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Acaricidal Activities of Apiol and Its Derivatives from *Petroselinum sativum* Seeds against *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Tyrophagus putrescentiae*

Ha Yun Song,[†] Ji Yeon Yang,[†] Joo Won Suh,[‡] and Hoi Seon Lee*[,†]

[†]Department of Bioenvironmental Chemistry and Institute of Agricultural Science and Technology, Chonbuk National University, Jeonju 561-756, Republic of Korea

[‡]Department of Biological Science, Myongji University, Yongin 449-728, Republic of Korea

ABSTRACT: The acaricidal effects of an active constituent derived from *Petroselinum sativum* seeds and its derivatives were determined using impregnated fabric disk bioassay against *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Tyrophagus putrescentiae* and compared with that of synthetic acaricide. The acaricidal constituent of *P. sativum* was isolated by various chromatographic techniques and identified as apiol. On the basis of LD_{50} values against *D. farinae* and *D. pteronyssinus*, apiol (0.81 and 0.94 $\mu g/cm^2$) was 12.4 and 10.2 times more toxic than benzyl benzoate (10.0 and 9.58 $\mu g/cm^2$), respectively. In acaricidal studies of apiol derivatives, 3,4-methylenedioxybenzonitrile (0.04, 0.03, and 0.59 $\mu g/cm^2$) was 250, 319, and 20.7 times more toxic than benzyl benzoate (10.0, 9.58, and 12.2 $\mu g/cm^2$) against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*. In structure−activity relationships, the acaricidal activities of apiol derivatives could be related to allyl ($-C_3H_5$) and methoxy ($-OCH_3$) functional groups. Furthermore, apiol and its derivatives could be useful for natural acaricides against these three mite species.

KEYWORDS: acaricidal activity, apiol, *Dermatophagoides* spp., essential oil, *Petroselinum sativum*, structure−activity relationship, *Tyrophagus putrescentiae*

INTRODUCTION

House dust and stored-food mites are currently designated as allergens causing allergic symptoms such as asthma, atopic dermatitis, and perennial rhinitis.[1,2] The house dust mites, *Dermatophagoides farinae* (Hughes) and *Dermatophagoides pteronyssinus* (Troussart), belong to the order Acari and the family Pyroglyphidae.[3] The stored-food mites, *Tyrophagus putrescentiae* (Schrank), belong to the family Acaridae.[4,5] They are found swarming in stored foods containing high fat and protein such as bacon, cereals, dried eggs, and peanuts.[6] Furthermore, *T. putrescentiae* has been recognized as an etiological factor in allergic diseases suffered by farmers and food workers.[7] *T. putrescentiae* can induce diarrhea, enteritis, and systemic anaphylaxis when contaminated foods are ingested or the mites themselves are inhaled.[8] Therefore, some attempts have been made to control mites and reduce their allergens; these attempts have involved chemical treatment using synthetic acaricides such as benzyl benzoate and *N*,*N*-diethyl-*m*-toluamide.[9] However, the repeated use of synthetic acaricides has some problems, including potential toxicity, deleterious environmental effects, and emergence of resistance in mites.[10] To find a solution of synthetic acaricides, new types of acaricides that are safe for nontarget organisms and generate lower residual toxicity than existing synthetic acaricides are clearly needed.[5] Several researchers are actively searching for such safer acaricides in plants.[5,10,11] The reason for this is that plants are known to generate an array of secondary metabolites such as alkaloids, monoterpenes, and quinones.[11] According to Lee et al.,[9] *Diospyros kaki*, which contains a variety of biological materials and naphthoquinones, proved to be an effective acaricidal agent against *D. farinae* and *D. pteronyssinus*. In particular, the essential oils tend to make excellent insecticides, due to their high volatility, selective toxicity to insects, and safety.[12−14] In a previous study, natural components derived from plants were shown to exert excellent effects against *Tetranychus urticae*.[15]

