

Full Length Research Paper

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

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Repellent activity of Thai essential oils derived from ylang-ylang (*Cananga odorata*), lemon grass (*Cymbopogon citratus*) and citronella grass (*Cymbopogon nardus*) were evaluated against female *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say) and compared a commercially available repellents (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 $\mu\text{l}/\text{cm}^2$ on the forearm of volunteers. All essential oils at 0.17 $\mu\text{l}/\text{cm}^2$ showed lower protection time and percentage of protection against two mosquito species than at 0.33 $\mu\text{l}/\text{cm}^2$. All essential oils exhibited higher repellent activity than chemical repellent. The essential oil of *C. citratus* at 0.33 $\mu\text{l}/\text{cm}^2$ exhibited excellent repellent activity with 98.67% protection from bites of *A. aegypti* for 116.67 \pm 55.75 min and 99.75% protection from bites of *C. quinquefasciatus* for 128.33 \pm 12.89 min. However, repellent activity in order of protection time and percentage of protection against two mosquito species in three essential oils was *C. citratus* oil > *C. odorata* oil > *C. nardus* oil. Our data showed that *C. citratus* oil is an effected green repellent for mosquitoes that is safe for humans and environmentally friendly.

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

INTRODUCTION

Mosquito is a serious insect to public health, which transmits 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 filariasis (Madhumathy et al., 2007;

Kumar et al., 2012). However, dengue worldwide disease is 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 shown an exponential increase over the last 30 years

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with the number of cases reported to the WHO between 2000 and 2007 doubling over those in the previous decade; Southeast Asian and Western Pacific countries bear the brunt of global disease burden due to dengue (WHO, 2009a). *Culex quinquefasciatus* is an important vector of filariasis in tropical and sub-tropical regions, about 90 million people worldwide suffer from 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 useful in 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 in 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, have no ill effect on non-target organisms (Govindarajan, 2011).

Many researchers have reported the repellent activity of plant essential oils against female mosquito vectors. Essential oils of *Cymbopogon citratus*, *Cinnamomum zeylanicum*, *Mentha piperita*, *Rosmarinus officinalis* and *Zingiber officinalis* showed repellent activity against *A. aegypti* and *C. quinquefasciatus* (Govindarajan, 2011; Khandagle et al., 2011; Kumar 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 was to investigate the repellency of Thai essential oils derived from *Cymbopogon citratus*, *Cymbopogon nardus* and *Cananga odorata* against females of *A. aegypti* and *C. quinquefasciatus* and to compare them with chemical repellent, IR3535 (12.5% w/w Ethyl butylacetylaminopropionate; Johnson's Baby Clear Lotion Anti-Mosquito®).

MATERIALS AND METHODS

Test mosquitoes

A. aegypti and *C. quinquefasciatus* eggs were obtained from the Armed Forces Research Institute of Medical Sciences (AFRIMS), Thailand. These eggs were brought to the laboratory of Entomology

and Environment Program, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok. The eggs were hatched in a glass cup with 250 ml of water. We then transferred batches of 200 larvae to white plastic trays (30 x 35 x 5 cm). Fish food (HIPRO®) was added (0.1 g for 1st and 2nd instar larvae, 0.3 g for 3rd instar larvae, and 0.5 g for 4th instar larvae at 0800 am and 0400 pm each day) to each tray for the successive two weeks until pupation of all larvae. The pupae were collected weekly and kept in a holding cage (size 30 x 30 x 30 cm) for emergence. Adult mosquitoes were reared at 25-30°C, and a relative humidity of 80±10% with a photoperiod of 12 h light followed by 12 h dark (12L:12D). We provided adults with soaked cotton balls containing a 5% glucose solution. Adult females, 5 to 7 days post eclosion, were used in these experiments. Prior to testing, the glucose-saturated cotton balls were removed from the holding containers for 12 h prior to blood feeding. Six hours prior to blood feeding, the water soaked cotton ball was removed.

Plant materials and herbal essential oils

Various parts of three species from Thai plants (flower of *Cananga odorata*, stem of *C. citratus* and stem of *C. nardus*) were collected from Nakhon Ratchasima province, Thailand. All plants were 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 dried 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 0.33µl/cm² 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., Thailand.

Repellent bioassay

The three essential oils were tested against *A. aegypti* and *C. 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 verbal/written 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 *A. aegypti* was tested during the day time from 0800 am to 0400 pm, while *C. 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 the left arm was used for treatment and the right arm for control. Both arms of volunteers were covered with rubber

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 repellent	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 0 a	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

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

sleeve with a window area of 3 x 10 cm on the ventral part of forearm. One hundred microlitres of each repellent was applied to the treatment area of left forearm of each volunteer and allowed to dry on the skin for 1 min. 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 s to mosquito cage (30 x 30 x 30 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 min 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. Percentage of protection was calculated for each repellent using the following formula (Manimaran et al., 2013):

$$\text{Percentage of protection} = 100 - \left(\frac{\text{No. of mosquitoes biting or landing} \times 100}{\text{No. of mosquitoes released}} \right)$$

Statistical analysis

The mean protection time was used as a standard measure of repellency of essential oil repellents and IR3535 (chemical repellent) against *A. aegypti* and *C. quinquefasciatus*. Differences in significance were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) comparisons by SPSS for windows (version 6.0).

