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Comparative mosquito repellency of essential oils against *Aedes aegypti* (Linn.), *Anopheles dirus* (Peyton and Harrison) and *Culex quinquefasciatus* (Say)

Siriporn Phasomkusolsil*, Mayura Soonwera

Entomology and Environment Program, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Lad Krabang, Chalong Krung Road, Lad Krabang, Bangkok, Thailand

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ABSTRACT

Objective: To assess the repellency to female *Aedes aegypti* (*Ae. aegypti*), *Anopheles dirus* (*An. dirus*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) of seven essential oils using two treatment methods. **Methods:** Topical applications of three dose concentrations (0.02, 0.10 and 0.21 mg/cm^2) were made on the forearms of volunteers. Dose-response study and protection time study were employed in the experiment. **Results:** In the dose-response test, *Cymbopogon citratus* (*C. citratus*), *Cymbopogon nardus* (*C. nardus*), *Syzygium aromaticum* (*S. aromaticum*) and *Ocimum basilicum* (*O. basilicum*) exhibited a high repellency against *Ae. aegypti* with ED_{50} at < 0.045 mg/cm^2, whereas *C. citratus*, *C. nardus* and *S. aromaticum* showed repellency against *An. dirus* with ED_{50} at <0.068 mg/cm^2. Furthermore, the essential oils of *C. citratus*, *C. nardus*, *S. aromaticum*, *O. basilicum* and *Cananga odorata* gave strong effective dose (ED_{50}) values at <0.003 mg/cm^2 when tested against *Cx. quinquefasciatus*. For testing by arm in cage method, at 0.21 mg/cm^2, protection time of *C. citratus* gave the longest lasting period against three mosquito species, 72 min for *Ae. aegypti*, 132 min for *An. dirus* and 84 min for *Cx. quinquefasciatus*. In addition, the two essential oils exhibited moderate repellency against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*, at 60, 90 and 78 min with *C. nardus*, and 54, 96 and 72 min with *S. aromaticum*, respectively. **Conclusions:** The percentage repellency increased when the concentration of essential oils increased. In contrast, biting rates decreased when the concentration of essential oils increased. *C. citratus* exhibited high efficiency for the protection time and the percentage of biting deterrent against all of 3 mosquito species.

1. Introduction

The mosquitoes which spread mosquito borne diseases in Thailand like dengue fever, malaria and Japanese encephalitis are dominated by three widespread vectors, *Aedes aegypti* (*Ae. aegypti*) (Linn.), *Anopheles dirus* (*An. dirus*)(Peyton and Harrison) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) (Say). The *Ae. aegypti* is the principal transmitter of dengue fever and dengue haemorrhagic fever (DHF) in Thailand but it also transmits Chikungunya fever[1]. In addition, *An. dirus* is the major vector of malaria in border of Thailand and other countries[2]. *Cx. quinquefasciatus* is a vector of Japanese encephalitis (JE) and it also causes annoyance and dermatitis[3]. Thus the risk of being infected with a mosquito-borne disease is caused by the risk of being bitten by the infected mosquito. Generally, mosquito bites are also very itchy and painful sometimes. The continuous scratching over the skin will irritate it even more and make you more desperate.

Therefore, insect repellent using is one of the most efficient ways to prevent disease transmission by biting insects, particularly by mosquitoes[4]. Currently, mosquito repellent containing diethyl toluamide (DEET) is recommended as the most effective form of bite-preventive treatment. The efficacy of DEET in providing long-lasting protection against a wide variety of mosquito species has been documented in several studies[5]. Although DEET is an effective repellent against mosquitoes, there are concerns associated with its use. Human toxicity has been reported with DEET, with symptoms varying from mild to severe[6]. It is irritating to mucous membranes, and concentrated formulations dissolve plastic. DEET may be unsafe for

*Corresponding author: Siriporn Phasomkusolsil, Entomology and Environment Program, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Lad Krabang, Chalong Krung Road, Lad Krabang, Bangkok 10520, Thailand.
Tel: +66 (0) 2326 4314
E-mail: msiriporn@hotmail.com
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children possibly causing encephalopathy[7]. Therefore, the development of alternative, environmentally friendly and sustainable approaches for mosquito control is with the use of natural products owning greater target specificity, lower bioaccumulation properties and reduction of malignancy in non-target animals[8].