Petroselinum sativum (Hoffm.) is a member of the Umbelliferae family and is also a widely used aromatic, culinary, and medicinal herb. Some parts of *P. sativum* are employed in a variety of industries, including the cosmetics, food, and pharmaceutical industries, because the plant generates a plethora of useful secondary metabolites.[16] *P. sativum* is used as an anticoagulant, antidiabetic, antihypertensive, antimicrobial, and antioxidant agent.[17] Apiol, well-known as the main component of *P. sativum* oil, has been shown to exhibit calcium channel blocking and antiproliferative activities.[18,19] In addition, phenylpropanoids derived from *P. sativum* showed potent insecticidal activities against larvae and pupae of *Aedes aegypti*, *Rhipicephalus microplus*, and *Sitophilus zeamais*.[20] Moreover, monoterpenes are more valuable as insect fumigants.[21] However, only a relatively few studies have been conducted thus far to assess the acaricidal activities of *P. sativum* materials against *Dermatophagoides* spp. and *T. putrescentiae*. Therefore, the goal of this study was to identify the bioactive components of *P. sativum* and to determine the structure−activity relationships of the main compound and its derivatives.

MATERIALS AND METHODS

Chemicals. Benzyl benzoate, camphene, 5-chloro-3,4-methylenedioxybenzene, 3,4-methylenedioxybenzene, 3,4-methylenedioxybenzonitrile,

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Table 1. Acaricidal Activities of *P. sativum* Oil and Synthetic Acaricide against Three Mite Species

sample	mite species	slope (±SE)	LD_{50} ($\mu g/cm^2$)	95% CL	RT[a]
P. sativum seed oil	*D. farinae*	7.64 (±0.15)	1.88	1.79−1.97	5.32
	D. pteronyssinus	12.01 (±0.19)	2.25	2.12−2.38	4.26
	T. putrescentiae	3.55 (±0.10)	–[b]	–	–
benzyl benzoate	*D. farinae*	4.98 (±0.65)	10.0	9.91−10.1	1.00
	D. pteronyssinus	6.13 (±0.71)	9.58	9.44−9.72	1.00
	T. putrescentiae	2.78 (±0.37)	12.2	11.5−12.9	1.00

[a] Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of each chemical. [b] No activity.

myrtenal, α-phellandrene, β-phellandrene, α-pinene, β-pinene, and terpinene were purchased from Aldrich (Milwaukee, WI). 1-Bromo-3,4-methylenedioxybenzene, limonene, and sabinene were provided by Fluka (Buchs, Switzerland). 1-Allyl-3,4-methylenedioxybenzene, 1-allyl-5-methoxy-3,4-methylenedioxybnezene, and α-thujene were supplied by Sigma (St. Louis, MO). All other chemicals were of reagent grade.

Sample Preparation. The air-dried seeds (1 kg) of *P. sativum* were purchased from a local market in Jeonju and extracted using steam distillation extraction. Anhydrous sodium sulfate was added to the extracted oil to precipitate H_2O, and the oil was concentrated by rotary evaporation (EYELA autojack NAJ-100, Japan) at room temperature (30 °C). The concentrated essential oil was placed in cold storage at 4 °C prior to isolation.

