
Mosquito repellent from Thai essential oils against dengue fever mosquito (*Aedes aegypti* L.) and filaria mosquito vector (*Culex quinquefasciatus* Say)

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Repellent activity of Thai essential oils derived from ylang-ylang (*Canaga odorata* Lamk), lemon grass (*Cymbopogon citratus* D.C., Stapf.) and citronella grass (*Cymbopogon nardus* L.) Rendle) were evaluated against female of *Aedes aegypti* L. and *Culex quinquefasciatus* Say and compared them with chemical repellent (IR3535, ethyl butylacetyl amino propionate 12.5% w/w; Johnson's Baby Clear Lotion Anti-Mosquito®). Each test repellent was applied at 0.17 and 0.33 µl/cm² on the fore arms of volunteers. All essential oil repellents at 0.17 µl/cm² showed the lower protection time and percentage of protection against two mosquito species than at 0.33 µl/cm². All essential oil repellents exhibited higher repellent activity than chemical repellent. The essential oil of *C. citratus* at 0.33 µl/cm² exhibited excellent repellent activity with 98.67% protection from bites of *Ae. Aegypti* till 116.67±55.75 min. and 99.75% protection from bites of *Culex quinquefasciatus* till 128.33±12.89 min. However, repellent activity indicated the order of protection time and percentage of protection against two mosquito species in three essential oils as *C. citratus* oil > *C. odorata* oil > *C. nardus* oil. Data showed that *C. citratus* oil is proved to be green repellent or friendly repellent in mosquito control, safe for human and friendly for environment.

Key words: Repellent, *Aedes aegypti*, *Culex quinquefasciatus*, Thai essential oil.

Introduction

Mosquitoes are a serious insect to public health, which transmit several dangerous diseases such as dengue, filariasis, malaria, yellow fever and Japanese encephalitis. Every year at least 500 million people in the world suffer from one or the other tropical diseases that include dengue, malaria and

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filariasis (Madhumathy *et al.*, 2007; Kumar *et al.*, 2012). However, dengue worldwide disease transmitted by *Aedes aegypti*, approximately 2.5 billion people from 100 countries live in areas infested with these mosquito vectors

(Borah *et al.*, 2010). Reported cases of dengue and dengue hemorrhagic fever have experienced an exponential increase over the last 30 years with the number of cases reported to the WHO between 2000 and 2007 over doubling those in the previous decade; Southeast Asian and Western Pacific countries bear the brunt of global disease burden due to dengue (WHO, 2009a). While, *Culex quinquefasciatus* is an important vector of filariasis in tropical and sub-tropical regions, about 90 million people worldwide are suffered with these diseases.

Thus, mosquito control and personal protection from mosquito bites are currently the most important measures to control mosquito transmitted diseases. However, repellents base on chemical insecticides are considered to be a useful of reducing and preventing the mosquito vectors. On the other hand, chemical repellents are not safe for human, especially children because they may cause skin irritation, hot sensation rashes or allergy (Das *et al.*, 2003). In recent years, there was an increase of public concern on the safety of many chemical products that instigated a renewed interest on the use of natural products from plant origin for mosquito vector management. In addition, plant essential oils in general have been recognized as an important natural resource of insecticides and insect repellents various essential oils have also been documented to exhibit acute toxic effects against insects, including mosquitoes (Pavela, 2008). The repellents base on plant essential oils are effective for mosquito control, environment-friendly, easily biodegradable, and readily available in many areas of the world, no ill effect on non-target organisms (Govindarajan, 2011).

Many researchers have reported the repellent activity of plant essential oils against female of mosquito vectors. Essential oils from *Cymbopogon citratus*, *Cinnamomum zeylanicum*, *Mentha piperita*, *Rosmarinus officinalis* and *Zingiber officinalis* showed repellent activity against *Ae. aegypti* and *Cx. quinquefasciatus* (Govindarajan, 2011; Kumar *et al.*, 2011; Khandagle *et al.*, 2011). Furthermore, the US Environmental Protection Agency (USEPA) has registered citronella, lemon and eucalyptus oil as insect repellent ingredients for application on the skin, these natural products are been frequently used due to their relative low toxicity, comparable efficacy, and customer approval (Katz *et al.*, 2008).

