

Toxicity of *Juniperus oxycedrus* oil constituents and related compounds and the efficacy of oil spray formulations to *Dermatophagoides farinae* (Acari: Pyroglyphidae)

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Abstract The American house dust mite (AHDM), *Dermatophagoides farinae* Hughes (Acari: Pyroglyphidae), is recognized as an important source of allergens in the domestic environment. This study was conducted to determine whether 19 constituents from essential oil of cade, *Juniperus oxycedrus* L. (Cupressaceae), eight structurally related compounds, and another 16 previously known cade oil constituents were toxic for adult AHDMs and to determine the route of acaricidal action of the test compounds, as well as to assess the control efficacy of four experimental spray formulations containing the oil (10–40 mg/L sprays). In a fabric-circle contact mortality bioassay, methyleugenol (LD₅₀, 5.82 µg/cm²) and guaiacol (8.24 µg/cm²) were the most toxic compounds against the mites, and the toxicity of these compounds and benzyl benzoate did not significantly differ. High toxicity was also observed with eugenol, *m*-cresol, and nerolidol (LD₅₀, 12.52–19.52 µg/cm²), and these compounds were significantly more toxic than *N,N*-diethyl-3-methylbenzamide (DEET) (LD₅₀, 37.67 µg/cm²). Cade applied as 30 or 40 mg/L experimental sprays provided 96 and 100% mortality against the mites, respectively, whereas permethrin (*cis:trans*, 25:75) 2.5 g/L spray treatment resulted in 17% mortality. In vapor-phase mortality tests, the compounds described were consistently more toxic in closed versus open containers, indicating that toxicity was achieved mainly through the

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action of vapor. Reasonable mite control in indoor environments can be achieved by spray formulation containing the 40 mg/L cade oil as potential contact-action fumigants.

Keywords *Dermatophagoides farinae* · *Juniperus oxycedrus* · Cade oil · Botanical acaricide · Spray formulation · Natural fumigant

Introduction

House dust mites (HDMs) are the most common cause of allergic symptoms in humans, affecting 65 million to 1.3 billion people worldwide (Arlian 2002; Colloff 2009; Hammad et al. 2009). The American house dust mite (AHDM), *Dermatophagoides farinae* Hughes (Acari: Pyroglyphidae), and the European house dust mite (EHDM), *Dermatophagoides pteronyssinus* Trouessart, are two of the most serious pyroglyphid mites because of their cosmopolitan occurrence and abundance in homes (Arlian and Morgan 2003; Colloff 2009; Thomas 2010). Pyroglyphid mites usually make up 60–90% of the house dust acarofauna in temperate climate regions of the world (Colloff 2009; Solarz 2010). HDMs are the primary sources of allergens causing various allergic diseases such as asthma, atopic dermatitis, conjunctivitis, eczema, and perennial rhinitis in sensitive patients (Arlian 2002; Milián and Díaz 2004; Bessor and Pauli 2011; Jacquet 2013; Calderón et al. 2015). Based on the medical importance of HDMs, numerous papers are published annually (Portnoy et al. 2013). Allergies to the pyroglyphid mites are increasing and closely associated with changes in the living environment of humans, such as central heating, space heating, more tightly sealed windows, poor ventilation, humidity control with humidifier, and fitted carpets, all of which allow the growth of house dust mites (Pollart et al. 1987; Milián and Díaz 2004; Thomas 2010; Calderón et al. 2015).

Increasing public concern for the environmental effects of acaricides, human health effects, and undesirable effects on nontarget organisms intensifies when conventional contact acaricides, such as benzyl benzoate, pirimiphos-methyl, and pyrethroids (Pollart et al. 1987; WHO 2006; Colloff 2009; Portnoy et al. 2013) are used repeatedly. In addition, the number of approved acaricides will probably be reduced soon in the United States (US EPA 2017) and European Union (2005) as reregistration is required. The removal of these conventional acaricide products from the markets due to increases in acaricide resistance or other concerns will have a serious impact on the proliferation of the pyroglyphid mites. Therefore, there is a pressing need to develop safe, effective alternatives with novel target sites to establish an efficient management strategy for the pyroglyphid mites, particularly those with fumigant action, that allow effective application in domestic environments that reach deep refuges missed by many commercially available acaricides.

Bioacaricides derived from essential oils (EOs) mostly extracted by steam distillation or hydrodistillation from plant parts (bark, flowers, fruit, leaves, seeds, stems, and roots) (Tongnuanchan and Benjakul 2014) may provide potential alternatives to conventional acaricides largely because EOs are a potential source of secondary bioactive substances that are perceived by the general public as being relatively safe and posing fewer risks to the environment (Isman and Machial 2006; Koul et al. 2008; Tripathi et al. 2009; Pavela and Benelli 2016). They can act at multiple and novel target sites (Enan 2001; Kostyukovsky et al. 2002; Priestley et al. 2003; Pavela and Benelli 2016), thereby reducing the potential for resistance (Koul et al. 2008; Perumalsamy et al. 2010; Pavela and Benelli 2016). Based on these benefits of botanical pesticides, numerous papers are published annually (Isman and Grieneisen 2014; Pavela and Benelli 2016). Phytochemicals are

regarded as potential sources for developing commercial acaricides as certain EO preparations, and their constituents meet the criteria for minimum-risk acaricides (Isman 2008; Koul et al. 2008). Previous studies have shown that EO from the cade plant, *Juniperus oxycedrus* L. (Cupressaceae), possessed good acaricidal activity against adult AHDMs. However, no previous studies have addressed the potential uses of the EO and its constituents for managing the pyroglyphid mites, despite its acaricidal activity against the poultry mite, *Dermanyssus gallinae* (De Geer) (Kim et al. 2004a).

