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Research article

Behavioral responses to five essential oils by *Aedes albopictus* (Skuse) (Diptera: Culicidae)

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Abstract

The behavioral responses using an excito-repellency test system were investigated of the laboratory strain of *Aedes albopictus* (Skuse) to four concentrations of different essential oils (0.5%, 1%, 2.5%, 5%) extracted from citronella (*Cymbopogon nardus* [L.] Rendle), eucalypt (*Eucalyptus camaldulensis* Dehnh), kaffir lime (*Citrus hystrix* DC.), guava (*Psidium gaujava* L.) and betel vine (*Piper betle* L.). DEET (N, N-diethyl-3-methylbenzamide) was used as a standard mosquito repellent. The escape and knockdown percentages were recorded of test mosquitoes in noncontact and contact chambers. The results showed that two oil extracts (from guava and betel vine) produced very promising test results. Higher escape responses of *Ae. albopictus* were observed in the contact and noncontact trials at 2.5% guava (100% and 90.92%, respectively) and 0.5% betel vine (96.49% and 84%, respectively). It was concluded that both these essential oils were very promising and further study is needed to identify the active ingredients from *Ps. gaujava* and *Pi. betle* for future development as plant-based repellents in commercialized products.

Introduction

Dengue and chikungunya are major public-health issues throughout tropical and sub-tropical regions of the world (Word Health Organization, 2011; Ministry of Public Health, 2013). They are considered as some of the most rapidly spreading mosquito-borne viral diseases, with a 30-fold increase in global incidence over the past 50 years (World Health Organization, 2012). In Thailand, dengue has been documented since 1949 with the different prominent peaks in 2005 (45,893 cases), 2013 (154,773 cases), 2015 (146,082 cases), and 2017 (53,916 cases) (Ministry of Public Health, 2017). For chikungunya, the first outbreak was reported in 1958 and then in 1975 and 1976 and again in 2008–2009 with 49,069 recorded cases in total (Le et al., 2020). The serious risk of dengue transmission has been found during May and June annually (Strickman and Kittayapong, 2003). In addition, there has been a tremendous growth in human population combined with demographic migrant movement to urban residential areas and an increase in tourism-based facilities (Patanarapeelet et al., 2016) These

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changes have influenced the population densities of *Aedes albopictus* (Skuse), an outdoor day biting mosquito, by creating more breeding larval habitats (Gubler, 1998).

Ae. albopictus is known as a dengue vector secondary in a rural-forest setting which has rapidly adapted to man-made container habitats in and around homes. For example, natural breeding habitats such as plant axils and outdoor artificial habitats containing organic debris have been documented as breeding sites of *Ae. albopictus* (Chareonviriyaphap et al., 2003). It has been reported that this species is invading many towns and suburban residential areas of the larger urban zones, especially Bangkok, one of the areas of greatest dengue transmission in Thailand and has also successfully adapted to different environments in many countries (Xu, et al., 2020). Despite decades of research activity, an efficacious and commercially viable dengue vaccine is not yet available. Therefore, the prevention and control of dengue and many other vector-borne diseases remains dependent on various vector control strategies to decrease the transmission risk.

Direct control of vectors is considered efficient using synthetic chemical insecticides (Mehlhorn et al., 2014). Chemical compounds can protect humans from mosquito bites by either causing mortality or excito-repellency that interrupts or inhibits normal feeding behavior (Roberts and Andre, 1994; Thanispong et al., 2009). Beside cost considerations, the use of insecticides carries risks associated with potential environmental contamination, reduction in non-target and beneficial organisms and the promotion of insecticide resistance in mosquito populations over time (Brown and Hebert, 1997; Qiu et al., 1998; Chareonviriyaphap et al., 2013). Therefore, it is desirable to use natural compounds extracted from plants as bio-pesticides to protect humans from mosquito bites.

A number of extracts and essential oils from botanical sources can repel mosquitoes (Tisgratog et al., 2016). Extracts from betel vine contain phenolic and terpenoid compounds that have been shown to provide safe and promising mosquito repellent activity (Alighiri et al., 2018; Twatsin et al., 2006). Extract from guava leaves (*Psidium guajava* L.) has proven repellent properties against mosquito vectors including *Aedes aegypti*, *Ae. albopictus*, *Anopheles dirus* and *Culex quinquefasciatus* (Twatsin et al., 2006). Investigating mosquito avoidance behavior using plant-derived essential oils has been evaluated using an excito-repellency (ER) test system (Nararak et al., 2016). However, the irritancy and repellency responses of many essential oils against *Ae. albopictus* have not been studied. In this study, essential oils from five plants were evaluated at four different concentrations for their contact irritant and noncontact repellent actions against *Ae. albopictus* mosquitoes.

