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Changes in Acaricidal Potency by Introducing Functional Radicals and an Acaricidal Constituent Isolated from *Schizonepeta tenuifolia*

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ABSTRACT: The acaricidal potential of an active constituent isolated from *Schizonepeta tenuifolia* oil and its structurally related derivatives was evaluated using filter paper and impregnated cotton fabric disk bioassays against house dust and stored food mites. The acaricidal constituent of *S. tenuifolia* oil was isolated by chromatographic techniques and identified as 2-isopropyl-5-methylcyclohexanone by GC-MS, ^{1}H-, and ^{13}C NMR spectra. 2-Isopropyl-5-methylcyclohexanone was a potent acaricide against house dust and stored food mites, based on the LD_{50} values from the filter paper and impregnated cotton fabric disk bioassays, followed by 4-isopropylcyclohexanone, 2-isopropylidene-5-methylcyclohexanone, 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, and benzyl benzoate. Furthermore, 4-isopropylcyclohexanone and 2-isopropyl-5-methylcyclohexanone, which were introduced on the isopropyl (C_3H_7) functional radical of the cyclohexanone skeleton, had the highest acaricidal potency. These results indicate that *S. tenuifolia* oil and 2-isopropyl-5-methylcyclohexanone structural analogues could be effective natural acaricides for managing house dust and stored food mites.

KEYWORDS: *house dust mite, 2-isopropyl-5-methylcyclohexanone, Schizonepeta tenuifolia, stored food mite, structure−activity relationship*

INTRODUCTION

The most serious pests are the American house dust mite, *Dermatophagoides farinae*, and the European house dust mite, *D. pteronyssinus*, because of their ubiquitous occurrence in the home.[1,2] In addition, they impact human health.[1,2] House dust mites are the most important source of indoor allergens that cause allergic diseases such as asthma and atopic dermatitis.[3] The stored food mite, *Tyrophagus putrescentiae* (Astigmata: Acaridae), is the dominant mite species found in stored products, such as cheese, ham containing high fat and protein, and different kinds of nuts.[4,5] Although the stored food mites live on the external surface of stored food products, they cause serious economic losses because they penetrate the surface.[5,6] *T. putrescentiae* have been also reported as etiological agents of several allergic symptoms among agro-industrialists and farmers.[7] House dust and stored food mites are increasingly associated with changes in the residential environment of humans, such as fitted carpets, more tightly sealed windows, and poor ventilation that allow their proliferation.[8] Populations of house dust and stored food mites are controlled by applying synthetic acaricides, such as benzyl benzoate and certain pyrethroids.[9] However, continued use of synthetic acaricides promotes concerns about the environment and human health.[9] These problems have aroused interest in the development of alternatives to control these mites, particularly those with contact and fumigant action, which allow applications in indoor living environments.[10]

Medicinal plants are known to possess various biological activities and are used to prevent and treat human disease and maintain health worldwide.[11,12] The essential oils derived from medicinal plants have been recommended as alternative materials to control house dust and stored food mites because they include bioactive chemicals that pose little risk to human health, thereby reducing the potential for resistance.[13] *Schizonepeta tenuifolia*, which is a South Korean medicinal plant, has analgesic, antifebrile, anti-inflammatory, and antispasmodic activities.[14] However, the essential oil of *S. tenuifolia* has not been evaluated against house dust and stored food mites. Therefore, this study was conducted to investigate the acaricidal properties of *S. tenuifolia* against house dust and stored food mites and establish the structure−activity relationships.

MATERIALS AND METHODS

Chemicals. 4-Isopropylcyclohexanone (98%), 2-isopropyl-5-methylcyclohexanone (97%), 2-methylcyclohexanone (98%), 3-methylcyclohexanone (98%), and 4-methylcyclohexanone (97%) were purchased from Aldrich (St, Louis, MO, USA). Benzyl benzoate (97%) and 2-isopropylidene-5-methylcyclohexanone (97%) were purchased from Fluka (Buchs, Switzerland). All other chemicals were of reagent grade.

Preparation of *S. tenuifolia* Oil. The aerial parts of dried *S. tenuifolia* (10 kg) were collected from a local market (Jeonju, South Korea). A specimen was authenticated by Dr. Jeongmoon Kim at College of Agriculture and Life Science, Chonbuk National University. The essential oil of *S. tenuifolia* was extracted using a steam-distillation extraction method,[13] added to anhydrous magnesium sulfate to remove molecular H_2O, and concentrated by a rotary evaporator (EYELA, NAJ-100, Tokyo, Japan) at 30 °C. The concentrated oil (14 g; yield, 0.14%) was stored in a sealed bottle at 4 °C to prevent volatilization of the constituents.

