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The effects of herbal essential oils on the oviposition-deterrent and ovicidal activities of *Aedes aegypti* (Linn.), *Anopheles dirus* (Peyton and Harrison) and *Culex quinquefasciatus* (Say)

Siriporn Phasomkusolsil[1*] and Mayura Soonwera[1]
[1]Entomology and Environment Program, Plant Production Technology Section,
Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang,
Chalong Krung Road, Ladkrabang, Bangkok 10520, Thailand
[*] Corresponding author email: msiriporn@hotmail.com
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Abstract. The effect of oviposition-deterrent and ovicidal of seven essential oils were evaluated towards three mosquito vectors, *Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus*. The oviposition activity index (OAI) values of six essential oils namely *Cananga odorata*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus citriodora*, *Ocimum basilicum* and *Syzygium aromaticum* indicated that there were more deterrent than the control whereas *Citrus sinensis* oil acted as oviposition attractant. At higher concentration (10%) of *Ca. odorata* (ylang ylang flowers) showed high percent effective repellency (ER) against oviposition at 99.4% to *Ae. aegypti*, 97.1% to *An. dirus* and 100% to *Cx. quinquefasciatus*, respectively. The results showed that mean numbers of eggs were lower in treated than in untreated water. In addition, there was an inverse relationship between essential oil concentrations and ovicidal activity. As the concentration of essential oil increased from 1%, 5% and up to 10% conc., the hatching rate decreased. The essential oil of *Ca. odorata* at 10% conc. gave minimum egg hatch of 10.4% (for *Ae. aegypti*), 0.8% (for *An. dirus*) and 1.1% (for *Cx. quinquefasciatus*) respectively. These results clearly revealed that the essential oil of *Ca. odorata* served as a potential oviposition-deterrent and ovicidal activity against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*.

INTRODUCTION

Mosquito vectors in Thailand transmit serious human diseases such as dengue fever, chigunkunya, malaria and Japanese Encephalitis (JE), causing many severe cases or deaths (WHO, 2010; Bureau of Epidemiology, 2011). Of the numerous mosquito species in Thailand, *Aedes aegypti* is the principal transmitter of dengue fever and dengue haemorrhagic fever (DHF) in Thailand and it also transmits Chikungunya fever (Thavara *et al.*, 2009). Additionally, *Anopheles dirus* is the major vector of malaria in border of Thailand and other countries (Rosenberg *et al.*, 1990). *Culex quinquefasciatus* is a vector of Japanese Encephalitis (JE) and it also causes annoyance and dermatitis (Nitatpattana *et al.*, 2008).

Mosquito control is a vital public-health practice throughout the world and especially in the tropics. Currently, synthetic insecticides can also be used to help control mosquitoes. It has been favorable so far, because of their speedy action and easy application. However, the environmental threat that these chemicals pose, effects on non-target organisms and the resistance of insects have all increased (Wattanachai & Tintanon, 1999). Thus, the environmental friendly and biodegradable natural insecticides of plants origin have been receiving attention as an alternative green measure for controlling arthropods of public health importance (Koul *et al*., 2008). Plant essential oils are natural volatile substances obtained from a variety of plants, in general have been recognized as an important natural resource of insecticides (Gbolade *et al*., 2000). In Thailand, many researchers have observed that biologically active materials derived from Thai indigenous plant sources can have repellent activities (Trongtokit *et al*., 2005; Phasomkusolsil & Soonwera, 2010a), insecticidal (Chaiyasit *et al*., 2006), larvicidal (Phasomkusolsil & Soonwera, 2010b; Kaewnang-O *et al*., 2011), ovicidal and oviposition-deterrent (Tawatsin *et al*., 2006) against mosquito vectors.

One of the successful strategies for mosquito control is focused on targeting breeding sites of mosquitoes for regulation of their population density (Gubler, 1989). Oviposition is one of the most important events in the life cycle of mosquitoes. If oviposition is prevented, the mosquito life cycle is disrupted and population growth reduced (Xue *et al*., 2001). Mosquitoes are known to select or reject their specific oviposition sites by sensing chemical signals that are detected by sensory receptors on the antenna (Davis & Bowen, 1994). Therefore, the aim of this study was to investigate the mosquito oviposition-deterrent and ovicidal activities of seven essential oils, *Cananga odorata*, *Citrus sinensis*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus citriodora*, *Ocimum basilicum* and *Syzygium aromaticum* against three mosquito species, *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Mosquitoes

Three species of lab-bred mosquito; *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*, were used in this study. *Aedes aegypti* and *An. dirus* eggs were obtained from the Department of Entomology, Armed Forces Research Institute of Medical Sciences (AFRIMS). *Culex quinquefasciatus* eggs were obtained from the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University. They were maintained in the laboratory of Entomology and Environment Programme, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok. The colonies of mosquitoes were maintained and all the experiments were carried out at conditions of 30-35ºC and 60-80% relative humidity. Larvae were kept in plastic trays (30x35 cm and 5 cm high and containing 1500 ml tap water) and they were fed with a diet of fish food; HIPRO®. Newly emerged pupae were transferred to screen cages (size 30x30x30 cm) and emerged as adults. Adults were provided with 5% glucose on saturated cotton pads. Females were blood-fed via membrane by artificial membrane methods (Nasirian & Ladonni, 2006).