Recently, the laboratory of the Plant Production Technology Section, King Mongkut Institute of Technology Lad Krabang (KMITL) has successfully developed the plant extracts and essential oils as one of the methods in controlling insect vector. Herbal shampoo from *Piper retrofractum* proved to be highly effective against all stage of human head lice and had no side effects on children, after treated[9]. Furthermore, Phosomkusolsil and Soonwera investigated the repellency of insect repellent, larvicidal and pupacidal activities of Thai indigenous plant oils against *Ae. aegypti*, *An. minimus* and *Cx. quinquefascatus* in the laboratory[10,11]. We have focused to develop plant-based repellents to increase long-lasting protection on registered commercially available repellent products. This study reports on repellency properties of the seven essential oils against mosquitoes, *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus* using dose-response study to determine effective dosages of essential oils and arm in cage method to estimate protection time and biting rate under laboratory conditions.

2. Materials and methods

2. 1. Mosquitoes

All three species of mosquitoes (*Ae. aegypti*, *An. dirus*, *Cx. quinquefasciatus*) were reared in the laboratory of the Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut Institute of Technology Lad Krabang (KMITL), Bangkok. *Ae. aegypti* and *An. dirus* eggs were obtained from the Armed Forces Research Institute of Medical Sciences (AFRIMS). *Cx. quinquefasciatus* eggs were obtained from the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University. Briefly, collected eggs were hatched in plastic trays (30×35×5 cm), which contained tap water (1500 mL). The hatched larvae were held in plastic trays and larval diet was added to each tray. Newly emerged pupae were transferred to screen cage (size 30×30×30 cm) and emerged as adults. Mosquitoes are kept in the room with temperature, relative humidity, and photoperiod range between 30-35 ℃, 70%-80%, and 12:12 hour (L:D) cycle, respectively. Adults continuously provided with 5% glucose solution in water soaked on cotton pads. In this study, 5- to 7-day-old female mosquitoes were starved by providing them with only water for 12 hours.

2. 2. Plant materials

The plant materials were collected from *Cananga odorata* (*C. odorata*) Lamk. (ylang ylang flowers), *Citrus sinensis* (*C. sinensis*) L. Osbeck (orange fruits), *Cymbopogon citratus* (*C. citratus*) DC. Stapf (lemongrass leaves and stems), *Cymbopogon nardus* (*C. nardus*) L (citronella grass leaves), *Eucalyptus citriodora* (*E. citriodora*) Hook (eucalyptus leaves), *Ocimum basilicum* L. (sweet basil leaves) and *Syzygium aromaticum* (*S. aromaticum*) L. (clove flowers). Each plant material was extracted for essential oils by steam distillation. The oils to be tested were diluted with soybean oil to concentrations of 0.02, 0.10 and 0.21 mg/cm^2. All formulations were kept at room temperature before testing.

2. 3. Repellent bioassay

There were two different treatment methods to determine the repellency activity of the seven essential oils against all three species of mosquito.

2.3.1. Dose-response study

The test procedure to determine initial effective dose was modified from the American Society for Testing and Materials Standard (ASTM E951-94)[12]. This is a standard laboratory method that was designed for testing non-commercial mosquito repellent formulations on human skin employing laboratory-reared mosquitoes. The laboratory bioassay for testing repellent efficacy consisted of a six-well feeding reservoir system similar to the K & D module[13]. It was made of Plexiglas and the base of the rectangular cage (26×5×5 cm) has six holes, each with rectangular 3×4 cm holes that opened and closed by a sliding door. The flexor region of the forearms of a human volunteer was outlined with four rectangular (3×4 cm) test areas. A volume of 0.025 mL of each concentration of the essential oils in soybean oil (0.02, 0.10 and 0.21 mg/cm^2) and 0.025 mL of the diluent (soybean oil as control) was applied randomly to the marked areas. After air drying for 5 minutes, a plastic cage with matching cutouts in its floor was secured over the treated areas by rubber bands. Each hole contained 5 nulliparous 5-7 days old mosquito females (totally 20 females per cage). The number of mosquitoes biting on each test site was recorded each minute for 5 min. Tests were conducted five times on each repellent-treated area and were completed within 25 min of repellent application. The effectiveness of essential oils was determined by the percentage repellency, using the formula described by Weaving and Sylvester[14]:

$$\% \text{ Repellency} = \frac{100 - \text{No. of bites on treated area}}{\text{No. of bites on control area}} \times 100$$

2.3.2. Protection time study

The seven essential oils were tested against 3 species of mosquitoes under laboratory conditions using a screened cage test method[15] following Thai Industrial Standards Institute (TISI) guidelines[16]. At 0.21 mg/cm2 seven essential

oils were the top dose tested in this study. Test times were determined by normal feeding times for each mosquito species. The *Ae. aegypti* testing time was between 8:00 AM to 4:00 PM, while the *Cx. quinquefasciatus* and *An. dirus* testing time was 4:00 PM and 12:00 PM.