Gas Chromatography−Mass Spectrometry (GC-MS) Analysis. The volatile constituents of *S. tenuifolia* oils were analyzed by the

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Table 1. Acaricidal Effects of *S. tenuifolia* Oil and a Synthetic Acaricide against House Dust and Stored Food Mites[a]

samples	bioassay	mite species	LD_{50} ± SD	95% CL	relative toxicity[b]
S. tenuifolia oil	filter paper ($\mu g/cm^2$)	*D. farinae*	2.56 ± 1.2	2.53−2.59	3.07
		D. pteronyssinus	2.50 ± 1.6	2.47−2.53	2.76
		T. putrescentiae	6.01 ± 2.0	5.98−6.04	1.57
	impregnated cotton fabric disk ($\mu g/cm^3$)	*D. farinae*	5.88 ± 1.5	5.85−5.91	2.08
		D. pteronyssinus	5.85 ± 1.8	5.82−5.88	1.72
		T. putrescentiae	10.25 ± 1.6	10.22−10.28	1.47
benzyl benzoate	filter paper ($\mu g/cm^2$)	*D. farinae*	7.86 ± 2.1	7.83−7.89	1.00
		D. pteronyssinus	6.90 ± 1.8	6.87−6.93	1.00
		T. putrescentiae	9.44 ± 1.5	9.41−9.47	1.00
	impregnated cotton fabric disk ($\mu g/cm^3$)	*D. farinae*	12.25 ± 1.4	12.22−12.28	1.00
		D. pteronyssinus	10.08 ± 1.2	10.05−10.11	1.00
		T. putrescentiae	15.07 ± 2.6	15.04−15.10	1.00

[a]Exposed for 24 h. [b]Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of *S. tenuifolia* oil.

GC-MS (6890 and 5973 IV; Agilent Technologies, Palo Alto, CA, USA) and separated using a DB-5 capillary column (0.25 mm i.d. × 3,000 mm length × 0.25 μm thickness; J&W Scientific, Folsom, CA, USA). The parameters for the DB-5 column were initial column temperature of 50 °C, which was increased to 210 °C at a rate of 2 °C/min, and ion source temperature of 230 °C. Helium was loaded as the carrier gas and flowed at 0.8 mL/min. The GC column effluents were introduced into the source of the mass spectrometer. Mass spectra (m/z) were obtained in electron ionization mode at 70 eV (range, 10−425 eV). The sector mass analyzer was set to scan from 50 to 600 amu for 2 s. The volatile constituents of *S. tenuifolia* oil were identified by comparing retention times, retention indices, and mass spectra of the chromatographic peaks and were confirmed by comparing the mass spectra fragmentation patterns with those in the literature.[15] The relative amount (%) of volatile constituents was obtained by calculating the known amount of the internal standard.

Isolation. The *S. tenuifolia* oil (10 g) was loaded on a silica gel column (Merck 70−230 mesh, 60 mm i.d. × 800 mm; Rahway, NJ, USA) and eluted gradually using hexane/ethyl acetate (9:1, 7:3, 5:5, v/v). Each fraction was combined by thin-layer chromatography, and six fractions (ST 1−ST 6) were obtained. The acaricidal activities of the six fractions were evaluated using a filter paper bioassay against house dust and stored food mites at 40 $\mu g/cm^2$. The ST 3 (5.95 g) fraction possessed an acaricidal effect against house dust and stored food mites, and was rechromatographed on a silica gel column using hexane/ethyl acetate (7:3, v/v) to give five fractions (ST 31−ST 35). The ST 33 (3.78 g) fraction was toxic to house dust and stored food mites. Thus, preparative high performance liquid chromatography (prep HPLC) (LC-908, Japan Analytical Industry Co., Ltd., Tokyo, Japan) was performed. The ST 33 fraction was separated into five fractions (ST 331−ST 335) using a Jaigel GS Series column (GS310 500 mm × 2) with methanol as the mobile phase (100%) at a flow rate of 3.5 mL/min. The ST 334 (1.29 g) fraction showed acaricidal activity against house dust and stored food mites among these fractions. Then, a Jaigel W Series column (W253 500 mm + W252 500 mm) with methanol (100%) as the mobile phase at a flow rate of 3.5 mL/min was used to give three fractions (ST 3341−ST 3343). Finally, the active compound (ST 3341, 790 mg) was isolated as a single peak. The structure of the ST 3341 fraction was identified using various spectroscopic analyses.