Materials and Methods

Mosquitoes

The laboratory strain of *Ae. albopictus* mosquitoes was received from the Bureau of Vector Borne Diseases, Ministry of Public Health, Nonthaburi, Thailand. The strain had been maintained at the insectary of Department of Entomology, Faculty of Agriculture, Thailand with conditions of 25 ± 5°C and 80 ± 10% relative humidity with a 12:12 daylight-to-darkness ratio for the photoperiod. Adults were reared in a screened cage and provided 10% sugar solution as food. Female mosquitoes were free-mated and permitted to feed on blood on the fourth day after emergence. Two days after blood feeding, 10 cm diameter oviposition dishes containing moist filter paper were placed in the cages with gravid females for egg deposition. Eggs in oviposition dishes were initially dried at room temperature for approximately 24–48 hr. The eggs were affixed to filter papers and immersed in fresh water in hatching trays. Then 1–2 d after hatching, approximately 250 larvae were transferred to individual plastic trays (20 cm × 30 cm × 5 cm) containing 1,500 mL of tap water and containing 5–6 Sakura goldfish food granules, of a medium size and specially colored (black, green and red pellets). Pupae were transferred to emergence cups and placed in screened cages. Adults were provided *ad libitum* with a 10% sugar meal until 24 hr prior to ER assays.

Essential oils and N, N-diethyl-3-methylbenzamide

Three plant- derived essential oils were conducted using a steam distillation method in stainless steel essential oil distiller at pressure of 1 kg/m^3. These plants were *Cymbopogon nardus* (CIT), *Eucalyptus camaldulensis* (EUC), and *Citrus hystrix* (KAF) (Mohamed and Morad, 2004). Two essential oils from plants, namely *Ps. guajava* (GUA) with product code 1812465-40027 and *Pi. betle* (BET) with product code 1812465-40025] were purchased from the Thai-China Flavors and Fragrances Industry (TCFF) Co., Ltd. Company, Phra Nakhon Si Ayutthaya, Thailand. These two oils were extracted from leaves using a steam distillation method and all essential oils were stored in amber-colored vials at 0°C before preparation for testing.

Impregnated papers

The four concentrations at 0.5, 1, 2.5 and 5% volume per volume (v/v) of each essential oil and DEET were diluted from individual stock solutions in absolute ethanol 99.9% for analysis and were impregnated onto filter paper (14.7 cm × 17.5 cm; Whatman® No.1), following the World Health Organization procedure for testing papers with insecticidal compounds (World Health Organization, 2006). All treatments involved 2.8 mL per paper. Control papers were treated separately with absolute ethanol only. All tested papers were prepared just prior to testing and were air-dried for 1 hr before testing (Nararak et al., 2017).

Bioassay system

The excito-repellency test assay was conducted according to Chareonviriyaphap et al. (2002). Briefly, sets of four test chambers were used simultaneously to evaluate noncontact spatial repellent and contact irritant behaviors, consisting of two treatment chambers (contact or noncontact) and two paired control chambers, respectively. An external receiving box was connected to each test chamber for collecting mosquitoes exiting the chamber. In the contact assay design, treated papers were attached in front of the screens inside the chamber, while the treated papers were placed behind the screen in the noncontact assay, thereby preventing the mosquito from making direct contact with the paper. A sample of 15 three-to-five day-old, non-blood-fed female mosquitoes were released into each of the four test chambers using a mouth aspirator. Mosquitoes were allowed to adjust (3 min) to the oil and environmental conditions inside the test chamber before the exit portal leading to the receiving box was opened. The number of escaping mosquitoes was recorded every minute for 30 min. At the end of the exposure period, escaped and non-escaped (those remaining in the chamber) mosquitoes were transferred to individual containers and provided 10% sugar solution during the holding period. Knockdown response (moribund mosquitoes, unable to fly) was recorded immediately after 30 min and for final mortality after 24 hr in both treatments and controls for escaped and non-escaped mosquitoes. Each repellent compound was tested in four replicates (n = 60) during daylight hours between 0800 hours and 1630 hours. The test chambers were carefully cleaned with 95% ethyl alcohol and allowed to dry at least 24 hr before beginning the next experiment.

