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Repellent activity of essential oils from rutaceae plants against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say)

Phukerd, U., M Soonwera[*] and O. Wongnet

Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

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Herbal essential oils derived from eight species of Rutaceae plants (*Citrus aurantifolia* (Christm.&Panz.) Swingle, *Citrus aurantium* L., *Citrus hystrix* DC., *Citrus maxima* (Burm.f.) Merr., *Citrus medica* L. var *sarcodaclylis* Swingle, *Citrus reticulate* Blanco, *Citrus sinensis* Osbeck and *Citrofortunella microcarpa* (Bunge) Wijnands were evaluated for repellent activity against adult female of *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say), and compared them with chemical repellent (DEET 20% w/w ; Sketolen Shield®). Each herbal essential oil in ethyl alcohol was applied at 0.33 $\mu l/cm^2$ on the forearms of volunteers. On the protection time (in minutes) and biting rate (%) revealed that essential oil of *C. aurantifolia* was effective as repellent and feeding against *Ae. aegypti* (65.0±22.91 minutes protection times and 1.47±0.46% biting rate) and *Cx. quinquefasciatus* (71.67±5.77 minutes protection times and 1.73±0.23% biting rate). Thus, repellent activity indicated the order of protection time and biting rate against two mosquito species in eight essential oils as *C. aurantifolia* > *C. microcarpa* > *C. maxima* > *C. reticulate* > *C. sinensis* > *C. hystrix* > *C. aurantium* >*C. medica* var *sarcodaclylis*. Unfortunately, the period of protection time against two mosquito species of all herbal essential oil was lower than DEET (155.0±7.07 minutes for *Ae. aegypti* and 180.0±14.14 minutes for *Cx. quinquefasciatus*).

Keywords: essential oils Repellency, *Aedes aegypti*, *Culex quinquefasciatus*

Introduction

Mosqutioes are responsible for spreading serious human diseases, especially *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say). *Ae. aegypti*, is the primary carrier for viruses that cause dengue fever, dengue hemorrhagic fever, yellow fever and chikungunya fever (Sakulku *et al*., 2009 ; Yang *et al*., 2009 ; WHO, 2011). Meanwhile, outbreaks of dengue fever and dengue hemorrhagic fever have been reported by WHO- South-East Asia Region (SEARO) from Bhutan, Bangladesh, Indonesia and Sri Lanka (in 2004-

[*] Corresponding author: Mayura Soonwera; e-mail: ksmayura@kmitl.ac.th

2005) (Tjahjani, 2008). In Thailand, it is estimated that more than 200,000 people suffer annually from dengue attacks (Juckkapan, 2009; Ministry of Public health, Thailand, 2013).

In addition, *Cx. quinquefasciatus* is the principal rector of Japanese encephalitis (JE), Lymphatic filariasis caused by *Wuchereria bancrofti*, heartworm in dogs and its also causes annoyance and dermatitis (Ramaiah *et al*., 2006; Nitatpattana *et al*., 2008; Du Ponte *et al*., 2009). Conseguently, *Ae. aegypti* and *Cx. quinquefasciatus* constitute one of the most serious pests for humans and animals.

The control of mosquito vectors and reducing the transmission of human pathogens are based on the chemical insecticides, especially chemical repellents. Thus, chemical repellents are considered to be a useful of reducing and preventing the mosquito vectors, deterring an insect from flying and landing, and biting human and animal skin. Unfortunately, chemical repellents are not safe for human, especially children because they may cause skin irritation, hot sensation rashes or allergy (Das *et al*., 2003), such as DEET (N,N-diethyl-M-methyl benzamide) may be unsafe for children possibly causing encephalopathy (Abdle-Rahman *et al*., 2001). Besides, DEET is also know to damage plastic and synthetic materials (Kang *et al*., 2009).

Therefore, there is an urgent need to develop new repellents for controlling mosquito vectors which are more environmentally friendly, biodegradable, non-toxic effects on human and domestic animals (Kumar *et al*., 2011; Rabha *et al*., 2012). The mosquito repellents base on plant extracts, or plant essential oils may be a possible alternative as one of the methods in preventing mosquito vectors also compatible with human life and environment. However, plant essential oils are reported to have repellency against mosquito adults, such as essential oils from *Citrus aurantifolia*, *Citrus sinensis*, *Cinnamomum xeylanicum*, *Cymbopogon nardus*, *Cymbopogon citratus*, *Curcuma aromatic*, *Eucalyptus citriodora*, *Eucalyptus globules*, *Mentha piperita*, *Ocimum basilicum*, *Piper aduncum*, *Syzygium aromaticum*, *Zanthoxylum limonella*, *Zingiber officinalis* and *Vitex negundo* (Das *et al*., 2003; Choochote *et al*., 2005; Gleiser *et al*., 2011; Karunamooethi *et al*., 2008, 2010; Prajapati *et al*., 2005; Yang and Ma, 2005; Phasomkusolsil and Soonwera, 2010a; Nour *et al*., 2009; Pushpanathan *et al*., 2008; Tjahjani, 2008).