Identification. To determine the number of carbons and protons in the ST 3341 fraction as the active compound of *S. tenuifolia* oil, ^{13}C and ^{1}H NMR were obtained using a JNM-ECA600 spectrometer (JEOL Ltd., Tokyo, Japan) at 600 and 150 MHz, respectively, in deuterochloroform ($CDCl_3$) using tetramethylsilane as the internal standard. Furthermore, DEPT NMR was recorded under identical conditions to determine the connection between carbons and protons. The absorption spectra were obtained using a UV spectrometer (DR/4000, HACH, Loveland, CO, USA), and the EI/MS spectra were investigated on a JEOL GSX 400 mass spectrometer.

Target Mites. The house dust and stored food mites were reared without exposure to commercial acaricides. The mites were maintained on dried feed (Korea Special Feed Meal Co. Ltd., Jeonju, Korea) and yeast (1:1 by weight) in a plastic case (15 × 12 × 6 cm^3). The cases were kept in an incubator at 25 °C and 75% relative humidity in the dark. The dried feed consisted of calcium (1.9%), cellulose (3.0%), lipid (4.0%), phosphorus (2.0%), and protein (49.0%). *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* were placed in Petri dishes (90 × 15 mm^2) for experiments and allowed to lay eggs for 48 h to synchronize their developmental cycles. The eggs laid were checked daily for developmental stage using an optical microscope until all adults died.

Filter Paper Bioassay. The acaricidal effects of the essential oil, active compound, and its structural analogues were investigated using a filter paper bioassay against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* using a bioassay method modified from Yang and Lee.[16] Various concentrations (40, 20, 10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, 0.08, 0.04, and 0.02 $\mu g/cm^2$) of each sample were dissolved in 30 μL of acetone and then applied to filter paper (50 mm i.d. × 55 μm thickness, Whatman Co., Maidenstone, UK). The negative control group was injected with acetone alone, and benzyl benzoate was used for the positive control. All filter papers were dried in a fume hood for 15 min, and each piece was placed in the bottom of a Petri dish (50 m i.d. × 8 mm length). Forty mites (males and females 1:1) were inoculated in a Petri dish, and the lid was sealed. Mortalities were determined under a binocular microscope after 24 h (20×, Olympus, Tokyo, Japan). The bioassays were replicated three times, and the total number of dead mites in the treatment group was compared with mites in the control group.

Impregnated Cotton Fabric Disk Bioassay. The acaricidal activities of samples were determined using an impregnated cotton fabric disk bioassay against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*. This method was modified from the bioassay described by Kim et al.[13] Each sample was dissolved in 30 μL of acetone at various concentrations (40, 20, 10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, and 0.08 $\mu g/cm^3$) and injected into a cotton fabric disk (8 mm i.d. × 1 mm thickness). The negative control was acetone, and the positive control was benzyl benzoate, which were used at the same concentrations. The solvent was evaporated under a fume hood for 15 min, and the cotton disk was inserted into the lid of a microtube. A piece of cotton fabric was inserted into the lid of the microtube to prevent contact of the mites with the applied fabric disk. Forty house dust and stored food mites (males and females 1:1) were inoculated in each microtube, and the cap was closed. The treatment and control groups were incubated at 25 ± 1 °C and 75% relative humidity in the dark for 24 h. Mortality rates were determined by observing the number of dead mites under a binocular microscope (20×, Olympus). The dead mites were verified by lack of movement when prodded with a pin. All bioassays were replicated three times.

Statistical Analysis. Mortalities in each group were determined under a binocular microscope (20×) after 24 h. House dust and stored food mites were regarded dead if they did not move when touched with a pin. The treatments were repeated three or four times, and the LD_{50} values were calculated by probit analysis.[17] When the

95% confidence limit (CL) of each LD_{50} value did not overlap, the acaricidal effects of samples were considered significantly different. Relative toxicity was determined as the ratio of synthetic acaricide LD_{50} value/each sample LD_{50} value.

