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

Siriporn Phasomkusolsil and Mayura Soonwera

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

Abstract. This study investigated insect bite protection and length of the protection with 30 repellents which were divided into 3 categories: plant oil, essential oil and essential oil with ethyl alcohol, tested against three mosquito species, *Aedes aegypti*, *Anopheles minimus* and *Culex quinquefasciatus*, under laboratory conditions. The plant oil group was comprised of Phlai (*Zingiber cassumunar*) and Sweet basil (*Ocimum basilicum*). Both substances were effective as repellents and feeding deterrents against *An. minimus* (205 minutes protection time and a biting rate of 0.9%), *Cx. quinquefasciatus* (165 minutes protection time and 0.9% biting rate) and *Ae. aegypti* (90 minutes protection time and 0.8% biting rate). Essential oil from citronella grass (*Cymbopogon nardus*) exhibited protection against biting from all 3 mosquito species: for *An. minimus, Cx. quinquefasciatus* and *Ae. aegypti*, the results were 130 minutes and 0.9%, 140 minutes and 0.8%, and 115 minutes and 0.8%, respectively. The period of protection time against *Ae. aegypti* for all repellent candidates tested was lower than the Thai Industrial Standards Institute (TISI) determined time of greater than 2 hours.

Key words: medicinal plant oil, repellent activity, *Aedes aegypti*, *Anopheles minimus*, *Culex quinquefasciatus*

INTRODUCTION

DEET, a synthetic mosquito repellent, is recognized as one of the few products effective against mosquitoes and biting flies. The efficacy of DEET in providing long-lasting protection against a wide variety of mosquito species has been documented in several studies (Fradin and Day, 2002; Roberts and Reigart, 2004). 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 (Briassoulis *et al*, 2001). It is irritating to mucous membranes, and

Correspondence: Siriporn Phasomkusolsil, Entomology and Environment Program, Plant Production Technology Section, 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

concentrated formulations dissolve plastic. DEET may be unsafe for children possibly causing encephalopathy (Abdel-Rahman *et al*, 2001). Research regarding insect repellents derived from plant extracts is needed to find alternatives that are safer but still effective. Some plant species contain insecticidal or repellent substances. Some plant extracts, such as neem (*Azadirachta indica*), sweet basil (*O. basilicum*), and lemon eucalyptus (*Corymbia citriodora*) have been studied as possible mosquito repellents and have demonstrated good efficacy against some mosquito species (Kirton, 2005; Sharma *et al*, 1993). Tawatsin *et al* (2001) reported the volatile oils extracted from turmeric (*Curcuma longa*), kaffir lime (*Citrus hystrix*), citronella grass (*Cy. winterianus*) and hairy basil (*O. americanum*) show strong repellency against three mosquito vectors (*Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus*) when applied to human skin.

However, the repellent effects of natural oils do not usually last as long as DEET which can protect from mosquito bites for up to 6 hours (Frances, 1987; Debboun *et al*, 2000; Barnard and Xue, 2004). Trongtokit *et al* (2005) found important factors regarding the effective time of repellency depended on concentrations, experimental design and mosquito species after they assessed repellent activity of 38 Thai essential oils. Prolonged protection time is the critical factor for mosquito repellent. Thailand has many plant oils with repellency against mosquito bites, but they do not give protection which lasts as long DEET. The aim of this study was to screen the repellency of various plant oils, essential oils and their combinations against *Ae. aegypti*, *An. minimus* and *Cx. quinquefasciatus* mosquitoes using a screen cage method to estimate protection time and biting rate under laboratory conditions.

MATERIALS AND METHODS

Mosquitoes

Ae. aegypti, *An. minimus* and *Cx. quinquefasciatus* lab bred mosquitoes were used in this study. They were maintained in the laboratory of the Entomology and Environment Program, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut Institute of Technology Lad Krabang (KMITL), Bangkok. Mosquitoes were reared at 26-28ºC at 70-80% relative humidity; adults were fed with 10% sucrose. Prior to testing, 5- to 7-day-old female mosquitoes were starved by providing them with only water for 12 hours.