Isolation and Identification. The essential oil of *P. sativum* seeds (10 g) was isolated by silica gel column chromatography (Merck 70−230 mesh, 600 g, 550 mm i.d. × 700 mm; Rahway, NJ) and continuously eluted with hexane/ethyl acetate (9:1; 8:2, and 6:4, v/v). Each fraction was analyzed to distinguish similar patterns of fractions using thin layer chromatography (TLC) and yielded four fractions designated SP1−SP4. Acaricidal activities of all fractions were evaluated against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* at 80 $\mu g/cm^2$. As a result, against *Dermatophagoides* spp., the SP2 fraction exhibited the strongest acaricidal activity among all fractions. However, in the case of *T. putrescentiae*, all fractions had no activity. Therefore, we selected *Dermatophagoides* spp. as target mites. Consequently, the bioactive SP2 fraction (5.6 g) was rechromatographed on a silica gel column using the same column conditions with hexane/ethyl acetate (7:3, v/v). In this step, the four fractions SP21−SP24 were obtained. We implement bioassay against target mites under the same conditions and checked out the acaricidal activity fraction. The bioactive SP23 (3.2 g) fraction was sequentially purified by preparative HPLC (Japan Analytical Industry Co., Ltd., Tokyo, Japan) and obtained the three fractions SP231−SP233. This method was carried out under the following conditions: column, GS 310 (21.5 mm i.d. × 1000 mm, Japan Analytical Industry Co., Ltd.); mobile phase, methanol 100%; flow rate, 3.5 mL/min; UV absorbance, 300 nm. As a result of the bioassay of purified fractions, SP233 had the highest acaricidal activity. Fraction SP233 was subsequently separated using a Jaigel W series column (W 253 + W 252, 20.0 mm i.d. × 1000 mm; Japan Analytical Industry Co., Ltd.) under the following conditions: mobile phase, chloroform 100%; flow rate, 3.5 mL/min; UV absorbance, 257 nm. Finally, the active component (SP2332, 840 mg) was isolated. The structure of the active compound was determined using spectroscopic analysis including ^1H and ^{13}C NMR. All spectra (^1H and ^{13}C NMR) were recorded on a JNM-ECA600 (JEOL Ltd., Tokyo, Japan) spectrometer at 600 and 150 MHz, respectively. The spectra were obtained using deuterochloroform as solvent, and the chemical shifts were measured using tetramethylsilane (TMS) as internal standard. Moreover, to simplify the complex structure, we analyzed the compound using 2D NMR ($^1H-^1H$ COSY and HMQC). In addition, the EI-MS spectra were determined on a JEOL GSX 400 mass spectrometer.

Mites. Cultures of *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* were maintained without exposure to any known acaricides. These mites were reared in cages (15 × 12 × 6 cm) containing 30 g of sterilized diet (fry feed 1 and dried yeast, 1:1 by weight) at 25 ± 1 °C and 75% relative humidity in the dark. The fry feed (Miropa) was purchased from Korea Special Feed Meal Co. Ltd., Jeonju, South Korea. The feed is composed of crude protein (44.0%), cellulose (4.0%), crude lipid (3.0%), P (2.0%), and Ca (1.8%).

Bioassay. The acaricidal activities of the samples were evaluated using an impregnated fabric disk bioassay. This bioassay was modified from the method described by Jeong et al.[5] and Collins.[22] Each concentration of test samples (80, 40, 20, 10, 5, 2.5, 1.25, 1.0, 0.5, 0.2, 0.1, 0.05, 0.025, and 0.0125 $\mu g/cm^2$) was dissolved in 20 μL of acetone and then applied to a paper disk (Advantec, 8 mm diameter, 1 mm thickness, Toyo Roshi, Tokyo, Japan). We applied acetone as a negative control and benzyl benzoate as a positive control at the same volume to the paper disks. The treated disks were dried under a fume hood for 10 min and then placed in the lid of a microtube (2 mL, Greiner bio-one GmbH, Germany). Thirty individual adults of *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* were inoculated in each microtube, and the lid was closed. Acetone was tested with acaricidal activity for the negative control, and benzyl benzoate was experimented as positive control. Each of the treated and control microtubes was maintained for 24 h at 25 ± 1 °C and 75% relative humidity in darkness. The mortality rate of every group was determined by observing the population number of mites under a binocular microscope (20×; Olympus, Tokyo, Japan). Mites were considered to be dead if their appendages did not move when prodded with a pin. All treatments were replicated three times. The LD_{50} values were calculated via probit analysis.

