LARVICIDAL AND PUPICIDAL ACTIVITIES OF ESSENTIAL OILS FROM ZINGIBERACEAE PLANTS AGAINST *AEDES AEGYPTI* (LINN.) AND *CULEX QUINQUEFASCIATUS* SAY MOSQUITOES

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Abstract. We conducted this study to investigate the efficacy of herbal essential oils from 12 species of Zingiberaceae plants to determine their larvicidal and pupicidal activity against fourth instar larvae and pupae of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes. Probit analysis was used to analyze the data. Larval mortality was recorded at 1, 5, 10, 15, 30 and 60 minutes and 24 hours. Pupal mortality was recorded at 15 and 30 minutes and 1, 3, 6, 12, 24 and 48 hours. All the essential oils tested showed larvicidal activity. *Zingiber cassumunar* and *Amomum biflorum* oils proved to have the greatest activity against *Ae. aegypti* larvae with LT_{50} of 1.4 minutes and 100% mortality at 5 and 10 minutes, respectively. *Boesenbergia rotunda*, *Curcuma zedoaria* and *Hedychium coronarium* essential oils had activity against *Cx. quinquefasciatus* larvae with LT_{50} of 1.7 minutes and 100% mortality at 10 minutes, 5 minutes and 15 minutes, respectively. All the herbal essential oils tested resulted in 100% mortality against *Ae. aegypti* and *Cx. quinquefasciatus* larvae at 60 minutes and 30 minutes, respectively. *Ae. aegypti* and *Cx. quinquefasciatus* pupae were susceptible to *Z. ottensii* oil (LT_{50} of 0.2 hour) and *Z. zerumbet* oil (LT_{50} of 0.6 hour) and had pupicidal activity with 100% mortality at 6 and 3 hours, respectively. All the essential oils test had pupicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* by inducing 100% mortality at 48 hours.

Keywords: herbal essential oil, larvicide, pupicide, *Aedes aegypti*, *Culex quinquefasciatus*

INTRODUCTION

Mosquitoes are a major public health problem worldwide. Vector borne diseases carried by mosquitoes cause major health problems in many countries (Kovendan and Murugan, 2011). *Aedes aegypti*, the primary vector for dengue and yellow fever is distributed widely in the tropics and subtropics (Paul *et al*, 2006). *Cx. quinquefasciatus* is a vector of Japanese encephalitis (JE) and causes annoyance, may cause dermatitis (Shultz *et al*, 2008) and is a vector for lymphatic filariasis. (Bernhard *et al*, 2003).

A common approach for mosquito vector control and to reduce vector

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borne diseases is chemical insecticide intervention measures (Paul *et al*, 2006). Synthetic insecticides may have adverse environmental effects, high cost and poor community acceptance (Sutthanont *et al*, 2010). Natural products used as insecticides may have less of an environmental impact due to shorter latency, possibly resulting in reduced resistance (Hardin and Jackson, 2009).

Larviciding is an effective method to reduce mosquito densities before they emerge as adults and synthetic insecticides have been widely used for this purpose (Tiwary *et al*, 2007). The larvicides may best be used in small breeding places, such as containers and coolers, where the water is stagnant (Ansari *et al*, 2005) because it is easier to kill larvae in stagnant water than to kill adult mosquitoes. Essential oils of many plants have been observed to have mosquito larvicidal and pupicidal properties (Tewtrakul *et al*, 1998; Komalamisra *et al*, 2005; Promsiri *et al*, 2006; Phasomkusolsil and Soonwera, 2010).

The purpose of this study was to investigate the larvicidal and pupicidal activities of essential oils from 12 species of Zingiberaceae plants against *Aedes aegypti* and *Culex quinquefasciatus* using a standard WHO method under laboratory conditions (WHO, 2005).

MATERIALS AND METHODS

Mosquitoes

In this study, *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes were used. Both types of mosquitoes were raised in the Entomology and Environment Laboratory, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. The mosquitoes were maintained and all experiments carried out at 26-28ºC with 70-80% relative humidity. Adult mosquitoes were maintained in cages (30x30x30 cm) and fed 5% glucose solution in water soaked on cotton pads. On days 5-7, the females were given a blood meal via artificial membrane method. Two to three days after the blood meal, the gravid mosquitoes laid their eggs. Larvae were reared in plastic trays (30x35x5 cm) containing 2 liters of tap water and fed on fine fish food. Fourth instar larvae and pupae were used for the experiments.