In this study, we aimed to assess the potential of cade essential oil (CA-EO), 19 CA-EO constituents identified, eight structurally related compounds, along with another 16 previously known CA-EO constituents (Boti et al. 2006) against adult AHDMs, using fabric-circle contact and vapor-phase mortality bioassays. The results were compared with those from the four conventional acaricides benzyl benzoate, *N,N*-diethyl-3-methylbenzamide (DEET), permethrin, and *d*-phenothrin. In addition, the efficacy of four experimental spray formulations containing CA-EO (10, 20, 30, and 40 mg/L sprays) against AHDMs was evaluated using a spray bioassay to determine the most effective formulations and compared to permethrin (*cis:trans*, 25:75) 2.5 g/L spray, which is a currently available synthetic acaricide, to determine whether they would be suitable for future commercial acaricides. The commercial product is registered as an acaricide for the control of dust mites in the Republic of Korea (ROK). Finally, quantitative structure–activity relationship (QSAR) and the route of acaricidal action of the test compounds are also discussed.

Materials and methods

Materials

CA-EO was purchased from Berjé (Carteret, NJ, USA). The 19 commercially available, pure organic compounds identified in CA-EO, eight structurally related compounds, and another 16 previously known CA-EO constituents (Boti et al. 2006) are listed in Table 1, along with their sources and purities. Benzyl benzoate ($\geq 99.0\%$ purity), DEET (99%), permethrin (99%), and *d*-phenothrin (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). A commercially available acaricide examined in this study was permethrin (*cis:trans*, 25:75) 2.5 g/L spray (Dongsung Pharmaceuticals, Asan, Chungnam, ROK). Polyoxyethylene + polyoxypropylene (9:1) styrenated phenyl ether (Koremul-SP-1008R), a surfactant, was a gift from Hannong Chemical (Anyang, Gyeonggi, ROK). All of the other chemicals used in this study were of reagent-grade quality and are available commercially.

House dust mite

The stock cultures of AHDM (Kim et al. 2003) have been maintained in a temperature-controlled incubator without exposure to any known acaricide for more than 10 years. Mites were reared in plastic containers (12.5 × 10.5 × 5.0 cm) containing 40 g of a sterilized diet (fry feed no. 1:dried yeast, 1:1 by weight). The containers were incubated at 25 ± 1 °C and 70–80% relative humidity in the dark. Fry feed and dried yeast were purchased from Korea Special Feed Meal (Inchon, ROK) and Daeheung Pharmaceutical (Seoul, ROK), respectively.

Table 1 The 43 organic pure compounds examined in this study for acaricidal activity

Compound	Source ^a	Purity (%)	Compound	Source ^a	Purity (%)
Anthracenedione ^b	S-A	97	α -Humulene ^{b,d}	S-A	96
(+)-Aromadendrene ^c	TCI	97	Isoeugenol ^b	S-A	98
β -Bisabolene ^c	TCI	90	Isolongifolene ^b	S-A	98
Camphor ^{b,d}	S-A	96	Isovanillin ^b	S-A	95
Camphene ^d	S-A	95	Methyl eugenol ^c	S-A	98
2-Carene ^c	S-A	97	Myristicin ^c	S-A	99
3-Carene ^d	S-A	90	Myrcene ^d	S-A	95
β -Caryophyllene ^{b,d}	TCI	90	Myrtenal ^d	S-A	97
β -Caryophyllene oxide ^{b,d}	S-A	95	Myrtenol ^d	S-A	95
(-)- α -Cedrene ^b	S-A	95	Nerolidol ^d	S-A	98
<i>m</i> -Cresol ^b	S-A	99	(<i>Z</i>)- β -Ocimene ^c	S-A	90
<i>o</i> -Cresol ^c	S-A	99	Phenol ^b	S-A	99
<i>p</i> -Cresol ^b	S-A	99	(+)- α -Pinene ^d	S-A	99
<i>p</i> -Cymene ^d	S-A	99	(-)- α -Pinene ^d	S-A	99
2,5-Dimethylphenol ^b	S-A	99	(+)- β -Pinene ^d	S-A	99
2,4-Di- <i>tert</i> -butylphenol ^b	S-A	99	(-)- β -Pinene ^d	S-A	99
Estragole ^b	S-A	98	Resorcinol ^b	S-A	99
Eugenol ^b	S-A	99	Terpinen-4-ol ^d	WK	90
(<i>E</i>)- β -Farnesene ^d	S-A	99	γ -Terpinene ^d	WK	80
(<i>E,E</i>)-Farnesol ^d	S-A	95	α -Terpinyl acetate ^d	TCI	85
Geraniol ^c	WK	97	<i>n</i> -Undecane ^b	TCI	99.5
Guaiacol ^b	S-A	99			

