Behavioral responses of *Aedes aegypti, Aedes albopictus, Culex quinquefasciatus,* and *Anopheles minimus* against various synthetic and natural repellent compounds

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ABSTRACT: The behavioral responses of colony populations of *Aedes aegypti*, *Aedes albopictus, Culex quinquefasciatus,* and *Anopheles minimus* to four essential oils (citronella, hairy basil, catnip, and vetiver), two standard repellents (DEET and picaridin), and two synthetic pyrethroids (deltamethrin and permethrin) were conducted in the laboratory using an excito-repellency test system. Results revealed that *Cx. quinquefasciatus* and *An. minimus* exhibited much stronger behavioral responses to all test compounds (65-98% escape for contact, 21.4-94.4% escape for non-contact) compared to *Ae. aegypti* (3.7-72.2% escape (contact), 0-31.7% (non-contact)) and *Ae. albopictus* (3.5-94.4% escape (contact), 11.2-63.7% (non-contact)). In brief, essential oil from vetiver elicited the greatest irritant responses in *Cx. quinquefasciatus* (96.6%) and *An. minimus* (96.5%) compared to the other compounds tested. The synthetic pyrethroids caused a stronger contact irritant response (65-97.8% escape) than non-contact repellents (0-50.8% escape for non-contact) across all four mosquito species. Picaridin had the least effect on all mosquito species. Findings from the current study continue to support the screening of essential oils from various plant sources for protective properties against field mosquitoes. ***Journal of Vector Ecology* 39 (2): 328-339. 2014.**

Keyword Index: Aedes aegypti, *Aedes albopictus, Culex quinquefasciatus, Anopheles minimus*, excito-repellency, non-contact repellent, contact irritant.

INTRODUCTION

There are various mosquito-borne diseases transmitted to humans by the bites of infected mosquitoes, including malaria (protozoa), dengue (virus), Japanese encephalitis (virus), chikungunya (virus), and yellow fever (virus). *Aedes aegypti* is the main vector of dengue and yellow fever viruses, whereas *Aedes albopictus* is a secondary dengue vector and a main vector of chikungunya virus (Thavara et al. 2009). *Culex quinquefasciatus* is an abundant nuisance mosquito in urban areas. It has been reported that *Cx. quinquefasciatus* in Thailand is a potential vector of Japanese encephalitis virus (JEV) (Nitatpattana et al. 2005). Moreover, *Cx. quinquefasciatus* in Thailand was first reported to be susceptible to the nocturnal periodic strain of Myanmar *Wuchereria bancrofti* that causes human lymphatic filariasis (Triteeraprapab et al. 2000). *Anopheles minimus* is now the most important malaria vector in Thailand and occurs in hilly, forested areas. Malaria is a serious and sometimes fatal disease that is routinely reported along the Thai-Cambodia and Thai-Myanmar borders as well as the southern part of Thailand (Chareonviriyaphap et al. 2013, Suwonkerd et al. 2013).

Among the many strategies that have been used for mosquito control are insecticides and repellents. Synthetic pyrethroids are widely used for controlling adult mosquitoes, especially deltamethrin and permethrin, as recommended by the World Health Organization. The Ministry of Public Health of Thailand has used these two pyrethroid insecticides for space spraying applied via fogging or misting machines and mosquito net treatments (Chareonviriyaphap et al. 1999, Jirakanjanakit et al. 2007). *Previous* studies have found that both deltamethrin and permethrin elicit strong contact irritant response in mosquito vectors (Chareonviriyaphap et al. 2004, 2012, 2013, Kongmee et al. 2004, Mongkalangoon et al. 2009).

The term "excito-repellency" is used to describe mosquito behavior that is triggered by the combination of either irritancy or repellency. Irritancy results from direct tarsal contact with an insecticide that can cause a mosquito to leave treated surfaces before acquiring a lethal dose, therefore repeated contact is required before mortality occurs (Roberts et al. 2000). On the other hand, repellency refers to the stimulation by a chemical that orients mosquito movement away from the treated surfaces without making tarsal contact (Roberts et al. 2000). These forms of behavioral responses can be quantitatively assessed by using an excito-repellency test system (Roberts et al. 1997).

For personal protection, DEET (N,N-Diethyl-meta-toluamide) is the most common active compound used as insect repellents with a strong effect against mosquitoes (Fradin and Day 2002, Klun et al. 2006). However, DEET has been shown to have toxic effects on humans, especially when misapplied at very high concentrations and used often or over a long period of time (Robbins and Cherniack 1986, Qui et al.

1998). Plant essential oils have become popular to use as safe mosquito repellents, such as citronella oil that is approved by the U.S. Environmental Protection Agency (EPA) (Fradin 1998). Besides citronella oil, numerous essential oils extracted from plants have been available for insect repellent activity for protection against mosquitoes. The objective of this research was to investigate the behavioral responses of four mosquito species to three different types of test compounds - essential oils, standard repellents, and synthetic chemicals.

MATERIALS AND METHODS

Test mosquitoes

Laboratory populations of *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus* and *An. minimus* were used in this study. The *Ae. aegypti* insecticide susceptible test population (United States Department of Agriculture laboratory [USDA]) was obtained as eggs from the Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL. The USDA population of *Ae. aegypti* has been reared in the laboratory for more than 40 years. *Aedes albopictus* has been maintained in the entomological laboratory at Kasetsart University for six years. A colony of *Cx. quinquefasciatus* were obtained from the National Institute of Health (NIH), Ministry of Public Health, Nonthaburi, Thailand, where the colony has been established since 1978. The *Anopheles minimus* colony has been maintained in the Department of Entomology, Kasetsart University for >15 years. The colony originated from the Malaria Division, Department of Communicable Disease Control (CDC), Ministry of Public Health, Nonthaburi, Thailand, in 1998.

