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Adult Repellency and Larvicidal Activity of Five Plant Essential Oils Against Mosquitoes

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Abstract

The larvicidal activity and repellency of 5 plant essential oils—thyme oil, catnip oil, amyris oil, eucalyptus oil, and cinnamon oil—were tested against 3 mosquito species: *Aedes albopictus*, *Ae. aegypti*, and *Culex pipiens pallens*. Larvicidal activity of these essentials oils was evaluated in the laboratory against 4th instars of each of the 3 mosquito species, and amyris oil demonstrated the greatest inhibitory effect with LC_{50} values in 24 h of 58 μg/ml (LC_{90} = 72 μg/ml) for *Ae. aegypti*, 78 μg/ml (LC_{90} = 130 μg/ml) for *Ae. albopictus*, and 77 μg/ml (LC_{90} = 123 μg/ml) for *Cx. p. pallens*. The topical repellency of these selected essential oils and deet against laboratory-reared female blood-starved *Ae. albopictus* was examined. Catnip oil seemed to be the most effective and provided 6-h protection at both concentrations tested (23 and 468 μg/cm^2). Thyme oil had the highest effectiveness in repelling this species, but the repellency duration was only 2 h. The applications using these natural product essential oils in mosquito control are discussed.

Keywords

Essential oils, mosquitoes, Aedes albopictus, Ae. Aegypti, Culex pipiens pallens, repellency, larvicides

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ADULT REPELLENCY AND LARVICIDAL ACTIVITY OF FIVE PLANT ESSENTIAL OILS AGAINST MOSQUITOES

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ABSTRACT. The larvicidal activity and repellency of 5 plant essential oils—thyme oil, catnip oil, amyris oil, eucalyptus oil, and cinnamon oil—were tested against 3 mosquito species: *Aedes albopictus*, *Ae. aegypti*, and *Culex pipiens pallens*. Larvicidal activity of these essentials oils was evaluated in the laboratory against 4th instars of each of the 3 mosquito species, and amyris oil demonstrated the greatest inhibitory effect with LC_{50} values in 24 h of 58 μg/ml (LC_{90} = 72 μg/ml) for *Ae. aegypti*, 78 μg/ml (LC_{90} = 130 μg/ml) for *Ae. albopictus*, and 77 μg/ml (LC_{90} = 123 μg/ml) for *Cx. p. pallens*. The topical repellency of these selected essential oils and deet against laboratory-reared female blood-starved *Ae. albopictus* was examined. Catnip oil seemed to be the most effective and provided 6-h protection at both concentrations tested (23 and 468 μg/cm^2). Thyme oil had the highest effectiveness in repelling this species, but the repellency duration was only 2 h. The applications using these natural product essential oils in mosquito control are discussed.

KEY WORDS Essential oils, mosquitoes, *Aedes albopictus*, *Ae. Aegypti*, *Culex pipiens pallens*, repellency, larvicides

INTRODUCTION

The control of mosquito vectors has become more difficult because some mosquitoes have rapidly become resistant to insecticides and synthetic repellents (Brown 1986, Chandre et al. 1998, Penilla et al. 1998). *N*,*N*-Diethyl-*m*-methylbenzamide (deet) has been considered one of the most effective synthetic repellents against mosquitoes. However, its toxic reactions have been reported in some circumstances, especially among children and the elderly (Moody et al. 1989, Clem et al. 1993). In addition, significant amounts of this chemical can be absorbed through the skin (Qiu et al. 1998). Deet also acts as a plasticizer and has an objectionable odor to some individuals. Therefore, research and development of alternative repellent compounds against mosquitoes with lower or no toxicity to humans are highly desired.

Plant-derived compounds (botanical insect repellents) are alternative sources for mosquito repellents (Sukumar et al. 1991, Watanabe et al. 1993, Wink 1993). The U.S. Environmental Protection Agency has ruled that 11 essential oils of plant origin are exempt from regulation under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (EPA 1996). Barnard (1999) and Cheng et al. (2004) have recently demonstrated that several plant essential oils not only have an inhibitory effect against bacteria, termites, and fungi but also exhibit strong mosquito repellency and larvicidal activity.

In this article, we report the topical repellency of five plant essential oils against the Asian tiger mosquito, *Aedes albopictus*, and their larvicidal activities on *Aedes aegypti*, *Ae. albopictus*, and *Culex pipiens pallens*. In addition, the repellency of different combinations of the two major ingredients of catnip oil, *Z*,*E*-nepetalactone and *E*,*Z*-nepetalactone, also were evaluated against female adults of *Ae. albopictus*.