Statistical Analysis. The 50% lethal dose (LD_{50}) values were calculated via probit analysis.[23] Relative toxicity (RT) was expressed as the ratio of commercial acaricide LD_{50} to each chemical LD_{50}, as described previously.[10]

RESULTS AND DISCUSSION

The acaricidal activity of the essential oil extracted from *P. sativum* seeds against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* was determined by the impregnated fabric disk method and compared with that of benzyl benzoate, which is a well-known synthetic acaricide (Table 1). On the basis of LD_{50} values, the essential oil (1.88 $\mu g/cm^2$) was 5.32 times more active than benzyl benzoate (10.0 $\mu g/cm^2$) against *D. farinae*. Against *D. pteronyssinus*, the acaricidal activity of the essential oil (2.25 $\mu g/cm^2$) was 4.26 times higher than that of benzyl benzoate (9.58 $\mu g/cm^2$). However, the essential oil exhibited no activity against *T. putrescentiae*. In other words, the essential oil of *P. sativum* seeds had selective toxicity against *Dermatophagoides* spp. and *T. putrescentiae*. These results exhibited the differences of the acaricidal activity on the species of insects. Actually, species-specific differences have been reported for a variety of insect species.[24]

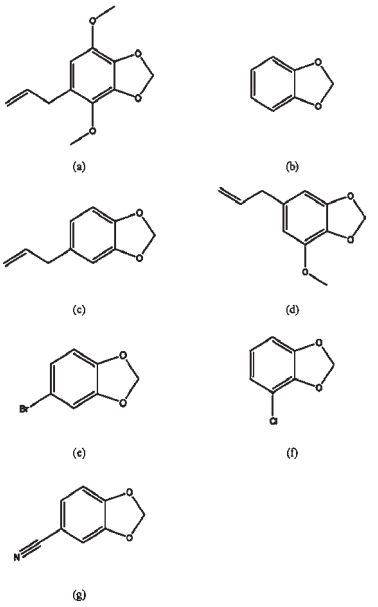

Figure 1. Structures of apiol and its derivatives: apiol (a), 3,4-methylenedioxybenzene (b), 1-allyl-3,4-methylenedioxybenzene (c), 1-allyl-5-methoxy-3,4-methylenedioxybenzene (d), 1-bromo-3,4-methylenedioxybenzene (e), 5-chloro-3,4-methylenedioxybenzene (f), and 3,4-methylenedioxybenzonitrile (g).

Table 2. Assignment of ^{1}H NMR and ^{13}C NMR[a] of SP2332

carbon	partial structure	δ_c	δ_H
1	C	125.753	
2	C	138.719	
3	C	137.327	
4	C	135.096	
5	C	139.056	
6	C−H	108.176	6.277 (s[b])
7	C−2H	101.500	5.861−5.936 (d, J = 45 MHz)
8	C−3H	60.123	3.826−3.852 (d, J = 15.6 MHz)
9	C−3H	56.839	3.826−3.852 (d, J = 15.6 MHz)
10	C−2H	34.060	3.280−3.290 (d, J = 6.0 MHz)
11	C−H	136.241	5.828−5.856 (d, J = 16.8 MHz)
12	C−2H	115.347	5.002−5.025 (t, J = 13.8 MHz)

[a] ^{1}H and ^{13}C spectra were measured in $CDCl_3$ at 600 and 150 MHz, respectively. [b] s, singlet; d, doublet; t, triplet.

Due to the excellent activity of the essential oil, the bioactive substance from the essential oil of *P. sativum* seeds was isolated by silica gel column chromatography and preparative HPLC using mixed organic solvents. This was confirmed by a variety of spectroscopic analysis including EI-MS and ^{1}H and ^{13}C NMR. The isolated compound was characterized as apiol (1-allyl-2,5-dimethoxy-3,4-methylenedioxybenzene) (Figure 1). Apiol ($C_{12}H_{14}O_4$, MW 222.23): EI-MS (70 eV), m/z M^+ 222, 197, 177, 149, 121, 101, 77, 53; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.277 (1H, s), 5.861−5.936 (2H, d), 5.828−5.856 (1H, d), 5.002−5.025 (2H, t), 3.826−3.852 (3H, d), 3.776−3.801 (3H, d), 3.280−3.290 (2H, d); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 139.056, 138.719, 137.327, 136.241, 135.096, 125.753, 115.347, 108.176, 101.500, 60.123, 56.839, 34.060 (Table 2). The analytical results for apiol were consistent with those from earlier studies.[25]

