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Toxicity and effects of essential oils and their components on *Dermanyssus gallinae* (Acari: Dermanyssidae)

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Abstract

The acaricidal activity of 30 essential oils against the poultry red mite, *Dermanyssus gallinae*, female adults and behavioral responses of the mites to these essential oils were investigated. Cinnamon bark oil and clove bud oil showed 100% acaricidal activity after 24 h in the 1.3 μg/m^2 treatment. In addition, four components in cinnamon bark oil and three components in clove bud oil were identified using gas chromatography-mass spectrometry. Cinnamon bark oil showed the highest LD_{50} value among all of the components, and eugenol showed 0.97-fold higher relative toxicity (RT) than the other components of clove bud oil. The fumigant effects of both essential oils and their seven components were observed using a vapor phase toxicity bioassay. All the substances showed repellent activity except for cinnamyl acetate, which did not show any repellent response even in the > 10 μg treatment. In the experiment using the T-tube olfactometer with the 10 μg treatment of each substance, *D. gallinae* female adults responded to all the substances except cinnamyl acetate. However, eugenol and eugenol acetate showed an attractant effect after 240 and 120 min of treatment, respectively. These results suggest that the two studied essential oils and their components may be used as control agents against *D. gallinae*.

Keywords *Dermanyssus gallinae* · Essential oils · Acaricidal activity · Repellency · Attractant

Introduction

The poultry red mite, *Dermanyssus gallinae* (De Geer), is an economically important hematophagous parasite that parasitizes birds around the world (Axtell and Arends 1990; Van Emous 2005; Sparagano et al. 2009). This mite is mainly known to suck the blood of chickens, wild birds, and rodents but sometimes causes skin diseases in humans (Gaaboub et al. 1982; Song and Song 1999; Rosen et al. 2002). *Dermanyssus gallinae* causes damage to poultry by

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serving as a vector for Newcastle disease virus, avian influenza A virus, avian spirochetosis, and salmonellosis and reduces the productivity and the quality of eggs due to stress (Arzey 1990; Chirico et al. 2003; Moro et al. 2009; George et al. 2015; Sommer et al. 2016).

Control of the poultry red mite is highly dependent on synthetic acaricides, such as organophosphates, carbamates, pyrethroids and spinosyns (Sparagano et al. 2014; Abbas et al. 2014). However, pesticide-based control is problematic because pesticide residues can be found in the eggs, including unregistered pesticides that may cause harm to the human body and that may pollute the environment (Pitasawat et al. 2007; Eladl et al. 2018). To reduce the use of pesticides and to solve the residue problem, the acaricidal effect can be increased through the application of fumigants, pheromones or attractants (Kim et al. 2004a, b; Hafsi et al. 2016; Morrison et al. 2016). *Dermanyssus gallinae* can also survive for weeks outside without hosts, and mites hide between cracks in chicken coops when they are not actively sucking blood; therefore, effective control methods must consider this behavior (Axtell and Arends 1990; Nordenfors et al. 1999). As an alternative to these methods and a solution to these problems, the use of essential oil has been shown to have effects as toxic substances, attractants, repellents, antifeedants and insect growth regulators used against many arthropods (Kwon and Ahn 2003; Kim et al. 2004a, b, 2007, 2018a, b; Pitasawat et al. 2007; Park et al. 2012). Some essential oils and their constituents meet the criteria of reduced-risk pesticides and are readily available for many purposes (US EPA 2004). Indeed, the use of several plant extracts, essential oils and related compounds derived from plants such as *Eugenia caryophyllata*, *Cinnamomum camphora*, *Asarum heterotropoides*, and *Cnidium officinale*, including cassia oil, cinnamon oil and the structurally related compound cinnamaldehyde, against *D. gallinae* has been reported in previous studies (Kim et al. 2004a, b, 2007, 2016, 2018a, b; George et al. 2010b).

