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Acaricidal Activity of Clove Bud Oil Compounds against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae)

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The acaricidal activity of clove (*Eugenia caryophyllata*) bud oil-derived eugenol and its congeners (acetyleugenol, isoeugenol, and methyleugenol) against adults of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* was examined using direct contact application and fumigation methods and compared with those of benzyl benzoate and *N*,*N*-diethyl-*m*-toluamide (DEET). Responses varied according to compound, dose, and mite species. On the basis of LD_{50} values, the compound most toxic to *D. farinae* adults was methyleugenol (0.94 μg/cm^2) followed by isoeugenol (5.17 μg/cm^2), eugenol (5.47 μg/cm^2), benzyl benzoate (9.22 μg/cm^2), and acetyleugenol (14.16 μg/cm^2). Very low activity was observed with DEET (37.59 μg/cm^2). Against *D. pteronyssinus* adults, methyleugenol (0.67 μg/cm^2) was much more effective than isoeugenol (1.55 μg/cm^2), eugenol (3.71 μg/cm^2), acetyleugenol (5.41 μg/cm^2), and benzyl benzoate (6.59 μg/cm^2). DEET (17.85 μg/cm^2) was least toxic. These results indicate that the lipophilicity of the four phenylpropenes plays a crucial role in dust mite toxicity. The typical poisoning symptom of eugenol and its congeners was a similar death symptom of the forelegs extended forward together, leading to death without knockdown, whereas benzyl benzoate and DEET caused death following uncoordinated behavior. In a fumigation test with both mite species, all four phenylpropenes were much more effective in closed containers than in open ones, indicating that the mode of delivery of these compounds was largely due to action in the vapor phase. Eugenol and its congeners merit further study as potential house dust mite control agents or as lead compounds.

KEYWORDS: Natural acaricide; natural fumigant; *Dermatophagoides farinae*; *Dermatophagoides pteronyssinus*; *Eugenia caryophyllata*; clove bud oil, mode of action; poisoning symptom

INTRODUCTION

The most important pyroglyphid mites are the American house dust mite, *Dermatophagoides farinae* (Hughes), and the European house dust mite, *Dermatophagoides pteronyssinus* (Trouessart), because of their cosmopolitan occurrence and abundance in homes (*1*, *2*), because they are a major source of multiple potent allergens (*3*, *4*), and because of their causal association with sudden infant death syndrome (*5*). Changes in living environments such as a rise in the number of apartment households with centrally installed heating, space heating, tighter windows, and fitted carpets have improved conditions for mite growth (*1*). Control of these mite populations has been principally through the use of chemicals such as γ-benzene hexachloride (γ-BHC), pirimiphos-methyl, benzyl benzoate, *N*,*N*-diethyl-*m*-toluamide (DEET), and dibutyl phthalate (*1*). Although effective, their repeated use has sometimes resulted in the development of resistance (*3*), has undesirable effects on nontarget organisms, and has fostered environmental and human health concerns (*1*, *6*). These problems have highlighted the need for the development of new strategies for selective house dust mite control.

Plant essential oils may be an alternative source for dust mite control because they constitute a rich source of bioactive chemicals and are commonly used as fragrances and as flavoring agents for food additives. Because of this, much effort has been focused on plant essential oils as potential sources of commercial pest control agents (*7*, *8*). In traditional Chinese medicine, clove (*Eugenia caryophyllata* Thunberg), belonging to the family Myrtaceae, has long been considered to have medicinal properties such as a stimulant against digestive disorders and diarrhea. It contains various compounds such as chavicol, α- and β-caryophyllene, eugenol, acetyleugenol, furfural, eugenin, tannins (eugeniin and 1-desgalloyleugeniin), chromone glycosides (eugenosides I and II), alkaloids (higenamine), flavonoids (rhamnetin and kaempferol), steroid glycosides (sitosterol and stigmasterol), and triterpenoids (oleanolic acid and methyl 2 α-hydroxyoleanoate) (*9*). Very little work has been done with respect to managing house dust mites, although it has been noted that clove bud oil has insecticidal activity against some stored

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product insects (*8*, *10*) and suppresses progeny production of *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* (Motsch.) (*10*).

