

Repellency of Volatile Oils from Plants against Three Mosquito Vectors

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Published in *Journal of Vector Ecology*, Vol. 26, No. 1: 76-82, 2001.

Abstract

Volatile oils extracted by steam distillation from four plant species turmeric (*Curcuma longa*), kaffir lime (*Citrus hystrix*), citronella grass (*Cymbopogon winterianus*) and hairy basil (*Ocimum americanum*), were evaluated in mosquito cages and in a large room for their repellency effects against three mosquito vectors, *Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus*. The oils from turmeric, citronella grass and hairy basil, especially with the addition of 5% vanillin, repelled the three species under cage conditions for up to eight hours. The oil from kaffir lime alone, as well as with 5% vanillin added, was effective for up to three hours. With regard to the standard repellent, deet alone provided protection for at least eight hours against *Ae. aegypti* and *Cx. quinquefasciatus*, but for six hours against *An. dirus*. However, deet with the addition of 5% vanillin gave protection against the three mosquito species for at least eight hours. The results of large room evaluations confirmed the responses for each repellent treatment obtained under cage conditions. This study demonstrates the potential of volatile oils extracted from turmeric, citronella grass and hairy basil as topical repellents against both day-and night-biting mosquitoes. The three volatile oils can be formulated with vanillin as mosquito repellents in various forms to replace deet (*N,N*-diethyl-3methylbenzamide), the most common chemical repellent currently available.

Keywords

Repellents, plant volatile oils, deet, mosquitoes

Introduction

Over two billion people, primarily in tropical countries, are at risk from mosquito-borne diseases, such as dengue hemorrhagic fever, malaria and filariasis (Service 1993). The search for effective vaccines against these diseases is still in progress. Mosquito control and personal protection from mosquito bites are currently the most important measures to control these diseases. The use of repellents is an obvious practical and economical means of preventing the transmission of these diseases to humans. The most common mosquito repellent formulations available on the market contain deet (*N,N*-diethyl-3-methylbenzamide), which has shown excellent repellency against mosquitoes and other biting insects (Yap 1986, Coleman et al. 1993, Walker et al. 1996). However, human toxicity reactions after the applications of deet vary from mild to severe (e.g., Zadikoff 1979, Robbins and Cherniack 1986, Edwards and Johnson 1987, Qiu et al. 1998). To avoid these adverse effects, research on repellents that are derived from plant extracts to replace deet has been conducted in many laboratories. Recently, extracts of several plants, including neem (*Azadirachta indica* A. Juss), basil (*Ocimum basilicum* L., *O. basilicum* L. fa. *citratum* Bach, *O. gratissimum* L., *O. americanum* L., *O. tenuiflorum* L.) citronella grass (*Cymbopogon nardus* Rendle), galingale (*Alpinia galanga* L.), clove (*Syzygium aromaticum* L.) and thyme (*Thymus vulgaris* L.), have been studied as possible mosquito repellents (Sharma et al. 1993, Chokechaijaroenporn et al. 1994, Suwonderd and Tantrarongroj 1994, Boonyabancha et al. 1997, Barnard 1999). These natural repellents have demonstrated good efficacy against some mosquito species but some were evaluated only by olfactometry or by using laboratory mice as hosts of *Aedes aegypti* (L.) under laboratory conditions. However, the evaluation of repellency should preferably be carried out using human subjects because laboratory animals may inadequately simulate the condition of human skin to which repellents will be eventually applied (WHO 1996).

This study investigates the repellency of volatile oils derived from four plant species against three mosquito vectors using human bait methods in mosquito cage and large room conditions. Also, the usefulness of the additive vanillin to increase the protection time of the oils was studied.