Data analysis

The mean values for the escape response and mortality percentages of mosquito species per test chamber (contact and noncontact) and for each essential oil were analyzed using Kaplan-Meier survival analysis (Roberts et al., 1997). The number of mosquitoes that successfully escaped in each paired contact was compared with those in the noncontact trial in order to exclude the repellency effect and to provide a better estimate of true contact irritancy (Sathantriphop et al., 2015). A log-rank method (Mantel and Haenzel, 1959) was used to compare the patterns of escape behavior between combinations of the five essential oils and the four concentrations. The escape time in minutes for 25% (ET_{25}), 50% (ET_{50}), and 75% (ET_{75}) of mosquitoes to escape was calculated for each oil and concentration. Test data were analyzed using the SAS Software version 9 (SAS Institute; Cary, NC, USA). Statistical significance for all tests was set at 5% ($p < 0.05$).

Results

The Ae. albopictus laboratory strain was exposed to five essential oils using the excito-repellency test system. The system was used to evaluate the two behavioral responses of contact irritancy and noncontact repellency. Percentage values for escape and mortality of Ae. albopictus during the 30-min exposure to the five essential oils and DEET at four various concentrations (0.5, 1, 2.5 and 5% v/v) are given in Table 1. With citronella, the greatest escape response was observed at 0.5% in the contact trial (54.6%) and at 1% in the noncontact trial (69.53%). High knockdown of non-escape mosquitoes was observed in chambers treated with 5% citronella oil in both the contact (89.36%) and noncontact trials (91.43%). The percentage mortality of non-escape mosquitoes was low (0–11.67%). With eucalypt, the greatest escape response was observed at 5% in both the contact trial (64.88%) and the noncontact trial (72.16%). High knockdown of non-escape mosquitoes was also observed in chambers treated with 5% eucalypt oil in both the contact (84.21%) and noncontact trials (66.67%). No mortality of non-escape and escape mosquitoes was observed. For kaffir lime, the greatest escape response was observed at 2.5% in both the contact trial (82.35%) and at 5% in the noncontact trial (66.76%). Knockdown of non-escape mosquitoes was observed in the chamber treated with 5% kaffir lime in the contact trial (50%). However, no mortality of non-escape and escape mosquitoes was observed.

Table 1 Escape response and mortality of *Aedes albopictus* after a 24 hr holding period following exposure in excito-repellency contact and noncontact trials

Compound	Dose (%)	Contact trials						Noncontact trials					
		% Escape		% KD		% Mortality		% Escape		% KD		% Mortality	
		N	Treated	Esc	Non Esc	Esc	Non Esc	N	Treated	Esc	Non Esc	Esc	Non Esc
CIT	0.5	61	54.6	0	0	0	0	60	59.26	0	0	0	0
	1	60	38.77	0	0	0	0	62	69.53	0	0	0	0
	2.5	59	32.33	0	0	0	0	60	21.57	0	0	0	0
	5	60	11.32	0	89.36	0	11.7	61	35.04	0	91.43	0	0
EUC	0.5	59	35.85	0	0	0	0	60	30.77	0	0	0	0
	1	60	44.44	0	0	0	0	60	32.69	0	0	0	0
	2.5	59	60.78	0	0	0	0	59	58.97	0	0	0	0
	5	60	64.88	0	84.21	0	0	61	72.16	0	66.67	0	0
KAF	0.5	60	57.63	0	0	0	0	61	25.93	0	0	0	0
	1	60	43.14	0	0	0	0	60	53.96	0	0	0	0
	2.5	60	82.35	0	0	0	0	61	66.63	0	0	0	0
	5	60	66.11	0	50	0	0	60	66.76	0	0	0	0
GUA	0.5	60	61.64	0	0	0	0	60	47.17	0	0	0	0
	1	61	94.72	0	0	0	0	59	63.35	0	0	0	0
	2.5	60	100	0	0	0	0	59	90.92	0	0	0	0
	5	60	58.18	0	100	0	0	60	94.35	0	100	0	0
BET	0.5	60	96.49	0	0	0	0	60	86.82	0	0	0	0
	1	60	60.71	0	0	0	0	62	82.11	0	0	0	0
	2.5	60	72.55	0	0	0	0	60	61.11	0	0	0	0
	5	61	45.73	0	90.63	0	0	60	36.36	0	77.14	0	0
DEET	0.5	60	76.92	0	0	0	0	60	29.76	0	0	0	0
	1	60	59.65	0	0	0	0	60	70.37	0	0	0	0
	2.5	60	69.14	0	0	0	0	60	57.48	0	0	0	0
	5	60	24.63	0	93.02	0	6.67	60	66.67	0	83.33	0	0

N = number; N = number of mosquito; CIT = citronella; EUC = eucalypt; KAF = kaffir lime; GUA = guava leaf; BET = betel vine; DEET = N, N-diethyl-3-methylbenzamide.
% Escape adjusted with paired controls using Abbott's formula; % KD = knock down at 30 min
Esc = escaped mosquitoes; Non Esc = non-escaped mosquitoes