^aS-A, Sigma-Aldrich (St. Louis, MO, USA); TCI, Tokyo Chemical Industry (Tokyo, Japan); WK, Wako (Osaka, Japan)

^bConstituents identified in this study

^cStructurally related compounds

^dConstituents of cade oil reported by Boti et al. (2006)

Experimental spray formulations

Four experimental spray formulations containing CA-EO were formulated to determine the effective acaricide products. The 10, 20, 30, and 40 mg/L spray formulations were, respectively, composed of 1, 2, 3, and 4 g of CA-EO, 3 g surfactant, 5 mL ethanol, and sterile distilled water at 91, 90, 89, and 88 mL in a 100 mL polyethylene containers with a pump spray nozzle (Homewell, Seoul). Single spray applications of CA-EO 10, 20, 30, and 40 mg/L formulations delivered approximately 51.34, 102.68, 152.26, and 210.20 $\mu\text{g}/\text{cm}^2$ of total material to a 5-cm diameter black cotton-fabric circle, respectively.

Gas chromatography analysis

An Agilent 7890A gas chromatography system (Agilent, Wilmington, DE, USA) equipped with a split injector and a flame ionization detection system was used to separate and detect the constituents of the CA-EO. These constituents were separated with a 30 m $\times$ 0.25 mm

i.d. ($d_f = 0.25 \mu\text{m}$) DB-5 MS capillary column (Agilent J&W Scientific, Folsom, CA, USA). The oven temperature was kept at 50 °C (5 min isothermal) and programmed to 280 °C at a rate of 4 °C/min, then isothermal at 280 °C for 10 min. The linear velocity of the nitrogen carrier gas was 36 cm/s (at 50 °C) at a split ratio of 50:1. The constituents were identified by the coelution of authenticated samples after coinjection.

Gas chromatography–mass spectrometry analysis

A gas chromatography–mass spectrometry (GC–MS) analysis was performed using an ISQ LT Single Quadrupole gas chromatograph-mass spectrometer (Thermo Scientific, Waltham, MA, USA). An Agilent 30 m $\times$ 0.25 mm i.d. ($d_f = 0.5 \mu\text{m}$) DB-5MS capillary column was used. The oven temperature was kept at 60 °C (1 min isothermal) and programed to 185 °C (held for 1 min) at a rate of 1.5 °C/min, and then to 275 °C (that was held for 2 min at the final temperature) at a rate of 9 °C/min. A helium carrier gas was used at a column head pressure of 15.7 psi (39.2 kPa). The ion source temperature was 250 °C, and mass spectra were obtained in EI-scan mode at 70 eV of electron energy. The sector mass analyzer was set to scan from 35 to 550 atomic mass units every 0.3 s. The chemical constituents were identified by comparing the mass spectra of each peak with those of the authentic samples in a mass spectrum library (Anonymous 2008).

Contact plus fumigant mortality bioassay

A fabric-circle contact mortality bioassay described by Yun et al. (2012) was used to evaluate the toxicity of CA-EO and all compounds to adult AHDMs. Based on the preliminary test results, 4–6 concentrations of the test materials, each in 50 μL of acetone (for permethrin and *d*-phenothrin) or ethanol (for the other materials), were applied to 5-cm-diameter black cotton-fabric circles. After drying in a fume hood for 90 s, each treated fabric circle was placed onto the bottom section of a disposable Petri dish (5-cm diameter $\times$ 1 cm). Groups of 40–50 adult mites (including both sexes, 7–10 days old) were separately placed onto the treated fabric circles. Each Petri dish was then sealed with the original tight-fitting lid and was wrapped with Parafilm M (Bemis, Neenah, WI, USA). Benzyl benzoate, DEET, and two pyrethroids (permethrin and *d*-phenothrin) served as positive controls and were similarly formulated. The negative controls (i.e., no test material or acaricide) consisted of 50 μL of acetone or ethanol only.

The treated and control (acetone or ethanol only) mites were held under the same conditions as those reared for colony maintenance. Mortalities were determined at 24 h after treatment under a dissecting microscope (20 $\times$). A mite was considered dead if its body and appendages did not move when it was 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 the 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 if the mortality of the 13 selected compounds against adult AHDMs was

attributable to either contact or fumigant action. Groups of 40–50 adult mites (both sexes, 7–10 days old) were placed separately onto the untreated cotton-fabric circles on the bottom section of polystyrene containers (5-cm diameter $\times$ 2 cm). Each container was then sealed with the original tight-fitting lid that had a fine wire screen covering a 4.5-cm diameter central hole. Twofold quantities of the fabric-circle contact LC₅₀ values of each test material were applied to the 5-cm diameter filter papers, as previously stated. Each treated filter paper was placed on top of the wire screen, thereby preventing direct contact of the adult mites with the test material. Each container was then sealed with either another solid lid (closed container treatment method) or sealed with another solid lid with a 4.5-cm diameter central hole (open container treatment method) to investigate the potential vapor-phase toxicity of the test materials. Control filter papers received 50 μ L of ethanol only. Mortalities were determined at 24 h post-treatment, as previously stated. All the bioassays were replicated three times using 40–50 adults per replicate.

