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Mite-Control Activities of Active Constituents Isolated from *Pelargonium graveolens* Against House Dust Mites

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The mite-control activities of materials obtained from *Pelargonium graveolens* oil against *Dermatophagoides farinae* and *D. pteronyssinus* were examined using an impregnated fabric disk bioassay and were compared with those shown by commercial benzyl benzoate and *N,N*-diethyl-*m*-toluamide (DEET). Purification of the biologically active constituents from *P. graveolens* oil was done by silica gel chromatography and high performance liquid chromatography. The structures of the active components were analyzed by EI/MS, ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{13}C COSY-NMR, and DEPT-NMR spectra, and were identified as geraniol ($C_{10}H_{18}O$, MW 154.25, *trans*-3,7-dimethyl-2,6-octadien-1-ol) and β-citronellol ($C_{10}H_{20}O$, MW 156.27, 3,7-dimethyl-6-octen-1-ol). Based on the LD_{50} values, the most toxic compound was geraniol (0.26 μg/cm^2), followed by β-citronellol (0.28 μg/cm^2), benzyl benzoate (10.03 μg/cm^2), and DEET (37.12 μg/cm^2) against *D. farinae*. In the case of *D. pteronyssinus*, geraniol (0.28 μg/cm^2) was the most toxic, followed by β-citronellol (0.29 μg/cm^2), benzyl benzoate (9.58 μg/cm^2), and DEET (18.23 μg/cm^2). These results suggest that *D. farinae* and *D. pteronyssinus* may be controlled more effectively by the application of geraniol and β-citronellol than benzyl benzoate and DEET. Furthermore, geraniol and β-citronellol isolated from *P. graveolens* could be useful for managing populations of *D. farinae* and *D. pteronyssinus*.

Keywords: Dust mite, *Pelargonium graveolens*, geraniol, β-citronellol

The most important of the pyroglyphid mites, the American dust mite (*Dermatophagoides farinae* [Hughes]) and the European dust mite (*D. ptertonyssinus* [Trouessart]), are cosmopolitan inhabitants of human dwellings in many parts of the world [17, 19, 24]. They are a major source of multiple potent allergens [7, 17, 31], and have been causally associated with sudden infant death syndrome [13, 18]. Exposure to house dust mite allergens can result in bronchoconstriction and inflammatory reaction of the airways [4]. Dust mite allergen avoidance measures are available in daily life. People spend approximately one-third of their lives in bed. Therefore, dust mites in mattresses, bedding, and pillows can contribute greatly to total house dust mite exposure [9, 22]. Most houses are co-inhabited by *D. farinae* and *D. pteronyssinus*. However, one species usually predominates in each home and makes up more than 70% of the total mite population. These findings illustrate the need for cross-antigenicity testing for both species when a patient undergoes skin testing, and when using immunotherapy [1]. Control of dust mites depends on environmental and chemical methods such as fumigation and spraying with acaricidal compounds [2, 8, 17, 19, 21]. However, repeated use of organophosphorus chemicals has resulted in the increase of resistance [17, 28], had undesirable effects on non-target organisms, and fostered environmental and human health concerns [16, 17]. Therefore, interests have been focused on more selective and new safer compounds against house dust mites.

The biological activities of essential oils derived from lower (Cryptogamae) and higher plants (Gymnospermae and Angiospermae) against several organisms, mainly bacteria, fungi, and arthropods, have been confirmed in many reports and are mainly due to monoterpenoids and sesquiterpenoids that represent their main components [3, 5, 24]. The essential oil of the rose-scented geranium (*Pelargonium* species, family, Geraniaceae) is widely used as a floral substitute of the rose scent, and is, therefore, one of the most valuable natural materials for the perfumery and cosmetic industries [27]. Among different essential oils, geranium oil, known as the "women's oil" because of its menstrual and menopausal benefits, has a wide variety of applications. Besides promoting women's health, it is

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also useful for skin problems like eczema and athlete's foot, and for respiratory tract health [15]. It contains various compounds, including geraniol, citronellol, nerol, linalool, geranyl acetate, and rose oxide [10, 25, 27, 29]. However, the acaricidal property of *P. graveolens* oil has not yet been investigated. This paper describes a laboratory study to examine the essential oil of the *P. graveolens* leaves for acaricidal constituents against *D. farinae* and *D. pteronyssinus*. Furthermore, the acaricidal activities of active compounds were compared with those of the commonly used benzyl benzoate and DEET.