This paper describes a laboratory study aimed at isolating acaricidal constituents from clove bud oil active against adults of *D. farinae* and *D. pteronyssinus* and determining their acaricidal mode of delivery. The structure−acaricidal activity relationships are also discussed.

MATERIALS AND METHODS

Chemicals. Benzyl benzoate and DEET were purchased from Aldrich (Milwaukee, WI). Acetyleugenol, eugenol, isoeugenol (*cis* + *trans*), and methyleugenol were supplied by Wako (Osaka, Japan), Sigma (St. Louis, MO), Fluka (Buchs, Swiss), and Merck (Mohenbrunn, Germany), respectively. All other chemicals were of reagent grade.

Mites. Cultures of *D. farinae* and *D. pteronyssinus* were maintained in the laboratory for 6 years without exposure to any known acaricide. They were reared in plastic containers (12.5 × 10.5 × 5.0 cm) containing 25 g of sterilized diet (fry feed no. 1/dried yeast, 1:1 by weight) at 25 ± 1 °C and 75% relative humidity in darkness. The fry feed was purchased from Korea Special Feed Meal Co. Ltd., Inchon, Korea.

Isolation and Identification. The essential oil of clove bud was purchased from Jin Aromatics, Anyang, Korea. A preparative HPLC (Spectra System P2000, Thermo Separation Products) was used for separation of the biologically active constituents from the oil (10 mg). The column was a μPorasil (7.8 mm i.d. × 300 mm, Waters) using hexane/ethyl acetate (8:2 by volume) at a flow rate of 2 mL/min and detected at 286 nm. Three fractions (F1, F2, and F3) were obtained and bioassayed at 50.9 $\mu g/cm^2$ as described below. The bioactive F2 fraction (4.6 mg, 100% mortality) was rechromatographed under the same conditions. Finally, an active principle (3 mg) at the retention time of 5.5 min was isolated.

The structure of the active isolate was determined by spectroscopic analyses. ^1H and ^{13}C NMR spectra were recorded in deuteriochloroform with a Bruker AM-500 spectrometer at 400 and 100 MHz, respectively. UV spectra were obtained in methanol with a Jasco V-550 spectrophotometer and mass spectra on a JEOL GSX 400 spectrometer.

Bioassays. An impregnated fabric disk bioassay was used for acaricidal activity of test materials. Amounts (1.6, 3.2, 4.8, 6.4, 9.6, 12.7, 25.5, 50.9, and 76.4 $\mu g/cm^2$) of each test material dissolved in 20 μL of ethanol were applied to disks of black cotton fabric (5 cm diameter). Control fabric disks received 20 μL of ethanol. After drying in a fume hood for 30 s, each piece was placed in the bottom of a Petri dish (5 cm diameter × 1.2 cm). Then 25 individuals of *D. farinae* (7−10 days old) and *D. pteronyssinus* (7−10 days old) were separately placed in each Petri dish and covered with a lid.

Treated and control mites were held at 25 ± 1 °C and 75% relative humidity in darkness. Mortalities were determined 24, 48, and 72 h after treatment under a binocular microscope (20×). Mites were considered to be dead if appendages did not move when prodded with a pin. All treatments were replicated three times. The LD_{50} values were calculated by probit analysis (*11*).

Acaricidal Route of Action. The susceptibility of adult *D. farinae* and *D. pteronyssinus* to the test compounds in the vapor phase was investigated according to the method of Kwon and Ahn (*12*). Briefly, groups of 25 adults (7−10 days old) were placed in the bottom of a Petri dish (5 cm diameter × 1.2 cm) and covered with a lid with a fine wire sieve (4.7 cm diameter) attached to the center hole (4.5 cm diameter). Each fabric disk (5 cm diameter), treated with 25.5 $\mu g/cm^2$ of each test compound dissolved in 20 μL of ethanol, was placed over the wire sieve. This prevented direct contact of test adults with the test compounds. Each Petri dish was then either covered with another lid (method A) to investigate the potential toxic effect of the vapor phase of the test compounds or left uncovered (method B). Control fabric disks received 20 μL of ethanol.

Treated and control mites were held at the same conditions used for colony maintenance. Mortalities were determined 24 h after treatment under a binocular microscope (20×). Mites were considered to be dead if appendages did not move when prodded with a pin. All treatments were replicated three times.