All four species of mosquitoes were reared in the insectary of the Department of Entomology, Faculty of Agriculture, Kasetsart University. All larvae and adults were held under laboratory conditions of 25 $\pm$ 5° *C* and 80 $\pm$ 10% *RH* with a 12:12 *L:D* photoperiod. Larvae were fed with fish food twice a day. Adults were reared in a screened cage and provided 10% sugar solution as food. Female mosquitoes aged three to five days old were starved for 24 h before testing.

Test compounds

Pyrethroid insecticides. Deltamethrin ([Cyano-[3-(phenoxy)phenyl]methyl] 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate) (98% purity) was obtained from BASF (Lot No: HDDLTK034). Permethrin (3-phenoxybenzyl (1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) (97.6% purity) was provided by Sherwood Chemicals Public Company Limited (Lot No: ABJFPAP118).

Repellents. DEET and picaridin were selected as representative synthetic repellents. DEET (N, N-diethyl-3-methylbenzamide) (97% purity) was obtained from the USDA, Beltsville, MD (Lot No: 0326/2009). Picaridin (2-(2-hydrozyethyl-1-piperidincarboxylic acid 1-methylpropyl ester) (98.4% purity) was obtained from Bayer Thai Company Limited (Lot No: CHCAEN0020).

Plant essential oils. Citronella, *Cymbopogon nardus* (Lot No: NO5410008-1/1910), hairy basil, *Ocimum americanum* (Lot No:5308093/0408), catnip, *Nepeta cataria* (Lot No: 01022011) and vetiver, *Vetiveria zizanoides* (Lot No: 5506713/2706) were selected from previous studies as *effective in repelling mosquitoes* (Tawatsin et al. 2001, Trongtokit et al. 2005, Phasomkusolsil and Soonwera 2010). Citronella, hairy basil and vetiver oils were supplied in 100% purity from Thai-China Flavours and Fragrances Industry Company Limited. Catnip was received from the Chemicals Affecting Insect Behavior Laboratory, United States Department of Agriculture, Beltsville, MD.

Paper treatment

Deltamethrin and permethrin were dissolved in a mixture of acetone and silicone (Dow Corning 556) to obtain doses of 25 mg/m^2 and 500 mg/m^2, respectively, the highest doses of insecticidal-treated nets (ITNs). DEET, picaridin, and four essential oils were diluted in ethanol to a concentration of 2.5%, based on optimal concentrations identified from previous mosquito behavioral studies (Polsomboon et al. 2008, Suwansirisilp et al. 2012). With a micropipette, 2.8 ml of the solution was dropped onto 14.7 × 17.5 cm^2 Whatman No.1 filter paper. Impregnated papers were allowed to air dry 1 h before testing (Licciardi et al. 2006). Control papers were treated with only ethanol or solvent mixture for insecticide tests.

Behavioral tests

The excito-repellency test assay used in this study has previously been described (Mongkalangoon et al. 2009, Suwansirisilp et al. 2012). Briefly, a set of four test chambers was used to evaluate both non-contact repellent and contact irritant behaviors. Each test chamber was connected with a receiving box for collecting mosquitoes exiting from the test chamber. A matched control was performed for each chemical treatment evaluation. Fifteen unfed female mosquitoes of three to five days old were released into each of four test chambers and mosquitoes were allowed to adjust themselves to environmental conditions inside the test chamber for 3 min before opening the exit door. The number of escaping mosquitoes was recorded every minute for 30 min during exposure to test repellent compounds. At the end of the exposure period, escaped and non-escaped mosquitoes were transferred to individual containers and provided 10% sugar solution. Knockdown was observed after 30 min and mortality after 24 h in both treatments and controls for both escaped and non-escaped cohorts. Each repellent compound was tested in four replicates between 08:00 am and 16:30.

Statistical analysis

Kaplan-Meier survival analysis was used to evaluate escaping mosquitoes from each test chamber of the excito-repellency test system (Roberts et al. 1997). The time in minutes for 25% (ET_{25}), 50% (ET_{50}), and 75% (ET_{75}) of assay populations to escape was calculated for each product assessed and the log-rank test used to compare the escape responses of test populations. Observed percentage escape was corrected with Abbott's formula. (Finney 1964).

RESULTS

The escape patterns during 30-min exposures for the four mosquito species are given in Figures 1 and 2. Results in contact trials indicate responses of *Ae. albopictus* to the synthetic pyrethroids (deltamethrin and permethrin) were significantly greater than DEET, picaridin, or essential oils (*P*<0.05). With deltamethrin, *Ae. aegypti* produced significantly greater responses than others (*P*<0.05), except hairy basil (*P*=0.0892). For permethrin, the repelling effect was not significantly different from that of all essential oils, *P>0.05* (Table 1). All compounds were found to elicit escape responses in both *Cx. quinquefasciatus* from 69.2% to 96.6% and *An. minimus* from 65.4% to 97.8%, except picaridin where minimal escape occurred (3.4% and 12.2%, respectively). Moreover, two test populations, *Cx. quinquefasciatus* and *An. minimus* showed high escape responses to three essential oils, citronella, hairy basil, and catnip in contact and non-contact trials (72.4% to 94.4%), whereas vetiver was associated with extremely high escape responses of both these species from the contact chambers (>95%). *Mortality rates were low overall, e*xcept in *An. minimus* when exposed to catnip where 76.7% and 89.9% mortality was reported in contact and non-contact, respectively.