Spray bioassay

A spray bioassay described by Lee et al. (2013) was used to elucidate the efficacy of the four experimental spray formulations against adult AHDMs. Each test solution was sprayed twice 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 stated previously. Groups of 40–50 adult mites (both sexes, 7–10 days 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/L spray served as a positive control. The negative controls consisted of the ethanol–polyoxyethylene + polyoxypropylene (9:1) styrenated phenyl ether solution in distilled water or water. Mortalities were recorded, as previously stated. Each treatment was replicated three times using 40–50 adults per replicate.

Data analysis

The data were corrected for control mortality using Abbott's (1925) formula. The percentages of mortality 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 2014). Student's *t* test was used to test for significant differences between the two treatment methods (SAS Institute 2014). The means $\pm$ standard errors (SEs) of untransformed data are reported. The concentration-mortality data were subjected to a probit analysis (SAS Institute 2014). The LD₅₀ values for the treatments were considered significantly different from one another when 95% confidence intervals did not overlap. A compound with an LD₅₀ > 500 μ g/cm² was considered ineffective (Lee et al. 2013).

Results

Chemical composition of cade oil

The CA-EO was composed of nine major ($\geq$ 3.0%) and 21 minor constituents by comparison of mass spectral data and the coelution of the authenticated samples following

coinjection (Table 2). The nine major constituents of the CA-EO were isovanillin, (–)- α -cedrene, camphor, β -caryophyllene, unknown peak number 24 compound, unknown peak

Table 2 Chemical composition of cade oil

Peak number	Compound	RT ^a (min)	% Area in GC-FID ^b	Identification	
				CI ^c	MS ^d
1	Camphor*	13.23	6.83	O ^e	O
2	Phenol	13.38	0.52	O	X ^f
3	Unknown	15.60	2.06		
4	<i>p</i> -Cresol*	16.24	3.51	O	O
5	<i>m</i> -Cresol	16.72	1.26	O	O
6	Guaiacol*	16.77	3.18	O	O
7	<i>n</i> -Undecane	17.07	0.12	O	X
8	Unknown	18.46	1.97		
9	Unknown	19.05	1.20		
10	Unknown*	19.81	3.88		
11	Estragole	20.03	0.15	O	O
12	2,5-Dimethylphenol	21.93	0.10	O	X
13	Resorcinol	22.21	2.00	O	X
14	Eugenol	24.55	0.69	O	O
15	Isoeugenol	24.90	0.72	O	O
16	Unknown	25.13	1.01		
17	Isolongifolene	25.29	0.56	O	O
18	(–)- α -Cedrene*	25.83	18.41	O	O
19	β -Caryophyllene*	25.99	5.45	O	O
20	α -Humulene	26.52	1.91	O	O
21	Unknown	26.82	0.95		
22	Unknown	27.26	2.51		
23	Unknown	27.41	1.56		
24	Unknown*	27.87	4.89		
25	2,4- <i>tert</i> -Butyl phenol	28.06	1.87	O	X
26	Isovanillin*	28.43	21.75	O	O
27	Unknown	28.79	1.15		
28	Caryophyllene oxide*	28.91	3.53	O	O
29	Unknown	30.33	1.07		
30	Anthracenedione	31.86	1.65	O	X

*Major constituent ($\geq 3\%$)

^aRetention time

^bGas chromatography with flame ionization detection

^cCoinjection with authentic samples

^dMass spectrometry

^eIdentified by coinjection or mass spectrometry

^fUnidentified by coinjection or mass spectrometry

number 10 compound, caryophyllene oxide, *p*-cresol, and guaiacol, and comprised 21.75, 18.41, 6.83, 5.45, 4.89, 3.88, 3.53, 3.51, and 3.18% of the oil, respectively.

Toxicity to adult American house dust mites

As judged by the 24 h LD₅₀ values from fabric-circle contact mortality bioassays, CA-EO was 13.4 and 3.0 times less toxic than benzyl benzoate (8.82 µg/cm²) and DEET (38.95 µg/cm²) against AHDMS, respectively (Table 3). The mortality in the ethanol–polyoxyethylene + polyoxypropylene (9:1) styrenated phenyl ether–water-treated controls was less than 3%.