MATERIALS AND METHODS

Chemicals and Plant Materials

Benzyl benzoate, citral, DEET, farnesol, geranyl acetate, and nerol were supplied from Aldrich (Milwaukee, WI, U.S.A.). All other chemicals were of reagent grade. The leaves of *P. graveolens* were purchased from a local market in Chonju. The essential oils tested were isolated by steam distillation with cohobation for 8 h as described by Cho et al. [6]. Essential oil yield was 0.2% from the steam distillation.

Dust Mites

Cultures of *D. farinae* and *D. pteronyssinus* were maintained in the laboratory for 7 years without exposure to any known acaricide. They were reared in plastic containers (15 cm×12 cm×6 cm) containing 30 g of sterilized diet (fry feed No. 1/dried yeast, 1:1 by weight) at 25±1°C and 75% relative humidity in continuous darkness. The fry feed (Miropa) was purchased from Korea Special Feed Meal Co. Ltd. (Chonju, South Korea).

Isolation and Identification

The oil (24 g) was chromatographed on a silica gel column (Merck 70–230 mesh, 600 g, 10 i.d. × 50 cm) and successively eluted with a stepwise gradient of hexane/ethyl acetate (10:0, 9:1, 8:2, 7:3, 5:5, and 0:10). The bioactive fraction (8.6 g) was successively rechromatographed on a silica gel column (Merck 70–230 mesh, 500 g, 5.5 i.d. × 70 cm) using hexane-ethyl acetate (7:3). Column fractions were analyzed by thin layer chromatography (TLC [silica gel 60 F_{254}]; SILC/UV 254, 0.25 mm; Macherey-Nagel, Germany), and fractions with similar streaking patterns on the TLC plates were pooled. The active H43 fraction was purified by Prep. HPLC (Recycling Preparative HPLC; Japan Analytical Industry Co., LTD, Japan) for separation of the biologically active constituent. The first column was a Jaigel GS Series Column (GS310 50 cm+GS310 30 cm×2, 21.5 mm i.d. × 500 mm; Japan Analytical Industry Co., LTD, Japan), using chloroform (100%) at a flow rate of 5 ml/min and detection at 299 nm. In this step, five fractions (H431-H435) were obtained and bioassayed at 80 µg/cm². The active H434 fraction (3.6 g) was further chromatographed on a Jaigel W Series Column (W-253 50 cm+W-252 50 cm, 20.0 mm i.d. × 500 mm; Japan Analytical Industry Co., LTD, Japan) using hexane:chloroform (7:3) at a flow rate of 5 ml/min and detection at 299 nm. Finally, the potent active principles (H43441 and H43442, 720 mg and 970 mg) were isolated. The structure of the active isolate was determined by instrumental analyses. ^{1}H- and ^{13}C-NMR spectra were recorded in

deuteriochloroform with a JNM-LA 400F7 spectrometer (JNM-ECA600; JEOL Ltd, Japan), at 600 and 150 MHz (TMS as an internal standard), respectively, and chemical shifts are given in δ (parts per million). Unambiguous ^{1}H- and ^{13}C-NMR chemical shifts were obtained using DEPT, ^{1}H-^{1}H COSY, and ^{1}H-^{13}H COSY spectra. UV spectra were obtained in chloroform with a Jasco V-550 spectrometer.

Gas Chromatography-Mass Spectrometry

The essential oil of *P. graveolens* leaves was analyzed on a gas chromatograph (6890; Agilent, Wilmington, DE, U.S.A.)-mass spectrometer (5973IV; Agilent) (GC-MS). The GC column was a 30 m×0.25 mm i.d. DB-5 (0.25-mm film) fused silica capillary column (J&W Scientific, Folsom, CA, U.S.A.). The GC conditions were as follows: injector temperature, 260°C; column temperature, isothermal at 70°C for 3 min, then programmed to rise to 300°C at 10°C/min and be held at this temperature for 5 min; ion source temperature, 230°C. Helium was used as the carrier gas at a rate of 0.8 ml/min. The effluent of the GC column was introduced directly into the source of the mass spectrometer. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 50 to 600 amu for 2 s. Compounds were identified by comparison with retention times, and the mass spectra obtained with the authentic standards on the GC-MS system were used for analysis. When an authentic sample was not available, the identification was carried out by comparison of experimentally obtained mass spectra with those in the mass spectra library (The Wiley Registry of Mass Spectral Data, 6th Ed.).

