

Food Protective Effect of Geraniol and Its Congeners against Stored Food Mites

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ABSTRACT

The acaricidal activities of compounds derived from the oil of *Pelargonium graveolens* leaves against the storage food mite, *Tyrophagus putrescentiae*, were compared with the activity of a commercial acaricide, benzyl benzoate, in an impregnated fabric disk bioassay. Purification of the active constituent from *P. graveolens* was accomplished by silica gel chromatography and high-performance liquid chromatography. Structural analysis of the active constituent by ^1H nuclear magnetic resonance (NMR), ^{13}C -NMR, ^1H - ^{13}C shift correlated spectroscopy NMR, and distortionless enhancement by polarization transfer NMR identified *trans*-3,7-dimethyl-2,6-octadien-1-ol (geraniol). Based on the 50% lethal dose values, the most toxic compounds against *T. putrescentiae* were geraniol ($1.95\ \mu\text{g}/\text{cm}^3$), which was followed by nerol ($2.21\ \mu\text{g}/\text{cm}^3$), citral ($9.65\ \mu\text{g}/\text{cm}^3$), benzyl benzoate ($11.27\ \mu\text{g}/\text{cm}^3$), and β -citronellol ($15.86\ \mu\text{g}/\text{cm}^3$). Our results suggest that geraniol is more effective in controlling *T. putrescentiae* than benzyl benzoate is. Furthermore, geraniol, which is used as a flavoring for beverages, candies, ice creams, and baked goods and congeners (citral and nerol), may be useful for managing populations of *T. putrescentiae*.

The storage food mite, *Tyrophagus putrescentiae* (Schränk), is very important in stored products (8). *T. putrescentiae* is frequently found in stored foods with high fat and protein contents such as bacon, cheese, dried eggs, flours, and peanuts (15). Although the mites generally live on the external surface of these products, they sometimes penetrate and contaminate the products, resulting in serious economic losses (10). In addition, *T. putrescentiae* can cause diarrhea, damage to the urinary tract, enteritis, and allergic reactions when contaminated food is ingested, inhaled, or handled (2). Furthermore, *T. putrescentiae* can also transport bacterial and fungal spores such as *Aspergillus* spp. and *Penicillium* spp. (19), which are carried onto the surface of grains by the food mite or deposited with its feces (8). *T. putrescentiae* is controlled by chemical methods such as fumigation treatments and spraying with organophosphorus compounds. However, the repeated use of organophosphorus compounds has sometimes resulted in resistance (10, 16, 19). As a result, there has been growing interest in the use of plant essential oils and phytochemicals as alternatives to synthetic agents for protection against stored food mites (20, 22, 23).

The biological activities of the essential oils derived from medicinal plants against arthropods, bacteria, fungi, and several organisms have been confirmed in many studies, activities primarily due to the presence of monoterpenoids (3, 4, 12). Monoterpenes are among the most important compounds in pharmaceutical sciences and biological products and include the antitumor agent perilla alcohol, the antifungal agent pinene, and the insecticidal agent pyrethrin

(12). The essential oil of *Pelargonium graveolens* (Geraniaceae) has a comparatively strong hydrophilic quality and contains a high percentage of monoterpene alcohols such as citronellol, geraniol, linalool, and nerol (6). Because of the volatile compounds present in the essential oil of the rose-scented geranium (*Pelargonium* sp.), it is widely used in aromatherapy, the cosmetic industry, and for flavoring food (5). However, the acaricidal property of materials derived from the essential oil of *P. graveolens* leaves against stored food mites has not yet been investigated. Therefore, in this study, the acaricidal activities of the essential oil of *P. graveolens*, its major constituent, and the congeners were compared with a commonly used synthetic acaricide, benzyl benzoate.

MATERIALS AND METHODS

Chemicals and plant materials. Benzyl benzoate, citral, farnesol, geranyl acetate, and nerol were obtained from Sigma (St. Louis, MO). All other chemicals used in this study were of reagent grade. The leaves of *P. graveolens* were purchased from a local market in Chonju. The oil of *P. graveolens* leaves was extracted by steam distillation with cohobation for 8 h, as described previously (5). The essential oil (yield of 0.2%) was dried over anhydrous sodium sulfate and stored at 4°C.