RESULTS AND DISCUSSION

Acaricidal Potential of *S. tenuifolia* oil. The acaricidal effects of *S. tenuifolia* oil against house dust and stored food mites were examined using filter paper and impregnated cotton fabric disk bioassays and compared with that of benzyl benzoate, which served as the positive control for acaricidal activity (Table 1). The LD_{50} values of *S. tenuifolia* oil in the filter paper bioassay were 2.56, 2.50, and 6.01 $\mu g/cm^2$ against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*, respectively. In the impregnated cotton fabric disk bioassay, the LD_{50} values of *S. tenuifolia* oil were 5.88, 5.85, and 10.25 $\mu g/cm^3$ against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*, respectively. The negative control treated with acetone was not toxic to the mites in either assay. The essential oil of *S. tenuifolia* in the filter paper bioassay was approximately 3.07, 2.76, and 1.57 times more active than that of benzyl benzoate (LD_{50} values, 7.86, 6.90, and 9.44 $\mu g/cm^2$) against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* (Table 1). In the impregnated cotton fabric disk bioassay, *S. tenuifolia* oil was about 2.08, 1.72, and 1.42 times more toxic than benzyl benzoate (LD_{50} values, 12.25, 10.08, and 15.07 $\mu g/cm^3$) against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* (Table 1). On the basis of the LD_{50} values, American and European house dust mites were slightly more sensitive than stored food mites to both *S. tenuifolia* oil and the acaricide benzyl benzoate using either assay. The acaricidal potential of *S. tenuifolia* oil is influenced by mite species and depends on biochemical (detoxification enzyme activity) and biological (size and weight of these mites) conditions.[16] Thus, the *S. tenuifolia* oil has potential as a natural acaricide to control house dust and stored food mites.

Composition of Volatile Constituents from *S. tenuifolia* Oil. The volatile constituents of *S. tenuifolia* oil were identified by GC-MS and compared with retention times, retention indices, and mass spectra of compounds in the literature[18] (Table 2). The identified volatile constituents accounted for 94.81% of the total *S. tenuifolia* oil. The relative amounts (%) of the volatile constituents were *trans*-caryophyllene (1.88%), caryophyllene oxide (0.52%), germacrene D (1.72%), 2-isopropylidene-5-methylcyclohexanone (33.88%), 2-isopropyl-5-methylcyclohexanone (50.21%), limonene (3.16%), piperitenone (1.73%), 1-octen-3-ol (1.16%), and *cis*-verbenol (0.55%). The volatile constituents were grouped as monoterpene alcohols (1-octen-3-ol and *cis*-erbenol), monoterpene hydrocarbons (limonene), monoterpene ketones (2-isopropylidene-5-methylcyclohexanone, 2-isopropyl-5-methylcyclohexanone, and piperitenone), and sesquiterpene hydrocarbons (*trans*-caryophyllene, caryophyllene oxide, and germacrene D). Therefore, the active component of *S. tenuifolia* oil may be expected to be 2-isopropyl-5-methylcyclohexanone. According to previous studies, the main compounds in *S. tenuifolia* oil extracted by headspace solid phase microextraction are 2-hydroxy-2-isopropenyl-5-methylcyclohexane (5.97%), (+)-menthone (14.32%), (−)-pulegone (47.73%), *cis*-pulegone oxide (4.12%), and schizonal (5.36%).[18] Furthermore, the active components of *S. tenuifolia* are cinnamic acid, flavonoids (diosmetin, hesperidin, and luteolin), monoterpenoids (schizonepetosides D, schizonepetosides E, schizonodiol, and schizonol), and ursolic acid.[19,20] In this study and previous studies, the quantities of volatile constituents depended on environmental conditions, harvest period, intraspecific variability, processing method, and storage time.[21] In addition to these factors, extraction method can also influence the quantities of bioactive compounds derived from plant extracts.[18]

Table 2. Composition of Volatile Constituents from *S. tenuifolia* Oil Identified by GC-MS

retention time (min)	constituents	RI[a]	mass spectra (m/z)[b]	relative amount (%)
4.055	1-octen-3-ol	978	128, 99, 85, 72, 57, 43, 29, 15	1.16
4.976	limonene	1025	136, 121, 107, 93, 80, 68, 53, 39	3.16
6.573	*cis*-verbenol	1125	152, 137, 121, 109, 94, 79, 67, 55	0.55
7.194	2-isopropyl-5-methylcyclohexanone	1235	154, 139, 125, 112, 97, 83, 69, 55	50.21
8.539	2-isopropylidene-5-methylcyclohexanone	1277	152, 137, 123, 109, 95, 81, 67, 53	33.88
9.958	Piperitenone	1342	150, 135, 121, 107, 91, 79, 67, 53	1.73
11.082	*trans*-caryophyllene	1417	204, 175, 147, 120, 105, 93, 79, 67, 55	1.88
11.868	germacrene D	1481	204, 161, 147, 133, 119, 105, 91, 67, 55, 41	1.72
13.121	caryophyllene oxide	2040	220, 191, 177, 161, 138, 121, 109, 91, 55, 41	0.52

[a]Retention indices were determined on a DB-5 capillary column. [b]Major fragmentation ions, base peak, and other ions in decreasing order of relative abundance.