Escape time in minutes (ET) 25%, 50%, and 75% during the 30-min exposure assay is shown in Table 2. *Aedes aegypti* showed the fastest response to permethrin with an ET_{25} value of 1 min and an ET_{50} value of 4 min in contact trials but no response was found in the non-contact trials. *Aedes albopictus* had low ET_{25} values in both contact and non-contact trials (≤1 min) against deltamethrin, DEET, catnip, and vetiver. For ET_{50}, this species displayed the values of <1 min only in contact trials for deltamethrin and permethrin and ET_{75} values of 2 min and 4 min appeared, respectively, for deltamethrin and permethrin in contact treatment chambers. *Culex quinquefasciatus* displayed ET_{25} values of <1 min in contact trials against all four essential oils tested and ≤1 min in non-contact trials for citronella, hairy basil, and catnip. *Anopheles minimus* showed fast escape responses of ≤1 min at ET_{25} to DEET, hairy basil, and catnip. Only catnip was found on *An. minimus* to have an ET_{25}, ET_{50} and ET_{75} for both contact and non-contact trials ≤4 min. *Anopheles minimus* had a delayed escape response to permethrin, DEET, and citronella with ET_{50} values of 6, 6, and 7 min, respectively.

A comparison of escape responses among the mosquito test populations between contact and non-contact trials is shown in Table 3. All species exhibited significant differences in response patterns between trials for deltamethrin and permethrin (*P*<0.0001). *Aedes aegypti* showed slightly different patterns among DEET (P=0.0460), hairy basil (P=0.0166), and catnip (P=0.0201), but there were no significant differences for picaridin, citronella, and vetiver (P>0.05). A significant difference was found between DEET and vetiver (P<0.0001) trials using *Culex quinquefasciatus.* Escape responses were also significantly different against citronella and vetiver trials using *An. minimus.*

Statistical comparisons between any two species exposed to deltamethrin, permethrin, DEET, picaridin, citronella, hairy basil, catnip, and vetiver in either contact or non-contact trials are shown in Table 4. For deltamethrin, there were significant differences in escape responses when *Ae. albopictus* was compared to the other species in both test conditions (*P*<0.0001). With permethrin, significance in escape responses were found when *Ae. aegypti* was compared to *Ae. albopictus* and *An. minimus* (*P*<0.0001) . In addition, the significant differences in escape responses between two species were most found in citronella, hairy basil, catnip and vetiver (*P*<0.05) (Table 4).

Statistical comparisons between pyrethroids and essential oils against four mosquito species are shown in Table 5. Significant differences in escape responses between pyrethroids and essential oils were most observed in *An. minimus* and *Ae. albopictus* (P<0.05). In contact trials, statistical significances in escape responses of all species were observed when catnip and vetiver were compared to deltamethrin (P<0.05). In non-contact trials, there were significant differences in escape responses when citronella and hairy basil were compared to deltamethrin for all species (*P*<0.0001).

DISCUSSION

Insecticides commonly used to control the vectors of human diseases are within the pyrethroid chemical group, mainly deltamethrin and permethrin, but several papers have reported pyrethroid resistance in *Ae. aegypti*, *Ae. albopictus,* and *An. minimus*, and in the nuisance-biting *Cx. quinquefasciatus* within Thailand (Ponlawat et al. 2005, Paeporn et al. 2006, Jirakanjanakit et al. 2007, Chareonviriyaphap et al. 2013). Thus, topical repellents could be used in combination with traditional indoor residual spraying (IRS) and bednet strategies for preventing mosquito bites. Plant-based mosquito repellents are more popular for self-protection because their natural scents are perceived as being safer to humans. Many plants have been studied for their efficacy in repelling mosquitoes. For example, Phasomkusolsil and Soonwera (2010) reported that citronella essential oil (*Cymbopogon nardus*) and phlai (*Zingiber cassumunar*), a famous Thai herbal medicine belonging to the same family as ginger and sweet basil (*Ocimum basilicum*), provided protection against *Cx. quinquefasciatus*, *An. minimus, and Ae. aegypti* when applied to the skin. Misni et al. (2008) also found there was no significant difference in efficacy between *Piper aduncum* essential oil (Malaysian plant) and DEET when tested against *Ae. aegypti*. Also, Tawatsin et al. (2001) reported turmeric, citronella grass, and hairy basil combined with 5% vanillin provided improved protection time against *Ae. aegypti*, *An. dirus,* and *Cx. quinquefasciatus*.