MATERIALS AND METHODS

Insects

Aedes albopictus (Beijing strain) and *Cx. p. pallens* were the 16th generation of mosquitoes originally collected in Beijing. *Aedes albopictus* (ISU strain) were the 10th to 20th generations of mosquitoes originally collected in Missouri and colonized by the Illinois Natural History Survey (Champaign, IL). *Aedes aegypti* were the 10th generation originally collected in Costa Rica and colonized at Iowa State University (Ames, IA) in 2000. All mosquitoes were maintained under controlled environmental conditions (27 ± 1°C and 80 ± 5% RH with a 16:8-h photoperiod). Mosquitoes were maintained on a 10% sucrose solution. Approximately 400 larvae reared in a tray (24 × 35 × 5 cm) containing 2,000 ml water were supplied with 0.5 g of ground Tetra-Min fish food (Tetra, Blacksburg, VA) and held under the same conditions as mosquito adults.

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Major compounds in essential oils tested as mosquito repellents

Five plant essential oils were used in the repellency and larvicidal activity bioassays. Thyme oil (*Thymus vulgaris*) was purchased from SelectOils Co. (Prairie Grove, AR), catnip oil (*Nepeta cataria*) and amyris oil (*Amyris balsamifera*) were purchased from Essential Oil University (Charles Town, IN), and eucalyptus lemon oil (*Eucalyptus citriodora*) was purchased online (www.wellnaturally.com). Cinnamon oil, *Cinnamomum cassia*, was purchased at Good Earth Natural Food Co. (Bloomingdale, IL). *Z*,*E*- and *E*,*Z*-nepetalactone was isolated from catnip oil and purified using the method described in Peterson (2001). The purity of the 2 nepetalactones was >97.5% based upon gas chromatography (GC) analyses.

Two- to 3-μl samples of essential oils were dissolved each in 5 ml of HPLC-grade hexane and analyzed on both DB-1 and DB-WAX columns (30 m × 0.25 mm i.d., 0.25 μm in thickness; J & W Scientific, Folsom, CA) in a Hewlett–Packard (HP) 5890 Series II gas chromatograph interfaced to an HP 5972 mass selective detector. The injector temperature was set at 200°C, and the split valve was opened 1 min after injection. The column temperature started at 40°C for 3 min after the injection and then linearly increased to 230°C at a rate of 5°C/min. Mass spectra were recorded from 30 to 450 a.m.u. after electronic impact ionization at 70 eV. The chemical structures of the putative components were tentatively identified by comparisons of the retention times and confirmed by comparisons of mass spectra with those of authenticated chemical standards and reference spectra in mass spectral libraries (NIST 98 and Wiley 138K; John Wiley & Sons, Inc., New York, NY).

Electroantennogram responses

Electroantennogram (EAG) recordings were made by connecting an electrogel-filled (Spectro 360; Park Laboratory Parker, NJ) glass electrode to the excised head of a mosquito. A recording electrode filled with the same electrogel was connected to the cut tip of one antenna. Mosquito antennae were exposed to a charcoal-filtered, humidified airstream at a rate of 0.5 m/sec, and EAGs were recorded at room temperature (23 ± 1°C). The EAG setup consisted of a high-impedance d.c. amplifier with automatic baseline drift compensation (Syntech®, Hilversum, The Netherlands), and an EAG program (version 2.4) was used to record and analyze the amplified EAG signals on a PC computer (Micron Inc., Simi Valley, CA). Antennal responses to various essential oil extracts were recorded. Dilutions of the tested oil samples were made in redistilled HPLC-grade hexane at a dose of 1 μg. The tested essential oil extracts (100 ng/μl) were applied to filter-paper strips (0.5 × 2.5 cm, Whatman No. 1) in 10 μl of solvent. The filter-paper strips were inserted into Pasteur pipettes (15 cm in length). Control puffs of air were applied after each puff of a test stimulus. The response to the solvent (hexane) puff was used to normalize mosquito antennal responses relative to the tested stimuli. The average EAG response to the solvent puff from antennae of *Cx. pipiens* was 6 ± 2.8 mV ($n = 4$). No significant differences in EAG responses to the air were observed (5.8 ± 1.2 mV; $n = 4$). The relative EAG response of each stimulus was normalized by dividing the amplitude of the test stimulus by the mean of the response to the solvent puff. The sequence of exposure of each stimulus to each antenna was randomized.

