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Ovicidal and Adulticidal Activities of *Origanum majorana* Essential Oil Constituents against Insecticide-Susceptible and Pyrethroid/Malathion-Resistant *Pediculus humanus capitis* (Anoplura: Pediculidae)

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The toxicity of essential oil constituents from marjoram, *Origanum majorana*, to eggs and adult females of the susceptible KR-HL and dual malathion- and permethrin-resistant BR-HL strains of human head louse, *Pediculus humanus capitis*, was examined using contact + fumigant mortality bioassay. Results were compared with those following treatment with two pyrethroid pediculicides, *d*-phenothrin or pyrethrum. As judged by the lethal time to 50% mortality (LT_{50}) values at the exposure rate of 0.25 mg/cm^2, 1,8-cineole (14.1 min) was the most toxic compound, followed by linalool (15.4 min) to KR-HL females. These compounds were faster acting than either *d*-phenothrin (24.1 min) or pyrethrum (33.4 min). Based on the lethal concentration causing 50% mortality (LC_{50}) values, (−)-camphor (0.022 mg/cm^2) was the most toxic compound, followed by linalool (0.035 mg/cm^2), (−)-terpinen-4-ol (0.040 mg/cm^2), α-terpineol (0.045 mg/cm^2), and 1,8-cineole (0.068 mg/cm^2) against KR-HL females. These monoterpenoids were less toxic than either *d*-phenothrin (LC_{50}, 0.0015 mg/cm^2) or pyrethrum (0.0013 mg/cm^2). However, the toxicities of these monoterpenoids were almost identical against females from either of the two strains, even though the BR-HL females exhibited high levels of resistance to *d*-phenothrin [resistance ratio (RR), 667] and pyrethrum (RR, 754). After a 24 h exposure to linalool, BR-HL egg hatch was inhibited 100 and 84% at 0.25 or 0.125 mg/cm^2, respectively, while (−)-terpinen-4-ol caused 94 and 69% inhibition of egg hatch at 0.25 and 0.125 mg/cm^2. α-Terpineol caused 88 and 76% inhibition of egg hatch at 0.5 and 0.25 mg/cm^2, respectively. Thus, certain monoterpenoids from *O. majorana* essential oil, particularly linalool, (−)-terpinen-4-ol and α-terpineol, merit further study as potential pediculicides and ovicides for the control of insecticide-resistant *P. h. capitis* populations as fumigants with contact action.

KEYWORDS: Botanical pediculicide; natural ovicide; *Pediculus humanus capitis*; *Origanum majorana*; insecticide resistance; mode of action; monoterpenoid

INTRODUCTION

The human head louse, *Pediculus humanus capitis* (De Geer), is an obligate ectoparasite, confined to the scalp and hair of humans. Infestations are prevalent worldwide and especially common among schoolchildren in both developed and developing countries (*1*, *2*). Head louse infections cause pruritus, skin irritation and sleep loss, as well as occasional secondary bacterial infection from scratching (*1*, *3*). Unlike the human body louse, *Pediculus humanus humanus* (L.), *P. h. capitis* has not been proven to be a vector of infectious diseases (*4*). Although the symptoms are relatively mild, *P. h. capitis* infestation has resulted in a variety of social, mental and economic problems. It is estimated that 6−12 million people in the United States (US) suffer from infestation, and estimated costs associated with pediculosis by lice exceeds 367 million USD annually (*1*). Infestations with *P. h. capitis* have been increasing in Korea in recent years (*5*, *6*). Control of head louse population worldwide has been provided principally by the use of organophosphorus (malathion), carbamate (carbaryl), pyrethrin, pyrethroid (per-

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methrin and *d*-phenothrin) and avermectin insecticides (*1, 3, 7*). Although these pediculicides are still effective, their continued or repeated use has often resulted in the development of resistance (*1, 2*), and increasing levels of resistance to the most widely used pediculicides have resulted in multiple treatments and excessive doses, fostering serious human health concerns (*1*). These problems substantiate the need for the development of selective control alternatives for *P. h. capitis*, particularly those with fumigant action because formulations such as powder or dust are usually less effective and inconvenient to apply (*3*).

Natural compounds extracted from plants, such as essential oils, have been suggested as alternative sources for insect control agents (*8*−*11*). This approach is appealing, in part, because they are a rich source of bioactive chemicals that often produce only minor adverse effects and often act at multiple and novel target sites, thereby reducing the potential for resistance. Additionally, plant essential oils are widely available with some being relatively inexpensive compared with plant extracts (*10, 11*). Thus, much effort has been focused on essential oils as potential sources of commercial pediculicidal products (*12*−*15*) largely because certain essential oils and their constituents meet the criteria of minimum risk pesticides (*16, 17*). In particular, we initially reported that the essential oil from marjoram, *Origanum majorana* L. (Lamiaceae, formerly Labiatae), had insecticidal activity that was more potent than either *d*-phenothrin or pyrethrum against female *P. h. capitis* (*18*). Very little information, however, exists in relation to the pediculicidal activity of the *O. majorana* essential oil and its constituents against insecticide-resistant *P. h. capitis*.

