



## Asian Pacific Journal of Tropical Biomedicine

Journal homepage: [www.apjtb.org](http://www.apjtb.org)

doi: 10.4103/2221–1691.250267

©2019 by the Asian Pacific Journal of Tropical Biomedicine.

## Toxicity of several botanical essential oils and their combinations against females of *Aedes albopictus* (Skuse) and *Anopheles minimus* (Theobald): Oviposition deterrent, ovicidal and adulticidal efficacies

Nuttavich Cotchakaew, Mayura Soonwera<sup>✉</sup>

Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

## ARTICLE INFO

## Article history:

Received 16 November 2018

Revision 7 December 2018

Accepted 8 January 2019

Available online 21 January 2019

## Keywords:

*Aedes albopictus**Anopheles minimus*

Plant essential oil

Ovicide

Oviposition deterrent

Adulticide

## ABSTRACT

**Objective:** To investigate the efficacies of 12 essential oil (EO) formulations from three Zingiberaceae plants (*Alpinia galanga*, *Curcuma zedoaria*, and *Zingiber cassumunar*) individually and in combination with an augmenting *Eucalyptus globulus* (*E. globulus*) EO against females of *Aedes albopictus* (*Ae. albopictus*) and *Anopheles minimus* (*An. minimus*). **Methods:** These formulations were evaluated for their ovicidal, oviposition deterrent and adulticidal activities against *Ae. albopictus* and *An. minimus* by a topical method, a double-choice method and a WHO susceptibility test, respectively. **Results:** It was found that all formulations of Zingiberaceae plants EOs augmented with *E. globulus* EO were more effective in oviposition deterrent, ovicidal, and adulticidal activities against the two mosquito species than all of the formulations used without *E. globulus* EO. Their oviposition deterrent, ovicidal and adulticidal activities were equivalent to those of 10% w/v cypermethrin. In contrast, 70% v/v ethyl alcohol as a control alone was not effective at all. The highest synergistic effect in effective repellency against *Ae. albopictus* was achieved by 5% *Alpinia galanga* EO + 5% *E. globulus* EO and against *An. minimus* was 5% *Zingiber cassumunar* EO + 5% *E. globulus* EO. Moreover, the highest synergistic effects in ovicidal activities against *Ae. albopictus* and *An. minimus* were achieved by 10% *Zingiber cassumunar* EO + 10% *E. globulus* EO and 5% *Curcuma zedoaria* EO + 5% *E. globulus* EO, respectively. For the adulticidal activities, the highest synergistic effect against two mosquitoes was achieved by 5% *Curcuma zedoaria* EO + 5% *E. globulus* EO. **Conclusions:** These results suggest that Zingiberaceae plant EOs augmented with *E. globulus* EO have a high potential to be developed into oviposition deterrent, ovicidal, and adulticidal agents for controlling populations of *Ae. albopictus* and *An. minimus*.

### 1. Introduction

Mosquitoes are small insects, with the body size of adults ranging from 2 mm to 10 mm. They belong to the Family Culicidae and Order Diptera. Normally, female adults feed on human bloods but male adults feed on nectar and other sources of sugar. In the

Culicidae family, *Aedes albopictus* (*Ae. albopictus*) (Skuse) and *Anopheles minimus* (*An. minimus*) (Theobald) are major insect pests. They are nuisance insect pests and disease vectors of dangerous human and animal diseases in Asia and other tropical and subtropical countries of the world[1–3].

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** [reprints@medknow.com](mailto:reprints@medknow.com)

©2019 Asian Pacific Journal of Tropical Biomedicine Produced by Wolters Kluwer-Medknow

**How to cite this article:** Cotchakaew N, Soonwera M. Toxicity of several botanical essential oils and their combinations against females of *Aedes albopictus* (Skuse) and *Anopheles minimus* (Theobald): Oviposition deterrent, ovicidal and adulticidal efficacies. Asian Pac J Trop Biomed 2019; 9(1): 29-39.

<sup>✉</sup>Corresponding author: Mayura Soonwera, Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

Tel: 66(0) 089–1246455; 66(0) 089–851–1106

Fax: 66(0) 2–329–8514; 66(0)–2–329–8512

E-mail: [mayura.soon@gmail.com](mailto:mayura.soon@gmail.com) ; [mayura.so@kmitl.ac.th](mailto:mayura.so@kmitl.ac.th)

Foundation project: This research was supported by Faculty of Agricultural Technology, KMITL, Bangkok, Thailand (Grant no 01–04–002) and the National Research Council of Thailand (GRAD 6007KMITL).

*Ae. albopictus*, commonly known as forest day mosquito or Asian tiger mosquito, is widely distributed in Thailand and other tropical countries in Africa, the Middle East, the Caribbean, North and South America, and Europe[2,4]. This mosquito species is a major vector of several viruses such as dengue, yellow fever, West Nile, chikungunya, Eastern equine encephalitis, Japanese encephalitis, St. Louis encephalitis, and Venezuelan equine encephalitis virus. It is also a serious vector of filariasis caused by *Dirofilaria immitis*, *Dirofilaria repens*, and *Setaria labiatopapillosa* as well as of parasitic roundworm that causes heart worm disease in domestic animals like cats and dogs[5,6]. Moreover, dengue is ranked by World Health Organization (WHO) as the most serious disease in the world. South-East Asia, Western Pacific and the Americas are most seriously affected by dengue. Approximately 50-100 millions of the world's population have been infected annually with dengue virus, and 2.5% of the 500 000 severe cases died. Most of the deaths were Latin American and Asian children[3,5,6].

*An. minimus* is a major vector of malaria in Southeast Asian and Asian countries such as Thailand, Cambodia, Lao PDR, Vietnam, Malaysia, Indonesia, the Philippines, Bangladesh, Sri Lanka, China, and India as well as several African countries[7–9]. WHO has reported that malaria disease infected more than 216 million cases in 2016 and caused 445 000 deaths in 91 countries; most of them were children under 5 years of age. The total funding for global malaria control was estimated as US 2.7 billions in 2016[8,9].

Unfortunately, there are no effective drugs or vaccines against the major parasites and pathogens transmitted by *Ae. albopictus* and *An. minimus*. Therefore, vector control is the best way for preventing these serious diseases today. Chemical control is the most common and oldest way of defense for mosquito bite protection and vector population control. Pyrethroid, carbamate, and organophosphate insecticides have been effective for mosquito vector control in the past[9,10]. However, currently, most mosquitocides in the groups of pyrethroids, carbamate, and organophosphates have lost their efficacy because mosquito vectors have developed resistance to them. Moreover, they are neurotoxic insecticides which are also highly toxic to human especially children and pregnant women, to mammals, and to the environment as well as non-target organisms[10,11]. Therefore, there is an urgent need to search for new, safe, and effective mosquitocides in order to protect humans and mammals from mosquito vectors as well as from the toxic side effects of chemical insecticides. A promising path for the search is to investigate ecofriendly mosquitocides that can reduce and even replace chemical insecticides. Mosquito control through botanical insecticides should be a better alternative to control by using chemical insecticides[12–14]. Botanical essential oils (EOs) are one of the best insecticides for mosquito vector control. They are virtually non-toxic to mammals, humans or non-target organisms as well as ecofriendly, safe for the environment and show potent insecticidal properties[15–17]. Researchers have reported that many botanical EOs such as EOs from *Cymbopogon citratus*, *Cymbopogon nardus*, *Citrus hystrix*, and *Curcuma aromatica* exhibited

larvicidal, pupicidal, and adulticidal activities against mosquito vectors such as *Aedes aegypti* (*Ae. aegypti*), *Anopheles dirus*, and *Culex quinquefasciatus*[18–24].

Many EOs from Zingiberaceae plants and *Eucalyptus* sp. have been used as insecticides for controlling many insect pests including mosquito vectors[25–28]. The three plant species tested here, *Alpinia galanga* (*A. galanga*), *Curcuma zedoaria* (*C. zedoaria*) and *Zingiber cassumunar* (*Z. cassumunar*) belong to the family Zingiberaceae. They are local plants found in all regions of Thailand and South East Asia[29]. The EOs from rhizomes of these three Zingiberaceae plants have shown toxicity against insect pests and have also been used to prevent and treat several human illnesses with properties including antimicrobial, antioxidant, antibacterial, antifungal, antiseptic, antidepressant, antispasmodic, anticancer and antineuralgic[30–34]. They also showed high toxicity against the larvae and pupae of *Ae. aegypti* and *Culex quinquefasciatus*[16,35]. EOs from *C. zedoaria* and *Z. cassumunar* showed a fair repellent activity against the females of *Ae. aegypti* and *Culex quinquefasciatus*[36]. In addition, EO from *C. zedoaria* rhizome has also shown a high adulticidal activity against laboratory-bred and field strains of *Ae. aegypti*[37]. The major monoterpene, 1-8-cineole, *p*-cymene and  $\alpha$ -phellandrene components from *C. zedoaria* EO showed a high larvicidal activity against *Ae. aegypti* and *Anopheles dirus*[26].

