Phytochemicals as Repellents against Mosquitoes in Thailand

Usavadee Thavara, Apiwat Tawatsin and Jakkrawarn Chompoosri

National Institute of Health, Department of Medical Sciences, Ministry of Public Health.

Published in Proceedings International Conference on Biopesticide 3, April 21-26, 2002, Kuala Lumpur, Malaysia, 244-250.

Abstract

Repellents are commonly used for personal protection against mosquitoes worldwide. They are one of the most effective products used in prevention and control of mosquito-borne tropical diseases. Although there are a number of effective repellents containing chemical active ingredients, such as deet, KBR 3023 and IR3535 there is in concern with regard to chemical toxicity. To overcome this concern phytochemicals extracted from various plants have been formulated as mosquito repellents to be sold in Thailand in recent years. Since the year 2000, 44 formulations of mosquito repellents containing plant extracts as active ingredients were evaluated for repellency against *Ae. aegypti* under laboratory conditions at the National Institute of Health (NIH), Thailand. These extracts included citronella oil, eucalyptus oil, tea tree oil, turmeric oil, bergamot oil, lavender extract, tobacco-leaves extract, clove extract and neem-leaves extract. The protection offered by these products was up to 6.3 hours. However, only 12 products were qualified for registration to be sold in the market since minimum protection time of 2 hours is the minimum in requirement. These qualified repellent products were formulated as lotion, spray, cream and balm, where citronella oil, eucalyptus oil and tea tree oil were the main active ingredients. On the other hand, the NIH also formulated a mosquito repellent containing turmeric oil and eucalyptus oil as active ingredients. It was found that this repellent provided protection time for 7 hours against *Ae. aegypti*, and at least 8 hours against *Culex quinquefasciatus* and *Anopheles dirus* under laboratory conditions. This study demonstrated and encouraged the development of alternative active ingredients derived from plants to be formulated as effective mosquito repellents.

Keywords

Repellents, mosquitoes, phytochemicals, biopesticides

Introduction

Repellents are commonly used for personal protection against mosquitoes. They are one of the most effective devices used in prevention and control of mosquito-borne diseases or for protection of mosquito bites. Although there are a number of effective mosquito repellents containing synthetic chemicals, such as deet (Smith 1953, Coleman et al. 1993), KBR 3023 (Yap et al. 1998) and IR 3535 (Thavara et al. 2001) which are currently available in the market, there is increasing concern with regard to their toxicity. Several researchers have reported adverse effects after uses of repellents containing deet, such as contact urticaria (Maibach and Johnson 1975), skin eruption (Yagupsky 1982) or toxic encephalopathy in children (Zadikoff 1979, Edwards and Johnson 1987). Although there is no report of any adverse effects of KBR 3023 and IR 3535 until now, the widespread use of these chemical repellents has been impeded by many people with the concern of possible effects similar to deet. In recent years attempts have then been placed in Thailand on extracting and evaluating extracts of plants for repellent activity against mosquitoes (Jaruwichitratana et al. 1988, Wasuwat et al. 1990, Chokechaijaroenporn et al. 1994, Suwonkerd and Tantrarongroj 1994, Boonyabancha et al. 1997, Tawatsin et al. 2001). Phytochemicals extracted from various plants, thus, have been formulated as mosquito repellent products for sale in Thailand. We initiated detailed studies on the evolution of plant-based products for use as mosquito repellents.

In this study we evaluated the repellency of mosquito repellent products containing plant extracts as active ingredients against mosquitoes in laboratory and under field conditions. The repellent formulation containing turmeric oil and eucalyptus oil was also developed by us and then evaluated under laboratory and field conditions.

Materials and methods

Test mosquitoes in laboratory

The mosquitoes used in this study were laboratory-reared female *Aedes aegypti* (L.), *Anopheles dirus* Peyton & Harrison, and *Culex quinquefasciatus* Say. The mosquitoes were bred and maintained according to the standard operation procedure of the Biology and Ecology Section, National Institute of Health (NIH), Thailand. In each test, 3-5 day-old female mosquitoes had no prior blood meal, were starved for 16 hours prior to testing.

Test repellents

The test repellents were the products proposed to be registered at the Food and Drug Administration (FDA) for sale in Thailand during the period from January 2000 to February 2002. Prior to registration, every product must be evaluated for repellency against *Ae. aegypti* at the NIH. A total of 44 repellent products containing plant extracts as active ingredients only were selected for this study. These extracts included citronella oil, eucalyptus oil, tea tree oil, turmeric oil, bergamot oil, lavender extract, tobacco-leaves extract, clove extract and neem-leaves extract. On the other hand, a repellent containing turmeric oil (5%) and eucalyptus oil (10%) as active ingredients with 5% vanillin added was formulated by us an evaluated for repellency in this study.