The current study measured irritant contact and repellent non-contact characteristics of eight compounds: citronella, hairy basil, catnip, and vetiver (plant essential oils), DEET and picaridin (synthetic repellents), and two synthetic pyrethroids (deltamethrin and permethrin), using the excito-repellency test system. The results show that both pyrethroid insecticides were effective in eliciting an irritant rather than a repellent response against test vector populations, similar to

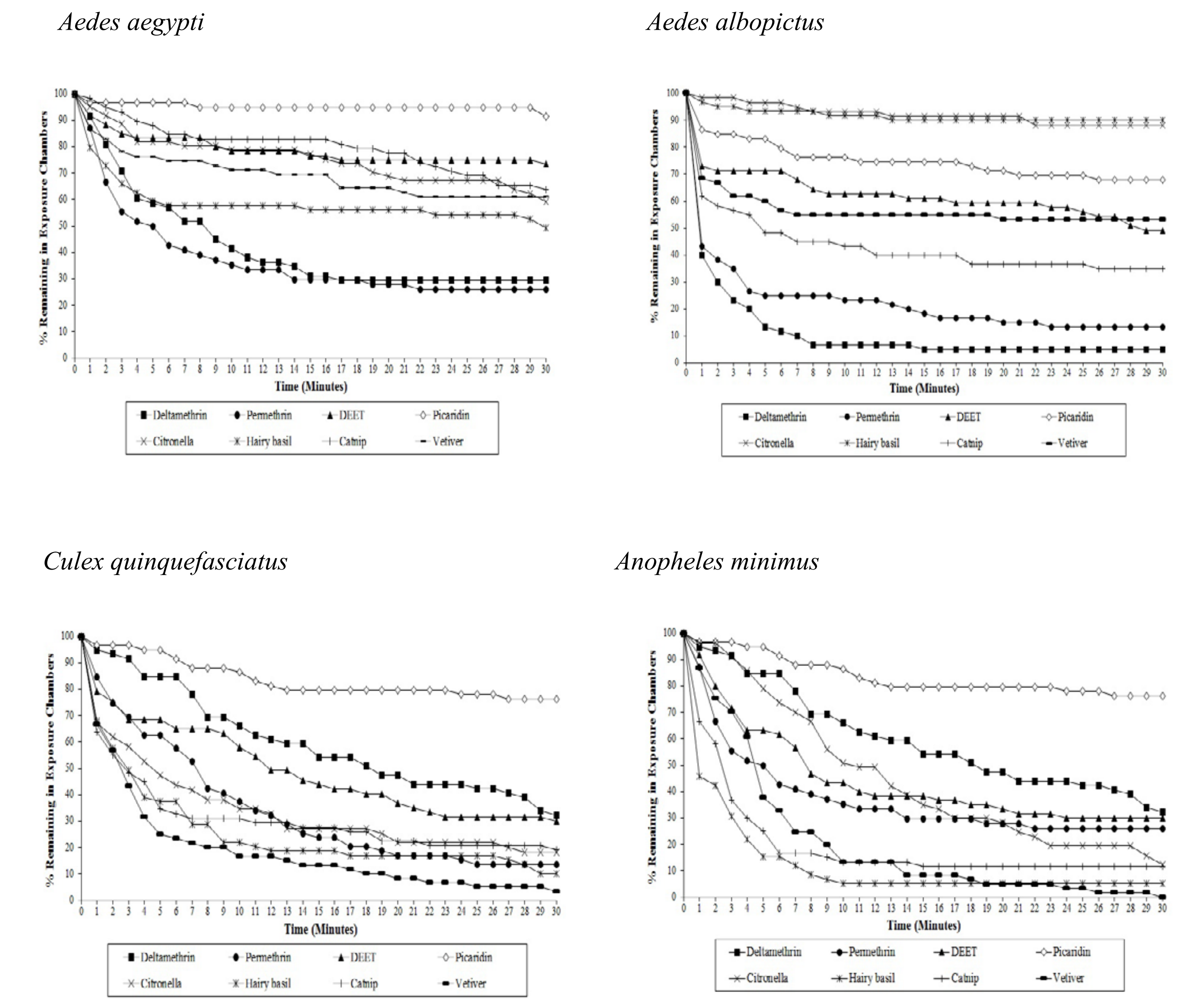

Figure 1. Kaplan-Meier survival curves indicating the percent of four mosquito species remaining inside a treated chamber during 30-min exposure to eight test compounds in contact behavioral assays.

