Essential oils from *Zingiber mekongense* Gagnep, *Myristica fragrans* Houtt and *Curcuma zedoaria* Roscoe as Larvicidal agents against *Aedes albopictus* (Skuse)

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Abstract Comparatively, *C. zedoaria* EO at 10% concentration provided the strongest larvicidal activity against *Ae. albopictus* and yielded the highest mortality rate of 100% with LC_{50} value at 1.09% and LT_{50} value of <0.01 h. The LT_{50} values for *Z. mekongense* and *M. Fragrans* EOs were 0.25, 0.52 h. respectively, while their LC_{50} value was the same at 3.00%. On the other hand, 1% w/w temephos (positive control) exhibited an LT_{50} value of 1.49 h., whereas 70% *v/v* ethyl alcohol (negative control) caused no mortality of *Ae. albopictus* larvae at all during the 48 hours of testing period. These results revealed that essential oils extracted from these plants can control mosquitoes well. They are good alternatives to temephos because they are friendly to the environment, humans and animals and should be further developed into commercial products.

Keywords: *Aedes albopictus*, Larvicidal activity, Plant essential oils

Introduction

Since the last few decades, it has been realized that mosquitoes not only transmit malaria but also many other serious pathological conditions such as dengue, chikungunya, yellow fever, Japanese Encephalitis (JE), filariasis and so on. Recently, Zika virus has created havoc in Africa, Southeast Asia, Pacific Island and Brazil (Hung *et al*., 2019). Botanical insecticides in general and essential oils, in particular, have emerged as promising, environmentally friendly alternatives. There are around 12 species of Erechtites (Asteraceae). They are indigenous in Australia, North America, South America, New Zealand, and West Indies. Many plant species have been found to contain toxic substances against insects during the last half of the century. These plants are from various families, and some chemicals that they contain have adverse effects on insects (Hung *et al*., 2019).

Mosquito-borne diseases are serious and prevail in countries where malaria is still a constant threat to 3.3 billion people (Benelli and Pavelec, 2018; Benelli and Beier, 2017). Most importantly, no medicines or vaccines are currently available for providing treatment against arboviruses, such as the virus of dengue fever. Drugs against Zika virus, malaria, and chikungunya currently employed in the fight against these parasites are seriously ineffective due to rapidly developing resistance by *Plasmodium parasites* (Benelli and Pavelec, 2018; Benelli *et al*., 2017b), while the freshly developed Plasmodium falciparum vaccine (RTS, S/AS01/Mosquirix) provides only temporary protection, as reported in a children treatment study by Gosling and Seidlein, (2016).

Adult, fully-grown mosquito population is harder to manage and control by insecticides, mainly because adult mosquitoes tend to develop resistance to insecticides after a short period of exposure. Therefore, the most effective control program is likely to be controlling mosquitoes when they are still in larvae or pupae stages (WHO, 2017; Soonwera and Phasomkusolsil, 2016). Insect management programs that apply insecticides to mosquito population in their larvae or pupae stages are called Larviciding and pupiciding methods. These methods also help preventing chikungunya dengue and other diseases vectored by mosquitoes.

Prevention from bites of sucking arthropods is based on the use of chemical pesticides. However, they hurt human health and the environment (Hicks *et al*., 2017; Silver *et al*., 2017). In addition, currently they are becoming ineffective, due to the rapidly developing resistance of the targeted vectors (Benelli and Pavelac., 2018; Naqqash *et al*., 2016).

Accordingly, it is urgent to expand a natural product vector management strategy for controlling mosquito populations. Lately, considerable attention has focused attention on essential oils as alternative agents due to their low toxicity to humans and the environment. and high biodegradability in the environment. EOs extracted from plants provide real-world applicability and high efficacy. They act as insecticides through several mechanisms of action (e.g., inhibiting P450 cytochromes, binding to GABA receptors, inhibiting cholinergic system or modulating octopaminergic system), but exert low toxicity to humans and animals including friendly to the environment (Pavela and Benelli, 2016).

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Manifold bioactive ingredients of EOs with varied action mechanisms can substantially decrease resistance in mosquitoes. It is reported that approximately 17,500 aromatic plants species have reported containing EOs; in particular, Asteraceae, Lauraceae, Lamiaceae, and Myrtaceae are major plant families is a plant extracting essential oils (Huang *et al.,* 2019).