Table 1. Acaricidal Activity of Clove Bud Oil against Adults of *D. farinae* and *D. pteronyssinus*, Using the Fabric Disk Bioassay

mite species	dose, $\mu g/cm^2$	mortality,[a] % (mean ± SE) 24 h	48 h	72 h
D. farinae	25.5	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	12.7	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	6.4	37 ± 4.8b	47 ± 3.5b	48 ± 2.3b
	3.2	11 ± 1.3c	12 ± 2.3c	16 ± 2.3cd
D. pteronyssinus	25.5	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	12.7	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	6.4	9 ± 2.7c	12 ± 2.3c	17 ± 1.3c
	3.2	5 ± 1.3c	8 ± 2.3c	9 ± 1.3d

[a] Means within a column followed by the same letter are not significantly different ($P < 0.05$, Scheffe test).

Table 2. Toxicity of Eugenol Congeners and Acaricides against *D. farinae* Adults, Using the Fabric Disk Bioassay[a]

compound	slope (± SE)	LD_{50}, $\mu g/cm^2$	95% confidence limit	RT[b]
eugenol	6.00 ± 0.67	5.47	5.04–5.89	1.7
isoeugenol[c]	6.29 ± 0.66	5.17	4.76–5.56	1.8
acetyleugenol	8.75 ± 1.32	14.16	13.31–14.86	0.7
methyleugenol	6.56 ± 0.85	0.94	0.86–1.01	9.8
DEET	5.02 ± 0.76	37.59	34.60–40.94	0.3
benzyl benzoate	6.54 ± 0.82	9.22	8.62–9.85	1.0

[a] Exposed for 24 h. [b] Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of each chemical. [c] Mixture of *cis*- and *trans*-isoeugenol.

Statistical Analyses. The percentage of mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe test at $P < 0.05$ (*11*). Means (± SE) of untransformed data are reported.

RESULTS

Acaricidal Activity of Clove Bud Oil. When clove bud oil was bioassayed by direct contact, significant differences were observed in toxicity to mite adults (**Table 1**). Responses varied according to dust mite species and dose rather than exposure time. In a test with *D. farinae* adults, the clove oil gave 100 and 37% mortality at 12.7 and 6.4 $\mu g/cm^2$, respectively, 24 h after treatment. Against *D. pteronyssinus* adults, the oil caused 100% mortality at 12.7 $\mu g/cm^2$, whereas the acaricidal activity was significantly decreased at 6.4 $\mu g/cm^2$. There was no mortality in the untreated controls.

Identification of Active Principle. Fabric disk bioassay-guided fractionation of clove bud oil afforded an active constituent identified by spectroscopic analyses, including MS and NMR, and by direct comparison with authentic compound. The active constituent was characterized as the phenylpropene eugenol.

Acaricidal Activity. The toxicity of eugenol and its congeners (acetyleugenol, isoeugenol, and methyleugenol) against *D. farinae* adults was compared with those of benzyl benzoate and DEET (**Table 2**). The commonly used benzyl benzoate served as a standard of comparison in toxicity tests. On the basis of 24-h LD_{50} values, the compound most toxic to *D. farinae* adults was methyleugenol followed by isoeugenol, eugenol, benzyl benzoate, and acetyleugenol. Very weak activity was observed with DEET. Methyleugenol, isoeugenol, and eugenol were about 10, 2, and 2 times more toxic than benzyl benzoate, respectively. There was no mortality in the untreated controls.

Table 3. Toxicity of Eugenol Congeners and Acaricides against *D. pteronyssinus* Adults, Using the Fabric Disk Bioassay[a]

compound	slope (± SE)	LD_{50}, μg /cm^2	95% confidence limit	RT[b]
eugenol	12.15 ± 2.42	3.71	2.13–4.45	1.8
isoeugenol[c]	3.25 ± 0.32	1.55	1.37–1.77	4.3
acetyleugenol	4.35 ± 0.48	5.41	4.89–5.89	1.2
methyleugenol	18.61 ± 2.14	0.67	0.65–0.68	9.8
DEET	2.35 ± 0.38	17.98	15.08–22.77	0.4
benzyl benzoate	3.05 ± 0.40	6.59	5.74–7.61	1.0

[a] Exposed for 24 h. [b] Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of each chemical. [c] Mixture of *cis*- and *trans*-isoeugenol.