In this paper, we assess the pediculicidal activity of the constituents that comprise *O. majorana* essential oil against eggs and adult females of an insecticide-susceptible strain and dual malathion- and permethrin-resistant strain of *P. h. capitis*. The adulticidal and ovicidal activities of the essential oil constituents were then compared with those of two commonly used pediculicides, *d*-phenothrin and pyrethrum.

MATERIALS AND METHODS

Materials. The 17 compounds examined in this study were as follows: (+)-borneol, camphene, (−)-camphor, β-caryophyllene, *p*-cymene, limonene, myrcene, α-pinene, β-pinene, γ-terpinene, and α-terpineol purchased from Sigma-Aldrich (St. Louis, MO); bornyl acetate, 1,8-cineole, and terpinyl acetate purchased from Wako (Osaka, Japan); and linalool, linalyl acetate, and (−)-terpinen-4-ol purchased from Fluka (Buchs, Switzerland). *O. majorana* essential oil (reference number 32297) was supplied by Berjé (Bloomfield, NJ). *d*-Phenothrin (92% purity) and 50% pyrethrum were obtained from Hanil and Biomist (Seoul, Korea), respectively. All other chemicals were of reagent grade and available commercially.

Gas Chromatography (GC). A Shimadzu GC 2010 gas chromatograph (Kyoto, Japan), equipped with splitless injector, was used to separate and detect the constituents of *O. majorana* essential oil. Analytes were separated with a 30 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-5 MS bonded-phase fused-silica capillary column (J&W Scientific, Ringoes, NJ). The flow velocity of the helium carrier gas was 1.1 mL/min. The oven temperature was kept at 50 °C (5 min isothermal) and programmed to 280 °C at a rate of 5 °C/min, then isothermal at 280 °C for 10 min. The injector temperature was 280 °C. Essential oil constituents were identified by coelution of authenticated samples following coinjection.

Gas Chromatography−Mass Spectrometry (GC−MS). GC−MS analysis was performed using a Shimadzu GC 2010 gas chromatograph-Shimadzu QP 2010 mass spectrometer. The capillary column and temperature conditions for the GC−MS analysis were the same as described above for GC analysis. Helium carrier gas was used at a column head pressure of 8.89 psi (61.3 kPa). The ion source temperature was 200 °C. The interface temperature was kept at 290 °C, and mass spectra were obtained at 70 eV. The sector mass analyzer was set to scan from 50 to 650 amu every 0.50 s. Chemical constituents were identified by comparison of mass spectra of each peak with those of authentic samples in a mass spectra library (*19*).

Table 1. Chemical Constituents of *O. majorana* Essential Oil Identified by Gas Chromatography and Gas Chromatography−Mass Spectrometry

compound	RT[b] (min)	% area
α-thujene[a]	7.73	2.40
α-pinene	10.71	2.52
camphene	11.29	0.13
sabinene[a]	12.08	0.45
β-pinene	12.27	3.43
p-cymene	13.90	0.87
limonene	14.03	6.38
1,8-cineole	14.21	50.96
linalool	16.42	24.04
borneol	18.73	0.97
α-terpineol	19.40	1.71
linalyl acetate	20.86	0.77
bornyl acetate	21.99	3.00
unknown	22.07	1.37
α-terpinyl acetate	23.69	0.36
β-caryophyllene	25.77	0.66

[a] Tentative identifications from mass spectra data. Other identifications were performed by comparison of GC−MS data and by GC with authentic sample coinjection. [b] Retention time.

Human Head Lice. A Korean strain of *P. h. capitis* (KR-HL), originally obtained from the hair of 78 infested children at a primary school in Songpa District, Seoul, in December 2001 (*13*), was maintained in vivo without exposure to any known insecticide. Because this strain was established in the laboratory prior to the extensive use of pyrethroid pediculicides, it is assumed to be pyrethroid susceptible. A dual malathion- and permethrin-resistant BR-HL strain of *P. h. capitis* was collected from Bristol (U.K.) and has been maintained using the in vitro rearing system with periodical selection with 0.5% malathion (*20, 21*). Based on LT_{50} values, the BR-HL strain was 3.6- and 3.7-fold more resistance to malathion and permethrin, respectively, than a susceptible EC-HL strain. The two strains were reared individually in tight plastic containers (5 × 1.2 cm) with 0.01 and 1.0 mm mesh screens fitted over central holes (4 cm diameter) inserted in the lid and bottom sections, respectively. Each plastic container contained a few strands of human hair and was placed on the bare leg of one of the authors (Y.-C.Y.) to provide the lice with blood meals for 16 h every day. In between blood feedings, the plastic container was placed at 25 °C and 50−60% RH for 8 h in darkness.