Materials and methods

Volatile oils

At least 20 kg of turmeric (*Curcuma longa* L.) rhizomes, kafir lime (*Citrus hystrix* DC.) leaves, citronella grass (*Cymbopogon winterianus* Jowitt) leaves, and hairy basil (*O. americanum*) leaves were extracted for volatile oils by steam distillation. One or two kg of fresh plant material at a time was cut into small pieces and placed in a distillation flask with approximately five times as much water and 10 glass beads. The distillation chamber was heated in a liquid paraffin bath at about 120 °C and allowed to boil until the distillation was completed. The distillate was collected in a separating funnel in which the aqueous portion was separated from the volatile oil. The water (lower) layer was slowly drawn off until only the oil layer remained. This procedure was repeated until at least 20 ml of oil had been recovered. The volatile oil was collected and kept in a stoppered cylinder at 4 °C until it was tested for mosquito repellency. For efficacy evaluation, each oil as well as deet was prepared in two formulations: 25% (v/v) in absolute ethanol with and without 5% vanillin.

Test mosquitoes

The mosquitoes used in this study were laboratory-reared female *Ae. aegypti* (dengue hemorrhagic fever vector), *Anopheles dirus* Peyton & Harison (malaria vector) and *Culex quinquefasciatus* Say (filariasis vector). These were reared according to the standard protocol of the Biology & Ecology Section, National Institute of Health, Thailand, and maintained in the insectary of the institute. Three to five-day-old females of these species were used for repellency tests.

Repellent test procedure

The repellency of the volatile oils was evaluated using the human-bait technique (Schreck and McGovern 1989, WHO 1996). The testing period lasted up to eight hours, depending on the efficacy. The timing of the tests depended on whether the target mosquitoes were day-or night-biters; *Ae. aegypti* was tested from 0800 h to 1600 h while *An. dirus* and *Cx. quinquefasciatus* were tested between 1800 h and 0200 h. Evaluations were carried out in a 6x6x3 m room, at 25-29 °C and relative humidity of 60-80%. An area 3x10 cm on each forearm of three human volunteers was marked out with a permanent marker.

Approximately 0.1 ml of test repellent was applied to the marked area of one forearm of each volunteer while the other forearm was treated with the same repellent with 5% vanillin added. As a blank control, a solution of 5% vanillin in ethanol was placed on one forearm of the same volunteer with the same process as the test repellents, whereas the other forearm was untreated. During the test, the forearm was covered by a paper sleeve with a hole corresponding to the marked area. Each volunteer put the test forearm in a mosquito cage (40x40x40 cm), containing 250 female mosquitoes (3-5 days old), for the first three minutes of every half-hour exposure. However, before the start of each exposure, the bare hand, used as control area of each volunteer, was exposed for up to 30 seconds. If at least two mosquitoes landed on or bit the hand, the repellency test was then continued. The test continued until at least two bites occurred in a three-minute period, or until a bite occurred and was followed by a confirmatory bite (second bite) in the following exposure period. The time between application of the repellents and the second successive bite was recorded as the protection time. Since two hours is the minimum protection time specified for mosquito repellents allowed to be sold in Thailand, repellents providing at least four hours of protection under mosquito cage conditions were then tested for efficacy under large room conditions.

Large room evaluations

The evaluations were conducted in a 6x6x3 m room that had a door and six glass-windows that were always closed during the tests. The room was lit with fluorescent lamps. Ten minutes before the start of each test, 250 avid female mosquitoes (3-5 days old) were released into the test room. To compare the data with the results from the mosquito cage, the same three volunteers were assigned to evaluate the volatile oils under the large room conditions. Assessment areas comprised each leg from knee to ankle, covering surface area of about 782-826 cm². Approximately 3 ml of the volatile oil were applied to the test area of one leg of each volunteer. The other leg was the control area. Immediately after oil application, the volunteers went into the test room and sat on chairs in a triangle 1.5 m from each other. Evaluation of the repellency was done by catching the mosquitoes that landed on or bit the assessment areas of the volunteers' legs. For the six hours of each repellent test, volunteers entered the room for 10 minutes each half-hour. Therefore, each test was based on 13 mosquito collections and 12 breaks. Volunteers' positions were rotated

on each collection occasion to allow for any variation among the positions. All mosquitoes caught during each collection were released again into the room to maintain the same number as at the start. The tests for each volatile oil against each mosquito species were conducted on separate days. Each test was carried out for 6 hours and the timing of the test depended on the target mosquitoes, i.e., 1000-1600 h for *Ae. aegypti* and 1800-2400 h for *An. dirus* and *Cx. quinquefasciatus*.