Plant materials

Twelve herbal essential oils (10%) in ethyl alcohol were used in this study (Table 1). These oils were provided by the medicinal plant laboratory, Faculty of Agricultural Technology, KMITL. All formulations were kept at room temperature before testing.

Bioassay procedures

The test procedures were conducted according to World Health Organization (2005) recommendations. One milliliter of test oil was added to 99 ml distilled water in a 250 ml plastic cup, which was shaken lightly to ensure a homogeneous test solution. Twenty-five specimens each of fourth instar larvae and pupae of *Ae. aegypti* and *Cx. quinquefasciatus* were divided into respective groups and placed in cups. No food was provided during the treatment. The larval mortality was recorded at 1, 5, 10, 15, 30 and 60 minutes and at 24 hours while pupal mortality was recorded at 15 and 30 minutes and 1, 3, 6, 12, 24 and 48 hours. Larvae were considered dead if they were incapable of rising to the surface or did not show the characteristic diving reaction when the water was disturbed (Tiwary *et al*, 2007). The mean mortality was recorded. Each experiment was performed in five repli-

Table 1
List of Zingiberaceae plants tested in this study.

Scientific name	Parts used	Perported therapeutic properties
Alpinia sp "ka luang"	Rhizomes	Anti-inflammatory, antioxidant, carminative, analgesic, anti-allergic, antimicrobial, antibacterial, antifungal, antidiabetic, anti-ulcer, immuno stimulating, anticancer, insecticide.
Amomum biflorum Jack	Leaves	Anti-inflammatory, antioxidant, carminative, tyrosinase inhibitory, antibacterial, cosmetic, relaxation.
Boesenbergia rotunda (L.) Mansf	Roots, rhizomes	Anti-inflammatory, antioxidant, analgesic, antimutagenic, antitumor, antibacterial, antifungal, antipyretic, anti-spasmodic, insecticidal, mosquito larvicidal.
Curcuma comosa Roxb	Rhizomes	Anti-inflammatory, antioxidant, nematocidal, herb for women, treat irregular menses.
Curcuma longa Linn	Rhizomes	Anti-inflammatory, antiseptic, antioxidant, carminative, anti-spasmodic, antimicrobial, antibacterial, antifungal, tonic, antimutagenic hepatoprotective, cosmetic, cytotoxicity, mosquito anti-repellant, mosquito larvicidal.
Curcuma xanthorrhiza Roxb	Rhizomes	Anti-inflammatory, antioxidant, nematocidal, antiviral, decreases cholesterol, herb for women, treat irregular menses, nematocidal, traditional medicine.
Curcuma zedoaria Rosc	Rhizomes	Anti-inflammatory, antioxidant, carminative, analgesic, antimicrobial, antitumor, anticlastogenic, anti-tyrosinase, cytotoxicity, perfumery, insecticidal.
Etlingera littoralis Gieseke	Leaves	Antioxidant, antibacterial, tyrosinase inhibition, lipid peroxidation inhibition, traditional medicine.
Hedychium coronarium J.Konig	Rhizomes	Anti-inflammatory, antibacterial, analgesic, antioxidant, antimicrobial, antifungal, antibacterial, cytotoxic, tyrosinase inhibition, mosquito larvicidal.
Zingiber cassumunar Roxb	Rhizomes	Anti-inflammatory, analgesic, anti-asthmatic, antiseptic, tonic, carminative, anti-hypotensive, antitoxic, anti-neuralgic, antimicrobial, antibacterial, anti-spasmodic, anti-viral, mosquitos repellent.
Zingiber ottensii Valeton	Rhizomes	Anti-inflammatory, antioxidant, carminative, anti-proliferative against fungi and human cancer, antibacterial.
Zingiber zerumbet (L.) Sm.	Rhizomes	Anti-inflammatory, antipyretic, anti-allergic, antiseptic, tonic, antioxidant, cytotoxic, antitumor, antiplatelet, antimicrobial, antibacterial, hepato-protective, tyrosinase inhibition, mosquito larvicidal.

Table 2
LT_{50} values of twelve essential oils from Zingiberaceae plants against fourth instar larvae and pupae of *Ae. aegypti* and *Cx. quinquefasciatus*.