Plant materials

Thirty types of repellent were used in this study as shown in Table 1. These oils were provided by the medicinal plant laboratory of KMITL. The repellents were formulated into 3 groups: plant oils, essential oils and essential oils in ethyl alcohol. All formulations were kept at room temperature before testing.

Laboratory test procedure

The oils were screened for repellency against 3 species of mosquitoes under laboratory conditions using a screened cage test method (Barnard, 2005) following TISI guidelines (TISI, 1986). Test times was 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 *An. minimus* and *Cx. quinquefasciatus* 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 x 10 cm on the forearm. One hundred µl of test material was applied to the treatment area and

Table 1
List of repellents tested in this study.

No	Name of material	Formulation
P1	Phlai *(Zingiber cassumunar)*	Plant oil
P2	Phlai *(Zingiber cassumunar)* + *Curcuma aromatica*	Plant oil
P3	Turmeric (*Curcuma longa*)	Plant oil
P4	Phlai *(Zingiber cassumunar)* + Turmeric (*Curcuma longa*)	Plant oil
P5	Phlai *(Zingiber cassumunar)* + Eucalyptus (*Eucalyptus citriodora*)	Plant oil
P6	Turmeric (*Curcuma longa*) + Eucalyptus (*Eucalyptus citriodora*)	Plant oil
P7	Phlai *(Zingiber cassumunar)* + †Clove (#1)(*Syzygium aromaticum*)	Plant oil
P8	Phlai *(Zingiber cassumunar)* + †Clove (#2)(*Syzygium aromaticum*)	Plant oil
P9	Phlai *(Zingiber cassumunar)* + Lavender (*Lavendula angustifolia*)	Plant oil
P10	Phla*i (Zingiber cassumunar)* + Sweet basil (*Ocimum basilicum*)	Plant oil
P11	Turmeric (*Curcuma longa*) + Peppermint (*Mentha piperita*)	Plant oil
P12	Phlai *(Zingiber cassumunar)* + Ginger (*Zingiber officinale*)	Plant oil
P13	Turmeric (*Curcuma longa*) + Ylang-ylang tree (*Cananga odorata*)	Plant oil
P14	Lemon Grass (*Cymbopogon citratus*) + Turmeric (*Curcuma longa*)	Plant oil
P15	Mah – Khwuaen (*Zanthoxylum limonella*)	Plant oil
E1	Citronella grass (#1) (*Cymbopogon nardus*)	Essential oil
E2	Citronella grass (#2) (*Cymbopogon nardus*)	Essential oil
E3	Orange oil (*Citrus sinensis*)	Essential oil
E4	Eucalyptus (#1) (*Eucalyptus citriodora*)	Essential oil
E5	Eucalyptus (#2) (*Eucalyptus citriodora*)	Essential oil
E6	Clove (*Syzygium aromaticum*)	Essential oil
E7	Orange oil (*Citrus sinensis*) + Eucalyptus (*Eucalyptus citriodora*)	Essential oil
E8	Clove (#2) (*Syzygium aromaticum*)	Essential oil
E9	Orange oil (*Citrus sinensis*) + Eucalyptus (*Eucalyptus citriodora*) + Citronella grass (*Cymbopogon nardus*)	Essential oil
EE1	Orange oil (*Citrus sinensis*) + Clove (*Syzygium aromaticum*)	Essential oil with ethyl alcohol
EE2	Clove (*Syzygium aromaticum*)	Essential oil with ethyl alcohol
EE3	Orange oil (*Citrus sinensis*)	Essential oil with ethyl alcohol
EE4	Orange oil (*Citrus sinensis*) + Clove (*Syzygium aromaticum*)	Essential oil with ethyl alcohol
EE5	Eucalyptus (*Eucalyptus citriodora*) + Clove (*Syzygium aromaticum*)	Essential oil with ethyl alcohol
EE6	Orange oil (*Citrus sinensis*) + Clove (*Syzygium aromaticum*)	Essential oil with ethyl alcohol

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, otherwise, the test was not stopped. The mosquito cage (30 x 30 x 30 cm) contained 250 nulliparous, 5-7 day old female mosquitoes; the test was conducted for 3 minutes. The total number of mosquitoes biting or landing on the treatment and control areas was recorded. If no mosquitoes bit or landed during the 3 minute study period, the arm was withdrawn from the cage and we waited 30 minutes before attempting to conduct the test again. This was continued until at least two mosquito bites/landings occurred during the 3-minute study period.