Therefore, the objective of this study investigated the repellency of Thai essential oils derived from *Cymbopogon citratus*, *Cymbopogon nardus* and

Cananga odorata against female of *Ae. aegypti* and *Cx. quinquefasciatus* and to compare them with chemical repellent (IR3535, ethyl butylacetylaminopropionate 12.5% w/w; Johnson's Baby Clear Lotion Anti-Mosquito®).

Materials and methods

Tested mosquitoes

Eggs of *Ae. aegypti* and *Cx. quinquefasciatus* were obtained from the Armed Forces Research Institute of Medical Sciences (AFRIMS), Thailand. All of mosquito was reared in Entomology Laboratory, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL). Adults of two mosquito species were fed on 5% glucose + 2% multivitamins and maintained at $30.50 \pm 1.20^\circ\text{C}$, $70.50 \pm 2.50\%$ RH and 12h : 12 H (light : dark) photoperiod. Nulliparous female of 4-5 days-old were used for repellency test.

Plant materials and Herbal essential oils

Various parts of three species from Thai Plants (flowers of *Cananga odorata*, Stem of *Cymbopogon citratus* and stem of *Cymbopogon nardus*) were collected from Nakhon Ratchasima province, Thailand. All of plant was identified, by plant taxonomist of Plant Production Technology Section, Faculty of Agricultural Technology, KMITL. The various plant parts were extracted for essential oils by water distillation. One kilogram of fresh and finely ground material from each plant was placed in an extraction column connected to a round-bottomed distillation flask containing distilled water. The flask was heated to approximately 100°C and allowed to boil until distillation was completed. The liquid formed, together with the distillate oils, were collected in a separating funnel. The mixture was then allowed to settle for 1 day, after which the water (lower) layer was slowly drawn off until only the oil remained. These essential oils were prepared at 0.17 and 0.33 $\mu\text{l}/\text{cm}^2$ in ethyl alcohol. All formulations were kept at room temperature before testing.

Chemical repellent

IR3535 (12.5%w/w Ethyl butylacetylaminopropionate; Johnson's Baby Clear Lotion Anti-Mosquito®), a common chemical repellent for children in Thailand, was purchased from IDS Manufacturing Co. Ltd. 21/7 Kukot, Lumlukka, Pathumthani province, Thailand.

Repellent bioassay

The three essential oils were tested against *Ae. aegypti* and *Cx. quinquefasciatus* females under laboratory conditions using the arm in cage method (Barnard, 2005) following WHO (2009b). Six human volunteers were recruited from the healthy students and lecturers of Entomology and

Environment Laboratory, Plant Production Technology Section, Faculty of Agricultural Technology, KMITL. The volunteers for the repellency test had no history of dermatological disease or allergic reaction to mosquito bites or repellents. All volunteers signed an informed consent form after having received a full explanation of the test objectives. The research proposal was approved by the research committee of Faculty of Agricultural Technology, KMITL. The timing of the tests depended on the mosquitoes, for *Ae. aegypti* was tested during the day time from 0800 am to 0400 pm, while *Cx. quinquefasciatus* was tested during night time from 0400 pm to 1200 pm. Before testing the volunteer's arms were washed and cleaned thoroughly with distilled water and used the left arm for treatment and the right arm for control. Both arms of volunteers were covered with rubber sleeve with a window area of 3x10 cm on the ventral part of forearm. One hundred µl of each repellent was applied to the treatment area of left forearm of each volunteer and allowed to dry on the skin for 1 minute. After applying the test repellent, the volunteer was instructed not to rub, touch or wet the treated area. The right arm acting as a control was exposed for up to 30 seconds to mosquito cage (30x30x30 cm) containing 250 nulliparous female mosquitoes (4-5 days old). If at least two mosquitoes landed on or bit the control arm, the repellency test was then continued. The test continued until at least two bites occurred in a three-minute period. However, if no mosquitoes bit during a three-minute period, the arm was withdrawn from the mosquito cage. The protection time or repellency test period was carried out every 15 minutes until at least two mosquitoes bit during the three-minute period and then the repellency test was stopped. The time between applications of the repellents and the two first mosquitoes bite was recorded as the protection time. For percentage of protection was calculated for each repellent using the following formula (Manimaran *et al.*, 2013).