For testing, a volunteer used the left arm for treatment and the right arm for control. Both arms were covered with a rubber sleeve with a window 3×10 cm on the forearm. 100 μL of test material was applied to the treatment area and allowed to dry for 5 minutes. The control arm was exposed to mosquitoes before the treated arm. If at least two mosquitoes landed on or bit the arm, the repellency test was continued. The mosquito cage of 30×30×30 cm contained 250 nulliparous, 5–7 day old female mosquitoes; the test was conducted for 3 minutes. The total number of mosquitoes biting on the treatment and control areas was recorded. If no mosquito bite occurred within 3 minutes, the forearm was then taken out and the test was repeated every 30 minute intervals. This was continued until at least two mosquito bites occurred during the 3-minute study period. The experiment was completed after two mosquitoes had bitten. The protection time was the time from repellent application until the study was stopped.

For comparison, a percentage of mosquito bitings was calculated for each test[17] using the following formula:

$$\%\ \text{Biting} = \frac{B}{250} \times 100$$

Where B is the total number of bitings by the end of the test. The test was carried out 5 times per sample.

2.4. Statistical analysis

For statistical analysis, ED_{50} values were calculated using probit analysis. The mean protection time was used to compare the seven essential oils. Differences in significance were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple comparisons by SPSS for Windows (version 16.0).

Table 1
Repellent activities of the seven essential oils in three concentrations (0.02, 0.10 and 0.21 mg/cm^2) against *Ae. aegypti*, *An. dirus*, *Cx. quinquefasciatus* adults.