Isolation and Identification of the ST 3341 Fraction. To isolate the active compound of *S. tenuifolia* oil, chromatographic analyses were conducted using a silica gel column and preparative HPLC. The ST 3341 fraction was isolated as the active compound of *S. tenuifolia* oil, and the structure of the ST 3341 fraction was identified by spectroscopic analyses such as GC-MS, ^1H NMR, ^{13}C NMR, and DEPT-NMR spectra. The isolated ST 3341 fraction was characterized as 2-isopropyl-5-methylcyclohexanone ($C_{10}H_{18}O$); EI/MS (70 eV) m/z M^+ 154.25; ^1H NMR ($CDCl_3$, 600 MHz) δ 0.91−0.92 (d, J = 3.0 Hz, 1H), 0.93−0.94 (d, J = 5.4 Hz, 1H), 0.98−0.99 (d, J = 4.8 Hz, 1H), 1.15−1.18 (m, J = 16.8 Hz, 1H), 1.37−1.41 (m, J = 25.6 Hz, 1H), 1.64−1.68 (dd, J = 21.0 Hz, 1H), 1.73- 1.75 (m, J = 12.7 Hz, 1H), 1.88−1.90 (m, J = 12.1 Hz, 1H), 2.13−2.18 (octet, J = 24.6 Hz, 1H), 3.30−3.32 (dd, J = 10.2 Hz, 2H); ^{13}C/DEPT NMR ($CDCl_3$, 150 MHz) δ 163.5, 49.6 (CH2), 47.8 (CH), 32.9 (CH), 32.4 (CH2), 29.4 (CH2), 26.5 (CH), 21.9 (CH3), 21.4 (CH3), 19.2 (CH3). These findings of 2-isopropyl-5-methylcyclohexanone were compared with those of a previous study.[22]

Toxicity of 2-Isopropyl-5-methylcyclohexanone Isolated from *S. tenuifolia* Oil. The acaricidal activities of 2-isopropyl-5-methylcyclohexanone isolated from *S. tenuifolia* oil were evaluated using the filter paper and impregnated cotton fabric disk bioassays against the house dust and stored food mites and compared with those of benzyl benzoate (Tables 3 and 4). The LD_{50} values of 2-isopropyl-5-methylcyclohexanone in the filter paper bioassay were 0.05, 0.04, and 0.12 $\mu g/cm^2$ against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*, respectively (Table 3). The LD_{50} values of 2-isopropyl-5-

Table 3. Acaricidal Potency of 2-Isopropyl-5-methylcyclohexanone Structural Analogues and a Synthetic Acaricide in the Filter Paper Bioassay[a]

compounds	mite species	LD_{50} ± SD ($\mu g/cm^2$)	95% CL	relative toxicity[b]
2-isopropyl-5-methylcyclohexanone	*D. farinae*	0.05 ± 2.11	0.02−0.08	157.20
	D. pteronyssinus	0.04 ± 1.61	0.01−0.07	172.50
	T. putrescentiae	0.12 ± 2.13	0.09−0.15	78.67
2-isopropylidene-5-methylcyclohexanone	*D. farinae*	0.20 ± 1.51	0.17−0.23	39.30
	D. pteronyssinus	0.19 ± 1.74	0.16−0.22	36.32
	T. putrescentiae	0.53 ± 0.91	0.50−0.56	17.81
2-methylcyclohexaone	*D. farinae*	2.50 ± 2.10	2.47−2.53	3.14
	D. pteronyssinus	2.45 ± 2.51	2.41−2.48	2.82
	T. putrescentiae	5.99 ± 1.24	5.96−6.02	1.58
3-methylcyclohexanone	*D. farinae*	4.17 ± 1.62	4.14−4.20	1.88
	D. pteronyssinus	4.15 ± 2.55	4.12−4.18	1.66
	T. putrescentiae	7.05 ± 2.42	7.02−7.08	1.34
4-methylcyclohexanone	*D. farinae*	5.76 ± 1.31	5.73−5.79	1.36
	D. pteronyssinus	5.55 ± 1.54	5.52−5.58	1.24
	T. putrescentiae	8.45 ± 1.53	8.42−8.48	1.12
4-isopropylcyclohexanone	*D. farinae*	0.16 ± 1.62	0.13−0.19	49.13
	D. pteronyssinus	0.15 ± 1.83	0.12−0.17	46.00
	T. putrescentiae	0.34 ± 2.44	0.31−0.37	27.76
benzyl benzoate[c]	*D. farinae*	7.86 ± 0.82	7.83−7.89	1.00
	D. pteronyssinus	6.90 ± 1.74	6.87−6.93	1.00
	T. putrescentiae	9.44 ± 1.93	9.41−9.47	1.00