Stored food mites. Cultures of *T. putrescentiae* were maintained in the laboratory for 7 years, without exposure to any known acaricides. The mites were maintained on fry feed no. 1 and dried yeast (1:1 [wt/wt]) and reared in plastic containers (15 by 12 by 6 cm) that held 30 g of a sterilized diet (Fig. 1). The rearing cages were kept in an incubator at $25 \pm 1^\circ\text{C}$ and 75% relative humidity in darkness, within a plastic tray (18 by 18 by 17 cm) that contained a saturated solution of potassium chloride to prevent the mites from escaping and to maintain high relative

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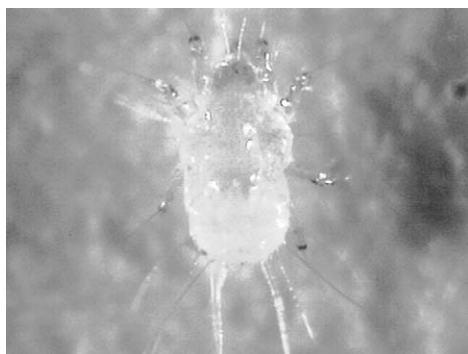


FIGURE 1. Stored food mite (*Tyrophagus putrescentiae*).

humidity. The fry feed was purchased from Korea Special Feed Meal Co., Ltd. (Chonju, Korea).

Isolation and identification. The essential oil (30 g) of *P. graveolens* was fractionated on a silica gel column (70-230 mesh, 700 g, 10-cm inside diameter, 60-cm height; Merck, Darmstadt, Germany) and then eluted with a stepwise gradient of hexane-ethyl acetate (10:1, 8:2, 7:3, 6:4, 5:5, and 1:9 [vol/vol]), which gave six fractions (C1 through C6) (13, 14). The bioactive fraction (C4, 10.8 g) was successively rechromatographed on a silica gel column (70-230 mesh, 600 g, 5.5-cm inside diameter, 80-cm height; Merck) by using hexane-ethyl acetate (7:3 [vol/vol]). The column fractions were analyzed by thin-layer chromatography (silica gel 60 F₂₅₄), and fractions with similar streaking patterns on the thin-layer chromatography plates were pooled. In this step, the five fractions (C41 through C45) were obtained and bioassayed at 80 µg/cm³. Next, the active fraction (C43) was purified by high-performance liquid chromatography (HPLC; recycling preparative HPLC, Japan Analytical Industry Co., Ltd., Tokyo) to separate the active constituent. The first column used was a Jaigel GS Series column (GS310 50 cm plus GS310 30 cm plus GS310 30 cm, 21.5-mm inside diameter, 500-mm height; Japan Analytical Industry Co., Ltd.), with chloroform (100%) applied at a flow rate of 5 ml/min as the mobile phase and detection at 299 nm. During this step, five fractions (C431 through C435) were obtained and bioassayed at a concentration of 80 µg/cm³. The active C434 fraction (4.6 g) was then further chromatographed on a Jaigel W Series column (W-253 50 cm plus W-252 50 cm, 20.0-mm inside diameter, 500-mm height; Japan Analytical Industry Co., Ltd.) with hexane-chloroform (8:2) at a flow rate of 5 ml/min as the mobile phase and detection at 299 nm. Finally, the potent active compound C43442 (921 mg) was isolated. The structures of the active principle were then determined by spectral analyses. ¹H nuclear magnetic resonance (NMR) and ¹³C-NMR spectra were recorded in deuteriochloroform by using a JNM-LA 400F7 spectrometer (JNM-ECA600, JEOL, Ltd., Japan) at 600 and 150 MHz (tetramethylsilane as the internal standard), respectively, and the chemical shift values (δ) are given in parts per million. Unambiguous ¹H- and ¹³C-NMR chemical shifts were obtained by using distortionless enhancement by polarization transfer, ¹H-¹H shift correlated spectroscopy, and ¹H-¹³C shift correlated spectroscopy spectra. UV spectra were obtained in chloroform by using a Jasco V-550 spectrometer.