Sodium Channel Mutation Detection. Genomic DNA was extracted from individual lice using DNAzol (MRC, Cincinnati, OH) as described previously (*22*). Genomic DNA fragments (548 or 561 bp due to intron size variation) of voltage-gated sodium channel α-subunit gene flanking the T917l and L920F mutation sites were PCR-amplified using a set of primers (5′CCC ACG TTA AAT TTA TTA ATT TCA A vs 5′GAT AAA CTA GAG GAA CCG AAA TT) as described previously (*23*). Presence or absence of the mutations was determined by cycle sequencing of the PCR product. The T917l mutation is primarily responsible for nerve insensitivity and permethrin resistance in head lice (*24*).

Filter-Paper Mortality Bioassay. A contact + fumigant mortality bioassay (*13*) was used to evaluate the toxicity of *O. majorana* essential oil and its constituents against females of the KR-HL and BR-HL strains because various essential oils and their constituents have fumigant action (*13*−*15, 18*). Briefly, appropriate quantities of each test chemical dissolved in 80 μL of acetone were applied to Whatman no. 2 filter papers (5 cm diameter; Whatman, Maidstone, U.K.). Control filter papers received 80 μL of acetone. After drying in a fume hood for 2 min, each filter paper was placed onto the bottom section of a tight plastic container (5 × 1.2 cm). Groups of 20 females (7−9 days old), each fed with human blood 4 h prior to the test, were placed onto the filter paper. A few strands of human hair were placed onto the filter paper, and the plastic container was sealed with its original lid.

Table 2. Comparative Toxicity of *d*-Phenothrin and Pyrethrum against Females of the KR-HL and BR-HL Strains of *P. h. capitis* Using Filter-Paper Contact Mortality Bioassay during a 12 h Exposure

	KR-HL strain		BR-HL strain		
insecticide	slope ± SE	LC_{50},[a] mg/cm^2 (95% CL[b])	slope ± SE	LC_{50},[a] mg/cm^2 (95% CL[b])	RR[c]
d-phenothrin	2.9 ± 0.32	0.0015 (0.0012−0.0017)	4.5 ± 0.60	1.000 (0.894−1.094)	667
pyrethrum	2.8 ± 0.31	0.0013 (0.0011−0.0015)	3.9 ± 0.57	0.980 (0.855−1.088)	754

[a] Median lethal concentration. [b] CL denotes confidence limit. [c] LC_{50} of females from the BR-HL strain of *P. h. capitis*/LC_{50} of females of the KR-HL strain.

Table 3. Toxicity of 17 Test Compounds and Two Pyrethroid Insecticides, *d*-Phenothrin and Pyrethrum, to KR-HL Females of *P. h. capitis* Using the Contact + Fumigant Mortality Bioassay during a 3 h Exposure to 0.25 mg/cm^2

compound	slope ± SE	LT_{50},[b] min (95% CL[c])	CT[d]
(+)-borneol[a]	8.0 ± 1.21	53.6 (48.44−57.41)	0.62
bornyl acetate[a]		>180	
camphene[a]	6.4 ± 0.74	37.8 (34.62−45.87)	0.88
(−)-camphor	9.0 ± 1.44	34.2 (31.34−40.56)	0.98
β-caryophyllene[a]		>180	
1,8-cineole[a]	8.1 ± 1.17	14.1 (11.33−17.33)	2.37
p-cymene[a]	14.3 ± 3.44	50.9 (47.97−53.60)	0.65
limonene[a]	7.3 ± 1.73	52.9 (47.99−59.60)	0.63
linalool[a]	7.0 ± 1.76	15.4 (12.77−20.38)	2.17
linalyl acetate[a]		>180	
myrcene		>180	
α-pinene[a]	6.8 ± 0.94	26.5 (21.34−31.11)	1.26
β-pinene[a]	8.4 ± 1.32	29.4 (24.51−34.63)	1.14
γ-terpinene	9.6 ± 1.75	60.3 (54.32−71.21)	0.55
(−)-terpinen-4-ol	16.2 ± 2.43	29.6 (28.60−30.76)	1.13
α-terpineol[a]	13.5 ± 2.89	26.6 (23.65−31.43)	1.26
terpinyl acetate[a]		>180	
d-phenothrin	3.1 ± 0.72	24.1 (21.39−29.88)	1.39
pyrethrum	2.9 ± 0.66	33.4 (30.81−39.64)	1.00

[a] Compounds identified in this study. The other compounds were reported by Vera and Chane-Ming (*36*) and Novak et al. (*37*). [b] Median lethal time. [c] CL denotes confidence limit. [d] LT_{50} value of pyrethrum/LT_{50} value of the test material.