The study period was carried out every 30 minutes until fewer than 2 mosquitoes land or bit during the 3 minute study period, at which time the study was stopped. The protection time was the time from repellent application until the study was stopped.

For comparison, a percentage of mosquito bitings/landings was calculated for each test (Amer and Mehlhorn, 2006) using the following formula:

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

Where *B* is the total number of bitings/landings by the end of the test. The test was carried out 3 times per sample.

Statistical analysis

The mean protection time was used to compare the 30 tested repellents. Differences in significance were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple comparisons by SPSS for Windows (version 16.0).

RESULTS

The results for plant oil repellency against mosquitoes are summarized in Table 2. There were significant differences in repellency among the repellents by mosquito species ($p<0.05$). The TISI standard determines the repellency time against *Ae. aegypti* mosquitoes should be >2 hours; none of the repellents met this requirement. Some repellents provided nearly 2 hours protection against *Aedes* mosquitoes while providing protection against *Anopheles* and *Culex* mosquito of >2 hours. The repellent P13 [Turmeric (*C. longa*) + Ylang-ylang tree (*Ca. odorata*)] had the best efficiency against *Ae. aegypti, An. minimus* and *Cx. quinquefasciatus*, in which the protection times were 100±34.6, 125.0±8.7 and 100±17.3 minutes, respectively. The protection times of P9 [Phlai (*Z. cassumunar*) + Lavender (*L. angustifolia*)] against *Ae. aegypti, An. minimus* and *Cx. quinquefasciatus* were 90±30.0, 145.0±8.7 and 125.0±8.7 minutes, respectively, and P10 [Phlai (*Z. cassumunar*) + Sweet basil (*O. basilicum*)] were 90±30.0, 205.0±8.7 and 165.0±0.0 minutes, respectively. Repellent P3: [Turmeric (*C. longa*)] and P12: [Phlai (*Z. cassumunar*) + Ginger (*Z. officinale*)] showed no repellency against *Ae. aegypti* (0 minute). The repellencies of the plant oils against the three mosquito species are shown in Fig 1.

The biting/landing percentages for all the tested plant oils are shown in Table 2. There were significant differences in protection against *Ae. aegypti* but not for *An. minimus* or *Cx. quinquefasciatus*. For *Ae. aegypti* the highest biting percentage was 2.3% seen with P4 [Phlai (*Z. cassumunar*) + Turmeric (*C. longa*)] and the lowest was 0.8%, seen with four plant oils: P5 [Phlai (*Z. cassumunar*) + Eucalyptus (*Eucalyptus citriodora*)], P10 [Phlai (*Z. cassumunar*) + Sweet basil (*O. basilicum*)], P11 [Turmeric (*C. longa*) + Peppermint (*Mentha piperita*)] and P14 [Lemon Grass (*Cy. citratus*) + Turmeric (*C. longa*)]. For *An. minimus* the biting percentage was 0.8% with five oils, 0.9% with seven oils and 1.2% with three oils among the fifteen plant oils tested. For *Cx. quinquefasciatus* the biting percentages ranged from 0.8% to 1.2%.

Table 3 shows the repellency for the 9 essential oils against the three mosquito species. There were significant differences in repellency among the mosquito species ($p<0.05$). The repellency of the 9 essential oils against *Ae. aegypti* was 0-115 minutes, against *An. minimus* was 30-135 minutes and against Cx. *quinquefasciatus* was 30-155 minutes (Fig 2). There was a significant difference between biting/landing of

Table 2
Protection time for each repellent in the plant oil group against three mosquito species (*Ae. aegypti, An. minimus, Cx. quinquefasciatus*) and biting percentages.