The toxicity of 43 organic pure compounds and four acaricides to adult AHDMS was likewise compared (Table 3). Based on the 24 h LD₅₀ values, methyl eugenol was the most toxic compound and significantly more toxic than benzyl benzoate. The toxicity of guaiacol and benzyl benzoate did not significantly differ. High toxicity was also observed with eugenol, *m*-cresol, 2,5-dimethylphenol, and nerolidol (LD₅₀, 12.52–19.52 µg/cm²), and these compounds were significantly more toxic than DEET. The LD₅₀ of *o*-cresol, phenol, myrtenal, 3-carene, *p*-cresol, myrtenol, 2-carene, terpinen-4-ol, and caryophyllene oxide was between 22.05 and 36.07 µg/cm². The LD₅₀ of camphor, 2,4-di-*tert*-butylphenol, isoeugenol, and geraniol was between 42.56 and 54.61 µg/cm². Low or very low toxicity was produced by five compounds (LD₅₀, 77.02–107.60 µg/cm²) and seven compounds (135.45–216.16 µg/cm²), respectively. No toxicity (LD₅₀, > 500 µg/cm²) was found when testing the other 12 compounds.

Acaricide mode of delivery

Because of their good acaricidal activity, the fumigant toxicity of the 13 selected compounds against adult AHDMS was evaluated using the vapor-phase mortality bioassay in two formats (Table 4). After 24 h of exposure to 11.64 mg/cm² methyl eugenol, there was a significant difference ($P < 0.0001$) in mortality between exposure in a closed container, which resulted in 100% mortality, and exposure in an open container, which resulted in 4% mortality against adult AHDMS. Similar differences in the response of adult AHDMS to the other 12 compounds in closed versus open container treatments were also observed.

Efficacy of experimental spray formulations

The control efficacy of four experimental spray formulations containing CA-EO and a commercial acaricide permethrin (*cis:trans* = 25:75) 2.5 g/L spray, which is a positive control, against adult AHDMS was examined using the spray bioassay (Table 5). The control efficacy of the test formulations significantly differed for the adult AHDMS ($F_{4,10} = 210.34$, $P < 0.0001$). The cade applied as 30 and 40 mg/L sprays provided 96 and 100% mortality against adult AHDMS, respectively. The mortality of the 20 and 10 mg/L sprays were 53 and 25%, respectively. The commercial permethrin spray treatment resulted in 17% mortality.

Table 3 Toxicity of cade oil, 31 organic pure compounds, and four acaricides to adult *Dermatophagoides farinae* using a contact plus fumigant mortality bioassay during a 24 h exposure

Material ^a	LD ₅₀ (μg/cm ²) (95% CI ^b)	Slope ± SE	χ ^{2c}	P
Cade oil	118.36 (104.99–131.73)	2.9 ± 0.30	4.33	0.93
Methyl eugenol	5.82 (4.89–6.88)	2.4 ± 0.29	3.99	0.95
Guaiacol ^d	8.24 (6.58–9.71)	2.2 ± 0.24	6.26	0.94
Eugenol ^d	12.52 (11.14–13.88)	3.4 ± 0.33	1.32	0.99
<i>m</i> -Cresol ^d	15.15 (13.47–16.90)	1.9 ± 0.18	1.62	1.0
2,5-Dimethylphenol ^d	17.55 (16.48–18.56)	5.2 ± 0.44	6.66	0.92
Nerolidol	19.52 (17.82–21.34)	4.1 ± 0.48	3.61	0.99
<i>o</i> -Cresol	22.05 (18.11–26.78)	2.1 ± 0.27	1.76	1.0
Phenol ^d	24.89 (23.09–26.82)	4.5 ± 0.42	2.62	0.92
Myrtenal	26.81 (20.71–33.24)	1.4 ± 0.15	4.79	0.90
3-Carene	26.94 (20.85–33.36)	1.4 ± 0.15	3.00	0.98
<i>p</i> -Cresol ^d	28.54 (25.31–31.77)	2.2 ± 0.24	6.43	0.93
Myrtenol	28.99 (22.07–36.40)	1.3 ± 0.16	1.92	1.0
2-Carene	30.18 (23.32–37.57)	1.5 ± 0.15	1.43	1.0
Terpinen-4-ol	30.55 (27.78–34.10)	3.1 ± 0.40	2.39	0.99
Caryophyllene oxide ^d	36.07 (28.93–51.79)	2.1 ± 0.31	2.60	0.99
Camphor ^d	42.56 (38.40–44.67)	4.3 ± 0.46	3.52	0.97
2 4-Di- <i>tert</i> -butylphenol ^d	46.69 (36.74–61.41)	1.1 ± 0.13	2.40	0.99
Isoeugenol ^d	47.76 (42.80–51.89)	3.5 ± 0.72	4.66	0.99
Geraniol	54.61 (45.55–68.06)	1.7 ± 0.21	3.08	0.98
Terpinyl acetate	77.02 (65.43–89.91)	1.8 ± 0.18	6.16	0.94
Estragole ^d	91.25 (77.80–106.79)	1.6 ± 0.22	2.28	0.99
α-Humulene ^d	97.45 (81.58–115.03)	2.1 ± 0.25	1.92	1.0
Isolongifolene ^d	104.60 (92.58–117.73)	2.3 ± 0.19	3.55	1.0
(+)-Aromadendrene	107.60 (90.76–125.28)	1.7 ± 0.18	4.05	0.95
(<i>E</i>)-β-Farnesene	135.45 (117.61–154.21)	2.4 ± 0.28	1.99	1.0
β-Caryophyllene ^d	156.24 (113.97–213.66)	1.2 ± 0.15	4.09	0.94
β-Bisabolene	176.18 (156.51–197.42)	2.1 ± 0.16	6.72	0.92
<i>n</i> -Undecane ^d	177.88 (157.80–199.70)	2.1 ± 0.16	5.32	0.97
(<i>E,E</i>)-Farnesol	185.41 (172.93–196.26)	5.5 ± 0.62	1.70	1.0
(–)-α-Cedrene ^d	200.30 (161.06–244.97)	1.4 ± 0.15	1.04	1.0
γ-Terpinene	216.16 (177.90–256.37)	1.7 ± 0.21	2.35	0.99
Benzyl benzoate ^c	8.82 (7.24–10.48)	1.6 ± 0.14	4.26	0.96
DEET ^c	38.95 (32.24–51.15)	1.6 ± 0.30	2.77	0.99
Permethrin ^c	> 500			
<i>d</i> -Phenothrin ^c	> 500			

