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Vapor Phase Toxicity of Marjoram Oil Compounds and Their Related Monoterpenoids to *Blattella germanica* (Orthoptera: Blattellidae)

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The toxicity of marjoram, *Origanum majorana* L., oil, 41 monoterpenoids, and 2 sesquiterpenoids against adult females of the German cockroach, *Blattella germanica* L., was examined using direct contact and vapor phase toxicity bioassays and compared with those of deltamethrin, dichlorvos, permethrin, and propoxur, four commonly used insecticides. In a filter-paper contact toxicity bioassay, the adulticidal activities of pulegone (0.06 mg/cm^2), (±)-camphor (0.07 mg/cm^2), and verbenone (0.07 mg/cm^2) were comparable to that of permethrin (0.05 mg/cm^2) but more pronounced than that of propoxur (0.18 mg/cm^2), as judged by the 24-h LC_{50} values. These compounds were less effective than either deltamethrin (0.013 mg/cm^2) or dichlorvos (0.007 mg/cm^2). The toxicity of marjoram oil, thymol, α-terpineol, (−)-α-thujone, linalool, 1,8-cineole, (−)-camphor, and (+)-carvone, ranging from 0.08 to 0.18 mg/cm^2, was higher than that of propoxur. In vapor phase toxicity tests, verbenone (11.48 mg/L air) was the most toxic compound followed by (−)-α-thujone (18.43 mg/L of air), thymol (18.76 mg/L of air), α-terpineol (21.89 mg/L of air), (±)-camphor (24.59 mg/L of air), linalool (26.20 mg/L of air), and marjoram oil (38.28 mg/L of air) on the basis of the 24-h LC_{50} values. Dichlorvos (0.07 mg/L of air) was the most potent fumigant. Structure−activity relationships indicate that structural characteristics, such as degrees of saturation and types of functional groups rather than types of carbon skeleton, and hydrophobicity and vapor pressure parameters appear to play a role in determining the monoterpenoid toxicities to adult *B. germanica*. Marjoram oil and the monoterpenoids described merit further study as potential fumigants or leads for the control of *B. germanica*.

KEYWORDS: Natural insecticide; natural fumigant; marjoram oil; *Origanum majorana*; *Blattella germanica*; monoterpenoids; mode of action; structure−activity relationship

INTRODUCTION

The German cockroach, *Blattella germanica* L., is the most important primary medical insect pests because of its even cosmopolitan occurrence and abundance in homes and other buildings as potential carriers of fecal pathogens and a major source of allergens (*1*−*4*). Additionally, cockroach exuviae are found to support large populations of *Dermatophagoides pteronyssinus* Trouessart, resulting in exacerbated cases of bronchial asthma (*1*−*3*). Control of cockroach populations worldwide is largely dependent on continued applications of residual insecticides such as chlorpyrifos, dichlorvos (DDVP), propoxur, pyrethrin, and pyrethroids, stomach poisons such as hydramethylnon and sulfluamid, and insect growth regulators such as flufenoxuron (*2*, *3*). Their repeated use has disrupted natural biological control systems and led to resurgences of the cockroach (*2*) and has often resulted in the development of resistance (*2*, *5*). Increasing levels of resistance to the most commonly used insecticides have caused multiple and overdosed treatments, fostering serious human health concerns (*2*). These problems have highlighted the need for the development of selective control alternatives for *B. germanica*, particularly with fumigant action because many insecticides are repellent to them and are therefore avoided (*2*, *6*) and it is difficult to reach deep, insecticide-free harborages and to apply insecticides to sensitive environments such as computer facilities, food industrial facilities, and hospitals (*2*).

Plant essential oils have been suggested as an alternative source of materials for insect control because some of them are selective to certain pests, often biodegrade to nontoxic products,

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and have little or no harmful effects on nontarget organisms (*7*−*10*). They can be applied to the resting and hiding places in the same way as other conventional insecticides. They also provide useful information on resistance management because certain plant extracts or phytochemicals can be highly effective against insecticide-resistant insect pests (*11*, *12*). In addition, some plant essential oils or their constituents have been proposed as an alternative to the commonly used synthetic insecticides because they were exempted for minimum risk pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requirements (*13*). Furthermore, plant essential oils are widely available, and some are relatively inexpensive compared with plant extracts (*7*). Because of this, much effort has been focused on plant essential oils or their constituents as potential sources of commercial cockroach control agents. Insecticidal activity against cockroaches has been reported for some essential oils such as dennettia, hiba oil, and catnip essential oils (*14*−*18*). In a preliminary experiment, the essential oil of marjoram, *Origanum majorana* L. (Lamiaceae, formerly Labiatae), had potent insecticidal activity against female *B. germanica*. Marjoram oil has been considered to have medicinal properties such as an analgesic, an anaphrodisiac, an antispasmodic, an expectorant, a sedative, and a stomachic (*19*). These properties are attributable to cadinene, carvacrol, citral, eugenol, geranyl acetate, linalool, linalyl acetate, ocimene, sabinenes, terpinenes, and terpineol (*19*). Very little work has been done with respect to managing *B. germanica* with marjoram oil compounds, although the insecticidal activity of essential oils has been well described by Isman (*7*, *20*) and Singh and Upadhyay (*21*).