For eggs of the BR-HL strain of *P. h. capitis*, one to four concentrations (0.0625−1.0 mg/cm^2) of each selected test compound in 80 μL of acetone were applied to filter papers as stated above. After drying for 2 min, eggs (3−4 days old) that were attached to hair were placed on the treated filter paper in each plastic container and sealed with a lid for 24 h.

Treated and control (acetone only) females and eggs were held at the same conditions used for colony maintenance. Adult mortalities were determined every 5 min for 3 h. Lice were considered to be dead if body and appendages did not move when they were prodded with fine wooden dowels. The toxicity of the test compounds and insecticides to the eggs was based on the number of unhatched eggs at 12 days post-treatment. *d*-Phenothrin and pyrethrum served as positive controls for comparison in mortality tests. All treatments were replicated three times using 20 females per replicate.

Data Analysis. Percentage of inhibition of egg hatch (PIH) was calculated from the formula PIH = $[(C - T)/C] \times 100$, where C is control percentage hatch and T is treated percentage hatch (*25*). The percentages of hatching were determined and transformed to arcsine square-root values for analysis of variance (ANOVA). The Bonferroni multiple-comparison method was used to test for significant differences among the test materials (*26*). Mean ± SE of untransformed data are reported. The LT_{50} or LC_{50} values were calculated by probit analysis (*26*). A resistance ratio (RR) was calculated according to the formula RR = LC_{50} of females from the BR-HL strain of *P. h. capitis*/LC_{50} of females of the KR-HL strain. The toxicity was considered to be significantly different when 95% confidence limits of the LT_{50} or LC_{50} values failed to overlap. Comparative toxicity (CT) was determined as the ratio of LT_{50} of pyrethrum/LT_{50} of test compound (*13*).

RESULTS

Chemical Constituents of *O. majorana* Essential Oil. *O. majorana* essential oil was composed of three major and 13 minor constituents based on the analysis of mass spectral data and retention times of authentic compounds (**Table 1**). The three major compounds, 1,8-cineole, linalool, and limonene, comprised 50.96, 24.04, and 6.38% of the oil, respectively. They constituted about 81% of total essential oil.

Detection of Sodium Channel Mutations. Genotyping of the voltage-gated sodium channel α-subunit gene fragments revealed that none of the lice specimens collected in Korea (KR-HL) had the mutations associated with knockdown resistance in *P. h. capitis* (*24*). In the absence of these mutations, the KR-HL strain should not be resistant to *d*-phenothrin and pyrethrum by the kdr mechanism.

Adulticidal Activity of Test Compounds. The comparative toxicity of two pediculicides against KR-HL and BR-HL females was evaluated using the contact + fumigant mortality bioassay (**Table 2**). As judged by LC_{50} values, BR-HL females exhibited high levels of resistance to *d*-phenothrin (RR, 667) and pyrethrum (RR, 754) compared to KR-HL females.

The lethality of 17 terpenoids and two pyrethroid insecticides, *d*-phenothrin and pyrethrum, against KR-HL females was evaluated by the contact + fumigant contact lethal-time bioassay (**Table 3**). Based on the LT_{50} values obtained at the 0.25 mg/cm^2 concentration of test compound, 1,8-cineole (14.1 min) was the fastest acting terpenoid, followed by linalool (15.4 min). These monoterpenoids were faster acting than either *d*-phenothrin (24.1 min) or pyrethrum (33.4 min). The pediculicidal activity of α-pinene, β-pinene, (−)-terpinen-4-ol, and α-terpineol was comparable with that of *d*-phenothrin. The pediculicidal activity of camphene and (−)-camphor was comparable with that of pyrethrum. Weak pediculicidal activity was produced by (+)-borneol, *p*-cymene, limonene, and γ-terpinene. The other five terpenoids exhibited no pediculicidal activity. There was no mortality for acetone-treated females over the observational interval of the contact + fumigant mortality bioassay.

O. majorana essential oil and seven selected terpenoids were likewise compared (**Table 4**). Based on LC_{50} values, (−)-camphor (0.022 mg/cm^2) was the most toxic pediculicide, followed by linalool (0.035 mg/cm^2), (−)-terpinen-4-ol (0.040 mg/cm^2), α-terpineol (0.045 mg/cm^2), 1,8-cineole (0.068 mg/cm^2), and *O. majorana* essential oil (0.077 mg/cm^2) against KR-HL females. Overall, the oil and five monoterpenoids were less toxic against KR-HL females than either *d*-phenothrin (LC_{50}, 0.0015 mg/cm^2, **Table 2**) or pyrethrum (0.0013 mg/cm^2, **Table 2**). β-Pinene and α-terpinene were weakly active against KR-HL females. Interestingly, all seven terpenoids and *O. majorana* essential oil were of equal toxicity against both the KR-HL and BR-HL females, indicating a lack of cross-resistance in the BR-HL.