Mosquito larvicidal activity studies

Thirty 4th instars were placed in 49.5 ml of distilled water, followed by the addition of 0.5 ml of dimethyl sulfoxide (DMSO) solution containing the test essential oils in a 100-ml glass beaker. The contents of the beaker were gently stirred with a glass rod to ensure a homogeneous test solution, after which the samples were incubated at room temperature. Each of the selected essential oils was tested at concentrations of 320, 160, 80, and 40 μg/ml. Controls were prepared with either 49.5 ml of distilled water with 0.5 ml of DMSO or 50 ml of distilled water only to which larvae were added. Each test was replicated at least 3 times. Larval mortality (observed with no physical movement after touching with a glass rod 3 times, 10 sec each) was recorded after 1-, 2-, 3-, 4-, 24-, and 48-h exposure. During the experimental period, food was not available to the larvae. Percentage of mortality was corrected for control mortality using Abbott's formula, and the results were plotted on log/probability paper using the method of Finney (1971). Toxicity and activity were reported as LC_{50} and LC_{90}, representing the concentration in micrograms per microliter that caused 50 and 90% larval mortality, respectively, in 24 h.

Repellent activity studies

One-week-old female unfed *Ae. albopictus* were used to evaluate the repellent activity of the essential oils, synthetic major ingredient compounds, and deet (Aldrich Chemical Co., Milwaukee, WI). Each compound was dissolved in 0.1% ethanol. A total volume of 37.5 μl of ethanol containing 2 concentrations (0.15 and 0.45 mg/cm^2) of selected repellents was applied

Table 1. Percentages of major chemicals identified from plant essentials oils tested as mosquito repellents and larvicides.

Compound[1]	THM	CAT	ECP	CIM	AMY
Cymene	27				
α-Terpinolene	10				
Thymol	61				
Caryophyllene		18			
Z,*E*-Nepetalactone		36			
E,*Z*-Nepetalactone		45			
Limonene			1		
p-Methane-3,8-diol			97		
Benzaldehyde				3	
Cinnamaldehyde				63	
Cinnamyl acetate				8	
O-Methoxycinnamaldehyde				24	
Elemol + eudesmols					80
β-Sesquiphellandrene					12
Identified components (%)	98	99	98	98	92

[1] THM, thyme oil, *Thymus vulgaris*; CAT, catnip oil, *Nepeta cataria*; ECP, eucalyptus oil, *Eucalyptus citriodora*; CIM, Cinnamon oil, *Cinnamomum cassi*; and AMY, amyris oil, *Amyris balsamifera*.

directly to 1 hand with an estimated area of exposed skin (through a 4- × 4-cm square hole on the back of a rubber glove). After applying the sample on the skin of the gloved hand, the sample was allowed to dry for 2 min. The same amount of ethanol without repellent was applied to the other hand (using the same method) to serve as the control. The skin was exposed for 3 min in a screened cage (40 × 40 × 45 cm) containing 300 blood-starved female mosquitoes. Immediately after the control exposure (3 min at 60-min intervals), the hand covered with the repellent candidate was inserted inside the cage. The number of tested mosquitoes biting on the skin was recorded. Four human volunteers were used for the repellency tests of each of the essential oils, individual compounds, and blends of major ingredients of 1 essential oil (catnip oil), except the test using a dose of 468 μg, for which only 3 human volunteers were used. The 2 blends tested were made up of 3:1 *Z*,*E*- and *E*,*Z*-nepetalactone (blend 1) and 1:1 of the same compounds (blend 2). The 2 nepetalactones were accumulated and purified from essential oils of *N. cataria* according to the method described in Peterson (2001). All tests were conducted at 26 ± 1°C and 65 ± 3% RH. Repellency was calculated according to the formula from Schreck et al. (1977): % repellency = [(Rc – Rt)] × 100, where Rc is the number of mosquitoes in the control group and Rt is the number of mosquitoes in the treated group.

Statistical analyses

Percentiles of repellency and larvicidal activity were determined and transformed to arcsine square-root values for analyses of variance (ANOVA). Significant differences at $P = 0.05$ (SAS version 9.1; SAS Institute, Cary, NC) were determined by the Scheffé test. The analysis of larval mortality involved the calculation of the median lethal concentration (LC_{50} or LC_{90}) of tested essential oils by using the POLO-PC program (Finney 1971, LeOra Software 1987).

RESULTS AND DISCUSSION

Chemical composition of essential oils

The major ingredients of 5 plant essential oils constituted 92–99% of the oils and were listed in Table 1. These major compounds identified were similar to those previously reported from thyme oil, catnip oil, eucalyptus lemon oil, and cinnamon oil (Peterson et al. 2002, Thompson et al. 2003, Cheng et al. 2004, Schultz et al. 2004). The identification of oxygenated sesquiterpenes, such as elemol and eudesmols, from amyris oil, also was reported from the fruit of the Osage orange tree, *Maclura pomifers* (Peterson et al. 2002).