In general, essential oils have been considered as useful insecticides, with low mammalian toxicity and rapid degradation in the environment. Essential oils derived from various plants show different bioactivities against mosquito species. These activities range from ovicidal, larvicidal, pupicidal activities to adulticidal activity as well as oviposition deterrence and repellency. Some plants that are known as excellent sources of essential oils with insecticidal properties include those in the Rutaceae family. In the Rutaceae family, plants in the genus Zanthoxylum provide a variety of secondary metabolites including alkaloids, aromatic and aliphatic amides, lignans and coumarins with important phytochemical and biological activities. (Soonwera. and Phasomkusolsil, 2017 ; Aurelie *et al.,* 2016).

Larvicidal activity is very important in vector management because larvae that are in the growth stage are the easiest to destroy. However, this kind of management program works well only when the water resource is a small area where larvae and pupae mosquitoes live in the stagnant water. Several previous papers report that essential oils extracted from various plants have larvicidal and pupicidal effects against mosquitoes. A lot of products from food, cosmetic and pharmaceutical industries are currently made from highly volatile essential oils extracted from plants (Soonwera and Phasomkusolsil, 2017). These essential oils have also been considered as crucial ingredients for natural insecticidal products that can be extracted from almost every part of a plant such as the seed, leaf, twig, flower, fruit, bark, bud, stem, and root. Since natural insecticides are made from plants oils, they are minimally toxic to mammalian species and quickly degrade in an environment with minimal impact to it.

This research aimed to determine the larvicidal and pupicidal properties against *Aedes albopictus* of essential oils extracted from three medicinal plants: *Zingiber mekongense* Gagnep, *Myristica fragrans* Houtt, and *Curcuma zedoaria* Roscoe.

Materials and methods

Mosquitoes

In this research, the *Ae. albopictus* mosquito subjects were reared in the laboratory of the Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. Their larvae were fed with fish food pellets (HIPRO®) and raised under the conditions of 26-28°C, 70-80% relative humidity, and 12:12 h. (light: dark) photoperiod. Stage-4 larvae were used in the tests for larvicidal and pupicidal activities.

Plant materials

Zingiber mekongense, Myristica fragrans and *Curcuma zedoaria* were obtained from sources in Thailand. All plant samples were correctly identified by a plant taxonomist from the Plant Production Technology Section, Faculty of Agricultural Technology, KMITL. Different plant parts were extracted for essential oils by a water distillation method. One kg of plant material was placed in an extraction column connected to a round-bottomed distillation flask containing distilled water. The flask was heated to almost 100̊ C, then the water was boiled until the distillation was complete at 4-6 h. The distilled fraction was removed of water with anhydrous sodium sulfate and kept in a refrigerator at 4 ℃ until further use. All of the extracted plant EOs were diluted with ethyl alcohol to 3 solutions at concentrations of 1%, 5%, and 10% and maintained under laboratory conditions before subsequent uses in various assays.

Chemicals

Chemical insecticide (positive control) and Ethyl Alcohol (negative control)

1) Temephos (1% *w/w* temephos; Sai GPO-1®) was employed as the positive control. Sai GPO-1®(1% *w/w* temephos) was a common chemical larvicide in Thailand. It was bought from the Government Pharmaceutical Organization, 75/1, Rama VI Rd, Ratchathewi, Bangkok 10400, Thailand.

2) Ethyl Alcohol 70% *v/v* (Alcohol Siribuncha®) was employed as the negative control. It was bought from Siribuncha Co., LTD, 50/4 Mu 7, Banggruay-sainoi Rd., Nonthaburi province, Thailand.

Larvicidal Bioassay

The larvicidal bioassay used was a standard assay of the World Health Organization (WHO,2017). Ten mosquito larvae (fourth instar larvae or pupal stage) of *Ae. albopictus* were put in a 150 ml glass jar containing 99 ml of distilled water and 1 ml of test solution. One percent *w/w* Temephos (Sai GPO-1®) was employed as the positive control and 10% ethyl alcohol was used as the negative control. During the assay, the larvae were not fed with anything. Larval mortality and pupal mortality were recorded at 0.08, 0.16, 0.5, 1, 6, 12 and 24 h. The Lethal time for 50% mortality (LT_{50}) was calculated according to probit analysis and significant differences were analyzed by one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) with SPSS for windows software version 16.0.