Cinnamon bark oil and clove bud oil have been reported to have acaricidal, insecticidal and repellent activity against *Tyrophagus putrescentiae*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae, Reticulitermes speratus*, *Sitophilus oryzae*, *Trialeurodes vaporariorum*, *Bemisia tabaci,* and Pediculus humanus (Kim et al. 2003, 2004a, b, 2011; Choi et al. 2003; Park and Shin 2005; Yang et al. 2005; Saad et al. 2006). These two essential oils are used in various fields such as medicinal pharmaceutical use and antibacterial activity (Tang and Eisenbrand 1992; Nuñez and Aquino 2012). They contain a variety of active substances, of which cinnamaldehyde and eugenol are the main constituents, which also affect many species of Acari, such as *T. putrescentiae*, *D. pteronyssinus*, *Dermacentor nitens* and *D. gallinae* (Kim et al. 2004a, b; Saad et al. 2006; Zeringóta et al. 2013; Sparagano et al. 2013).

This experiment was conducted to evaluate (1) the acaricidal activity of 30 essential oils and their components against adult female poultry red mites, *D. gallinae*; (2) the vapor-phase toxicities (contact or fumigation) of two highly active essential oils (cinnamon bark oil and clove bud oil) and their seven components; and (3) the olfactory response of this mite species.

Materials and methods

Mites

The adult female *D. gallinae* used in this experiment were reared using chicken at Biogenoci (Suwon, Korea), and the experiment was established in a starving state within 2 days after the mites were obtained from chickens. Identification of the poultry red mites was performed in the same manner as in a previous study according to the method of Potenza et al. (2009) using polymerase chain reaction (PCR) (Kim et al. 2018a, b).

Plant essential oils and chemicals

The 30 essential oils used in this study were as follows: acerola cherry purchased from Kimex (Yongin, Korea); bergamot peel, rosemary, and sandalwood from Seoul Perfumery (Seoul, Korea); cedarwood from Kanto Chemical (Tokyo, Japan); cinnamon bark, cinnamon leaf, and grapefruit from Aura Cacia (Benton, IL, USA); garlic and onion from Aromaticos Quimicos Potosinos (Zapopan, Mexico); gurjun balsam and pine from French Korean Aromatics (Yongin, Korea); hysope from New Directions Aromatics (Mississauga, Canada); lemon peel from Firmenich (Genève, Switzerland); mustard from Allin Exporters (Noida, India); peanut from Greenwood Associates (Niles, IL, USA); and other 14 essential oils (caraway, citronella java, clary sage, clove bud, ginger, lavender, lemon, lemongrass, lime, marjoram, *Mentha arvensis*, pennyroyal, peppermint and thyme white) from Jinarome (Anyang, Korea).

Cinnamyl acetate, cinnamic aldehyde, 2-methoxycinnamaldehyde, acetyl eugenol and coumarin were purchased from Sigma-Aldrich (St. Louis, MO, USA), and β-caryophyllene was purchased from Tokyo Chemical Industry (Tokyo, Japan).

Gas chromatography-mass spectrometry

The composition of the cinnamon bark oil and clove bud oil, which showed a very high acaricidal effect against adult female of *D. gallinae*, was analyzed with gas chromatography-mass spectrometry (GC–MS, Agilent Technologies 7890A/5975C, Santa Clara, CA, USA). The column used for the analysis was a DB-1 column (0.25 µm, 0.25 mm × 30 m, J&W Scientific, Folsom, CA, USA) with fused-silica capillary tubing and helium (He) as the carrier gas at a flow rate of 1.2 ml/min. The initial oven temperature was maintained at 70 °C for 3 min and was increased at a rate of 5 °C/min to 325 °C. The injector temperature was 250 °C, and spectra were obtained at 70 eV (ionization voltage). The two target essential oil components were identified by comparing the mass spectrum of each peak with a mass spectra library (Wiley Registry of Mass Spectral Data, 7th edn, John Wiley & Sons, NY, USA).