Antennal responses to repellents

McIver (1982) and Sutcliffe (1994) suggested that there are 2 types of olfactory sensilla (sensilla trichodea and sensilla coeloconica) on female mosquito antennae that are involved in host location and finding oviposition sites. Electrophysiological studies showed that significant EAG responses are elicited in responding to attractants and certain repellents (Lacher 1967, Davis and Rebert 1972, Davis 1977, Qiu et al. 2004). In *C. p. pallens*, we found that female antennae responded to all tested essential oils and deet, although no significant differences were observed in EAG amplitudes to the tested repellent candidates (Fig. 1; F = 0.789, df 4, $P > 0.05$).

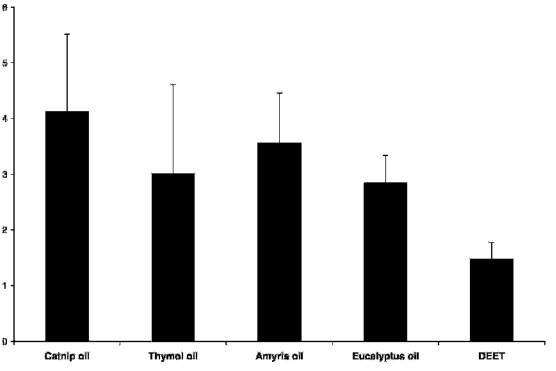

Fig. 1. Relative EAG responses of female *Culex pipiens pallens* mosquitoes to various essential oils of natural products and deet ($n = 4$).

Larvicidal activity tests

Mosquito larvicidal activities of 4 plant essential oils against 4th instars of 3 species are shown in Fig. 2 (*Ae. albopictus*), Fig. 3 (*Cx. p. pallens*), and Fig. 4 (*Ae. aegypti*). Differences in larval mortality varied by mosquito species, essential oil, concentration, and time that had elapsed after topical application. At the concentration of 40 µg/ml, mortality resulted in <20% for all 3 mosquito species. In *Ae. aegypti*, eucalyptus oil showed >80% mortality at concentrations of 80–320 µg/ml. High mortalities of cinnamon oil and amyris oil (>160 µg/ml) were observed against *Ae. albopictus* and *Cx. pipiens* at 24 h. At the highest concentration (320 µg/ml), eucalyptus oil was the only oil that caused 100% mortality to larvae of all mosquito species in 2 h. Table 2 summarizes the toxicity results of 4 plant essential oils against 4th instars of the 3 mosquito species at 24 h. *Aedes aegypti* is the most susceptible species, with the lowest LC_{50}s (52–80 µg/ml). Amyris oil showed an excellent toxicity against *Ae. aegypti* and *Ae. albopictus*. Cinnamon oil was the most toxic oil against *Cx. p. pallens* larvae, with an LC_{50} of 66 µg/ml and an LC_{90} of 99 µg/ml.

Cinnamon oil from the bark of *C. cassia* has been commonly used in the food and beverage industry, and its antibacterial activity has been reported against meat spoilage organisms (Ouattara et al. 1997). Cheng et al. (2004) reported that the essential oil from leaves of the indigenous cinnamon (*C. osmophloeum*) was an effective larvicide for *Ae. aegypti*. It was shown that the major constituent of this essential oil, cinnamaldehyde, exhibited the strongest activity against mosquito larvae (Cheng et al. 2004). Although 4 of 5 plant essential oils tested in this study have previously been reported to contain pesticidal compounds that are toxic to adult mosquitoes or are active as a chemical defense against other insect pests, this is the first report on larvicidal activity of essential oils from lemon eucalyptus, catnip, and amyris on mosquitoes.