Data analysis

The median protection time was used as a standard measure of the repellency of the volatile oils and deet against the three mosquito species in the laboratory. The repellency among the oils was compared using the Kruskal-Wallis one-way ANOVA. The effects of vanillin in prolonging the protection time of the repellents were analyzed using the Mann-Whitney U Test. Percentage repellency in the semi-field trial was calculated as follows (Sharma and Ansari 1994, Yap et al. 1998):

$$\% \text{ Repellency} = \frac{C - T}{C} \times 100$$

Where *C* is the number of mosquitoes collected from control areas and *T* is the number collected from the treated areas of volunteers. The total numbers of mosquitoes caught during each exposure at all seat positions for the semi-field evaluation of each mosquito species was compared using a Kruskal-Wallis one-way ANOVA.

Results

The relative repellency of the four volatile oils and deet with and without vanillin against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus* is shown in Figure 1. There were significant overall differences in repellency among the repellents against each of the mosquito species ($P < 0.01$). There was no repellency against the three mosquito species of the blank control (5% vanillin in ethanol); i.e., biting frequency did not differ from that on the control (untreated) arm. Among the four oils without vanillin, citronella and hairy basil provided repellency against *Ae. aegypti* for three hours while turmeric and kaffir lime gave only one hour (Figure 1A). However, vanillin significantly increased the repellency of these oils against *Ae. aegypti* ($P < 0.05$). As a result, citronella and hairy basil with vanillin could repel *Ae. aegypti* for up to 6.5 h,

whereas turmeric and kaffir lime with vanillin had extended repellency to 4.5 and 3 hours respectively. Deet, with and without vanillin, provided repellency against *Ae. aegypti* for at least eight h.

For *An. dirus* (Figure 1B), turmeric oil alone showed outstanding efficacy among the test repellents without vanillin. In fact, turmeric oil provided protection for at least eight hours, whereas deet gave only six hours for this species. The other three oils all had a repellency of less than four hours. However, vanillin prolonged the repellency of citronella, hairy basil and deet to at least eight hours. Vanillin extended the repellency of kaffir lime from 0.5 to 1.5 hours. Again, vanillin significantly increased the repellency of the extracts against *An. dirus* ($P < 0.05$).

In contrast with *An. dirus*, citronella and hairy basil oils without vanillin provided as good a repellency as did deet against *Cx. quinquefasciatus* of at least eight hours, whereas turmeric oil alone gave repellency for five hours and kaffir lime only 0.5 h (Figure 1C). With vanillin added, turmeric, citronella and hairy basil oils repelled *Cx. quinquefasciatus* for at least eight hours, but kaffir lime was extended to only 2.5 h. There was no significant difference in the effect of vanillin in prolonging the protection by the four oils against *Cx. quinquefasciatus* ($P > 0.05$).

The results of repellency against the mosquitoes under large room conditions are given in Table 1. There were no bites by the mosquitoes for at least four hours after the application of all extracts. Citronella + vanillin demonstrated a repellency equivalent to the standard repellents, deet and deet + vanillin, with at least six hours complete protection against *Ae. aegypti*. Turmeric + vanillin and hairy basil + vanillin were less effective than the deet standard, with repellencies of about 60% and 85.7% six hours after application. In contrast, the four volatile oil formulations, turmeric, turmeric + vanillin, citronella + vanillin and hairy basil + vanillin, showed greater protection against *An. dirus* than did deet. In fact, the four formulations as well as deet + vanillin could completely repel the anopheline mosquito for at least six hours, whereas six hours after application deet alone gave repellency of about 58.3% (Table 1). All repellents demonstrated equally good repellency against *Cx. quinquefasciatus* that lasted for at least six hours after application.

The numbers of *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus* biting on the control and treated areas are shown in Table 1. There were no significant

differences in the number of mosquitoes caught among the control groups for each mosquito species ($P>0.05$).