	Repellents	*Ae. aegypti*		*An. minimus*		*Cx. quinquefasciatus*	
		Mean±SD[1] (min)	%Biting[1]	Mean±SD[1] (min)	%Biting[2]	Mean±SD[1] (min)	%Biting[2]
P1	Phlai (*Z. cassumunar*)	70.0 ± 17.3^{cde}	2.1^{de}	120.0 ± 0.0^{cd}	0.80	90.0 ± 0.0^{cd}	1.20
P2	Phlai (*Z. cassumunar*) + *C. aromatica*	50.0 ± 17.3^{bc}	1.7^{e}	80.0 ± 17.3^{b}	1.20	70.0 ± 17.3^{bc}	0.90
P3	Turmeric (*C. longa*)	0	1.9^{ab}	60.0 ± 0.0^{ab}	1.20	145.0 ± 8.7^{fg}	0.80
P4	Phlai (*Z. cassumunar*) + Turmeric (*C. longa*)	30.0 ± 0.0^{ab}	2.3^{abcd}	110.0 ± 17.3^{c}	0.90	120.0 ± 0.0^{ef}	0.80
P5	Phlai (*Z. cassumunar*) + Eucalyptus (*Eucalyptus citriodora*)	50.0 + 17.3^{bc}	0.8^{a}	110.0 ± 17.3^{c}	0.80	100.0 ± 17.3^{dc}	1.10
P6	Turmeric (*C. longa*) + Eucalyptus (*Eucalyptus citriodora*)	40.0 ± 17.3^{bc}	1.2^{cde}	140.0 ± 8.7^{d}	0.80	50.0 ± 34.6^{ab}	0.90
P7	Phlai (*Z. cassumunar*) + Clove (#1) (*Syzygium aromaticum*)	60.0 ± 0.0^{bcd}	1.2^{abc}	120.0 ± 0.0^{cd}	1.20	100.0 ± 17.3^{dc}	1.10
P8	Phlai (*Z. cassumunar*) + Clove (#2) (*Syzygium aromaticum*)	60.0 ±0.0^{bcd}	1.6^{a}	70.0 ± 17.3^{b}	0.90	90.0 ± 0.0^{cd}	0.90
P9	Phlai (*Z. cassumunar*) + Lavender (*L. angustifolia*)	90.0 ± 30.0^{de}	1.2^{a}	145.0 ± 8.7^{d}	0.80	125.0 ± 8.7^{ef}	1.20
P10	Phlai (*Z. cassumunar*) + Sweet basil (*O. basilicum*)	90.0 ± 30.0^{de}	0.8^{bcde}	205.0 ± 8.7^{e}	0.90	165.0 ± 0.0^{g}	0.90
P11	Turmeric (*C. longa*) + Peppermint (*Mentha piperita*)	70.0 ± 17.3^{cde}	0.8^{cde}	130.0 ± 8.7^{cd}	0.90	90.0 ± 0.0^{cd}	1.20
P12	*Phlai (Z. cassumunar*) + Ginger (*Z. officinale*)	0	1.5^{abc}	80.0 ± 17.3^{b}	0.80	30.0 ± 0.0^{a}	0.90
P13	Turmeric (*C. longa*) + Ylang-ylang tree (*Ca. odorata*)	100.0 ± 34.6^{e}	0.9^{abc}	125.0 ± 8.7^{cd}	0.90	100.0 ± 17.3^{dc}	0.80
P14	Lemon Grass (*Cy. citratus*) + Turmeric (*C. longa*)	30.0 ± 0.0^{ab}	0.8^{a}	120.0 ± 30.0^{cd}	0.90	125.0 ± 8.7^{ef}	0.80
P15	Mah – Khwuaen (*Zanthoxylum limonella*)	30.0 ± 0.0^{ab}	0.9^{ab}	40.0 ± 17.3^{a}	0.90	50.0 ± 17.3^{ab}	0.80

[1]Means in each column against each mosquito species followed by the difference letter 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).

Ae. aegypti and the other 2 species (*An. minimus* and *Cx. quinquefasciatus*), but there was no significant difference between these 2 species. The essential oil E2 [Citronella grass (*Cy. nardus*)] exhibited good efficiency for adequate times against *An. minimus, Cx. quinquefasciatus* and *Ae. aegypti*: for 130 minutes with a 0.9% bite rate, 140 minutes with a 0.8% bite rate, and 115 minutes with a 0.8% bite rate.