Protection (%) = 100 - number of mosquitoes biting or landing/ number of mosquitoes released X 100.

Statistical analysis

The median protection time was used as a standard measure of repellency of essential oil repellents and IR3535 (chemical repellent) against *Ae. aegypti* and *Cx. quinquefasciatus*. Differences in significance were analyzed by one-way analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DMRT) comparisons by SPSS for windows (version 6.0).

Results

Table 1 and Figure 1 showed the protection time in minute and percentage of protection of herbal essential oil repellents at 0.17 $\mu\text{l}/\text{cm}^2$ and IR3535 (chemical repellent) against *Ae. aegypti* and *Cx. quinquefasciatus* in the laboratory. There were differences in repellency among the herbal essential oil repellents and IR3535 by mosquito species. The essential oils of *C. citratus* and *C. odorata* gave the highest repellency for the longest lasting period and percentage protection against *Ae. aegypti* and *Cx. quinquefasciatus* for 60 to 90 min with 98.80 to 98.67% protection. All herbal essential oils provided 96.92 to 98.80% protection from *Ae. aegypti* for 53 to 60 min of protection time, and also provided 98.94 to 99.20% protection from *Cx. quinquefasciatus*. On the other hand, the chemical repellent showed 75.73 to 77.54% protection from both mosquito species for 3 min. For the results of 0.33 $\mu\text{l}/\text{cm}^2$ of herbal essential oil repellents and IR3535 were tested against two mosquito species as shown in Table 2 and Figure 2. *C. citratus* oil gave the highest repellency with 98.67% protection from bites of *Ae. aegypti* up to a mean time of 116 min and with 99.75% protection from bites of *Cx. quinquefasciatus* up to a mean time of 128 min. IR3535 gave protection for only 3 min and 78.80 to 77.30% protection from bites of two mosquito species. However, all herbal essential oil repellents provided higher protection time and percentage of protection against two mosquito species than IR3535. All herbal essential oil repellent provided lower repellency activity (97.07 to 98.67% protection for 78 to 116 min) against *Ae. aegypti* than *Cx. quinquefasciatus* (98.94 to 99.75% protection for 86 to 128 min).

Discussions

In our study clearly revealed that all essential oils from Thai herbs at 0.33 $\mu\text{l}/\text{cm}^2$ offered the protection time against the mosquito species tested more than 80 min. *C. citratus* oil exhibited the highest repellent activity with 98.0 to 99.0% protection from bites of two mosquitoes for more than 120 min. The result coincides with the earlier result of other researchers, reported that essential oils from *C. citratus* showed repellent activity against *Ae. aegypti*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Anopheles subpictus* and *An. dirus* (Govindaraja, 2011; Sritabutra and Soonwera, 2013). Besides, *C. citratus* oil also showed the insecticidal effect against housefly (*Musca domestica*),

showing 100% knockdown at 30 min and KT50 values of 5.14 min (Sinthusiri and Soonwera, 2013). However, *C. odorata* oil also showed the excellent

Table 1. Repellency of herbal essential oils and chemical repellent against *A. aegypti* and *C. quinquefasciatus* at 0.17 $\mu\text{l}/\text{cm}^2$.