Table 3. Acaricidal Activities of Apiol Derived from *P. sativum* Oil and 11 Volatile Compounds against Three Mite Species

		mortality (mean ± SE, %)		
sample	dose (μg/cm^2)	*D. farinae*	*D. pteronyssinus*	*T. putrescentiae*
apiol	40	100	100	—[a]
(isolated compound)	20	100	100	
	10	100	100	
	5	100	100	
	2.5	100	100	
myristicin	40	100	100	—
	20	100	100	
	10	100	100	
	5	94.3 ± 0.6	81.0 ± 0.8	
	2.5	91.2 ± 1.3	74.0 ± 2.1	
camphene	40	—	—	—
limonene	40	—	—	—
myrtenal	40	—	—	—
α-phellandrene	40	—	—	—
β-phellandrene	40	—	—	—
α-pinene	40	—	—	—
β-pinene	40	—	—	—
sabinene	40	—	—	—
terpinene	40	—	—	—
α-thujene	40	—	—	—

[a] No activity.

According to Kurowska and Galazka,[26] 12 volatile compounds from the essential oil of *P. sativum* seeds were analyzed by GC-MS. The volatile compounds identified from *P. sativum* oil consist of 65.2% phenylpropanoids (apiol and myristicin), 34.4% terpenoids (camphene, limonene, α-phellandrene, β-phellandrene, α-pinene, β-pinene, sabinene, terpinene, and α-thujene), and 0.4% aldehyde (myrtenal). Furthermore, Diaz-Maroto et al.[27] have reported that the major components of *P. sativum* leaves are apiol, isopropenyl-4-methylbenzene, *p*-mentha-1,3,8-triene, myrcene, myristicin, and β-phellandrene. In our study, the acaricidal activities of apiol isolated from *P. sativum* oil (Figure 1) and 11 commercial available components (camphene, limonene, myristicin, myrtenal, α-phellandrene, β-phellandrene, α-pinene, β-pinene, sabinene, terpinene, and α-thujene)[26,27] were evaluated using impregnated fabric disk bioassay against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* (Table 3). Against *D. farinae* and *D. pteronyssinus*, apiol (100% at 2.5 μg/cm^2) and myristicin (94.3 and 91.2% at 5 μg/cm^2, respectively) had potent acaricidal activity. In the case of *T. putrescentiae*, apiol and myristicin had no activity at 40 μg/cm^2. The other components (camphene, limonene, myrtenal, α-phellandrene, β-phellandrene, α-pinene, β-pinene, sabinene, terpinene, and α-thujene) had no activity against *Dermatophagoides* spp. and *T. putrescentiae* at 40 μg/cm^2. Therefore, our results indicated that apiol and myristicin may be acaricidal components of *P. sativum* oil against *Dermatophagoides* spp.

Table 4. Acaricidal Activities of Apiol, Its Derivatives, and Synthetic Acaricide against Three Mite Species

chemical	mite species	slope (±SE)	LD_{50} ($\mu g/cm^2$)	95% CL	RT[a]
apiol (1-allyl-2,5-dimethoxy-3,4-methylenedioxybenzene)	*D. farinae*	4.88 (±0.18)	0.81	0.72−0.90	12.4
	D. pteronyssinus	5.04 (±0.16)	0.94	0.84−1.04	10.2
	T. putrescentiae	—[b]	—	—	—
3,4-methylenedioxybenzene	*D. farinae*	4.24 (±0.72)	18.6	17.7−19.6	0.54
	D. pteronyssinus	3.97 (±0.66)	14.6	13.9−15.3	0.66
	T. putrescentiae	5.25 (±0.77)	9.40	8.51−10.3	1.30
1-allyl-5-methoxy-3,4-methylenedioxybenzene	*D. farinae*	4.68 (±0.22)	2.75	2.11−3.39	3.65
	D. pteronyssinus	6.60 (±0.16)	4.75	3.83−5.67	2.02
	T. putrescentiae	—	—	—	—
1-allyl-3,4-methylenedioxybenzene	*D. farinae*	2.52 (±0.62)	10.5	9.80−11.2	0.96
	D. pteronyssinus	3.33 (±0.12)	14.5	13.6−15.4	0.66
	T. putrescentiae	5.87 (±0.22)	4.25	3.72−4.78	2.87
1-bromo-3,4-methylenedioxybenzene	*D. farinae*	4.56 (±0.12)	4.06	3.22−4.90	2.47
	D. pteronyssinus	5.61 (±0.34)	3.56	2.94−4.18	2.69
	T. putrescentiae	13.28 (±0.16)	3.75	2.91−4.59	3.25
5-chloro-3,4-methylenedioxybenzene	*D. farinae*	1.23 (±0.89)	5.75	4.85−6.65	1.74
	D. pteronyssinus	2.22 (±0.54)	6.50	5.72−7.28	1.47
	T. putrescentiae	2.17 (±0.13)	3.25	2.53−3.97	3.75
3,4-methylenedioxybenzonitrile	*D. farinae*	3.33 (±0.45)	0.04	0.03−0.05	250
	D. pteronyssinus	2.11 (±0.21)	0.03	0.02−0.04	319
	T. putrescentiae	5.21 (±0.33)	0.59	0.49−0.69	20.7
benzyl benzoate	*D. farinae*	4.98 (±0.65)	10.0	9.91−10.1	1.00
	D. pteronyssinus	6.13 (±0.71)	9.58	9.44−9.72	1.00
	T. putrescentiae	2.78 (±0.37)	12.2	11.5−12.9	1.00