Mite-control activity. An impregnated fabric disk method was used to determine the ability of the test materials to control mites. An impregnated fabric disk method slightly modified from that utilized by Jeong et al. (14) was used to determine the toxicity of mites against test materials. Twenty microliters of the various concentrations of test materials (80, 60, 40, 20, 10, 5.0, 2.0, and

1.0 µg/cm³) was applied to the paper disks by a pipettor (Advantec, Toyo Roshi, Ltd., Tokyo, Japan), with acetone alone being used as a control. The disks were then allowed to air dry in a fume hood (19°C) for 30 s, after which they were individually placed in the cap of a microtube (2 ml; Greiner Bio-One GmbH, Frickenhausen, Germany). Mites (7 to 10 days old) were placed individually in each microtube. There were three replications of 20 mites each per treatment. The microtubes were then sealed with the caps containing the treated paper disks. The treated and control mites were incubated at 25 ± 1°C and 75% relative humidity in the darkness for 24 h, after which the mortality was determined by observation under a binocular microscope (×20; Olympus, Tokyo, Japan). The mites were considered dead if their appendages did not move when stimulated with a fine pin. The stored food mite-control activity was then evaluated based on the mortality of the mites. Each treatment was replicated three times, and all assays were performed in an incubator with a constant temperature and humidity of 25 ± 1°C and 75% relative humidity, respectively, in dark conditions. The 50% lethal dose (LD₅₀) values were then calculated by probit analysis (7).

RESULTS AND DISCUSSION

Isolation and identification. Due to the strong activity of the essential oil extracted from *P. graveolens*, the active component was isolated. The purified active constituent was then identified by spectroscopic analyses, including HPLC, gas chromatography-mass spectrometry, electron-ionization mass spectrometry, and ¹³C- and ¹H-NMR, as well as by direct comparison with an authentic reference compound. The results of these analyses indicated that the active compound was *trans*-3,7-dimethyl-2,6-octadien-1-ol (geraniol; C₁₀H₁₈O, molecular weight of 154.25). Specifically, geraniol was identified based on the following evidence: electron-ionization mass spectrometry (70 eV) *m/z* (percent relative intensity) M⁺ 154 (100, base peak), 136 (21), 123 (18), 111 (16), 93 (14), 81 (8), 53 (5); ¹H-NMR (CD₃OD, 600 MHz); δ 5.40 to 5.43 (1H, m), 5.10 (1H, m), 4.15~4.16 (2H, d), 2.09~2.11 (2H, d), 2.02~2.03 (2H, d), 1.68~1.69 (3H, t), 1.61 (3H, t), 1.40 ppm (3H, t); ¹³C-NMR (CD₃OD, 150 MHz); δ 139.7, 131.7, 123.9, 123.3, 59.3, 39.5, 26.4, 25.6, 17.6, and 16.2 ppm. The spectroscopic data of geraniol were found to be the same as those for geraniol from the mesocarp of *Vitis vinifera* L. (18).

Mite-control activity. Evaluation of the essential oil extracted from *P. graveolens* leaves in an impregnated fabric disk bioassay indicated strong toxic activity against *T. putrescentiae* at various doses (Table 1). Benzyl benzoate, a widely used commercial acaricide, was used as a positive control. Mortality was 100% at doses ≥10 µg/cm³ of the essential oil. Mortality was 67.5, 37.5, and 7.5% at doses of 5.0, 3.0 and 2.0 µg/cm³, respectively. There was no mortality in the untreated controls. Mortality of the mites against the active constituent, geraniol, and its congeners (citral, β-citronellol, farnesol, geranyl acetate, and nerol) are shown in Table 1. Structural formulas of the congeners are given in Figure 2. Based on the LD₅₀ values, geraniol was the most active compound (LD₅₀ of 1.95 µg/cm³), which was followed by nerol (2.21 µg/cm³), citral (9.65 µg/cm³), and benzoate (11.27 µg/cm³). Only β-citronellol (15.86 µg/cm³) was less effective than benzyl benzoate

TABLE 1. Toxicity of *Pelargonium graveolens* oil, geraniol congeners, and synthetic acaricide against *Tyrophagus putrescentiae* adults, as determined by an impregnated fabric disk bioassay^a