Herbal essential oils	Mosquito spp.	Conc 0.02 mg/cm^2		Conc 0.10 mg/cm^2		Conc 0.21 mg/cm^2		ED_{50} (mg/cm^2)
		Mean No. of mosquito biting ± SD	% Repellency	Mean No. of mosquito biting ± SD	% Repellency	Mean No. of mosquito biting ± SD	% Repellency	
C. odorata oil	*Ae. aegypti*	2.70 ± 1.42^{b}	46	2.10 ± 1.37^{b}	58	1.70 ± 1.06^{b}	66	0.045
	An. dirus	0.30 ± 0.48^{g}	94	0.40 ± 0.70^{f}	92	0.40 ± 0.70^{d}	92	2.149
	Cx. quinquefasciatus	2.10 ± 0.99^{h}	58	0.60 ± 0.70^{i}	88	0.50 ± 0.53^{e}	90	<0.003
C. sinensis oil	*Ae. aegypti*	2.50 ± 1.18^{b}	50	2.50 ± 1.43^{b}	50	2.60 ± 1.51^{a}	48	0.049
	An. dirus	3.00 ± 1.63^{d}	40	2.30 ± 2.11^{e}	54	0.80 ± 1.14^{d}	84	0.068
	Cx. quinquefasciatus	2.20 ± 0.63^{h}	56	1.70 ± 0.67^{g}	66	0.60 ± 0.70^{e}	88	0.003
C. citratus oil	*Ae. aegypti*	1.00 ± 0.67^{c}	80	0.30 ± 0.48^{d}	94	0.00^{c}	100	<0.045
	An. dirus	1.20 ± 1.03^{fg}	76	0.90 ± 1.29^{f}	82	0.10 ± 0.32^{d}	98	<0.068
	Cx. quinquefasciatus	0.40 ± 0.70^{i}	92	0.10 ± 0.32^{i}	98	0.10 ± 0.32^{e}	98	<0.003
C. nardus oil	*Ae. aegypti*	2.00 ± 1.56^{bc}	60	0.70 ± 0.82^{cd}	86	0.60 ± 0.70^{c}	88	<0.045
	An. dirus	0.40 ± 0.70^{g}	92	0.40 ± 0.97^{f}	92	0.10 ± 0.32^{d}	98	<0.068
	Cx. quinquefasciatus	0.90 ± 0.88^{i}	82	0.80 ± 0.79^{hi}	84	0.30 ± 0.48^{e}	94	<0.003
E. citriodora oil	*Ae. aegypti*	4.50 ± 0.97^{a}	10	4.20 ± 0.79^{a}	16	3.20 ± 1.40^{a}	36	0.285
	An. dirus	2.40 ± 1.26^{de}	52	1.30 ± 1.25^{ef}	74	0.70 ± 1.25^{d}	86	1.043
	Cx. quinquefasciatus	0.60 ± 0.70^{i}	88	0.70 ± 0.82^{hi}	86	0.70 ± 0.82^{e}	86	2.526
O. basilicum oil	*Ae. aegypti*	2.30 ± 1.70^{bc}	54	1.50 ± 1.58^{bc}	70	0.80 ± 0.79^{c}	84	<0.045
	An. dirus	1.70 ± 0.82^{ef}	66	1.30 ± 0.48^{ef}	74	0.20 ± 0.42^{d}	96	0.628
	Cx. quinquefasciatus	1.20 ± 1.32^{i}	76	1.10 ± 0.88^{gh}	78	0.50 ± 0.53^{e}	90	<0.003
S. aromaticum oil	*Ae. aegypti*	1.70 ± 1.57^{bc}	66	0.30 ± 0.67^{d}	94	0.20 ± 0.42^{c}	96	<0.045
	An. dirus	0.90 ± 0.74^{fg}	82	0.40 ± 0.52^{f}	92	0.10 ± 0.32^{d}	98	<0.068
	Cx. quinquefasciatus	1.20 ± 0.92^{i}	76	0.90 ± 0.74^{h}	82	0.40 ± 0.70^{e}	92	<0.003
CV (%)	*Ae. aegypti*	56.21	–	66.19	–	74.88	–	–
	An. dirus	72.10	–	117.11	–	215.52	–	–
	Cx. quinquefasciatus	73.52	–	85.74	–	136.14	–	–

In the column of Mean No. of mosquito biting ± SD, Data followed by the different superscripted letters are significantly different (P<0.05, by one-way ANOVA and Duncan's Multiple Range Test); Tested with different oils within the same species.

3. Results

3.1. Dose-response study

In dose-response study for determining effective dose, the results of ED_{50} values, the mean number of mosquito bites and the percentage repellency of seven essential oils against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus* adults at various concentration are shown in Table 1. Meanwhile the percentage of repellency of seven essential oils for three species of mosquitoes is presented in Figure 1. ED_{50} values of four essential oils, *C. citratus*, *C. nardus*, *O. basilicum* and *S. aromaticum* against *Ae. aegypti* were less than 0.045 mg/cm^2. In addition, the oil of *C. citratus* at three concentrations (0.02, 0.10 and 0.21 mg/cm^2) provided the highest repellency with 80, 94 and 100%, respectively. Likewise the percentage repellency of *C. nardus*, *O. basilicum* and *S. aromaticum* increased when the concentration of these essential oils increased, in contrast, biting rates decreased when the concentration increased. The results showed significant differences in both the percentage of repellency and the number of mosquitoes biting (P<0.05).