Laboratory evaluation

The test repellents were evaluated for repellency under laboratory conditions using human-bait method (Tawatsin et al. 2001, Thavara et al. 2001). The test period was up to 8 hours, depending on duration of repellency of each repellent. Our turmeric and eucalyptus repellent and the 44 other repellent products were tested for repellency against day biting mosquitoes, *Ae. aegypti*, from 0800 h to 1600 h. Moreover, our turmeric and eucalyptus oil repellent was tested against night biting mosquitoes, *Cx. quinquefasciatus* and *An. dirus* during the period between 1800 h and 0200 h. All evaluations were carried out in a 6x6x3 m^3 room, at 25-29 °C with relative humidity of about 60-80%. One of each forearm of three volunteers was marked out for an area of 3x10 cm^2 with a permanent marker. An amount of 0.1 ml of test repellent was applied to the marked area of one forearm of each volunteer. Prior to each exposure, the marked forearm was covered by a paper sleeve, having a rectangular hole corresponding to the marked area. Then, each volunteer inserted the marked forearm into a mosquito cage (40x40x40 cm), containing 250 female mosquitoes for three minutes every half-hour. The test continued until at least two bites occurred in a three-minute exposure period, or until a bite in the previous exposure period followed by the second bite in the following exposure period. Repellency or protection time of each repellent was the time between application of the test repellent and the second successive bite.

Field evaluation

Ten out of 44 repellent products and our repellent (5% turmeric oil and 10% eucalyptus oil with 5% vanillin) were evaluated for repellency against wild population of mosquitoes under field conditions. Among the 10 selected repellent products, half of them were chosen from qualified products (protection time 2.5-5.3 hours) and the rest were from unqualified ones (protection time 0.5-1.8 hours). Field evaluations were conducted by four volunteers (aged 25-57 years) at a research station located in Bang Bua Thong District, Nonthaburi Province. This place was chosen to be the study site as it had developmental sites for a variety of mosquito species. The evaluations were carried out at locations with minimal wind disturbance in an opened-building (6x24 m^2), which had only roof and there were some water jars placed in the building. Mosquitoes therefore could come from surrounding areas to feed on volunteers. Each volunteer was treated with 3 ml of the test repellent on one leg (from knee to ankle) whereas the other leg was left as control. The volunteers were positioned in a row, 5 m apart from each other and they caught all of the mosquitoes landing on or biting both their legs in the desired area (from knee to ankle) within a 10-minute period. Each mosquito collection was followed by a 20-minute break before the next collection was conducted again. As a result, there were two mosquito collections and two breaks during each hour of the test. The tests were carried out for 4 hours from 1800 h to 2200 h. The collected mosquitoes then were identified to species in laboratory. Repellency of each repellent was assessed through comparisons of mosquitoes collected on control (untreated) and treaded legs. The reduction in biting was calculated as follow (Yap et al. 1998):

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

Where *C* denotes the number of mosquitoes collected by the control group and *T* stands for the number collected on the treated ones.

Results and Discussion

Laboratory evaluation

The duration of repellency against *Ae. aegypti* under laboratory conditions obtained from 44 mosquito repellent products ranged from 0 to 6.3 hours, with an average of about 2 hours. However, only 12 products (27.3%) were qualified for registration at the FDA for marketing since minimum repellency of 2 hours

is required for labeling. The repellency of these qualified repellent products ranged from 2.5 hours to 6.3 hours (Table 1). These products were formulated as lotion, spray, cream and balm, whereas citronella oil, eucalyptus oil and tea tree oil were the main active ingredients. The concentrations of the 12 qualified products used were citronella oil 6-13%, whereas those of eucalyptus and tea tree oil were 10-15% and 5%, respectively. The repellency of these qualified products, containing citronella oil, eucalyptus oil and tea tree oil were 2.5-6.3 hours, 5.3-5.8 hours and 3 hours, respectively. However, some products consisting of citronella oil, eucalyptus oil and tea tree oil were also unqualified. It is interesting to note that citronella oil was the most common active ingredient used in both qualified (66.7%) and unqualified (65.6%) products. These imply clearly that repellency of each repellent product depends on not only main active ingredient but also other synergists added in each formulation. Moreover, the same concentration of the same active ingredient obtained from different plant sources could exhibit different repellency. The rest of the products in this study containing turmeric oil, bergamot oil, lavender extract, tobacco-leaves extract, clove extract and neem-leaves extract were found to be unqualified as they provided repellency against *Ae. aegypti* for less than 2 hours (data omitted).