Another EO tested, *Eucalyptus globulus* (*E. globulus*), belongs to the family Myrtaceae. *E. globulus* EO has antifungal, antineuralgic, antiseptic, antibacterial, and insecticidal properties[38,39]. Its major component, 1,8-cineole, a monoterpene, has shown a high activity as oviposition repellent and antifeedant against the adults of *Ae. aegypti*[40] and head lice, *Pediculus humanus capitis*[39]. Several researchers have reported synergistic effects between phytochemical substances and EOs combined at a suitable ratio, which resulted in higher insecticidal or oviposition deterrent activities than those provided by a single EO or phytochemical alone[27,41]. Their suggestion has been confirmed by the result from a study by Auysawasdi *et al.*[42] that 25% *E. globulus* EO mixed with 5% vanillin gave a longer mosquito protection time from 66 to 144 and 204 to 390 min against *Ae. aegypti* and *Anopheles dirus* females, respectively. Many researchers have reported some efficacies of individual plant EOs against insects in the literature. However, reports on synergistic effect of combinations of plant EOs against insect pests are limited. Chauhan *et al.*[43] reported that synergistic combinations of EOs not only exerted more effects against insect pests but also let the insects develop less resistance to them than to individual EOs.

Therefore, the objectives of this study were to determine the efficacy and toxicity of three EOs from Zingiberaceae plants, *A. galanga*, *C. zedoaria*, and *Z. cassumunar* against the eggs and adults of *Ae. albopictus* and *An. minimus* as well as their oviposition deterrent activity and to investigate the EOs in combination with an augmenting substance, *E. globulus* EO, to find out whether the augmentation increased the efficacy of the three EOs in killing

the eggs and adults of these two mosquito species. If some of the combinations showed a synergistic effect, the combinations would be more successful at controlling female mosquito vectors at their breeding sites and could be used for total eradication of the whole populations of mosquito vectors.

## 2. Materials and methods

### 2.1. Plant materials

The plant materials used in this study were fresh rhizomes of three kinds of plants. They are one-year-old Zingiberaceae plants: *A. galanga* (KMITL-1AG), *C. zedoaria* (KMITL-2CZ) and *Z. cassumunar* (KMITL-3ZC). They were collected from a farm in Rayong province (12.7074°N, 101.1474°E), Thailand. Fresh leaves of *E. globulus* (KMITL-4EG) from five-year-old trees were collected from a farm in Chachoengsao province (13.6904°N, 101.0780°E), Thailand. Both were collected during the summer season of March, 2017 to April, 2017.

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 leaves were cleaned, cut into small pieces, and extracted for 7-8 h for their 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 °C for later experiments. Gas chromatography and gas chromatography/mass spectrometry were used to analyze the composition of the four plant EOs. The analyses were carried out by the Scientific Instrument Center, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand (Table 1). All prepared EOs were stored under normal laboratory conditions [(27.7±2.3) °C and (73.8±2.2)% relative humidity] for subsequent experiments. The formulations containing these EOs in this study are presented in Table 2.

**Table 2**

Formulations and details of Zingiberaceae plant EOs with and without augmenting *E. globulus* EO, cypermethrin and ethyl alcohol.

Formulation	Details
F1 5% <i>A. galanga</i> EO	5% EO from rhizomes of % <i>A. galanga</i> + 95% ethyl alcohol
F2 5% <i>A. galanga</i> EO + 5% <i>E. globulus</i> EO	5% EO from rhizomes of % <i>A. galanga</i> + 5% <i>E. globulus</i> EO + 90% ethyl alcohol
F3 10% <i>A. galanga</i> EO	10% EO from rhizomes of <i>A. galanga</i> + 90% ethyl alcohol
F4 10% <i>A. galanga</i> EO + 10% <i>E. globulus</i> EO	10% EO from rhizomes of <i>A. galanga</i> + 10% <i>E. globulus</i> EO + 80% ethyl alcohol
F5 5% <i>C. zedoaria</i> EO	5% EO from rhizomes of <i>C. zedoaria</i> + 95% ethyl alcohol
F6 5% <i>C. zedoaria</i> EO + 5% <i>E. globulus</i> EO	5% EO from rhizomes of <i>C. zedoaria</i> + 5% <i>E. globulus</i> EO + 90% ethyl alcohol
F7 10% <i>C. zedoaria</i> EO	10% EO from rhizomes of <i>C. zedoaria</i> + 90% ethyl alcohol
F8 10% <i>C. zedoaria</i> EO + 10% <i>E. globulus</i> EO	10% EO from rhizomes of <i>C. zedoaria</i> + 10% <i>E. globulus</i> EO + 80% ethyl alcohol
F9 5% <i>Z. cassumunar</i> EO	5% EO from rhizomes of <i>Z. cassumunar</i> + 95% ethyl alcohol
F10 5% <i>Z. cassumunar</i> EO + 5% <i>E. globulus</i> EO	5% EO from rhizomes of <i>Z. cassumunar</i> + 5% <i>E. globulus</i> EO + 90% ethyl alcohol
F11 10% <i>Z. cassumunar</i> EO	10% EO from rhizomes of <i>Z. cassumunar</i> + 90% ethyl alcohol
F12 10% <i>Z. cassumunar</i> EO + 10% <i>E. globulus</i> EO	10% EO from rhizomes of <i>Z. cassumunar</i> + 10% <i>E. globulus</i> EO + 80% ethyl alcohol
F13 cypermethrin (positive control)	10% w/v cypermethrin
F14 ethyl alcohol (negative control)	70% v/v ethyl alcohol

### 2.2. Positive control

A common mosquitocide, cypermethrin (Dethriod 10<sup>®</sup>, 10% w/v cypermethrin), was used as a positive control in this study. It was manufactured by Pentacheme Co. Ltd, 214-216 Charoenakhon Road, Khongsan, Bangkok 10600, Thailand.

### 2.3. Negative control

Ethyl alcohol at 70% v/v (Siribuncha<sup>®</sup>) in aqueous solution was used as a negative control in this study. It was manufactured by Siribuncha Co., LTD. 50/4 Mu7 Banggruay-Sainoi Rd. Nonthaburi province, Thailand; www.siribuncha.com.

**Table 1**

Chemical compositions of EOs from four plant species, expressed as relative percentage of each constituent as determined by chromatography.

Constituent <sup>a</sup>	<i>A. galanga</i>	<i>C. zedoaria</i>	<i>Z. cassumunar</i>	<i>E. globulus</i>
1-8-cineole	45.1	12.2	-	42.6
camphor	18.7	43.2	-	-
camphene	-	15.8	-	-
caryophyllenr	-	-	2.1	-
α-fenchyl acetate	15.5	-	-	-
γ-terpinene	-	-	8.3	-
α-terpineole	10.3	-	-	-
limonene	3.3	-	-	-
E-methylcinamate	2.8	-	-	-
zingiberene	-	12.3	-	-
β-pinene	-	-	6.2	18.7
isoborneol	-	10.7	-	-
sabinaene	-	-	40.8	-
terpinen-4-ol	-	-	28.2	-
caryophyllene oxide	-	-	11.7	-
α-pinene	-	-	-	21.2
terpineol	-	-	-	11.3

<sup>a</sup>Main constituents of EOs.

### 2.4. Mosquitoes

Two species of laboratory-bred mosquitoes, *Ae. albopictus* and *An. minimus*, were used in this study. The eggs of *Ae. albopictus*

and *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: (27.7±2.3) °C and (73.8±2.2)% relative humidity with a photoperiod of 12-h light and 12-h dark (12L:12D). The mosquito eggs of each species were brought to hatch in a plastic tray (the size of 28 cm×35 cm×4 cm) containing 2 000 mL of clean water. One plastic tray was used to rear 400 larvae. The larvae were fed with ground fish food for *An. minimus* and fish food pellets (SAKURA®, 32% protein) for *Ae. albopictus* for 7-14 d until pupation occurred. The pupae were not fed with any 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 lying until they developed into adults. The male and female adults were provided with 5% glucose solution food soaked in cotton wool. 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. Two days after blood feeding, the 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 the females were prepared for oviposition, the eggs were collected to be used in the next ovicidal bioassay.