Table 4. Acaricidal Activity of Test Compounds against *D. farinae* Adults, Using the Fabric Disk Bioassay

compound	dose, μg/cm^2	mortality, % (mean ± SE) 24 h	48 h	72 h
eugenol	8.0	85 ± 7.4	85 ± 7.4	85 ± 7.4
	6.4	50 ± 2.3	50 ± 2.3	50 ± 2.3
isoeugenol[a]	8.0	92 ± 1.3	92 ± 1.3	92 ± 1.3
	6.4	66 ± 1.3	66 ± 1.3	66 ± 1.3
acetyleugenol	19.1	88 ± 2.3	88 ± 2.3	88 ± 2.3
	15.9	65 ± 3.5	65 ± 3.5	65 ± 3.5
methyleugenol	1.2	71 ± 1.3	71 ± 1.3	71 ± 1.3
	0.8	35 ± 4.8	39 ± 3.5	41 ± 3.5
DEET	50.9	79 ± 3.5	83 ± 2.3	85 ± 1.3
	38.2	41 ± 1.3	41 ± 1.3	41 ± 1.3
benzyl benzoate	12.7	85 ± 1.3	96 ± 2.3	96 ± 2.3
	9.6	47 ± 1.3	47 ± 1.3	47 ± 1.3

[a] Mixture of *cis*- and *trans*-isoeugenol.

Toxic effects in the fabric bioassay of the test compounds on *D. pteronyssinus* adults are reported in **Table 3**. On the basis of 24-h LD_{50} values, methyleugenol was much more effective than isoeugenol, eugenol, acetyleugenol, and benzyl benzoate. DEET was least toxic. Methyleugenol, isoeugenol, and eugenol were about 10-, 4-, and 2-fold more active than benzyl benzoate, respectively. The acaricidal activity of acetyleugenol was comparable to that of benzyl benzoate.

The acaricidal activity of the test compounds against *D. farinae* adults was evaluated 24, 48, and 72 h after treatment (**Table 4**). Potencies varied according to dose rather than exposure time. Similar results were also obtained from *D. pteronyssinus* adults (data not shown). No mortality was observed in the untreated controls.

Poisoning Symptoms. Typical poisoning symptoms in both *Dermatophagoides* mites to eugenol (6.4 μg/cm^2), isoeugenol (6.4 μg/cm^2), acetyleugenol (12.7 μg/cm^2), and methyleugenol (0.8 μg/cm^2) were compared with those of benzyl benzoate (50.9 μg/cm^2) and DEET (50.9 μg/cm^2) using the fabric disk bioassay. The treated amounts of each test compound gave ∼80% mortality 24 h after treatment. All test compounds resulted in a similar death symptom of the forelegs extended forward together without knockdown. Initial intoxication symptoms by the test compounds, however, were distinguishable among compounds. Acetyleugenol caused rapid and active movements of treated mites before death, whereas the mites treated with eugenol showed leg movements as their unique initial intoxication symptom. Both isoeugenol and methyleugenol resulted in lethargy of treated mites, leading to death. Loss of glossiness of the cuticle was observed in the mites treated with methyleugenol but not in those treated with the other compounds.

Acaricidal Route of Action. The response of *D. farinae* adults to vapors of eugenol, isoeugenol, acetyleugenol, and methyleugenol varied with the treatment method (**Table 5**). After 24 h of exposure to 25.5 μg/cm^2, there was a significant difference ($P < 0.05$) in acaricidal activity of eugenol between exposure in a closed container (method A), which resulted in 100% mortality, and exposure in an open container (method B), which resulted in 11% mortality against *D. farinae* adults. Similar differences in the response of *D. farinae* adults to isoeugenol, acetyleugenol, and methyleugenol in treatments A and B were observed. There was no mortality in the untreated controls.