[a]Exposed for 24 h. [b]Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of each chemical. [c]Benzyl benzoate served as the positive control (synthetic acaricide).

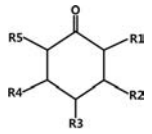
Table 4. Acaricidal Potency of 2-Isopropyl-5-methylcyclohexanone Structural Analogues and a Synthetic Acaricide in the Impregnated Cotton Fabric Disk Bioassay[a]

compounds	mite species	LD_{50} ± SD ($\mu g/cm^3$)	95% CL	relative toxicity[b]
2-isopropyl-5-methylcyclohexanone	*D. farinae*	0.17 ± 2.22	0.14−0.19	72.06
	D. pteronyssinus	0.16 ± 1.52	0.13−0.18	63.00
	T. putrescentiae	0.42 ± 1.41	0.39−0.44	35.88
2-isopropylidene-5-methylcyclohexanone	*D. farinae*	0.88 ± 1.63	0.86−0.91	13.92
	D. pteronyssinus	0.75 ± 1.52	0.73−0.78	13.44
	T. putrescentiae	4.26 ± 1.84	4.23−4.28	3.54
2-methylcyclohexaone	*D. farinae*	4.85 ± 2.13	4.83−4.88	2.53
	D. pteronyssinus	4.00 ± 0.92	3.97−4.02	2.52
	T. putrescentiae	8.55 ± 2.14	8.52−8.58	1.76
3-methylcyclohexanone	*D. farinae*	8.41 ± 2.22	8.39−8.44	1.46
	D. pteronyssinus	7.54 ± 1.62	7.52−7.57	1.34
	T. putrescentiae	10.13 ± 1.83	10.01−10.16	1.49
4-methylcyclohexanone	*D. farinae*	9.25 ± 1.94	9.22−9.28	1.32
	D. pteronyssinus	8.13 ± 2.11	8.10−8.15	1.24
	T. putrescentiae	13.52 ± 1.73	13.49−13.55	1.11
4-isopropylcyclohexanone	*D. farinae*	0.73 ± 0.93	0.70−0.75	16.78
	D. pteronyssinus	0.69 ± 2.15	0.66−0.71	14.61
	T. putrescentiae	1.34 ± 1.84	1.31−1.36	11.25
benzyl benzoate[c]	*D. farinae*	12.25 ± 2.42	12.22−12.28	1.00
	D. pteronyssinus	10.08 ± 1.23	10.05−10.11	1.00
	T. putrescentiae	15.07 ± 2.54	15.04−15.10	1.00

[a]Exposed for 24 h. [b]Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of each chemical. [c]Benzyl benzoate was served as a positive control (synthetic acaricide).

methylcyclohexanone in the impregnated cotton fabric disk bioassay were 0.17, 0.16, and 0.42 $\mu g/cm^3$ against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*, respectively (Table 4). Compared with the LD_{50} values of benzyl benzoate in the filter paper bioassay, 2-isopropyl-5-methylcyclohexanone was approximately 157.20, 172.50, and 78.67 times more active than benzyl benzoate (LD_{50} values, 7.86, 6.90, and 9.44 $\mu g/cm^2$) against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* (Table 3). In the impregnated cotton fabric disk bioassay, 2-isopropyl-5-methylcyclohexanone was about 72.06, 63.00, and 35.88 times more toxic than benzyl benzoate (LD_{50} values, 12.25, 10.08, and 15.07 $\mu g/cm^3$) against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* (Table 4). Taken together, the acaricidal activity of 2-isopropyl-5-methylcyclohexanone was greater than