This paper describes a laboratory study aimed at isolating insecticidal constituents from marjoram oil active against female *B. germanica* and determining their insecticide route of action. Also, the contact and vapor phase toxicities of marjoram oil, 41 monoterpenoids, and 2 sesquiterpenoids were compared with those of four commonly used insecticides, deltamethrin, dichlorvos, permethrin, and propoxur. The structure−activity relationships of monoterpenoids are discussed.

MATERIALS AND METHODS

Chemicals. Forty-four terpene compounds used in this study were as follows: (−)-camphor, carvacrol, (−)-carveol, α-humulene, menthone, (+)-perillaldehyde, α-phellandrene, pulegone, and (−)-α-thujone purchased from Fluka (Buchs, Switzerland); (+)-borneol, camphene, (±)-camphor, 2-carene, 3-carene, (*E*)-cinnamaldehyde, (*E*)-cinnamic acid, cinnamyl acetate, cinnamyl alcohol, linalyl acetate, β-myrcene, α-pinene, β-pinene, thymol, and verbenol from Aldrich (Milwaukee, WI); (+)-carvone, citral, citronellal, citronellol, citronellic acid, linalool, menthol, fenchone, terpinen-4-ol, and verbenone from Sigma (St. Louis, MO); geraniol and β-caryophyllene from Tokyo Kasei (Tokyo, Japan); and 1,8-cineole, geranyl acetate, (−)-limonene, paeonol, α-terpinene, γ-terpinene, and α-terpineol from Wako (Osaka, Japan). Structures of these monoterpenoids are given in **Figure 1**. Values of hydrophobic and vapor pressure parameters for the tested monoterpenoids were calculated using ACD/Log P v 8.02 and ACD/Boiling Point and Vapor Pressure v 8.02 (ACD/I-Lab, Montreal, Canada), respectively. The results of ACD/Log P v 8.02 and ACD/Boiling Point and Vapor Pressure v 8.02 were obtained using the ACD/I-Lab service. Marjoram oil was purchased from Polarome International (Jersey, NJ). Deltamethrin (98% purity) and dichlorvos (DDVP, 99% purity) were obtained from the Department of Agricultural Biology, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon, Korea. Permethrin (95% purity) and propoxur (98% purity) were obtained from the National Institute of Health, Korea Center for Disease Control and Prevention, Seoul, Korea.

Cockroaches. Cultures of *B. germanica* were maintained in the laboratory for 9 years without exposure to any known insecticide. They were reared with calf chow pellets (Samyang, Seoul) in glass jars (30 cm diameter × 30 cm) at 27 ± 1 °C and 55 ± 5% relative humidity (RH) under a 12:12 h light/dark cycle.

Gas Chromatography−Mass Spectroscopy (GC-MS) Analysis of Marjoram Oil. GC-MS analysis of marjoram oil was performed using a GC-MS spectrometer (QP 2010), equipped with a splitless injector. Analytes were separated with a 0.32 mm i.d. × 60 m DB-1MS capillary column (Agilent/J&W Scientific) with a film thickness of 0.25 μm. The temperature program used for the analysis was as follows: initial temperature at 80 °C, held for 5 min, ramped at 5.0 °C/min to 280 °C, and held for 10 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The ion source temperature was set to 200 °C, and the injector was set to 210 °C. The interface was kept at 280 °C, and mass spectra were obtained at 70 eV. The effluent of the capillary column was introduced directly into the ion source of the mass spectrometer. The sector mass analyzer was set to scan from 50 to 500 amu every 0.5 s. Components of marjoram oil were identified by comparison of mass spectra of each peak with those of authentic samples in a mass spectra library (The Wiley Registry of Mass Spectral Data, 7th ed.).

Contact Toxicity Bioassay. A filter-paper contact toxicity bioassay (*22*) was used to evaluate the toxicity of marjoram oil, 41 monoterpenoids, 2 sesquiterpenoids and four insecticides, deltamethrin, dichlorvos, permethrin, and propoxur, to adult female *B. germanica*. Cockroaches were exposed to appropriate amounts of materials, each of which were dissolved in 50 μL of methanol or acetone and applied to filter papers (Whatman no. 2; 5 cm diameter). Control filter papers received 50 μL of methanol or acetone. After drying in a fume hood for 2 min, each filter paper was placed on the bottom of a polyvinyl chloride (PVC) container (120 mL). Groups of 10 females (7−8 days old) were separately placed on each container containing calf chow pellets and covered with a lid.

Treated and control (methanol or acetone only) females were held at the same conditions used for colony maintenance. Adult mortalities were determined 24 h after treatment. Adults were considered to be dead if appendages did not move when they were prodded with a wooden dowel. All treatments were replicated three times. The LC_{50} values were calculated by probit analysis (*23*). The toxicity was considered to be significantly different when 95% confidence limit levels of the LC_{50} values failed to overlap.