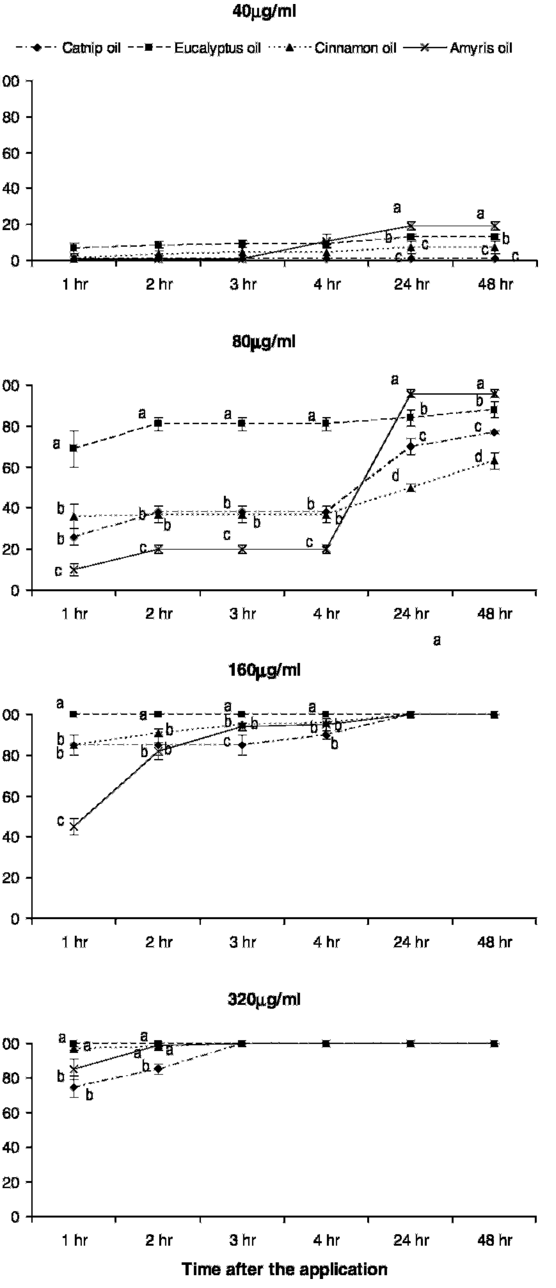

Fig. 2. Larval mortality in *Aedes albopictus* caused by 4 different concentrations of essential oils. Means followed by different letters are significantly different at $P < 0.05$ level, according to the Scheffé test (SAS, version 9.1). Error bars represent the standard error range.

Repellency of essential oils

Repellent activity of plant essential oils against female *Ae. albopictus* varied according to plant species, concentration, and duration of contact (Table 3). No differences in repellency were found at the 1st h after the topical application,

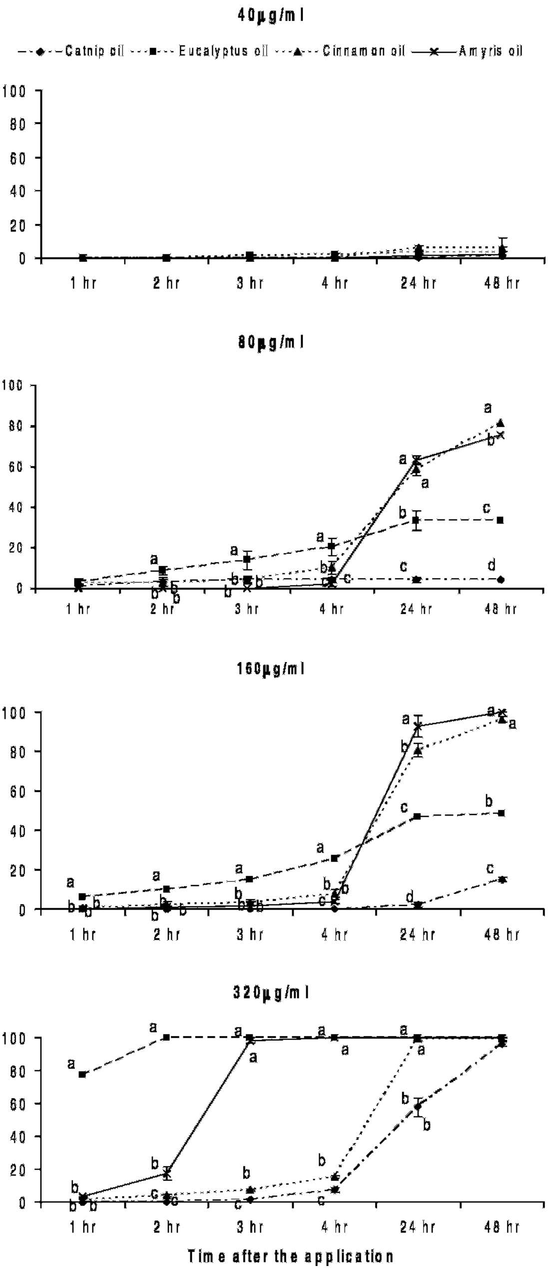

Fig. 3. Larval mortality in *Culex pipiens pallens* caused by four different concentrations of essential oils. Means followed by different letters are significantly different at $P < 0.05$ level, according to the Scheffé test (SAS, version 9.1). Error bars represent the standard error range.

