumigant toxicity of 11 selected compounds to adult American house dust mite was examined using a vapor-phase mortality bioassay in two formats (Table 4). After 24 h of exposure to 2.54 μg/cm³ citral, there was a significant difference ($P < 0.0001$) in lethality between exposure in a closed container, which resulted in 98% mortality, and exposure in an open container, which resulted in 3% mortality against adult American house dust mite. Similar differences in the response of adult American house dust mite to the other 10 compounds in closed versus open container treatments were also observed.

Efficacy of Experimental Spray Formulations. The control efficacy of four experimental spray formulations containing basil oil and a commercial permethrin (cis:trans = 25:75) 2.5 g/liter spray against adult American house dust mite was evaluated using a spray bioassay (Table 5). The control efficacy was significantly affected by formulation ($F = 53.88$; $df = 3, 8$; $P < 0.0001$). Basil applied as 3 (BSO-3) and 4% sprays (BSO-4) provided 97 and 100% mortality, respectively. The lethality of the 2 (BSO-2) and the 1% sprays (BSO-1) were 73 and 33%, respectively. A commercial permethrin (cis:trans = 25:75) 2.5 g/liter spray treatment resulted in 17% mortality. There was no mortal-

Table 4. Fumigant toxicity of 11 selected compounds to adult *D. farinae* using a vapor-phase mortality bioassay during a 24-h exposure

Compound	Conc. ($\mu\text{g}/\text{cm}^3$)	Mortality (%) ($\pm$ SE)		ANOVA <i>F</i> ; <i>df</i> ; <i>P</i>
		Vapor in closed container	Vapor in open container	
Citral	2.54	98 $\pm$ 2.1	3 $\pm$ 1.9	398.98; 1, 4; < 0.0001
Menthol	3.38	96 $\pm$ 4.4	11 $\pm$ 2.9	581.04; 1, 4; < 0.0001
Methyl eugenol	11.56	100	4 $\pm$ 2.1	380551; 1, 4; < 0.0001
Eugenol	25.04	90 $\pm$ 1.9	1 $\pm$ 1.1	315.22; 1, 4; < 0.0001
Menthone	34.56	100	8 $\pm$ 2.1	14767.0; 1, 4; < 0.0001
Spathulenol	35.96	96 $\pm$ 4.4	2 $\pm$ 2.4	5263.47; 1, 4; < 0.0001
α -terpineol	37.48	96 $\pm$ 3.7	6 $\pm$ 1.1	1561.92; 1, 4; < 0.0001
Nerolidol	37.90	93 $\pm$ 3.3	3 $\pm$ 1.9	653.68; 1, 4; < 0.0001
Zerumbone	38.84	100	8 $\pm$ 1.1	3151.35; 1, 4; < 0.0001
Nerol	42.88	87 $\pm$ 4.1	6 $\pm$ 1.1	2396.85; 1, 4; < 0.0001
(+)-terpinen-4-ol	59.10	100	1 $\pm$ 0.7	822.62; 1, 4; < 0.0001

According to Student's *t*-test.

ity for the ethanol-surfactant-water-treated or water-treated dust mites in the spray bioassay.

Discussion

It is well recognized that some essential oils can be developed into products suitable for integrated arthropod pest management because they can be selective, have few harmful effects on nontarget organisms, and are environmentally nonpersistent (Ahn et al. 2006, Isman 2006). They also can be used in conjunction with biological control (Isman 2006). Essential oils consist of highly complex mixtures of hydrocarbons (usually terpenoids) and oxygenated compounds (alcohols, aldehydes, esters, ketones, oxides, and phenols; Lawless 2002, Isman 2006). The oil constituents jointly or independently contribute to toxicity to various types of pest complex (Isman 2006). Bioacaricide products can also be applied to dust mite nests such as mattresses, pillows, carpets, rugs, and upholstered furniture in the same manner as other conventional acaricides (Ahn et al. 2006). Naturally occurring acaricidal constituents against American house dust mite and European house dust mite have been well described by Ahn et al. (2006). In the current study, the acaricidal principles of basil oil were determined to be the monoterpenoids citral, linalool, and α -terpineol. Of the test compounds, citral and menthol were more toxic than benzyl benzoate against adult American house dust mite, whereas