Insecticide Route of Action. Experiments were conducted to determine whether the lethal activity of marjoram oil and 10 selected monoterpenoids against adult *B. germanica* was attributable to contact or vapor phase toxicity. Groups of 10 females (7−8 days old) were separately introduced into the PVC containers (120 mL). Then, the container was covered with gauze. Appropriate amounts (3.6−30 mg) of each marjoram oil and monoterpenoid in 50 μL of methanol or acetone were applied to filter papers (Whatman no. 2; 4.25 cm diameter), which is equivalent to 30−250 mg/L of air. Dichlorvos served as a standard insecticide for comparison in toxicity tests. After drying in a fume hood for 2 min, each treated filter paper was attached to the inner side of a lid with a small amount of solid glue. It did not affect adversely adult *B. germanica*. Control filter papers received 50 μL of methanol or acetone. This prevented direct contact of adult females with the tested materials. Each container was then either covered with a lid (method A) to investigate the potential vapor phase toxicity of the tested materials or left uncovered (method B). Mortalities were determined 24 h after treatment. All treatments were replicated three times.

Vapor Phase Toxicity Bioassay. Fumigant toxicity of marjoram oil, 41 monoterpenoids, and 2 sesquiterpenoids against adult female *B. germanica* was investigated using the vapor phase toxicity bioassay as above. Groups of 10 females (7−8 days old) were separately placed on the bottom of a PVC container (120 mL). The container was then covered with gauze. Appropriate amounts (0.63−96 mg) of each tested material in 50 μL of methanol or acetone were applied to filter papers (Whatman no. 2; 4.25 cm diameter), which is equivalent to 5.25−800 mg/L of air. Dichlorvos served as a standard for comparison in fumigant toxicity tests. After drying in a fume hood for 2 min, each treated paper was attached to the inner side of a lid with a small amount of solid glue and the container was covered with the lid. Control filter papers received 50 μL of methanol or acetone.

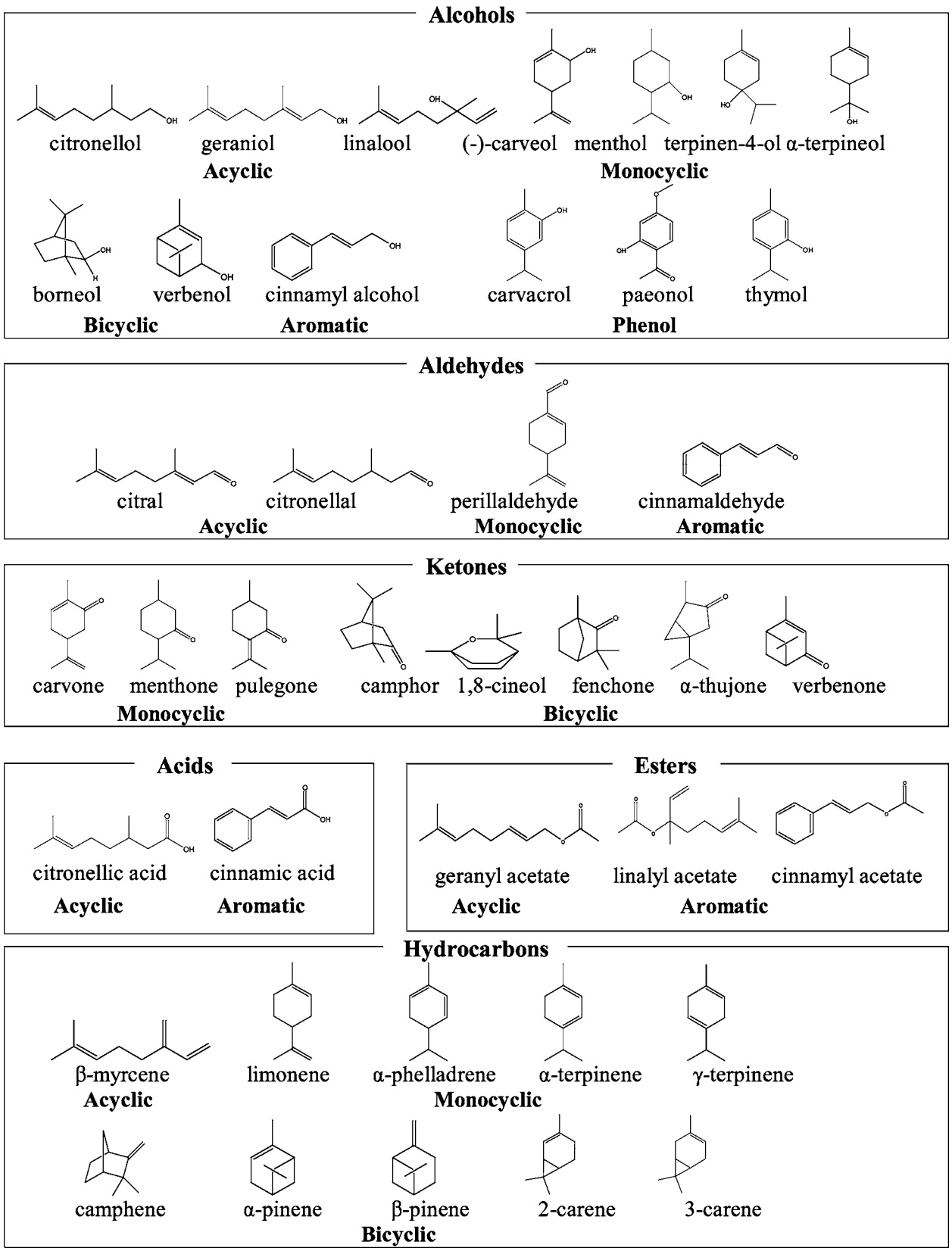

Figure 1. Structures of the 41 monoterpenoids tested.