Herbal essential oils	LT_{50} (min) in larval stage		LT_{50} (hr) in pupal stage	
	Ae. aegypti	*Cx. quinquefasciatus*	*Ae. aegypti*	*Cx. quinquefasciatus*
Alpinia sp	13.2 (12.6-14.0)	3.7 (-)	0.4 (0.1-0.7)	1.0 (0.8-1.3)
Am. biflorum oil	1.4 (1.0-1.8)	2.2 (1.9-2.6)	1.0 (0.9-1.1)	1.9 (1.8-2.1)
B. rotunda oil	2.3 (2.0-2.7)	1.7 (1.4-2.1)	1.7 (<2.7-4.6)	4.1 (-)
C. comosa oil	7.5 (7.0-8.0)	8.1 (7.6-8.7)	6.6 (4.1-10.2)	2.4 (2.2-2.6)
C. longa oil	8.3 (-)	3.8 (3.3-4.2)	8.4 (6.3-11.5)	3.5 (3.1-3.9)
C. xanthorrhiza oil	6.5 (5.9-7.0)	5.7 (4.9-6.5)	29.6 (26.8-33.1)	15.0 (13.3-17.2)
C. zedoaria oil	4.0 (3.4-4.3)	1.7 (1.4-2.0)	9.3 (6.8-12.8)	1.9 (1.6-2.2)
E. littoralis oil	3.2 (2.9-3.5)	3.7 (3.0-4.3)	3.1 (<0.1-5.8)	1.0 (0.9-1.1)
H. coronarium oil	6.1 (-0.9-10.3)	1.7 (1.1-2.2)	0.7 (-)	10.8 (7.6-14.9)
Z. cassumunar oil	1.4 (1.0-1.7)	3.0 (2.7-3.3)	23.9 (21.5-27.0)	6.8 (6.1-7.7)
Z. ottensii oil	2.6 (2.3-2.9)	2.2 (1.9-2.8)	0.2 (<0.7-0.6)	2.4 (2.2-2.6)
Z. *zerumbet* oil	2.6 (2.3-3.0)	3.6 (3.3-4.0)	4.8 (1.4-9.5)	0.6 (0.6-0.7)

LT_{50}, lethal time for 50% mortality at 95% confidence limit.

cates with a simultaneous control (1 ml ethyl alcohol in 99 ml water). Lethal time for 50% mortality (LT_{50}) values were calculated using probit analysis. The mortality data were analyzed by Duncan's multiple range test using SPSS for Windows, version 16.0 (SPSS, Chicago, IL).

RESULTS

The LT_{50} values of the essential oils of 12 species of Zingiberaceae plants against fourth instar larvae and pupae of *Ae. aegypti* and *Cx. quinquefasciatus* are shown in Table 2. *Z. cassumunar* and *Amomum biflorum* oils proved to be the most effective larvicides against *Ae. aegypti* larvae with the lowest LT_{50} (1.4 minutes) while the essential oils most active against *Cx. quinquefasciatus* larvae were *B. rotunda* oil, *C. zedoaria* oil and *H. coronarium* oil (LT_{50} 1.7 minutes). The least effective essential oils against *Ae. aegypti* and *Cx. quinquefasciatus* larvae with the highest LT_{50} were *Alpinia* sp oil and *C. comosa* oil (13.2 minutes and 8.1 minutes, respectively). The essential oils most effective against *Ae. aegypti* and *Cx. quinquefasciatus* pupae were *Z. ottensii* oil and *Z. zerumbet* oil with LT_{50} values of 0.2 and 0.6 hour, respectively, while *C. xanthorrhiza* oil was the least effective against *Ae. aegypti* and *Cx. quinquefasciatus* pupae with LT_{50} values of 29.6 and 15.0 hours, respectively.

All the essential oils induced 100% mortality against *Ae. aegypti* larvae by 60 minutes (Table 3). *Am. biflorum* oil and *Z. cassumunar* oil had the greatest larvicidal activity at 1 minute with 43.2% mortality; a significant difference from the other essential oils. *Z. cassumunar* oil gave 100% larval mortality at 5 minutes. The essential oils from *Am. biflorum*, *B. rotunda*, *Z. ottensii* and *Z. zerumbet* were highly effective against *A. aegypti* larva with 97.6% and 100% mortality at 5 and 10 minutes, re-

Table 3
Larvicidal activity of herbal essential oils against fourth instar larvae of *Ae. aegypti*.