Toxicity bioassay

The acaricidal activity of the 30 essential oils against adult female *D. gallinae* was assessed with a direct contact assay. Three concentrations (127.4, 12.7 and 1.3 µg/cm^2) of each essential oil in 40 µl of ethanol were applied to filter paper (55 mm diameter; Hyundai Micro, Korea) and dried under a hood at room temperature for 3 min. The treated filter paper was laid on the bottom of a Petri dish (50 mm diameter × 10 mm), and 50 µl of distilled water was added to maintain the humidity. Fifteen adult female mites were placed in each Petri dish with non-vented conditions, and their mortality was assessed 24 h after treatment. All the experiments were replicated 3 ×.

The two most effective acaricidal essential oils (cinnamon bark oil and clove bud oil) and their seven components were evaluated with various doses (eugenol: 1.0; eugenol acetate: 78.9; β-caryophyllene: 28.7; cinnamaldehyde: 1.7; 2-methoxycinnamaldehyde: 7.1; cinnamyl acetate: 4.1; and coumarin: 4.5 μg/cm^2) to identify the lethal dose, and the bioassay was performed as above.

The vapor-phase toxicities of the two essential oils and their components were identified on the basis of the LD_{90} values of each compound. The fumigant activity of the compounds towards adult female *D. gallinae* was determined using a modification of the closed- and open-container treatment methods (Kim et al. 2007). Briefly, this experiment was conducted in the same way as the experiment assessing acaricidal activity except the lid of the Petri dish had a fine wire screen (200 mesh) covering a center hole (30 mm diameter). Fifteen to 20 adult mites were placed on the bottom of the Petri dish, which was covered with the fine wire-screened lid to prevent contact with the tested compound. Filter paper was treated with the concentration of the test compound in 40 μl of ethanol corresponding to the LD_{90} value and then placed on top of the wire screen lid. Each Petri dish was sealed with a solid lid to determine the fumigant activity of the compounds under the closed-container treatment or sealed with a lid with a center hole for the open-container treatment. Mortalities were determined 24 h after treatment. All the experiments were replicated 3 ×.

T-tube olfactometer bioassay

Olfactory response to the two essential oils (cinnamon bark oil and clove bud oil) and their components was investigated by dissolving 10 μg of the each substance in 100 μl ethanol using a T-tube olfactometer (Kim et al. 2018a, b). The structure of the glass T-tube olfactometer is a 4.5 cm stem, 12 cm arms at 180° and a 1.5 cm internal width. Air was supplied at 5 L/min and was filtered with activated charcoal, a molecular sieve and silica gel blue. Ethanol was used as a control and dried for 3 min at room temperature. Observations of the response of the mites for the median repellent dose (RD_{50}) value began 30 min after treatment. The response rate of mites was measured after 30, 60, 120 and 240 min after drying for 3 min after treatment. The mites were considered as responding when they moved more than 3 cm, and the other mites were regarded as nonresponding. The experiments were maintained at 25 ± 1 °C and 50–60% relative humidity in darkness. All experiments were replicated 3 × with 10–25 adult female mites per replicate without feeding for 2 days. In each experiment, a new glass T-tube was used.

Mite response rate was calculated as: (number of responsive mites in the untreated arm/total number of responsive mites) × 100.

Data analysis

The acaricidal activity of the 30 essential oils against *D. gallinae* adults was analyzed using Tukey's test (SAS Institute 2009). The lethal dose (LD) and repellent dose (RD) of the two essential oils and their components were calculated using probit analysis (SAS Institute 2009). The relative susceptibility (RS) was calculated as the ratio of the LD_{50} value of the compounds based on the LD_{50} value of each essential oil. The differences in vapor-phase toxicity between the closed-container treatment and open-container treatment were analyzed using *t*-tests (SAS Institute 2009).