Treated and control (methanol or acetone) females were held at the same conditions used for colony maintenance. Evaluation of adulticidal activity was made 24 h after treatment. All treatments were replicated three times. The LC_{50} values were calculated by probit analysis (*23*). The toxicity was considered to be significantly different when 95% confidence limit levels of the LC_{50} values failed to overlap.

RESULTS

Chemical Constituents of Marjoram Oil. Marjoram oil was composed of 4 major and 22 minor constituents by comparison of mass spectral data and retention times of authentic compounds (**Table 1**). The four major constituents, 1,8-cineole, linalool,

Table 1. Chemical Constituents of Marjoram Oil Identified by GC-MS

compound	retention time (min)	relative %
α-thujene	8.73	0.26
α-pinene	8.96	1.59
sabinene	9.90	3.25
β-pinene	10.07	0.38
β-myrcene	10.28	0.93
α-phellandrene	10.78	0.40
α-terpinene	11.14	3.49
p-cymene	11.21	2.54
1,8-cineole	11.50	41.50
cis-ocimene	11.59	0.59
trans-ocimene	11.94	0.29
γ-terpinene	12.35	6.48
trans-sabinene hydrate	12.49	1.89
α-terpinolene	13.29	1.46
linalool	13.39	11.11
cis-*p*-menth-2-en-1-ol	14.16	0.56
trans-*p*-menth-2-en-1-ol	14.66	0.40
terpinen-4-ol	15.88	10.60
α-terpineol	16.18	1.82
β-citronellol	17.25	0.66
geraniol	17.98	3.18
linalyl acetate	18.16	1.31
thymol	18.94	0.52
β-caryophyllene	23.27	1.54
α-humulene	24.11	0.10
biocyclogermacrene	25.14	0.82

Table 2. Toxicity of Marjoram Oil and Four Insecticides against Adult Female *B. germanica* Using the Filter-Paper Contact Toxicity Bioassay during a 24-h Exposure

material	n^a	slope (± SE)	LC_{50} (mg/cm^2)	95% cl[b]	RT[c]
marjoram oil	240	3.13 ± 0.61	0.08	0.06–0.09	2.25
deltamethrin	240	1.87 ± 0.66	0.013	0.006–0.018	13.85
dichlorvos	240	2.18 ± 0.53	0.007	0.005–0.010	25.71
permethrin	240	4.60 ± 0.82	0.05	0.04–0.06	3.60
propoxur	240	2.99 ± 0.64	0.18	0.14–0.23	1.00

[a] Number of *B. germanica* females tested. [b] Confidence limit. [c] Relative toxicity = LD_{50} value of propoxur/LD_{50} value of the other compound.

terpinen-4-ol, and γ-terpinene, comprised 41.50, 11.11, 10.60, and 6.48% of the oil, respectively.

Contact Toxicity of Marjoram Oil and Its Constituents. The toxicity of marjoram oil and four insecticides, deltamethrin, dichlorvos, permethrin, and propoxur, to adult female *B. germanica* was evaluated by comparing the LC_{50} values estimated from direct contact toxicity bioassay (**Table 2**). As judged by the 24-h LC_{50} values, marjoram oil (0.08 mg/cm^2) was more active than propoxur (0.18 mg/cm^2) but less effective than either dichlorvos (0.007 mg/cm^2), deltamethrin (0.013 mg/cm^2), or permethrin (0.05 mg/cm^2). There was no mortality in the solvent-treated controls.

The toxic effects of 16 marjoram oil compounds on adult female *B. germanica* were compared with those of the 4 insecticides as above (**Table 3**). On the basis of the 24-h LC_{50} values, potent adulticidal activity (LC_{50}, 0.09−0.13 mg/cm^2) was observed in 1,8-cineole, linalool, α-terpineol, and thymol. These compounds were more toxic than propoxur but less effective than either dichlorvos, deltamethrin, or permethrin. Moderate adulticidal activity (LC_{50}, 0.28−0.50 mg/cm^2) was obtained from α-phellandrene, γ-terpinene, and terpinen-4-ol. Weak adulticidal activity (LC_{50}, 1.23−2.81 mg/cm^2) was produced from β-myrcene, α-pinene, β-pinene, and α-terpinene. β-Caryophyllene, citronellol, geraniol, α-humulene, and linalyl acetate were ineffective.