[a] Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of each chemical. [b] No activity.

Due to the excellent activity of apiol, the acaricidal activities of apiol derivatives were determined and compared with that of a synthetic acaricide (benzyl benzoate) against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* (Table 4). On the basis of the LD_{50} values against *D. farinae*, 3,4-methylenedioxybenzonitrile (0.04 $\mu g/cm^2$) was 250 times more toxic than benzyl benzoate (10.0 $\mu g/cm^2$), followed by apiol (0.81 $\mu g/cm^2$), 1-allyl-5-methoxy-3,4-methylenedioxybenzene (2.75 $\mu g/cm^2$), 1-bromo-3,4-methylenedioxybenzene (4.06 $\mu g/cm^2$), 5-chloro-3,4-methylenedioxybenzene (5.75 $\mu g/cm^2$), 1-allyl-3,4-methylenedioxybenzene (10.5 $\mu g/cm^2$), and 3,4-methylenedioxybenzene (18.6 $\mu g/cm^2$). Against *D. pteronyssinus*, 3,4-methylenedioxybenzonitrile (0.03 $\mu g/cm^2$) was 319 times more toxic than benzyl benzoate (9.58 $\mu g/cm^2$), followed by apiol (0.94 $\mu g/cm^2$), 1-bromo-3,4-methylenedioxybenzene (3.56 $\mu g/cm^2$), 1-allyl-5-methoxy-3,4-methylenedioxybenzene (4.75 $\mu g/cm^2$), 5-chloro-3,4-methylenedioxybenzene (6.50 $\mu g/cm^2$), 1-allyl-3,4-methylenedioxybenzene (14.5 $\mu g/cm^2$), and 3,4-methylenedioxybenzene (14.6 $\mu g/cm^2$). In the case of *T. putrescentiae*, 3,4-methylenedioxybenzonitrile (0.59 $\mu g/cm^2$) was 20.7 times more effective than benzyl benzoate (12.2 $\mu g/cm^2$), followed by 5-chloro-3,4-methylenedioxybenzene (3.25 $\mu g/cm^2$), 1-bromo-3,4-methylenedioxybenzene (3.75 $\mu g/cm^2$), and 1-allyl-3,4-methylenedioxybenzene (4.25 $\mu g/cm^2$). However, apiol and 1-allyl-5-methoxy-3,4-methylenedioxybenzene had no activity against *T. putrescentiae* at 80 $\mu g/cm^2$. Taken together, these results show that the acaricidal activities of apiol and its derivatives are higher than that of the synthetic acaricide.