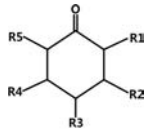
Table 5. Structure−Activity Relationships of 2-Isopropyl-5-methylcyclohexanone Analogues in the Filter Paper Bioassay



Cyclohexanone

						LD_{50} ($\mu g/cm^2$)		
compounds	R_1	R_2	R_3	R_4	R_5	*D. farinae*	*D. pteronyssinus*	*T. putrescentiae*
2-isopropyl-5-methylcyclohexanone	C_3H_7	H	H	CH_3	H	0.05 ± 2.12	0.04 ± 1.62	0.12 ± 2.11
2-isopropylidene-5-methylcyclohexanone	C_3H_6	H	H	CH_3	H	0.20 ± 1.51	0.19 ± 1.73	0.53 ± 0.90
2-methylcyclohexaone	CH_3	H	H	H	H	2.50 ± 2.14	2.45 ± 2.51	5.99 ± 1.20
3-methylcyclohexanone	H	CH_3	H	H	H	4.17 ± 1.65	4.15 ± 2.54	7.05 ± 2.43
4-methylcyclohexanone	H	H	CH_3	H	H	5.76 ± 1.32	5.55 ± 1.53	8.45 ± 1.52
4-isopropylcyclohexanone	H	H	C_3H_7	H	H	0.16 ± 1.61	0.15 ± 1.82	0.34 ± 2.44

Table 6. Structure−Activity Relationships of 2-Isopropyl-5-methylcyclohexanone Analogues in the Impregnated Cotton Fabric Disk Bioassay



Cyclohexanone

						LD_{50} ($\mu g/cm^3$)		
compounds	R_1	R_2	R_3	R_4	R_5	*D. farinae*	*D. pteronyssinus*	*T. putrescentiae*
2-isopropyl-5-methylcyclohexanone	C_3H_7	H	H	CH_3	H	0.17 ± 2.20	0.16 ± 1.53	0.42 ± 1.42
2-isopropylidene-5-methylcyclohexanone	C_3H_6	H	H	CH_3	H	0.88 ± 1.62	0.75 ± 1.53	4.26 ± 1.83
2-methylcyclohexaone	CH_3	H	H	H	H	4.85 ± 2.14	4.00 ± 0.92	8.55 ± 2.11
3-methylcyclohexanone	H	CH_3	H	H	H	8.41 ± 2.20	7.54 ± 1.60	10.13 ± 1.80
4-methylcyclohexanone	H	H	CH_3	H	H	9.25 ± 1.92	8.13 ± 2.12	13.52 ± 1.72
4-isopropylcyclohexanone	H	H	C_3H_7	H	H	0.73 ± 0.91	0.69 ± 2.11	1.34 ± 1.84

that of a synthetic acaricide against house dust and stored food mites.