Table 3. Toxicity of Marjoram Oil Compounds against Adult Female *B. germanica* Using the Filter-Paper Contact Toxicity Bioassay during a 24-h Exposure

compound	n^a	slope (± SE)	LC_{50} (mg/cm^2)	95% cl[b]	RT[c]
β-caryophyllene	80		>10		
1,8-cineole	240	5.45 ± 1.05	0.13	0.11–0.15	1.38
citronellol	80		>10		
geraniol	80		>10		
α-humulene	80		>10		
linalool	240	3.49 ± 0.60	0.12	0.10–0.15	1.50
linalyl acetate	80		>10		
β-myrcene	240	7.77 ± 2.03	2.81	2.47–3.15	0.06
α-phelladrene	150	6.72 ± 1.29	0.28	0.25–0.31	0.64
α-pinene	240	9.53 ± 2.77	2.77	2.50–3.05	0.06
β-pinene	240	3.03 ± 0.79	1.23	1.00–1.79	0.15
α-terpinene	240	6.49 ± 1.99	2.61	2.08–2.95	0.07
γ-terpinene	240	2.80 ± 0.61	0.50	0.38–0.64	0.36
terpinen-4-ol	150	3.80 ± 0.81	0.42	0.35–0.51	0.43
α-terpineol	240	2.68 ± 0.67	0.10	0.07–0.14	1.80
thymol	240	2.93 ± 0.68	0.09	0.08–0.13	2.00

[a] Number of *B. germanica* females tested. [b] Confidence limit. [c] Relative toxicity = LD_{50} value of propoxur in **Table 2**/LD_{50} value of the other compound.

Contact Toxicity of 41 Monoterpenoids. The insecticidal activity of 41 monoterpenoids used against adult female *B. germanica* was compared with those of deltamethrin, dichlorvos, permethrin, and propoxur as above (**Table 4**). Potencies varied according to compound tested. As judged by the 24-h LC_{50} values, the adulticidal activity of pulegone (LC_{50}, 0.06 mg/cm^2), (±)-camphor (0.07 mg/cm^2), and verbenone (0.07 mg/cm^2) was comparable to that of permethrin. The toxicity of linalool, α-terpineol, thymol, (+)-perillaldehyde, (−)-camphor, 1,8-cineole, and (−)-α-thujone, ranging from 0.09 to 0.18 mg/cm^2, was higher than that of propoxur. Moderate contact toxicity (LC_{50}, 0.23−0.56 mg/cm^2) was observed with (*E*)-cinnamic acid, (−)-carveol, menthol, terpinen-4-ol, verbenol, carvacrol, citral, citronellal, (*E*)-cinnamaldehyde, (+)-carvone, menthone, fenchone, α-phellandrene, γ-terpinene, and 2-carene. Weak or no contact toxicity was produced from the other 16 monoterpenoids. No mortality was observed in the solvent-treated controls.

Insecticide Route of Action. The fumigant toxicity of marjoram oil, 10 selected monoterpenoids, and dichlorvos to adult female *B. germanica* was investigated using a vapor phase toxicity bioassay in two formats (**Table 5**). Responses were dependent on treatment method. After 24 h of exposure to 80 mg/L of air, there was a significant difference in lethal activity of marjoram oil between exposure in a closed container (method A), which resulted in 100% mortality, and exposure in an open container (method B), which resulted in 0% mortality against female *B. germanica*. Similar differences in the response of female *B. germanica* to 2-carene, 1,8-cineole, fenchone, linalool, menthone, (+)-perillaldehyde, γ-terpinene, α-terpineol, thymol, and verbenone in treatments A and B were likewise observed. Dichlorvos exhibited potent fumigant toxicity.

Fumigant Toxicity of Marjoram Oil and Its Constituents. Because of the fumigant activity of marjoram oil and 10 selected monoterpenoids, the fumigant toxicity of marjoram oil and its constituents to adult female *B.* germanica was examined (**Table 6**). As judged by the 24-h LC_{50} values, potent fumigant toxicity was observed with marjoram oil (LC_{50}, 38.28 mg/L of air). Of the marjoram oil constituents, potent fumigant toxicity was observed in thymol (LC_{50}, 18.76 mg/cm^2), α-terpineol (21.89 mg/cm^2), and linalool (26.20 mg/cm^2). Moderate fumigant toxicity (LC_{50}, 56.75−92.97 mg/cm^2) was obtained from 1,8-

Table 4. Insecticidal Activity of 41 Monoterpenoids (MT) against Adult Female *B. germanica* Using the Filter-Paper Contact Toxicity Bioassay during a 24-h Exposure