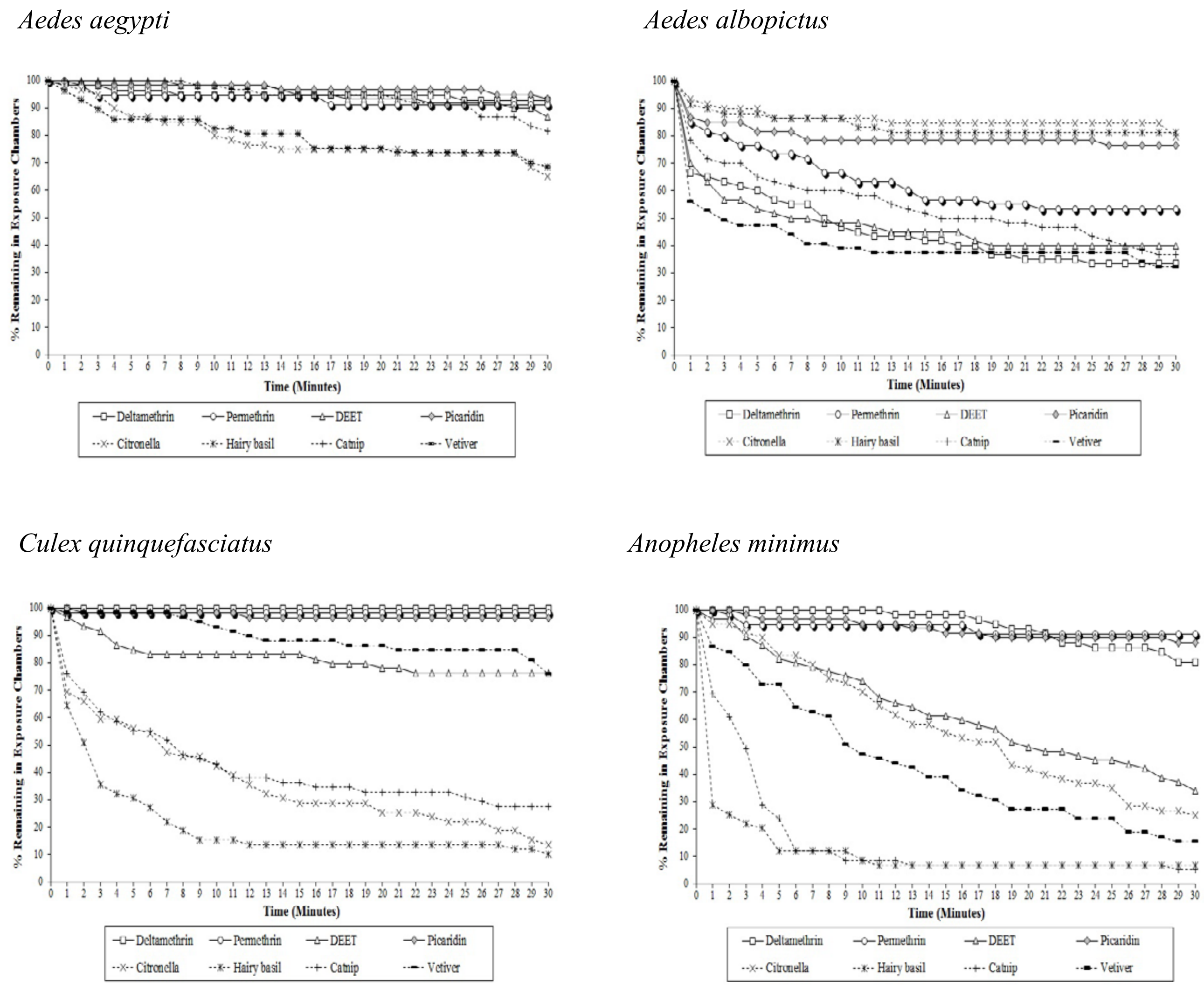

Figure 2. Kaplan-Meier survival curves indicating the percent of four mosquito species remaining inside a treated chamber during 30-min exposure to eight test compounds in non-contact behavioral assays.

Table 1. Knockdown, mortality, and escape response of four mosquito species exposed to deltamethrin (0.025 g/m^2), permethrin (0.5 g/m^2), DEET, picaridin, citronella, hairy basil, catnip, and vetiver each at 2.5% (2.72 g/m^2).

Compound[1]	*Aedes aegypti*					*Aedes albopictus*					*Culex quinquefasciatus*					*Anopheles minimus*				
	% Esc[2]	% KD		%Mort (24h)		% Esc	% KD		%Mort (24h)		% Esc	% KD		%Mort (24h)		% Esc	% KD		%Mort (24h)	
		Es[2]	NEs[3]	Es	NEs		Es	NEs	Es	NEs		Es	NEs	Es	NEs		Es	NEs	Es	NEs
Contact																				
Deltamethrin	67.9	46.6	22.4	46.6	27.6	94.4	36.7	5.0	13.3	5.0	72.4	0	0	0	0	65.4	5.1	5.1	15.3	10.2
Deltamethrin-C	8.6	0	0	0	0	10.0	0	0	0	0	3.3	0	0	0	0	10.0	0	0	0	0
Permethrin	72.2	27.8	24.1	24.1	24.1	85.2	25.0	8.3	33.3	10.0	85.9	0	1.7	0	0	97.8	0	3.4	6.9	0
Permethrin-C	7.0	0	0	0	0	10.0	0	0	0	0	3.4	0	0	0	0	10.0	0	0	0	0
DEET	21.4	0	0	0	0	47.3	0	1.7	0	1.7	69.2	0	0	0	0	80.7	0	0	0	0
DEET-C	6.7	0	0	0	0	6.7	0	0	0	0	3.3	0	0	0	0	6.7	0	0	0	0
Picaridin	3.7	0	0	0	0	28.6	0	0	0	0	3.4	0	0	0	0	12.2	0	1.7	0	1.7
Picaridin-C	5.0	0	0	0	0	5.0	0	0	0	1.7	3.4	0	0	0	0	5.0	0	0	0	0
Citronella	37.9	0	0	0	0	5.6	0	76.3	0	0	80.8	0	0	0	0	86.3	0	0	0	0
Citronella-C	0	0	0	0	0	6.7	0	0	0	0	5.0	0	0	0	0	6.7	0	0	0	0
Hairy basil	47.3	0	0	0	0	3.5	0	88.3	0	1.7	89.3	0	0	0	0	90.8	0	6.9	0	3.4
Hairy basil-C	6.7	0	0	0	1.7	6.7	0	0	0	0	5.1	0	0	0	0	6.7	0	0	0	0
Catnip	32.8	5.2	32.8	0	0	61.1	0	8.3	0	1.7	80.7	0	8.6	0	5.2	87.9	0	10.0	76.7	0
Catnip-C	5.1	0	0	0	0	10.0	0	0	0	0	1.7	0	0	0	0	10.0	0	0	3.4	0
Vetiver	37.8	0	0	0	0	42.9	0	0	0	1.7	96.6	0	0	0	0	96.5	0	0	0	0
Vetiver-C	1.7	0	0	0	0	6.7	0	0	0	1.7	1.7	0	0	0	0	6.7	0	0	0	0

Continued.