Test Repellents	Mosquito sp.	Protection Time (Mean $\pm$ SD) (min.)	% Protection
<i>C. odorata</i> oil	<i>A. aegypti</i>	53.20 $\pm$ 5.79c ^{1/}	98.80
	<i>C. quinquefasciatus</i>	90.0 $\pm$ 0a	98.67
<i>C. citratus</i> oil	<i>A. aegypti</i>	60.67 $\pm$ 6.45b	98.80
	<i>C. quinquefasciatus</i>	65.0 $\pm$ 2.35b	99.20
<i>C. nardus</i> oil	<i>A. aegypti</i>	58.33 $\pm$ 7.64bc	96.92
	<i>C. quinquefasciatus</i>	65.0 $\pm$ 2.35b	98.94
IR3535	<i>A. aegypti</i>	3.0 $\pm$ 0d	77.54
	<i>C. quinquefasciatus</i>	3.0 $\pm$ 0d	75.73

^{1/}Means of protection time in each column, followed by the same letter are not significantly different (one-way ANOVA and Duncan's multiple Range Test, P<0.05)

Table 2. Repellency of herbal essential oils and chemical repellent against *A. aegypti* and *C. quinquefasciatus* at 0.33 $\mu\text{l}/\text{cm}^2$.

Test Repellents	Mosquito sp.	Protection Time (Mean $\pm$ SD) (min.)	% Protection
<i>C. odorata</i> oil	<i>A. aegypti</i>	86.67 $\pm$ 10.40b ^{1/}	98.94
	<i>C. quinquefasciatus</i>	126.0 $\pm$ 15.77a	99.20
<i>C. citratus</i> oil	<i>A. aegypti</i>	116.67 $\pm$ 55.75a	98.67
	<i>C. quinquefasciatus</i>	128.33 $\pm$ 12.89a	99.75
<i>C. nardus</i> oil	<i>A. aegypti</i>	80.33 $\pm$ 16.07b	97.07
	<i>C. quinquefasciatus</i>	86.67 $\pm$ 10.40b	98.94
IR3535	<i>A. aegypti</i>	3.0 $\pm$ 0c	78.80
	<i>C. quinquefasciatus</i>	3.0 $\pm$ 0c	77.30

^{1/}Means of protection time in each column, followed by the same letter are not significantly different (one-way ANOVA and Duncan's multiple Range Test, P<0.05)

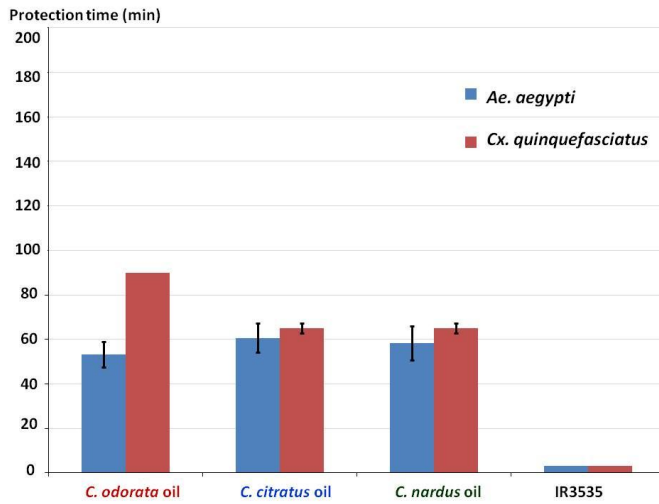


Figure 1. Comparison of protection times for repellent testing at 0.17 µl/cm² against mosquito species

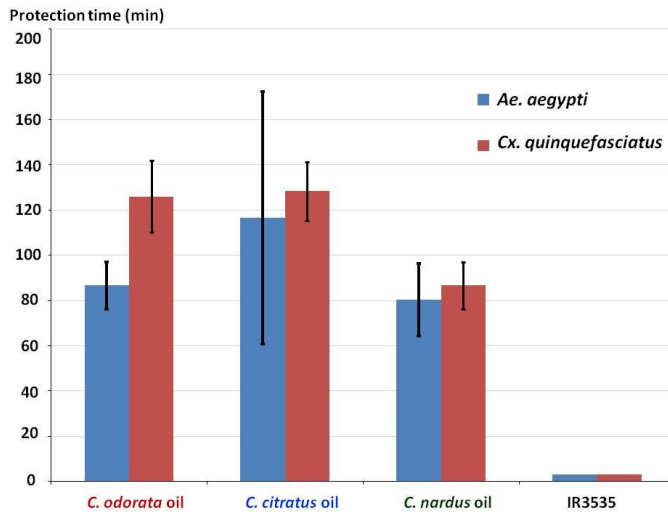


Figure 2. Comparison of protection times for repellent testing at 0.33 µl/cm² against mosquito species

