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REPELLENT ACTIVITIES OF ESSENTIAL OILS AND MONOTERPENES AGAINST *CULEX PIPIENS PALLENS*

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ABSTRACT. Essential oils of *Eulcalyptus globulus, Lavender officinalis, Rosemarinus officinalis,* and *Thymus vulgaris* were examined for their repellent activities against *Culex pipiens pallens.* All 4 essential oils effectively repelled adult mosquitoes on hairless mice. Essential oil of *T. vulgaris* (thyme) had potent repellent activity within the tested materials, with a protection rate of 91% at a concentration of 0.05% topical treatment. Thyme essential oil significantly extended the duration of protection until 3 bites by mosquitoes. With gas chromatography–mass spectrometry analysis, thyme essential oil was a rich source of 5 monoterpenes, including in descending order thymol, *p*-cymene, carvacrol, linalool, and α-terpinene. These 5 monoterpenes also were assessed to determine their repellent activities to the mosquitoes. α-Terpinene had a potent repellent activity with a protection rate of 97% at a concentration of 0.05% topical treatment. Additionally, carvacrol and thymol showed an equivalent level of repellency. A spray-type solution containing 2% α-terpinene was tested for its repellent activity against *Cx. pipiens.* This solution showed stronger repellent activity than the currently used repellent *N,N*-diethyl-*m*-methylbenzamide (deet).

KEY WORDS *Thymus vulgaris,* essential oil, *Culex pipiens,* repellents, α-terpinene

INTRODUCTION

The control of mosquito vectors is becoming difficult because of resistance to insecticides (Chandre et al. 1998, Penilla et al. 1998). As agents of chemical control, the use of repellents can be effective for protecting humans from mosquitoes. One way to prevent mosquito bites is to apply the repellent *N,N*-diethyl-*m*-methylbenzamide (deet) to the skin. However, toxic reactions to deet have been found in some circumstances and age groups, although these reactions do not seem to present serious problems for deet use (Mody et al. 1989, Clem et al. 1993). Nevertheless, there is a need to find alternatives to the currently used repellents.

Phytochemicals derived from various botanical sources have provided numerous compounds with potential use as repellents (Sukumar et al. 1991, Watanabe et al. 1995). Essential oils and monoterpenes are used as fragrances in cosmetics, food additives, household products, medicine, and insecticides. Some of these generally are recognized as safe by the U.S. Food and Drug Administration (FDA).

In this paper, we report the mosquito repellency of *Thymus vulgaris* (thyme) essential oil and its primary constituents against *Culex pipiens pallens* Coquillett, by using the mouse bioassay system (Rutledge et al. 1994).

MATERIALS AND METHODS

Chemicals: N,N-Diethyl-*m*-methylbenzamide; 2-cyano-1-methyl-3-(2-(5-methyl-imidazol-4-yl-methyl-thio)ethyl)guanidine (eudragit E100; a histamine antagonist); polyvinyl-pyrrolidone (povidone); and polyethylene glycol 100 (PEG 100) were kindly provided by the Dong-Wha Co. (Seoul, Korea). Four essential oils, *Eucalyptus globulus* (eucalyptus), *Lavender officinalis* (lavender), *Rosemarinus officinalis* (rosemary), and *Thymus vulgaris* (thyme), were purchased from the Nature Co. (Sydney, Australia). Five monoterpenes, carvacrol, *p*-cymene, linalool, α-terpinene, and thymol, were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals and husbandry: All experiments were conducted in accordance with the Korean Food and Drug Administration (KFDA) guidelines (98-116) in this study. All experimental animals were treated according to U.S. Environmental Protection Agency guidelines.

Mice: Twenty-five male SKH-1 HRBR hairless mice (7 wk old, 26–38 g, Charles River Laboratories, Wilmington, MA) were used after acclimatization for 13 days. Animals were allocated 5 per polycarbonate cage in a temperature- and humidity-controlled room. The daily light cycle was 12:12 h light : dark, and feed (Samyang Co., Seoul, Korea) and water were supplied continuously. Animals were identified by marking the tail with a marking pen.

Insects: Five hundred female mosquitoes (*Cx. pipiens pallens* F_{11} adults) were acquired from KFDA for use in this study. Mosquitoes were reared at 27 ± 2°C and 75–80% relative humidity in a 12:12 h light : dark cycle and fed an 8% sugar solution in water. For each test, 100 6- to 7-day-old nulliparous females were placed into a wire screen cage measuring 30 × 30 × 30 cm. The sugar solution pad was removed from this cage at least 12 h before testing.