^aNo toxicity was produced by anthracenedione, camphene, *p*-cymene, isovanillin, myrcene, myristicin, (Z)-β-ocimene, (+)-α-pinene, (–)-α-pinene, (+)-β-pinene, (–)-β-pinene and resorcinol (LD₅₀ > 500 μg/cm²)

^bCI denotes confidence interval

^cPearson's χ² goodness-of-fit test

^dCompounds identified in this study

^eAcaricides

Table 4 Fumigant toxicity of 13 selected compounds to adult *Dermatophagoides farinae* using a vapor-phase mortality bioassay during a 24 h exposure

Compound	Conc. ($\mu\text{g}/\text{cm}^2$)	Mortality (%) ($\pm$ SE)		Student's <i>t</i> test <i>P</i>
		Vapor in closed container	Vapor in open container	
Methyl eugenol	11.64	100	4 $\pm$ 2.1	< 0.0001
Guaiacol	16.48	100	5 $\pm$ 1.6	< 0.0001
<i>m</i> -Cresol	30.30	99 $\pm$ 0.8	2 $\pm$ 0.7	< 0.0001
2,5-Dimethylphenol	35.10	90 $\pm$ 1.8	4 $\pm$ 1.3	< 0.0001
Nerolidol	39.04	95 $\pm$ 2.6	5 $\pm$ 2.9	< 0.0001
<i>o</i> -Cresol	44.10	98 $\pm$ 0.8	2 $\pm$ 0.7	< 0.0001
Phenol	49.78	98 $\pm$ 0.7	2 $\pm$ 0.9	< 0.0001
Myrtenal	53.62	62 $\pm$ 4.4	2 $\pm$ 1.7	< 0.0001
3-Carene	53.88	92 $\pm$ 3.3	2 $\pm$ 1.7	< 0.0001
<i>p</i> -Cresol	57.08	98 $\pm$ 1.7	2 $\pm$ 0.7	< 0.0001
Myrtenol	57.98	57 $\pm$ 3.3	2 $\pm$ 1.7	< 0.0001
2-Carene	60.36	88 $\pm$ 4.4	3 $\pm$ 1.7	< 0.0001
Caryophyllene oxide	72.14	75 $\pm$ 1.2	4 $\pm$ 0.7	< 0.0001

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

Spray treatment	Mortality (%) ($\pm$ SE)
Cade oil	
10 mg/L	25 $\pm$ 1.2 c
20 mg/L	53 $\pm$ 1.8 b
30 mg/L	96 $\pm$ 1.0 a
40 mg/L	100 a
Permethrin (<i>cis:trans</i> , 25:75)	
2.5 g/L	17 $\pm$ 0.8 d

Means followed by different letters are significantly different (Bonferroni method: $P < 0.05$)

Discussion

Approximately 67 species in the genus *Juniperus* are widely distributed throughout the northern hemisphere (Adams 2014). Cade is a common evergreen shrub 2–3 m tall or a small erect tree 10–15 m tall. CA-EO contains various constituents such as terpenic hydrocarbons, alcohols, and ketones, although the chemical composition of cade oil depends on the origin of the plant and the subspecies (Boti et al. 2006). No information can be found about managing the pyroglyphid mites with CA-EO, despite its excellent pharmacological actions (Leung and Foster 1996; Lawless 2002; Derwich and Chabir 2011). In

the current study, CA-EO exhibited good acaricidal activity against adult AHDMs. The essential oil of *Juniperus chinensis* (var. *globosa*) leaves has been also reported to possess the acaricidal activity against adult AHDMs (Lee et al. 2009).