Dust Mite Bioassay

An impregnated fabric disk bioassay was used to assess the acaricidal activity of test samples at 80, 60, 40, 20, 10, 5, 2.0, 1.0, 0.5, 0.2, 0.1, and 0.05 µg/cm² [17, 19, 21]. A 20 µl aliquot of test material was applied to disks made of black cotton fabric (0.5 g, 5 cm diameter: 700 mesh). Control fabric disks were treated with only 20 µl of acetone. After the disks were dried in a fume hood (19°C) for 30 s, each disk was placed individually in the bottom of a Petri dish (5 cm diameter×1.2 cm) [17]. Thirty individuals of *D. farinae* (7–10 days old) and *D. pteronyssinus* (7–10 days old) were 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 the dark [17, 19, 21]. 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. LD_{50} values were calculated by probit analysis [28]. The percentage mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test at $P=0.05$ (SAS Institute) [28].

RESULTS AND DISCUSSION

When the essential oil extracted from the *P. graveolens* leaves was bioassayed using impregnated fabric disks, the acaricidal activity of the essential oil was observed in various doses against *D. farinae* and *D. pteronyssinus* (Table 1). The widely used benzyl benzoate and DEET

Table 1. Acaricidal activity of *Pelargonium graveolens* oil against *Dermatophagoides farinae* and *D. pteronyssinus*, using the impregnated fabric disk bioassay.

| Mite species | 24 h mortality (mean±SE, %)[a] | | | | | | LD_{50} ($\mu g/cm^2$) |
| | Concentration ($\mu g/cm^2$) | | | | | | |
	40	20	10	5.0	2.5	1.25	
D. farinae	100	100	100	62.5±1.3	27.5±1.5	5.0±1.9	2.82
D. pteronyssinus	100	100	100	53.1±2.7	15.2±1.4	2.3±1.7	3.13

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

compounds served as standards for comparison in the toxicity tests. At doses of 40, 20, and 10 $\mu g/cm^2$, the essential oils of *P. graveolens* gave 100% mortality against house dust mites. In the test with *D. farinae* adults, the *P. graveolens* oil gave a 62.5%, 27.5%, and 5.0% mortality of *D. farinae* adults at 5, 2.5, and 1.25 mg/cm^2, respectively, 24 h after treatment. For *D. pteronyssinus* adults, the essential oil caused 53.1%, 15.2%, and 2.3% mortality at 5, 2.5, and 1.25 mg/cm^2, respectively. No mortality was seen in the untreated controls. Because of the strong activity of the oil, the isolation of the active component was pursued (Fig. 1). Purification of the biologically active constituents were identified by spectroscopic analyses, including HPLC, GC-MS, EI-MS, and ^{13}C- and ^1H-NMR, through direct comparison with an authentic reference compound. The active constituents were characterized as geraniol and β-citronellol. These compounds were

Rose geranium essential oil, 24 g

Fig. 1. Isolation procedure of the acaricidal constituent from the essential oil extracted from *Pelargonium graveolens* leaves.

