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Efficacies of Essential Oils from Illiciaceae and Zingiberaceae Plants as Oviposition Deterrent, Ovicidal, and Adulticidal Agents against Females *Aedes albopictus* (Skuse) and *Anopheles minimus* (Theobald)

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Abstract At present, serious mosquito vectors throughout the world have developed resistance to chemical insecticides.Therefore, safe natural products for controlling mosquitoes are absolutely necessary. This study investigated the efficacy of herbal essential oil (EO) from Illiciaceae and Zingiberaceae plants, *Alpinia galanga* (*A. galanga*), *Amomum krervanh* (*A. krervanh*), *Curcuma zedoaria* (*C. zedoaria*), *Illicium verum* (*I. verum*), *Zingiber cassumunar* (*Z. cassumunar*) and *Zingiber mekongense* (*Z. mekongense*) against the females of *Aedes albopictus* (*Ae. albopictus*) and *Anopheles minimus* (*An. minimus*). All EOs at the high concentration (10%) were highly effective in oviposition deterrent, ovicidal, and adulticidal activities. Ten percent of *C. zedoaria* EO showed 100% effective repellency with an oviposition activity index (OAI) of -1.0 against the females of the two mosquito species, and 10% *I.verum* EO showed a high inhibition rate of 100% against the eggs of the two mosquitoes. Ten percent of *A. galanga* EO showed a high adulticidal activity against the females of *Ae. albopictus* and *An. minimus* with KT_{50} of 0.7 and 1 min at 1h, respectively, and 100% mortality at 24h as well as an LC_{50} of 7.5 and 2.9%, respectively. When compared with temephos and cypermethrin, the EOs from *C. zedoaria*, *I.verum* and *A. galanga* were more effective in oviposition deterrent and ovicidal activities than temephos and were equivalent in adulticidal activity to cypermethrin. To conclude, our data show that EOs from *C. zedoaria*, *I.verum* and *A. galanga* can be used as an oviposition deterrent, ovicidal and adulticidal agents against *Aedes albopictus* and *Anopheles minimus*.

Keywords: Herbal essential oil, Oviposition deterrent, Ovicidal activity, Adulticidal activity, *Aedes albopictus, Anopheles minimus.*

Introduction

Mosquito vectors are major medical and veterinary insect pests in Thailand, throughout Southeast Asia, and other tropical and subtropical areas of

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the world. They are serious vectors of human diseases such as dengue fever, dengue hemorrhagic fever, and malaria that transmit serious pathogens to more than 700,000,000 people annually and cause 700,000 deaths per year (Pompon *et al*., 2017; Akiner *et al*., 2016; The United Nations Children's Fund, 2004). *Aedes albopictus* (Skuse) (*Ae. albopictus*) also known as Asian tiger mosquito, is a major vector of dengue fever, yellow fever, zika and chikungunya diseases. It is widely distributed in Thailand, Southeast Asia, and other tropical and subtropical countries. (Akiner *et al*., 2016; The United Nations Children's Fund, 2004; Baskar *et al*., 2018; Leta *et al*., 2015). *Anopheles minimus* (Theobald) (*An. minimus*), also known as malarial mosquito vector, is a major vector of malaria in Indonesia, Bangladesh, Vietnam, China, Malaysia, Pakistan, Sri Lanka, Philippines, Lao, India, and Thailand (Sonkong *et al*., 2015; Sriwichai *et al*., 2016; Kounnavong *et al*., 2017). Malaria causes more than 400,000 deaths annually; most of them were children under 5 years of age (The United Nations Children's Fund, 2004; Sonkong *et al*., 2015; Sriwichai *et al.*, 2016). Presently, there are no effective vaccines for dengue fever, dengue hemorrhagic fever, and malaria. Chemical control is the first line of defense for mosquito vector control, especially during an epidemic of the mentioned diseases. Previously, organochlorine and synthetic parathyroid insecticides such as DDT, cypermethrin and permethrin have been effective insecticides for mosquito vector control. Unfortunately, most chemical insecticides have lost their efficacy because mosquitoes have developed resistance to them. Moreover, these chemical insecticides also have toxic side effects to humans, domestic animals, non-target organisms, and the environment. Not only are they persistent, they are also non-biodegradable and biomagnified through the food chain (Omondi *et al*., 2017; Killeen and Ranson, 2018; Dallegrave *et al*., 2018; Aguirre *et al*., 2016). Therefore, botanical insecticide is one of the best alternatives to chemical insecticide because it is ecofriendly and nontoxic to humans and non-target organisms. Plant essential oils (EOs) are several of the best botanical insecticides for mosquito vector control. They have been shown to be easily biodegraded in the environment and non-toxic to mammals, fishes, and birds (Benelli *et al*., 2018a; Benelli *et al*., 2018b). Researchers have reported that essential oils from the following plants exhibited high toxicity to larvae, pupae and adults of mosquito vectors (*Anopheles dirus*, *Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus*, *Aedes albopictus*) and house fly (*Musca domestica*): neem (*Azadirachta indica*), galanga (*Alpinia galanga*), ylang-ylang (*Canaga odorata*), star anise (*Iillicium verum*), cinnamon (*Cinnamomum verum*), clove (*Syzygium aromaticum*), peppermint (*Mentha piperita*), lemongrass (*Cymbopogon citratus*), lavender (*Lavandula angustifolia*), calamodin (*Citrus madurensis*), sweet orange (*Citrus sinensis*), eucalyptus (*Eucalyptus globulus*), *Zanthoxylum limonella*, ginger (*Zigiber officinale*), garlic (*Allium sativum*), and phlai (*Zigiber cassumunar*) (Govindarajan *et al*., 2018; Muturi *et al*., 2018; Shaalan and Canyon, 2018; Benelli *et al*., 2015; Soonwera and Phasomkusolsil, 2017; Pavela, 2015; Soonwera, 2015; Elango *et al*., 2009). However, published papers related to oviposition deterent, ovicidal and adulticidal activities of essential oils from Illiciaceae and Zingiberaceae plants against females and eggs of *Ae. albopictus* and *An. minimus* are still limited. Plant EOs from Illiciaceae and Zingiberaceae families, especially EOs from *Alpinia galanga*, *Amomum krervanh*, *Curcuma zedoaria*, *Zingiber cassumunar*, *Zingiber mekongense*, and *Illicium verum*, have been used to prevent and treat human illness with medicinal properties such as antimicrobial, antioxidant, antibacterial, antifungal, antiseptic, antidepressant, antispasmodic, anticancer, and antneuralgic. They have been shown to be highly toxic to larvae and pupae of *Aedes aegypti* and *Culex quinquefasciatus*, but their oviposition deterrent, ovicidal and adulticidal activities against *Ae. albopictus* and *An. dirus* (Soonwera and Phasomkusolsil, 2017; Pavela, 2015; Soonwera, 2015) have not been reported yet. Data on oviposition deterrent, ovicidal and adulticidal activities against *Ae. albopictus* and *An. minimus* are important for successful mosquito control at the breeding sites of female mosquito vectors to regulate strictly the density of new mosquito generation and completely eradicate mosquito vectors (Elango *et al*., 2009). Therefore, the objective of this study was to determine the oviposition deterent, ovicidal, and adulticidal activities against the females and eggs of *Aedes albopictus* and *Anopheles minimus* of six essential oils from *Alpinia galanga*, *Amomum krervanh*, *Curcuma zedoaria*, *Zingiber cassumunar*, *Zingiber mekongense*, and *Illicium verum* as well as these three activities of two synthetic insecticides, cypermethrin (Dethriod10®, 10% w/v) manufactured by Pentacheme Co.Ltd, 214 -216 Charoenakhon Road, Khongsan, Bangkok 10600,Thailand) and temephos (SaiGPO-1®, 1.0% w/w) manufactured by 138 Government Pharmaceutical Organization, Rangsit-NakhonNayok Road, Pathumthani province, Thailand).