### 2.5. Oviposition deterrence bioassay

The oviposition deterrence bioassay performed was a double-choice method. The female adults were ready for use as subjects in an oviposition deterrence bioassay after two days of blood feeding. Fifteen of five-day-old gravid females per group 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 each formulation or 1 mL of 10% w/v cypermethrin or 70% v/v ethyl alcohol. The non-treatment and treatment cups were placed at the opposite corners of the cage and the cups were switched to the other position in each next replication of the experiment. After 48 h, the number of eggs laid in the non-treatment and treatment cups was counted under a stereomicroscope. The oviposition activity index (OAI), percentage effective repellency (ER%) and percentage effective repellency change (ERC%) were determined. The OAI was calculated by the following formula as described by Govindarajan *et al.*[44] and Soonwera and Phasomkusolsil[45]:

$$OAI = \frac{TT-TN}{TT+TN}$$

Where TT is the total number of mosquito eggs laid in the treatment cup and TN 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 signifies a neutral response (N); an OAI from 0 to +1.0 signifies 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 signifies 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 successfully deterred the female mosquitoes from spawning eggs.

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

$$ER\% = \frac{TN-TT}{TN} \times 100$$

ERC% as *E. globulus* EO was added to the formulation was calculated as follows:

$$ERC\% = \frac{ER\% \text{ with } E. \text{ globulus EO} - ER\% \text{ without } E. \text{ globulus EO}}{ER\% \text{ with } E. \text{ globulus EO}} \times 100$$

### 2.6. Ovicidal activity test

The ovicidal bioassay performed was a topical method. Twenty-five eggs of each species of mosquitoes in every group were placed on a filter paper, topically treated with 0.005 mL of each formulation or with the positive control (10% w/v cypermethrin) or negative control (70% v/v ethyl alcohol), then left lying for 3 h. After that, all mosquito eggs were rinsed with clean water and put in a 200-mL plastic cup containing 100 mL of clean water. The ovicidal 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 10% w/v cypermethrin (positive control) and 70% v/v ethyl alcohol (negative control). The percentage inhibition rate (IR%) of eggs was calculated by the following formula:

$$IR\% = \frac{TD}{TT} \times 100$$

Where TD is the total number of dead eggs (not hatched within 48 h); and TT is the total number of treated eggs.

The percentage inhibition rate change (IRC%) as *E. globulus* EO was added to the formulation was calculated as follows:

$$IRC\% = \frac{IR\% \text{ with } E. \text{ globulus EO} - IR\% \text{ without } E. \text{ globulus EO}}{IR\% \text{ with } E. \text{ globulus EO}} \times 100$$

### 2.7. WHO susceptibility test

The knockdown and mortality tests against mosquito females were performed using the WHO[46] susceptibility test. Two-day old female adults (not yet fed with blood meal) were collected as

subjects for a WHO susceptibility test. Twenty-five two-day-old female mosquitoes were exposed to 2 mL of each formulation that was dropped onto a filter paper (Whatman® No.1) with the size of 12 cm×15 cm for one h in the treatment tube (44 mm in diameter and 125 mm in length) then transferred to the non-treatment tube. The knockdown rate (KD%) was recorded at 1 h and the mortality rate (MR%) was recorded at 24 h after the exposure. Each treatment was performed in five replicates. Ten percent (w/v) cypermethrin and 70% v/v ethyl alcohol were used as positive control and negative control, respectively. The criterion for knockdown and mortality was no movement of any of the mosquitoes' body parts. KD% and MR% were calculated by the following formulas:

$$\text{KD\%} = \frac{\text{NK}}{\text{NT}} \times 100$$

$$\text{MR\%} = \frac{\text{ND}}{\text{NT}} \times 100$$

Where NK is the total number of knocked-down adults; ND is the total number of dead adults; and NT is the total number of treated adults.

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. According to these criteria, any substances that cause less than 98% mortality are considered to be at least 'possible resistant' which in reality, mosquitoes may not develop a resistance to them at all. They are just not 100% effective in the first place. The susceptibility status should be considered as an indicator for only a chemical insecticide that has been 100% effective in the past but not as effective at the present due to developed resistance.

The percentage knockdown rate change (KDC%) as *E. globulus* EO was added to the formulation was calculated as follows:

$$\text{KDC\%} = \frac{\text{KD\% with } E. \text{ globulus EO} - \text{KD\% without } E. \text{ globulus EO}}{\text{KD\% with } E. \text{ globulus EO}} \times 100$$

The percentage mortality rate change (MRC%) as *E. globulus* EO was added to the formulation was calculated as follows:

$$\text{MRC\%} = \frac{\text{MR\% with } E. \text{ globulus EO} - \text{MR\% without } E. \text{ globulus EO}}{\text{MR\% with } E. \text{ globulus EO}} \times 100$$

## 2.8. Statistical analysis

Five replications were performed in oviposition deterrence bioassay and the results were compared by a paired *t*-test. Moreover, all of the means of inhibition, knockdown and mortality rates were analyzed and compared by analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). *P* value less than 0.5 was considered statistically significant.

## 3. Results

Table 3 presents the oviposition deterrent activities at 48 h exposure of 12 formulations of EOs from Zingiberaceae plants and *E. globulus* as well as of 10% w/v cypermethrin and 70% v/v ethyl alcohol in terms of OAI, ER% and ERC% against the females of *Ae. albopictus*. It was found that the OAI and ER% of formulations of Zingiberaceae plant EOs (*A. galanga* EO and *Z. cassumunar* EO) augmented with *E. globulus* EO (F2, F4, F10, and F12) were higher than those of corresponding formulations without *E. globulus*. The highest ERC% at 34.4% was achieved by F2 (5% *A. galanga* EO + 5% *E. globulus* EO) followed by F10 (5% *Z. cassumunar* EO + 5% *E. globulus* EO), F4 (10% *A. galanga* EO + 10% *E. globulus* EO), and F12 (10% *Z. cassumunar* EO + 10% *E. globulus* EO) with ERC% at 19.7%, 1.4%, and 1.2%, respectively. The lowest ERC% of 0% was from F6 (5% *C. zedoaria* EO + 5% *E. globulus* EO), and F8 (10% *C. zedoaria* EO + 10% *E. globulus* EO), while F5 (5% *C. zedoaria* EO) and F7

**Table 3**

Oviposition deterrent effect of 12 formulations from Zingiberaceae plant EOs with and without augmenting *E. globulus* EO as well as cypermethrin and ethyl alcohol against the females of *Ae. albopictus*.

Formulation	No. of eggs ± SD		OAI	ER%	ERC%
	Treatment cup	Non-treatment cup			
F1 5% <i>A. galanga</i> EO	175.6±49.8	509.8±66.3	-0.488	65.6	
F2 5% <i>A. galanga</i> EO + 5% <i>E. globulus</i> EO	0	34.8±26.8*	-1.000	100.0	34.4
F3 10% <i>A. galanga</i> EO	10.4±9.8	724.8±68.3*	-0.972	98.6	
F4 10% <i>A. galanga</i> EO + 10% <i>E. globulus</i> EO	0	677.3±58.7*	-1.000	100.0	1.4
F5 5% <i>C. zedoaria</i> EO	0	728.5±78.7*	-1.000	100.0	
F6 5% <i>C. zedoaria</i> EO + 5% <i>E. globulus</i> EO	0	578.2±48.3*	-1.000	100.0	0.0
F7 10% <i>C. zedoaria</i> EO	0	638.5±58.7*	-1.000	100.0	
F8 10% <i>C. zedoaria</i> EO + 10% <i>E. globulus</i> EO	0	548.8±55.4*	-1.000	100.0	0.0
F9 5% <i>Z. cassumunar</i> EO	157.8±25.4	799.4±68.3	-0.670	80.3	
F10 5% <i>Z. cassumunar</i> EO + 5% <i>E. globulus</i> EO	0	534.7±65.8*	-1.000	100.0	19.7
F11 10% <i>Z. cassumunar</i> EO	9.8±5.7	805.2±70.3*	-0.976	98.8	
F12 10% <i>Z. cassumunar</i> EO + 10% <i>E. globulus</i> EO	0	675.7±67.8*	-1.000	100.0	1.2
F13 10% w/v cypermethrin	0	20.5±7.8*	-1.000	100.0	
F14 70% v/v ethyl alcohol	685.2±77.8	695.5±88.9	-0.007	1.5	

OAI = oviposition activity index, ER% = effective repellency, ERC% = effective repellency change as *E. globulus* EO was added.

\*A significant difference between the treatment cup and non-treatment cup by paired *t* test (*P*<0.05).

(10% *C. zedoaria* EO) showed the highest OAI and ER% at -1.0 and 100%, respectively. It was also found that the efficacies of 5% and 10% *C. zedoaria* EO augmented with *E. globulus* EO were equivalent in terms of oviposition deterrent activity to those that were not augmented. On the other hand, 5% and 10% *A. galanga* EO as well as 5% and 10% *Z. cassumunar* EO exhibited a considerable increase in effective repellency after being augmented with *E. globulus* EO. They also exhibited an equivalent oviposition deterrent activity and effective repellency to cypermethrin (positive control) and an obviously higher oviposition deterrent activity than ethyl alcohol did (negative control).