Bioassay: Bioassays were conducted according to the method of Rutledge et al. (1994). Test materials (0.05% in 0.1% ethanol) were deposited on

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Table 1. Spray formulation of test repellent topically applied to hairless mice.

Raw materials (%)	Content (g)
α-terpinene (2.0%)	0.5
Eudragit E100 (5.0%)	1.25
Povidone (2.0%)	0.5
PEG400 (0.2%)	0.05
Ethanol (90.8%)	22.7

5 hairless mice and application of 0.1% ethanol served as a control. Treatments were applied with absorbent (sterile) cotton over the whole body to point of runoff. After drying, treated mice were transferred to a mosquito cage containing 100 female mosquitoes. During each test, the cage was supported 10 cm above the surface of the worktable to permit air circulation. The number of mosquitoes biting each mouse was recorded at 5-min intervals for 1 h. Total bites for 1 h on each mouse were calculated at end of the test. Tests were replicated 5 times. Total numbers of 5 tests were converted to mean ± standard deviation. Mean totals were converted to percentage of the totals with the following equation:

$$\text{protection rate (\%)} = [1 - (\text{total biting of treatment} \div \text{total biting of control})] \times 100.$$

In addition, mouse skin tests for each active monoterpene were examined to minimize the high volatility of the commercial formulae, as described in Table 1. The experimental procedures were exactly same as described as above. The control was an identical treatment, but without any mosquito-repellent compound in the spray solution. Riferan-S™ (a commercial deet product available as a spray; Shin-Shin Pharmaceutical Co., Seoul, Korea) was used as a positive control. Two percent α-terpinene in a spray solution described in Table 1 was tested and compared with the control.

Tests for the determination of duration of protection were conducted according to the methods described by Frances et al. (1993). Duration of protection was the time (minutes) until 3 bites were recorded. Tests were replicated 5 times. Total numbers of 5 tests were converted to mean ± standard deviation.

Identification of constituents in essential oils was determined with electron-impact mass spectra obtained by gas chromatography–mass spectrometry (GC-MS) as described by Lee et al. (2000).

Statistical analyses: Data collected during evaluation of biting numbers and duration of protection were subjected to Scheffe's test ($P = 0.05$; SAS 1995).

Table 2. Repellent activities of essential oils (EO) topically applied on hairless mice against *Culex pipiens pallens* Coquillett.

Tested EO	Total bites[1]	Protection rate (%)	Duration of protection (min)[2]
Control	17.2 ± 1.64a[3]	—	17.8 ± 1.92ab
Eucalyptus	5.2 ± 0.84b	70	33.2 ± 4.09ac
Lavender	6.0 ± 1.00b	65	31.0 ± 3.67ac
Rosemary	4.0 ± 1.71b	77	47.0 ± 8.28ac
Thymus	1.6 ± 1.34c	91	65.4 ± 12.2bc

[1] Total bites were measured at 1 h after exposure to mosquitoes.
[2] Mice were considered protected until 3 bites were recorded.
[3] Means within a column followed by a different letter are significantly different ($P \leq 0.05$; Scheffe's test [SAS 1995]).

RESULTS

Four essential oils (0.5% in 0.1% ethanol) prevented mosquito biting in comparison with controls (Table 2). Numbers biting control mice at 5 min after exposure to mosquitoes were zero, but after 1 h total numbers of bites were 17.2 ± 1.64. On the other hand, total bites by mosquitoes at 1 h after exposure to mice treated with eucalyptus, lavender, rosemary, and thyme were 5.2, 6.0, 4.0, and 1.6, respectively. Thyme essential oil was the most effective repellent among the test materials and provided 91% protection from mosquito bites. Thyme oil also showed 3.7-fold stronger protection time (65.4 min) than the control (17.8 min) until 3 bites by mosquitoes. With GC-MS analysis, thyme essential oil was a rich source of 5 monoterpenes, including thymol, *p*-cymene, carvacrol, linalool, and α-terpinene, in descending order. These 5 primary components of thyme essential oil were assessed for repellent activities against mosquitoes. Two principal monoterpenes, α-terpinene and thymol, had potent repellent activities with 96 and 97% protection, respectively, from mosquito bites when compared with deet, which provided 89% protection (Table 3).

A spray-type solution (Table 4) was developed to minimize volatilization and to prolong the repellency of α-terpinene to mosquitoes. The spray-type solution containing 2% α-terpinene resulted in 80.3% repellency after 3 h of exposure, whereas the repellency of the control was 66.6%. The solution also had 1.5-fold and 6-fold higher protection time than the control and the positive-control Riferan-S, respectively (Fig. 1).