Materials and methods

Plant materials

The plant materials used in this study were fresh rhizomes of one-year-old *Alpinia galanga*, *Amomum krervanh*, *Curcuma zedoaria*, *Zingiber cassumunar*, and *Zingiber mekongense*. They were collected from Rayong province, Thailand. The material from another plant, dried fruits of *Illium* *verum*, were purchased from Chao Krom Poe pharmacy, Bangkok, Thailand (Table 1). All plant specimens were positively identified by a botanical taxonomist from the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. Fresh rhizomes and dried fruits of the plants mentioned above were cleaned, cut into small pieces, and extracted for their essential oils (EOs) by a water distillation method. After the distillation was completed, all EOs were collected from the separating funnel, stored in airtight bottles, and kept at 4 ℃ for later experiments. All EOs were diluted to 1, 5 and 10% concentrations with ethyl alcohol and kept under laboratory conditions (27.3±2.3 ℃ and 70.5±2.5 %RH) before they were used in subsequent tests.

Mosquito rearing

The eggs of *Aedes albopictus* (*Ae. albopictus*) and *Anopheles minimus* (*An.minimus*) were provided by the Entomological Laboratory, Department of Plant Production Technology, Faculty of Agricultural Technology, KMITL, Bangkok.The laboratory colony was kept under the following conditions: 27.3±2.3 ℃ and 70.5±2.5 %RH with a photoperiod of 12-h light and 12-h dark (12L:12D). Mosquito eggs of each species were brought to hatch in a plastic tray (the size of 28×35×4 cm) containing 2,000 ml clean water. One plastic tray was used to rear 400 larvae. The larvae were fed with fish food pellets (SAKURA®, 32% protein) for 14 days until pupation occurred. The pupae were not fed with food. One hundred new pupae were collected in a 250 ml plastic cup containing 200 ml clean water, transferred to an insect cage (the size of 30 cm×30 cm×30 cm), and left until they developed into adults. The male and female adults were provided with 5% glucose solution soaked in cotton wool. Two-day old female adults (not yet fed with blood meal) were collected as subjects for a World Health Organization (WHO) susceptibility test. Two hundred and fifty 5-day old female adults in one insect cage were fed with blood meal by an artificial membrane feeding method for 60 min. The female adults were ready for use in an oviposition deterrence bioassay after two days of blood feeding. Three days after blood feeding, 250 female adults were transferred to an insect cage to oviposition. A 250-ml plastic cup containing 200 ml of clean water was placed inside the cage with a filter paper as a support for *Ae. albopictus* females to lay their eggs on. For *An. minimus* females that needed no support for laying their eggs on, a 250-ml plastic cup with just 200 ml clean water was placed in the insect cage. Two days after oviposition, the eggs were collected for an ovicidal bioassay.

Table 1. List of Illiciaceae and Zingiberaceae plants tested in this study.

Scientific name Common name Family	Therapeutic properties	Chemical constituents	References
Alpinia galanga (L.) Willd. Galanga Zingiberaceae	Antituberculosis, stimulant properties and carminative.	1,8-cineole, β-pinene, α -pinene, α- terpineol, β –elemene, camphene, camphor, guaiol and fenchly acetate	Voravuthikunchai *et al*., 2007; Rao *et al*., 2010
Amomum krervanh Pierre ex Gagnep. Siam Cardamom Zingiberaceae	Antioxidant, antimicrobial, anti-inflammatory and against allergy.	1,8-Cineole, pinene, α-terpinene, and β-pinene	Diao *et al*., 2014
Curcuma zedoaria (Christm.) Roscoe Zedoary Zingiberaceae	Antiviral, treat inflammation, antioxidant, platelet aggregation inhibitory and analgesic.	Monoterpenoids, camphor, α-zingiberene, camphene, α-curcumene, isoborneol, germacrone, borneol, α-pinene, β-pinene and 2-nonanol	Suthisut *et al*., 2011; Hamdi *et al*.,2015
Illicium verum Hook.f. Star Anise Illiciaceae	Carminative, anti-spasmodic, antifungal, antibacterial, stimulant, properties, diuretic, rheumatism, stomachic and insecticide.	E-anethole, limonene, linanool, methyl chavicol, α-pinene, anis aldehyde, and terpinen-4-ol.	Peng *et al*., 2014; Wang *et al*., 2011
Zingiber cassumunar Roxb. Phlai Zingiberaceae	Antispasmodic pills, anti-inflammatory rheumatism, muscular pain and asthma,	Sabinene, α-terpinene, γ- terpinene, terpinen 4-ol, 2,6,9,9-Tetramethyl-2,6,10-, α –caryophyllene and caryophyllene oxide	Kamazeri *et al*., 2012
Zingiber mekongense Gagnep. - Zingiberaceae	Antiseptic, diuretic, antioxidant, carminative antiseptic and even cancer deterrent	Camphene, α -pinene, nerol, geranyl acetate, linalool, borneol (-)-zingiberene, (+)-ar-curcumene, (-)-β -sesquiphellandrene, and β -bisabolene,	Chareonkla *et al*., 2011

Oviposition deterrence bioassay

The performed oviposition deterrence bioassay was a double-choice method. Fifteen five-day-old gravid females were transferred into an insect cage (the size of 30 cm × 30 cm × 30 cm) containing two 250-ml plastic cups. The 1st cup, non-treatment cup, was filled with 100 ml of clean water, while the 2nd cup, treatment cup, was filled with 99 ml clean water and added with 1 ml of 1 or 5 or 10% of each EO or 0.01 g of temephos. The non-treatment and treatment cups were placed at the opposite corners of the cage and the positions of the cups were switched in each next replication of the experiment. After 48 hours, the number of eggs laid in the non-treatment and treatment cups were counted under a stereomicroscope. Five replications were performed and the results from the two types of cups were compared by a paired t-test ($P<0.05$). The oviposition activity index (OIA), percentage effective repellency (ER%), and percentage effective attractantcy (EA%) were determined.