Test material	Molecular wt	LD ₅₀ (μg/cm ³)	95% confidence limit	Relative toxicity ^b
Oil	ND ^c	4.17	1.11–4.25	2.7
Geraniol	154.24	1.95	1.92–1.98	5.8
β-Citronellol	156.27	15.86	15.82–19.00	0.7
Nerol	154.24	2.21	2.18–2.26	5.1
Citral	152.23	9.65	9.62–9.68	1.2
Geranyl acetate	196.29	— ^d	—	—
Farnesol	222.37	—	—	—
Benzyl benzoate	212.25	11.27	11.01–11.57	1.0

^a Twenty *T. putrescentiae* (7–10 days old) individuals (×3) were separately placed in each microtube (2 ml). Exposure time was 24 h.

^b Relative toxicity = LD₅₀ value of benzyl benzoate/LD₅₀ value of each test material.

^c ND, no data.

^d Not active at 80 μg/cm³ (initial testing concentration).

was. Our studies have shown that geraniol and most of its congeners were more active than the commercially available acaricide benzyl benzoate was. Geraniol, nerol, and citral were 5.8, 4.1, and 1.2 times more toxic than benzyl benzoate was, respectively. However, geranyl acetate and farnesol exerted no acaricidal activity at 80 μg/cm³. This study is the first to report the acaricidal properties of geraniol extracted from *P. graveolens* leaves against *T. putrescentiae* adults.

Structure-activity relationships. The results of this study also provided information regarding the relationship between the structure of the tested compounds and their acaricidal activity against stored food mites. Specifically, monoterpenoid alcohols (geraniol, citronellol, and nerol) and monoterpenoid aldehydes (citral) exerted greater acaricidal activity than did geranyl acetate and farnesol. One might speculate that β-citronellol in individual testing against stored food mite was not active; however, according to some reports, its role in mixtures of other compounds can vary. β-Citronellol was reported to be a more active acaricide than benzyl benzoate was in an earlier study against *Dermatophagoides* spp. (13). Based on prior studies, closely related species can vary widely in their responses to pure allelochemicals (1, 11). In particular, geraniol and its congeners, which are derived from plant essential oils, are known to induce the insecticidal effects of *Anisakis* larvae (9), housefly (17), and stored-rice pests (21). However, few studies have been produced to evaluate the effects of *P. graveolens* leaf-derived materials on mite control, in spite of its excellent insecticide effects.

In conclusion, these results indicate that geraniol and its congeners are more effective than synthetic acaricide is, and they may be useful for the control *T. putrescentiae*, due to their selective actions and minimal toxic effects against mammals. Indeed, in a previous study, the skin LD₅₀ values of geraniol for rabbits were reported to be >5,000 mg/kg (24). Moreover, geraniol is used as a food additive, and in

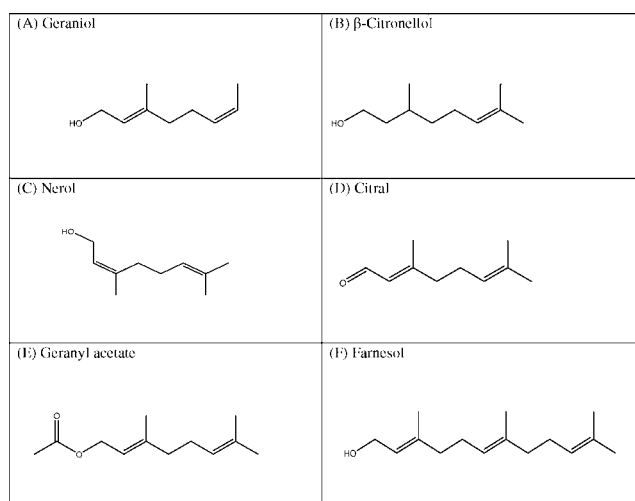


FIGURE 2. Chemical structures of geraniol and its congeners.

cosmetics and detergents, at a volume >80,000 lb (36,287 kg) annually (24). Furthermore, materials derived from the oil of *P. graveolens* may be very useful as fumigants for the control of stored food mites. Further studies should be conducted to determine if the indoor use of these chemicals is safe. In addition, the acaricidal mode of action, and the formulation that optimizes the acaricidal potency and stability of geraniol should be determined. Finally, immunological testing of geraniol against humans should be conducted.

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