Results

Acaricidal activity of 30 plant essential oils

The acaricidal activity of the 30 tested essential oils was evaluated against female adults of *D. gallinae* (Table 1). In the 127.4 μg/cm² treatment, 100% acaricidal activity was observed in 24 essential oils; the exceptions were cedarwood, ginger, grapefruit, lemon, peanut, and sandalwood. In the 12.7 μg/cm² treatment, 100% mortality was observed for 13 essential

Table 1 Acaricidal activity of 30 plant essential oils against adult female *Dermanyssus gallinae* using the filter paper contact bioassay for 24 h exposure

Essential oil	Mortality (%, mean ± SE)					
	n[a]	127.4 μg/cm²	*n*	12.7 μg/cm²	*n*	1.3 μg/cm²
Acerola Cherry	45	100 a	45	0 c		–
Bergamot peel	45	100 a	45	4.4 ± 4.4 c		–
Caraway	45	100 a	45	100 a	45	4.5 ± 2.2 b
Cedarwood	45	48.9 ± 15.6 b		–		–
Cinnamon Bark	45	100 a	45	100 a	45	100 a
Cinnamon leaf	45	100 a	45	100 a	46	85.3 ± 11.5 a
Citronella java	45	100 a	45	86.7 ± 10.2 ab	47	76.7 ± 1.7 a
Clary sage	45	100 a	45	11.1 ± 5.9 c		–
Clove bud	45	100 a	45	100 a	45	100 a
Garlic	45	100 a	45	100 a	45	0 b
Ginger	45	42.2 ± 4.5 b		–		–
Grape fruit	45	8.9 ± 4.4 c		–		–
Gurjun balsam	45	100 a	45	64.4 ± 12.4 b		
Hysope	45	100 a	45	100 a	45	2.2 ± 2.2 b
Lavender	45	100 a	45	2.2 ± 2.2 c		–
Lemon	45	33.3 ± 24.0 bc		–		–
Lemon peel	45	100 a	45	2.2 ± 2.2 c		–
Lemongrass	45	100 a	45	100 a	45	0 b
Lime	45	100 a	45	6.7 ± 6.7 c		–
Marjoram	45	100 a	45	100 a	45	0 b
Menta avensis	45	100 a	45	100 a	45	2.2 ± 2.2 b
Mustard	45	100 a	45	2.2 ± 2.2 c		–
Onion	45	100 a	45	6.7 ± 3.8 c		–
Peanut	45	8.9 ± 4.4 c		–		–
Pennyroyal	45	100 a	45	100 a	45	0 b
Peppermint	45	100 a	45	100 a	45	6.7 ± 3.8 b
Pine	45	100 a	45	100 a	45	24.5 ± 2.2 b
Rosemary	45	100 a	45	17.8 ± 11.1 c		–
Sandalwood	45	20.0 ± 3.9 bc		–		–
Thyme white ND	45	100 a	45	100 a	45	28.9 ± 17.4 b

[a]*n* indicates total number of mites tested

Means within a column followed by a different letter are significantly different (Tukey's test: $p < 0.01$)

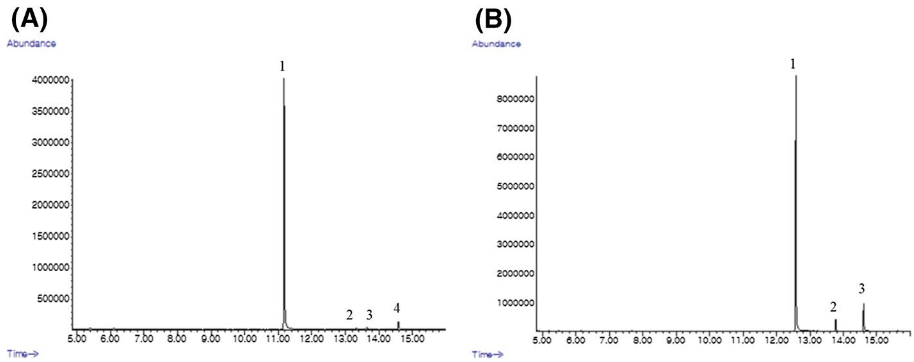

Fig. 1 GC–MS analysis of essential oils of **a** cinnamon bark and **b** clove bud

Table 2 Quantitative composition of two essential oils using GC-MS

EsRTsential oil	Peak no.	RT (min)[a]	Compound	Area (%)
Cinnamon bark	1	11.171	Cinamaldehyde	94.6
	2	13.331	Coumarin	0.9
	3	13.642	Trans-cinnamyl acetate	0.9
	4	14.586	2-methoxycinnamaldehyde	3.7
Clove bud	1	12.578	Eugenol	85.4
	2	13.773	β-caryophyllene	4.2
	3	14.606	Eugenol acetate	10.4

[a]*RT* retention time

oils. Only two essential oils (cinnamon bark and clove bud) showed 100% mortality at the 1.3 μg/cm^2 initial dose of the 100-fold dilution.