The OAI was calculated by the following formula (Govindarajan *et al*., 2018; Shaalan and Canyon, 2018; Soonwera and Phasomkusolsil, 2017)

$$OAI= TC-UC/TC+UC,$$

where TC is total number of mosquito eggs laid in the treatment cup and UC is the total number of mosquito eggs laid in the non-treatment cup. The values of OAI ranged from -1.0 to +1.0 where an OAI=0 signified a neutral response (N); an OAI from 0 to +1.0 signified an attractant (A), i.e., that more mosquito eggs were laid in the treatment cup than in the non-treatment cup; and an OAI from 0 to -1.0 signified a repellent (R), i.e., that more mosquito eggs were laid in the non-treatment cup than in the treatment cup. A highly negative index was what we were looking for which would show that the test solution deterred the female mosquitoes from spawning eggs.

ER% was calculated (for the case of positively repellent and deterrent) by the following formula:

$$ER\%= [UC-TC/UC] \times 100,$$

EA% was calculated (for the case of positively attractant) by the following formula:

$$EA\%= [TC-UC/TC] \times 100,$$

where TC is the total number of mosquito eggs laid in the treatment cup and UC is the total number of mosquito eggs laid in the non-treatment cup.

Ovicidal bioassay

The performed ovicidal bioassay was a topical method. Twenty five eggs of each species of mosquitoes were placed on a filter paper and topically treated for 3 hours with 0.005ml of each EO at each of the three concentrations. After the topical treatment was completed, all mosquito eggs were rinsed with clean water and put into a 200-ml plastic cup containing 100 ml of clean water. In the case of temephos, 0.01g of it was added to 100 ml of clean water in a 200 ml plastic cup then the solution was used as a positive control. One hundred milliliters of clean water in a 200 ml plastic cup was used as a negative control. The results were recorded at 48 hours after the topical treatment was completed. Each treatment was replicated five times and the results were compared to those from temephos (positive control) and clean water (negative control). The percentage inhibition rate of eggs was calculated by the following formula:

$$\text{Inhibition rate (\%)} = [NT/NC] \times 100,$$

where NT is the total number of dead eggs (not hatched within 48 hours) and NC is the total number of treated eggs.

World Health Organization (WHO) Susceptibility Test

The knockdown rate and mortality rate tests of mosquito females were performed using the World Health Organization (WHO) Susceptibility Test. Twenty-five two-day-old female mosquitoes (not yet fed with blood meal) were exposed to each EO at each concentration (2 ml) that was dropped onto a filter paper (Whatman No1®) the size of 12×15 cm for one hour in the treatment tube (44 mm in diameter and 125 mm in length) then transferred to the non-treatment tube. The knockdown rates were recorded at 1, 5, 10, 15, 30, and 60 min and the mortality rates were recorded 24 h after the exposure. Each treatment was performed in five replicates. Cypermethrin (common chemical insecticide for mosquito control) and clean water were used as positive and negative control, respectively. The criterion for knockdown and mortality was that all of the mosquitoes' body parts did not move. The distinction between knock down and mortality was that knock down was a symptom that was recorded 1 hour after exposure while mortality was recorded 24 hours after exposure. Knockdown rate and mortality rate were calculated by the following formula:

Knockdown rate (%) = [NT/NC] x 100,

Mortality rate (%) = [NT/NC] x 100,

where NT is the total number of knocked down/dead adults and NC is the total number of treated adults.

All data means were analyzed and compared by analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). In addition, 50% knockdown time (KT_{50}) and 50% lethal concentration (LC_{50}) of all treatments were analyzed by standard probit analysis. The susceptibility levels were classified according to WHO criteria: Susceptible (S) means 98-100% of mosquito mortality; Possible Resistant (PR) means 80-97% of mosquito mortality; and Resistant (R) means less than 80% of mosquito mortality.

Results

Table 2 showed the oviposition deterrent activities of six EOs at three concentrations (1, 5 and 10%) in terms of OAI (oviposition activity index), ER% (percentage effective repellency) and EA% (percentage effective attractantcy) against female adults of *Ae. albopictus* at 48 h exposure. A bar chart of OAIs of all six EOs at 1, 5 and 10% concentrations (conc.) are presented in Figure1. All EOs at high concentration (10%) showed a high ER% and a high oviposition deterrent effect than the EOs at lower concentrations (5 and 1%). At 5 and 10% conc., *Curcuma zedoria* (*C.zedoaria*) EO exhibited the most effective oviposition deterrent activity against *Ae. albopictus* females with the highest deterrent activity of 100% ER and -1.0 OAI. At 1% concentration, it showed 80.3% ER and -0.6 OAI. In addition, *C.zedoaria* EO at all tested concentrations showed significant differences in the mean numbers of eggs laid in the treatment and non-treatment cups ($P<0.05$). *Zingiber mekongense* (*Z.mekongense*) at all tested concentrations showed the second most effective oviposition deterrent activity against *Ae.albopictus* females with a high deterrent activity of 65.0 to 98.0% ER and -0.4 to -0.9 OAI. They also showed significant differences in the mean numbers of eggs laid in the treatment and non-treatment cups ($P<0.05$). EOs from *Alpinia galanga* (*A.galanga*), *Zingiber cassumunar* (*Z.cassumunar*) *Illciium verum* (*I. verum*) and *Amomum krervanh* (*A. krervanh*) showed low oviposition deterrent activities with %ER of 7.4 to 98.5%, 27.2 to 84.8%, 21.0 to 74.0%, and 12.5 to 66.9 %, respectively, and OAI of -0.03 to -0.9%, -0.1 to -0.7%, -0.1 to -0.5%, and -0.06 to -0.5%, respectively. The mean numbers of eggs laid in all treatment cups were lower than those laid in the non-treatment cups. In addition, all EOs at 10% conc.

showed higher oviposition deterrent activities (66.9 to 100% ER, -0.5 to-1.0 OAI) than temephos did (32.3%ER and -0.1 OAI).