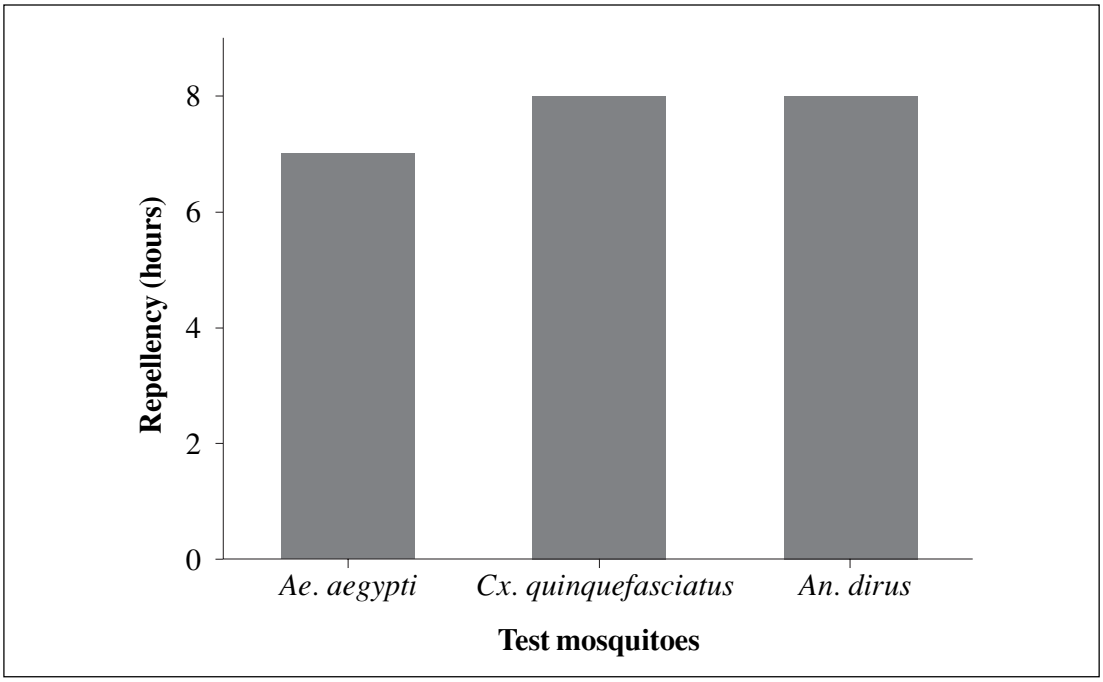

Figure 1. Repellency of repellent (turmeric oil 5% and eucalyptus oil 10%) against *Ae. aegypti, Cx. quinquefasciatus* ***and*** *An. dirus* ***under laboratory conditions.***

Figure 1. shows repellency of our repellent containing turmeric oil 5% and eucalyptus oil 10% with 5% vanillin added, against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. dirus* under laboratory conditions. As can be seen, this repellent showed a high degree of repellency against both day- and night-biting mosquitoes as it provided protection time for 7 hours against *Ae. aegypti*, and at least 8 hours against both *Cx. quinquefasciatus* and *An. dirus*. In comparison with the repellent (20% turmeric oil with 5% vanillin added) as described by Tawatsin et al. (2001), our present repellent composition shows equal repellency against *Cx. quinquefasciatus* and *An. dirus*, but better repellency against *Ae. aegypti*. Previously, we also evaluated the repellency of our repellents consisting of each single oil (i.e. turmeric oil and eucalyptus oil) with different concentrations from 5% to 10% (with 5% vanillin added). However, all of them showed repellency against *Ae. aegypti* for up to 4 hours only. With the same concentrations, turmeric oil provided longer protection time than did eucalyptus oil, but turmeric oil was approximately four times more expensive than eucalyptus oil. To minimize repellent cost, an idea of combinations of both oils with various concentrations then was carried out to study for extended repellency. Finally, the combination of turmeric oil 5% and eucalyptus oil 10% was the most cost-effective formulation, which provided a high degree of repellency against the mosquitoes as mentioned above. This suggests that the eucalyptus oil played an important role as a synergist for the extended repellency.