Table 1. Continued.

Compound[1]	*Aedes aegypti*					*Aedes albopictus*					*Culex quinquefasciatus*					*Anopheles minimus*				
	% Esc[2]	% KD		%Mort (24h)		% Esc	% KD		%Mort (24h)		% Esc	% KD		%Mort (24h)		% Esc	% KD		%Mort (24h)	
		Es[2]	NEs[3]	Es	NEs		Es	NEs	Es	NEs		Es	NEs	Es	NEs		Es	NEs	Es	NEs
Non-contact																				
Deltamethrin	3.7	0	10.7	0	12.5	62.3	0	0	0	0	0	0	0	0	0	16.2	0	0	3.4	3.4
Deltamethrin-C	3.5	0	0	3.5	0	3.3	0	0	0	0	0	0	0	0	0	3.3	0	0	0	0
Permethrin	0	0	0	0	3.6	40.8	0	0	0	0	0	0	0	0	0	50.8	0	1.7	1.7	3.3
Permethrin-C	8.9	0	0	0	1.8	15.3	0	0	0	0	1.7	0	0	0	0	15.3	0	0	0	1.7
DEET	8.7	0	0	0	0	57.9	0	0	0	1.7	21.1	0	0	0	0	77.7	0	0	0	0
DEET-C	5.0	0	0	0	0	10.3	0	0	0	0	3.3	0	0	0	0	10.3	0	0	0	0
Picaridin	0	0	0	0	0	14.8	0	0	0	0	1.8	0	0	0	0	2.1	0	0	1.7	0
Picaridin-C	6.7	0	0	0	0	10.0	0	0	0	0	1.7	0	0	0	0	10.0	0	0	0	0
Citronella	30.4	0	0	0	0	14.6	0	52.5	0	1.7	85.9	0	1.7	0	1.7	73.2	0	0	0	0
Citronella-C	3.4	0	0	0	0	6.6	0	0	0	0	3.3	0	0	0	0	6.6	0	0	0	0
Hairy basil	30.4	0	17.5	1.8	1.8	11.2	0	72.9	0	5.1	89.6	0	0	0	0	78.1	1.8	17.5	1.8	3.5
Hairy basil-C	1.7	0	0	0	1.7	11.7	0	0	0	1.7	1.7	0	0	0	0	11.7	0	0	0	0
Catnip	18.6	1.7	35.0	0	0	60.0	0	6.7	0	0	72.4	0	3.4	0	0	94.4	18.6	5.1	89.8	0
Catnip-C	1.7	0	0	0	0	8.3	0	0	1.7	0	0	0	0	0	0	8.3	0	0	5.0	0
Vetiver	31.7	0	0	0	0	63.7	0	0	3.3	0	24.1	0	0	0	0	66.0	0	0	3.3	4.9
Vetiver-C	0	0	0	0	0	3.4	0	0	1.7	1.7	0	0	0	0	0	3.4	0	0	0	0

Fifteen sugar-unfed female mosquitoes aged three to five days old were used in each of the four replications for the behavioral test. [1]C = control test without insecticide/repellent/essential oil. [2]Esc or Es = escaped mosquitoes. [3]NEs = not escaped mosquitoes. All treatment escapes were adjusted for control escapes by Abbott's formula (Finney 1964).

Table 2. Time in minutes for 25% (ET_{25}), 50% (ET_{50}), and 75% (ET_{75}) of four mosquitoes test populations to escape from test chambers treated with deltamethrin, permethrin, DEET, picaridin, citronella, hairy basil, catnip, and vetiver. Fifteen sugar-unfed female mosquitoes aged three to five days old were used in each of the four replications for the behavioral test.

Compound	*Aedes aegypti*			*Aedes albopictus*			*Culex quinquefasciatus*			*Anopheles minimus*		
	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}
Contact												
Deltamethrin	2	8	-	<1	<1	2	4	9	-	7	18	-
Permethrin	1	4	-	<1	<1	4	2	7	14	2	6	12
DEET	17	-	-	<1	28	-	2	12	-	1	6	21
Picaridin	-	-	-	10	-	-	-	-	-	-	-	-
Citronella	15	-	-	-	-	-	<1	4	19	5	7	22
Hairy basil	1	29	-	-	-	-	<1	3	8	<1	2	4
Catnip	21	-	-	<1	15	-	<1	3	18	<1	2	4
Vetiver	6	-	-	<1	-	-	<1	2	5	3	5	11
Non-contact												
Deltamethrin	-	-	-	<1	24	-	-	-	-	-	-	-
Permethrin	-	-	-	5	-	-	-	-	-	10	22	-
DEET	-	-	-	<1	6	-	-	-	-	5	16	27
Picaridin	-	-	-	-	-	-	-	-	-	-	-	-
Citronella	26	-	-	-	-	-	<1	6	22	8	10	30
Hairy basil	15	-	-	-	-	-	<1	2	6	<1	1	16
Catnip	-	-	-	1	4	-	1	7	-	<1	2	4
Vetiver	9	-	-	<1	2	-	-	-	-	3	19	-

Table 3. Comparison of escape responses between paired contact and non-contact trials for four mosquito species exposed to deltamethrin, permethrin, DEET, picaridin, citronella, hairy basil, catnip, and vetiver using the log rank test. Fifteen sugar-unfed female mosquitoes aged three to five days old were used in each of the four replications for the behavioral test.