Table 2. Oviposition deterrent activities of six EOs at three concentrations (1%, 5% and 10%) against females of *Ae. albopictus*.

Treatment	Conc. (%)	Number of eggs±SD		OAI*	ER%	EA%	No. of tested eggs laid per female
		Treatment cup	Non-treatment cup				
Alpinia galanga	1	310±80.47	334.8±76.96	-0.03	7.4	-	20.6
	5	175.2±49.85*	509.8±66.30	-0.4	65.6	-	11.6
	10	10.4±18.24*	724.8±73.39	-0.9	98.5	-	0.6
Amomum krervanh	1	493.2±104.97	563.8±86.79	-0.06	12.5	-	32.8
	5	494.8±88.02	591±177.12	-0.08	16.2	-	32.9
	10	242.2±70.21*	733.4±48.69	-0.5	66.9	-	16.1
Curcuma zedoaria	1	134.8±34.03*	686.8±125.20	-0.6	80.3	-	8.9
	5	0±0*	784.4±224.86	-1	100	-	0.0
	10	0±0*	762.4±133.03	-1	100	-	0.0
Illicium verum	1	484.8±116.82	614±78.74	-0.1	21.0	-	32.3
	5	321.4±112.34*	688.4±97.83	-0.3	53.3	-	21.4
	10	208.8±82.56*	803.4±133.127	-0.5	74.0	-	13.9
Zingiber cassumunar	1	385.2±127.36	529.6±111.75	-0.1	27.2	-	25.6
	5	147.8±54.89*	704.8±122.67	-0.6	79	-	9.8
	10	94.2±29.69*	620.2±101.79	-0.7	84.8	-	6.2
Zingiber mekongense	1	237.4±97.06*	678.8±168.13	-0.4	65	-	15.8
	5	157.8±99.92*	799.4±93.81	-0.6	80.2	-	10.5
	10	9.2±11.43*	804.2±110.25	-0.9	98.8	-	0.6
temephos	w/w	308±83.58	455±171.98	-0.1	32.3	-	20.5

* Significant different results between the treatment solution and the non-treatment solution from paired t-test (P< 0.05)
OAI = Oviposition Active Index; ER% = Percentage effective repellency; EA% = Percentage effective attractancy

The oviposition deterrent results of six EOs against *Anopheles minimus* (*An.minimus*) females are summarized in Table 3 and Figure 1. *C. zedoaria* EO at 5 and 10% conc. exhibited the highest oviposition deterrent activity against *An.minimus* females with 100% ER and -1.0 OAI. This EO at 1% conc. also showed a high oviposition deterrent activity at 94.7% ER and -0.8 OAI. In fact, *C.zedoaria* EO at all tested concentrations showed significant differences in the mean numbers of eggs laid in the treatment cups and non-treatment cups (P<0.05). Moreover, 10%. EOs of *I.verum*, *Z.cassumunar* and *A. galanga* showed the highest oviposition deterrent activity against *An. minimus* females with 100% ER and -1.0 OAI, and the three EOs at all concentrations showed significant differences in the mean numbers of eggs laid in the treatment cups and non-treatment cups (P<0.05). The three EOs at 5% conc. showed a highly deterrent activity with 88.7 to 99.7% ER and -0.7 to -0.9 OAI, while at 1% conc., they exhibited a moderate deterrent activity with 49.0 to 70.4% ER and -0.3 to -0.5 OAI. EOs from *A.krervanh* and *Z.mekongense* at all tested conc. showed a moderate deterrent activity with ER ranging from 75.3 to

98.8% and OAI ranging from -0.6 to -0.9. They showed significant differences in the mean numbers of eggs laid in the treatment cups and non-treatment cups (P<0.05). On the other hand, temephos was an attractant with 24.9% EA and 0.1 OAI.

Table 3. Oviposition deterrent activities of six EOs at three concentrations (1%, 5% and 10%) against females of *An. minimus*.

Treatment	Conc. (%)	Number of eggs ±SD		OAI	ER%	EA%	No. of tested eggs laid per female
		Treatment cup	Non-treatment cup				
Alpinia galanga	1	213.6±63.4*	425.8±68.89	-0.3	49.8	-	14.2
	5	1.2±2.68*	569.6±102.75	-0.9	99.7	-	0.08
	10	0±0*	600±91.73	-1	100	-	0
Amomum krervanh	1	145.6±172.2*	590.2±132.22	-0.6	75.3	-	9.7
	5	76±105.55*	575.6±75.43	-0.7	86.7	-	5.0
	10	56.2±61.07*	567.8±121.85	-0.8	90.1	-	3.7
Curcuma zedoaria	1	32.6±30.42*	617±67.90	-0.8	94.7	-	2.1
	5	0±0*	583.8±167.45	-1	100	-	0
	10	0±0*	540.4±82.66	-1	100	-	0
Illicium verum	1	140.8±106.4*	476.4±57.23	-0.5	70.4	-	9.3
	5	40.6±39.67*	477.4±60.08	-0.8	91.4	-	2.7
	10	0±0*	513.4±64.87	-1	100	-	0
Zingiber cassumunar	1	198.4±142.22*	405.6±129.32	-0.3	51	-	13.2
	5	68±17.64*	509.2±124.26	-0.7	86.6	-	4.5
	10	0±0*	573.8±85.76	-1	100	-	0
Zingiber mekongense	1	117.2±64.0*	525±90.79	-0.6	77.6	-	7.8
	5	76.2±19.74*	569.2±152.91	-0.7	86.6	-	5.0
	10	6.6±9.73*	592.2±166.88	-0.9	98.8	-	0.4
temephos	*w/w*	395±172.30	297±129.31	0.1	-	24.9	26.3

* Significant different results between the treatment solution and the non-treatment solution from paired t-test ($P < 0.05$)
OAI = Oviposition Active Index; ER% = Percentage effective repellency; EA% = Percentage effective attractantcy.