DISCUSSION

Application of thyme essential oil to experimental mice resulted in a 91% protection rate against female *Cx. pipien pallens,* whereas the essential oils of eucalyptus, lavender, and rosemary showed 70, 65, and 77% protection, respectively. All essential oils exhibited significantly greater repellency than the control. This suggests that the repellency of the

Table 3. Repellent activities of the principal monoterpenes (MT) of thyme essential oil and deet topically on hairless mice against *Culex pipiens pallens* Coquillett.

Tested MT	Total bites[1]	Protection rate (%)	Duration of protection (min)[2]
Control	17.2 ± 1.64a[3]	—	17.8 ± 1.92ab
p-Cymene	2.6 ± 0.98b	85	59.1 ± 9.22ac
Thymol	1.7 ± 1.30b	90	62.7 ± 10.3ac
Linalool	1.9 ± 1.54b	89	60.3 ± 11.2ac
Carvacrol	1.1 ± 1.23c	96	70.0 ± 13.2bc
α-Terpinene	1.0 ± 1.43c	97	72.1 ± 12.3bc
Deet	2.0 ± 0.71b	88	72.1 ± 12.3bc

[1] Total bites were measured at 1 h after exposure to mosquitoes.
[2] Mice were considered protected until 3 bites were recorded.
[3] Means within a column followed by a different letter are significantly different ($P = 0.05$; Scheffe's test [SAS 1995]).

thyme essential oil resulted from compounds that were absent in the other 3 essential oils. This result was relatively similar to that reported by Barnard (1999). Barnard showed that of 15 essential oils tested, clove and thyme essential oils were the most potent repellents. However, Barnard did not analyze which components contributed to repellency. Other reports have shown essential oils to be potent alternatives to deet (Ansari and Razdan 1995, Matsuda et al. 1996). The GC-MS analyses revealed the presence of 5 monoterpenes in thyme oil, with α-terpinene exhibiting potent repellency to *Cx. pipiens pallens*. Klocke et al. (1987) reported the mosquito feeding and ovipositional repellency of the major monoterpenoids present in the volatile oil of *Hemizonia fitchii* (Asteraceae). The compound

Table 4. Repellent activities of the 3 different treatments in spray solution developed for minimizing volatilization for α-terpinene.

Time after application (h)	Group[1]	Total bites[2]	Protection rate (%)
0	Control	18.0 ± 2.24a[3]	—
	Positive control	2.33 ± 1.33b	87
	2% α-Terpinene	0.67 ± 0.58b	96
1	Control	16.7 ± 2.08a	—
	Positive control	4.33 ± 0.58b	74
	2% α-Terpinene	1.33 ± 1.15b	92
3	Control	17.0 ± 1.00a	—
	Positive control	5.67 ± 1.53b	67
	2% α-Terpinene	3.33 ± 0.58b	80
6	Control	16.7 ± 1.15a	—
	Positive control	10.7 ± 0.58b	36
	2% α-Terpinene	9.33 ± 2.08b	44

[1] Control indicates a treatment without any mosquito-repellent compound in a spray solution. Riferan-S (Shin-Shin Pharmaceutical Co., Seoul, Korea) was used as a positive control. Two percent α-terpinene means a spray solution containing 2% α-terpinene solution described in Table 1.
[2] Total bites were measured at 1 h after exposure to mosquitoes.
[3] Means within a column followed by a different letter are significantly different ($P \leq 0.05$; Scheffe's test [SAS 1995]).

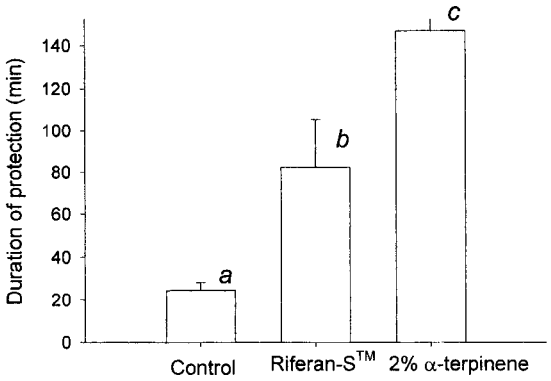

Fig. 1. Protection time after 3 different treatments in spray solution on hairless mice until 3 bites by *Culex pipiens* were recorded. Control is the spray solution without α-terpinene. The positive control is the commercial product Riferan-S™ (deet; Shin-Shin Pharmaceutical Co., Seoul, Korea). Same letter on the bars is not significantly different ($P \leq 0.05$; Scheffe's test).