Components of the two main essential oils

The peaks showed the main constituents of the cinnamon bark and clove bud oil using GC–MS (Fig. 1). Four main components of cinnamon bark oil and three components of clove bud oil were identified, and their retention time (RT) and composition were established by GC–MS (Table 2). Cinnamaldehyde (94.6%) and eugenol (85.4%) were the main components of the cinnamon bark and clove bud oils, respectively. The retention time (RT) of cinnamaldehyde and eugenol was 11.171 and 12.578 min, respectively.

Toxicity of the two essential oils and their components

The median lethal dose (LD_{50}) of the acaricidal activity of cinnamon bark oil, clove bud oil and their components against *D. gallinae* was statistically analyzed (Table 3). The

Table 3 Acaricidal activities of two essential oils and their compounds to adult female *Dermanyssus gallinae* after 24 h of treatment

Essential oil	Compound	n^a	Slope (±SE)	LD_{50} (μg/cm^2) (95% CL[b])	*Wald* x^2	RS[c]
Cinnamon bark		289	3.68 (±0.749)	0.542 (0.311–0.789)	24.20	1
	Cinnamaldehyde	191	2.81 (±0.303)	0.586 (0.522–0.663)	86.08	1.08
	2-methoxycinnamaldehyde	306	2.90 (±0.686)	2.602 (1.186–3.812)	17.93	4.80
	Cinamyl acetate	237	2.36 (±0.248)	1.194 (1.062–1.369)	90.71	2.20
	Coumarin	190	2.54 (±0.281)	1.325 (1.172–1.524)	81.63	2.45
Clove bud		279	7.49 (±0.614)	0.693 (0.661–0.723)	148.7	1
	Eugenol	193	7.69 (±1.277)	0.717 (0.576–0.890)	36.20	1.04
	Eugenol acetate	213	2.23 (±0.297)	20.607 (13.842–29.327)	56.39	29.74
	β-caryophyllene	235	1.94 (±0.301)	6.575 (4.286–9.399)	41.71	9.49

LD_{50} values are significantly different if the 95% CLs do not overlap

[a]*n* total number of mites tested

[b]*CL* confidence limit

[c]*RS* relative susceptibility

cinnamon bark oil and its components showed the highest LD_{50} values with a value of 0.542 μg/cm^2 for the crude essential oil against female adults of *D. gallinae*, and the cinnamaldehyde showed a value of 0.586 μg/cm^2. These materials also showed similar activity, with an RS value of 1.08. However, the *D. gallinae* adults showed 4.8-fold higher susceptibility to 2-methoxycinnamaldehyde than to the crude essential oil. In terms of clove bud oil, the crude essential oil (LD_{50}=0.693 μg/cm^2) showed the highest activity, followed by eugenol (LD_{50}=0.717 μg/cm^2). The mites were 29.7-fold more susceptible to eugenol acetate than to the crude essential oil.

In the vapor-phase toxicity experiment evaluating the two essential oils and their seven components at their LD_{90} values, the two essential oils showed 100% mortality in the closed-container treatment (Table 4). In addition, all seven components were observed to cause over 90% mortality in the closed-container treatment (A), whereas less than 8% mortality was observed in the open-container treatment (B). All the materials were statistically confirmed to differ in terms of mortality between the closed- and open-container treatments.

Olfactory response of *Dermanyssus gallinae*

The RD_{50} value of crude cinnamon bark oil was observed to be < 0.1 μg, and coumarin showed the highest repellent activity as a single compound (Table 5). However, the RD_{50} value of cinnamyl acetate was never confirmed, even in the > 10 μg treatment. In terms of the clove bud oil (0.192 μg), even though the crude essential oil showed high repellent activity, the RD_{50} value of eugenol was the lowest, at < 0.1 μg.