repellent activity against the mosquito species tested with 98.94 to 99.20% protection for 86 to 126 min. Moreover, *C. odorata* oil also exhibited high potential for oviposition-deterrent and ovicidal action against *Ae. aegypti*, *Cx.*

quinquefasciatus and *An. dirus* (Phasomkusolsil and Soonwera, 2012). The essential oil of *C. odorata* flowers has been shown to possess repellency against mosquito bites (*Ae. aegypti*) and two grain storage insects, *Sitophilus zeamias* and *Tribolium castaneum* (Trongtokit *et al.*, 2005; Nerio *et al.*, 2009; Caballero-Gallardo *et al.*, 2011). However, these essential oils in this study exhibited the protection time against *Ae. aegypti* bites of nearly 1 hour but less than 2 hours. Even though, essential oil repellents can be short-lived in their effectiveness, since essential oils can evaporate completely. Thus, 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). 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).

In addition, *C. citratus* oil have been traditionally used in Thai medicine for analgesic, antifungal, anti-inflammatory, antiseptic, antiviral, bactericidal digestive and tonic. While, *C. odorata* oil is used topically as a sedative, antiseptic, hypotensive, and aphrodisiac. In addition, it is used in foods and beverages as a flavoring agent and in consumer product manufacturing as a fragrance for cosmetics and soaps (Burdock and Carabin, 2008). However, *C. nardus* oil also has been traditionally used to Thai medicine and repel mosquitoes in rural of Thailand and this oil produces the most used natural repellents in the world (Trongtokit *et al.*, 2005). Citronella oil's mosquito repellency has also been verified by Kim *et al.* (2005), including effectiveness in repelling *Ae. aegypti*, but requires reapplication after 30 to 60 minutes. Research also indicates that citronella oil is an effective repellent for body louse, head louse and stable flies (Baldacchino *et al.* 2013, Mumcuoglu *et al.* 1996; 2004).

Fortunately, all herbal essential oil repellents provide higher repellency activity than IR3535. Moreover, allergic reaction in this study clearly indicated that all herbal essential oil repellents did not show any allergic effects such as headache, itching, breathing difficulty, skin irritation and hot sensation rashes. While, IR3535 repellent maybe unsafe for children, the physiological mechanism of IR3535 action is a neuroexcitatory effect (Faulde *et al.*, 2010). Therefore, essential oils from three species of Thai herbs in this study can be considered for their use in green repellent or friendly repellents in mosquito control instead of chemical repellent could reduce mosquito resistance, safe repellents for humans and friendly for environment.

Mosquito repellent is one of the success methods in controlling mosquito transmitted diseases. While, chemical repellents are not safe for human and unfriendly for environment. All essential oils in this study exhibited high repellent activity against two mosquito vectors (*Ae. aegypti* and *Cx. quinquefasciatus*) and safe for human skin, friendly for environment. Thus, three essential oils from Thai herbs showed high potential for used as environmental friendly repellent against mosquito vectors.

Conclusion

Mosquito repellent is one of the success methods in controlling mosquito transmitted diseases. While, chemical repellents are not safe for human and unfriendly for environment. All essential oil repellent in this study exhibited high repellent activity against two mosquito vectors (*Ae. aegypti* and *Cx. quinquefasciatus*) and safe for human skin, friendly for environment. Thus, three essential oils from Thai herbs showed high potential for used as environmental friendly repellent against mosquito vectors.

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