The oviposition deterrent activities at 48 h exposure of the 12 formulations of EOs from Zingiberaceae plants and *E. globulus* as well as 10% w/v cypermethrin and 70% v/v ethyl alcohol against the females of *An. minimus* are summarized in Table 4. It was found that all formulations of EOs from Zingiberaceae plants augmented with *E. globulus* EO (F2, F4, F6, F8, F10, and F12) showed the highest oviposition deterrent activity against the females of *An. minimus* with 100% ER and -1.0 OAI. The four formulations of pure EOs from Zingiberaceae plants (F3, F5, F7, and F11) exhibited the highest oviposition deterrent activity similar to that of all formulations of Zingiberaceae EOs that were augmented with *E. globulus* EO. The F1 and F9 formulations provided an OAI at -0.996, -0.792, and ER% at 99.8%, 86.6%, respectively. The maximum change in effective repellency after the EO was augmented with *E. globulus* EO was from F10 (5% *Z. cassumunar* EO + 5% *E. globulus* EO) formulation which exhibited an increase in effective repellency with 13.4% ERC, followed by F2 (5% *A. galanga* EO + 5% *E. globulus* EO) with 0.2% ERC. Similarly, cypermethrin (F13) exhibited -1.0 OAI and 100% ER. In contrast, ethyl alcohol (F14) showed only -0.003 OAI and 0.5% ER. In addition, all formulations of EOs from Zingiberaceae plants either augmented or non-augmented with *E. globulus* EO showed an equivalent oviposition deterrent activity and effective repellency to cypermethrin except F1 and F9 formulations. As expected, all formulations exhibited a higher oviposition deterrent

activity and effective repellency than ethyl alcohol did.

Table 5 shows the ovicidal activities in terms of inhibition rate of the 12 formulations of EOs from Zingiberaceae plants with and without augmenting *E. globulus* EO as well as of 10% w/v cypermethrin and 70% v/v ethyl alcohol against the eggs of *Ae. albopictus* and *An. minimus*. It was found that the inhibition rates against the *Ae. albopictus* eggs of all formulations of EOs from Zingiberaceae plants augmented with *E. globulus* EO were higher than those of pure Zingiberaceae EOs. The highest inhibition rate at 100% was achieved by F4 (10% *A. galanga* EO + 10% *E. globulus* EO), F8 (10% *C. zedoaria* EO + 10% *E. globulus* EO), and F12 (10% *Z. cassumunar* EO + 10% *E. globulus* EO). This highest rate was equivalent to that of cypermethrin. The lowest inhibition rate of 11.2% was from F9 (5% *Z. cassumunar* EO). In addition, F1 (5% *A. galanga* EO), and F3 (10% *A. galanga* EO) provided an inhibition rate of 73.7% and 92.7%, respectively, while F5 (5% *C. zedoaria* EO) and F7 (10% *C. zedoaria* EO) provided an inhibition rate of 25.9% and 46.3%, respectively. These inhibition rates were lower than that from cypermethrin. As expected, the 12 formulations of EOs from Zingiberaceae plants with and without augmenting *E. globulus* EO showed significantly higher inhibition rates when compared to that provided by ethyl alcohol ( $P < 0.05$ ). The maximum change in inhibition rate as a result of augmentation with *E. globulus* EO occurred in F12 (10% *Z. cassumunar* EO + 10% *E. globulus* EO), which showed an increase of 88.5% in inhibition rate after augmentation, followed by F10 (5% *Z. cassumunar* EO + 5% *E. globulus* EO), F6 (5% *C. zedoaria* EO + 5% *E. globulus* EO), F8 (10% *C. zedoaria* EO + 10% *E. globulus* EO), F2 (5% *A. galanga* EO + 5% *E. globulus* EO), and F4 (10% *A. galanga* EO + 10% *E. globulus* EO) with an increase of 88.4%, 72.8%, 53.7%, 24.7%, and 7.3%, respectively. As expected, the negative control ethyl alcohol showed 0% inhibition rate (100% hatching rate).

Regarding the inhibition rate against the *An. minimus* eggs, it was found that after they were treated with the 12 formulations of EOs from Zingiberaceae plants augmented and not augmented with *E. globulus* EO, the inhibition rates were quite similar to those against

**Table 4**

Oviposition deterrent effects of 12 formulations of Zingiberaceae plant EOs with and without augmenting *E. globulus* EO as well as cypermethrin and ethyl alcohol against the females of *An. minimus*.

Formulation	No. of eggs $\pm$ SD		OAI	ER%	ERC%
	Treatment cup	Non-treatment cup			
F1 5% <i>A. galanga</i> EO	1.2 $\pm$ 0.7	569.6 $\pm$ 72.8*	-0.996	99.8	
F2 5% <i>A. galanga</i> EO + 5% <i>E. globulus</i> EO	0	458.7 $\pm$ 63.7*	-1.000	100.0	0.2
F3 10% <i>A. galanga</i> EO	0	605.5 $\pm$ 54.5*	-1.000	100.0	
F4 10% <i>A. galanga</i> EO + 10% <i>E. globulus</i> EO	0	487.2 $\pm$ 62.7*	-1.000	100.0	0.0
F5 5% <i>C. zedoaria</i> EO	0	583.8 $\pm$ 82.3*	-1.000	100.0	
F6 5% <i>C. zedoaria</i> EO + 5% <i>E. globulus</i> EO	0	438.5 $\pm$ 53.8*	-1.000	100.0	0.0
F7 10% <i>C. zedoaria</i> EO	0	550.5 $\pm$ 62.7*	-1.000	100.0	
F8 10% <i>C. zedoaria</i> EO + 10% <i>E. globulus</i> EO	0	532.8 $\pm$ 65.2*	-1.000	100.0	0.0
F9 5% <i>Z. cassumunar</i> EO	68.0 $\pm$ 27.5	509.2 $\pm$ 82.3*	-0.792	86.6	
F10 5% <i>Z. cassumunar</i> EO + 5% <i>E. globulus</i> EO	0	438.7 $\pm$ 75.6*	-1.000	100.0	13.4
F11 10% <i>Z. cassumunar</i> EO	0	537.8 $\pm$ 65.8*	-1.000	100.0	
F12 10% <i>Z. cassumunar</i> EO + 10% <i>E. globulus</i> EO	0	575.4 $\pm$ 77.9*	-1.000	100.0	0.0
F13 10% w/v cypermethrin	0	28.7 $\pm$ 12.5*	-1.000	100.0	
F14 70% v/v ethyl alcohol	587.4 $\pm$ 87.8	590.5 $\pm$ 78.9	-0.003	0.5	

OAI = oviposition activity index, ER% = effective repellency, ERC% = effective repellency change as *E. globulus* EO was added.

\*A significant difference between the treatment cup and non-treatment cup by paired *t* test ( $P < 0.05$ ).

the *Ae. albopictus* eggs except for formulation with 5% and 10% *Z. cassumunar* (Table 5). The highest inhibition rate at 100% was provided by F1 (5% *A. galanga* EO), F2 (5% *A. galanga* EO + 5% *E. globulus* EO), F3 (10% *A. galanga* EO), F4 (10% *A. galanga* EO + 10% *E. globulus* EO), F6 (5% *C. zedoaria* EO + 5% *E. globulus* EO), F8 (10% *C. zedoaria* EO + 10% *E. globulus* EO), F10 (5% *Z. cassumunar* EO + 5% *E. globulus* EO), and F12 (10% *Z. cassumunar* EO + 10% *E. globulus* EO). This inhibition rate was equivalent to that provided by cypermethrin. As expected, all of the 12 formulations were more effective in ovicidal activity than ethyl alcohol was (0% inhibition rate). The lowest inhibition rate at 15.5% was found from F5 (5% *C. zedoaria* EO) while F7 (10% *C. zedoaria* EO) provided a 39.2% inhibition rate. F9 (5% *Z. cassumunar* EO) and F11 (10% *Z. cassumunar* EO) showed an inhibition rate of 89.9% and 93.3%, respectively. The 12 formulations of EOs from Zingiberaceae plants with and without augmenting *E. globulus* EO

showed significantly higher inhibition rates when compared to that provided by ethyl alcohol ( $P < 0.05$ ). The maximum increase in inhibition rate as a result of augmentation with *E. globulus* EO was 84.5% in F6 (5% *C. zedoaria* EO + 5% *E. globulus* EO), followed by F8 (10% *C. zedoaria* EO + 10% *E. globulus* EO), F10 (5% *Z. cassumunar* EO + 5% *E. globulus* EO), and F12 (10% *Z. cassumunar* EO + 10% *E. globulus* EO) that got an increase of 60.8%, 10.1% and 6.7%, respectively.