monoterpenoid	n^a	slope (± SE)	LC_{50} (mg/cm^2)	95% cl[b]	RT[c]
MT acids					
citronellic acid	80		>10		
(*E*)-cinnamic acid	150	6.67 ± 1.29	0.56	0.50–0.62	0.32
MT alcohols					
citronellol	80		>10		
geraniol	80		>10		
linalool	240	3.49 ± 0.60	0.12	0.10–0.15	1.50
(−)-carveol	150	6.30 ± 2.09	0.30	0.26–0.43	0.60
menthol	150	3.36 ± 0.89	0.29	0.23–0.45	0.62
terpinen-4-ol	150	3.80 ± 0.81	0.42	0.35–0.51	0.43
α-terpineol	240	2.68 ± 0.67	0.10	0.07–0.14	1.80
(+)-borneol	240	4.04 ± 1.15	2.67	2.15–3.15	0.07
verbenol	150	3.53 ± 0.66	0.26	0.22–0.32	0.69
cinnamyl alcohol	80		>10		
carvacrol	150	4.44 ± 0.82	0.29	0.25–0.33	0.62
paeonol	80		>10		
thymol	240	2.93 ± 0.68	0.09	0.08–0.13	2.00
MT aldehydes					
citral	150	3.68 ± 0.78	0.50	0.42–0.59	0.36
citronellal	150	7.74 ± 2.10	0.28	0.25–0.33	0.64
(+)-perillaldehyde	150	3.44 ± 0.76	0.18	0.15–0.22	1.00
(*E*)-cinnamaldehyde	150	4.21 ± 0.78	0.23	0.19–0.27	0.82
MT ketones					
(+)-carvone	150	3.42 ± 0.84	0.25	0.21–0.35	0.60
menthone	150	4.91 ± 1.90	0.25	0.21–0.34	0.72
pulegone	150	3.88 ± 1.16	0.06	0.05–0.08	3.00
(±)-camphor	240	2.47 ± 0.65	0.07	0.05–0.09	2.57
(−)-camphor	240	2.30 ± 0.42	0.13	0.10–0.17	1.38
1,8-cineole	240	5.45 ± 1.05	0.13	0.11–0.15	1.38
fenchone	150	6.30 ± 2.09	0.30	0.26–0.43	0.60
(−)-α-thujone	120	3.47 ± 0.68	0.09	0.07–0.11	2.00
verbenone	150	3.17 ± 1.12	0.07	0.05–0.11	2.57
MT hydrocarbons					
β-myrcene	240	7.77 ± 2.03	2.81	2.47–3.15	0.06
(−)-limonene	240	7.56 ± 2.72	2.58	2.05–2.86	0.07
α-phelladrene	150	6.72 ± 1.29	0.28	0.25–0.31	0.64
α-terpinene	240	6.49 ± 1.99	2.61	2.08–2.95	0.07
γ-terpinene	240	2.80 ± 0.61	0.50	0.38–0.64	0.36
camphene	240	4.79 ± 0.83	0.97	0.86–1.12	0.19
α-pinene	240	9.53 ± 2.77	2.77	2.50–3.05	0.06
β-pinene	240	3.03 ± 0.79	1.23	1.00–1.79	0.15
2-carene	240	4.24 ± 0.85	0.31	0.27–0.38	0.58
3-carene	150	5.25 ± 1.23	1.26	1.11–1.48	0.14
MT esters					
geranyl acetate	80		>10		
linalyl acetate	80		>10		
cinnamyl acetate	80		>10		

[a] Number of *B. germanica* females tested. [b] Confidence limit. [c] Relative toxicity = LD_{50} value of propoxur in **Table 2**/LD_{50} value of the other compound.

cineole, α-phellandrene, and terpinen-4-ol. Weak fumigant toxicity was produced from β-myrcene, α-pinene, β-pinene, α-terpinene, and γ-terpinene. β-Caryophyllene, citronellol, geraniol, α-humulene, and linalyl acetate were ineffective.

Fumigant Toxicity of 41 Monoterpenoids. The fumigant toxicity of the 41 monoterpenoids tested to adult female *B. germanica* was compared with that of dichlorvos (**Table 7**). On the basis of 24-h LC_{50} values, verbenone (11.48 mg/L of air) was the most toxic fumigant followed by (−)-α-thujone (18.43 mg/L of air), thymol (18.76 mg/L of air), α-terpineol (21.89 mg/L of air), (±)-camphor (24.59 mg/L of air), and linalool (26.20 mg/L of air). Moderate toxicity (LC_{50}, 42.71−92.97 mg/L air) was observed with terpinen-4-ol, (+)-perillaldehyde, menthone, pulegone, (−)-camphor, 1,8-cineole, fenchone, and α-phellandrene. The other 27 monoterpenoids exhibited weak or no fumigant activity. All tested compounds were less effective than dichlorvos (LC_{50}, 0.07 mg/L of air).

Table 5. Route of Insecticidal Action of Majoram Oil, Monoterpenoids, and Dichlorvos against Adult Female *B. germanica* Using the Vapor Phase Toxicity Bioassay during a 24-h Exposure

material	dose (mg/L of air)	mortality (%, mean ± SE) n^a	A[b]	n^a	B[b]
majoram oil	80	30	100 ± 0.0	30	0 ± 0.0
2-carene	250	30	100 ± 0.0	30	0 ± 0.0
1,8-cineole[c]	200	30	100 ± 0.0	30	0 ± 0.0
fenchone	100	30	100 ± 0.0	30	0 ± 0.0
linalool[c]	80	30	100 ± 0.0	30	0 ± 0.0
menthone	150	30	100 ± 0.0	30	0 ± 0.0
(+)-perillaldehyde	150	30	100 ± 0.0	30	0 ± 0.0
γ-terpinene[c]	80	30	100 ± 0.0	30	0 ± 0.0
α-terpineol[c]	80	30	100 ± 0.0	30	0 ± 0.0
thymol[c]	80	30	100 ± 0.0	30	0 ± 0.0
verbenone	30	30	100 ± 0.0	30	0 ± 0.0
dichlorvos	0.4	30	93 ± 3.3	30	30 ± 3.3

[a] Number of *B. germanica* females tested. [b] A, vapor in closed containers; B, vapor in open containers. [c] Compounds identified in this study.