To establish the structure−activity relationships between apiol derivatives and toxicity against three mite species, the LD_{50} values of apiol derivatives were compared to those of functional radicals (allyl and methoxy groups). The acaricidal activity of 1-allyl-3,4-methylenedioxybenzene containing an allyl group in 3,4-methylenedioxybenzene was 1.77 and 2.21 times more toxic than that of 3,4-methylenedioxybenzene against *D. farinae* and *T. putrescentiae*, respectively. Against *D. pteronyssinus*, the acaricidal activity of 1-allyl-3,4-methylenedioxybenzene was similar to that of 3,4-methylenedioxybenzene. Moreover, the acaricidal activity of apiol (1-allyl-2,5-dimethoxy-3,4-methylenedioxybenzene) including a methoxy group in 1-allyl-3,4-methylenedioxybenzene was 12.96 and 15.43 times more toxic than that of 1-allyl-3,4-methylenedioxybenzene against *D. farinae* and *D. pteronyssinus*, and 1-allyl-5-methoxy-3,4-methylenedioxybenzene was 3.82 and 3.05 times more effective than 1-allyl-3,4-methylenedioxybenzene against *D. farinae* and *D. pteronyssinus*, respectively. In this regard, when the number of allyl or methoxy

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groups was gradually added to 3,4-methylenedioxybenzene, the acaricidal activities of 1-allyl-3,4-methylenedioxybenzene, 1-allyl-5-methoxy-3,4-methylenedioxybenzene, and apiol were increased against *Dermatophagoides* spp. Additionally, the introduction of the bromo (−Br), chloro (−$CHCl_3$), or nitrile (−CN) functional groups (1-bromo-3,4-methylenedioxybenzene, 5-chloro-3,4-methylenedioxybenzene, 3,4-methylenedioxybenzonitrile) to 3,4-methylenedioxybenzene resulted in an increase of acaricidal activity against three mite species. In a similar study, Kerr[28] reported that the insecticidal activity against *Musca domestica* depends on the number of methoxy functional groups in the benzene ring (eugenol, methyleugenol, and elemicin). Furthermore, Lim et al.[29] reported that acaricidal activities of isothiocyanate derivatives containing allyl (−C_3H_5), acetyl (−OCH_3), benzoyl (−OC_6H_5), and methyl (−CH_3) in isothiocyanate were determined using an impregnated fabric disk bioassay against *Dermatophagoides* spp. In comparison with benzyl benzoate, LD_{50} values of allyl isothiocyanate (1.36 and 2.88 $\mu g/cm^2$) were 7.4- and 3.3-fold more toxic than benzyl benzoate (10.03 and 9.58 $\mu g/cm^2$) against *D. farinae* and *D. pteronyssinus*, respectively, followed by methyl isothiocyanate (2.92 and 3.17 $\mu g/cm^2$), benzoyl isothiocyanate (13.58 and 7.63 $\mu g/cm^2$), and acetyl isothiocyanate (195.01 and 168.82 $\mu g/cm^2$). These results indicated that the allyl functional group may increase the acaricidal activity against *Dermatophagoides* spp. In comparison with apiol derivatives, the acaricidal activity of 3,4-methylenedioxybenzonitrile was 34.0 and 96.0 times more toxic than that of allyl isothiocyanate, and apiol was 1.7 and 3.1 times more effective than allyl isothiocyanate. In this regard, our results indicated that the acaricidal activity against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* could be related to allyl (−C_3H_5) and methoxy (−OCH_3) functional groups.

According to the Science Lab MSDS[30] and Koul et al.,[21] the oral LD_{50} values of *P. sativum* oil were 3300 and 1520 mg/kg against rats and mice, respectively, and the intravenous LD_{50} value of apiol was 500 mg/kg against dogs. These results indicate that *P. sativum* oil and apiol have a relatively low acute toxicity toward mammals. In this regard, our results and those of previous studies suggest that apiol and its derivatives may be especially effective at reducing typical *Dermatophagoides* spp. in habitation. Further studies should be conducted to establish the acaricidal mode of action and formulations to improve the acaricidal potency.

AUTHOR INFORMATION

Corresponding Author

*Phone: +82 63 270 2544. Fax: +82 63 270 2550. E-mail: hoiseon@jbnu.ac.kr.

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