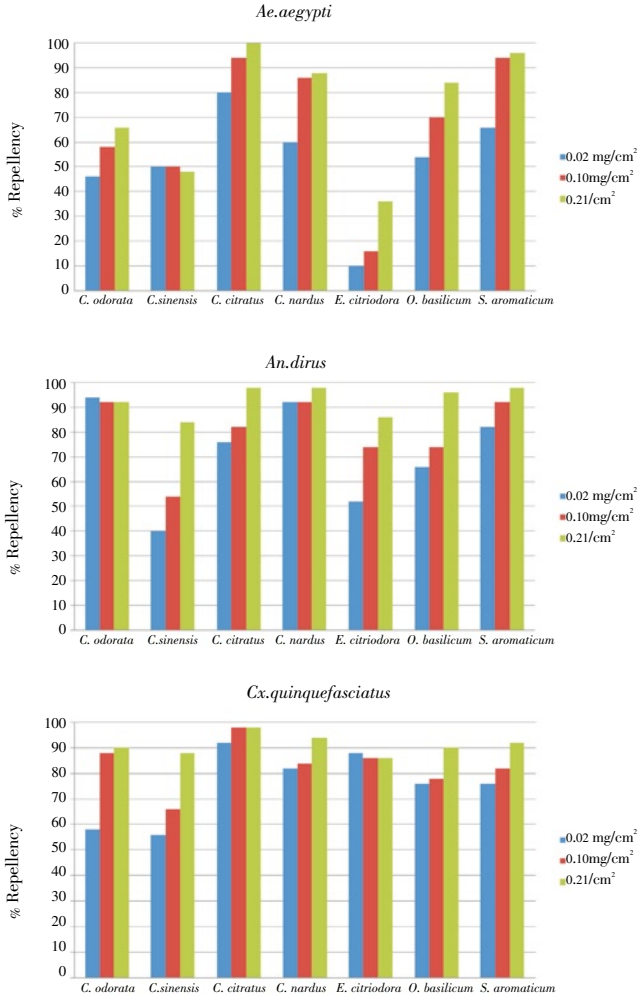

Figure 1. Comparison of repellency percentages for each repellent plant oils against three mosquito species (*Ae. aegypti*, *An. dirus and Cx. quinquefasciatus*).

Table 1 shows the repellents activity against *An. dirus* by using the modified K&D module in the laboratory. The essential oils of *C. citratus*, *C. nardus*, *S. aromaticum* gave strong effective dose (ED_{50}) values at <0.068 mg/cm^2. The strongest value was shown by *C. nardus*, it showed significant percentage repellency (92%-98%) and the mean number of mosquito biting was (0.40-0.10) bite/min at various concentrations. Furthermore, repellency evaluation of the essential oils exhibited ED_{50} values ranging from <0.003 to 2.526 mg/cm^2 when tested against *Cx. quinquefasciatus* (Table 1). The most effective essential oil was *C. citratus* (ED_{50} at <0.003 mg/cm^2; % repellency = 92%-98 %; (0.1-0.4 bite/min of no. of biting) at various concentrations. One-way ANOVA showed a significant difference of both % repellency and the number of mosquitos biting (P<0.05).

3.2. Protection time study

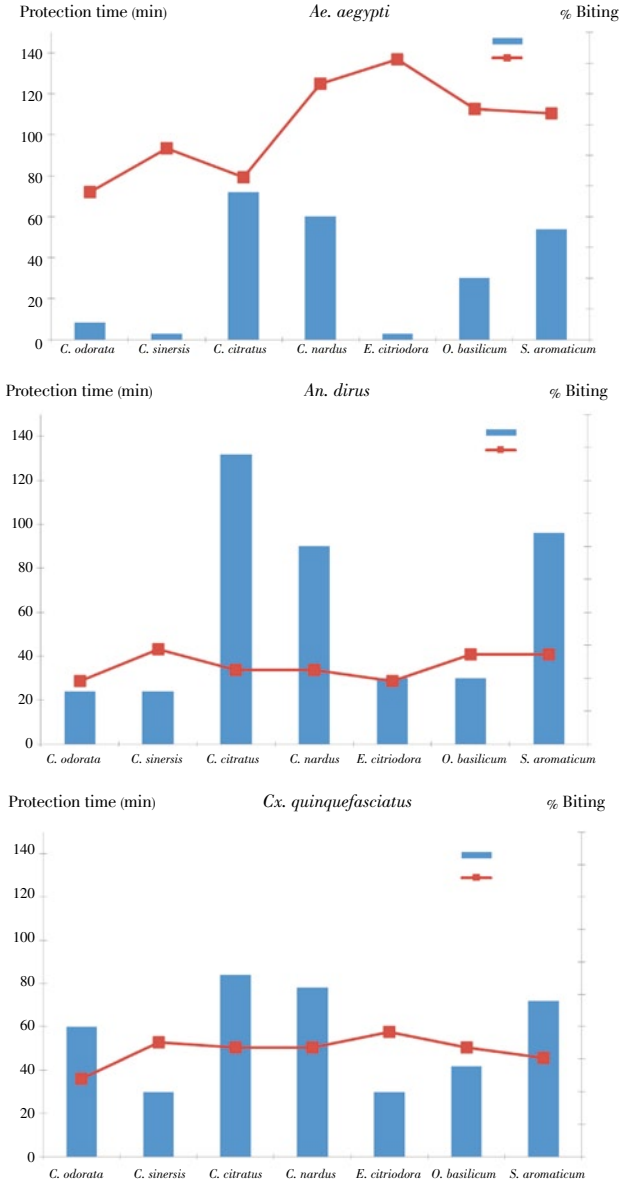

Figure 2. Comparison of protection times (minutes) and biting percentages for each repellent plant oils against three mosquito species (*Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*).