identified based on the following evidence. Geraniol (*trans*-3,7-dimethyl-2,6-octadien-1-ol), ($C_{10}H_{18}O$; MW: 154.25); EI-MS (70 eV) *m/z* (% relative intensity) M^+ 154 (100, base peak), 136 (21), 123 (18), 111 (16), 93 (14), 81 (8), 53 (5); ^1H-NMR (CD_3OD, 600 MHz); δ 5.404–5.426 (1H, *m*), 5.095 (1H, *m*), 4.145–4.156 (2H, *d*), 2.087–2.112 (2H, *d*), 2.019–2.030 (2H, *d*), 1.678–1.685 (3H, *t*), 1.606 (3H, *t*), 1.402 (3H, *t*); ^{13}C-NMR (CD_3OD, 150 MHz); δ 139.68, 131.69, 123.86, 123.31, 59.33, 39.51, 26.35, 25.63, 17.64, 16.21. β-Citronellol (3,7-dimethyl-6-octen-1-ol), ($C_{10}H_{20}O$; MW: 156.27); EI-MS (70 eV) *m/z* (% relative intensity) M^+ 156 (100, base peak), 138 (20), 123 (18), 109 (16), 95 (14), 82 (9), 55 (6); ^1H-NMR (CD_3OD, 600 MHz); δ 5.086–5.109 (1H, *m*), 1.554–1.643 (1H, *m*), 3.636–3.717 (2H, *d*), 1.950–2.034 (2H, *d*), 1.683 (2H, *d*), 1.318–1.414 (2H, *d*), 1.462 (3H, *t*), 1.159–1.209 (3H, *t*), 0.913 (3H, *t*); ^{13}C-NMR (CD_3OD, 150 MHz); 131.22, 124.68, 61.14, 39.87, 37.19, 29.15, 25.67, 25.42, 19.48, 17.59.

The substances in the oil extract of the *P. graveolens* leaves were identified by GC-MS. Analysis led to identification of 22 components from the essential oil of the *P. graveolens* leaves. The main constituents were β-citronellol (relative percent=26.61%), geraniol (14.91%), 2-naphthalenemethanol (7.16%), citronellyl formate (6.83%), cyclohexanone (6.03%), linalool (5.60%), δ-cadinene (2.99%), geraniol formate (2.94%), *trans*-caryophyllene (1.71%), butanoic acid (1.61%), 2-butanoic acid (1.60%), germacrene (1.57%), geranyl propionate (1.50%), geranyl tiglate (1.49%), 1-menthone (1.47%), β-myrcene (1.32%), β-bourbonene (1.31%), α-cubebene (1.31%), *trans*-rose oxide (1.17%), 2-pentanone (1.13%), τ-cadinol (1.13%), and 1,3,6-octatriene (1.03%). Together, β-citronellol, geraniol, 2-naphthalenemethanol, citronellyl formate, cyclohexanone, and linalool made up 67.1% of the essential oil derived from the *P. graveolens* leaves. In previous studies, Gomes *et al.* [10] reported that the main constituents of supercritical fluid extraction were citronellol (24.8%), citronellyl formate (10.2%), guaia-6,9-diene (8.8%), geraniol (8.5%), geranyl formate (7.9%), germacrene-D (4.6%), isomethone (3.5%), geranyl tiglate (3.3%), 2-phenylethyl tiglate (1.8%), rose oxide (0.4%), and linalool (0.1%). Peterson *et al.* [25] determined that the compositions of steam-distilled Chinese geranium oil
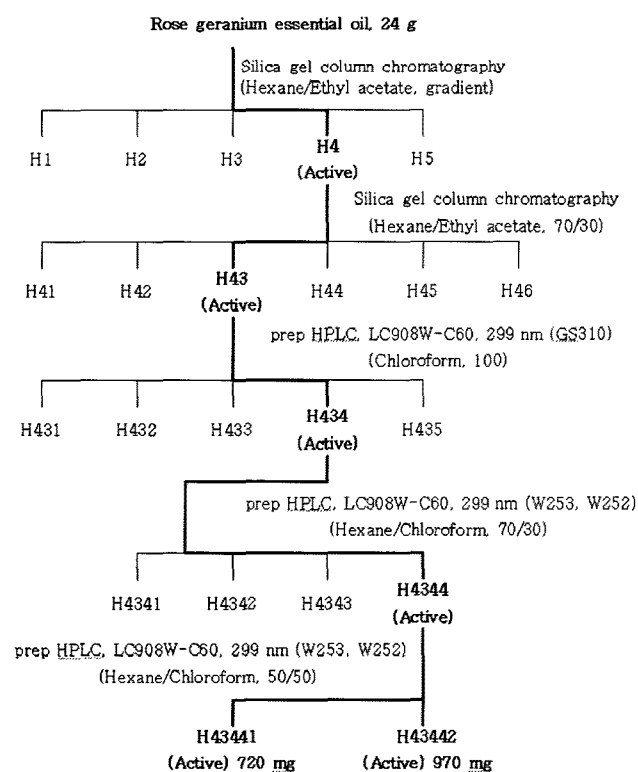
Table 2. Toxicity of geraniol and β-citronellol congeners and acaricides against *Dermatophagoides farinae* and *D. pteronyssinus* adults, using the fabric disk bioassay.[a]