For guava oil, the greatest escape response was observed at 2.5% in the contact trial (100%) and at 5% in the noncontact trial (94.35%). Complete (100%) knockdown of non-escape mosquitoes was observed in chambers treated with 5% guava oil in both the contact and noncontact trials. No mortality of non-escape and escape mosquitoes was observed. With betel vine oil, the greatest escape response was observed at 0.5% in both the contact trial (96.49%) and the noncontact trial (86.82%). Again, high knockdown of non-escape mosquitoes was observed in chambers treated with 5% betel vine oil in both the contact trial (90.63%) and the noncontact trial (77.14%). No mortality of non-escape and escape mosquitoes was observed. DEET produced its greatest escape response at 0.5% in the contact trial (76.92%) and at 1% in the noncontact trial (70.36%). The percentage mortality of non-escape mosquitoes was low (0–6.67%).

The statistical comparisons of escape responses between control versus contact, control versus noncontact and contact versus noncontact, exposed to the five essential oils are shown in Table 2. Highly significant ($p < 0.0001$) differences in escape responses were observed when control was compared to contact and noncontact for all five test essential oils, except for control versus contact with 5% citronella oil ($p > 0.1413$). Significant differences in escape responses were observed for all pairs of control and noncontact regardless of concentrations and test compounds. No significant differences were observed in the escape responses between contact and noncontact with eucalypt oil, regardless of the test concentration. Significant escape responses were observed when contact was compared to noncontact for guava oil, except at 0.5%.

Multiple comparisons of escape patterns between the four doses of essential oils in the contact and noncontact trials were

Table 2 Log-rank comparisons of escape responses between paired control and contact trials, control and noncontact trials and paired contact and noncontact trials for *Aedes albopictus* exposed to five essential oils and N, N-diethyl-3-methylbenzamide (DEET)

Compound	Concentration (%)	Control *vs* Contact	Control *vs* Noncontact	Contact *vs* Noncontact
CIT	0.5	<.0001	<.0001	0.8793
	1	<.0001	<.0001	0.0069
	2.5	0.0002	0.0235	0.4943
	5	0.1413	<.0001	0.0143
EUC	0.5	<.0001	0.0011	0.7507
	1	<.0001	0.0005	0.2832
	2.5	<.0001	<.0001	0.3074
	5	<.0001	<.0001	0.5007
KAF	0.5	<.0001	0.0026	0.0098
	1	<.0001	<.0001	0.0254
	2.5	<.0001	<.0001	0.0323
	5	<.0001	<.0001	0.8994
GUA	0.5	<.0001	<.0001	0.3339
	1	<.0001	<.0001	0.0135
	2.5	<.0001	<.0001	0.0135
	5	<.0001	<.0001	<.0001
BET	0.5	<.0001	<.0001	0.127
	1	<.0001	<.0001	0.0004
	2.5	<.0001	<.0001	0.1349
	5	<.0001	<.0001	0.444
DEET	0.5	<.0001	0.0008	<.0001
	1	<.0001	<.0001	0.387
	2.5	<.0001	<.0001	0.1029
	5	0.0039	<.0001	<.0001

CIT = citronella; EUC = eucalypt; KAF = kaffir lime; GUA = guava leaf; BET = betel vine

analyzed using the log-rank test method at the 0.05 level of probability (Table 3). In the contact trial with guava oil, there were significant differences in all cases, except for 0.5% versus 5% comparisons. For DEET, significant differences were observed when 5% was compared with 0.5%, 1.0% or 2.5%. There were significant differences when 0.5% betel vine was compared to 1%, 2.5% or 5%. For the noncontact trials with eucalypt and betel vine, only two of the paired-dose comparisons failed to show a significant difference (0.5% versus 1%). For guava, two of the paired-dose comparisons also not significantly different (0.5% versus 1% and 2.5% versus 5%). For DEET, there were significant differences in escape responses when 0.5% was compared with 1%, 2.5% or 5% (Table 3). Statistical comparisons between DEET and each essential oil in the contact and noncontact trials were analyzed and the results are presented in Table 4. There were significant differences in escape responses in all paired-dose comparisons when DEET was compared to guava. In the noncontact trial, there were no significant differences between all paired-dose comparisons for DEET and kaffir lime. Two of the paired-dose comparisons at 1% (DEET versus citronella and DEET versus guava) were not significantly different.