The results of repellency of essential oils with ethyl alcohol are given in Table 4. None of the essential oils with alcohol had repellency for at least 2 hours against

Table 3
Repellency of essential oils against *Ae. aegypti*, *An. minimus* and *Cx. quinquefasciatus*.

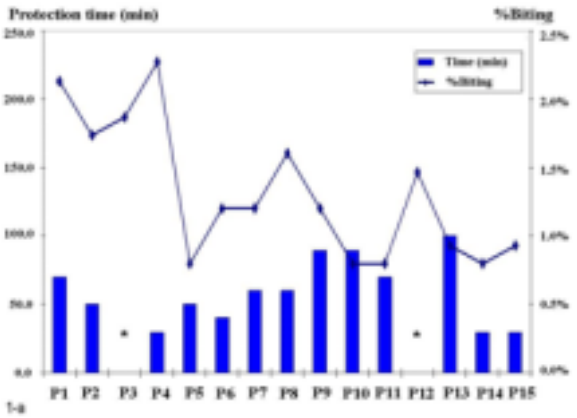
	Repellents	*Ae. aegypti*		*An. minimus*		*Cx. quinquefasciatus*	
		Mean±SD[1] (min)	%Biting[1]	Mean±SD[1] (min)	%Biting[2]	Mean±SD[1] (min)	%Biting[2]
E1	Citronella grass (#1) (*Cy. nardus*)	50.0 ± 17.3^{ab}	0.8^{a}	130.0 ± 8.7^{c}	0.90	100.0 ± 17.3^{c}	1.10
E2	Citronella grass (#2) (*Cy. nardus*)	115.0 ± 22.9^{d}	0.8^{a}	130.0 ± 17.3^{c}	0.90	140.0 ± 22.9^{d}	0.80
E3	Orange oil (*Citrus sinensis*)	30.0 ± 0.0^{a}	0.8^{a}	50.0 ± 17.3^{ab}	0.90	60.0 ± 0.0^{ab}	0.80
E4	Eucalyptus (#1) (*Eucalyptus citriodora*)	0	1.2^{a}	30.0 ± 0.0^{a}	0.80	30.0 ± 0.0^{a}	0.80
E5	Eucalyptus (#2) (*Eucalyptus citriodora*)	30.0 ± 0.0^{a}	2.0^{b}	60.0 ± 0.0^{b}	0.80	80.0 ± 17.3^{bc}	0.80
E6	Clove (*Syzygium aromaticum*)	80.0 ± 17.3^{c}	0.8^{a}	120.0 ± 26.0^{c}	0.90	90.0 ± 0.0^{bc}	0.80
E7	Orange oil (*Citrus sinensis*) + Eucalyptus (*Eucalyptus citriodora*)	40.0 ± 17.3^{a}	0.8^{a}	30.0 ± 0.0^{a}	0.80	40.0 ± 17.3^{a}	1.20
E8	Clove (#2) (*Syzygium aromaticum*)	70.0 ± 17.3^{bc}	1.1^{a}	60.0 ± 0.0^{b}	1.30	130.0 ± 17.3^{d}	0.80
E9	Orange oil (*Citrus sinensis*) + Eucalyptus (*Eucalyptus citriodora*) + Citronella grass (*Cy. nardus*)	30.0 ± 0.0^{a}	1.1^{a}	135.0 ± 0.0^{c}	0.80	155.0 ± 31.2^{d}	0.80

[1]Means in each column against each mosquito species followed by the difference letter are significantly different ($p<0.05$, by one-way ANOVA and Duncan's multiple tange yest).
[2]Means between groups are not significantly different ($p>0.05$, by one-way ANOVA).