Compound	*Aedes aegypti* (*P*)	*Aedes albopictus* (*P*)	*Culex quinquefasciatus* (*P*)	*Anopheles minimus* (*P*)
Deltamethrin	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
Permethrin	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
DEET	0.05*	0.22	< 0.0001*	0.06
Picaridin	0.71	0.31	0.44	0.08
Citronella	0.42	0.20	0.97	0.04*
Hairy basil	0.02*	0.12	0.46	0.20
Catnip	0.02*	0.45	0.13	0.74
Vetiver	0.38	0.04*	< 0.0001*	< 0.0001*

*Indicates significant differences ($P<0.05$) between contact and non-contact trials.

Table 4. Comparison of escape responses between two species from the four mosquito species exposed to deltamethrin, permethrin, DEET, picaridin, citronella, hairy basil, catnip, and vetiver using the log rank test.

Compound	Mosquito species	Contact (*P*)	Non-contact (*P*)
Deltamethrin	*Ae. aegypti* and *Ae. albopictus*	< 0.0001*	< 0.0001*
	Ae. aegypti and *Cx. quinquefasciatus*	0.71	0.04*
	Ae. aegypti and *An. minimus*	0.11	0.07
	Ae. albopictus and *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. albopictus and *An. minimus*	< 0.0001*	< 0.0001*
	Cx. quinquefasciatus and *An. minimus*	0.15	0.00*
Permethrin	*Ae. aegypti* and *Ae. albopictus*	< 0.0001*	< 0.0001*
	Ae. aegypti and *Cx. quinquefasciatus*	< 0.0001*	0.08
	Ae. aegypti and *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus and *Cx. quinquefasciatus*	0.07	< 0.0001*
	Ae. albopictus and *An. minimus*	0.59	0.52
	Cx. quinquefasciatus and *An. minimus*	0.12	< 0.0001*
DEET	*Ae. aegypti* and *Ae. albopictus*	0.01*	< 0.0001*
	Ae. aegypti and *Cx. quinquefasciatus*	< 0.0001*	0.11
	Ae. aegypti and *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus and *Cx. quinquefasciatus*	0.05	< 0.0001*
	Ae. albopictus and *An. minimus*	0.05	0.52
	Cx. quinquefasciatus and *An. minimus*	0.76	< 0.0001*
Picaridin	*Ae. aegypti* and *Ae. albopictus*	0.00*	0.01*
	Ae. aegypti and *Cx. quinquefasciatus*	0.72	0.45
	Ae. aegypti and *An. minimus*	0.02*	0.32
	Ae. albopictus and *Cx. quinquefasciatus*	0.0004*	0.00*
	Ae. albopictus and *An. minimus*	0.26	0.08
	Cx. quinquefasciatus and *An. minimus*	0.01*	0.10
Citronella	*Ae. aegypti* and *Ae. albopictus*	0.00*	0.09
	Ae. aegypti and *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. aegypti and *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus and *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. albopictus and *An. minimus*	< 0.0001*	< 0.0001*
	Cx. quinquefasciatus and *An. minimus*	0.26	0.01*
Hairy basil	*Ae. aegypti* and *Ae. albopictus*	< 0.0001*	0.15
	Ae. aegypti and *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. aegypti and *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus and *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. albopictus and *An. minimus*	< 0.0001*	< 0.0001*
	Cx. quinquefasciatus and *An. minimus*	0.03*	0.04*
Catnip	*Ae. aegypti* and *Ae. albopictus*	0.0001*	< 0.0001*
	Ae. aegypti and *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. aegypti and *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus and *Cx. quinquefasciatus*	0.08	0.19
	Ae. albopictus and *An. minimus*	0.00*	< 0.0001*
	Cx. quinquefasciatus and *An. minimus*	0.20	< 0.0001*
Vetiver	*Ae. aegypti* and *Ae. albopictus*	0.27	< 0.0001*
	Ae. aegypti and *Cx. quinquefasciatus*	< 0.0001*	0.23
	Ae. aegypti and *An. minimus*	< 0.0001*	< 0.0001*
	Ae. albopictus and *Cx. quinquefasciatus*	< 0.0001*	< 0.0001*
	Ae. albopictus and *An. minimus*	< 0.0001*	0.33
	Cx. quinquefasciatus and *An. minimus*	0.37	< 0.0001*

*Indicates significant differences ($P<0.05$) between two species.

Table 5. Comparison of contact irritant and non-contact repellent of pyrethroids and synthetic repellents or essential oils against the four mosquito species by using the log rank test.