In addition, statistical comparisons between each essential oil in the contact and noncontact trials were analyzed and the results are presented in Table 4. There were significant differences in escape response in all paired-dose comparisons of 2.5% and 5% when citronella was compared to the other three oils (eucalypt, kaffir lime or guava), regardless of the test conditions. Statistical differences were also found when betel vine at 0.5% was compared to citronella, eucalypt, kaffir lime or guava in the contact and noncontact trials. The escape patterns in minutes and percentages exiting the exposure chambers were expressed in 1 min intervals for 25%, 50% and 75% (ET_{25}, ET_{50} and ET_{75}) of the test population leave the treated chambers (Table 5). Ae. albopictus had the fastest response to ET_{25} values (< 1 min) in the contact trial with 0.5% betel vine and in the noncontact trial with 0.5% guava oil. In addition, citronella oil at 0.5% produced ET_{25} values of 1 min for both the contact and noncontact trials. Kaffir lime oil had an ET_{50} value of < 1 min for 2.5% and 5% concentrations in the contact and noncontact trials. Betel vine oil had an ET_{50} value of 2 min at 0.5% concentration. Among the five essential oils, the greatest ET_{50} value (28 min) was produced by 1% kaffir lime oil in the contact trial. At 5%, all four essential oils had very rapid escape responses (ET_{25} values < 8) compared to DEET.

Table 3 Log-rank comparisons of escape responses between different concentrations of essential oils and N, N-diethyl-3-methylbenzamide (DEET) in *Aedes albopictus* exposed to contact and noncontact trials in excito-repellency test system

Compound	Concentration (%)	Contact	Noncontact
CIT	0.5 *vs* 1	0.0972	0.4835
	0.5 *vs* 2.5	0.0076	0.0004
	0.5 *vs* 5	<.0001	0.0427
	1 *vs* 2.5	0.3445	<.0001
	1 *vs* 5	0.0047	0.0061
	2.5 *vs* 5	0.0514	0.2043
EUC	0.5 *vs* 1	0.3159	0.8035
	0.5 *vs* 2.5	0.004	0.0238
	0.5 *vs* 5	0.0011	<.0001
	1 *vs* 2.5	0.0559	0.0468
	1 *vs* 5	0.0176	<.0001
	2.5 *vs* 5	0.7746	0.027
KAF	0.5 *vs* 1	0.0085	0.003
	0.5 *vs* 2.5	<.0001	<.0001
	0.5 *vs* 5	0.0276	<.0001
	1 *vs* 2.5	<.0001	0.2734
	1 *vs* 5	0.4527	0.0799
	2.5 *vs* 5	0.1803	0.5767
GUA	0.5 *vs* 1	<.0001	0.1297
	0.5 *vs* 2.5	<.0001	<.0001
	0.5 *vs* 5	0.5071	<.0001
	1 *vs* 2.5	<.0001	<.0001
	1 *vs* 5	<.0001	<.0001
	2.5 *vs* 5	<.0001	0.8743
BET	0.5 *vs* 1	<.0001	0.1901
	0.5 *vs* 2.5	<.0001	0.0001
	0.5 *vs* 5	<.0001	<.0001
	1 *vs* 2.5	0.0598	0.0012
	1 *vs* 5	0.1116	<.0001
	2.5 *vs* 5	0.0009	0.0058
DEET	0.5 *vs* 1	0.1617	<.0001
	0.5 *vs* 2.5	0.8227	0.0002
	0.5 *vs* 5	<.0001	<.0001
	1 *vs* 2.5	0.2367	0.1625
	1 *vs* 5	0.0001	0.5801
	2.5 *vs* 5	<.0001	0.3827

CIT = citronella; EUC = eucalypt; KAF = kaffir lime; GUA = guava leaf; BET = betel vine

Table 4 Log-rank comparison of escape responses of *Aedes albopictus* exposed compared to other essential oils and N, N-diethyl-3-methylbenzamide (DEET) in contact (CT) and noncontact (NT) trials