Compound	Molecular weight	Mite species	LD$_{50}$ μg/cm^2	95% Confidence limit	RT[b]
Geraniol	154.24	*D. farinae*	0.26	0.24–0.28	38.58
		D. pteronyssinus	0.28	0.26–0.31	34.21
β-Citronellol	156.27	*D. farinae*	0.28	0.26–0.31	35.82
		D. pteronyssinus	0.29	0.26–0.33	33.03
Nerol (*cis*-geraniol)	154.24	*D. farinae*	1.57	1.52–1.61	6.39
		D. pteronyssinus	1.85	1.80–1.89	5.18
Citral	152.23	*D. farinae*	0.35	0.33–0.38	28.66
		D. pteronyssinus	0.32	0.30–0.34	29.94
Geranyl acetate	196.29	*D. farinae*	–	–	–
		D. pteronyssinus	–	–	–
Farnesol	222.37	*D. farinae*	–	–	–
		D. pteronyssinus	–	–	–
Benzyl benzoate	212.25	*D. farinae*	10.03	9.98–10.06	1.00
		D. pteronyssinus	9.58	9.50–9.62	1.00
DEET	191.27	*D. farinae*	37.12	36.12–39.25	0.27
		D. pteronyssinus	18.23	17.47–19.05	0.52

[a]Exposed for 24 h.
[b]Relative toxicity=LD$_{50}$ value of benzyl benzoate/LD$_{50}$ value of each chemical.

were citronellol (34.0%), citronellyl formate (11.18%), geraniol (7.38%), methone (6.34%), linalool (3.12%), rose oxide (1.97%), nerol (1.87%), caryophyllene (1.51%), and geranyl tiglate (1.38%). Comparing the three reported compositions of geranium oil, the amounts of citronellyl formate and methone were smaller in our sample (around 2–4% less), whereas the geraniol content increased 1.75 and 2.02 times, respectively. The linalool content was also quite different. Our sample has the highest linalool content because the high temperatures of water vapor turned geraniol into linalool [11].

The toxicities of geraniol, β-citronellol, and its congeners (nerol, citral, geranyl acetate, and farnesol) against *D. farinae* and *D. pteronyssinus* adults were compared with those of benzyl benzoate and DEET (Table 2) (Fig. 2). On the basis of 24 h LD$_{50}$ values, geraniol (0.26 μg/cm^2) was the most toxic against *D. farinae*, followed by β-citronellol (0.28 μg/cm^2), citral (0.35 μg/cm^2), nerol (1.57 μg/cm^2), benzyl benzoate (10.03 μg/cm^2), and DEET (37.12 μg/cm^2). Against *D. pteronyssinus*, geraniol (0.28 μg/cm^2) showed the most toxicity, followed by β-citronellol (0.29 μg/cm^2), citral (0.32 μg/cm^2), nerol (1.85 μg/cm^2), benzyl benzoate (9.58 μg/cm^2), and DEET (18.23 μg/cm^2). However, no activity was observed for geranyl acetate and farnesol at 80 μg/cm^2. These results indicate that the acaricidal activity of the essential oil of *P. graveolens* leaves can be mostly attributed to geraniol and β-citronellol, because geraniol was approximately 39 and 34 times more toxic than benzyl benzoate against *D. farinae* and *D. pteronyssinus*, respectively. Additionally, β-citronellol was approximately 36 and 33 times more toxic to *D. farinae* and *D. pteronyssinus* than benzyl benzoate. These results suggest that *D. farinae*