The responses of poultry red mites to the two essential oils and their seven components were divided by three types (response to the treated side, response to untreated side and no response) with a 10 μg treatment (Fig. 2). The mites showed various responses to cinnamon bark oil and its four constituents (Fig. 2a). When the cinnamon bark oil was applied, all the mites moved to the untreated side, where they remained

Table 4 Fumigant activity of cinnamon bark and clove bud essential oil and their compounds against *Dermanyssus gallinae* using the vapor phase toxicity bioassay with exposure for 24 h at LD_{90}

Essential oil	Compound	LD_{90} (μg/cm^2)	Method[a]	n[b]	Mortality (%, mean ± SE)
Cinnamon bark		1.027	A	47	100 a
			B	49	1.9 ± 3.2 b
	Cinnamaldehyde	1.672	A	51	95.8 ± 4.2 a
			B	48	4.1 ± 2.1 b
	2-methoxycinnamaldehyde	7.189	A	43	100 a
			B	58	5.4 ± 3.2 b
	Cinamyl acetate	4.169	A	44	100 a
			B	51	0 b
	Coumarin	4.231	A	43	93.1 ± 3.7 a
			B	50	7.9 ± 4.8 b
Clove bud		1.207	A	53	100 a
			B	49	0 b
	Eugenol	1.052	A	45	91.1 ± 4.5 a
			B	39	5.8 ± 2.9 b
	Eugenol acetate	77.548	A	50	94.2 ± 3.2 a
			B	44	7.2 ± 1.1 b
	β- caryophyllene	30.084	A	45	95.8 ± 4.2 a
			B	51	3.7 ± 3.7 b

Means followed by a different letter are significantly different (t test: $p < 0.01$)

[a]A, vapor in close container; B, vapor in open container

[b]n indicates total number of mites tested

Table 5 Repellency of essential oils and their compounds to adult female *Dermanyssus gallinae* 30 min after exposure

Essential oil	Compound	n[a]	Slope (± SE)	RD_{50} (μg, 95% CL[b])	*Wald* x^2
Cinnamon bark		135		< 0.1	
	Cinnamaldehyde	131	0.75 (± 0.105)	0.206 (0.102–0.343)	51.24
	2-Methoxycinnamaldehyde	133	1.58 (± 0.193)	2.876 (2.234–3.601)	67.15
	Cinamyl acetate	146	–	> 10	
	Coumarin	144	0.58 (± 0.100)	0.114 (0.034–0.234)	33.51
Clove bud		187	0.96 (± 0.129)	0.192 (0.113–0.296)	55.2
	Eugenol	170		< 0.1	
	Eugenol acetate	190	0.81(± 0.093)	0.389 (0.227–0.624)	76.04
	β-caryophyllene	153	1.01 (± 0.116)	0.294 (0.187–0.430)	76.11