Compound	0.5%		1%		2.5%		5%	
	CT	NT	CT	NT	CT	NT	CT	NT
CIT *vs* EUC	0.0172	0.0042	0.7498	0.0005	0.0016	0.003	<.0001	0.0008
CIT *vs* KAF	0.3483	0.0006	<.0001	0.2048	<.0001	<.0001	<.0001	0.0013
CIT *vs* GUA	0.8958	0.2901	<.0001	0.6309	<.0001	<.0001	<.0001	<.0001
CIT *vs* BET	0.0001	0.0074	0.1811	0.0827	<.0001	0.0008	0.0044	0.5192
EUC *vs* KAF	0.1046	0.5287	0.0002	0.0299	0.0175	0.2153	0.5096	0.9885
EUC *vs* GUA	0.0088	0.0914	<.0001	0.0022	<.0001	<.0001	0.1965	0.0008
EUC *vs* BET	<.0001	<.0001	0.3586	<.0001	0.5403	0.7042	0.0046	<.0001
KAF *vs* GUA	0.317	0.023	<.0001	0.3883	<.0001	0.0006	0.0992	0.0074
KAF *vs* BET	<.0001	<.0001	0.0473	0.004	0.0384	0.3193	0.0022	<.0001
GUA *vs* BET	<.0001	0.0002	<.0001	0.0292	<.0001	<.0001	0.1334	<.0001
CIT *vs* DEET	0.1631	<.0001	0.0843	0.9998	0.0001	0.0016	0.4262	0.0184
EUC *vs* DEET	<.0001	0.4291	0.1405	0.0004	0.4684	0.8016	<.0001	0.2433
KAF *vs* DEET	0.0047	0.9065	0.0448	0.1919	0.1104	0.2663	<.0001	0.2358
GUA *vs* DEET	0.0667	0.0104	<.0001	0.6	<.0001	<.0001	0.0003	<.0001
BET *vs* DEET	0.0002	<.0001	0.5899	0.0632	0.8927	0.9369	0.0303	0.0004

CIT = citronella; EUC = eucalypt; KAF = kaffir lime; GUA = guava leaf; BET = betel vine; DEET = N, N-diethyl-3-methylbenzamide

Table 5 Escape time (minutes) for 25% (ET_{25}), 50% (ET_{50}) and 75% (ET_{75}) of *Aedes albopictus* to escape from exposure chambers of excito-repellency tests

Test	Compound	0.5%			1%			2.5%			5%		
		ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}	ET_{25}	ET_{50}	ET_{75}
CT	CIT	1	3	-	1	-	-	4	-	-	-	-	-
	EUC	5	-	-	2	14	-	-	2	-	-	3	-
	KAF	3	13	-	< 1	28	-	-	<1	7	-	< 1	-
	GUA	2	8	-	-	2	5	-	-	2	1	17	-
	BET	< 1	2	4	2	13	-	-	7	20	3	-	-
	DEET	2	4	15	-	3	-	-	< 1	-	23	-	-
NT	CIT	1	4	-	1	3	-	6	-	-	1	-	-
	EUC	5	-	-	2	-	-	< 1	11	-	-	2	22
	KAF	8	-	-	-	-	-	-	<1	-	-	< 1	-
	GUA	1	13	-	1	6	-	-	-	1	-	-	2
	BET	< 1	2	5	-	2	6	2	9	-	8	-	-
	DEET	14	-	-	-	5	-	2	7	-	1	5	-

CIT = citronella; EUC = eucalypt; KAF = kaffir lime; GUA = guava leaf; BET = betel vine; DEET = N, N-diethyl-3-methylbenzamide; CT = contact trial; NT = noncontact trial

Figs 1 and 2 show the proportions of mosquitoes remaining in the exposure chambers under different test conditions. The patterns were indicative of the escape probabilities among the five oils and DEET at 0.5% in the contact (Fig 1B) and noncontact trials (Fig 1A), at 1% in the contact (Fig 1D) and noncontact trials (Fig 1C), at 2.5% in the contact (Fig 2B) and noncontact trials (Fig 2A) and at 5% in the contact (Fig 2D) and noncontact trials (Fig 2C). In the contact trial, there was a greater escape rate at the lowest dose (0.5%citronella and betel vine) compared to the higher doses (1%, 2.5%, 5%). Guava at 1% and 2.5% had a high escape rate compared to at 0.5% and 5%. In the noncontact trial, there were large escape patterns from guava at 2.5% and 5%.