Plant materials

The plant materials were collected from *Cananga odorata* Lamk. (ylang ylang flowers), *Citrus sinensis* L. Osbeck (orange fruits), *Cymbopogon citratus* DC. Stapf (lemongrass stems), *Cymbopogon nardus* L (citronella grass stems), *Eucalyptus citriodora* Hook (eucalyptus leaves), *Ocimum basilicum* L. (sweet basil leaves) and *Syzygium aromaticum* L. (clove flowers) (Table 1). Each plant material was extracted for essential oils by steam distillation (Peter & Amala, 1998). One kilogram of dried and finely ground material of each plant was placed in an extraction column connected to a round bottomed distillation flask containing distilled water. The flask was heated to about 100ºC and allowed to boil until distillation was completed. The liquid formed, together with distillate oil, was collected in a separating funnel. The mixture was allowed to settle for 1 day, after which the water (lower) layer was slowly drawn off until only the oil layer remained. Amount of 1 kg of each plant in this study was extracted which yielded essential oil quantities as follow: 2.5% (w/v) *Ca. odorata*, 2.0% (w/v) *Ci. sinensis*, 2.6% (w/v) *C. citratus*, 2.2% (w/v) *C. nardus*, 2.4% (w/v) *E. citriodora*, 1.3% (w/v) *O. basilicum* and 3.0% (w/v) *S. aromaticum.* The volatile

Table 1. List of herbal essential oils tested in this study

Common name	Scientific name	Family	Oil properties	Therapeutic property
Citronella grass oil	*Cymbopogon nardus* L.	Poaceae	Citronella oil has a slightly sweet, lemony smell.	Antiseptic, bactericidal, deodorant, diaphoretic, parasitic, tonic, stimulant, insecticide.
Clove oil	*Syzygium aromaticum* L.	Myrtaceae	Clove oil has a warm, strong, spicy smell and the oil is colourless to pale yellow with a medium to watery viscosity.	Analgesic, antiseptic, antispasmodic, anti-neuralgic, carminative, anti-infectious, disinfectant, stimulant, stomachic, uterine, tonic, insecticide.
Eucalyptus oil	*Eucalyptus citriodora* Hook.	Myrtaceae	Eucalyptus has a clear, sharp, fresh and very distinctive smell, is pale yellow in colour and watery in viscosity.	Antifungal, antimicrobial, antiseptic, anti-inflammatory, bactericidal
Lemongrass oil	*Cymbopogon citratus* DC. Stapf	Poaceae	Lemongrass oil has a lemony, sweet smell and is dark yellow to amber and reddish in colour, with a watery viscosity.	Analgesic, antifungal, anti-inflammatory, antiseptic, antiviral, bactericidal, digestive, febrifuge, tonic, insecticidal
Sweet basil oil	*Ocimum basilicum* L.	Lamiaceae	The oil has a watery viscosity and is pale greenish-yellow in colour. The aroma is clear, light and peppery and gives a sweet, green top note to blends.	Antiemetic, antiseptic, antispasmodic, carminitve, cephalic, expectorant, immune support, insecticide
Orange oil	*Citrus sinensis L.* Osbeck	Rutaceae	Sweet orange oil has a sweet, fresh and tangy smell, is yellow to orange in colour and watery in viscosity. The shelf life is approximately 6 months.	Antiseptic, anti-depressant, antispasmodic, anti-inflammatory, carminative, diuretic, cholagogue, sedative and tonic
Ylang ylang oil	*Cananga odorata* Lamk	Annonaceae	It has an exotic, sweet smell and is slightly yellow in colour.	Antidepressant, anti-seborrhoeic, antiseptic, aphrodisiac, hypotensive, nervine and sedative.

oil was stored in airtight bottle and kept at 4°C for later experiments. These essential oils were prepared as 1%, 5% and 10% solutions in soybean oil.