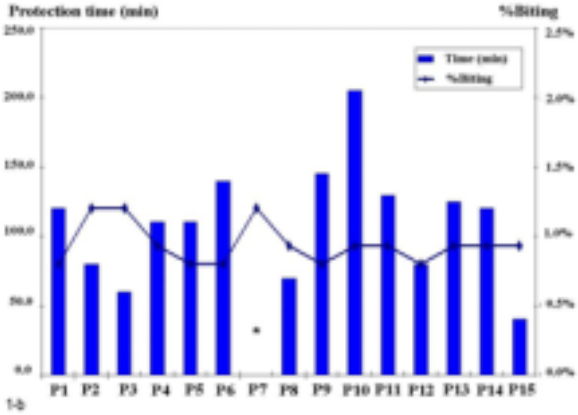
Table 4
Repellency of essential oils with ethyl alcohol against *Ae. Aegypti*, *An. minimus* and *Cx. quinquefasciatus*.

	Repellents	*Ae. aegypti*		*An. minimus*		*Cx. quinquefasciatus*	
		Mean±SD[1] (min)	%Biting[1]	Mean±SD[1] (min)	%Biting[2]	Mean±SD[1] (min)	%Biting[2]
EE1	Orange oil (*Citrus sinensis*) + Clove (*Syzygium aromaticum*)	40.0 ± 17.3^{a}	0.8^{a}	80.0 ± 17.3^{b}	0.80	90.0 ± 0.0^{b}	1.90
EE2	Clove (*Syzygium aromaticum*)	70.0 ± 17.3^{b}	0.8^{a}	120.0 ± 0.0^{c}	0.80	90.0 ± 0.0^{b}	0.90
EE3	Orange oil (*Citrus sinensis*)	0	0.8^{a}	0	0.80	0	0.80
EE4	Orange oil (*Citrus sinensis*) + Clove (*Syzygium aromaticum*)	0	0.8^{a}	30.0 ± 0.0^{a}	0.80	50.0 ± 17.3^{a}	0.80
EE5	Eucalyptus (*Eucalyptus citriodora*) + Clove (*Syzygium aromaticum*)	0	1.3^{b}	120.0 ± 0.0^{c}	0.90	50.0 ± 17.3^{a}	1.30
EE6	Orange oil (*Citrus sinensis*) + Clove (*Syzygium aromaticum*)	0	0.8^{a}	130.0 ± 8.7^{c}	0.90	70.0 ± 17.3^{ab}	1.20

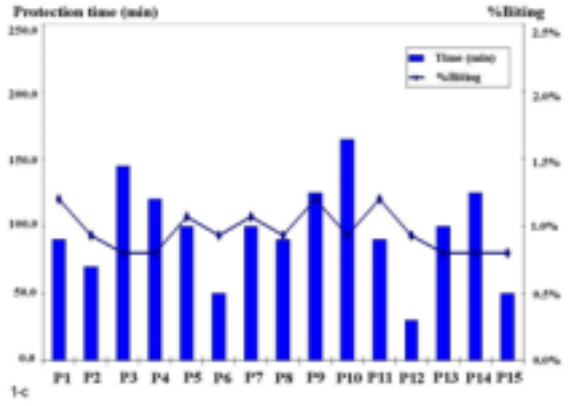
[1]Means in each column against each mosquito species followed by the difference letter are significantly different ($p<0.05$, by one-way ANOVA and Duncan's multiple range test).
[2]Means between groups are not significantly different ($p>0.05$, by one-way ANOVA).