Table 2
Protection time for each repellent against three mosquito species (*Ae. aegypti*, *An. dirus*, *Cx. quinquefasciatus*) and biting percentages.

Herbal essential oils	*Ae. aegypti*		*An. dirus*		*Cx. quinquefasciatus*	
	Protection time (min)	% Biting[1/]	Protection time (min)	% Biting[2/]	Protection time (min)	% Biting[2/]
C. odorata oil	8.4^{d}	2.40 ± 0.63^{b}	24.0^{c}	0.96 ± 0.22	60.0^{bc}	1.20 ± 0.49
C. sinensis oil	3.0^{d}	3.12 ± 0.91^{ab}	24.0^{c}	1.44 ± 0.73	30.0^{d}	1.76 ± 1.15
C. citratus oil	72.0^{a}	2.64 ± 0.61^{b}	132.0^{a}	1.12 ± 0.33	84.0^{a}	1.68 ± 1.18
C. nardus oil	60.0^{ab}	4.16 ± 1.28^{a}	90.0^{b}	1.12 ± 0.33	78.0^{ab}	1.68 ± 1.18
E. citriodora oil	3.0^{d}	4.56 ± 1.56^{a}	30.0^{c}	0.96 ± 0.22	30.0^{d}	1.92 ± 1.11
O. basilicum oil	30.0^{c}	3.76 ± 0.96^{ab}	30.0^{c}	1.36 ± 0.54	42.0^{cd}	1.68 ± 0.95
S. aromaticum oil	54.0^{b}	3.68 ± 0.91^{ab}	96.0^{b}	1.36 ± 0.67	72.0^{ab}	1.52 ± 0.77
CV (%)	–	29.71	–	61.57	–	40.02

[1/] Means in each column against each mosquito species followed by the difference letters are significantly different ($P<0.05$, by one-way ANOVA and Duncan's Multiple Range Test).
[2/] Means between groups are not significantly different ($P>0.05$, by one-way ANOVA).

In the test by arm in cage method, the results of the protection time and the biting percentage of 0.21 mg/cm^2 of seven essential oils against the three mosquito species are shown in Table 2. There were significant differences in repellency among the repellents by mosquito species ($P<0.05$). Furthermore, there was a significant difference between the biting percentages of *Ae. aegypti* but there was no significant difference between the other 2 species (*An. dirus* and *Cx. quinquefasciatus*). The results indicated that *C. citratus* gave the highest repellency for the longest lasting period against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*: for 72 min with a 2.64% bite rate, 132 min with a 1.68% bite rate and 84 min with a 1.12% bite rate, respectively. Furthermore, *C. nardus* and *S. aromaticum* provided moderate protection time against *Aedes* mosquitoes, 60 min and 54 min, respectively. The relationship between protection time and biting percentage of the plant oils against the three mosquito species are shown in Figure 2.

4. Discussion

In our study, the seven essential oils show action as a topical repellent that is effective over a short time period. The high repellent activity of *C. citratus*, *C. nardus* and *S. aromaticum* oil against *Ae. aegypti* adults was lower than that of same plant oils when compared with other researches[18-20]. However, our result is not difference from some reports[10,21]; these essential oils exhibited the protection time against *Ae. aegypti* bites of nearly 1 hour but less than 2 hours. To evaluate the efficacy of repellent, Amer and Mehlhorn defined that if the protection time of a compound is long and the percentage of biting is low, the compound had good efficiency in repelling mosquitoes and deters biting[17]. If the protection time is short but the percentage of biting is low, then the compound is more a feeding deterrent than a repellent. Conversely, if the protection time is long but the biting rate is high, then the compound is more a repellent than a feeding deterrent. As a result, *C. citratus* exhibited a high protection time of 72 min against *Ae. aegypti*, while the biting rate decreased to 2.64%. This means that this repellent had attributes of a repellent and a feeding deterrent. Even though the protection time against *Ae. aegypti* of *C. nardus* and *S. aromaticum* were 60 min and 54 min, but the biting rate increased up to 4.16% and 3.68%, respectively. This shows that both essential oils are rather repellents than feed deterrents.