Oviposition deterrent activity assay

The bioassay was evaluated by using the following method (Prajapati *et al*., 2005). Two black plastic cups (250 ml in capacity) were filled with 99 ml of well water and were placed at diagonally opposite corners of the cage. An aliquot (1 ml) of essential oil dissolved in soybean oil (1%, 5% and 10%) was added to one bowl whilst an equivalent quantity of pure soybean oil was added to the second (control). Their positions were switched between replicates to avoid positional effects. A piece of filter paper was placed on the internal surface of each bowl to provide a support for oviposition. The paper was located in each cup so as the lower half of the paper was submerged in water. Meanwhile fifteen gravid female mosquitoes (5-7 days old) were mated by artificial mating technique (Lardeux *et al*., 2007) for *An. dirus* which was not required for *Ae. aegypti* and

Cx. quinquefasciatus because natural mating usually occurs. They were simultaneously exposed, in a bioassay cage (30 x 30 x 30 cm). After 48 hours, the eggs laid in each cup were counted after removal of the oviposition paper. Five replicates were performed. The oviposition experiments were expressed as mean number of eggs and oviposition activity index (OAI), which was calculated using the following formula (Kramer & Mulla, 1979).

$$OAI = \frac{NT-NC}{NT+NC}$$

Where NT is the total number of eggs in the test solution, and NC is the total number of eggs in the control solution. The OAI ranges from -1 to +1, with 0 indicating neutral response. The positive index values indicate that more eggs were deposited in the test cups than in the control cups, and that the test solutions were attractive. Conversely, more eggs in the control cups than in the test cups result in negative index values and the test solutions were a deterrent.

The percent effective repellency (ER%) for each essential oil was calculated in case of the test solution as a deterrent using the following formula (Rajkumar & Jebanesan, 2009).

$$ER\% = \frac{NC-NT}{NC} \times 100$$

In addition, the percent effective attractancy (EA%) was calculated in case of the test solution as a attractant using the following formula (Govindarajan *et al.*, 2008).

$$EA\% = \frac{NT-NC}{NT} \times 100$$

Where ER is effective repellency/EA is effective attractancy, NC is the total number of eggs in the control solution and NT is the total number of eggs in the test solution.

Ovicidal bioassay

In ovicidal activity, the method of Pushpanathan *et al.* (2006) was followed. Twenty five eggs of *Ae. aegypti*, *An. dirus* and one egg raft containing a minimum of 100 eggs of *Cx. quinquefasciatus* were treated topically with 50 µl of each concentration (1%, 5% and 10% in soybean oil) of essential oils. After 3 hours of treatment, the eggs were sieved through muslin cloth, thoroughly rinsed with tap water, and left in plastic cups filled with dechlorinated water for hatching assessment after counting the eggs under microscope. In these assays, each test comprised of five replicates and negative controls (pure water and pure soybean oil) were carried out in parallel for comparison. The hatched larvae were counted after 98 h post-treatment and the percent hatch was calculated by the following formula. The percent hatch in the control was compared with the percent hatch in each oil concentration.

$$\frac{\text{Number of hatched larvae x 100}}{\text{Total number of egg in treated water}}$$

Statistical analysis

The mean number of eggs deposited in test and control cups were analyzed using a paired *t*-test. One-way analysis of variance (ANOVA) and Duncan's multiple comparisons were used for the hatching percentage to determine significant treatment differences by SPSS for Windows (version 16.0). All levels of statistical significance were determined at $P<0.05$. Non egg hatchability data was analyzed statistically for effective concentration to inhibit egg viability (EC_{50}) was calculated by Probit analysis.

RESULTS

Oviposition deterrent activity assay

For oviposition deterrent test, the results were summarized in Table 1-3. Additionally, the oviposition activity index (OAI) of seven essential oils at three concentrations (10%, 5% and 1%) has been illustrated in Figure 1. The present results showed that the different concentration of essential oils reduced number of eggs deposited by gravid *Ae.*