Results

The results of the larvicidal assay for 1%, 5% and 10 % essential oils were recorded as mortality rate listed in table 2, 3 and 4, respectively, and LT_{50} as well as LC_{50} are listed in table 1.

The mortality rate was higher with increasing concentration of essential oils. *C. zedoaria* provided an LT_{50} of less than 0.01 h. against *Ae. albopictus*, while *Z. mekongense* and *M. fragrans* EOs provided an LT_{50} of 0.25 and 0.52 h, respectively. At 5% concentration, *C. zedoaria* and *Z. mekongense* provided an LT_{50} of 0.02 and 0.36 h, respectively, while *M. fragrans* EO was the least effective with an LT_{50} of 0.48 h. At 1% concentration, *Z. mekongense* EO did not induce any mortality against *Ae. albopictus* larvae (Table 2), but *C. zedoaria* EO and *M. fragrans* EO provided an LT_{50} of 1.06 and 6.04 h, respectively. Ten percent essential oil from *C. zedoaria* provided the highest larvicidal effect against the larvae of *Ae. albopictus* within the shortest period (LT_{50} < 0.01 h.) and had the lowest LC_{50} of 1.09%, followed by the essential oils from *Z.mekongense* and *M. fragrans* that had an identical LC_{50} of 3.00%.

Table 1. 50% lethal concentration (LC_{50}) and 50% lethal time of three essential oils, negative, and postsitive control against fourth instar larvae of *Ae. albopictus*

Treatment	LC_{50} (%)	LT_{50} (h)(LCL-UCL)1// concentration(%)		
		1 %	5 %	10 %
70% *v/v* Ethyl alcohol (Negative control)	NA2/	NA	NA	NA
1% *w/w* Temephos (Postsitive control)	NA	1.49 (1.36-1.62)	1.49 (1.36-1.62)	1.49 (1.36-1.62)
C. zedoaria EO	1.09 (-)	1.06 (0.97-1.20)	0.02 (-0.25-1.41)	<0.01(-1.07-0.03)
Z.mekongense EO	3.00 (-)	NA	0.36 (0.30-0.44)	0.25 (0.15-0.35)
M. fragrans EO	3.00 (-)	6.04 (5.49-6.78)	0.48 (0.42-0.55)	0.52 (-0.17-0.16)

1/ 95% CL means 95% confidence limit; LCL= lower confidence limit; UCL= upper confidence limit

2/ NA means not available

Table 2. Larvicidal activity of three essential oils at 1% concentration , negative and postsitive control against fourth instar larvae of *Ae.albopictus*

Treatment	% Mortality ± SD at specified time(h)						
	0.08	0.16	0.5	1	6	12	24
70% *v/v* Ethyl alcohol	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0b1/	0.0 ±0.0c1/	0.0 ±0.0	0.0 ±0.0
1% *w/w* Temephos	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	20.0 ±27.4b	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0
C. zedoaria EO	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	46.0 ±15.2a	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0
Z.mekongense EO	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0b	0.0 ±0.0c	0.0 ±0.0	0.0 ±0.0
M. fragrans EO	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0b	48.0 ±38.4b	100.0 ±0.0	100.0 ±0.0
C.V.(%)	NA2/	NA	NA	60.30	28.80	NA	NA

1/ Mean mortality in a different column followed by a different letter is significantly different (*P*< 0.05 by ANOVA and DMRT)

2/ NA= not computed from this Probit analysis.