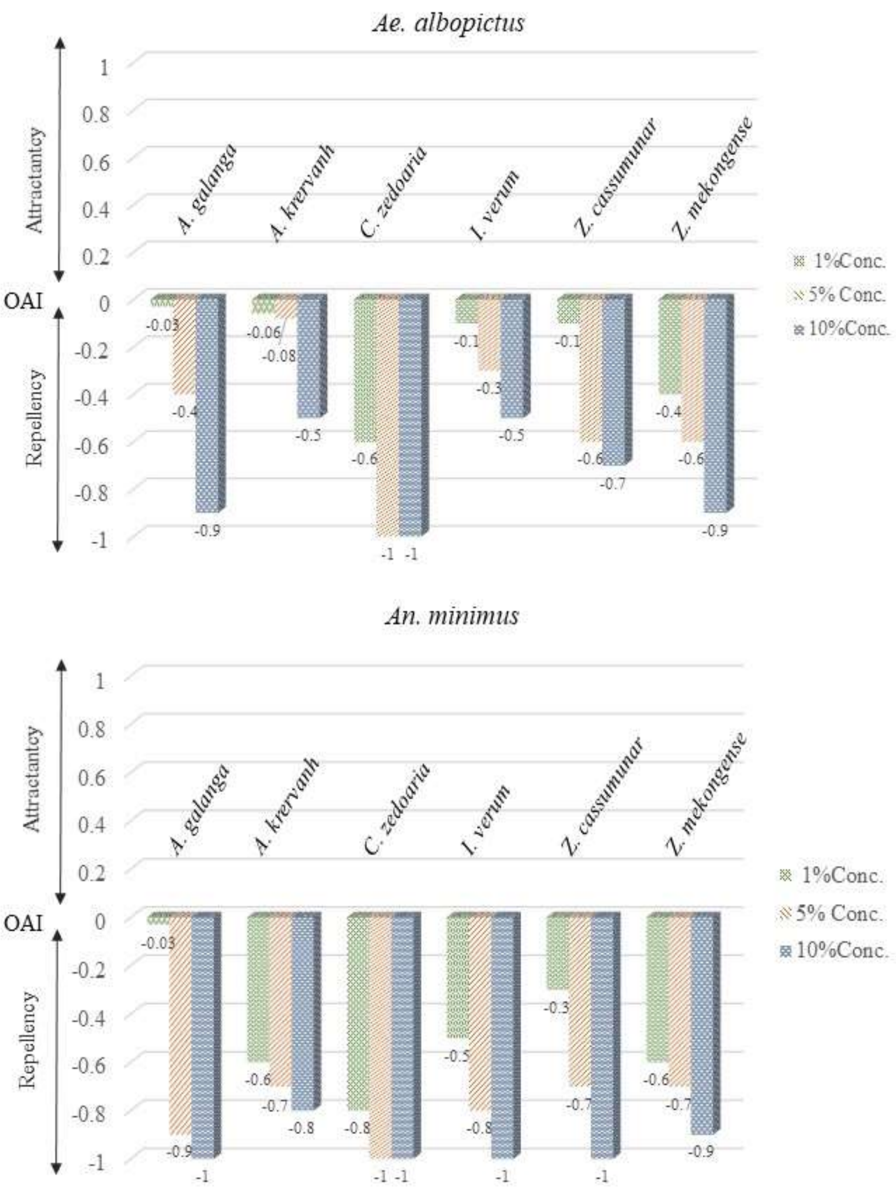

Figure 1. Oviposition activity index (OAI) values of six essential oils against *Ae. albopictus* and *An. minimus* females.

Table 4 shows various ovicidal activities of six EOs at 1, 5 and 10% conc. against *Ae. albopictus* and *An. minimus* eggs in terms of inhibition rate and LC_{50} value against the eggs at 48 h exposure. *I.verum* EO at 10% conc. was highly toxic to *Ae albopictus* eggs with 100% inhibition rate and an LC_{50} value of 1.7%. Moreover, this EO at all tested conc. exhibited the highest toxicity against *An. minimus* eggs with 100% inhibition rate and an LC_{50} value of 0.1%. In addition, EOs from *A.krervanh* and *A.galanga* at 10%conc.were highly toxic against *Ae. albopictus* eggs with 94.5 and 92.7 % inhibition rates and LC_{50} values of 4.2 and 4.6%, respectively, followed by EOs from *Z. cassumunar*, *C. zedoaria* and *Z. mekongense* at 10% conc. which showed inhibition rates of 74.0, 46.3 and 11.5 % and LC_{50} values of 6.5, 10.7 and 24.0%, respectively. *A.krervanh* and *A.galanga* EOs at 1 and 5% conc. exhibited a moderate to low toxicity against the eggs of the two mosquito species with an inhibition rate ranging from 74.1 to 73.7% and 11.4 to 24.6 %, respectively, while EOs from the other plants at 10% conc. showed a moderate to low toxicity against the eggs with inhibition rates ranging from 6.7 to 47.8 %.

In addition, *A.galanga* EO at 5 and 10 % conc. also showed the highest toxicity against *An. minimus* eggs with 100% inhibition rate and an LC_{50} value of 1.8%, while this EO at 1% conc. gave a moderate inhibition rate of 46.5%. *Z. cassumunar* and *A. krervanh* EOs at 5 and 10% conc. exhibited high inhibition rates ranging from 93.3 to 80.9 % and 24.7 to 91.0 % with LC_{50} values of 3.6% and 6.2 %, respectively, while at 1%conc., these two EOs gave moderate to low inhibition rates of 43.9 and 23.4 %, respectively. All of the other EOs showed LC_{50} values ranging from 11.1 to 19.3 % and inhibition rates ranging from 3.9 to 39.2 %. In contrast, temephos (positive control) showed only 9.3% and 12.1% inhibition rate against *Ae. albopictus* and *An. minimus* eggs, respectively, while clean water (negative control) was not effective at all against the eggs of these two mosquito species, i.e., it showed 0% inhibition rate.

The knockdown rate, 50% knockdown time (KT_{50}), mortality rate, 50% lethal concentration (LC_{50}) and susceptibility status of *Ae*. *albopictus* and *An. minimus* females against six EOs at 1, 5 and 10% conc. and their comparative efficacy to cypermethrin are presented in Table 5. *A.galanga* EO at 5 and 10% conc. were the most efficient against *Ae. albopictus* females with a knockdown rate of 100% and KT_{50} values of 11.9 and 0.7 min, respectively, at 1 h after exposure. These results were considerably better than those of cypermethrin (100% knockdown rate and KT_{50} values of 5.3 min). At 10% conc., *I. verum*, *Z. cassumunar*, *Z. mekongense* and *C. zedoaria* EOs exhibited a 100% knockdown rate against *Ae. albopictus* females and KT_{50} values of 5.7, 6.5, 6.7 and 8.4 min, respectively, while *A.krervanh* EO at 10% conc. showed a 57.6 % knockdown rate and a KT_{50} value of 41.5 min. On the other hand, the five EOs at 5% conc. showed knockdown rates ranging from 57.6 to 96.8 %, less effective than cypermethrin.

Table 4. Ovicidal activities of six EOs at three concentrations (1%, 5% and 10%) against *Ae. albopictus* and *An. minimus* eggs.