Certain EOs and their constituents can be developed into products that are suitable for integrated pest management because they can be target-specific, have few harmful effects on nontarget organisms, and be environmentally nonpersistent (Pillmore et al. 1993; Isman and Machial 2006; Koul et al. 2008; Tripathi et al. 2009; Pavela and Benelli 2016). They also can be used in conjunction with biological control (Isman and Machial 2006; Miresmailli and Isman 2006). EOs consist of highly complex mixtures of terpenic hydrocarbons (monoterpenoids, sesquiterpenoids, and diterpenoids) and oxygenated compounds (alcohols, aldehydes, esters, ketones, oxides, and phenols) (Bakkali et al. 2008; Shaabana et al. 2012). The EO constituents, either alone or in combination, contribute to an acute toxicity to various types of pest complexes (Hummelbrunner and Isman 2001; Isman and Machial 2006). Natural acaricidal compounds against the pyroglyphid mites have been well documented by Ahn et al. (2006). The acaricidal constituents derived from plants against adult AHDMs include aldehydes [e.g., *p*-anisaldehyde, $LD_{50} = 1.13 \mu\text{g}/\text{cm}^2$ (Lee 2004a)], organosulfur compounds [e.g., allylthiocyanate, $LD_{50} = 2.52 \mu\text{g}/\text{cm}^2$ (Yun et al. 2012)], benzofuranoids [e.g., butylidenephthalide, $LD_{50} = 6.77 \mu\text{g}/\text{cm}^2$ (Kwon and Ahn 2002)], terpenoids [e.g., redioides A, C, E and F, $LD_{50} = 5.40\text{--}16.55 \mu\text{g}/\text{cm}^2$ (Soonthornchareonnon et al. 2005); (+)-fenchone and thymol, $LD_{50} = 3.89$ and $9.03 \mu\text{g}/\text{cm}^2$ (Lee 2004a); 3-carene, $LD_{50} = 42.10 \mu\text{g}/\text{cm}^2$ (Lee 2004b)]; β -thujaplicine and cedrol, $LD_{50} = 7.22$ and $9.88 \mu\text{g}/\text{cm}^2$ (Jang et al. 2005); bisabolangelone, $LD_{50} = 1.88 \mu\text{g}/\text{cm}^2$ (Kang et al. 2006); atractylon and atractylenolide III, $LD_{50} = 13.62$ and $10.33 \mu\text{g}/\text{cm}^2$ (Kim et al. 2007); bornyl acetate and linalool, $LD_{50} = 22.81$ and $36.13 \mu\text{g}/\text{cm}^2$ (Lee et al. 2013); (–)-thujopsene and carvacrol, $LD_{50} = 9.82$ and $12.05 \mu\text{g}/\text{cm}^2$ (Kim et al. 2015)], phenylpropanoids [e.g., cinnamaldehyde, $LD_{50} = 2.58 \mu\text{g}/\text{cm}^2$ (Kim et al. 2008); estragole, $LD_{50} = 41.33 \mu\text{g}/\text{cm}^2$ (Lee 2004a); eugenol, $LD_{50} = 5.47 \mu\text{g}/\text{cm}^2$ (Kim et al. 2003)], and carboxylic acid [e.g., 2-(1,3-dihydroxy-but-2-enylidene)-6-methyl-3-oxoheptanoic acid, $LD_{50} = 2.04 \mu\text{g}/\text{cm}^2$ (Wu et al. 2012)].

In the current study, CA-EO was mainly composed of isovanillin, (–)- α -cedrene, camphor, β -caryophyllene, caryophyllene oxide, *p*-cresol, and guaiacol. The acaricidal constituents of CA-EO were determined to be the phenylpropanoid eugenol, and the phenols *m*-cresol and guaiacol. The LD_{50} of these constituents was between 8.24 and $15.15 \mu\text{g}/\text{cm}^2$, although the LD_{50} of the natural compounds previously stated was between 1.13 and $42.10 \mu\text{g}/\text{cm}^2$. Guaiacol and benzyl benzoate did not significantly differ in toxicity from each other against AHDMs. Eugenol and *m*-cresol were more toxic than DEET. In addition, the 20 and 30 mg/L sprays containing CA-EO resulted in potent control efficacy compared to permethrin (*cis:trans* = 25:75) 2.5 g/L spray. This original finding indicates that CA-EO preparations containing active constituents hold promise for the development of novel, effective naturally occurring acaricides for the control of the pyroglyphid mites. Akhtar et al. (2012) studied the effect of the chemical complexity of EOs on feeding deterrence in cabbage looper larvae. They reported that a blend containing all the known constituents of EOs is the most active feeding deterrent, and proposed that the minor constituents may act as synergists, enhancing the effect of the major constituents through various mechanisms. Therefore, an interaction between various constituents of CA-EO may contribute to enhancement of acaricidal activity of the oil against AHDMs.