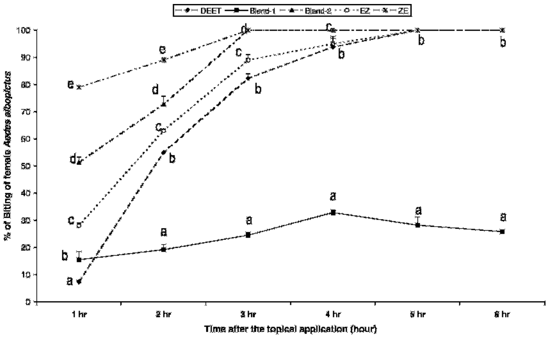

Fig. 4. Larval mortality in *Aedes aegypti* caused by 4 different concentrations of selected essential oils. Means followed by different letters are significantly different at $P < 0.05$ level, according to the Scheffé test (SAS, version 9.1). Error bars represent the standard error range.

except for eucalyptus oil. At a lower dose (0.375 mg), both deet and thyme oil started to lose repellency after 3 h. At a higher dose (7.5 mg), differences in repellency after 4 h were catnip oil (98%) > deet (86%) > elemol (57%). Catnip oil was the only repellent to provide protection for 6 h (Table 3). These results are consistent with a previous Y-tube olfactometer bioassay (Peterson 2001), indicating that catnip oil is an effective mosquito repellent. More recently, catnip oil was shown to inhibit host-finding capability of *Ae. aegypti* (Bernier et al. 2004). Repellent activity of thyme oil has been reported for *Ae. aegypti* and *Anopheles albimanus* (Barnard 1999). In the present study, we found that female *Ae. albopictus* are repelled by this oil at a lower concentration (Table 3). It is surprising that no repellent activity was observed against *Ae. albopictus* from eucalyptus oil, which Yang et al. (2004) showed to have the highest repellency among other tested plant essential oils against *Ae. aegypti*, although a relatively high concentration was used. Choi et al. (2002) also reported that this oil is effective against mosquito bites (*Cx. pipiens*) on hairless mice. We found that a higher dosage of deet is required for it to be an effective repellent while using lower dosages of nepetalactones. Peterson (2001) reported similar results in laboratory olfactometer bioassays.

Repellency of blends and individual compounds of catnip oil

The skin test for evaluating the repellency of the two major active ingredients of the catnip oil, *Z,E*-nepetalactone and *E,Z*-nepetalactone, showed that a blend containing the 2 compounds at a 3:1 ratio has the highest and longest repellent activity (Fig. 5). One hour after the application, repellent activity among individual or blends of nepetalactones compared with deet was blend 1 > *E,Z*-nepetalactone > blend 2 > *Z,E*-nepetalactone. More than 70% of repellency from blend 1 was observed after 6 h. The higher repellency of *E,Z*-nepetalactone compared with *Z,E*-nepetalactone demonstrated here against mosquitoes also

Table 2. LC_{50}s and LC_{90}s of essential oils of 4 plant species against 4th instars of 3 mosquito species (24 h).

	LC_{50} (µg/ml) (95% CI)				LC_{90} (µg/ml) (95% CI)			
	CIN	CAT	ECP	AMY	CIN	CAT	ECP	AMY
Aedes aegypti	80 (70–88)	70 (65–76)	58 (53–63)	52 (48–56)	123 (109–151)	97 (88–112)	88 (78–104)	72 (65–82)
Ae. albopictus	84 (72–96)	298 (274–328)	127 (100–158)	78 (64–95)	177 (147–231)	488 (404–753)	274 (211–440)	130 (106–193)
Culex pipiens pallens	66 (58–74)	122 (NA)[2]	113 (71–147)	77 (68–84)	99 (89–121)	178 (NA)	264 (198–516)	123 (110–147)

[1] CIN, cinnamon oil, *Cinnamomum cassia*; CAT, catnip oil, *Nepeta cataria*; ECP, eucalyptus oil, *Eucalyptus citriodora*; and AMY, amyris oil, *Amyris balsamifera*.
[2] NA, not applicable.

Table 3. Repellent activity of plant essential oils at 2 concentrations against female *Aedes albopictus*.