Ovicidal Activity of Test Compounds. The ovicidal effects of the *O. majorana* essential oil and eight selected terpenoids were evaluated by measuring egg hatch of the eggs from BR-HL females using the filter-paper mortality bioassay (**Table 5**). Responses varied according to compound and exposure concentration. After a 24 h exposure to *O. majorana* essential oil, BR-HL egg hatch was inhibited 58% at 1.0 mg/cm^2. Linalool

Table 4. Toxicity of *O. majorana* Essential Oil and Seven Selected Constituents of the Oil to Females of the KR-HL and BR-HL Strains of *P. h. capitis* Using the Contact + Fumigant Mortality Bioassay during a 12 h Exposure

material	KR-HL strain		BR-HL strain	
	slope ± SE	LC_{50},[a] mg/cm^2 (95% CL[b])	slope ± SE	LC_{50},[a] mg/cm^2 (95% CL[b])
O. majorana oil	7.0 ± 1.99	0.077 (0.053−0.111)	5.6 ± 0.63	0.072 (0.066−0.078)
(−)-camphor	5.4 ± 0.71	0.022 (0.019−0.024)	5.8 ± 1.49	0.020 (0.014−0.030)
1,8-cineole	4.7 ± 0.56	0.068 (0.061−0.077)	3.7 ± 0.48	0.066 (0.058−0.076)
linalool	7.3 ± 0.98	0.035 (0.032−0.038)	6.8 ± 1.72	0.040 (0.028−0.056)
β-pinene	3.1 ± 0.50	0.107 (0.090−0.137)	3.5 ± 0.37	0.101 (0.089−0.116)
γ-terpinene	6.1 ± 0.73	0.129 (0.117−0.143)	4.6 ± 0.55	0.122 (0.108−0.137)
(−)-terpinen-4-ol	10.7 ± 1.17	0.040 (0.038−0.042)	11.4 ± 1.18	0.042 (0.042−0.044)
α-terpineol	5.4 ± 0.71	0.045 (0.040−0.050)	4.7 ± 0.61	0.043 (0.038−0.049)

[a] Median lethal concentration. [b] CL denotes confidence limit.

Table 5. Ovicidal Action of *O. majorana* Essential Oil and Eight Selected Constituents of the Oil as Judged by Egg Hatch Using BR-HL Eggs and the Filter-Paper Mortality Bioassay during a 24 h Exposure

material	dose, mg/cm^2	% egg hatch (mean ± SE)[a]	% inhibition of egg hatch
O. majorana oil	1.0	37 ± 4.4 ghi	58
	0.5	60 ± 2.9 cdef	32
	0.25	75 ± 2.9 abc	15
(+)-borneol	1.0	47 ± 4.4 efg	47
	0.5	78 ± 1.7 abc	11
(−)-camphor	1.0	87 ± 1.7 ab	1
	0.5	90 ± 2.9 a	0
1,8-cineole	1.0	70 ± 2.9 bcd	20
linalool	0.25	0 L	100
	0.125	13 ± 1.7 jkl	85
	0.0625	43 ± 4.4 fgh	51
β-pinene	1.0	67 ± 3.3 cd	24
γ-terpinene	1.0	63 ± 3.3 cde	28
(−)-terpinen-4-ol	0.25	5 ± 2.9 kl	94
	0.125	27 ± 1.7 hij	69
	0.0625	53 ± 4.4 defg	40
α-terpineol	0.5	10 ± 2.9 jkl	89
	0.25	20 ± 2.9 ijk	77
	0.125	52 ± 4.4 defg	41
	0.0625	73 ± 4.4 abc	17
control (acetone)		83 ± 3.3 ab	

[a] Means within a column followed by the same letter are not significantly different ($P = 0.05$, Bonferroni method).

treatment resulted in 100, 84, and 48% inhibition of egg hatch at 0.25, 0.125, and 0.0625 mg/cm^2, respectively. (−)-Terpinen-4-ol caused 94, 69, and 40% inhibition of egg hatch at 0.25, 0.125, and 0.0625 mg/cm^2, respectively. α-Terpineol treatment resulted in 88, 76, and 37% inhibition of egg hatch at 0.5, 0.25, and 0.125 mg/cm^2, respectively. Only weak inhibition of egg hatch was observed with (+)-borneol, 1,8-cineole, β-pinene, and γ-terpinene at 1.0 mg/cm^2. (−)-Camphor did not affect egg hatch at any tested concentration.