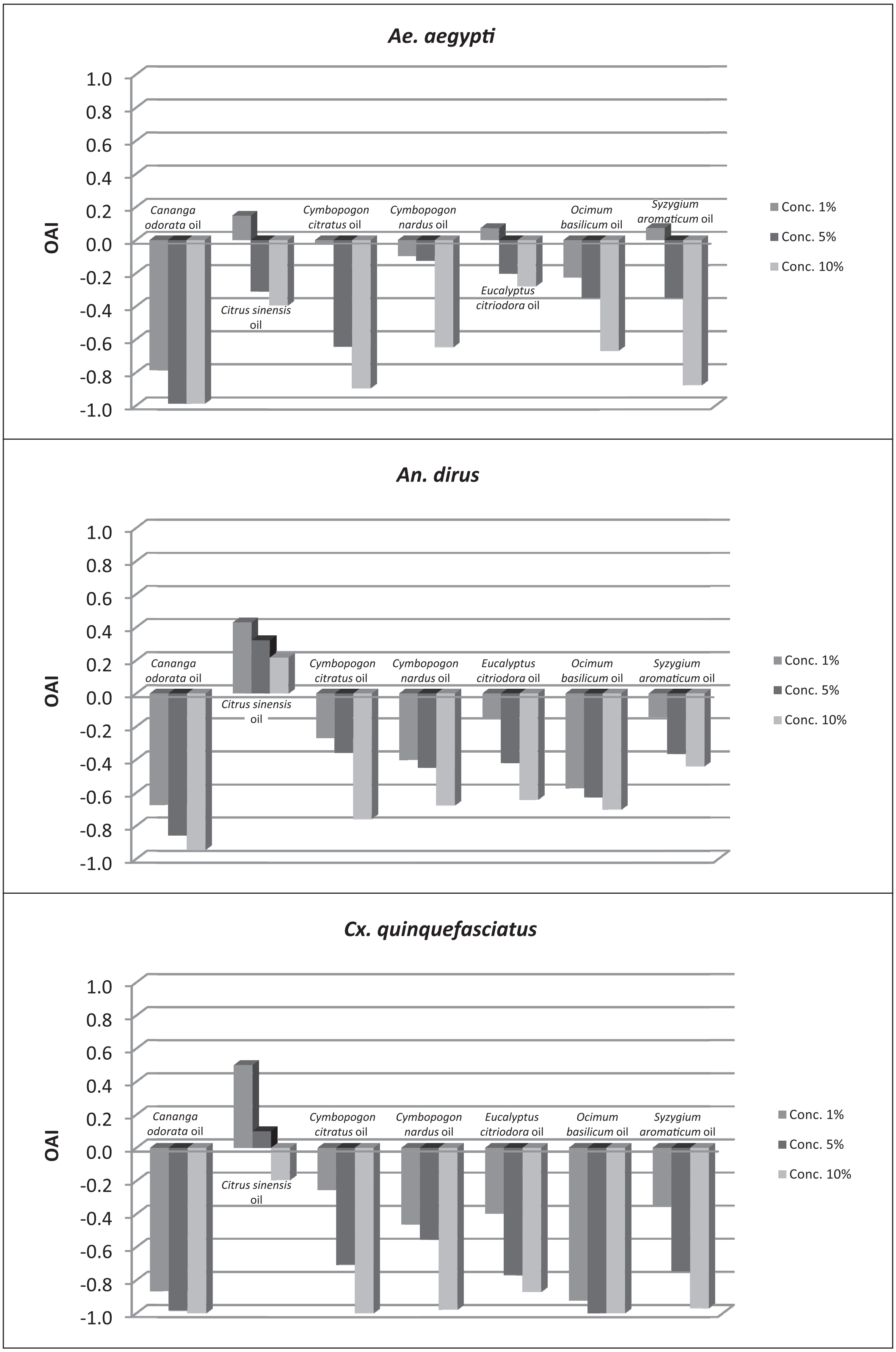

Figure 1. Oviposition activity index (OAI) values of seven essential oils of *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*

aegypti of treatment concentrations at 10%, 5% and 1% as compared with soybean oil control (Table 2). The mean number of eggs laid in three concentrations (10%, 5% and 1%) of *Ca. odorata* showed 0.3±0.8, 3.6±3.9 and 32.6±22.3 eggs per cup, respectively showed significant difference with controls ($P<0.05$). Furthermore, the highest percentage of effective repellency (ER%) of *Ca. odorata* against oviposition was 99.4% in 10% conc. and 5% conc. followed by 87.5% in 1% conc., respectively. The oviposition activity index (OAI) of seven essential oils at three concentrations (10%, 5% and 1%) when being paired with control ranged from -1 to -0.8 (*Ca. odorata*), -0.4 to 0.1 (*Ci. sinensis*), -0.9 to 0 (*C. citratus*), -0.6 to -0.1 (*C. nardus*), -0.3 to 0.1 (*E. citriodora*), -0.7 to -0.2 (*O. basilicum*) and -0.9 to 0.1 (*S. aromaticum*), respectively. It showed that gravid *Ae. aegypti* females preferred to lay eggs in control cups than in the cups treated with solvent extracts of seven essential oils except only in *Ci. sinensis*, *E. citriodora* and *S. aromaticum.*