1,8-cineole was moderately effective as a feeding repellent and highly effective as an ovipositional repellent against adult *Aedes aegypti* (L.). Watanabe et al. (1993) reported that *p*-methane-3,8-diols isolated from *Eucalyptus camaldulensis* were potent mosquito repellents and they synthesized a new mosquito repellent, eucamalol. This compound was effective (75%) until 3 h after exposure, whereas the deet did not show repellency. Hwang et al. (1985) reported that the mugwort *Artemisia vulgaris* (Anthemideae) contains insect repellents. Terpinen-4-ol was the most active constituent, and was as effective as dimethyl phthalate against *Ae. aegypti*.

In conclusion, α-terpinene and its spray-type solution developed in this study could be an alternative repellent to deet for personal protection against mosquitoes. However, terpenes are known to irritate the skin and mucous membranes and prolonged exposure has caused contact dermatitis and chronic impairment of lung function in humans. Reports of eye and skin irritancy from exposure to monoterpenes are rare. Thus, we plan to make additional studies of eye and skin toxicity of the spray solution containing 2% α-terpinene, even though this compound is considered as generally reported as safe by the U.S. FDA.

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REFERENCES CITED

Ansari MA, Razdan RK. 1995. Relative efficacy of various oils in repelling mosquitoes. *Indian J Malariol* 32: 104–111.

Barnard DR. 1999. Repellency of essential oils to mosquitoes (Diptera: Culicidae). *J Med Entomol* 36:625–629.

Chandre F, Darriet F, Darder M, Cuany A, Doannio JM, Pasteur N, Guillet P. 1998. Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Med Vet Entomol* 12:359–366.

Clem JR, Havemann DF, Raebel MA. 1993. Insect repellent (*N,N*-diethyl-*m*-toluamide) cardiovascular toxicity in an adult. *Ann Pharmacother* 27:289–293.

Frances SP, Eikarat N, Sripongsai B, Eamsila C. 1993. Response of *Anopheles dirus* and *Aedes albopictus* to repellents in the laboratory. *J Am Mosq Control Assoc* 9:474–476.

Hwang YS, Wu KH, Kumamoto J, Axelrod H, Mulla MS. 1985. Isolation and identification of mosquito repellents in *Artemisia vulgaris*. *J Chem Ecol* 11:1297–1306.

Klocke JA, Darlington MV, Barlandrin MF. 1987. 1,8-Cineol (eucalyptol), a mosquito repellent from volatile oil of *Hemizonia fitchii* (Asteraceae). *J Chem Ecol* 13: 2131–2141.

Lee SE, Choi WS, Lee HS, Park BS. 2000. Cross-resistance of a chlorpyrifos-methyl resistant strain of *Oryzaephilus surinamensis* (Coleoptera: Cucujidae) to fumigant toxicity of essential oil extracted from *Eucalyptus globulus* and its major monoterpene, 1,8-cineole. *J Stored Prod Res* 36:383–389.

Matsuda BM, Surgeoner GA, Heal JD, Tucker AO, Maciarello MJ. 1996. Essential oil analysis and field evaluation of the citrosa plant *Pelagonium citrosum* as a repellent against populations of *Aedes* mosquitoes. *J Am Mosq Control Assoc* 12:69–74.

Mody RP, Benoit FM, Riedel R, Ritter L. 1989. Dermal absorption of the insect repellent deet (*N,N*-diethyl-*m*-toluamide) in rats and monkeys; effect of anatomical site and multiple exposure. *J Toxicol Environ Health* 26:137–147.

Penilla RP, Rodriguz AD, Hemingway J, Torres JL, Arredondo-Jimenez JI, Rodriguez MH. 1998. Resistance management strategies in malaria vector mosquito control. Baseline data for a large-scale field trial against *Anopheles albimanus* in Mexico. *Med Vet Entomol* 12: 217–233.

Rutledge LC, Gupta RK, Wirtz RA, Buescher MD. 1994. Evaluation of the laboratory mouse model for screening topical mosquito repellents. *J Am Mosq Control Assoc* 10:565–571.

SAS. 1995. *SAS user's guide: statistics* Cary, NC: SAS Institute.

Sukumar K, Perich MJ, Boobar LR. 1991. Botanical derivatives in mosquito control: a review. *J Am Mosq Control Assoc* 7:210–237.

Watanabe K, Shono Y, Kakimizu A, Okada A, Matsuo N, Satoh A, Nishimura H. 1993. New mosquito repellent from *Eucalyptus camalduensis*. *J Agric Food Chem* 41: 2164–2166.

Watanabe K, Tanaka Y, Mastuo N, Nishimura H. 1995. Rotundial, a new natural mosquito repellent from the leaves of *Vitex rotundifolia*. *Biosci Biotechnol Biochem* 59:1979–1980.