Table 5. Fumigant Activity of Test Compounds against Adults of *D. farinae* and *D. pteronyssinus*

compound[b]	method[c]	mortality,[a] % (mean ± SE) *D. farinae*	*D. pteronyssinus*
eugenol	A	100 ± 0.0a	100 ± 0.0a
	B	11 ± 1.3b	21 ± 1.3b
isoeugenol	A	100 ± 0.0a	100 ± 0.0a
	B	21 ± 3.5b	32 ± 2.3b
acetyleugenol	A	100 ± 0.0a	100 ± 0.0a
	B	12 ± 2.3b	17 ± 2.7b
methyleugenol	A	100 ± 0.0a	100 ± 0.0a
	B	5 ± 3.5b	9 ± 1.3b

[a] Means within a column followed by the same letter are not significantly different ($P < 0.05$, Scheffe test). [b] Exposed for 24 h at a dose of 25.5 μg/cm^2. [c] Method A, vapor in closed containers; method B, vapor in open containers.

The toxic effects of the vapors of the test compounds on *D. pteronyssinus* adults were examined at 25.5 μg/cm^2 (**Table 5**). There was a significant difference ($P < 0.05$) in the toxicity of eugenol between closed (A, 100% mortality) and open containers (B, 21% mortality). Similar differences in the response of *D. pteronyssinus* adults to isoeugenol, acetyleugenol, and methyleugenol in treatments A and B were observed.

DISCUSSION

Plant essential oils are potential products for dust mite control because many of them are selective to pests, have no or little harmful effects on nontarget organisms and the environment (*7*, *8*), and may be applied to dust mite nests such as beds, sofas, furnitures, and carpeted floors in the same way as other conventional acaricides (*1*). Many plant essential oils and phytochemicals are known to possess acaricidal activity against house dust mites (*12*−*18*). The reported naturally occurring acaricidal compounds against house dust mites include butylidenephthalide from the rhizome of *Cnidium officinale* Makino (*12*); *O*-anisaldehyde, citronellal, and perillaldehyde derived from perilla oil (*14*); isosericenine, acryophyllene oxide, and α-cadinol from the essential oil of the leaves from *Neolitsea sericea* Blume (*15*); pisiferic acid from the leaves of *Chamaecyparis pisiferta* Sieb. et Zucc. (*16*); sericealactone from the heartwood of *N. sericea* (*17*); and cinnamaldehyde, cinnamyl alcohol, and salicylaldehyde from the bark of *Cinnamomum cassia* Blume (*18*). It has been reported that susceptibility to some plant essential oils such as almond bitter oil, caraway oil, and perilla oil was greater in *D. farinae* adults than in *D. pteronyssinus* adults (*14*, *15*). However, *D. farinae* adults are found to be more tolerant to the wood oils of *Thuga heteropylla* Sarg. and *Cryptomeria japonica* D. Don than *D. pteronyssinus* adults (*13*). No significant difference in toxicity of either (*E*)-cinnamaldehyde, cinnamyl alcohol, or salicylaldehyde between *D. farinae* and *D. pteronyssinus* has been noted (*18*). Similar results have been also reported in butylidenephthalide (*12*). Additionally, the toxicity of (*E*)-cinnamaldehyde, cinnamyl alcohol, and salicylaldehyde (*18*) as well as butylidenephthalide (*12*) varied with the dose rather than the exposure time. El-Nahal et al. (*19*) stated that exposure time appears to be a more important factor affecting the efficiency of the vapors of *Acorus calamus* L. essential oil to adults of five stored-product insect species than the dosage.

In the present study, clove bud oil exhibited potent acaricidal activity against adults of *D. farinae* and *D. pteronyssinus*. The acaricidal constituent of the oil was identified as the phenylpropene eugenol. Eugenol congeners (acetyleugenol, isoeugenol, and methyleugenol) also exhibited potent acaricidal activity against both mite species. Potencies varied according to compound, dose, and mite species. The acaricidal activity was more pronounced in eugenol and its three congeners than in benzyl benzoate and DEET against both mite species. Additionally, *D. farinae* adults were more tolerant to acetyleugenol, eugenol, isoeugenol, methyleugenol, benzyl benzoate, and DEET than *D. pteronyssinus* adults. Eugenol possesses attractant effects for *Diabrotica barberi* (Smith and Lawrence) adults (*20*, *21*) and insecticidal activity against *T. castaneum* and *S. zeamais* (*10*), whereas methyleugenol has potent attractant effects for *Dacus dorsalis* (Hendel) male (*22*) and insecticidal activity against *T. castaneum* and *S. zeamais* (*10*). Isoeugenol has insecticidal activity against *T. castaneum* and *S. zeamais* (*10*).

