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Mosquito-repellent activity of Star anise (*Illicium verum* Hook.f.), Bustard cardamom *(Amomum villosum* Lour.) and Best cardamom *(Amomum krervanh* Pierre.) essential oils against *Aedes albopictus* (Skuse)

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Abstract The mosquito repellent activities of essential oils derived from *Illicium verum* Hook.f., *Amomum villosum* Lour. and *Amomum krervanh* Pierre. against *Aedes albopictus* females were compared with that of a chemical repellent (DEET; N, N-diethyl-3-methylbenzamide). The results showed that the three essential oils at the concentrations of 10% and 5% provided the same 15-minute protection from *Ae. albopictus*. The essential oils at 1% also provided some 3-minute protection. Comparing the essential oils, it was found that the essential oils at 5% concentration provided the most effective and safe protection against *Ae. albopictus*, with a protection time of 15minutes, the higher concentration, 10%, provided the same protection rate. DEET at concentrations of 1%, 5% and 10% provided protection times of 15, 30 and 45 minutes, respectively. It can be concluded that the essential oils extracted from *I. verum, A. villosum, A.krervanh* have a full potential as effective protection against *Ae. albopictus* females. Although the essential oils were less effective than the synthetic chemical repellent, they are safer to use. It is hereby recommeded that these essential oils were good alternatives and should be further developed into commercial repellent products.

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

Introduction

Mosquito infestation is among the most serious public health problems in Thailand, especially those that vector mosquito-borne diseases, including severe dengue fever and Chikununya diseases. According to the situational report from the Epidemiological surveillance by the Bureau of Epidemiology (2016) Control of dengue virus (DENV), disease transmission is particularly problematic because no vaccines or medicines are effective against DENV. A tight control of mosquito vectors during epidemics is considered the only option for preventing disease transmission.

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Mosquitoes are vectors of many serious pathogens for example DENV, Zika virus, malaria, and filariasis (Benelli *et al*., 2016). Dengue virus is being spread by infected female mosquitoes biting humans. Upon biting, the infection will enter humans. (WHO, 2018). Among various mosquitoes, *Aedes aegypti* and *Aedes albopictus* are predominant feature species for a successful encroachment. Their habitat is around human dwellings. Their eggs are resistance to prolonged desiccation and their larvae live in small water sources in the area of human habitat, which contributes to their success transmission of the pathogens. Mosquitoes have an important vector status owing to numbers and types of diseases transmitted by them. It is a failure should they affect public health. Notwithstanding the technological evolution in healthcare, mosquito-borne diseases are still a major problem. Encephalitis is another mosquito bourne illness for human. The species of Culex mosquito vectors of encephalitis and various other viral diseases are widespread in the world. Common viral illnesses such as chikungunya, DENV and Zika virus have been elevated to be major public health issues during the recent past. Many *Aedes* spp. have been identified as the primary carriers of these pathogens. (WHO, 2016; Bhat and Aravind, 2017).

Dengue virus (Dengue fever virus, DENV) is a disease of which both the virus and vector are worldwide. It incurred in an epidemiologic and is expanding since 1998. In the twentieth century, dengue fever spread became the second rank, after malaria, causing around 25,000 deaths each year. DENV is very because of its mortality and morbidity to humans. Because there is no effective vaccine against the disease, the most probable way to contain the virus is to control the mosquito. Normally, mosquito control is accomplished by applying synthetic organophosphates, pyrethroid insecticides or DEET (Souza *et al*., 2019). Among improved synthetic chemicals, DEET provides the widest of repellency, keeping mosquitoes away. It is the most favored and effective for humans. Initially, since 1946 the US Department of Agriculture had improved DEET (DEET; N, N-diethyl-3-methylbenzamide) to protect the army. Approved for public use in 1957 n the United States, it was estimated that nearly 75 million people use DEET to repel mosquitoes periodically (Navayan *et al*., 2017). Human and animal studies have shown that DEET to be not dangerous. But a report from other sources revealed that the use of DEET has bad effects. Dermal absorption of DEET has occurred in kids subsequently using pharmaceutical formulations comprised of DEET (Navayan *et al*., 2017). There have been cases of mortality and toxicity including encephalopathy, cardiovascular and skin diseases, and seizure. Although they have been used extensively, it is related to affecting the environment and human beings. Accordingly, there have been development of natural repellent substances and