Moreover, among the seven essential oils screened for oviposition deterrent activity, essential oil of *Ca. odorata* was found to be highly effective in preventing egg laying of *An. dirus* (Table 3). The present results showed that the *Ca. odorata*-treated cups showed means of 32.2±39.8, 103.4±151.0 and 162.8±71.6 eggs per cup at 10%, 5% and 1% conc., while the control cups showed means of 1060.0±63.8, 1030.2±275.1 and

Table 2. The oviposition deterrent/ attractant/ neutral of the essential oils in three concentrations (1%, 5% and 10%) against *Ae. aegypti*

Herbal essential oils	Conc.	Number of eggs±SD		OAI**	ER%	EA%	No. of tested eggs laid per female
		Tested	Control				
Cananga odorata oil	1%	32.6±22.3*	282.4±96.4	-0.8	87.5	–	2.2
	5%	3.6±3.9*	513.6±186.0	-1.0	99.4	–	0.2
	10%	0.3±0.8*	92.5±84.3	-1.0	99.4	–	0
Citrus sinensis oil	1%	204.2±63.4	146.0±30.4	0.1	–	21.6	13.6
	5%	119.2±50.8	256.8±117.0	-0.3	34.5	–	7.9
	10%	105.4±50.9*	235.6±56.1	-0.4	55.3	–	7
Cymbopogon citratus oil	1%	341.6±78.4	348.6±40.0	0	0.8	–	22.8
	5%	89.2±28.9*	406.0±37.2	-0.6	77.9	–	5.9
	10%	25.6±24.9*	494.8±64.4	-0.9	94.2	–	1.7
Cymbopogon nardus oil	1%	316.0±85.1	375.8±48.6	-0.1	13.5	–	21.1
	5%	292.4±97.2	376.4±107.4	-0.1	12.9	–	19.5
	10%	122.2±59.1*	557.6±63.3	-0.6	77.7	–	8.1
Eucalyptus citriodora oil	1%	304.0+84.5	255.0+31.3	0.1	–	9.3	37.3
	5%	227.2±67.9	338.0±56.3	-0.2	30.9	–	15.1
	10%	124.4±82.4	215.8±93.9	-0.3	30.7	–	8.3
Ocimum basilicum oil	1%	267.8±106.5	411.8±66.3	-0.2	30.4	–	17.9
	5%	94.0±36.4*	201.2±88.0	-0.3	48.8	–	6.3
	10%	86.0±55.4*	409.8±76.0	-0.7	79.4	–	5.7
Syzygium aromaticum oil	1%	337.2±114.7	292.2±96.0	0.1	–	3.6	22.5
	5%	180.4±105.6	382.8±129.9	-0.3	41.8	–	12
	10%	36.6±29.1*	520.6±103.7	-0.9	93.3	–	2.4

* Significant differences between tested and control by paired *t*-test ($P< 0.05$)
** The OAI ranges from -1 to +1; the positive index values (+) indicated that the test solutions were attractants; the negative index values (–) indicated that the test solutions were deterrents and 0 indicating neutral response
OAI = Oviposition Active Index; *ER* = Effective Repellency; *EA* = Effective Attractancy

Table 3. The oviposition deterrent/ attractant/ neutral of the essential oils in three concentrations (1%, 5% and 10%) against *An. dirus*

Herbal essential oils		Number of eggs±SD		OAI**	ER%	EA%	No. of tested eggs laid per female
		Tested	Control				
Cananga odorata oil	1%	162.8±71.6*	903.8±259.0	-0.7	79.5	–	10.9
	5%	103.4±151.0*	1030.2±275.1	-0.9	91.3	–	6.9
	10%	32.2±39.8*	1060.0±63.8	-0.9	97.1	–	2.1
Citrus sinensis oil	1%	358.5±123.6	307.8±456.7	0.4	–	32.0	23.9
	5%	513.4±184.3*	273.0±130.7	0.3	–	42.9	34.2
	10%	482.2±361.0	279.4±170.6	0.2	–	14.3	32.1
Cymbopogon citratus oil	1%	228.0+288.3	303.0+124.9	-0.3	14.1	–	15.2
	5%	275.6±286.9	503.6±226.3	-0.4	32.5	–	18.4
	10%	109.8±46.5*	859.6±228.4	-0.8	85.6	–	7.3
Cymbopogon nardus oil	1%	252.2+164.0	645.4+272.8	-0.4	33.6	–	16.8
	5%	266.2±102.8*	716.8±229.4	-0.4	59.1	–	17.7
	10%	124.6±152.3*	962.8±479.1	-0.7	71.9	–	8.3
Eucalyptus citriodora oil	1%	279.0+192.5	331.4+124.4	-0.1	12.4	–	18.6
	5%	132.2±36.8*	375.6±236.0	-0.4	57.3	–	8.8
	10%	129.0±138.8*	494.2±69.3	-0.6	75.3	–	8.6
Ocimum basilicum oil	1%	109.6±107.6*	341.8±148.3	-0.6	66.1	–	7.3
	5%	66.2±42.1*	419.0±261.1	-0.6	73.3	–	4.4
	10%	44.0±28.6*	294.6±156.4	-0.7	81.6	–	2.9
Syzygium aromaticum oil	1%	304.4+204.6*	374.2+180.2	-0.1	22.7	–	20.3
	5%	137.2+51.8*	291.2+63.3	-0.4	52.5	–	9.1
	10%	199.2±131.0*	514.2±138.3	-0.4	54	–	13.3