LD_{50} values are significantly different if the 95% CLs do not overlap

[a]n total number of tested mites

[b]*CL* confidence limit

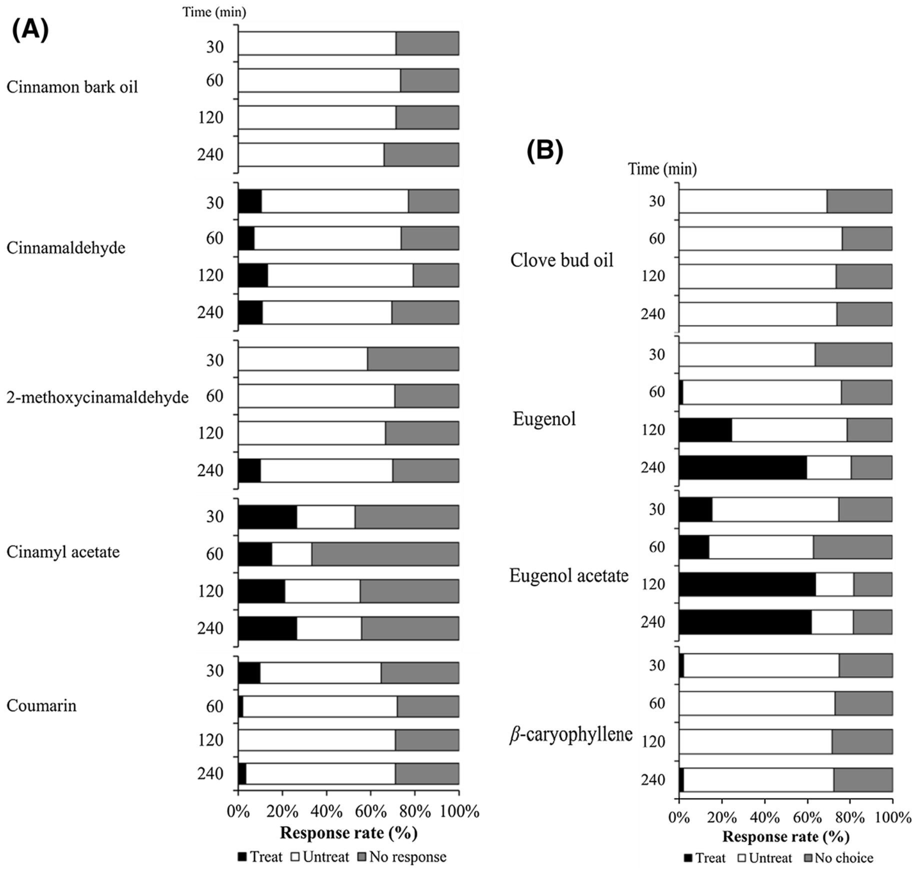

Fig. 2 Response to two essential oils and their derived components

even after 240 min, indicating that this oil has a repellent effect. Most of the mites were repelled by cinnamaldehyde, 2-methoxycinnamaldehyde and coumarin by choosing the untreated side. For example, in the 2-methoxycinnamaldehyde treatment, the mites did not select the treated side until 120 min after the beginning of the trial. There were many nonresponsive mites to cinnamyl acetate, as they did not show any characteristic response to the treated or untreated side. The response of *D. gallinae* to clove bud oil and its three constituents was also investigated (Fig. 2b). For the crude clove bud oil, no mites chose the treated side after 240 min. For β-caryophyllene, most mites had selected the untreated side by 240 min after the trial had begun, and nonresponsive mites were observed in approximately 20% of the trials. The mites were attracted by eugenol and eugenol acetate, as shown by their selection of the treated side more often than the untreated side with increasing duration of the trial. Specifically, the eugenol acetate attracted the *D. gallinae* mites, as the majority of the mites had selected the treated side after 120 min.

Discussion

This study was conducted to develop an acaricidal agent using essential oils as an alternative to chemical pesticides for poultry red mite control, as the resistance of *D. gallinae* to various acaricides (organophosphates, pyrethroids, neonicotinoids, carbamates) has been reported in many countries (Beugnet et al. 1997; Nordenfors et al. 2001; Fiddes et al. 2005; Abdel-Ghaffar et al. 2009; Abbas et al. 2014; Murano et al. 2015; Pavlicevic et al. 2016; Lee et al. 2017; Sigognault Flochlay et al. 2017).