DISCUSSION

Highly complex mixtures of terpenoids, particularly monoterpenoids, and related phenols exist in essential oils, and they jointly or independently contribute to behavioral efficacy, such as repellency and feeding deterrence, and physiological efficacy, such as acute toxicity and developmental disruption, against various insect species (*8*, *10*, *11*). In the current study, constituents of *O. majorana* essential oil exhibited strong adulticidal (females) and ovicidal activities. This original finding supports the contention that selected terpenoids from *O. majorana* essential oil may hold promise as novel and effective pediculicidal products even against currently insecticide-resistant *P. h. capitis* populations.

Elucidation of the modes of delivery of natural insecticidal products and insecticides is of practical importance for insect control because it may give useful information on the most appropriate formulations and delivery means (*27*). Naturally occurring adulticidal and ovicidal compounds against *P. h. capitis* include eugenol and methyl salicylate (*13*); α-pinene, β-pinene, (*E*)-pinocaveol, and α-terpineol (*14*); and salicylaldehyde and benzaldehyde (*15*). Likewise, many essential oils and their constituents, particularly monoterpenoids, act as fumigants against female *P. h. capitis* (*13*−*15*, *18*). In the present study, we used a contact + fumigant mortality bioassay to identify the pediculicidal constituents of *O. marjorana* essential oil. The monoterpenoids, camphene, (−)-camphor, 1,8-cineole, linalool, α-pinene, β-pinene, (−)-terpinen-4-ol, and α-terpineol, were the most effective, and this finding suggests that their mode of delivery was, in part, a result of vapor action. The pediculicidal activity of these monoterpenoids was comparable with that of either *d*-phenothrin or pyrethrum based on the LT_{50} values, although these monoterpenoids were less effective than either *d*-phenothrin or pyrethrum as judged by the LC_{50} values. Additionally, (−)-terpinen-4-ol, α-terpineol and linalool were highly ovicidal against the eggs from insecticide-resistant BR-HL females. The dual contact + fumigant action of *O. majorana* essential oil and its constituents as demonstrated through our contact + fumigant bioassay is of practical importance not only because volatile compounds, when the treated hair and scalp is tightly sealed with hair cap or hat, can easily reach deep harborages in hair, resulting in good control effect, but because volatile compounds usually have ovicidal action (*13*−*15*, *18*). This system has an advantage because exposure to volatile compounds can be easily controlled.

Investigations on the modes of action and the resistance mechanisms of natural insecticidal products are of practical importance for *P. h. capitis* control because it may give useful information for future resistance management. Mechanisms of resistance to insecticides currently used to control *P. h. capitis* are target site insensitivity that reduces sodium channel sensitivity to the pyrethrins and permethrin and enhanced metabolism of malathion (*20*, *22*−*24*, *28*, *29*). Alternative control agents with novel modes of action, low mammalian toxicity and little environmental impact are badly needed. Certain plant preparations or their constituents are highly effective against insecticide-resistant insect pests (*30*−*32*). For example, guineensine possesses remarkable insecticidal activity against a pyrethroid-resistant strain of *Musca domestica* (L.) (*30*). Our current finding that camphene, (−)-camphor, 1,8-cineole, linalool, β-pinene, (−)-terpinen-4-ol, and α-terpineol are virtually equal in toxicity to both insecticide-susceptible and pyrethroid-resistant strains of *P. h. capitis* suggests that these monoterpenoids and the pyrethrum/pyrethroid insecticides do not share a common mode

of action or elicit cross-resistance. Although not yet proven, the octopaminergic and γ-aminobutyric acid (GABA) receptors have been suggested as novel target sites for some essential oil constituents by Kostyukovsky et al. (*33*) and Priestley et al. (*34*), respectively.

In conclusion, *O. majorana* essential oil and its constituents, particularly linalool, (−)-terpinene-4-ol and α-terpineol, could be useful as fumigants with contact action in the control of *P. h. capitis* populations, particularly due to their activity against insecticide-resistant *P. h. capitis* adults and eggs. For the practical use of *O. majorana* essential oil and its constituents as novel pediculicides or ovicides to proceed, further research is needed to establish their human safety. However, *Origanum* essential oil is widely used as fragrance components in detergents, soaps, cosmetics, and perfumes (*35*). Additionally, their adulticide and ovicide modes of action need to be established and formulations for improving pediculicidal potency and stability, thereby reducing costs, need to be developed.

LITERATURE CITED

(1) Gratz, N. G. *Human Lice: Their Prevalence, Control and Resistance to Insecticides-a Review 1985−1997*; World Health Organization (WHO): Geneva, Switzerland, 1997; WHO/CTD/WHOPES/97.8.