Herbal essential oils	% Mortality±SD at specified time						
	1 min	5 min	10 min	15 min	30 min	60 min	24 hr
Alpinia sp oil	0.0^{c}	2.4±3.6^{e}	22.4±8.3^{d}	66.4±8.3^{c}	100.0±0.0^{a}	100.0±0.0^{ns}	100.0±0.0^{ns}
Am. biflorum oil	43.2±12.8^{a}	97.6±2.2^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0
B. rotunda oil	21.6±4.6^{b}	97.6±2.2^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0
C. comosa oil	0.0^{c}	33.6±6.1^{d}	68.8±9.1^{c}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0
C. longa oil	0.0^{c}	36.0±10.6^{d}	62.4±9.2^{c}	93.6±8.3^{ab}	99.2±1.8^{a}	100.0±0.0	100.0±0.0
C. xanthorrhiza oil	0.0^{c}	24.0±25.1^{d}	96.0±5.7^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0
C. zedoaria oil	0.0^{c}	82.4±7.3^{b}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0
E. littoralis oil	3.2±4.4^{c}	93.6±4.6^{ab}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0
H. coronarium oil	0.0±0.0^{c}	64.0±17.2^{c}	81.6±12.2^{b}	89.6±12.5^{b}	94.4±8.8^{b}	100.0±0.0	100.0±0.0
Z. cassumunar oil	43.2±11.1^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0
Z. ottensii oil	9.6±6.1^{c}	97.6±2.2^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0
Z. zerumbet oil	9.6±13.4^{c}	97.6±3.6^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0
Control (Ethyl alcohol)	0.0^{c}	0.0^{e}	0.0^{e}	0.0^{d}	0.0^{c}	0.0	0.0
CV%	64.49	15.05	7.15	5.38	2.70	NA	NA

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). ns, not significant.

Table 4
Larvicidal activity of herbal essential oils against the fourth instar larvae of *Cx. quinquefasciatus*.

Herbal essential oils	% Mortality ± SD at specified time						
	1 min	5 min	10 min	15 min	30 min	60 min	24 hr
Alpinia sp oil	0.0^{d}	95.2±4.4^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{ns}	100.0±0.0^{ns}	100.0±0.0^{ns}
Am. biflorum oil	21.6±6.1^{b}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0	100.0±0.0
B. rotunda oil	34.4±7.3^{a}	99.2±1.8^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0	100.0±0.0
C. comosa oil	0.0^{d}	17.6±4.6^{d}	77.6±12.5^{c}	95.2±4.4^{b}	100.0±0.0	100.0±0.0	100.0±0.0
C. longa oil	10.4±.2.2^{c}	75.2±1.8^{b}	97.6±2.2^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0	100.0±0.0
C. xanthorrhiza oil	2.4±2.2^{d}	54.4±30.1^{c}	85.6±10.4^{b}	100.0±0.0^{a}	100.0±0.0	100.0±0.0	100.0±0.0
C. zedoaria oil	35.2±7.7^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0	100.0±0.0
E. littoralis oil	13.6±5.4^{c}	77.6±8.8^{b}	95.2±6.6^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0	100.0±0.0
H. coronarium oil	39.2±3.3^{a}	90.4±4.6^{a}	99.2±1.8^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0	100.0±0.0
Z. cassumunar oil	0.0^{d}	92±2.8^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0	100.0±0.0
Z. ottensii oil	11.2±5.2^{c}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0	100.0±0.0
Z. zerumbet oil	0.0^{d}	89.6±6.7^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0	100.0±0.0	100.0±0.0
Control (Ethyl alcohol)	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{c}	0.0	0.0	0.0
CV%	32.24	12.10	5.56	1.32	NA	NA	NA

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).
ns, not significant.

Table 5
Pupicidal activity of herbal essential oils against the pupae of *Ae. aegypti*.