* Significant differences between tested and control by paired *t*-test ($P< 0.05$)
** The OAI ranges from -1 to +1; the positive index values (+) indicated that the test solutions were attractants; the negative index values (–) indicated that the test solutions were deterrents and 0 indicating neutral response
OAI = Oviposition Active Index; *ER* = Effective Repellency; *EA* = Effective Attractancy

903.8±259.0 eggs per cup, respectively. Furthermore, significant differences in means of numbers of eggs laid on tested and control were statistically analysed by using ANOVA ($P<0.05$). Additionally, the number of eggs laid was observed in water with 10% *Ca. odorata* oil which established its maximum oviposition deterrence with 97.1% effective repellency and -0.9 OAI. On the other hand *Ci. sinensis* provided no repellency, the oviposition activity index (OAI) indicated that *Ci. sinensis* acted as oviposition attractant.

Meanwhile the oviposition deterrent effects of seven essential oils against *Cx. quinquefasciatus* are shown in Table 4. At 10% concentration, *Ca. odorata*, *C. citratus* and *O. basilicum* exhibited oviposition deterrent activity against *Cx. quinquefasciatus* with degree of repellency (ER%) at 100% and -1 OAI. There were significant differences in the numbers of eggs laid on test and control ($P<0.05$). *Culex quinquefasciatus* did not lay any eggs in the 10% *Ca. odorata*, *C. citratus* and *O. basilicum* cups. A mean±SD of 909.8±490.8 eggs, 1112.8±285.7 eggs and 150.6±102.9 eggs were laid in the control cups, respectively.

Ovicidal activity

The percentage of egg hatchability of three mosquito species, *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus* with the essential oils of *Ca. odorata*, *Ci. sinensis*, *C. citratus*, *C. nardus*, *E. citriodora*, *O. basilicum* and *S.*

Table 4. The oviposition deterrent/ attractant/ neutral of the essential oils in three concentrations (1%, 5% and 10%) against *Cx. quinquefasciatus*

Herbal essential oils		Number of eggs±SD		OAI**	ER%	EA%	No. of tested eggs laid per female
		Tested	Control				
Cananga odorata oil	1%	19.4±43.4	277.8±244.9	-0.9	89.9	–	1.3
	5%	5.0±11.2*	446.4±313.1	-1	99.2	–	0.3
	10%	0.0*	909.8±490.8	-1	100	–	0.0
Citrus sinensis oil	1%	228.0+183.8	163.4+223.8	0.5	–	6.2	15.2
	5%	119.2+88.0	79.6+79.8	0.1	–	24.3	7.9
	10%	159.4+59.2	247.2+91.2	-0.2	16.6	–	10.6
Cymbopogon citratus oil	1%	336.4±54.2	689.0±360.2	-0.3	23.5	–	22.4
	5%	145.0±85.3*	841.0±166.4	-0.7	82.1	–	9.7
	10%	0.0*	1112.8±285.7	-1	100	–	0.0
Cymbopogon nardus oil	1%	155.4±97.9*	382.2±81.7	-0.5	59.4	–	10.4
	5%	123.0±178.3	277.6±161.5	-0.6	40	–	8.2
	10%	11.0±24.6*	1222.6±142.6	-1	98.9	–	0.7
Eucalyptus citriodora oil	1%	41.4±68.5	345.4±367.1	-0.4	58.1	–	2.8
	5%	77.4±74.0*	720.8±216.1	-0.8	84.9	–	5.2
	10%	48.0±63.5*	710.4±468.1	-0.9	92.3	–	3.2
Ocimum basilicum oil	1%	11.0±24.6	376.4±466.8	-0.9	95.2	–	0.7
	5%	0.0*	477.6±332.2	-1	100	–	0.0
	10%	0.0*	150.6+102.9	-1	100	–	0.0
Syzygium aromaticum oil	1%	104.6±59.5	223.0±125.6	-0.3	34.6	–	7.0
	5%	15.4±23.1	337.0±325.5	-0.7	74.3	–	1.0
	10%	7.8±11.4*	359.0±134.0	-1	98.4	–	0.5