	% repellency (±SE)						
		Oils of plants[1] (23.425 µg/cm^2)			Oils of plants[1] (468.5 µg/cm^2)		
Time (h)	THM	CAT	ECP	Deet	AMY	CAT	Deet
1	96 ± 2 a[2]	84 ± 3 a	3 ± 0.5 b	93 ± 2 a	95 ± 2.2 A	100 ± 0 A	100 ± 0 A
2	88 ± 4 a	81 ± 1 a	1 ± 0.2 b	45 ± 2 a	86 ± 14 A	100 ± 0 A	100 ± 0 A
3	39 ± 3 b	75 ± 2 a	2 ± 0.3 c	0 ± 0 c	82 ± 18 A	100 ± 0 A	93 ± 3 A
4	13 ± 1 b	69 ± 1 a	0 ± 0 c	0 ± 0 c	57 ± 7 C	98 ± 2 A	86 ± 1 B
5	22 ± 2 b	67 ± 3 a	0 ± 0 c	0 ± 0 c	47 ± 18 C	92 ± 3 A	71 ± 7 B
6	14 ± 1 b	70 ± 1 a	0 ± 0 c	0 ± 0 c	34 ± 16 B	73 ± 8 A	45 ± 2 B

[1] THM, thyme oil, *Thymus vulgaris*; CAT, catnip oil, *Nepeta cataria*; ECP, eucalyptus oil, *Eucalyptus citriodora*; and AMY, amyris oil, *Amyris balsamifera*.
[2] Means within a row followed by the same letter are not significantly different ($P > 0.05$, Scheffé test; SAS Institute, version 9.1).

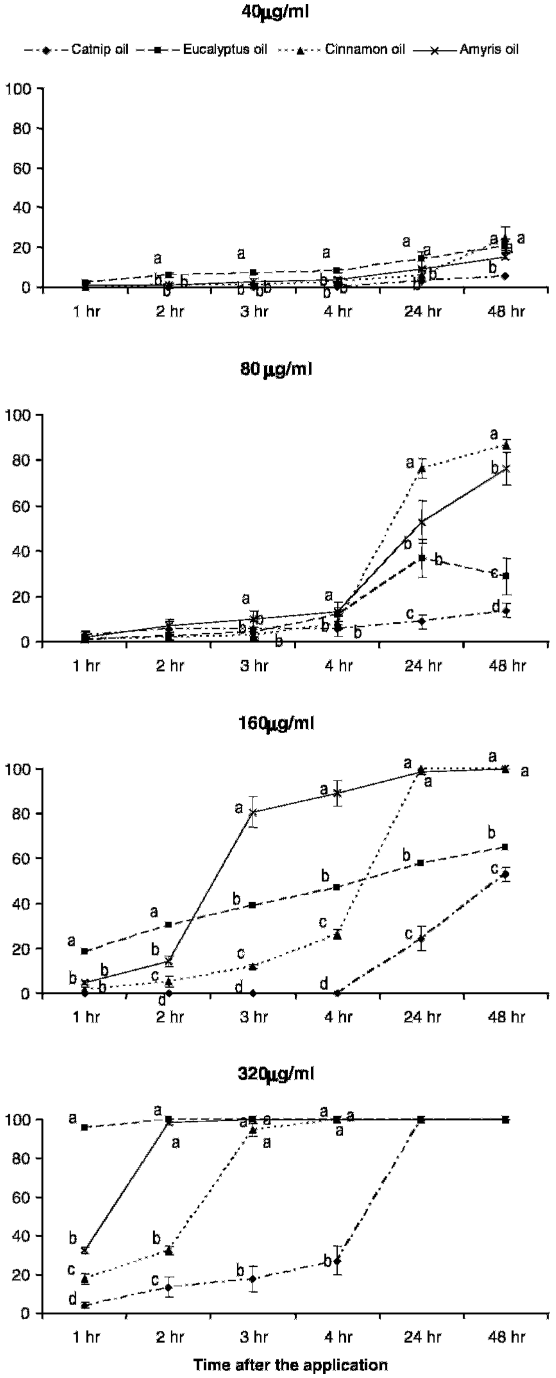

Fig. 5. Duration of protection from different blends of the major ingredients of catnip oil (*Nepeta cataria*), *Z*,*E*-nepetalactone and *E*,*Z*-nepetalactone, and deet against female *Aedes albopictus* tested at 0.468 µg/cm^2. Different letters next to means (± SE) indicate significances ($P < 0.05$, Scheffé test, SAS, version 9.1).

was observed in cockroaches (Peterson et al. 2002). In insect pheromone systems, many male moths use specific ratios of pheromone compounds for conspecific female attraction. However, for insect repellency, this report provides the first demonstration of differences in effectiveness of repellency related to ratios of repellent compounds. More studies are needed to explore the underlying mechanisms of the mixture of repellents and their applications in mosquito control.

These results indicate that several plant essential oils can be useful for preventing mosquito bites and also act as effective larvicides for the control of mosquitoes, because most of the essential oils are considered to be minimum risk pesticides and are exempted under section 25(b) of FIFRA. However, for practical use of these plant essential oils, further studies on their safety to human health are necessary. In addition, research on the different formulations may lead to improvement in the effectiveness of topical repellency and potency in larvicidal activity against mosquitoes.