a. *Ae. aegypti*



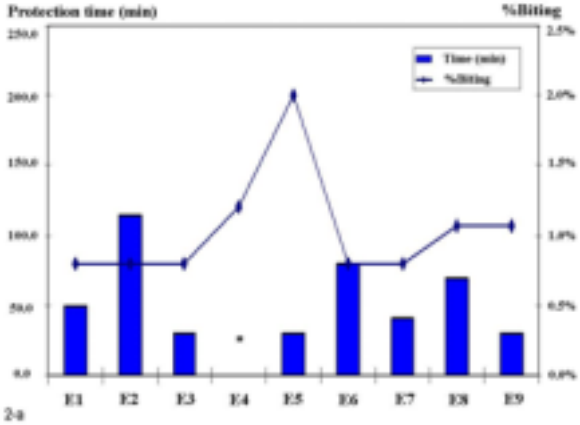
b. *An.minimus*



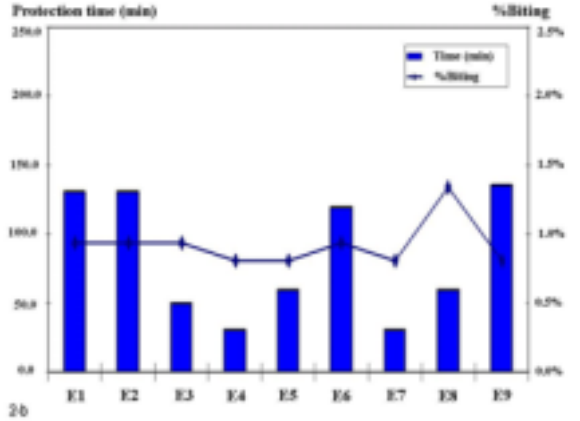
c. *Cx. quinquefasciatus*

*There were two mosquito bites after inserting the treated arm in cage at 0 minute.

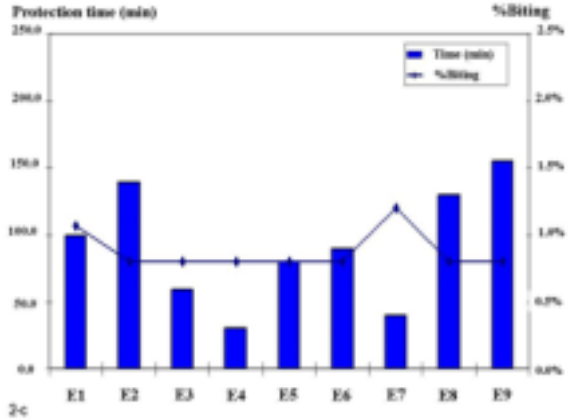
Fig 1–Comparison of protection times (minutes) and biting percentages for each repellent plant oil group against three mosquito species.



a. *Ae. aegypti*



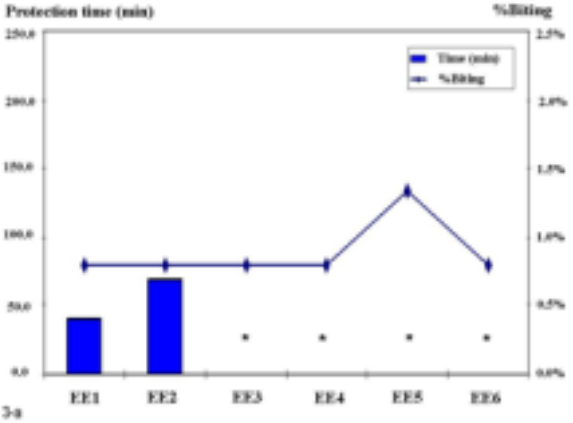
b. *An.minimus*



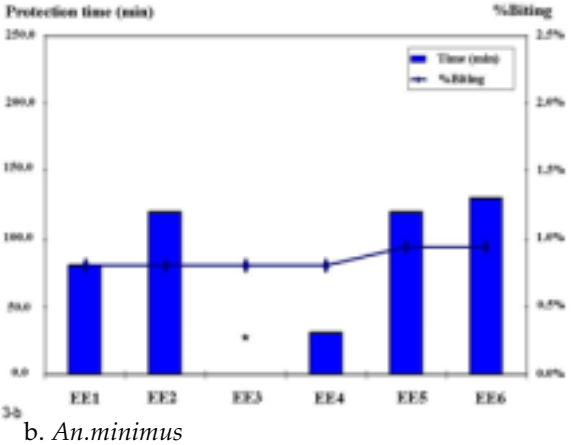
c. *Cx. quinquefasciatus*

*There were two mosquitoe bites after inserting the treated arm in cage at 0 minute.