Moreover, mosquito repellents based on Thai indigenous plant oils have demonstrated good efficacy against *Ae. aegypti*, *Anophele minimus* and *Culex quinquefasciatus* in the laboratory condition (Phasomkusolsil and Soonwera, 2010b, 2011).

This study investigates the repellency of essential oils derived from eight species of Rutaceae plants against *Ae. aegypti* and *Cx. quinquefasciatus* and to compare them with chemical repellent (DEET) under laboratory conditions.

Materials and methods

Plant materials and essential oils

The eight species of Rutaceae plants were used in this study, as shown in Table 1. All of plants were identified, authenticated and submitted at Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL). Each plant material was extracted for essential oils by water distillation. All essential oil was dissoled in ethyl alcohol and were kept at room temperature defore testing.

Table 1. List of Rutaceae plants were extracted for essential oils and used in this study

Scientific Name	Part Used	Location
Citrus aurantifolia (Christm. X Panz.) Swingle	fruit	Phetchaburi, Thailand
Citrus aurantium L.	fruit	Phetchaburi, Thailand
Citrus hystrix DC.	fruit	Nakhonratchasima, Thailand
Citrus maxima (Burm.f.) Merr.	fruit	Nakhon Nayok, Thailand
Citrus medica L. var *sarcodactylis* Swingle	fruit	Chumphon, Thailand
Citrus reticulata Blanco	fruit	Chiangmai, Thailand
Citrus sinensis Osbeck	fruit	Chiangmai, Thailand
Citrofortunella microcarpa (Bunge) Wijnands	fruit	Chumphon, Thailand

Chemical repellent

DEET (20% w/w DEET; Sketolene Shield®), a common chemical repellent in Thailand, was purchased from DKSH (Thailand) Co. Ltd., 2535, Sukumvit Road, Bangkok, Phrakanong, Bangkok 10260, Thailand.

Test mosquitoes

Ae. aegypti and *Cx. quinquefasciatus* eggs were obtained from the Armed Forces Research Institute of Medical Sciences (AFRIMS). All of mosquito was reared in Entomology and Environmental Laboratory, Plant Production Technology Section, Faculty of Agricultural Technology, KMITL. Adults of two mosquito species were fed on 5% glucose solution and maintained at 32.50±1.20 ºc, 64.80±3.50% RH and 12h : 12h (light : dark) photoperiod. Nulliparous female of 4-5 days-old were used for repellency tests.

Repellency test

The repellency of eight essential oils were evaluated using the human-bait technique following Thai Industrial Standards Institute Guidelines (TISI, 2009). Human volunteers were recruited from the Healthy students and staff of Entomology and Environment Laboratory, Plant Production Technology Section, Faculty of Agricultural Technology, KMITL. The volunteers signed an informed consent from after having received a full explanation of the lest objectives. The research proposal 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 daytime from 8.00 am to 4.00 pm, while *Cx. quinquefasciatus* was tested during night time from 4.00pm to 12.00 pm (Phasomkusolsil and Soonwera, 2010, 2011).

Before testing the volunteers arms were washed and cleaned thoroughly with distilled water and used the left arm for treatment and the night arm for control. Both arms of volunteers were covered with rubber sleeve with a window area of 30 cm^2 (3x10 cm) on the ventral part of forearm 0.1 ml of each test repellent was applied to the treatment area of left forearm of each volunteer and allowed to dry on the skin for 1.0 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 them continued. The test continued unit as least two bites occurred in a three-minute period. However, if no mosquito bit or landed during a three-minute period the arm was with draw from the mosquitoed cage. The protection time or repellency test period was carried out every 15.0 minutes until fewer than two mosquito bit or landed during the three-minute period and then the repellency test was stopped. The time between application of the repellents was recorded as the protection time.