* Significant differences between tested and control by paired *t*-test ($P < 0.05$)
** The OAI ranges from -1 to +1; the positive index values (+) indicated that the test solutions were attractants; the negative index values (–) indicated that the test solutions were deterrents and 0 indicating neutral response
OAI = Oviposition Active Index; *ER* = Effective Repellency; *EA* = Effective Attractancy

aromaticum are presented in Table 5. The hatching rates of *Ae. aegypti* eggs exposed to *Ca. odorata* and *O. basilicum* were significantly lower from those of the others essential oils. Hatching rate at 1% conc. *Ca. odorata* and *O. basilicum* were 20.8% and 22.4%, reducing to 12.8% and 16.8% at 5% conc. and down to 10.4% and 16.8% at 10% conc., respectively. There was an inverse relationship between concentration and the magnitude of hatching rate. With probit analysis, the EC_{50} was calculated as less than 1.9% for both *Ca. odorata* and *O. basilicum*.

The same trends in *An. dirus*, the concentration of *Ca. odorata* and *O. basilicum* oils increased while the hatching rates decreased from 33.6% and 28.8% at 1% conc. to 3.2% and 26.4% at 5% conc. until to 0.8% and 5.6% at 10% conc., respectively. ED_{50} values of both *Ca. odorata* and *O. basilicum* were less than 1.4%.

While the result showed that only the ovicidal effect of 1%, 5% and 10% of *Ca. odorata* highly exhibited significant effects against *Cx. quinquefasciatus* ($P<0.05$), producing 13.3%, 3.9% and 1.1%, respectively. The six essential oils except *E. citriodora* gave strong effective dose (ED_{50}) values at less than 0.5%. Furthermore, the result suggests that the hatching rates of three mosquito species eggs exposed to various concentrations of essential oils were significantly lower than those of control eggs. Pure soybean oil and distilled water that served as a control showed 85.6-94.2% and 90.4-97.4% (for *Ae. aegypti*), 73.6-83.2% and

Table 5. Ovicidal activity of seven essential oils against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*

Herbal essential oils	*Ae. aegypti*				*An. dirus*				*Cx. quinquefasciatus*			
	%Hatching±SD[1/]			EC_{50} (%)	%Hatching±SD[1/]			EC_{50} (%)	%Hatching±SD[1/]			EC_{50} (%)
	1%	5%	10%		1%	5%	10%		1%	5%	10%	
Cananga odorata oil	20.8±20.3^{d}	12.8±18.2^{c}	10.4±9.2^{b}	<1.9	33.6±7.8^{d}	3.2±3.3^{d}	0.8±1.8^{d}	<1.4	13.3±12.5^{d}	3.9±7.8^{d}	1.1±2.4^{d}	<0.5
Citrus sinensis oil	69.6±11.5^{ab}	43.2±20.1^{b}	26.4±9.6^{b}	4.5	72.0±11.0^{b}	46.4±8.3^{b}	28.8±1.8^{c}	5.1	44.8±15.1^{bc}	23.7±6.1^{bc}	22.5±12.9^{bc}	<0.5
Cymbopogon citratus oil	28.8±22.5^{cd}	20.0±17.4^{bc}	18.4±7.3^{b}	<1.9	72.0±22.3^{b}	47.2±17.1^{b}	23.2±7.7^{c}	4.8	28.6±17.0^{cd}	11.4±8.5^{cd}	9.8±8.0^{cd}	<0.5
Cymbopogon nardus oil	46.4±36.8^{bcd}	25.6±23.6^{bc}	22.4±24.3^{b}	<1.9	53.6±5.4^{c}	37.6±11.9^{bc}	35.2±6.6^{bc}	1.4	32.3±24.4^{bc}	24.1±14.0^{bc}	12.2±9.1^{cd}	<0.5
Eucalyptus citriodora oil	88.0±11.0^{a}	86.4±10.8^{a}	85.6±10.0^{a}	96.6	66.4±14.3^{bc}	48.8±16.3^{b}	44.8±19.1^{b}	6.8	50.8±11.2^{b}	23.2±10.6^{bc}	16.0±13.3^{bc}	0.5
Ocimum basilicum oil	22.4±3.6^{d}	16.8±3.3^{bc}	16.8±1.8^{b}	<1.9	28.8±15.3^{d}	26.4±12.5^{c}	5.6±4.6^{d}	<1.4	33.2±8.9^{bc}	22.2±20.7^{bc}	19.7±12.5^{bc}	<0.5
Syzygium aromaticum oil	52.8±33.4^{bc}	40.8±46.0^{bc}	26.4±36.2^{b}	1.9	65.6±14.6^{bc}	44.8±10.4^{b}	31.2±5.9^{c}	4.6	32.7±7.4^{bc}	28.4±15.3^{b}	26.7±8.6^{b}	<0.5
Control (Water)	97.4±1.5^{a}	90.4±4.6^{a}	95.2±5.2^{a}		89.6±8.3^{a}	84.8±6.6^{a}	78.4±9.6^{a}		93.2±5.7^{a}	92.6±6.4^{a}	87.2±3.7^{a}	
Control (Soybean Oil)	94.2±0.8^{a}	88.0±6.3^{a}	85.6±8.3^{a}		80.0±5.7^{ab}	83.2±11.1^{a}	73.6±9.6^{a}		86.6±5.3^{a}	90.7±6.1^{a}	81.7±8.3^{a}	
CV (%)	34.9	44.2	37.4		20.4	24.7	24.9		24.9	32.7	30.8	