The knockdown rate, mortality rate, susceptibility status, KDC and MRC against *Ae. albopictus* females of the 12 formulations of EOs from Zingiberaceae plants with and without augmenting *E. globulus* as well as of 10% w/v cypermethrin and 70% v/v ethyl alcohol are presented in Table 6. All formulations of Zingiberaceae plant EOs with augmenting *E. globulus* EO showed the highest efficacy against the *Ae. albopictus* females with a knockdown rate of 100% at 1 h after exposure. Moreover, the formulations of Zingiberaceae

**Table 5**

Ovicidal effect of 12 formulations from Zingiberaceae plant EOs with and without augmenting *E. globulus* EO as well as cypermethrin and ethyl alcohol against the eggs of *Ae. albopictus* and *An. minimus*.

Formulation	<i>Ae. albopictus</i>		<i>An. minimus</i>	
	Inhibition rate (%) $\pm$ SD	IRC%	Inhibition rate (%) $\pm$ SD	IRC%
F1 5% <i>A. galanga</i> EO	73.7 $\pm$ 5.8 <sup>b</sup>		100 <sup>a</sup>	
F2 5% <i>A. galanga</i> EO + 5% <i>E. globulus</i> EO	97.9 $\pm$ 4.8 <sup>a</sup>	24.7	100 <sup>a</sup>	0
F3 10% <i>A. galanga</i> EO	92.7 $\pm$ 3.5 <sup>a</sup>		100 <sup>a</sup>	
F4 10% <i>A. galanga</i> EO + 10% <i>E. globulus</i> EO	100 <sup>a</sup>	7.3	100 <sup>a</sup>	0
F5 5% <i>C. zedoaria</i> EO	25.9 $\pm$ 8.5 <sup>d</sup>		15.5 $\pm$ 6.2 <sup>d</sup>	
F6 5% <i>C. zedoaria</i> EO + 5% <i>E. globulus</i> EO	95.3 $\pm$ 7.5 <sup>a</sup>	72.8	100 <sup>a</sup>	84.5
F7 10% <i>C. zedoaria</i> EO	46.3 $\pm$ 6.2 <sup>c</sup>		39.2 $\pm$ 8.5 <sup>c</sup>	
F8 10% <i>C. zedoaria</i> EO + 10% <i>E. globulus</i> EO	100 <sup>a</sup>	53.7	100 <sup>a</sup>	60.8
F9 5% <i>Z. cassumunar</i> EO	11.2 $\pm$ 5.7 <sup>c</sup>		89.9 $\pm$ 2.7 <sup>b</sup>	
F10 5% <i>Z. cassumunar</i> EO + 5% <i>E. globulus</i> EO	96.5 $\pm$ 7.8 <sup>a</sup>	88.4	100 <sup>a</sup>	10.1
F11 10% <i>Z. cassumunar</i> EO	11.5 $\pm$ 4.7 <sup>c</sup>		93.3 $\pm$ 2.3 <sup>ab</sup>	
F12 10% <i>Z. cassumunar</i> EO + 10% <i>E. globulus</i> EO	100 <sup>a</sup>	88.5	100 <sup>a</sup>	6.7
F13 10% w/v cypermethrin	100 <sup>a</sup>		100 <sup>a</sup>	
F14 70% v/v ethyl alcohol	0 <sup>f</sup>		0 <sup>c</sup>	

Mean percent inhibition rates in each column followed by the same letter are not significantly different (one way ANOVA and Duncan's multiple range test,  $P < 0.05$ ).

IRC% = Inhibition rate change as *E. globulus* EO was added.

**Table 6**

Knockdown and mortality rates as well as susceptibility status of the *Ae. albopictus* females against 12 formulations of EOs from Zingiberaceae plants with and without augmenting *E. globulus* EO, cypermethrin and ethyl alcohol.

Formulation	Knockdown rate (%) $\pm$ SD	KDC (%)	Mortality rate (%) $\pm$ SD	MRC (%)	Susceptibility
F1 5% <i>A. galanga</i> EO	100 <sup>a</sup>		10.5 $\pm$ 3.7 <sup>c</sup>		R
F2 5% <i>A. galanga</i> EO + 5% <i>E. globulus</i> EO	100 <sup>a</sup>	0	100 <sup>a</sup>	89.5	S
F3 10% <i>A. galanga</i> EO	100 <sup>a</sup>		100 <sup>a</sup>		S
F4 10% <i>A. galanga</i> EO + 10% <i>E. globulus</i> EO	100 <sup>a</sup>	0	100 <sup>a</sup>	0	S
F5 5% <i>C. zedoaria</i> EO	48.5 $\pm$ 10.3 <sup>c</sup>		0 <sup>f</sup>		R
F6 5% <i>C. zedoaria</i> EO + 5% <i>E. globulus</i> EO	100 <sup>a</sup>	51.5	100 <sup>a</sup>	100.0	S
F7 10% <i>C. zedoaria</i> EO	100 <sup>a</sup>		40.3 $\pm$ 8.7 <sup>d</sup>		R
F8 10% <i>C. zedoaria</i> EO + 10% <i>E. globulus</i> EO	100 <sup>a</sup>	0	100 <sup>a</sup>	59.7	S
F9 5% <i>Z. cassumunar</i> EO	78.6 $\pm$ 8.3 <sup>b</sup>		75.2 $\pm$ 7.3 <sup>c</sup>		R
F10 5% <i>Z. cassumunar</i> EO + 5% <i>E. globulus</i> EO	100 <sup>a</sup>	21.4	100 <sup>a</sup>	24.8	S
F11 10% <i>Z. cassumunar</i> EO	100 <sup>a</sup>		100 <sup>a</sup>		S
F12 10% <i>Z. cassumunar</i> EO + 10% <i>E. globulus</i> EO	100 <sup>a</sup>	0	100 <sup>a</sup>	0	S
F13 10% w/v cypermethrin	100 <sup>a</sup>		95.2 $\pm$ 7.8 <sup>b</sup>		PR
F14 70% v/v ethyl alcohol	0 <sup>d</sup>		0 <sup>f</sup>		R

Mean percent knockdown and mortality rates in each column followed by the same letter are not significantly different (one way ANOVA and Duncan's multiple range test,  $P < 0.05$ ).

KDC% = Knockdown rate change as *E. globulus* EO was added; MRC% = Mortality rate change as *E. globulus* EO was added; S, Susceptible, means 98%–100% of mosquito mortality; PR, Possible resistant, means 80%–97% of mosquito mortality; R, Resistant, means less than 80% of mosquito mortality.

plant EOs without augmenting *E. globulus* EO (F1: 5% *A. galanga* EO; F3: 10% *A. galanga* EO; F7: 10% *C. zedoaria* EO; F11: 10% *Z. cassumunar* EO) also showed high toxicity to the *Ae. albopictus* females with a knockdown rate of 100%. This highest knockdown rate was equivalent to that provided by 10% w/v cypermethrin. F5 (5% *C. zedoaria* EO) and F9 (5% *Z. cassumunar* EO) provided a knockdown rate of 48.5% and 78.6%, respectively. In contrast, 70% v/v ethyl alcohol did not cause any knockdown at all and was non-toxic to the *Ae. albopictus* females. The maximum increase in KDC as a result of augmentation with *E. globulus* EO was 51.5% for F6 (5% *C. zedoaria* EO + 5% *E. globulus* EO), followed by F10 (5% *Z. cassumunar* EO + 5% *E. globulus* EO) that showed an increase of 21.4%. The *Ae. albopictus* females were susceptible to all formulations of Zingiberaceae plant EOs with augmenting *E. globulus* EO. F3 (10% *A. galanga* EO) and F11 (10% *Z. cassumunar* EO) provided a mortality rate of 100% at 24 h after exposure. The susceptibility status of the *Ae. albopictus* females against them was 'S=Susceptible'. Other formulations used without *E. globulus* EO showed mortality rates ranging from 0.0% to 75.2% with an 'R = Resistance' status. Meanwhile, 10% w/v cypermethrin showed 95.2% mortality rate and the susceptibility status of *Ae. albopictus* females against it was 'PR = Possible Resistance' while 70% v/v ethyl alcohol did not cause any mortality at all and was non-toxic to the *Ae. albopictus* females. The maximum change in mortality rate as a result of augmentation with *E. globulus* EO was 100% for F6 (5% *C. zedoaria* EO+ 5% *E. globulus* EO) followed by F2 (5% *A. galanga* EO + 5% *E. globulus* EO), F8 (10% *C. zedoaria* EO + 10% *E. globulus* EO) and F10 (5% *Z. cassumunar* EO + 5% *E. globulus* EO) that showed an increase of 89.5%, 59.7% and 24.8%, respectively.