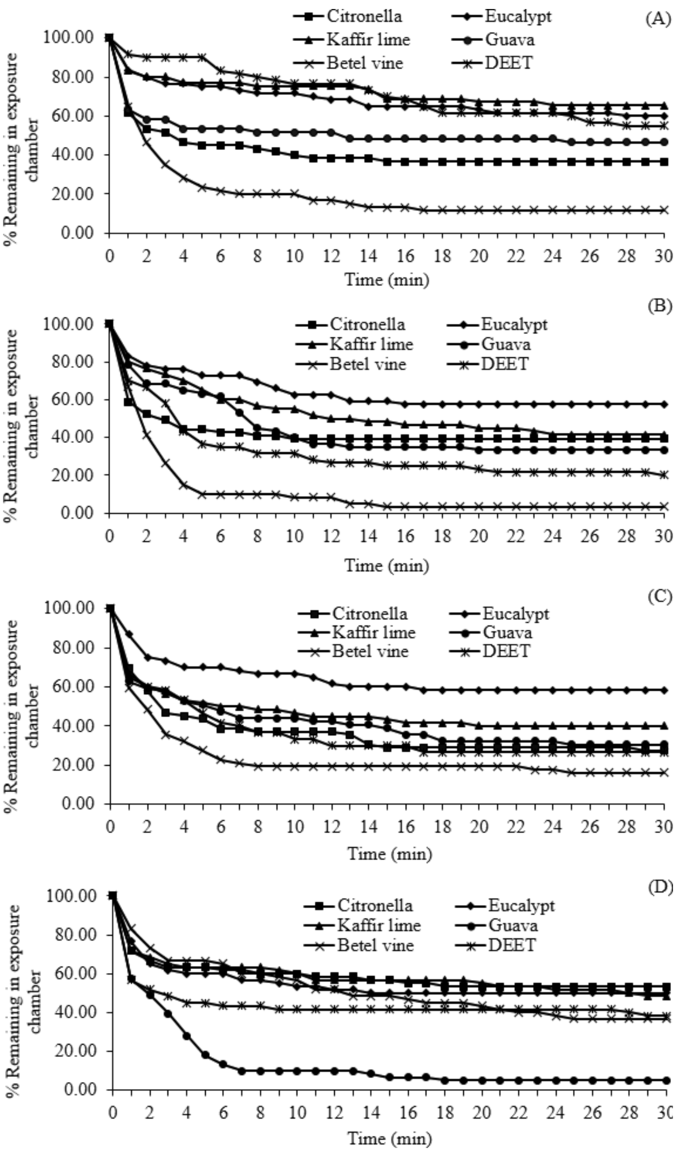

Fig. 1 Survival curves of *Aedes albopictus* escaping from contact chamber during 30 min exposure to essential oils and N, N-diethyl-3-methylbenzamide (DEET) at: (A) 0.5% concentration and noncontact; (B) 0.5% concentration and contact, (C) 1% concentration and noncontact; (D) 1% concentration and contact

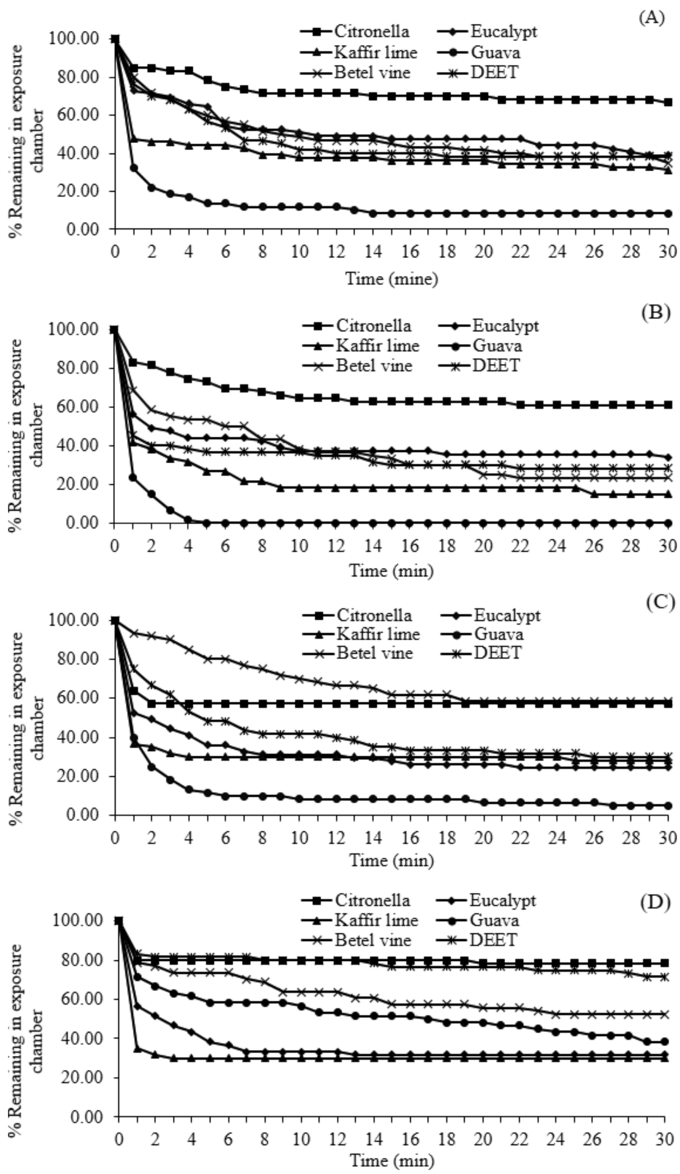

Fig. 2 Survival curves of *Aedes albopictus* escaping from contact chamber during the 30 min exposure to essential oils and N, N-diethyl-3-methylbenzamide (DEET) at: (A) 2.5% concentration and noncontact; (B) 2.5% concentration and contact, (C) 5% concentration and noncontact; (D) 5% concentration and contact