Table 3. Larvicidal activity of three essential oils at 5% concentration , negative and postsitive control against fourth instar larvae of *Ae.albopictus*

Treatment	% Mortality ± SD at specified time(h)						
	0.08	0.16	0.5	1	6	12	24
70% *v/v* Ethyl alcohol	0.0 ±0.0b1/	0.0 ±0.0	0.0 ±0.0c	0.0 ±0.0c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
1% *w/w* Temephos	0.0 ±0.0b	0.0 ±0.0	0.0 ±0.0c	20.0 ±27.4b	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
C. zedoaria EO	46.0 ±21.9a	100.0 ±0.0	100.0 ±0.0a	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
Z.mekongense EO	0.0 ±0.0b	0.0 ±0.0	84.0 ±13.4b	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
M. fragrans EO	0.0 ±0.0b	0.0 ±0.0	100.0 ±0.0c	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
C.V.(%)	51.00	NA2/	12.80	17.00	NA	NA	NA

1/ Mean mortality in a different column followed by a different letter is significantly different (*P*<0.05 by ANOVA and DMRT)

2/ NA= not computed from this Probit analysis.

Table 4. Larvicidal activity of three essential oils at 10% concentration , negative and postsitive control against fourth instar larvae of *Ae.albopictus*

Treatment	% Mortality ± SD at specified time(h)						
	0.08	0.16	0.5	1	6	12	24
70% *v/v* Ethyl alcohol	0.0 ±0.0c1/	0.0 ±0.0c	0.0 ±0.0	0.0 ±0.0c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
1% *w/w* Temephos	0.0 ±0.0c	0.0 ±0.0c	0.0 ±0.0	20.0 ±27.4b	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
C. zedoaria EO	88.0 ±26.8a	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
Z.mekongense EO	0.0 ±0.0c	72.0 ±21.7b	100.0 ±0.0	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
M. fragrans EO	36.0 ±5.5b	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0a	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
C.V.(%)	35.20	15.10	NA2/	16.60	NA	NA	NA

1/ Mean mortality in a different column followed by a different letter is significantly diffent (*P*< 0.05 by ANOVA and DMRT)

2/ NA= not computed from this Probit analysis.

Discussion

The results of this study demonstrate that these essential oils had a significant larvicidal activity against *Ae. albopictus* mosquitoes. Nowadays, mosquito vector control programs focus more on elimination of mosquitoes at the larval stage by reason of insecticides can reduce the adult population only for a while. Therefore would the larvicidal activity is an alternative to reduce the population of mosquitoes. (Soonwera Phasomkusolsil. 2017). *C. zedoaria* EO exhibited a highly toxic effect towards mosquitoes. The highest activity with 100% mortality against the adults of *Ae. albopictus* was found in 10% of *C. zedoaria* EO with LT_{50} of < 0.01 h.) and LC_{50} of 1.09%. Alonso-Amelot (2016) reported that the *C. zedoaria* EO contained Curzerenone (22.3%) is an important element, followed by 1,8-cineole (15.9%) and germacrone (9.0%). The author also reported that 1,8-cineole has been found in many abundant plants, for instance, krervanh Thai (*Amomum krervanh*), greater galangal (*Alpinia galanga*), and basil (*Ocimum basilicum*). Earlier studies showed that 1-8cineole was toxic to *Plutella xylostella* and exerted a moderate toxicity to *Cotesia pultellae*. 1,8-cineole and thymol have been reported to be insecticides against varroa codling moth (*Cydia pomonella*), larvae of American cockroach (*Periplaneta americana*), and northern house mosquito (*Culex pipiens*) larvae (Bullangpoti, 2018). Essential oil-based repellents are safe for human health, animals, and the environment. Temephos, an organophosphate, was an approved chemical larvicide that was commonly being used in Malaysia, In the past 43 years, Temephosto has been used to control the mosquito larva. (Rahim *et al*., 2017). In Malaysia, malathion, permethrin, deltamethrin, and temephos were commonly being used for controlling mosquito vectors. In Malaysia, has reported a metabolic resistance mechanism, overproduction of cytochrome P450, responsible for pyrethroid resistance in *A. albopictus* (Ishak *et al*., 2016). Development in the activity of the metabolic resistance mechanism developed into cross-resistance between pyrethroids and organophosphate. Several studies have reported insecticides resistance of Aedes albopictus (Rahim *et al*., 2017). Herbal products are one of the best alternatives to synthetic chemicals for mosquito control. EOs can be safe and eco-friendly alternatives to synthetic pesticides. These results are encouraging for prospective development of new natural mosquitocidal products from plant oils (Soonwera and Phasomkusolsil, 2017).

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