For the *An. minimus* females (Table 7), it was found that knockdown and mortality rates, susceptibility status, KDC and MRC for *An. minimus* females after they were treated with 12 formulations of EOs from Zingiberaceae plants with and without

augmenting *E. globulus* EO were similar to those results for the *Ae. albopictus* females. All formulations of Zingiberaceae plant EOs augmented with *E. globulus* EO showed the highest efficacy against the females of *An. minimus* with a knockdown rate of 100% at 1 h after exposure. In addition, the formulations of Zingiberaceae plant EOs without augmenting *E. globulus* EO-F1 (5% *A. galanga* EO), F3 (10% *A. galanga* EO), F9 (5% *Z. cassumunar* EO), and F11 (10% *Z. cassumunar* EO)-also showed the highest efficacy against *An. minimus* females with a knockdown rate of 100%. This knockdown rate result was equivalent to that of 10% w/v cypermethrin. F5 (5% *C. zedoaria* EO) and F7 (10% *C. zedoaria* EO) provided a knockdown rate of 62.8% and 94.4%, respectively. In contrast, 70% v/v ethyl alcohol did not cause a knockdown at all, thus it was non-toxic to the *An. minimus* females. The maximum change in knockdown rate as a result of augmentation with *E. globulus* EO was 37.2% for F6 (5% *C. zedoaria* EO + 5% *E. globulus* EO), followed by F8 (10% *C. zedoaria* EO + 10% *E. globulus* EO) that showed an increase of 5.6%. In addition, the highest mortality rate against *An. minimus* females reached 100% at 24 h after exposure and the susceptibility status of the *An. minimus* females against them was 'S = Susceptible' except F5 (5% *C. zedoaria* EO), F7 (10% *C. zedoaria* EO), and F9 (5% *Z. cassumunar* EO). F7 (10% *C. zedoaria* EO), F9 (5% *Z. cassumunar* EO), and F5 (5% *C. zedoaria* EO) provided a mortality rate of 76.3%, 55.2%, and 38.5%, respectively, and the susceptibility status of *An. minimus* females against them was 'R = Resistance'. Meanwhile, 10% w/v cypermethrin provided a 96.2% mortality rate and the susceptibility status of *An. minimus* females against it was 'PR = Possible Resistance'. In contrast, 70% v/v ethyl alcohol did not cause any mortality at all and was non-toxic to the *An. minimus* females. The maximum change in mortality rate as a result of augmentation with *E. globulus* EO was 61.5% for F6 (5% *C. zedoaria* EO + 5% *E. globulus* EO), followed by F10 (5% *Z. cassumunar* EO + 5% *E. globulus* EO) and F8 (10% *C. zedoaria* EO +10% *E. globulus*

**Table 7**

Knockdown and mortality rates and susceptibility status of the *An. minimus* females against 12 formulations of EOs from Zingiberaceae plants with and without *E. globulus* EO, cypermethrin and ethyl alcohol.

Formulation	Knockdown rate (%) ± SD	KDC%	Mortality rate (%) ± SD	MRC%	Susceptibility
F1 5% <i>A. galanga</i> EO	100 <sup>a</sup>		100 <sup>a</sup>		S
F2 5% <i>A. galanga</i> EO + 5% <i>E. globulus</i> EO	100 <sup>a</sup>	0	100 <sup>a</sup>	0	S
F3 10% <i>A. galanga</i> EO	100 <sup>a</sup>		100 <sup>a</sup>		S
F4 10% <i>A. galanga</i> EO + 10% <i>E. globulus</i> EO	100 <sup>a</sup>	0	100 <sup>a</sup>	0	S
F5 5% <i>C. zedoaria</i> EO	62.8±10.3 <sup>c</sup>		38.5±5.7 <sup>e</sup>		R
F6 5% <i>C. zedoaria</i> EO + 5% <i>E. globulus</i> EO	100 <sup>a</sup>	37.2	100 <sup>a</sup>	61.5	S
F7 10% <i>C. zedoaria</i> EO	94.4±9.1 <sup>b</sup>		76.3±7.2 <sup>c</sup>		R
F8 10% <i>C. zedoaria</i> EO + 10% <i>E. globulus</i> EO	100 <sup>a</sup>	5.6	100 <sup>a</sup>	23.7	S
F9 5% <i>Z. cassumunar</i> EO	100 <sup>a</sup>		55.2±5.3 <sup>d</sup>		R
F10 5% <i>Z. cassumunar</i> EO + 5% <i>E. globulus</i> EO	100 <sup>a</sup>	0	100 <sup>a</sup>	44.8	S
F11 10% <i>Z. cassumunar</i> EO	100 <sup>a</sup>		100 <sup>a</sup>		S
F12 10% <i>Z. cassumunar</i> EO + 10% <i>E. globulus</i> EO	100 <sup>a</sup>	0	100 <sup>a</sup>	0	S
F13 10% w/v cypermethrin	100 <sup>a</sup>		96.2±8.8 <sup>b</sup>		PR
F14 70% v/v ethyl alcohol	0 <sup>d</sup>		0 <sup>f</sup>		R

Mean percent knockdown and mortality rates in each column followed by the same letter are not significantly different (one way ANOVA and Duncan's multiple range test,  $P < 0.05$ ).

KDC% = Knockdown rate change as *E. globulus* EO was added; MRC% = Mortality rate change as *E. globulus* EO was added; S, Susceptible, means 98%–100% of mosquito mortality; PR, Possible resistant, means 80%–97% of mosquito mortality; R, Resistant, means less than 80% of mosquito mortality.



EO) that showed an increase of 44.8% and 23.7%, respectively.

#### 4. Discussion

All of the combinations of three Zingiberaceae plant EOs (*A. galanga*, *C. zedoaria*, and *Z. cassumunar*) augmented with *E. globulus* EO clearly exhibited highly effective repellency, inhibition rate, knockdown rate and mortality rate against the females of both *Ae. albopictus* and *An. minimus*. Moreover, all of the combinations showed a synergistic effect. These results confirmed that *E. globulus* EO acted as a synergist agent with the three Zingiberaceae plant EOs. Researchers have reported that several combinations of different EOs or phytochemical groups showed a higher insecticidal activity, repellency or ovicidal activity than the individual EO or phytochemical group[27,41,47]. Our results are in agreement with the result from another research study in which a combination of EOs from mentha [*Mentha piperrita* (*M. piperrita*)] + eucalyptus (*E. globulus*) at 50:50 ratio was more toxic to the larvae of *Anopheles stephensi* and house fly [*Musca domestica* (*M. domestica*)] than *E. globulus* EO alone[46]. A result from another study of another insect pest was that combinations of EOs from mentha (*M. piperrita*) + eucalyptus (*E. globulus*) at 70:30 and 50:50 ratio showed a higher repellency activity against the adults of house fly (*M. domestica*) than individual *E. globulus* EO did[48]. In the same vein, another study showed that combinations of EOs from *Lippia origanoides* + *Swinglea glutinosa* and *Turnera diffusa* + *Swinglea glutinosa* showed higher larvicidal activities against *Ae. aegypti* larvae than the individual EOs did[23]. In addition, a study indicated that the combinations of EOs from *M. piperrita* EO + *E. globulus* EO showed higher larvicidal activities against *Anopheles stephensi* and house fly (*M. domestica*) than *E. globulus* EO alone did. However, there has been a report that *M. piperrita* EO alone showed a higher larvicidal activity against the larvae of *Anopheles stephensi* and *M. domestica* than its combination with an EO from *Cymbopogon citratus* did[43].

A combination of seven major components of *Hyptis suaveolens* EO (sabinene +  $\alpha$ -pinene +  $\beta$ -pinene + limonene + terpinolene +  $\beta$ -carophyllene + 4-terpineol) exhibited a synergistic effect and showed high toxicity to *Ae. albopictus* larvae[49]. In addition, a combination of five major components of geranium EO (geraniol + citronellol + citronellyl + formate + linalool) at a ratio of 1:1:1:1:1 exhibited a synergistic effect and high toxicity to head louse females (*Pediculus humanus capitis*)[50]. Combinations of tea tree oil and nerolidol also showed high toxicity to all stages of head louse[51].