Structure–activity relationships of plant compounds against arthropod pests have been well studied. Kim et al. (*23*) studied the structure–activity relationship between cinnamaldehyde and its 11 congeners and acaricidal activity against *Tyrophagus putrescentiae* (Schrank) adults: hydrophobicity appears to play a crucial role in mite toxicity, but a conjugated double bond and a length of CH chain outside the ring appear not to be responsible for toxicity. Regnault-Roger and Hamraoui (*24*) studied the structure–activity relationship between monoterpenoids and fumigant activity against *Acanthoscelides obtectus* (Say): the oxygenated structures proved to be the most active compounds, especially carvacrol, linalool, and terpineol. It has been reported that adulticidal activity against *S. oryzae*, *C. chinensis*, and *L. serricorne* was much more pronounced in (*Z*)-asarone than in (*E*)-asarone (*25*). In our study, the order of lethal effect against adults of *D. farinae* and *D. pteronyssinus* was methyleugenol > eugenol > isoeugenol > acetyleugenol. These results indicate that the order of the toxicity of the phenylpropenes might be due, in part, to their lipophilicity.

Observation of poisoning symptoms of chemicals is of practical importance for dust mite control. Five types of poisoning symptoms of chemicals against mites have been reported: a knockdown-type death caused by *N. sericea* leaf oil in adults of *D. farinae* and *D. pteronyssinus* (*15*); death related with uncoordinated behavior without knockdown by (*E*)-cinnamaldehyde, cinnamyl alcohol, salicylaldehyde, benzyl benzoate, and DEET in adults of *D. farinae* and *D. pteronyssinus* (*18*); death associated with desiccation by several monoterpenes such as fenchone, linalool, menthone, and pulegone in *T. putrescentiae* adults (*26*); death related with a characteristic depression of the dorsal surface of the idiosoma by tricalcium phosphate and ferric phosphate in *T. putrescentiae* adults (*27*); and death associated with lethargy by butylidenephthalide in adults of *D. farinae* and *D. pteronyssinus* (*12*). In this study, all of the compounds resulted in a similar death symptom of the forelegs extended forward together without knockdown, despite different initial intoxication symptoms. Acetyleugenol caused rapid and active movements of treated mites before death, whereas the mites treated with eugenol showed leg movements as their unique initial intoxication symptom. Both isoeugenol and methyleugenol resulted in lethargy of treated mites, being directly connected to death. It is worth noting that loss of glossiness of the cuticle was observed only in the mites treated with methyleugenol and not in ones treated with other compounds.

Volatile compounds of many plant extracts and essential oils are composed of alcohols, aldehydes, alkanes, and terpenoids, especially monoterpenoids, and exhibit fumigant activity (*12*, *18*, *23*, *25*, *28*, *29*). Fumigant activity against adults of *D. farinae* and *D. pteronyssinus* has been reported in (*E*)-cinnamaldehyde, cinnamyl alcohol, and salicylaldehyde (*18*) as well as butylidenephthalide (*12*). In this study, acetyleugenol, eugenol, isoeugenol, and methyleugenol were much more effective in closed containers than in open ones against adults of *D. farinae* and *D. pteronyssinus*. These results indicate that the mode of delivery of these compounds was largely due to action in the vapor phase: they may be toxic when they penetrate the dust mite body via the respiratory system. However, the exact acaricidal mode of action remains unknown.

Results of this and earlier studies indicate that clove bud oil-derived materials could be useful as fumigants for *D. farinae* and *D. pteronyssinus*. Methyleugenol causes hepatic tumors in mice and rats (*30*), induces intrachromosomal recombination in a yeast assay (*31*), and elicits a positive response in a bacterial DNA repair test (*32*) and a negative response in the Ames mutagenicity test (*31*), although this compound is a food flavoring agent and appears on the Flavor and Extract Manufactures' Generally Regarded as Safe (GRAS) list. Eugenol is found to give a positive response in the yeast assay and is carcinogenic in mice and rats (*30*). For practical use of these compounds as novel fumigants, further research should be done on safety issues of these compounds for human health, their acaricidal mode of action, and formulations improving the acaricidal potency and stability.

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