No skin irritation, hot sensations or rashes were observed on the arms and legs of the test volunteers treated with the volatile oils during five months of the study period or in the following three months, after which time observations ceased.

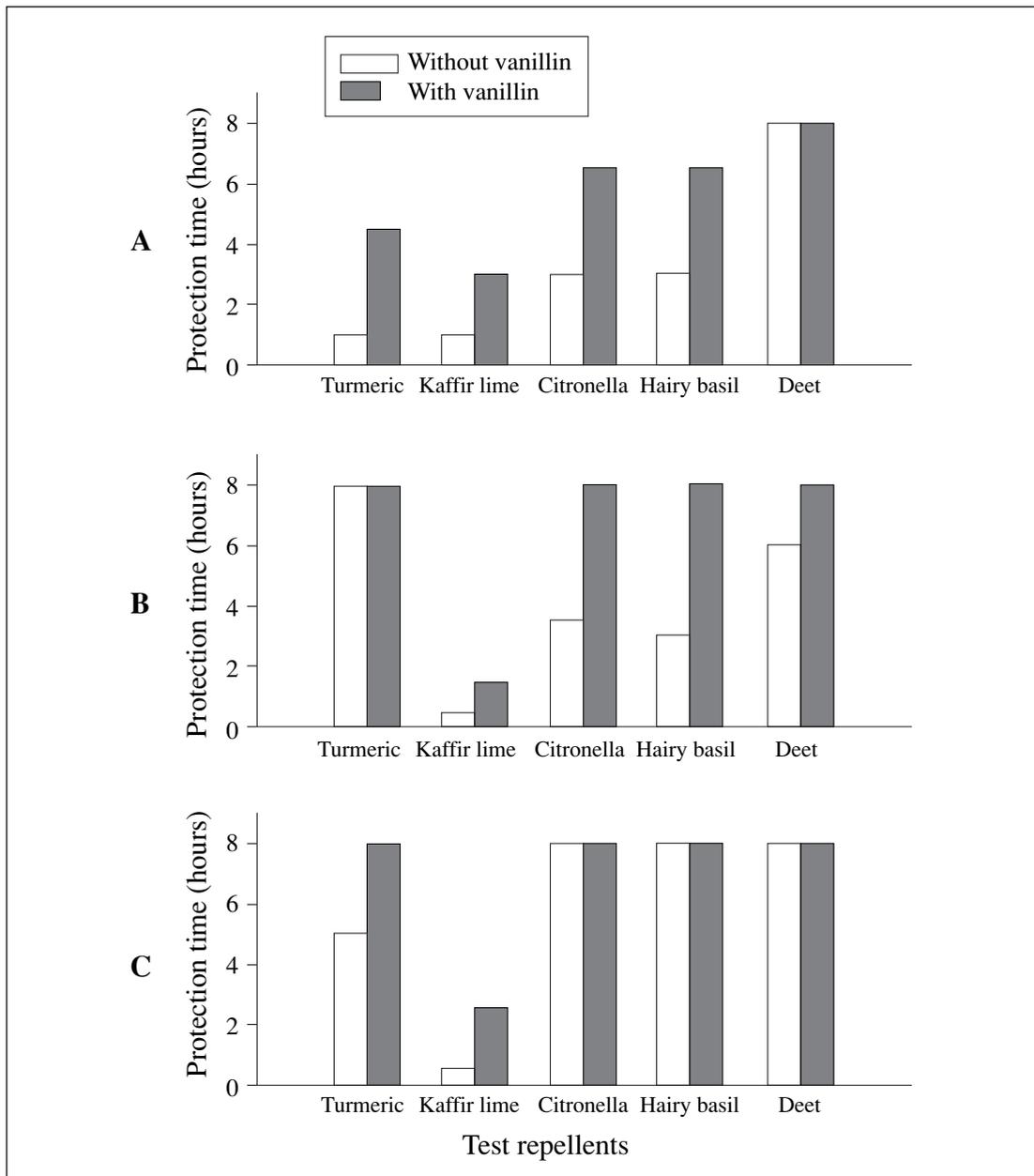


Figure 1. Relative repellency (median protection time) of volatile oils and deet against (A) *Ae. aegypti*, (B) *An. dirus*, and (C) *Cx. quinquefasciatus* under laboratory conditions.