Data analysis

The median protection time was used as a standard measure of repellency of the essential oils and DEET 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). Percentage of mosquito biting or landing was calculated for each test using the following formula (Phalsomkusolsil and Soonwera, 2010).

$$\% \text{ Biting} = [B/250] \times 100$$

Where B is the total number of biting or landing by the end of the test. The test was carried out 3 times per test solution.

Results

The repellency results (as show in minute of protection time) for eight essential oils repellents, chemical repellent (DEET 20% w/w, positive control) and ethyl alcohol 70% (negative control) assessed by mean protection time against *Ae. aegypti* under laboratory conditions is show in Table 2. The essential oils from *C. aurantifolia* and *C. microcarpa* had the best efficiency against *Ae. aegypti* ; in which the mean protection times were 65.0±22.91 and 61.67±2.89 minutes and 1.47±0.46 and 1.60±0.69 % biting rate, respectively. The mean protection times of essential oil from *C. maxima*, *C. reticulate*, *C. sinensis*, *C. hystrix*, *C. medica* and *C. aurantium* against *Ae. aegypti* were 45.0 ±6.93, 21.0±6.93, 20.95±8.67, 20.50±8.66, 11.67±5.75 and 10.0±8.66 minutes and 2.27±0.16, 1.33±0.23, 1.20±0, 1.47±0.21, 1.46±0.28 and 1.73±0.23 % biting rate, respectively. The period of protection time against *Ae. aegypti* of all essential oil was higher than ethyl alcohol (negative control, 0 minute), but was lower than DEET (positive control, 155.0±7.07 minutes). For ethyl alcohol (negative control) showed no repellency against *Ae. aegypti*, in contrast ethyl alcohol showed the highest biting percentage at 24.67±0.68%.

The mean repellency in minutes for eight essential oils, negative control (ethyl alcohol) and positive control (DEET) against *Cx. quinquefasciatus* as show in Table 3. There were significant differences in repellency among essential oils and chemical repellent (DEET) ($P<0.05$). The essential oils from *C. aurantifolia* and *C. microcarpa* also showed the best effiency against *Cx. quinquefasciatus,* in which the protection times were 71.67±5.77 and 70.0±8.67 minutes and 1.73±0.23 and 1.43±0.33 % biting rate, respectively. The protection times of essential oils from *C. medica*, *C. hystrix*, *C. sinensis*, *C. reticulate*, *C. aurantium* and *C. maxima* against *Cx. quinquefasciatus* were 45.95±1.25, 45.0±8.67, 42.80±7.83, 40.85±8.55, 21.58±8.08 and 15.95±1.25 minutes, and 1.33±0.23, 1.67±0.23, 1.33±0.23, 1.43±0.34, 1.43±0.33 and 1.33±0.23 % biting rate, respectively. However, the mean protection times against *Cx. quinquefasciatus* of all essential oils was lower than chemical repellent (DEET) 180.0±14.14 minutes and 1.33±0.33 % biting rate). For negative control (ethyl alcohol) showed no repellency against *Cx.*

quinquefasciatus (0 minute), but exhibited the highest biting percentage (15.33±1.18 %). In contrast, all essential oil showed a low biting percentage (1.33–1.43 %).

Table 2. Repellency of eight essential oils from Rutaceae plants, positive control (DEET) and negative control (ethyl alcohol) against adult female of *Aedes aegypti* at 0.33 µl/cm^2

Test repellents	**Protection time (mean±SD) (min)**	**Biting rate (mean±SD) (%)**
C. aurantifolia	65.0±22.91b[1/]	1.47±0.46b[1/]
C. aurantium	10.0±8.66e	1.73±0.23b
C. hystrix	20.50±8.66d	1.47±0.21b
C. maxima	45.0±6.93c	2.27±0.16b
C. medica	11.67±5.75e	1.46±0.28b
C. reticulata	21.0±6.93d	1.33±0.23b
C. sinensis	20.95±8.67d	1.20±0b
C. microcarpa	61.67±2.89b	1.60±0.69b
Negative control (ethyl alcohol 70%)	0f	24.67±0.68a
Positive control (DEET 20% w/w)	155.0±7.07a	1.46±0.65b

[1/]means of protection time / biting rate 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 3. Repellency of eight essential oils from Rutaceae plants, positive control (DEET) and negative control (ethyl alcohol) against adult female of *Culex quinquefasciatus* at 0.33 µl/cm^2