Many researchers improved repellency which provide a repellent that is effective over several hours with a base or fixative materials, such as vanillin, salicylic acid, and mustard and coconut oils[22-24]. However, the effectiveness and duration of repellency chemicals depend on multiple factors, including the type of repellents (active ingredients and formulation), the mode of application, environmental factors (temperature, humidity, and wind), the attractiveness of individual people to insects, loss due to removal by perspiration and abrasion, the sensitivity of the insects to repellents, and the biting density[25-32]. Moreover, differences in the mosquito species tested and body size, adult density in test cages, and mosquito age can affect test results[33].

We can conclude that the average protection time for seven essential oils against *Ae. aegypti* was noted the order of effectiveness as follows: *C. citratus* (72 min)> *C. nardus* (60 min)> *S. aromaticum* (54 min)> *O. basilisum* (30 min) > *C. odorata* (8.4 min) > *C. sinensis* and *E. citriodora* (3 min). However, the TISI standard determines the repellency time against *Ae. aegypti* mosquitoes should be >2 hours; none of the repellents met this requirement[16].

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Thavara U, Tawatsin A, Pengsakul T, Bhakdeenuan P, Chanama S, Anantapreecha S, et al. Outbreak of chikungunya fever in Thailand and virus detection in field population of vector mosquitoes, *Aedes aegypti* (L.) and *Aedes albopictus* Skuse (Diptera: Culicidae). *Southeast Asian J Trop Med Public Health* 2009; **40**: 951-962.

[2] Rosenberg R, Andre RG, Somchit L. Highly efficient dry season transmission of malaria in Thailand. *Trans R Soc Trop Med Hyg* 1990 **84**: 22-28.

[3] Nitatpattana N, Dubot-Peres A, Gouilh MA, Souris M, Barbazan P, Yoksan S, et al. Change in Japanese encephalitis virus distribution, Thailand. *Emerg Infect Dis* 2008; **14**: 1762-1765.

[4] Gupta RK, Rutledge LC. Role of repellents in vector control and disease prevention. *Am J Trop Med Hyg* 1994; **50**: 82-86.

[5] Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquito bites. *N Engl J Med* 2002; **347**: 13-18.

[6] Briassoulis G, Narlioglou M, Hatzis T. Toxic encephalopathy associated with use of DEET insect repellents: a case analysis of its toxicity in children. *Hum Exp Toxicol* 2001; **20**: 8-14.

[7] Abdel-Rahman A, Shetty AK, Abou-Donia MB. Subchronic dermal application of N, N-diethyl m-toluamide (DEET) and permethrin to adult rats, alone or in combination, causes diffuse neuronal cell death and cytoskeletal abnormalities in the cerebral cortex and the hippocampus, and Purkinje neuron loss in the cerebellum. *Exp Neurol* 2001; **172**: 153-171.

[8] Benner JP. Pesticidal compounds from higher plants. *Pestic Sci* 1993. **39**: 95-102.

[9] Rassami W, Soonwera M. Effect of herbal shampoo from long pepper fruit extract to control human head louse of the Ladkrabang childrens, Bangkok, Thailand. *J Agri Tech* 2011; **7**: 331-338.

[10] Phasomkusolsil S, Soonwera M. Insect repellent activity of medicinal plant oils against *Aedes aegypti* (Linn.), *Anopheles minimus* (Theobald) and *Culex quinquefasciatus* Say based on protection time and biting rate. *Southeast Asian J Trop Med Public Health* 2010a; **41**: 831-840.

[11] Phasomkusolsil S, Soonwera M. Potential larvicidal and pupacidal activities of herbal essential oils against *Culex quinquefasciatus* Say and *Anopheles minimus* (Theobald). *Southeast Asian J Trop Med Public Health* 2010b; **41**: 1342-1351.

[12] American Society for Testing and Materials (ASTM). Standard test methods for laboratory testing of non-commercial mosquito repellent formulations on the skin, ASTM-E951-94. Pennsylvania: ASTM International; 2006, p. 6.