methyl eugenol and benzyl benzoate did not differ significantly in toxicity from each other. Eugenol, menthone, spathulenol, α -terpineol, nerolidol, zerumbone, and nerol were more toxic than DEET. Interestingly, the sesquiterpenoid α -humulene, lacking only the α,β -unsaturated carbonyl group present in zerumbone, was less effective than zerumbone. This finding indicates that the α,β -unsaturated carbonyl group of zerumbone is a prerequisite component for toxicity against American house dust mite. In addition, the 3 and 4% sprays containing basil oil resulted in potent control efficacy compared with permethrin (cis:trans = 25:75) 2.5 g/liter spray. This original finding indicates that the basil oil and the compounds described may hold promise for the development of novel and effective products for the control of house dust mites.

Investigations on the modes of action and delivery of bioacaricides provide important practical information for mite control, such as the most appropriate formulations and delivery means to be adopted for their future commercialization and for the development of selective control alternatives with novel target sites (Ahn et al. 2006, Isman 2006, Kim et al. 2006). Essential oil volatile constituents, such as alcohols, aldehydes, alkanes, esters, ketones, oxides, phenols, and terpenoids (particularly monoterpenoids; Lawless 2002) primarily act as fumigants with additional contact action against American house dust mite and European house dust mite (Ahn et al. 2006). Fumigant toxicity to both dust mite species has been reported for butylidenephthalide (Kwon and Ahn 2002), paeonol (Kim et al. 2004), atractylon and atractylenolide III (Kim et al. 2007), and ally isothiocyanate (Yun et al. 2007). In a separate study, bisabolangelone was found to be largely toxic through contact action (Kang et al. 2006). In the current study, the 11 selected compounds described were consistently more effective in closed versus open containers. These results indicate that the route of acaricidal action of these compounds was largely a result of vapor action. The dual contact + fumigant action of basil oil and its constituents described, as demonstrated through the current vapor-phase mortality and spray bioassays, is of practical importance because volatile constituents can easily

Table 5. Effectiveness of four experimental spray formulations containing basil oil and commercial permethrin against adult *D. farinae* using a spray bioassay during a 24-h exposure

Spray treatment	Mortality % ($\pm$ SE)
BSO-1 ^a	33 $\pm$ 3.3c
BSO-2 ^a	73 $\pm$ 5.8b
BSO-3 ^a	97 $\pm$ 3.3a
BSO-4 ^a	100a
Permethrin 2.5 g/liter ^b	17 $\pm$ 3.3d

Means within a column followed by the same letter are not significantly different at *P* = 0.05 (Bonferroni method).

^a Basil oil 1, 2, 3, and 4%.

^b Mixture (cis:trans, 25:75).

reach deep refuges in a closed space such as a building, wardrobe, pet house, or storage bin, resulting in good control. This system has advantages because exposure to volatile constituents can be easily controlled in a closed space using appropriate automatic control equipment or dispenser (Kim et al. 2006). Because of high volatility of the basil oil constituents and the test compounds described, the binary mixtures of these compounds and compounds with contact action (e.g., bisabolangelone; Kang et al. 2006) could be useful in a space, i.e., where a window is open. The odors of the 3 or 4% spray containing basil oil are fragrant, which is appropriate for commercialization. Detailed tests are needed to fully understand the mode of action of the active basil oil constituents, although the octopaminergic and γ -aminobutyric acid receptors have been suggested as novel target sites for some monoterpene essential oil constituents in *Helicoverpa armigera* (Hübner) (Kostyukovsky et al. 2002) and *Drosophila melanogaster* Meigen (Priestley et al. 2003), respectively.

In conclusion, bioacaricides derived from basil oil containing citral, linalool, α -terpineol, and other compounds described could be useful as contact-action fumigants for protection of humans from various allergic diseases caused by house dust mites. For the practical use of the basil oil-derived materials as novel acaricides to proceed, further research is needed to establish their human safety, although historically basil oil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunction (Simon et al. 1999). In addition, detailed tests are needed to understand how to improve acaricidal potency and stability for eventual commercial development.

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