Treatment	*Ae.albopictus* %inhibition rate±SD[1]			LC_{50}[2] (%)	*An.minimus* %inhibition rate±SD[1]			LC_{50}[2] (%)
	1%	5%	10%		1%	5%	10%	
Alpinia galanga	11.4±5.3[c]	73.7±5.8[b]	92.7±3.5[a]	4.6	46.5±12.0[b]	100[a]	100[a]	1.8
Amomum krervanh	24.6±8.7[b]	74.1±4.6[b]	94.5±3.5[a]	4.2	23.8±8.4[c]	24.7±6.8[c]	91.0±3.3[b]	6.2
Curcuma zedoaria	9.2±13.0[c]	25.9±22.3[d]	46.3±12.5[c]	10.7	3.9±20.2[d]	15.2±12.4[d]	39.2±8.9b[c]	11.1
Illicium verum	87.6±43.0[a]	97.9±1.3[a]	100a	1.7	100[a]	100[a]	100[a]	0.1
Zingiber cassumunar	22.8±16.0[b]	47.8±6.5[c]	74.0±4.1[b]	6.5	43.9±4.3[b]	80.9±2.5[b]	93.3±2.3[b]	3.6
Zingiber mekongense	6.7±20.4[d]	11.2±10.8[e]	11.5±5.7[d]	24.0	11.9±16.4[d]	13.0±9.3[d]	17.3±18.9[c]	19.2
temephos	9.3±8.6[c]	9.3±8.6[e]	9.3±8.7[d]	ns[3]	12.1±12.0[d]	12.1±12.1[d]	12.1±12.2[c]	ns[3]
Clean water	0.0[c]	0.0[f]	0.0[e]	ns	0.0[e]	0.0[c]	0.0[d]	ns
CV %	17.46	14.19	29.97		17.01	14.48	24.06	

[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.
[2] LC_{50} = 50% lethal concentration.
[3] ns – not computed by Probit analysis.

Ae. albopictus females were resistant to all EOs at 1 and 5% conc. with mortality rates ranging from 0 to 75.2 %. *A.galanga*, *Z. cassumunar* and *Z. mekongense* EOs at 10% conc. showed the highest mortality rate at 100% against *Ae. albopictus* females at 24 h after exposure with LC_{50} values of 7.5, 6.1 and 4.4 %, respectively. Moreover, the susceptibility status of *Ae. albopictus* against these EOs was 'susceptible' which was a higher level than that against cypermethrin at 10% conc. *I. verum* and *C. zedoaria* EOs at 10% conc. gave 95.2 and 40.0 % mortality rates and LC_{50} values of 5.3 and 10.3%, respectively, while the susceptibility status against them was 'resistance suspected'. On the other hand, *A.krervanh* EO at all conc. did not show an effective LC_{50} value or mortality rate against *Ae. albopictus* females. At the same time, cypermethrin at 10% conc. showed a 95.2% mortality rate and an LC_{50} value of 8.5%. The susceptibility status of *Ae. albopictus* against it was 'possible resistance'.

Against *An. minimus* females, the results showed that *A. galanga* and *Z. mekongense* EOs at 5 and 10% conc. and *I. verum* and *Z. cassumunar* EOs at 10% conc. gave the highest knockdown rate of 100% after 1 h of exposure with KT_{50} values ranging from 1.0 to 5.0 min. *C. zedoaria* EO at 5 and 10% conc. gave knockdown rates of 62.4 and 94.4 % with KT_{50} values of 47.7 and 26.1 min, respectively. On the other hand, *A.krervanh* EO at all tested conc. and the other EOs at the low 1% conc. did not show an effective KT_{50} value or knockdown rate against *An. minimus* females. At 5 and 10% conc., cypermethrin gave a 100% knockdown rate and KT_{50} values of 10.2 and 3.7

min, respectively, whereas at 1%, cypermethrin did not show an effective KT_{50} value or knockdown rate against *An. minimus* females at all.

Table 5. Knockdown rate, KT_{50}, mortality rate, LC_{50} and susceptibility status of *Ae. albopictus* and *An. minimus* females against six EOs at three concentrations (1%, 5% and 10%) and cypermethrin.

Treatment	Conc. (%)	*Ae. albopictus* Knockdown rate at 1h (%)	*Ae. albopictus* KT_{50} (min)	*Ae. albopictus* Mortality rate at 24 h (%)	*Ae. albopictus* LC_{50} (%) at 24 h	*Ae. albopictus* Susceptibility status	*An.minimus* Knockdown rate at 1h (%)	*An.minimus* KT_{50} (min)	*An.minimus* Mortality rate at 24 h (%)	*An.minimus* LC_{50} (%) at 24 h	*An.minimus* Susceptibility status
Alpinia galanga	1	$0.0^{b1/}$	$ns^{2/}$	$0.0^{b1/}$		R	$0.0^{b1/}$	$ns^{2/}$	$0.0^{b1/}$		R
	5	100^{a}	11.9	0.0^{b}	7.5	R	100^{a}	1.1	100^{a}	2.9	S
	10	100^{a}	0.7	100^{a}		S	100^{a}	1.0	100^{a}		S
Amomum krervanh	1	0.0^{c}	ns	0.0		R	0.0	ns	0.0^{b}		R
	5	8.8^{b}	76.2	0.0	ns	R	0.0	ns	0.0^{b}	7.5	R
	10	57.6^{a}	41.5	0.0		R	0.0	ns	100^{a}		S
Curcuma zedoaria	1	0.0^{c}	ns	0.0^{b}		R	0.0^{c}	ns	12.8^{c}		R
	5	48.0^{b}	55.1	0.0^{b}	10.3	R	62.4^{b}	47.7	38.4^{b}	6.4	R
	10	100^{a}	8.4	40.0^{a}		R	94.4^{a}	26.1	76.8^{a}		R
Illicium verum	1	0.0^{c}	ns	0.0^{c}		R	0.0^{c}	ns	0.0^{c}		R
	5	92.8^{b}	21.3	52.8^{b}	5.3	R	67.2^{b}	40.4	31.2^{b}	5.5	R
	10	100^{a}	5.7	95.2^{a}		PR	100^{a}	4.1	100^{a}		S
Zingiber cassumunar	1	3.2^{b}	88.3	0.0^{c}		R	0.0^{b}	ns	0.0^{c}		R
	5	96.8^{a}	12.2	15.2^{b}	6.1	R	0.0^{b}	ns	19.2^{b}	5.9	R
	10	100^{a}	6.5	100^{a}		S	100^{a}	5.0	100^{a}		S
Zingiber mekongense	1	16.8^{c}	70.3	0.0^{c}		R	0.0^{b}	ns	0.0^{c}		R
	5	76.8^{b}	30.4	75.2^{b}	4.4	R	100^{a}	3.0	56.0^{b}	4.8	R
	10	100^{a}	6.7	100^{a}		S	100^{a}	4.6	100^{a}		S
cypermethrin	1	0.0^{b}	ns	0.0^{b}		R	0.0^{b}	ns	0.0^{b}		R
	5	100^{a}	17.1	0.0^{b}	8.5	R	100^{a}	10.2	100^{a}	2.9	S
	10	100^{a}	5.3	95.2^{a}		PR	100^{a}	3.7	100^{a}		S

KT_{50}- 50% knockdown time; LC_{50}- 50% lethal concentration.