Table 1. Relative repellency of volatile oils and deet against three mosquito vectors under large room conditions.

Mosquito species	Test repellents	No. of mosquito bites (mean \pm S.E.)		% Repellency after application				
		Control	Treated	4.0 h	4.5 h	5.0 h	5.5 h	6.0 h
<i>Ae. aegypti</i>	Turmeric + Vanillin	45.3 \pm 2.7	1.2 \pm 0.6	100	94.4	76.9	62.5	60
	Citronella + Vanillin	44.8 \pm 4.2	0.0 \pm 0.0	100	100	100	100	100
	Hairy basil + Vanillin	48.4 \pm 2.8	0.3 \pm 0.2	100	100	92.3	90.9	85.7
	Deet	38.2 \pm 3.5	0.0 \pm 0.0	100	100	100	100	100
	Deet + Vanillin	46.1 \pm 5.0	0.0 \pm 0.0	100	100	100	100	100
<i>An. dirus</i>	Turmeric	29.1 \pm 1.7	0.0 \pm 0.0	100	100	100	100	100
	Turmeric + Vanillin	35.5 \pm 2.3	0.0 \pm 0.0	100	100	100	100	100
	Citronella + Vanillin	36.1 \pm 3.0	0.0 \pm 0.0	100	100	100	100	100
	Hairy basil + Vanillin	33.0 \pm 2.2	0.0 \pm 0.0	100	100	100	100	100
	Deet	30.6 \pm 2.5	0.6 \pm 0.4	100	100	88.9	75	58.3
	Deet + Vanillin	34.1 \pm 2.2	0.0 \pm 0.0	100	100	100	100	100
<i>Cx. quinquefasciatus</i>	Turmeric	33.0 \pm 1.9	0.0 \pm 0.0	100	100	100	100	100
	Turmeric + Vanillin	26.8 \pm 1.8	0.0 \pm 0.0	100	100	100	100	100
	Citronella	27.4 \pm 2.2	0.0 \pm 0.0	100	100	100	100	100
	Citronella + Vanillin	28.6 \pm 2.6	0.0 \pm 0.0	100	100	100	100	100
	Hairy basil	30.3 \pm 1.8	0.0 \pm 0.0	100	100	100	100	100
	Hairy basil + Vanillin	31.1 \pm 2.3	0.0 \pm 0.0	100	100	100	100	100
	Deet	27.2 \pm 1.8	0.0 \pm 0.0	100	100	100	100	100
	Deet + Vanillin	33.2 \pm 1.9	0.0 \pm 0.0	100	100	100	100	100

Discussion

The volatile oils derived from turmeric, citronella grass and hairy basil, especially with 5% vanillin added, were very effective against the three mosquito species, and that from kaffir lime alone or with 5% vanillin added showed the least repellency. The results of large room evaluations clearly confirmed these results. The protection time of the four oils was significantly increased by the incorporation of 5% vanillin. These results agree with those of Khan et al. (1975) that vanillin could prolong protection time against *Ae. aegypti* by more than 100% in most cases. However, the volatile oil of kaffir lime is not suitable as a mosquito repellent because of its low repellency; only those of turmeric, citronella and hairy basil should be considered further as topical mosquito repellents. The results of this study are similar to those of