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REFERENCES CITED

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

Bernier UR, Furman KD, Kline DL, Allan SA, Barnard DR. 2004. Comparison of contact and spatial repellency of catnip oil and *N*,*N*-diethyl-3-methylbenzamide (Deet) against mosquitoes. *J Med Entomol* 42:306–311.

Brown AWA. 1986. Insecticide resistance in mosquitoes: pragmatic review. *J Am Mosq Control Assoc* 2: 123–140.

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.

Cheng SS, Liu JY, Tsai KH, Chen WJ, Chang ST. 2004. Chemical composition and mosquito larvicidal activity of essential oils from leaves of different *Cinnamomum osmophloeum* provenances. *J Agric Food Chem* 52:4395–4400.

Choi WS, Park BS, Ku SK, Lee SU. 2002. Repellent activities of essential oils and monoterpenes against *Culex pipiens pallens*. *J Am Mosq Control Assoc* 18: 348–351.

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

Davis EE. 1977. Response to the antennal receptors of the male *Aedes aegypti* mosquito. *J Insect Physiol* 23:613–617.

Davis EE, Rebert CS. 1972. Elements of olfactory receptor coding in the yellow fever mosquito. *J Econ Entomol* 65:1058–1061.

[EPA] Environmental Protection Agency. 1996. 40 CFR 152.25 (g). Exemption of certain pesticide substances from Federal Insecticide, Fungicide, and Rodenticide Act requirements. Final Rule. EPA, May 6.

Finney DJ. 1971. *Probit analysis*. London, England: Cambridge Univ. Press.

Lacher V. 1967. Elektrophysiologische untersuchungen an einzenlen geruchsrezeptoren auf den antennen weiblicher moskitos (*Aedes aegypti* L). *J Insect Physiol* 13:L1461–L1470.

LeOra Software. 1987. *POLO-PC, a user's guide to probit and logit analysis*. Berkeley, CA: LeOra Software.

McIver SB. 1982. Sensilla of mosquitoes (Diptera: Culicide). *J Med Entomol* 19:489–535.

Moody 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.

Ouattara B, Simard RE, Holley RA, Piette GJP, Begin A. 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *Int J Food Microbiol* 37:155–162.

Penilla PR, Rodriguez 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.

Peterson CJ. 2001. Insect repellent of natural origin: catnip and osage orange. Ph.D. dissertation. Iowa State University: Ames, IA.

Peterson C, Zhu J, Coats JR. 2002. Identification of components of osage orange fruit (*Maclura pomifera*) and their repellency to German cockroaches. *J Essent Oil Res* 14:233–236.

Qiu H, Jun HW, McCall JW. 1998. Pharmacokinetics, formulation and safety of insect repellent N,N-diethyl-3-methylbenzamide (deet): a review. *J Am Mosq Control Assoc* 14:12–27.

Qiu YT, Smallengange RC, Hoppe S, van Loon JJA, Bakker EJ, Takken WT. 2004. Behavioral and electrophysiological responses of the malaria mosquito *Anopheles gambiae* Giles *sensu stricto* (Diptera: Culicidae) to human skin emanations. *Med Vet Entomol* 18:429–438.

Schreck CE, Posey K, Smith D. 1977. Repellent activity of compounds submitted by Walter Reed Army Institute of Research. 1. Protection time and minimum effective dosage against *Aedes aegypti* mosquitoes. *Tech Bull USDA* 1549:215.

Schultz G, Simbro E, Belden J, Zhu J, Coats J. 2004. Catnip, *Nepeta cataria* (Lamiales: Lamiaceae)—a close look: seasonal occurrence of nepetalactone isomers and comparative repellency of the terpenoids to insects. *Environ Entomol* 33:1562–1569.

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

Sutcliffe JF. 1994. Sensory base of attractancy: morphology of mosquito olfactory sensilla—a review. *J Am Mosq Control Assoc* 10:309–315.

Thompson JD, Chalchat JC, Michet A, Linhart YB, Ehlers B. 2003. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. *J Chem Ecol* 29:859–880.

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

Wink M. 1993. Production and application of phytochemicals from an agricultural perspectives. In: van Beek TA, Breteler H, eds. *Phytochemistry and agriculture*. Oxford, United Kingdom: Clarendon Press. p 171–213.

Yang YC, Lee EH, Lee HS, Lee DK, Ahn YJ. 2004. Repellency of aromatic medicinal plant extracts and a steam distillate to *Aedes aegypti*. *J Am Mosq Control Assoc* 20:146–149.