Species	Compound	Contact		Non-contact	
		Deltamethrin	Permethrin	Deltamethrin	Permethrin
Aedes aegypti	DEET	< 0.0001*	0.0118*	0.30	0.50
	Picaridin	< 0.0001*	< 0.0001*	0.90	0.64
	Citronella	0.00*	0.24	0.00*	0.00*
	Hairy basil	0.09	0.59	0.00*	0.00*
	Catnip	< 0.0001*	0.07	0.08	0.16
	Vetiver	0.00*	0.30	0.00*	0.00*
Aedes albopictus	DEET	< 0.0001*	< 0.0001*	0.63	0.05
	Picaridin	< 0.0001*	< 0.0001*	< 0.0001*	0.01*
	Citronella	< 0.0001*	< 0.0001*	< 0.0001*	0.00*
	Hairy basil	< 0.0001*	< 0.0001*	< 0.0001*	0.00*
	Catnip	< 0.0001*	0.00*	0.46	0.10
	Vetiver	< 0.0001*	< 0.0001*	0.77	0.01*
Culex quinquefasciatus	DEET	0.73	0.03*	< 0.0001*	0.00*
	Picaridin	< 0.0001*	< 0.0001*	0.15	0.54
	Citronella	0.07	1.00	< 0.0001*	< 0.0001*
	Hairy basil	0.00*	0.12	< 0.0001*	< 0.0001*
	Catnip	0.02*	0.60	< 0.0001*	< 0.0001*
	Vetiver	< 0.0001*	0.00*	< 0.0001*	0.00*
Anopheles minimus	DEET	0.17	0.00*	< 0.0001	0.42
	Picaridin	< 0.0001*	< 0.0001*	0.34	< 0.0001*
	Citronella	0.01*	0.00*	< 0.0001*	0.07
	Hairy basil	< 0.0001*	0.00*	< 0.0001*	< 0.0001*
	Catnip	< 0.0001*	0.14	< 0.0001*	< 0.0001*
	Vetiver	< 0.0001*	0.12	< 0.0001*	0.0001*

*Indicates significant differences (P<0.05) between pyrethroids and repellents/essential oils.

previous findings reported by Chareonviriyaphap et al. (2004), Kongmee et al. (2004), and Dusfour et al. (2009). In these papers, pyrethroids had an irritant effect when mosquitoes came into contact with the treated surface. Additionally, our results indicated that pyrethroids, particularly deltamethrin, induced a knockdown effect and mortality against *Ae. aegypti* and *Ae. albopictus* in contact trials. In general, synthetic pyrethroids have a knockdown and killing effect through direct contact (Elliott et al. 1965).

Most essential oils were effective against four mosquito vector species, especially *Cx. quinquefasciatus* and *An. minimus*, but some of them were much weaker than DEET and/or deltamethrin and permethrin when tested against *Ae. aegypti* and *Ae. albopictus*. Previous studies using the same excito-repellency test system found that *Cx. quinquefasciatus* had a strong behavioral escape response to clove, citronella, and cinnamon, whereas *Ae. aegypti* was less responsive and some were knocked down with clove (Suwansirisilp et al. 2012). Phasomkusolsil et al. (2010) found that *An. minimus* and *Cx. quinquefasciatus* were more sensitive to several different oils than were *Ae. aegypti*. Polsomboon et al. (2008) reported that *Ae. aegypti* showed increased escape rates in the contact chamber with 5% catnip, but *An. harrisoni* had a greater irritancy escape response to 2.5% catnip. Thus, the irritant/repellent efficacy of essential oils depends upon the active ingredient, concentration, and mosquito species tested. Moreover, some plants are toxic to mosquitoes. Deletre et al. (2013) reported that 1% of cinnamon, citronella, and thyme essential oils exhibited a toxic effect to *An. gambiae*, and Phasomkusolsil and Soonwera (2011b) found that 10% lemongrass produced 100% mortality for *Ae. aegypti, Cx. quinquefasciatus* and *An. dirus*. In our study, hairy basil and citronella achieved over 50% knockdown of *Ae. albopictus*. Similarly, Boonyuan et al. (2013), using the excito-repellency test system, demonstrated that 10% hairy basil gave almost 90% mortality for *Ae. aegypti* in a contact assay and 69% mortality for a non-contact assay, while 10% citronella showed very high knockdown in *Ae. aegypti*. Therefore, hairy basil and citronella are potential candidates for development as natural insecticides.

Behavioral responses against 2.5% DEET in the present study indicate that this compound elicited both a moderate repellent action against *Ae. albopictus* and *An. minimus* and an irritant effect against *Ae. albopictus*, *Cx. quinquefasciatus* and *An. minimus*. Tisgratog et al. (2011) showed the result of DEET at 5% compared to bifenthrin with a field dose of 25 mg/m^2, that *An. minimus* had a greater escape response to DEET than bifenthrin, and also showed that DEET was both a contact irritant and spatial repellent.

Overall, this study showed synthetic pyrethroids to be the most effective against all four mosquito species tested. In addition, each mosquito species responded in different escape patterns to the various test compounds. Understanding how alternative natural products function to prevent mosquito human contact is vital in optimizing tools for personal protection. Insecticide resistance phenotypes in many important vector species will continue to pressure the scientific community to develop new and improved vector control strategies. Combination approaches are a good way to ensure the beneficial qualities of the various tools implemented (Gratz 1993, Yap et al. 1994, Lee et al. 2010). Essential oils are one such area for exploration. As a result of the current study, citronella, hairy basil, catnip, and vetiver essential oils could serve as potential mosquito repellent products against *Cx. quinquefasciatus* and *An. minimus*. Further testing with higher concentrations of all essential oils should be performed to find an effective repellent dose against *Ae. aegypti*. According to Phasomkusolsil and Soonwera (2011a), increasing concentrations of essential oils can increase their repellent activity. However, development of a natural repellent formulation with long-lasting protection is necessary to be comparable to the effectiveness of the standard topical repellent DEET. The irritant and/or repellent effects of test compounds described here must be validated with tests on field mosquitoes for confirmation.

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