QSAR analysis of chemicals against many arthropod pests has been well noted. Not only can the QSAR information contribute to the search for additional compounds with higher activity, it can also promote the understanding of the mechanism of acaricidal action

of phytochemicals, as described by Dias and Moraes (2014). For example, Tsao et al. (1995) found that the transformation of the hydroxyl group in selected monoterpenoids and phenols enhanced biological activity. This was suggested to result from an increase in vapor pressure, which increased the fumigation activity and/or increased the lipophilicity, which led to better penetration of the insect cuticle and availability in the insect's body. Yun et al. (2012) studied the toxicity of the 28 isothiocyanate ($RN=C=S$) compounds against adult AHDMS. They reported that aromatic isothiocyanates appeared to be more toxic than aliphatic isothiocyanates and structural characteristics, such as carbon skeleton, types of functional groups, side chain length of aliphatic isothiocyanate, and structural isomerism, appear to play a role in determining the isothiocyanate toxicity to adult AHDMS. In the current study, among the eugenol derivatives (Fig. 1), methyleugenol was 2.2 and 8.2 times more toxic than eugenol and isoeugenol, respectively. This finding indicates that the methyl group of methyleugenol is a prerequisite component for toxicity to AHDMS. Among the phenol derivatives (Fig. 1), guaiacol was 1.8, 2.7, and 3.5 times more toxic than *m*-, *o*-, and *p*-cresol, respectively. This finding indicates that the methoxy group of guaiacol is a prerequisite component for toxicity to AHDMS and the methyl group substitution onto the ring of phenol is also important for toxicity. In chemical structures of cresol, *meta* form appears to play a role in determining the cresol toxicities to AHDMS.

An investigation about the modes of action and delivery for botanical acaricides provide important practical information for HDM control, such as the most appropriate formulations and delivery means to be adopted for their future commercialization (Ahn et al. 2006; Kim et al. 2006). EO volatile constituents against adult AHDMS and EHDMS primarily act as fumigants with additional contact action. Fumigant toxicity to the pyroglyphid mites has been reported for atracylon and atracylenolide III (Kim et al. 2007), allylisothiocyanate (Yun et al. 2012), butylidenephthalide (Kwon and Ahn 2002), and paeonol (Kim et al. 2004b). In a separate study, bisabolangelone was reported to be largely toxic through contact action (Kang et al. 2006).

In the current study, the 13 compounds 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 plus fumigant action of CA-EO constituents, as demonstrated through the current vapor-phase mortality and spray bioassays, is of practical importance because it allows the EO volatile constituents to reach deep refuge in closed spaces such as a building, a wardrobe, a pet house, or a storage bin, resulting in good control of the pyroglyphid mites. This dual system has advantages compared to contact system because exposure to volatile constituents can be easily

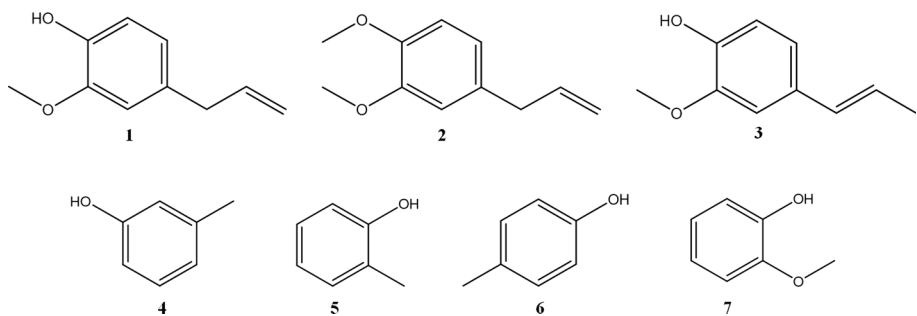


Fig. 1 Structures of the three phenylpropanoids eugenol (1), methyl eugenol (2), and isoeugenol (3) and the four phenols, *m*-cresol (4), *o*-cresol (5), *p*-cresol (6), and guaiacol (7)

controlled in a closed space using an appropriate automatic control equipment or dispenser (Kim et al. 2006). Because of high volatility of the CA-EO constituents and the test compounds described, the binary mixtures of these volatile constituents 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 30 or 40 mg/L spray containing CA-EO are fragrant, which is appropriate for commercialization. Detailed tests are needed to understand fully the mode of action of the active CA-EO constituents, although the octopaminergic and γ -aminobutyric acid receptors have been suggested as novel target sites for some monoterpenoid EO constituents in *Periplaneta americana* (L.) (Enan 2001) and *Helicoverpa armigera* (Hübner) (Kostyukovsky et al. 2002), and *Drosophila melanogaster* Meigen (Priestley et al. 2003), respectively.

In conclusion, bioacaricides derived from CA-EO containing guaiacol, eugenol, and *m*-cresol, and other compounds could be useful as contact-action fumigants to protect humans from various allergic diseases caused by the pyroglyphid mites. Further research is needed regarding the practical applications of CA-EO-derived preparations as novel acaricide products. Specifically, it will be essential to establish safety profiles for these oil-derived preparations in humans. Cade oil is used in the treatment of cutaneous diseases, such as chronic eczema, parasites, scalp, disease, and hair loss (Lawless 2002). In addition, detailed tests are needed to understand how to improve the acaricidal potency and stability of the constituents isolated from cade oil for eventual product development.

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