[13] Klun JA, Debboun M. A new module for quantitative evaluation of repellent efficacy using human subjects. *J Med Entomol* 2000; **37**: 177-181.

[14] Weaving AJS, Sylvester NK. Pyrethrum as an insect repellent, part II: a laboratory technique for its evaluation as a mosquito repellent and the influence formulation on persistence. *Pyrethrum Post* 1967; **9**: 31-35.

[15] Barnard DR. Biological assay methods for mosquito repellents. *J Am Mosq Control Assoc* 2005; **21**: 12-16.

[16] Thai Industrial Standards Institute (TISI). Mosquito repellents. Bangkok, Thailand: Thai Industrial Standards Institute Ministry of Industry; 1986, p. 15.

[17] Amer A, Mehlhorn H. Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. *Parasitol Res* 2006; **99**: 478-490.

[18] Karunamoorthi K, Ilango K, Murugan K. Laboratory evaluation of traditionally used plant-based insect repellent against the malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae). *Parasitol Res* 2010; **106**: 1217-1223.

[19] Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwathnasorn C. Comparative repellency of 38 essential oils against mosquito bites. *Phytother Res* 2005; **19**: 303-309.

[20] Nuchuchua O, Sakulku U, Uawongyart N, Puttipipatkhachorn S, Soottitantawat A, Ruktanonchai U. *In vitro* characterization and mosquito (*Aedes aegypti*) repellent activity of essential-oils-loaded nanoemulsions. *AAPS PharmSciTech* 2009; **10**: 1234-1242.

[21] Aisien MSO, Imasuen AA, Wagbatsoma VA, Ayinde BA. Preliminary evaluation of the repellent activity of some plant essential oils against *Simulium damnosum* S.L., the vector of human onchocerciasis. *Int J Trop Insect Sci* 2004; **24**: 196-199.

[22] Stuart AE, Brooks CJ, Prescott RJ, Blackwell A. Repellent and antifeedant activity of salicylic acid and related compounds against the biting midge, *Culicoides impunctatus* (Diptera: Ceratopogonidae). *J Med Entomol* 2000; **37**: 222-227.

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

[24] Das NG, Baruah I, Talukdar PK, Das SC. Evaluation of botanicals as repellents against mosquitoes. *J Vector Borne Dis* 2003; **40**: 49-53.

[25] Rozendaal JA. Vector control: Methods for use by individuals and communities. Geneve: World Health Organization; 1997, p. 7-177.

[26] Govindarajan M, Sivakumar R, Amsath A, Niraimathi S. Mosquito larvicidal properties of *Ficus benghalensis* L. (Family: Moraceae) against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; **4**(7): 505-509.

[27] Hossain E, Rawani A, Chandra G, Mandal SC, Kumar JG. Larvicidal activity of *Dregea volubilis* and *Bombax malabaricum* leaf extracts against the filarial vector *Culex quinquefasciatus*. *Asian Pac J Trop Med* 2011; **4**(6): 436-441.

[28] Wabo PJ, Ngankam NJD, Bilong BCF, Mpoame M. A comparative study of the ovicidal and larvicidal activities of aqueous and ethanolic extracts of pawpaw seeds *Carica papaya* (Caricaceae) on *Heligmosomoides bakeri*. *Asian Pac J Trop Med* 2011; **4**(6): 447-450.

[29] Singha S, Chandra G. Mosquito larvicidal activity of some common spices and vegetable waste on *Culex quinquefasciatus* and *Anopheles stephensi*. *Asian Pac J Trop Med* 2011; **4**(4): 288-293.

[30] Ahmad N, Fazal H, Abbasi BH, Iqbal M. *In vitro* larvicidal potential against *Anopheles stephensi* and antioxidative enzyme activities of *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous*. *Asian Pac J Trop Med* 2011; **4**(3): 169-175.

[31] Govindarajan M. Evaluation of *Andrographis paniculata* Burm. f. (Family: Acanthaceae) extracts against *Culex quinquefasciatus* (Say.) and *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; **4**(3): 176-181.

[32] Barnard DR. Repellents and toxicants for personal protection. Geneve: World Health Organization; 2000, p. 23-27.

[33] Barnard DR, Posey KH, Smith D, Schreck CE. Mosquito density, biting rate and cage size effects on repellent tests. *Med Vet Entomol* 1998; **12**: 39-45.