Test repellents	**Protection time (mean±SD) (min)**	**Biting rate (mean±SD)(%)**
C. aurantifolia	71.67±5.77b[1/]	1.73±0.23b[1/]
C. aurantium	21.58±8.08d	1.43±0.33b
C. hystrix	45.0±8.67c	1.67±0.23b
C. maxima	15.95±1.25e	1.33±0.23b
C. medica	45.95±1.25c	1.33±0.23b
C. reticulata	40.85±8.55c	1.43±0.34b
C. sinensis	42.80±7.83c	1.33±0.23b
C. microcarpa	70.0±8.67b	1.43±0.33b
Negative control (ethyl alcohol 70%)	0f	15.33±1.18a
Positive control (DEET 20% w/w)	180.0±14.14a	1.33±0.33b

[1/]means of protection time / biting rate in each column, followed by the same letter are not significantly different (one-way ANOVA and Duncan's multiple Range Test, P<0.05)

Discussions

In our study showed that all essential oil from Rutaceae plants gave mean protection times and mean biting percentage against *Ae. aegypti* for 65.0±22.91 – 10.0±8.66 minutes and 2.27±0.16-1.20±0 % and against *Cx. quinquefasciatus* for 71.67±5.77-15.95±1.25 minutes and 1.73±0.23 – 1.33±0.23 %. However, the responses of two mosquito species to the eight essential oils were differcnt, *Cx. quinquefasciatus* was sensitive to all essential oil than *Ae. aegypti*. Moreover, Tawatsin *et al*., (2006) have reported that the essential oils extracted from 18 plant species, belonging to 11 families were more effective against the night-biting mosquitoes (*Anopheles dirus*, *Cx. quinquefasciatus*) exhibiting repellency for 4.5–8.0 hrs. than against *Ae. aegypti* (0.3–2.8 hrs). The essential oil from *C. aurantifolia* was highly effective as repellent and feeding deterrents, this essential oil exhibited the protection time against two mosquito species more than 1 hour (65.0–71.67 minutes), but biting percentage was less than 1.80%. Unfortunately, the mean protection times against *Ae. aegypti* and *Cx. quinquefasciatus* for all essential oil was lower than the Thai Industrial Standards Institute determined time of greater than 2 hrs (120 minutes) (TISI, 2010). Besides, Amer and Mehlhorn (2006) defined that if the protection time of repellent is long and the biting percentage is low, the repellent had good efficiency in repelling mosquitoes and deters biting. In contrast, the protection time is short but the biting percentage is low, than the repellent is more a feeding deterrent than a repellent. If the protection time is long but the biting percentage is high, then the repellent is more a repellent than a feeding deterrent. In the result of this study, essential oil from *C. aurantifolia* showed a low protection time (< 2 hours.) against two mosquito species, while the biting percentage exhibited less than 1.80%, this indicate that *C. aurantifolia* oil is rather feed deterrent than repellent. However, essential oils from other Rutaceae plants in this study also rather feed deterrent (< 2.30 % biting rate) than repellent (< 1 hour protection time). Moreover, plant essential oils have been reported to have repellent activity against mosquito vectors include citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cinnamon, rosemary, basil, thyme and peppermint. Most of these essential oils provided short-lasting protection usually lasting less than 2 hours (Koul *et al*., 2008). The essential oils from clove (*Syzygium aromaticum*) and *Zanthoxylum limonella* were the most effective and provided 2 hours of complete repellency against mosquito vectors (Das *et al*., 2003; Shapiro, 2012).

Besides, many researchers suggested the synergistic effects among constituents of plant essential oils and mixtures of oils as well as the search of new additives that could make longer the protection times, represents an important tool to replace the chemical repellents used today (Nerio *et al*., 2010).

Although, none of the essential oils in this study provided protection for more than DEET., Nevertheless, human toxicity has been reported with DEET, with symptoms varying from mild to severe and may cause dermal toxicity in infants and children (Kang *et al*., 2009). On the other hand, the mosquito repellent base on plant essential oils exhibited low toxicity to human, animal other non-target organisms, environmental friendly and mosquito resistance will develop more slowly to mosquito repellent base on plant essential oils (Koul *et al*., 2008; Regnault-Roger *et al*., 2012; Isman, 2006).

In conclusion, this study demonstrated the potential of essential oils derived from *C. aurantifolia*, *C. aurantium*, *C. hystrix*, *C. maxima*, *C. medica*, *C. reticulate*, *C. sinensis* and *C. microcarpa* for use as mosquito repellents against *Ae. aegypti* and *Cx. quinquefasciatus*. However, all essential oil repellents showed higher feeding deterrent activity than repellency activity.

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