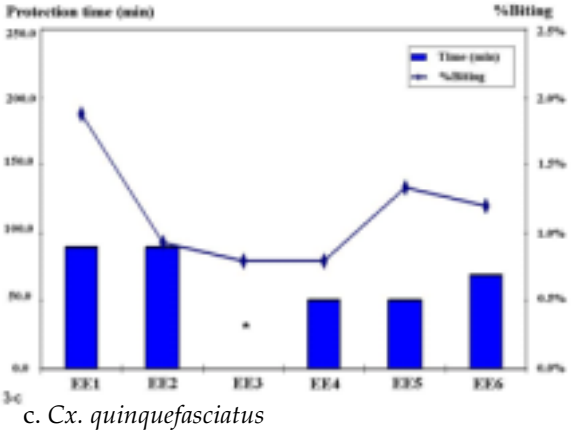
Fig 2–Comparison of protection times (minutes) and biting percentages for each repellent essential oil group against three mosquito species.



a. *Ae. aegypti*



b. *An.minimus*



c. *Cx. quinquefasciatus*

*There were two mosquito bites after inserting the treated arm in cage at 0 minute.

Fig 3–Comparison of protection times (minutes) and biting percentages for each repellent essential oil with ethyl alcohol group against three mosquito species.

Ae. aegypti or *Cx. quinquefasciatus*. *An. minimus* was repelled by EE2 [Clove (*S. aromaticum*)], EE5 [Eucalyptus (*E. citriodora*) + Clove (*S. aromaticum*)] and EE6 [Orange oil (*Ci. sinensis*) + Clove (*S. aromaticum*)] for 120, 120 and 130 minutes, respectively (Fig 3).

DISCUSSION

The aim of this study was to investigate repellency based on protection time in terms of the percentage of biting/landing. Even though the biting percentages in this experiment were not high (0.8-2.3%) due to the limited duration of the test (3 minutes exposure), it can be determined these repellents have efficiency against mosquito biting/landing (Amer and Mehlhorn, 2006). Some researchers have defined a repellent as a chemical that causes insects to orientate away from the source (Miller *et al*, 2009), and a deterrent as a chemical that inhibits feeding or oviposition (Bentley and Day, 1989).

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. 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. P13 [Turmeric (*C. longa*) + Ylang-ylang tree (*Ca. odorata*)] exhibited high repellent efficiency and inhibited feeding by *Ae. aegypti*, as evidenced by the protection time of 100 minutes and the biting rate of 0.9%. P10 [Phlai (*Z. cassumunar*) + Sweet basil (*O. basilicum*)] exhibited a protection time of 90 minutes against *Ae. aegypti* with a biting rate of 0.8%. Both these repellents had attributes of a repellent and a feeding

deterrent. E2 [Citronella grass (*Cy. nardus*)] from the essential oil group acted as both a repellent and a feeding deterrent against all 3 species of mosquitoes. P9 [Phlai (*Z. cassumunar*) + Lavender (*L. angustifolia*)] exhibited only the efficacy of a repellent but not a feeding deterrent. There are more examples in Tables 2-4.

The responses of the three mosquito species to the oil preparations were different. *An. minimus* and *Cx. quinquefasciatus* were sensitive to many oils in each group, while *Ae. aegypti* was tolerant to many oils. None of the oils from any of the 3 tested groups provided protection for more than 2 hours. The short protective duration of oils, such as P13 [Turmeric (*C. longa*) + Ylang-ylang tree (*Ca. odorata*)], P10 [Phlai (*Z. cassumunar*) + Sweet basil (*O. basilicum*)] and E2 [Citronella grass (*Cy. nardus*)] may be improved by developing a formulation that would prolong the retention time of the aromatic constituents of the oil on the skin. Many researchers have demonstrated improved repellency of plant-derived topical repellent products after formulating with a base or fixative materials, such as vanillin, salicylic acid, and mustard and coconut oils (Stuart *et al*, 2000; Tawatsin *et al*, 2001; Das *et al*, 2003).

The present study evaluated various repellents under laboratory conditions. There are many factors that affect the efficacy of repellent against mosquitoes, such as species and density of mosquito (Barnard *et al*, 1998), age of person, sex and biochemical attractiveness to biting mosquitoes (Golenda *et al*, 1999), ambient temperature, humidity, and wind speed (Service, 1980).

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