[1]Mean % knockdown rates and mortality rates followed by the same letter in the same column are not significantly different (one-way ANOVA and Duncan's Multiple Range Test) [2]ns = not computed by Probit analysis.

S = Susceptible is defined as 98-100% mortality; *PR* = Possible resistant is defined as 80-97% mortality; *R* = Resistant is defined as <80% mortality.

At 5 and 10%conc, *A.galanga* EO was also the most toxic against *An.minimus* females exhibiting 100% mortality rate at 24 h after exposure and an LC_{50} value of 2.9% as well as a 'susceptible' status. This high mortality rate was equal to that achieved by cypermethrin (100% mortality rate and an LC_{50} value of 2.9%). The other EOs at 10% conc. also showed a high mortality rate of 100% and LC_{50} values ranging from 4.4 to 7.5 % with a 'susceptible' status. On the other hand, five EOs at 1 and 5% conc. (except *A.galanga* EO) gave mortality rates ranging from 0 to 56.0% with a 'resistant' status. Incidentally, the susceptibility status defined by WHO was based on the declining mortality rate effected by prevailing synthetic chemicals at the time to which several mosquito species were starting to develop resistance. In this study, the EOs at lower conc. were assigned a 'resistant' status because they caused low mortality rate, not because the mosquitoes had developed resistance to them as in the case of synthetic chemicals.

Discussion

Our results demonstrate that *C. zedoaria*, *I. verum* and *A. galanga* EOs at a high 10% conc., are effective oviposition deterrent, ovicidal, and adulticidal agents against *Ae. albopictus* and *An. minimus* mosquitoes. In addition, at both 5 and 10% conc., *C. zedoaria* EO gave the highest oviposition deterrent activity with 100%ER and -1.0 OAI against the two mosquitoes. These results demonstrate that all 3 EOs are more effective as oviposition deterrent agents than temephos. This is not a coincidence. These results are in agreement with the results reported in two papers published in 2013 that *C. zedoaria* EO at 10% conc. showed a good oviposition deterrent activity against *Aedes aegypti* (*Ae. aegypti*) females with 98.0%ER and -1.0 OAI. Moreover, these EOs also exhibited high larvicidal and pupicidal activities against *Ae.aegypti* and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) with 100% mortality at 10 and 6 h and LT_{50}values ranging from 1.4-4 min. and 1.9 to 9.3 h, respectively (Phukerd and Soonwera, 2013a; Phukerd and Soonwera, 2013b). In addition, *C. zedoaria* EO showed a moderate repellent activity against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes with a protection time ranging from 16 -155 min and a biting rate of 0.9% (Phukerd and Soonwera, 2015). *C. zedoaria* EO has also been shown to be effective against another species of mosquito. Several papers have reported that it was highly toxic against the larvae of *Ae. aegypti* and *Anopheles dirus* (*An. dirus*) (Pitasawat *et al*., 2007). It is also effective across some other insect families; there is a report that *C. zedoaria* EO showed a repellent activity against two species of serious store product pests, *Sitophilus zeamais* and *Tribolium castaneum* (Suthisut *et al*., 2011a; Suthisut *et al*., 2011b). The main chemical components of *C.zedoaria* EO are monoterpenoids, camphor, α-zingiberene, camphene, α-curcumene, isoborneol, germacrone, borneol, α-pinene, β-pinene and 2-nonanol (Table 1; Hamdi *et al*., 2015; Voravuthikunchai, 2007). Monoterpenoids from plant EOs are toxic against insects due to their inhibitory action on acetyl cholinesterase enzyme in insects' nervous system. More importantly, they are non-toxic or less toxic to humans and other mammals as well as show only a short persistence in the environment (Regnault-Roger *et al*., 2013; George *et al*., 2014; El-Wakeil, 2013). Therefore, it stands to reason that *C. zedoaria* EO was repellent and oviposition deterrent against *Ae. albopictus* and *An. minimus* females. Hence, this EO can be used as an oviposition deterrent agent against these two mosquito vectors at their breeding sites such as a residential or industrial area for strict regulation of the density of new mosquito generation and their complete eradication.

C. zedoaria is an important medicinal plant and widely used as food additive in many Southeast Asia countries. Its common name is "zedoary", but it is well known in Thailand as "Khamin oi" (Phukerd and Soonwera, 2013b). *C. Zedoaria* plant belongs to the family Zingiberaceae. It is distributed mainly in East Asian countries including Thailand, Loa RP, Cambodia, Vietnam, Malaysia, Indonesia, India, Bangladesh, Japan, Korea and China (Phukerd and Soonwera, 2013b; Tholkappiyavathi *et al*., 2013; Chen *et al*., 2008). The rhizome of this plant has been extensively used for more than two hundred years in traditional Thai medicine with therapeutic effects such as antidiarrheal, anti-emetic, antipyretic and for an external use as astringent for wound (Faculty of Pharmacy, Mahidol University, 1992).