Table 6. Fumigant Activity of Marjoram Oil and Its Constituents against Female Adult *B. germanica* Using the Vapor Phase Toxicity Bioassay during a 24-h Exposure

material	n^a	slope (± SE)	LD_{50} (mg/L of air)	95% cl[b]
marjoram oil	180	3.95 ± 0.79	38.28	31.79–45.81
β-caryophyllene	80		>800	
1,8-cineole	150	3.18 ± 0.75	92.97	73.55–115.85
citronellol	80		> 800	
geraniol	80		> 800	
α-humulene	80		>800	
linalool	150	4.42 ± 0.81	26.20	22.90–30.15
linalyl acetate	80		>800	
β-myrcene	150	6.84 ± 1.14	310.49	287.91–337.51
α-phellandrene	150	7.06 ± 1.36	80.61	72.88–89.86
α-pinene	150	3.85 ± 0.87	218.17	182.96–269.87
β-pinene	150	3.05 ± 0.82	143.76	115.22–226.97
α-terpinene	150	6.57 ± 1.14	332.29	307.44–336.91
γ-terpinene	150	2.84 ± 0.78	206.86	162.98–278.63
terpinen-4-ol	150	2.96 ± 0.48	56.75	45.96–69.99
α-terpineol	150	5.90 ± 1.24	21.89	19.43–24.80
thymol	150	4.25 ± 0.72	18.76	16.23–21.85

[a] Number of *B. germanica* females tested. [b] Confidence limit.

Structure−Activity Relationships. Comparisons were made to determine contact and fumigant toxicity differences involving the skeletal structure, degree of unsaturation, and functional groups of monoterpenoids using the toxicity data obtained. In both bioassays, ketones appear to be more toxic than analogous alcohols (verbenone versus verbenol). Aldehydes were more effective fumigants than their corresponding alcohols (cinnamaldehyde versus cinnamyl alcohol; citral versus geraniol; citronellal versus citronellol), acids (citronellal versus citronellic acid; cinnamaldehyde versus cinnamic acid), and an analogous ketone [(+)-perilladehyde versus (+)-carvone]. The monocyclic ketone pulegone containing one carbon−carbon double bond was a more active insecticide than the saturated monocyclic ketone menthone or the monocyclic ketone (+)-carvone containing two double bonds. The toxicity of the bicyclic ketone verbenone containing one double bond was more pronounced than that of the saturated bicyclic ketone fenchone. The toxicity of the diunsaturated aldehyde citronellal was more pronounced than that of the monosaturated aldehyde citral. With the exception of carvacrol, thymol containing three double bonds and α-terpineol with one double bond were more active than carveol with two double bonds and menthol with no double bonds. No apparent structure−activity relationships were found

Table 7. Fumigant Activity of 41 Monoterpenoids (MT) and Dichlorvos against Female Adult *B. germanica* Using the Vapor Phase Toxicity Bioassay during a 24-h Exposure

monoterpenoid	n^a	slope (± SE)	LD_{50} (mg/L of air)	95% cl[b]
MT acids				
citronellic acid	80		>800	
(*E*)-cinnamic acid	150	5.18 ± 1.27	367.11	323.22–454.14
MT alcohols				
citronellol	80		>800	
geraniol	80		>800	
linalool	150	4.42 ± 0.81	26.20	22.90–30.15
(–)-carveol	150	4.11 ± 0.92	264.91	223.93–340.17
menthol	150	4.25 ± 0.73	105.47	88.45–126.59
terpinen-4-ol	150	2.96 ± 0.48	56.75	45.96–69.99
α-terpineol	150	5.90 ± 1.24	21.89	19.43–24.80
(+)-borneol	150	2.42 ± 0.79	291.99	218.31–431.80
verbenol	150	3.15 ± 0.67	148.95	120.01–202.28
cinnamyl alcohol	80		>800	
carvacrol	150	2.87 ± 0.75	235.83	188.36–329.61
paeonol	80		>800	
thymol	150	4.25 ± 0.72	18.76	16.23–21.85
MT aldehydes				
citral	150	3.21 ± 0.75	196.25	157.10–246.04
citronellal	150	3.96 ± 0.70	106.10	88.06–128.50
(+)-perillaldehyde	150	2.48 ± 0.60	64.71	49.74–92.52
(*E*)-cinnamaldehyde	150	6.46 ± 1.30	254.72	228.52–286.46
MT ketones				
(+)-carvone	150	3.96 ± 0.70	102.86	85.38–124.53
menthone	150	3.59 ± 0.68	63.61	52.35–80.00
pulegone	150	3.77 ± 1.18	47.02	38.09–72.84
(±)-camphor	150	5.89 ± 0.91	24.59	22.04–27.35
(–)-camphor	150	2.39 ± 0.41	84.03	65.53–110.07
1,8-cineole	150	3.18 ± 0.75	92.97	73.55–115.85
fenchone	150	2.36 ± 0.62	42.71	32.20–74.78
(–)-α-thujone	150	2.85 ± 0.64	18.43	13.04–23.29
verbenone	150	2.29 ± 0.59	11.48	7.98–15.38
MT hydrocarbons				
β-myrcene	150	6.84 ± 1.14	310.49	287.91–337.51
(–)-limonene	150	6.65 ± 1.63	341.08	309.72–418.21
α-phellandrene	150	7.06 ± 1.36	80.61	72.88–89.86
α-terpinene	150	6.57 ± 1.14	332.29	307.44–336.91
γ-terpinene	150	2.84 ± 0.78	206.86	162.98–278.63
camphene	150	5.54 ± 1.24	126.37	105.63–142.49
α-pinene	150	3.85 ± 0.87	218.17	182.96–269.87
β-pinene	150	3.05 ± 0.82	143.76	115.22–226.97
2-carene	150	4.84 ± 1.19	157.95	137.08–185.51
3-carene	150	3.98 ± 0.81	213.57	179.30–259.24
MT esters				
geranyl acetate	80		>800	
linalyl acetate	80		>800	
cinnamyl acetate	80		>800	
insecticide				
dichlorvos	240	2.18 ± 0.53	0.007	0.005–0.010

[a] Number of *B. germanica* females tested. [b] Confidence limit.

among the types of carbon skeleton of each alcohol, aldehyde, ketone, and hydrocarbon.