and *D. pteronyssinus* may be controlled more effectively by the application of the above compounds (geraniol and β-citronellol). Moreover, similar results have been exhibited in its congeners (nerol and citral). The volatile compounds of geraniol, citronellol, nerol, and citral were more effective than geranyl acetate and farnesol. Furthermore,
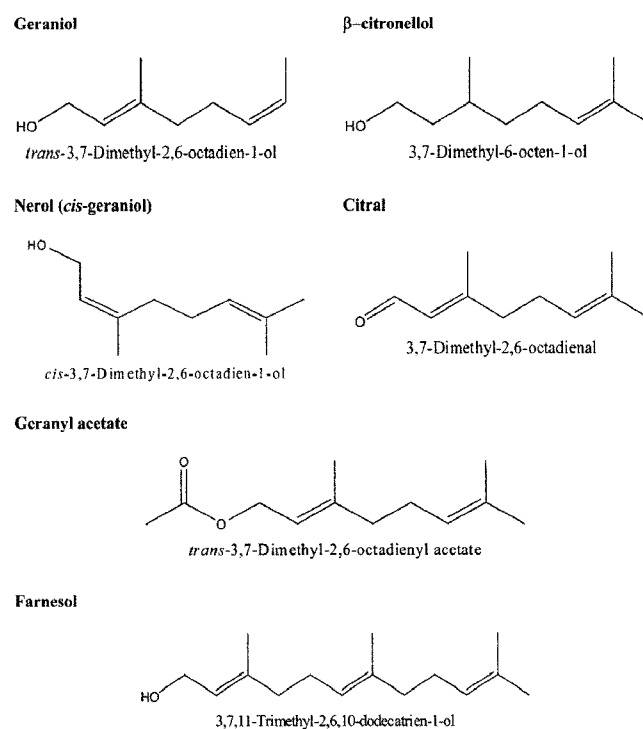
Fig. 2. Chemical structures of geraniol, β-citronellol, and their derivatives.

as for the relationship between structure and acaricidal activity against house dust mites, the monoterpenoid alcohols (geraniol, citronellol, nerol) and monoterpenoid aldehyde (citral) revealed more effective acaricidal activity than geranyl acetate and farnesol, which had four methyl groups and showed no activity against house dust mites. In addition, Lee and Lee [18] reported an acaricidal activity constituent of 5-hydroxy-1,4-naphthoquinone isolated from *Caesalpinia sappan* heartwoods against house dust mites. However, the 1,4-naphthoquinone congener (2-methyl-1,4-naphthoquinone) had a methyl group, and was found to be ineffective against house dust mites. These results indicate that the lack of methyl functional groups in a compound seems to result in effective activity against house dust mites.

Plant products are potential sources for dust mite control because many of them are selective to pests, with a few, if any, harmful effects on non-target organisms and the environment [14, 17, 30]. Furthermore, many plant extracts and essential oils are known to possess acaricidal activity against house dust mites [16–19, 21, 22]. The differential responses to arthropod pests are influenced by extrinsic and intrinsic factors, such as the plant species, parts of the plant, the solvent used for extraction, the geographical location where the plants were grown, and application methods [23]. As the essential oils and their purified constituents have a long history of global use by the food and fragrance industries, and recently in the field of aromatherapy, many of the oils and/or constituents that are pesticidal are readily available in great quantity at low to moderate cost [14]. Our study is the first to report the acaricidal properties of components derived from *P. graveolens* leaves against *D. farinae* and *D. pteronyssinus*. In a previous study, the oral LD_{50} values of geraniol and β-citronellol for rats were reported to be 3,600–4,800 mg/kg and 3,450 mg/kg, respectively [32]. Geraniol is commonly used in the fragrance industry and as a flavoring for alcoholic and non-alcoholic beverages, ice creams, candies, and baked goods. Annually, over 80,000 lb of the compound is used in cosmetics, soaps, and detergents. Geraniol, β-citronellol, nerol, and geranyl acetate are currently recognized by the U.S. Food and Drug Administration as GRAS ("generally regarded as safe") for their intended use as flavoring substances [12].

From this point of view, geraniol, β-citronellol, and their derivatives are most promising for possible use against *D. farinae* and *D. pteronyssinus* owing to their selective actions and their safety for mammals. Therefore, *P. graveolens* oil-derived materials could be useful as fumigants for house dust mites, and can be very useful in removing allergens. The key considerations in the use of chemicals indoors relate to their safety, the efficacy of the active ingredient, immunological testing for humans, and the formulation that optimizes the acaricidal potency and stability.

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