In our study, two combinations of EOs (1:1 ratio of % 5% *A. galanga* EO + 5% *E. globulus* EO and 5% *Z. cassumunar* EO + 5% *E. globulus* EO) showed the highest synergistic effect at effective repellency change against the females of *Ae. albopictus* and *An. minimus*, respectively. In addition, three combinations of EOs (1:1 ratio of 10% *A. galanga* EO + 10% *E. globulus* EO, 10% *C. zedoaria* EO + 10% *E. globulus* EO, and 10% *Z. cassumunar* EO

+ 10% *E. globulus* EO) exhibited the highest synergistic effect at 100% inhibition rate against the eggs of the two mosquito species. Moreover, two combinations of EOs (1:1 ratio of 10% *A. galanga* EO + 10% *E. globulus* EO and 10% *Z. cassumunar* EO + 10% *E. globulus* EO) also showed the highest synergistic effect at 100% knockdown and mortality rates. Several researchers have reported that the major constituents of EOs from *A. galanga*, *C. zedoaria*, and *Z. cassumunar* were monoterpenes, 1-8-cineole, camphor, terpinen-4-ol,  $\alpha$ -pinene,  $\beta$ -pinene, and terpineol and the major constituents of *E. globulus* EO were also monoterpenes, 1-8-cineole, terpinen-4-ol,  $\alpha$ -pinene, and  $\beta$ -pinene[38,52,53]. Monoterpenes from plant EOs have been reported to cause mortality of insect pests by inhibition of GABA (gamma aminobutyric acid) receptor, acetylcholinesterase, octopamine and neuromodulator of the insect nervous system[54]. Monoterpenes from plant EOs (geraniol, linalool and citronella) showed high repellency against the females of *Ae. aegypti*[55]. Similarly, 1-8-cineole, camphor, and terpinen-4-ol from *C. zedoaria* and *Zingiber zerumbet* EOs were highly toxic to *Sitophilus zeamais* and *Tribolium castaneum* (major insect pests of stored products)[52,53]. Camphor from plant EO also showed high toxicity to housefly adults[56]. The results from this study pointed out that the synergistic effect between EOs from *A. galanga*, *C. zedoaria*, and *Z. cassumunar* and *E. globulus* resulted in high toxicity to the nervous system of the two mosquito species. More importantly, all combinations of EOs were either more or equivalently effective in oviposition deterrent, ovicidal, and adulticidal activities than 10 % w/v cypermethrin. Therefore, the combinations of *A. galanga*, *C. zedoaria*, and *Z. cassumunar* with *E. globulus* EOs have a high potential for developing into a new ovicidal and adulticidal formulations against these two mosquito vectors which are especially vectors of dengue and malaria. An important and necessary future study is to perform a rigorous field experiment on the toxicity of these combinations of EOs before they can be used with confidence as a botanical ovicide and adulticide for mosquito vector control.

#### Conflict of interest statement

We declare that there is no other conflict of interest regarding the content of this article with any other parties whatsoever.

#### Acknowledgements

We would like to thank the botanist and entomologist at King Mongkut's Institute of Technology Ladkrabang (KMITL) for their help in identifying the species of Zingiberaceae plants and the mosquitoes. We also would like to thank Mr. Pratana Kangsadal, the KMITL Proofreader, for commenting on and reviewing the English of this manuscript.

## Funding

This research was supported by Faculty of Agricultural Technology, KMITL, Bangkok, Thailand (Grant no 01-04-002) and the National Research Council of Thailand (GRAD 6007KMITL). These funding sources had no involvement in the study design, interpretation, collection and analysis of data, in the writing of manuscript and in the decision to submit the article for publication.

## References

- [1] Pompon J, Manuel M, Ng GK, Wong B, Shan C, Manokaran G, et al. Dengue subgenomic flaviviral RNA disrupts immunity in mosquito salivary glands to increase virus transmission. *PLoS Pathog* 2017; **13**(7): e1006535.
- [2] Akiner MM, Demirci B, Babuadze G, Robert V, Schaffner F. Spread of the invasive mosquitoes *Aedes aegypti* and *Aedes albopictus* in the black sea region increases risk of chikungunya, dengue, and zika outbreaks in europe. *PLoS Negl Trop Dis* 2016; **10**(4): e0004664.
- [3] World Health Organization (WHO). *Dengue: Guidelines for diagnosis, treatment, prevention and control*. Geneva: WHO; 2009, p. 160.
- [4] Leta S, Beyene TJ, De Clercq EM, Amenu K, Kraemer MUG, Revie CW. Global risk mapping for major diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. *Int J Infect Dis* 2018; **67**: 25-35.
- [5] Attaway DF, Waters NM, Geraghty EM, Jacobsen KH. Zika virus: Endemic and epidemic ranges of *Aedes* mosquito transmission. *J Infect Public Health* 2017; **10**(1): 120-123.
- [6] Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R, Goindin D, et al. Differential susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to zika virus. *PLoS Negl Trop Dis* 2016; **10**(3): e0004543.
- [7] Sonkong K, Chaiklieng S, Neave P, Suggaravetsiri P. Factors affecting delay in seeking treatment among malaria patients along Thailand-Myanmar border in Tak Province, Thailand. *Malar J* 2015; **14**: 1-8. Doi: 10.1186/1475-2875-14-3.
- [8] Sriwichai P, Samung Y, Sumruayphol S, Kiattibutr K, Kumpitak C, Payakkapol A, et al. Natural human *Plasmodium* infections in major *Anopheles* mosquitoes in western Thailand. *Parasit Vectors* 2016; **9**: 17. Doi: 10.1186/s13071-016-1295-x.
- [9] Kounnavong S, Gopinath D, Hongvanthong B, Khamkong C, Sichanthongthip O. Malaria elimination in Lao PDR: The challenges associated with population mobility. *Infect Dis Poverty* 2017; **6**: 1-9. Doi: 10.1186/s40249-017-0283-5.
- [10] Aguirre-Obando OA, Anna Julia Pietrobon AJ, Bona ACD, Navarro-Silva MA. Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in *Aedes aegypti* populations from Jacarezinho (Brazil) after a Dengue Outbreak. *Rev Bras Entomol* 2016; **60**(1): 94-100.
- [11] Killeen GF, Ranson H. Insecticide-resistant malaria vectors must be tackled. *Lancet* 2018; **391**(10130): 1551-1552.
- [12] McAllister JC, Adams MF. Mode of action for natural products isolated from essential oils of two trees is different from available mosquito adulticides. *J Med Entomol* 2010; **47**(6): 1123-1126.
- [13] Pavela R. Essential oils for the development of eco-friendly mosquito larvicides: A review. *Ind Crops Prod* 2015; **76**: 174-187.
- [14] Gerwick BC, Sparks TC. Natural products for pest control: An analysis of their role, value and future. *Pest Manag Sci* 2014; **70**(8): 1169-1185.
- [15] Baskar K, Sudha V, Nattudurai G, Ignacimuthu S, Duraipandiyar V, Jayakumar M, et al. Larvicidal and repellent activity of the essential oil from *Atalantia monophylla* on three mosquito vectors of public health importance, with limited impact on non-target zebra fish. *Physiol Mol Plant Pathol* 2018; **101**: 197-201.
- [16] Benelli G, Pavela R. Beyond mosquitoes-Essential oil toxicity and repellency against bloodsucking insects. *Ind Crops Prod* 2018; **117**: 382-392.
- [17] Benelli G, Rajeswary M, Govindarajan M. Towards green oviposition deterrents? effectiveness of *Syzygium lanceolatum* (Myrtaceae) essential oil against six mosquito vectors and impact on four aquatic biological control agents. *Environ Sci Pollut Res Int* 2018; **25**(11): 10218-10227.
- [18] Dias CN, Moraes DF. Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: Review. *Parasitol Res* 2014; **113**(2): 565-592.
- [19] Benelli G, Bedini S, Cosci F, Toniolo C, Conti B, Nicoletti M. Larvicidal and ovideterrent properties of neem oil and fractions against the filariasis vector *Aedes albopictus* (Diptera: Culicidae): A bioactivity survey across production sites. *Parasitol Res* 2015; **114**(1): 227-236.
- [20] Soonwera M. Efficacy of essential oil from *Cananga odorata* (Lamk.) Hook.f. & Thomson (Annonaceae) against three mosquito species *Aedes aegypti* (L.), *Anopheles dirus* (Peyton and Harrison), and *Culex quinquefasciatus* (Say). *Parasitol Res* 2015; **114**(12): 4531-4543.
- [21] Soonwera M, Phasomkusolsil S. Effect of *Cymbopogon citratus* (lemongrass) and *Syzygium aromaticum* (clove) oils on the morphology and mortality of *Aedes aegypti* and *Anopheles dirus* larvae. *Parasitol Res* 2016; **115**(4): 1691-1703.
- [22] Chantawee A, Soonwera M. Efficacies of four plant essential oils as larvicide, pupicide and oviposition deterrent agents against dengue fever mosquito, *Aedes aegypti* Linn. (Diptera: Culicidae). *Asian Pac J Trop Biomed* 2018; **8**(4): 217-225.
- [23] Ríos N, Stashenko EE, Duque JE. Evaluation of the insecticidal activity of essential oils and their mixtures against *Aedes aegypti* (Diptera: Culicidae). *Med Vet Entomol* 2017; **61**(4): 307-311.
- [24] Chellappandian M, Vasanth-Srinivasan P, Senthil-Nathan S, Karthi S, Thanigaivel A, Ponsankar A, et al. Botanical essential oils and uses as mosquitocides and repellents against dengue. *Environ Int* 2018; **113**: 214-230.
- [25] Kumar P, Mishra S, Malik A, Satya S. Compositional analysis and insecticidal activity of *Eucalyptus globulus* (family: Myrtaceae) essential oil against housefly (*Musca domestica*). *Acta Trop* 2012; **122**(2): 212-218.
- [26] Pitasawat B, Champakaew D, Choochote W, Jitpakdi A, Chaithong U, Kanjanapothi D, et al. Aromatic plant-derived essential oil: An alternative larvicide for mosquito control. *Fitoterapia* 2007; **78**(3): 205-210.
- [27] El-Wakeil NE. Botanical pesticides and their mode of action. *Gesunde Pflanz* 2013; **65**: 125-149.
- [28] George DR, Finn RD, Graham KM, Sparagano OA. Present and future