Field evaluation

The results of 10 selected repellent products and our repellent (turmeric oil 5% and eucalyptus oil 10%) that were evaluated under field conditions are shown in Table 1. The relative repellency is demonstrated in terms of biting reduction. The qualified products substantially provided substantial protection where average biting reduction, ranged from 90.6% to 100%, whereas those of the unqualified products were between 58.2% and 99.4%. It is interesting to note that two qualified repellent products, containing citronella oil 13% and eucalyptus 15% and our repellent (5% turmeric oil and 10% eucalyptus oil with 5% vanillin) completely prevented biting by mosquitoes (Table 1). Actually, these repellents provided protection time against *Ae. aegypti* in laboratory for 4.5, 5.3 and 7 hours, respectively. This indicates that a repellent, which possesses a high degree of repellency against *Ae. aegypti* under laboratory

conditions could also prevent biting of night biting mosquitoes. However, some of the unqualified products also provided a high degree of biting reduction with average ranging from 87.3% to 99.4%, except the product containing eucalyptus 15% provided 58.2% reduction only. In other words, the unqualified repellents that failed to prevent biting of *Ae. aegypti* under laboratory conditions were able to substantially reduce biting of night biting mosquitoes. This may be explained by two possibilities: *Ae. aegypti* is more aggressive than the night biting mosquitoes or *Ae. aegypti* is less sensitive to the phytochemicals than are the night biting mosquitoes.

Table 1. Biting reduction of test repellents that were evaluated under field conditions at Bang Bua Thong District, Nonthaburi Province, during the period between March 30, 2002 and April 18, 2002 (from 1800 h to 2200 h).

Active ingredient (%)	Lab. Repellency* (hours)	Biting reduction field (%)		Total mosquitoes Caught	Predominant species (%)
		Average	Range		
Qualified products					
Citronella oil 6	3.8	98.6	96.3-100	328	*Cx. vishnui* (73), *Cx. gelidus* (12), *Cx. quinquefasciatus* (7), *Ma. uniformis* (4), *Ma. indiana* (3)
Citronella oil 10	2.5	97.3	95.6-100	295	*Cx. vishnui* (42), *Cx. gelidus* (31), *Cx. quinquefasciatus* (14), *Cx. tritaeniorhynchus* (9), *Ma. annulifera* (2)
Citronella oil 13	4.5	100	100-100	237	*Cx. gelidus* (51), *Cx. quinquefasciatus* (19), *Cx. vishnui* (15), *Ma. indiana* (8), *Ma. uniformis* (5)
Eucalyptus oil 15	5.3	100	100-100	272	*Cx. vishnui* (38), *Cx. quinquefasciatus* (29), *Cx. gelidus* (15), *Cx. tritaeniorhynchus* (8), *Ma. uniformis* (4), *Ma. annulifera* (3)
Tea tree oil 5	3.0	93	88.9-100	353	*Cx. quinquefasciatus* (32), *Cx. gelidus* (26), *Cx. vishnui* (25), *Ma. indiana* (9), *Cx. tritaeniorhynchus* (4), *Ma. annulifera* (3)

Table 1. Biting reduction of test repellents that were evaluated under field conditions at Bang Bua Thong District, Nonthaburi Province, during the period between March 30, 2002 and April 18, 2002 (from 1800 h to 2200 h). (Cont.)

Active ingredient (%)	Lab. Repellency* (hours)	Biting reduction field (%)		Total mosquitoes Caught	Predominant species (%)
		Average	Range		
Unqualified products					
Citronella oil 5.6	0.5	96.3	86.7-100	245	*Cx. vishnui* (42), *Cx. gelidus* (31), *Cx. quinquefasciatus* (14), *Cx. tritaeniorhynchus* (9), *Ma. annulifera* (2)
Citronella oil 12	0.5	99.4	97.9-100	325	*Cx. gelidus* (45), *Cx. quinquefasciatus* (18), *Cx. vishnui* (17), *Ma. annulifera* (10), *Ma. indiana* (5), *An. barbirostris* (2)
Citronella oil 15	1.0	87.3	80-100	274	*Cx. vishnui* (38), *Cx. gelidus* (29), *Cx. quinquefasciatus* (15), *Cx. tritaeniorhynchus* (8), *Ma. uniformis* (4), *Ma. annulifera* (3)
Eucalyptus oil 15	1.8	58.2	40-100	259	*Cx. gelidus* (29), *Cx. quinquefasciatus* (25), *Cx. vishnui* (22), *Ma. annulifera* (9), *Ma. indiana* (7), *Ma. uniformis* (6)
Tea tree oil 5	1.5	92.9	71.4-100	314	*Cx. vishnui* (34), *Cx. gelidus* (25), *Cx. quinquefasciatus* (19), *Ma. uniformis* (11), *Ma. indiana* (6), *Cx. tritaeniorhynchus* (3)
Our repellent Turmeric oil 5 and eucalyptus oil 10	7.0	100	100-100	347	*Cx. quinquefasciatus* (41), *Cx. vishnui* (35), *Cx. gelidus* (12), *Ma. annulifera* (5), *Ma. indiana* (4), *Cx. tritaeniorhynchus* (2)