Discussion

Several plant-based essential oils have been evaluated for insect repellent activity for protection against mosquitoes and other arthropod pests in Thailand. These have included: *Ocimum* spp. (Twatsin et al., 2001); *Nepeta cataria*, *Ci. hystrix* (Thavara et al., 2007); *Melaleuca leucadendron*, *Litsea cubeba* and *Litsea salicifolia* (Noosidam et al., 2008); *Citrus aurantium*, *Cinnamomum verum*, *Cymbopogon winteri*anus, and *Syzygium aromaticum* (Suwansirisilp et al., 2013); citronella, hairy basil, catnip and vetiver (Sathantriphop et al., 2015); and *Cy. nardus*, *Oc. americanum, Oc. basilicum* and *Vetiveria zizanioides* (Nararak et al., 2016). Over 90 plant species were evaluated as potential repellents or toxicants against blood-feeding invertebrates and pestiferous flies in Lao PDR (De Boer et al., 2010). Although a number of essential oils have exhibited significant repellent activity against insects, there has been comparatively little done to differentiate the two primary types of behavioral responses of irritancy and repellency (Polsomboon et al., 2008; Noosidam et al., 2008; Nararak et al. 2016). This is unfortunate because the role of irritant (contact excitation) and repellent actions against target mosquitoes would greatly assist operational programs to estimate their impact and to make better-informed decisions on allocating disease control resources.

The current study investigated five plant-based insect repellents based on citronella, eucalypt, kaffir lime, guava and betel vine essential oils against the *Ae. albopictus* laboratory strain based on the two forms of behavioral responses (irritability and repellency). The results demonstrated that guava and betel vine at ≤2.5% v/v were highly effective in eliciting an irritant rather than a repellent response against *Ae. albopictus*. Guava at 5% demonstrated promising mosquito repellency activity. Kaffir lime at 2.5% acted as an irritant rather than as a repellent against *Ae. albopictus*. Citronella at ≤1% was effective in eliciting a repellent rather than an irritant response. Thus, all five plant-based insect repellents could serve as alternative natural mosquito repellents and would also avoid using toxic and unpleasant-odored chemical repellents such as DEET. The higher concentrations of citronella (2.5% and 5%) in the current study demonstrated low excito-repellency activity. Similarly, citronella at 2.5% was less effective against *Ae. albopictus* as reported by Sathantriphop et al. (2014) However, Nararak et al. (2016) showed that citronella at <5% v/v had strong irritant effects against *Anopheles. minimus*.

Essential oils have been shown to contain several chemical compounds which have mosquito repellent activity (De Souza et al., 2019). The oils that were used in the current study were evaluated using mass spectroscopy/gas chromatography to identify the constituents of each oil by the TCFF essential oil company. The main chemical components of the guava were α-pinene (8.46%), D-limonene (12.31%), 1,8 cineole (4.96%), copaene (3.69%), caryophyllene (11.77%), β-bisabolene and caryophyllene oxide (6.73%), while in betel vine they were chavicol, cineol, eugenol (28.33%), β-sitosterol, isoeugenol and eugenyl acetate (14.86%). The chemicals found in plants and their characteristic odors could be effective as mosquito repellents (Maia and Moore, 2011). Mosquitoes have olfactory (smell) receptors on their antennae or maxillary palps, and gustatory (contact) receptors on the labella and tarsi that respond to some chemicals in plant essential oils (Das et al., 2003). Mosquitoes dislike the odor and taste of some essential oils derived from plants, so these oils can repel or act as barriers against mosquitoes.

In summary, five essential oils and DEET were tested as potential repellents against *Ae. albopictus* in the laboratory. Excito-repellency properties were evaluated using excito-repellency system. Among these oils, two (guava leaf oil and betel vine oil) displayed a promising effect on the test mosquitoes. The findings showed clearly that guava leaf oils and betel vine oil are volatile bioactive compounds that have potential as natural-based repellents and they should be explored further as active ingredients in various commercial mosquito repellent products or with synthetic-based mosquito repellents. The advantages for using both plants are the ease and low cost of cultivation and the high yields from leaves. The repellent products could be further screened and improved by increasing the amount of essential oils or adding some active components of plant essential oils. Formulations of products based on repellents from plant-derived essential oils could perform well against insect disease vectors and hence deserve further research and development.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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