- potential of plant-derived products to control arthropods of veterinary and medical significance. *Parasit Vectors* 2014; **7**: 28. Doi: 10.1186/1756-3305-7-28.
- [29]Phukerd U, Soonwera M. Insecticidal effect of essential oils from *Boesenbergia rotunda* (L.) Mansf. and *Curcuma zedoaria* Rosc against dengue vector mosquito, *Aedes aegypti* L. *J Agri Tech* 2013; **9**(6): 1573-1583.
- [30]Chen IN, Chang CC, Ng CC, Wang CY, Shyu YT, Chang TL. Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. *Plant Foods Hum Nutr* 2008; **63**(1): 15-20.
- [31]Lobo R, Prabhu KS, Shirwaikar A, Shirwaikar A. *Curcuma zedoaria* Rosc. (white turmeric): A review of its chemical, pharmacological and ethnomedicinal properties. *J Pharm Pharmacol* 2009; **61**(1): 13-21.
- [32]Rao K, Ch B, Narasu LM, Giri A. Antibacterial activity of *Alpinia galanga* (L) Willd crude extracts. *Appl Biochem Biotechnol* 2010; **162**(3): 871-884.
- [33]Tholkappiyavathi K, Muthamizh Selvan K, Neyanila SK, Prakash Yoganandam G, Gopal V. A concise review on *Curcuma zedoaria*. *J Phytother* 2013; **3**(1): 1-4.
- [34]Hamdi OAA, Ye LJ, Kamarudin MNA, Hazni H, Paydar M, Looi CY, et al. Neuroprotective and antioxidant constituents from *Curcuma zedoaria* rhizomes. *Rec Nat Prod* 2015; **9**(3): 349-355.
- [35]Phukerd U, Soonwera M. Larvicidal and pupicidal activities of essential oils from Zingiberaceae plants against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* Say mosquitoes. *Southeast Asian J Trop Med Public Health* 2013; **44**(5): 761-771.
- [36]Phukerd U, Soonwera M. Repellency of essential oils extracted from Thai native plants against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say). *Parasitol Res* 2014; **113**(9): 3333-3340.
- [37]Chaiyasit D, Choochote W, Rattanachanpichai E, Chaithong U, Chaiwong P, Jitpakdi A, et al. Essential oils as potential adulticides against two populations of *Aedes aegypti*, the laboratory and natural field strains, in Chiang Mai province, northern Thailand. *Parasitol Res* 2006; **99**(6): 715-721.
- [38]Barbosa LC, Filomeno CA, Teixeira RR. Chemical variability and biological activities of *Eucalyptus* spp. essential oils. *Molecules* 2016; **21**(12): E1671.
- [39]Tolozza AC, Lucía A, Zerba E, Masuh H, Picollo MI. *Eucalyptus* essential oil toxicity against permethrin-resistant *Pediculus humanus capitis* (Phthiraptera: Pediculidae). *Parasitol Res* 2010; **106**(2): 409-414.
- [40]Lucia A, Licastro S, Zerba E, Masuh H. Yield, chemical composition, and bioactivity of essential oils from 12 species of *Eucalyptus* on *Aedes aegypti* larvae. *Entomol Exp Appl* 2008; **129**(1): 107-114.
- [41]Regnault-Roger C, Vincent C, Arnason JT. Essential oils in insect control: Low-risk products in a high-stakes world. *Annu Rev Entomol* 2012; **57**: 405-424.
- [42]Auysawasdi N, Chuntranuluck S, Phasomkusolsil S, Keeratinijakal V. Improving the effectiveness of three essential oils against *Aedes aegypti* (Linn.) and *Anopheles dirus* (Peyton and Harrison). *Parasitol Res* 2016; **115**(1): 99-106.
- [43]Chauhan N, Malik A, Sharma S, Dhiman RC. Larvicidal potential of essential oils against *Musca domestica* and *Anopheles stephensi*. *Parasitol Res* 2016; **115**(6): 2223-2231.
- [44]Govindarajan M, Vaseeharan B, Alharbi NS, Kadaikunnan S, Khaled JM, Al-Anbr MN, et al. High efficacy of (Z)- $\gamma$ -bisabolene from the essential oil of *Galinsoga parviflora* (Asteraceae) as larvicide and oviposition deterrent against six mosquito vectors. *Environ Sci Pollut Res Int* 2018; **25**(11): 10555-10566.
- [45]Soonwera M, Phasomkusolsil S. Adulticidal, larvicidal, pupicidal and oviposition deterrent activities of essential oil from *Zanthoxylum limonella* Alston (Rutaceae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). *Asian Pac J Trop Biomed* 2017; **7**(11): 967-978.
- [46]World Health Organization. *Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets*. Geneva: World Health Organization; 2006. [Online]. Available from: <http://www.who.int/iris/handle/10665/69296> [Accessed on 17 December, 2017].
- [47]Sritabutra D, Soonwera M, Waltanachanobon S, Pongjai S. Evaluation of herbal essential oil as repellents against *Aedes aegypti* (L.) and *Anopheles dirus* Peyton & Harrison. *Asian Pac J Trop Biomed* 2011; **1**(1): S124-S128.
- [48]Chauhan N, Malik A, Sharma S. Repellency potential of essential oils against housefly, *Musca domestica* L. *Environ Sci Pollut Res Int* 2018; **25**(5): 4707-4714.
- [49]Conti B, Benelli G, Flamini G, Cioni PL, Profeti R, Ceccarini L, et al. Larvicidal and repellent activity of *Hyptis suaveolens* (Lamiaceae) essential oil against the mosquito *Aedes albopictus* Skuse (Diptera: Culicidae). *Parasitol Res* 2012; **110**(5): 2013-2021.
- [50]Gallardo A, Mougabure-Cueto G, Vassena C, Picollo MI, Toloza AC. Comparative efficacy of new commercial pediculicides against adults and eggs of *Pediculus humanus capitis* (head lice). *Parasitol Res* 2012; **110**(5): 1601-1606.
- [51]Di Campli E, Di Bartolomeo S, Delli Pizzi P, Di Giulio M, Grande R, Nostro A, et al. Activity of tea tree oil and nerolidol alone or in combination against *Pediculus capitis* (head lice) and its eggs. *Parasitol Res* 2012; **111**(5): 1985-1992.
- [52]Suthisut D, Fields PG, Chandrapatya A. Contact toxicity, feeding reduction, and repellency of essential oils from three plants from the ginger family (Zingiberaceae) and their major components against *Sitophilus zeamais* and *Tribolium castaneum*. *J Econ Entomol* 2011; **104**(4): 1445-1454.
- [53]Suthisut D, Fields PG, Chandrapatya A. Fumigant toxicity of essential oils from three Thai plants (Zingiberaceae) and their major compounds against *Sitophilus zeamais*, *Tribolium castaneum* and two parasitoids. *J Stored Prod Res* 2011; **47**(3): 222-2230.
- [54]Pavela R, Benelli G. Essential oils as ecofriendly biopesticides? challenges and constraints. *Trends Plant Sci* 2016; **21**(12): 1000-1007.
- [55]Müller GC, Junnila A, Butler J, Kravchenko VD, Revay EE, Weiss RW. Efficacy of the botanical repellents geraniol, linalool, and citronella against mosquitoes. *J Vector Ecol* 2009; **34**(1): 2-8.
- [56]Zhang Z, Xie Y, Wang Y, Lin Z, Wang L, Li G. Toxicities of monoterpenes against housefly, *Musca domestica* L. (Diptera: Muscidae). *Environ Sci Pollut Res Int* 2017; **24**(31): 24708-24713.