[1/] Means in each column followed by the same letter are not significantly different (P>0.05, by one-way ANOVA and Duncan's Multiple Range Test)

78.4-89.6% (for *An. dirus*) and 81.7-90.7% and 87.2-93.2% (for *Cx. quinquefasciatus*), respectively.

DISCUSSION

This study has revealed that the essential oil of *Ca. odorata* showed both oviposition-deterrent and ovicidal activities, towards the three mosquito species. *Cananga odorata*, known as ylang–ylang, is a large tree (up to 33-50 feet tall). Its leaves are long, smooth and glossy. Its flowers are greenish yellow and curly, each one resembling a starfish, and they yield a highly fragrant essential oil (Nagashima *et al.*, 2010). The tree is native to Malaysia and Indonesia, but is now naturalized to most of the larger Pacific Island groups, northern Australia, Thailand, and Vietnam (Clifford & Kobayashi, 2010). *Cananga odorata* oil is primarily extracted from the flowers by water or water-and-steam distillation and the fraction will vary in physical/chemical properties (Burdock & Carabin, 2008). The phytochemical constituents of *Ca. odorata* are appreciable such as phenols, eugenol, methyleugenol, isoeugenol, limonene, geraniol, cinnamaldehyde (Burdock & Carabin, 2008). These constituents have properties to act as toxins, feeding deterrents and oviposition deterrents to a wide variety of insect pests (Koul *et al.*, 2008). Unfortunately, studies reported in the literature of the toxicity of *Ca. odorata* oil, as a whole, are very limited. Chu *et al.* (1998) reports that extracts of the flowers of *Ca. odorata* obtained from Southeast Asia exhibited amebicidal activity against three species of Acanthamoeba. Additionally, Maudsley & Kerr (1999) investigated the sterility and antibacterial activity of eight commercially available essential oil products including *Ca. odorata* oil. The result showed that *Ca. odorata* oil exhibited modest inhibitory activity against bacterial strains, coagulase-negative Streptococcus, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Stenotrophomonas maltophilia* and *Candida albicans*. Furthermore, Caballero-Gallardo *et al.* (2011) also reports that essential oils from *Ca. odorata* and *Lippia alba* showed the most active repellent properties against *Tribolium castaneum*.

However, many researches of oviposition-deterrent and ovicidal don't succeed as expected. Also the lack of documents of *Ca. odorata* oil exhibiting both activities against mosquito have not been investigated to any great extent because there are many factors involved from both plant and mosquito eggs. In term of plants, these affecting factors are the quality of essential oils include plant species, cultivating conditions, maturation of harvested plants, plant storage, plant preparation and methods of extraction. (Tawatsin *et al.*, 2001). Furthermore, the eggs of mosquitoes are found to be much more tolerant to the action of insecticides compared to larval stage. Mosquito eggshell are hardened and studies conducted on freshly laid eggs were limited (Chenniappan & Kadarkarai, 2008). Insect eggs are covered with shell, which differs biochemically from the integument of the larvae, and the difference in penetration of the insecticide through the eggshell (Kuppusamy & Murgan, 2009).

In the present study it was found that 10% *Ca. odorata* in soybean oil showed high potential for oviposition-deterrent and ovicidal action against three mosquito species, *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*. This plant oil has potential for the development of new and safe control product for mosquito vectors. Thus, effective oviposition-deterrent and ovicidal could be useful and developed further in the integrated approach to mosquito control programmes against container-inhabiting mosquitoes (Xue *et al.*, 2005). These results could encourage the search for new active natural compounds offering an alternative to synthetic ovicidal and oviposition-deterrent from other Thai indigenous plants.

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