Linear regression analyses of the contact and fumigant toxicities of monoterpenoids against female *B. germanica* were determined using their LC_{50} values and the values of the physical parameters for the tested compounds. Compounds showing LC_{50} values of >10 mg/cm^2 or >800 mg/L of air were excluded for the analysis. Neither hydrophobicity ($r^2 = 0.35$, $n = 33$) nor vapor pressure ($r^2 = 0.10$, $n = 33$) parameters were significantly related to the observed toxicities of the tested compounds in the contact and vapor phase toxicity bioassays.

DISCUSSION

In the laboratory study with *B. germanica*, marjoram oil exhibited potent contact and fumigant toxicity against adult females. The contact toxicity of the essential oil was higher than that of propoxur but lower than those of deltamethrin, dichlorvos, and permethrin. This is apparently the first report on the insecticidal activity of marjoram oil against *B. germanica*.

Various compounds, including phenolics, terpenoids, and alkaloids, exist in plant essential oils and jointly or independently they contribute to bioefficacy such as insecticidal, ovicidal, repellent, and antifeeding activities against various insect species (*7*, *18*, *20*, *24*, *25*). Much effort has been focused on the determination of the distribution, nature, and practical use of plant essential oil-derived chemical substances that have insecticidal activity. Ngoh et al. (*25*) reported the contact and fumigant toxicity of benzene derivatives eugenol, methyleugenol, isosafrole, and safrole but neither contact nor fumigant toxic effects of the terpenoids cineole, *p*-cymene, limonene, and α-pinene of essential oils against adult female *Periplaneta americana* L. In the current study, four marjoram oil constituents, 1,8-cineole, linalool, α-terpineol, and thymol, showed potent insecticidal activity against female *B. germanica*. Of the monoterpenoids, the contact toxicity of pulegone, (±)-camphor, and verbenone was comparable to that of permethrin. The toxicity of thymol, α-terpineol, thujone, linalool, 1,8-cineole, (−)-camphor, and (+)-carvone was higher than that of propoxur. These compounds were less effective than either deltamethrin or dichlorvos.

Elucidation of the mode of action of essential oils and their constituents is of practical importance for insect control because it may give useful information on the most appropriate formulation, delivery means, and resistance management. Volatile compounds of many plant extracts and essential oils consist of alkanes, alcohols, aldehydes, and terpenoids, particularly monoterpenoids (*26*, *27*), and exhibit fumigant activity (*15*, *22*, *24*, *28*). In the present study, marjoram oil, 2-carene, 1,8-cineole, fenchone, linalool, menthone, (+)-perilladehyde, γ-terpinene, α-terpineol, thymol, and verbenone were much more effective in closed versus open containers against female *B. germanica*. These results indicate that the mode of delivery of these compounds was likely by vapor action via the respiratory system, although the exact mode of action remains unknown. Of the mono- and sesquiterpenoids used, potent fumigant toxicity was observed in verbenone, (−)-α-thujone, thymol, α-terpineol, camphor, and linalool. However, these compounds were less effective than dichlorvos.

Structure−activity relationships of plant compounds against insect pests have been well studied. Rice and Coats (*29*) and Tsao et al. (*30*) attempted to enhance the potency of selected monoterpenes and phenols through derivatization of the hydroxyl group. They found that enhanced bioactivity of the derivatives appeared to result from increased vapor pressure, leading to greater fumigant action, and/or increased lipophilicity, leading to better penetration and bioavailability in the insect's body. In the current study, structural characteristics such as degrees of saturation and types of functional groups rather than types of carbon skeleton appear to play a role in determining the monoterpenoid toxicities to female *B. germanica*. Neither hydrophobicity nor vapor pressure parameters were significantly related to the observed monoterpenoid toxicities. The difference between our present results and previous other studies might be attributed to the difference in either application method (filter paper versus topical application) or different physiological/biochemical characteristics between cockroach and housefly.

The results of the present study indicate that marjoram oil and test monoterpenoids [1,8-cineole, (±)-camphor, linalool,

α-terpineol, (−)-α-thujone, thymol, and verbenone] could be useful as insect control fumigants for *B. germanica* in enclosed spaces such as inaccessible cockroach hiding places such as crevices or electrical or plumbing ducts, buildings, and cabinet voids because of their fumigant action. For the practical use of marjoram oil and these monoterpenoids as novel fumigants to proceed, further research is required on the safety issues for human health. Other areas requiring attention are insecticide mode of action and formulations, including a carrier giving a slow release of active material, to improve insecticidal potency and stability and to reduce cost.

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