Herbal essential oils	% Mortality ± SD at specified time							
	15 min	30 min	1 hr	3 hr	6 hr	12 hr	24 hr	48 hr
Alpinia sp oil	21.6±23.6^{b}	56.8±28.1^{a}	91.2±5.9^{a}	96.8±1.8^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{ns}
Am. biflorum oil	0.0^{c}	16.8±6.6^{c}	50.4±20.1^{d}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
B. rotunda oil	8.8±3.3^{bc}	36.8±9.5^{b}	62.4±5.4^{cd}	73.6±6.1^{cd}	78.4±7.3^{abc}	80±6.3^{b}	83.2±7.2^{c}	100.0±0.0
C. comosa oil	0.0^{c}	0.0^{c}	3.2±3.3^{f}	57.6±31.1^{de}	77.6±20.7^{bc}	84.0±10.6^{b}	93.6±5.4^{abc}	100.0±0.0
C. longa oil	0.0^{c}	0.8±1.8^{c}	2.4±2.2^{f}	26.4±15.1^{f}	51.2±43.0^{d}	83.2±15.6^{b}	94.4±5.4^{abc}	100.0±0.0
C. xanthorrhiza oil	0.0^{c}	0.0^{c}	0.0^{f}	0.0^{g}	0.0^{e}	11.2±10.4^{cd}	15.2±14.0^{e}	100.0±0.0
C. zedoaria oil	0.0^{c}	0.0^{b}	0.8±1.8^{f}	47.2±15.3^{e}	62.4±17.1^{cd}	76.8±16.3^{b}	84.0±18.8^{bc}	100.0±0.0
E. littoralis oil	0.0^{c}	15.2±4.4^{c}	54.4±10.8^{d}	78.4±15.4^{bc}	86.4±11.2^{ab}	89.6±7.3^{ab}	93.6±6.7^{abc}	100.0±0.0
H. coronarium oil	20±8.5^{b}	42.4±6.7^{ab}	72±13.0^{bc}	96.0±6.9^{a}	97.6±3.6^{ab}	98.4±3.6^{a}	100.0±0.0^{a}	100.0±0.0
Z. cassumunar oil	0.0^{b}	0.0^{b}	0.0^{c}	0.8±1.8^{g}	8.8±10.0^{e}	20.8±15.3^{c}	40.8±11.1^{d}	100.0±0.0
Z. ottensii oil	43.2±34.3^{a}	54.4±39.3^{ab}	82.4±17.1^{ab}	92.8±10.0^{ab}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
Z. zerumbet oil	0.0^{c}	0.8±1.8^{c}	21.6±7.8^{e}	73.6±17.6^{cd}	84.0±6.9^{b}	89.6±4.6^{ab}	95.2±3.3^{ab}	100.0±0.0
Control (Ethyl alcohol)	0.0^{c}	0.0^{c}	0.0^{f}	0.0^{g}	0.0^{e}	0.0^{d}	0.0^{f}	0.0
CV%	148.35	81.00	27.45	22.57	22.98	12.76	10.41	NA

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).
ns, not significant.

Table 6
Pupicidal activity of herbal essential oils against the pupae of *Cx. quinquefasciatus*.

Herbal essential oils	% Mortality ± SD at specified time							
	15 min	30 min	1 hr	3 hr	6 hr	12 hr	24 hr	48 hr
Alpinia sp oil	11.2±5.2^{a}	31.2±16.1^{b}	68.0±20.8^{b}	92.8±7.2^{ab}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{ns}
Am. biflorum oil	0.8±1.8^{bc}	9.6±6.1^{c}	28.8±3.3^{de}	87.2±7.7^{ab}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
B. rotunda oil	2.4±3.6^{bc}	12.8±11.8^{c}	25.6±20.7^{de}	61.6±18.2^{d}	90.4±8.3^{a}	94.4±4.6^{a}	96.0±4.0^{a}	100.0±0.0
C. comosa oil	0.0^{c}	1.6±2.2^{c}	8.0±5.7^{efg}	74.4±11.5^{bcd}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
C. longa oil	0.0^{c}	0.0^{c}	4.8±4.4^{fg}	60.8±19.5^{d}	86.4±10.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
C. xanthorrhiza oil	2.4±2.2^{bc}	3.2±3.3^{c}	4.8±5.2^{fg}	8.0±9.4^{e}	13.6±17.8^{d}	26.4±13.4^{c}	90.4±12.8^{a}	100.0±0.0
C. zedoaria oil	1.6±3.6^{bc}	2.4±5.4^{c}	34.4±23.6^{cd}	82.4±21.7^{abc}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
E. littoralis oil	2.4±5.4^{bc}	4.0±6.9^{c}	52.0±33.8^{bc}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
H. coronarium oil	5.6±3.6^{b}	10.4±6.1^{c}	23.2±9.5^{def}	55.2±19.9^{d}	58.4±17.8^{b}	60.0±17.2^{b}	67.2±19.7^{b}	100.0±0.0
Z. cassumunar oil	0.0^{c}	3.2±3.3^{c}	8.0±9.8^{efg}	12.0±12.3^{e}	31.2±21.8^{c}	92.8±4.4^{a}	100.0±0.0^{a}	100.0±0.0
Z. ottensii oil	4.0±2.8^{bc}	6.4±3.6^{c}	16.0±9.4^{defg}	66.4±19.9^{c}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
Z. zerumbet oil	11.2±6.6^{a}	48.8±20.5^{a}	90.4±8.2^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0
Control (Ethyl alcohol)	0.0^{c}	0.0^{c}	0.0^{g}	0.0^{e}	0.0^{e}	0.0^{d}	0.0^{c}	0.0
CV%	107.42	85.68	54.06	22.31	13.15	7.63	7.49	NA