Structure−Activity Relationships. To establish the structure−activity relationships of 2-isopropyl-5-methylcyclohexanone analogues, 4-isopropylcyclohexanone, 2-isopropylidene-5-methylcyclohexanone, 2-methylcyclohexanone, 3-methylcyclohexanone, and 4-methylcyclohexanone were selected to test their acaricidal activities, and their acaricidal activities against house dust and stored food mites were evaluated using the filter paper and impregnated cotton fabric disk bioassays (Tables 3 and 4). 4-Isopropylcyclohexanone (0.16 $\mu g/cm^2$) was approximately 49.13 times more active than benzyl benzoate (7.86 $\mu g/cm^2$) in the filter paper bioassay against *D. farinae*, followed by 2-isopropylidene-5-methylcyclohexanone (0.20 $\mu g/cm^2$), 2-methylcyclohexanone (2.50 $\mu g/cm^2$), 3-methylcyclohexanone (4.17 $\mu g/cm^2$), and 4-methylcyclohexanone (5.76 $\mu g/cm^2$) compared with that of benzyl benzoate. 4-Isopropylcyclohexanone (0.15 $\mu g/cm^2$) was about 46.0 times more toxic than benzyl benzoate (6.90 $\mu g/cm^2$) against *D. pteronyssinus*, followed by 2-isopropylidene-5-methylcyclohexanone (0.19 $\mu g/cm^2$), 2-methylcyclohexanone (2.45 $\mu g/cm^2$), 3-methylcyclohexanone (4.15 $\mu g/cm^2$), and 4-methylcyclohexanone (5.55 $\mu g/cm^2$). 4-Isopropylcyclohexanone (0.34 $\mu g/cm^2$) was roughly 27.76 times more effective than benzyl benzoate (9.44 $\mu g/cm^2$) against *T. putrescentiae*, followed by 2-isopropylidene-5-methylcyclohexanone (0.53 $\mu g/cm^2$), 2-methylcyclohexanone (5.99 $\mu g/cm^2$), 3-methylcyclohexanone (7.05 $\mu g/cm^2$), and 4-methylcyclohexanone (8.45 $\mu g/cm^2$) (Table 3). In accordance with the results of the impregnated cotton fabric disk bioassay against *D. farinae*, 4-isopropylcyclohexanone (0.73 $\mu g/cm^3$) was approximately 16.78 times more active than benzyl benzoate (12.25 $\mu g/cm^3$), followed by 2-isopropylidene-5-methylcyclohexanone (0.88 $\mu g/cm^3$), 2-methylcyclohexanone (4.85 $\mu g/cm^3$), 3-methylcyclohexanone (8.41 $\mu g/cm^3$), and 4-methylcyclohexanone (9.25 $\mu g/cm^3$). 4-Isopropylcyclohexanone (0.69 $\mu g/cm^3$) was about 14.61 times more toxic than benzyl benzoate (10.08 $\mu g/cm^3$) against *D. pteronyssinus*, followed by 2-isopropylidene-5-methylcyclohexanone (0.75 $\mu g/cm^3$), 2-methylcyclohexanone (4.00 $\mu g/cm^3$), 3-methylcyclohexanone (7.54 $\mu g/cm^3$), and 4-methylcyclohexanone (8.13 $\mu g/cm^3$). 4-Isopropylcyclohexanone (1.34 $\mu g/cm^3$) was roughly 11.25 times more effective than benzyl benzoate (15.07 $\mu g/cm^3$) against *T. putrescentiae*, followed by 2-isopropylidene-5-methylcyclohexanone (4.26 $\mu g/cm^3$), 2-methylcyclohexanone (8.55 $\mu g/cm^3$), 3-methylcyclohexanone (10.13 $\mu g/cm^3$), and 4-methylcyclohexanone (13.52 $\mu g/cm^3$) (Table 4). Taken together, 4-isopropylcyclohexanone possessed the highest activity among 2-isopropyl-5-methylcyclohexanone isomers against house dust and stored food mites.

Monoterpene ketone (2-isopropyl-5-methylcyclohexanone) contained pendant isopropyl and methyl functional groups on the cyclohexanone skeleton. The structure−activity relationships of 2-isopropyl-5-methylcyclohexanone analogues were determined by comparing the LD_{50} values from the filter paper and impregnated cotton fabric disk bioassays against house dust and stored food mites (Tables 5 and 6). In the filter paper and

impregnated cotton fabric disk bioassays, 4-isopropylcyclohexanone and 2-isopropyl-5-methylcyclohexanone, which had an isopropyl (C_3H_7) functional group on cyclohexanone, showed potent effects against house dust and stored food mites, but 2-, 3-, and 4-methylcyclohexanone, containing a methyl (CH_3) functional group on cyclohexanone, showed no acaricidal activity against house dust and stored food mites. Interestingly, the acaricidal responses were different among these mites. In particular, *D. pteronyssinus* was more sensitive to the essential oil and the 2-isopropyl-5-methylcyclohexanone structural analogues than has been reported in many insect species.[23,24]

In conclusion, our results indicate that *S. tenuifolia* oil and the 2-isopropyl-5-methylcyclohexanone structural analogues appear to be potential agents to control house dust and stored food mites and may protect humans from indoor allergens. Introducing an isopropyl functional radical on the cyclohexanone skeleton resulted in the highest acaricidal potency. We must develop new and safe strategies to avoid side effects, such as biological magnification and residual toxicity to the environment.[25] Monoterpenoids have low acute toxicity compared with that of conventional insecticides; however, the acute toxicities of *S. tenuifolia* oil and the 2- isopropyl-5-methylcyclohexanone analogues have not been reported in mammals.[26] Nevertheless, these results indicate that *S. tenuifolia* oil and the 2- isopropyl-5-methylcyclohexanone structural analogues could be effective natural acaricides to control house dust and stored food mites. Further research should be conducted on the environmental and human health safety of *S. tenuifolia* oil and the 2-isopropyl-5-methylcyclohexanone analogues to enhance acaricidal potency and stability.

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Notes

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