Jaruwichitratana et al. (1988), Wasuwat et al. (1990), Chokechaijaroenporn et al. (1994), Suwonderd and Tantrarongroj (1994) and Boonyabancha et al. (1997), but are potentially more useful in many respects, such as the method used (see below), length of assessment and a wider range of mosquito species tested. It is important to note that all those other studies were with citronella oil obtained from a different species of citronella grass (*Cy. nardus*). In fact, Jaruwichitratana et al. (1988) conducted an efficacy test on 14% citronella cream against *Culex* mosquitoes under field conditions for only one hour and showed that the cream could prevent at least 90% of mosquito attacks in thirteen out of twenty volunteers who applied enough cream (1.2 g or more per whole forearm). Wasuwat et al. (1990) demonstrated under laboratory conditions that repellency of a cream containing 14% citronella oil was about two hours against *Ae. aegypti*. On the other hand, Suwonderd and Tantrarongroj (1994) showed that a repellent cream containing less than 10% citronella oil provided only two hours protection against *An. minimus* Theobald mosquitoes under laboratory conditions while a 10% formulation could repel this species for at least four hours. Additionally, cream containing a combination of 2.5% citronella oil, 5% galangale oil and 0.5% vanillin could prevent biting by *An. minimus* for at least six hours. Unfortunately, this laboratory study was conducted during the day whereas *An. minimus* is a night-biter. In the field, the repellency of a cream containing a combination of 2.5% citronella oil, 5% galangale oil and 0.5% vanillin against *Cx. quinquefasciatus* was six hours from 1800 h to 2400 h.

In contrast to these previous studies, Boonyabancha et al. (1997) used a modified olfactometer incorporating laboratory mice and demonstrated that the EC95 concentrations against *Ae. aegypti* for citronella oil and hairy basil oil were approximately 5.3% and 1.5%. They also showed that at fifteen minutes post-application, a 1% concentration of those two oils provided about 75% and 90% protection against *Ae. aegypti* biting human arms during a single three-minute exposure under laboratory conditions. In contrast, again using mice, Chokechaijaroenporn et al. (1994) showed that the volatile oil obtained from hairy basil exhibited the least repellency among the oils from five *Ocimum* spp., being only fifteen minutes against *Ae. aegypti*, but those of *O. gratissimum*, *O. basilicum*, *O. basilicum* L. fa. *citratum* and *O. tenuiflorum*, were 135, 75, 75 and 105 minutes respectively. On the other hand, in a study in Guinea Bissau,

West Africa, fresh *O. canum* Sims (syn. *O. americanum*) could reduce biting by anopheline mosquitoes by about 63.6%, mostly *An. gambiae* Giles and *An. pharoensis* Theobald, under field conditions between 2000 h and 2200 h (Palsson and Jaenson 1999). There is clearly inconsistent repellency of volatile oils derived from *O. americanum* among the methods used for evaluation. It would therefore be very valuable to compare the repellency, by evaluations based on human skin, among the different plant species, such as citronella grass (*Cy. nardus* and *Cy. winterianus*), basil (*O. americanum*, *O. gratissimum*, *O. basilicum*, *O. bsilicum* fa. *citratum* and *O. tenuiflorum*).

This study could not describe the rearing details, e.g., soil, water, and nutritional conditions of the plant materials used for oil extraction because the study plants were purchased from the local market. However, it is important to obtain the most appropriate conditions for growing each plant in order to obtain the best yield, and further studies should emphasize this point. The quality of volatile oils depends on many factors, e.g., plant species, rearing conditions, maturation of harvested plants, plant storage, plant preparation and methods of extraction. Thus, these factors should be carefully considered and standardized when the extraction of volatile oils is being planned.

In conclusion, this study clearly demonstrated the potential of volatile oils derived from turmeric, citronella and hairy basil, for use as topical repellents against both diurnal and nocturnal mosquitoes. To improve their repellent efficacy, these three oils should be formulated with vanillin and could replace deet, currently the most common chemical repellent available. However, testing in the field will be necessary.

Acknowledgments

This study was supported by the Ministry of Foreign Affairs and Trade, New Zealand, Lincoln University, New Zealand, and the National Institute of Health, Thailand. The authors are grateful to Dr. Mir S. Mulla, University of California, Riverside, USA, for his valuable comments on the manuscript. We appreciate the guidance on statistical analysis of Mr. Preecha Asavadachanukorn, Chulalongkorn University, Thailand, and Dr. Christ Frampton, Lincoln University, New Zealand. We also would like to thank Mr. Jakkrawarn Chomposri, Mr. Dusit Noree and Mr. Navy Srivarom for their assistance in repellent tests.

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