I.verum EO at 10% conc. is another EO that exhibited the highest ovicidal activity against *Ae. albopictus* and *An. minimus* eggs with 100% inhibition rate and an LC_{50} value ranging from 0.1 to 1.7 %. These results were better than those achieved by temephos and in good agreement with the results on another insect family reported by (Sinthusiri and Soonwera, 2014) that 10% *I. verum* EO showed a 97.33% inhibition rate against house fly (*Musca domestica*) eggs with an LC_{50}value of 6.85%. In addition, *I.verum* EO also showed high toxicity to house fly larvae, *Culex pipiens* larvae, larvae and pupae of *Ae. albopictus* and *Cx. quinquefasciatus*, museum insect pest, *Demestes maculatus* and other insect pests of store products (Sinthusiri and Soonwera, 2014 ;Kimbaris *et al*., 2012 ;Zhang, 2009). *I. verum* is a local plant in Vietnam, Southern China and other countries in Asia. It is well known and widely used as spice in Asian cuisine (Peng *et al*., 2014; Wang *et al*., 2011). *I. verum* plant belongs to the family Illiciaceae. Its common name is star anise. The fruit of this plant is an important ingredient in traditional Chinese medicine for abdominal colic, stomach ache, vomiting, skin inflammation, rheumatic pain, and common cold. Traditional star anise tea is used to treat nervousness and sleep disorder (Peng *et al*., 2014; Wang *et al*., 2011). The main chemical components of *I. verum* EO are *E*-anethole (86.3%), limonene (7.8), linanool (1.4%), methyl chavicol (1.1%), α-pinene (0.8%), anis *aldehyde* (0.8%), and terpinen-4-ol (0.7%) (Wang *et al*., 2011). It has been reported that the monoterpene, anethole ($C_{10}H_{12}O_{10}$), from *I. verum* EO showed a high larvicidal activity against *Cx. pipiens* larvae with an LC_{50}value of 16.56% (Kimbaris *et al*., 2012). In a recent study, it was suggested that anethole might penetrate into the cuticle of mosquito eggs and block their oxygen uptake, causing embryo mortality.

In contrast, temephos was a less effective oviposition deterrent and ovicidal agent than *C. zedoaria* and *I.verum* EOs. It is a synthetic organophosphate insecticide that is very widely used as a larvicidal agent for mosquito control in residential and industrial sites throughout the world (Melo-Santos *et al*., 2010). One percent w/w temephos has been used as a larvicide for mosquito control in the urban and rural residential areas in Thailand since 1967 (Paeporn *et al.*, 2013).Unfortunately, there have been reports that temephos is harmful to humans and can caused acute and subacute toxicity (Paeporn *et al.*, 2003). Temephos has an adverse effect on the neurons of human nervous system, lymphocytes and hepatoma (Hussain, 2010). Moreover, temephos has several other adverse side effects such as asthenia, headache, stomachache, diarrhea, inability to walk, angina pectoris, unconsciousness, muscular spasm, rhinorrhea, choking, and even death (Paeporn *et al.*, 2003; Hussain, 2010). Most importantly, there has been reports that the larvae of *Ae. aegypti, Cx. quinquefasciatus,* and *An. stephensi* in Thailand and several countries have developed resistance to temephos (Melo-Santos *et al.*, 2012; Paeporn *et al.*, 2003, Bhan *et al.*, 2015).

As for the results of adulticidal activity, *A. galanga* EO at 10% conc. exhibited the highest adulticidal activity against *Ae. albopictus* and *An. minimus* females with KT_{50} values ranging from 0.7 to 1.0 min and LC_{50} values ranging from 2.9 to 7.5 % while the susceptibility status of these two mosquito species against it was classified as 'susceptible'. All of these results show that *A. galanga* EO at 10% conc was more effective than cypermethrin in this respect. Similarly, two papers published in 2013 and 2015 reported that 10% *A. galanga* EO showed high toxicity to the larvae and pupae of *Ae. aegypti* and *Cx. quinquefasciatus* (Phukerd and Soonwera, 2013b; Phukerd and Soonwera, 2015). In addition, Sinthusiri and Soonwera, (2013) reported that 10% *A. galanga* EO showed an adulticidal activity against house fly (*M. domestica*) with a KT_{50} value of 84.31 min and an LC_{50} value of 21.41 %.

A. galanga plant is a native plant of Southeast Asia and Southern China. It belongs to the family Zingiberaceae. The common name of *A. galanga* is "galanga" but it is well known in Thailand as 'Kha" (Phukerd Soonwera, 2013b; Voravuthikunchai, 2007). *A. galanga* rhizome has a spicy and hot taste like ginger rhizome and is commonly used in Thai and Southeast Asian cuisine such as Thai chicken soup and Thai curry paste. In addition, *A. galanga* rhizome is used as Thai folk medicine for bad breath, allergy, bronchial catarrh, dyspepsia, stomachache, fever, rheumatism, toothache and infections (Faculty of Pharmacy, Mahidol University, 1992). The therapeutic properties of this plant are presented in Table1. The main chemical components of *A. galanga* EO are 1, 8-cineole, β-pinene, α -pinene, α- terpineol, β –elemene, camphene, camphor, guaiol and fenchyl acetate (Voravuthikunchai, 2007; Rao *et al*., 2010). Its monoterpenes: 1,8-cineole, β-pinene, α -pinene, α- terpineol, β – elemene, linalool, geraniol, and limonene. These monoterpenes show high toxicity to the larvae of *Anopheles gambiae* mosquito and adult hourse flies, inhibiting the acetylcholinesterase enzyme activity in the insects' nervous system. They also showed a high repellency activity against mosquitoes such as *Ae. aegypti*, *Ae. mediovittatus*, *Ae. sollicitans* and *Cx. nigripalpus* (Zhang *et al*., 2017; Müller *et al*., 2009; Kweka *et al*., 2016). The *A. galanga* EO and main chemical components in this study might toxic to nervous system of *Ae albopictus* and *An. minimus* females. On the other hand, cypermethrin showed high toxic to two mosquito females, but these results were less effective than *A. galanga* EO.

Cypermethrin is a synthetic pyrethriod. It is a broad spectrum insecticide in wide scale use for controlling agricultural insect pests as well as medical and veterinary insect pests (Sallam *et al*., 2015). However, cypermethrin is defined as moderately hazardous by the World Health Organization, (2013). Cypermethrin is highly toxic to fish and beneficial insects such as bee, bumble bee, carpenter bee and other predatory and parasitic insects (Sallam *et al*., 2015; Ullah *et al*., 2018). Most importantly, it is harmful to human health. It causes cytogenesis effects on human lymphocytes, induces apoptosis in human neuroblastoma cells and causes neuroblastoma in children (Sallam *et al*., 2015; Ullah *et al*., 2018; Naqqash *et al*., 2016). To make matters worse, *Ae. aegypti* and *Cx.tritaeniorhynchus* development of resistance to cypermethrin has been reported in several countries (Wu *et al*., 2016; Aguirre *et al*., 2016).

For these reasons, EOs of *C. zedoaria*, *I. verum* and *A. galanga* are a better alternative to cypermethrin and temephos. These EOs were demonstrated to be effective against *Ae. albopictus* and *An. minimus* mosquitoes. They have a good potential to be developed into safe insecticidal agents for controlling mosquito vector population because they not only exhibited a high adulticidal activity but also showed high ovicidal and oviposition deterrent activities; therefore, they can act against mosquitoes throughout all stages of their life cycle thus are more effective at controlling the population of *Ae.albopictus* and *An. minimus*.

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