(2) Downs, A. M.; Stafford, K. A.; Coles, G. C. Head lice: prevalence in schoolchildren and insecticide resistance. *Parasitol. Today* **1999**, *15*, 1–4.

(3) Rozendaal, J. A. *Vector Control. Methods for Use by Individuals and Communities*; World Health Organization: Geneva, Switzerland, 1997; pp 7−177.

(4) de Berker, D.; Sinclair, R. Getting ahead of head lice. *Australas. J. Dermatol.* **2000**, *41*, 209–212.

(5) Ree, H. I.; Yong, T. S.; Shin, H. J.; Shin, C. O.; Lee, I. Y.; Seo, S. A.; Seo, J. H.; Chang, J. K.; Lee, D. H.; Im, K. I. Mass treatment of head louse infestation with Sumithrin powder in primary schools in Korea. *Korean J. Parasitol.* **1992**, *30*, 349–354.

(6) Hong, H. K.; Kim, C. M.; Lee, J. S.; Lee, W. J.; Yang, Y. C. Infestation rate of head lice in primary school children in Inchon, Korea. *Korean J. Parasitol.* **1995**, *33*, 243–244.

(7) Dolianitis, C.; Sinclair, R. Optimal treatment of head lice: is a no-nit policy justified? *Clin. Dermatol.* **2002**, *20*, 94–96.

(8) Singh, G.; Upadhyay, R. K. Essential oils: A potent source of natural pesticides. *J. Sci. Ind. Res.* **1993**, *52*, 676–683.

(9) Wink, M. Production and application of phytochemicals from an agricultural perspective. In *Phytochemistry and Agriculture*; van Beek, T. A., Breteler, H., Eds.; Clarendon: Oxford, 1993; Vol. 34, pp 171−213.

(10) Isman, M. B. Plant essential oils for pest and disease management. *Crop Prot.* **2000**, *19*, 603–608.

(11) Isman, M. B. Botanical insecticides, deterrents, and repellents in modern agriculture and increasingly regulated world. *Annu. Rev. Entomol.* **2006**, *51*, 45–66.

(12) Mumcuoglu, K. Y.; Miller, J.; Zamir, C.; Zentner, G.; Helbin, V.; Ingber, A. The in vivo pediculicidal efficacy of a natural remedy. *Isr. Med. Assoc. J.* **2002**, *4*, 790–793.

(13) Yang, Y. C.; Lee, S. H.; Lee, W. J.; Choi, D. H.; Ahn, Y. J. Ovicidal and adulticidal effects of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. *J. Agric. Food Chem.* **2003**, *51*, 4884–4888.

(14) Yang, Y. C.; Choi, H. Y.; Choi, W. S.; Clark, J. M.; Ahn, Y. J. Ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus capitis* (Anoplura: Pediculidae). *J. Agric. Food Chem.* **2004**, *52*, 2507–2511.

(15) Yang, Y. C.; Lee, H. S.; Lee, S. H.; Clark, J. M.; Ahn, Y. J. Ovicidal and adulticidal activities of *Cinnamomum zeylanicum* bark essential oil compounds and related compounds against *Pediculus humanus capitis* (Anoplura: Pediculidae). *Int. J. Parasitol.* **2005**, *35*, 1595–1600.

(16) U.S. EPA. Exemption of certain pesticide substances from federal insecticide, fungicide, and rodenticide act requirements. U.S. Environmental Protection Agency, 1996, Final Rule, 40 CFR 152.25 (g), May 6, (Accessed: August 08, 2008).

(17) U.S. EPA. Biopesticides-25b Minimum risk pesticides. U.S. Environmental Protection Agency, 2006. http://www.epa.gov/oppbppd1/biopesticides/regtools/25b_list.htm (Accessed: August 08, 2008).

(18) Yang, Y. C.; Lee, H. S.; Clark, J. M.; Ahn, Y. J. Insecticidal activity of plant essential oil against *Pediculus humanus capitis* (Anoplura: Pediculidae). *J. Med. Entomol.* **2004**, *41*, 699–704.

(19) *The Wiley Registry of Mass Spectral Data*, 7th ed.; John Wiley & Sons: New York, 2000.

(20) Gao, J. R.; Yoon, K. S.; Frisbie, R. K.; Coles, G. C.; Clark, J. M. Esterase-mediated malathion resistance in the human head louse, Pediculus capitis (Anoplura: Pediculidae). *Pestic. Biochem. Physiol.* **2006**, *85*, 28–37.

(21) Yoon, K. S.; Strycharz, J. P.; Gao, J. R.; Takano-Lee, M.; Edman, J. D.; Clark, J. M. An improved in vitro rearing system for the human head louse allows the determination of resistance to formulated pediculicides. *Pestic. Biochem. Physiol.* **2006**, *86*, 195–202.