RESULTS

Table 1 and Figure 1 shows 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 *A. aegypti* and *C. 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 *A. aegypti* and *C. 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 *A. aegypti* for 53 to 60 min of protection time, and also provided 98.94 to 99.20% protection from *C. quinquefasciatus*. On the other hand, the chemical repellent showed 75.73 to 77.54% protection from both mosquito species for 3 min. The results of 0.33 $\mu\text{l}/\text{cm}^2$ of herbal essential oil repellents and IR3535 against two mosquito species are shown in Table 2 and Figure 2. *C. citratus* oil gave the highest repellency with 98.67% protection from bites of *A. aegypti* up to a mean time of 116 min and with 99.75% protection from bites of *C. 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 *A. aegypti* than *C. quinquefasciatus* (98.94 to 99.75% protection for 86 to 128 min).

DISCUSSION

Our study clearly revealed that all essential oils from Thai herbs at 0.33 $\mu\text{l}/\text{cm}^2$ offered protection against the mosquito species tested for 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, which reported that essential oils from *C. citratus* showed repellent activity against *A. aegypti*, *C. quinquefasciatus*, *Culex tritaeniorhynchus*, *Anopheles subpictus* and *Anopheles 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 KT_{50} values of 5.14 min (Sinthusiri and

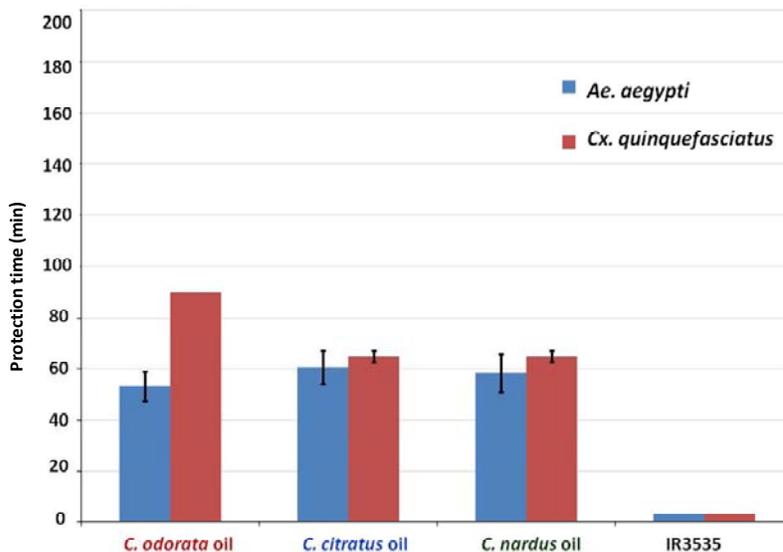


Figure 1. Comparison of protection times for repellent testing at 0.17 µl/cm² against mosquito species

Table 2. Repellency of herbal essential oils and chemical repellent against *A. aegypti* and *C. quinquefasciatus* at 0.33 µl/cm².

Test repellents	Mosquito sp.	Protection time (mean ± SD) (min)	Protection (%)
<i>C. odorata</i> oil	<i>A. aegypti</i>	86.67±10.40b ¹	98.94
	<i>C. quinquefasciatus</i>	126.0±15.77a	99.20
<i>C. citratus</i> oil	<i>A. aegypti</i>	116.67±55.75 a	98.67
	<i>C. quinquefasciatus</i>	128.33±12.89a	99.75
<i>C. nardus</i> oil	<i>A. aegypti</i>	80.33±16.07b	97.07
	<i>C. quinquefasciatus</i>	86.67±10.40b	98.94
IR3535	<i>A. aegypti</i>	3.0±0c	78.80
	<i>C. quinquefasciatus</i>	3.0±0c	77.30

¹Means 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).

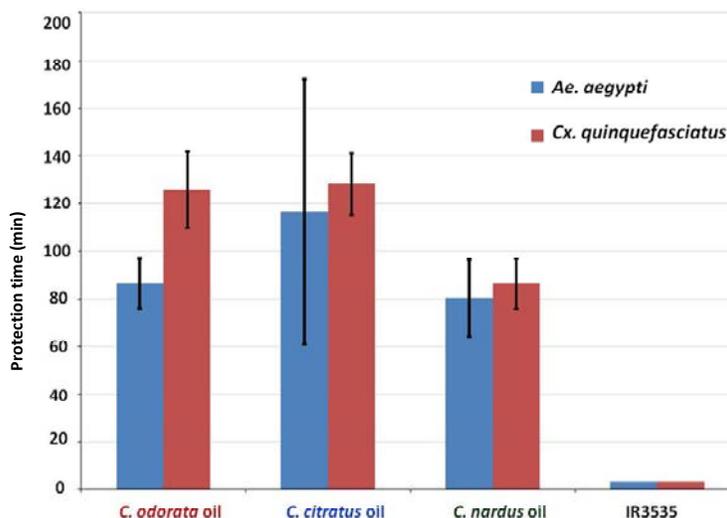


Figure 2. Comparison of protection times for repellent testing at 0.33 µl/cm² against mosquito species.

Soonwera, 2013). However, *C. odorata* oil also showed excellent 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 *A. aegypti*, *C. quinquefasciatus* and *A. dirus* (Phasomkusolsil and Soonwera, 2012). The essential oil of *C. odorata* flowers has been shown to possess repellency against mosquito bites (*A. 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 protection time against *A. aegypti* bites of nearly 1 h and less than 2 h. Even though, essential oil repellents can be short-lived in their effectiveness, they 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 in Thai medicine to repel mosquitoes in rural of Thailand and this oil produces the most used natural repellents in the world (Trongtokit et al., 2005). Citronella oil mosquito repellency has also been verified by Kim et al. (2005), including effectiveness in repelling *A. aegypti*, but requires reapplication after 30 to 60 min. 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 that could reduce mosquito

resistance, safe repellents for humans and friendly to the environment.

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

Conflict of Interests

The author(s) have not declared any conflict of interests.

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