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).
ns, not significant.

spectively. For *Cx. quinquefasciatus* larvae, all the essential oils induced 100% mortality at 30 minutes (Table 4). The essential oils from *Am. biflorum*, *C. zedoaria* and *Z. ottensii* caused 100% larval mortality at 5 minutes and the essential oils from *Alpinia* sp, *B. rotunda*, *Z. cassumunar* and *Z. zerumbet* caused 100% mortality at 10 minutes.

All the essential oils caused 100% mortality against *Ae. aegypti* pupae by 48 hours (Table 5). *Z. ottensii* oil had the best pupicidal activity by 15 minutes with 43.2% mortality; a significant difference from the other essential oils. *Alpinia* sp oil caused greater pupicidal activity than the other essential oils at 30 minutes and 1 hour with 56.8% and 91.2% mortality, respectively. *Am. biflorum* oil caused 100% mortality at 3 hours and *Alpinia* sp oil and *Z. ottensii* oil both caused 100% mortality at 6 hours.

All the essential oils cause 100% mortality among *Cx. quinquefasciatus* pupae at 48 hours (Table 6). *Z. zerumbet* oil caused 11.2, 48.8, 90.4 and 100% mortality at 15 and 30 minutes and 1 and 3 hours, respectively; a significant difference from the other essential oils. *E. littoralis* oil caused 100% mortality at 3 hours. The essential oils of *Alpinia* sp, *Am. biflorum*, *C. comosa*, *C. zedoaria*, *Z. zerumbet* and *Z. ottensii* all caused 100% mortality at 6 hours.

DISCUSSION

Essential oils from plants may be of potential benefit for mosquito control, since they have a rich source of bioactive compounds that may be biodegradable into nontoxic products and are potentially suitable for use in integrated management programs for mosquito control. All the essential oils from Zingiberaceae plants tested had larvicidal and pupicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus*. All the essential oils tested induced 100% mortality against *Ae. aegypti* and *Cx. quinquefasciatus* larvae at 60 minutes and 30 minutes, respectively, and had pupicidal activity against both *Ae. aegypti* and *Cx. quinquefasciatus* pupae by causing 100% mortality at 48 hours.

The essential oil from *Z. cassumunar* and *Am. biflorum* proved to be the most effective against *Ae. aegypti* larvae (LT_{50} value of 1.4 minutes). Jantan *et al* (2003) also found *Z. cassumunar* oil to be effective against mosquito larvae with a LC_{50} value less than 200 g/ml. Furthermore, the essential oils from *B. rotunda*, *C. zedoaria*, *E. littoralis*, *Z. ottensii* and *Z. zerumbet* also exhibited high larvicidal activity against *Ae. aegypti* larvae. Isa *et al* (2012) also found *B. rotunda* exhibited insecticidal properties. The larvicidal activity of *H. coronarium* oil seen in our study supports the finding of Ho (2011) who reported the leaf and rhizome oils of *H. coronarium* exhibited mosquito larvicidal activity.

Many researchers have reported the effectiveness of plant essential oils against mosquito larvae. Pitasawata *et al* (2007) and Champakaew *et al* (2007) found *C. zedoaria* had larvicidal activity against *Ae. aegypti*. *Z. zerumbet* oil was also found to have larvicidal activity (Tewtrakul *et al*, 1998). Sutthanont *et al* (2010) recommended the use of that oil to develop larvicides to against the vectors of mosquito-borne disease.

Tewtrakul *et al* (1998) found the rhizomes of *Z. zerumbet* had larvicidal and pupicidal activities. Kamaraj *et al* (2010) reported the hexane extract of *Z. zerumbet* had larval activity against *Cx. quinquefasciatus* and had potential to be used as an eco-friendly agent to control *Cx. quinquefasciatus*.

In conclusion, the twelve essential oils studied here from Zingiberaceae

plants displayed larvicidal and pupicidal activities. The Zingiberaceae plants are well known for their purported medicinal value and are distributed widely throughout the tropics, particularly in Southeast Asia (Jantan *et al*, 2003). These results may be useful for developing newer and possibly safer and more effective larvicidal and pupicidal products against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes.

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