*Against *Ae. aegypti*

The mosquitoes collected from this study in the field evaluation included several species belonging to 3 predominant genera: *Culex*, *Mansonia* and *Anopheles*. These were *Cx. vishnui* Theobald, *Cx. gelidus* Theobald, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus* Giles, *Ma. annulifera* (Theobald), *Ma. indiana* (Edwards), *Ma. uniformis* (Theobald) and *An. barbirostris* Van der Wulp (Table 1.). Among these species, *Cx. vishnui*, *Cx. gelidus* and

Cx. quinquefasciatus were the most common ones collected. However, some other species, such as *Armigeres subalbatus* (Coquillett), *Ma. bonneae* (Edwards), *An. sundaicus* Rodenwaldt, *An. vagus* Donitz, *Ae. aegypti*, *Ae. albopictus* (Skuse) and *Cx. whitmorei* Giles were also collected in very small numbers. Regarding the mosquitoes captured on the treated areas of volunteers' legs, they were mostly *Ar. subalbatus*, *Cx. gelidus*, *Ma. annulifera*, *Ma. bonneae*, *Ae. aegypti* and *An. vagus*.

Acknowledgements

The authors are grateful to Dr.Mir S. Mulla, University of California, Riverside, U.S.A., for his comments on this manuscript. We thank Ms.Ruthairath Srithommarat, Ms.Thanittha Chumphonrak, Mr.Nares Juntornnual, Mr.Chumphon Chumphonrak and Mr.Sumas Juntamas for their assistance in fieldwork.

References

Boonyabancha S, Suphapathom K, Srisurapat A. 1997. Repellent effect of volatile oils on *Aedes aegypti*, Bull. Dept. Med. Sci. 39: 61-66.

Chokechaijaroenporn O, Bunyapraphatsara N, Kongchuensin S. 1994. Mosquito repellent activities of ocimum volatile oils. Phytomedicine. 1: 135-139.

Coleman RE, Robert LL, Roberts LW, Glass JA, Seeley DC, Laughinghouse A, Perkins PV, Wirtz RA. 1993. Laboratory evaluation of repellents against four anopheline mosquitoes (Diptera: Culicidae) and two phebotomine sand flies (Diptera: Psychodidae). J. Med. Entomol. 30: 499-502.

Edwards DL, Johnson CE. 1987. Insect-repellent-induced toxic encephalopathy in a child. Clin. Pharm. 6: 496-498.

Jaruwichitratana S, Tanyasittisuntorn P, Timpatanapong P. 1988. Comparison of citronella cream and placebo cream in protection of mosquito biting. J. Rama Med. 11: 94-97.

Maibach HI, Johnson HL. 1975. Contact urticaria syndrome. Arch. Dermatol. 111: 726-730.

Smith CN. 1957. Insect repellents. Soap Chem. Spec. 34: 105-122, 126-133.

Suwonkerd W, Tantrarongroj K. 1994. Efficacy of essential oil against mosquito biting. Commun. Dis. J. 20: 4-11.

Tawatsin A, Wratten SD, Scott RR, Thavara U, Techadamrongsin Y. 2001 Repellency of volatile oils from plants against three mosquito vectors. J. Vector Ecol. 26: 76-82.

Thavara U, Tawatsin A, Chompoosri J, Suwankerd W, Chansang U, Asavadachanukorn P. 2001. Laboratory and field evaluation of the insect repellent 3535 (Ethyl butylacethylaminoproprionate) and deet against mosquito vectors in Thailand. J. Am. Mosq. Control Assoc. 17: 190-195.

Wasuwat S, Sunthornthanasart T, Jarikasem S, Putsri N, Phanrakwong A, Janthorn S, Klongkarn-ngan I. 1990. Mosquito repellent efficacy of citronella cream. J. Sci. Tech. 5: 62-68.

Yap HH, Jahangir K, Chong ASC, Adanan CR, Chong NL, Malik YA, Rohaizat B. 1998. Field efficacy of a new repellent, KBR 3023, against *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* (Say) in a tropical environment. J. vector Ecol. 23: 62-68.

Zadikoff CM. 1979. Toxic encephalopathy associated with use of insect repellent. J. Pediatr. 95: 140-142.