Plant extracts and plant-derived materials are known to be effective against chemical pesticide-resistant pests, and their use may be an effective means of controlling such pests (Lindquist and Casey 1990; Ahn et al. 1997). Essential oils contain various active substances with different modes of action that can delay the development of resistant pest populations (Sparagano et al. 2013; Pavela and Benelli 2016). Many plant-derived essential oils are relatively safe for contact with human bodies; they are used as food additives and in cosmetics, and they also contain various constituents with various effects, such as acaricidal, repellent and attractant effects (Ahn et al. 2006; Isman and Machial 2006; US EPA 2011). There have been many studies on the effects of various essential oils and compounds against *D. gallinae*, but the results of some of these studies differ from those found here (Kim et al. 2004a, b, 2007, 2015, 2018a, b; Na et al. 2011). For example, mustard oil (93%) was shown to have higher acaricidal activity against *D. gallinae* than cinnamon oil (42%) in a 0.04 mg/cm^2 treatment (Kim et al. 2004a; Paes et al. 2012). However, in the current experiment, cinnamon bark oil showed 100% mortality at 12.7 μg/cm^2, whereas mustard oil only showed 2.2% mortality. This discrepancy suggests that the difference in the toxicity of essential oils is based upon the pesticides used on the poultry farms where the mites are and the developmental stage of the mites (Kim et al. 2004b; Lee et al. 2017). The difference in the mode of action according to the class of pesticides used on the farm showed differences in pesticide tolerance of the mites, and it is also thought that the modes of action differ among essential oils (El-Wakeil 2013; Lee et al. 2017). Essential oils sometimes exhibit similar or higher acaricidal activity than their constituents, and the use of essential oils is efficient because of the low economic costs due to the elimination of purification processes and the delayed development of resistance due the presence of various substances (Osanloo et al. 2017). Many essential oils and plant extracts contain various volatile compounds, such as alkanes, alcohols, aldehydes, and especially terpenes and monoterpenes, which show fumigation effects (Kim et al. 2004a). In this study, two essential oils and their constituents showed fumigant activity against *D. gallinae*, and it is therefore thought to be possible to develop to mite control agents using repellent or attractant effects. However, the plant components may differ due to various factors such as extraction method, cultivation area and harvesting time, and this factor should be considered in their use (Azuma et al. 2001; Jerković et al. 2001; Park et al. 2012; Kim et al. 2016).

The acaricidal effects against poultry red mites are important, but the development of repellent agents against mites is also necessary for the health of poultry workers. Because mites sometimes attack workers and cause skin diseases, it is necessary to develop a repellent agent that can prevent the access of mites to workers' clothing (Rosen et al. 2002). *Dermanyssus gallinae* responded to cinnamon bark oil, clove bud oil and their constituents, which all showed repellent effects except cinnamyl acetate. In particular, eugenol has been reported to show repellent activity against various arthropods, such as *Ocimum suave*, *Periplaneta americana*, *Aedes albopictus*, *Rhipicephalus microplus* and *D. nitens* (Obeng-Ofori and Reichmuth 1997; Ngoh et al. 1998; Hao et al. 2008; Zeringóta et al. 2013).

However, eugenol and eugenol acetate were found to change from having repellent to attractant effects on *D. gallinae* over time in this experiment. As time passes after the treatment, the substance will volatilize and the concentration will decrease, and when the concentration is reduced, the acaricidal activity is decreased, and the repellency is also thought to be affected by the concentration. The essential oils from *Amomum villosum*, camphene and bornyl acetate for *Tribolium castaneum* and *Lasioderma serricorne* showed repellent activity at a very high concentration (78.63 nL/cm^2) but were attractants at low concentrations, and *T. castaneum* larvae were also attracted to a low concentration (0.4 g/L) of oil of *Cymbopogon citratus* (Stefanazzi et al. 2011; Chen et al. 2018). These two compounds can be more effective in controlling mites if they are mixed at appropriate treatment dosages and used with the appropriate control methods (spray or fumigation). In addition, volatile compounds and 2.5% CO_2 have controlling effects against poultry red mites because the mites cluster together in response to aggregation pheromones after sucking blood and can be controlled using an attractant, such as kairomones (George et al. 2010a; Koenraadt and Dicke 2010). The lure and kill method using pheromones is a safe and effective method for mite control (Chirico and Tauson 2002; Carr and Roe 2017). In experiments on five compounds (nerolidol, guanine, 2,6-dichlorophenol, farnesol and geraniol), nerolidol and guanine showed the highest attractant activity among blood-fed *D. gallinae* females, especially nerolidol, which attracted more than 90% of the female mites, whereas males showed a low attractive response (Entrekin and Oliver 1982). Eugenol and eugenol acetate showed various effects depending on the concentration and time, and more experiments should be conducted on these compounds because they are likely to be used for the development of effective repellents or attractants in the future.

Therefore, this study suggests that the two studied essential oils and their components could be used for the development of fumigants, repellents and attractants for the control of the poultry red mite, *D. gallinae*.

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