(22) Yoon, K. S.; Gao, J. R.; Lee, S. H.; Clark, J. M.; Brown, L.; Taplin, D. Permethrin-resistant human head lice, *Pediculus capitis*, and their treatment. *Arch. Dermatol.* **2003**, *139*, 994–1000.

(23) Lee, S. H.; Gao, J. R.; Yoon, K. S.; Mumcuoglu, K. Y.; Taplin, D.; Edman, J. D.; Takano-Lee, M.; Clark, J. M. Sodium cahnnel mutations associated with knockdown resistance in the human head louse, *Pediculus capitis* (De Geer). *Pestic. Biochem. Physiol.* **2003**, *75*, 79–91.

(24) Yoon, K. S.; Symington, S. B.; Lee, S. H.; Soderlund, D. M.; Clark, J. M. Three mutations identified in the voltage-sensitive sodium channel α-subunit gene of permethrin-resistant human head lice reduce the permethrin sensitivity of house fly Vsscl sodium channels expressed in *Xenopus* oocytes. *Insect Biochem. Mol. Biol.* **2008**, *38*, 296–306.

(25) Sharma, R. N.; Saxena, K. N. Orientation and developmental inhibition in the housefly by certain terpenoids. *J. Med. Entomol.* **1974**, *11*, 617–621.

(26) SAS Institute. *SAS OnlineDoc®*, version 8.01; Statistical Analysis System Institute: Cary, NC, 2004.

(27) Kim, S. I.; Kim, H. K.; Koh, Y. Y.; J, M.; Ahn, Y. J. Toxicity of spray and fumigant products containing cassia oil to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *Pest Manag. Sci.* **2006**, *62*, 768–774.

(28) Lee, S. H.; Williamson, M. S.; Yoon, K. S.; Takano-Lee, M.; Edman, J. D.; Devonshire, A. L.; Clark, J. M. Molecular analysis of *kdr*-like resistance in insecticide-resistant strains of head lice, *Pediculus capitis*. *Pestic. Biochem. Physiol.* **2000**, *66*, 130–143.

(29) Gao, J. R.; Yoon, K. S.; Lee, S. H.; Takano-Lee, M.; Edman, J. D.; Meinking, T. L.; Taplin, D.; Clark, J. M. Increased frequency of the T9291 and L932F mutations associated with knockdown resistance in permethrin-resistant populations of the human head louse, *Pediculus capitis*, from California, Florida, and Texas. *Pestic. Biochem. Physiol.* **2003**, *77*, 115–124.

(30) Miyakado, M.; Nakayama, I.; Ohno, N. Insecticidal unsaturated isobutylamides from natural products to agrochemical leads. In *Insecticides of Plant Origin*; Arnason, J. T., Philogène, B. J. R., Morand, P., Eds.; ACS Symposium Series 387; American Chemical Society: Washington, DC, 1989; pp 173−187.

(31) Ahn, Y. J.; Kwon, M.; Park, H. M.; Han, C. G. Potent insecticidal activity of *Ginkgo biloba*-derived trilactone terpenes against *Nilaparvata lugens*. In *Phytochemical Pest Control Agents*; Hedin, P. A., Hollingworth, R. M., Masler, E. P., Miyamoto, J., Thompson, D. G., Eds.; ACS Symposium Series 658; American Chemical Society: Washington, DC, 1997; pp 90−105.

(32) Kim, N. J.; Byun, S. G.; Cho, J. E.; Chung, K.; Ahn, Y. J. Larvicidal activity of *Kaempferia galanga* rhizome phenylpropanoids towards three mosquito species. *Pest Manag. Sci.* **2008**, *64*, 857–862.

(33) Kostyukovsky, M.; Rafaeli, A.; Gileadi, C.; Demchenko, N.; Shaaya, E. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests. *Pest Manag. Sci.* **2002**, *58*, 1101–1106.
(34) Priestley, C. M.; Williamson, E. M.; Wafford, K. A.; Sattelle, D. B. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human $GABA_A$ receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. *Br. J. Pharmacol.* **2003**, *140*, 1363–1372.
(35) Lawless, J. *The Encyclopedia of Essential Oils*. Thorsons: London, U.K., 2002.
(36) Vera, R. R.; Chane-Ming, J. Chemical composition of the essential oil of marjoram (*Origanum majorana* L.) from Reunion Island. *Food Chem.* **1999**, *66*, 143–145.
(37) Novak, J.; Langbehn, J.; Pank, F.; Franz, C. M. Essential oil compounds in a historical sample of marjoram (*Origanum majorana* L., Lamiaceae). *Flav. Frag. J.* **2002**, *17*, 175–180.

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