environmentally friendly formulas. In the past 50 years, Various types of plants have been selected for mosquito repellent activity. Some plant formulations and natural products are more effective than synthetic chemicals. The repellent activirty of essential oils has a short span usually takes action because of their evaporation. There are several preparations from plants that control certain insects. On the contrary, synthetic chemicals have harmful adverse effects on humans. To avoid the adverse effects of DEET, many laboratories have tried to replace it with repellents from plant extracts. (Navayan *et al*., 2017).

Plants naturally produce a wide variety and remarkable features such as steroids, terpenoids, alkaloids, and phenolic compounds. One of the low-cost substances is the Essential oils (EOs) from plants and usually used as alternative to commercial synthetic insecticides. EOs are considered to be safe for human health and in history, humans use EOs for perfume and medicines. Major plant families like Solanaceae and Asteraceae with sub-families Rutaceae, Poaceae, Cupressaceae, Lamiaceae, Labiatae, Zingiberaceae, and Piperaceae have been shown to contain EOs with can control mosquitoes (Chellappandian *et al*., 2018;Govindarajan *et al*., 2016a, 2016b, 2016c, 2016d; Baskar *et al*., 2016; Soonwera and Phasomkusolsi, 2016; Benelli *et al*., 2016). Therefore, essential oils (EOs) are natural products that are becoming a good choice because they are biodegradable and do not have adverse affect to the environment (Reichert *et al*., 2018). The purpose of this research was to evaluate the mosquito repellent activities of three essential oils: Star anise (*Illicium verum* Hook.f), Bustard cardamom (*Amomum villosum* Lour) and Best cardamom (*Amomum krervanh* Pierre) against *Aedes albopictus* mosquitoes.

Materials and methods

Tested mosquitoes

All *Ae.albopictus* mosquito subjects were reared in the laboratory of the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang,(KMITL) Bangkok, Thailand. The eggs on filter paper were hatched in a plastic container (30 x 35 x 5 cm) subduing 1,500 milliliters of water. After they hatched, the filter paper was removed. The mosquito larvae were fed with Fish food (HIPRO®), and the adults were fed with 5% glucose under a condition of 12h:12 H (light: dark) photoperiod. Four to five days old female mosquitoes were used in repellency tests. Before the commencement of each test, the mosquitoes were starved, i.e., they were only provided with water for 1 h.

Plant materials

Star anise (*Illicium verum* Hook.f), Bustard cardamom (*Amomum villosum* Lour) and Best cardamom (*Amomum krervanh* Pierre) plant parts were extracted for EOs by water distillation. The formed liquid with the distillate oils were collected in a separatory funnel. The mixture was kept to settle for 24 h, after which the water (lower) layer was slowly pulled out until only the oil layer remained. All extracted oils were diluted with ethyl alcohol in 3 concentrations: 1%, 5%, and 10% then kept under laboratory conditions.

Chemicals

DEET, a common synthetic chemical in Thailand, was purchased from The British Dispensary (L.P.) Co., Ltd., Bangkok, Thailand.

Repellent bioassay

EOs solutions were tested for their repellent activitiy against *Ae. albopictus* females using an arm-in-cage method. Five human volunteers were recruited from the Faculty of Agricultural Technology, KMITL. Those volunteers had no history of skin diseases. Our research was completely safe to human subjects and our proposal had been accepted by the research committee of the Faculty of Agricultural Technology, KMITL. The duration of the tests depended on the species of the mosquitoes. For *Ae. albopictus*, the tests were conducted in the day time from 08.00 am to 4:00 pm. The oil solutions at 1, 5, and 10 % concentrations were used as treatments. The positive control was DEET. Before the employment of the repellents, the volunteer's arms were cleaned. Their skin was not applied with any lotion, perfume, oil or perfumed soap before the time of the assay and was washed with water. Both arms were covered with rubber gloves, each with a window of 3 cm x 10 cm at the ventral part of the forearm. The left arm was used for treatment and the right arm was for control. Before every test, the control arm was used to test the readiness of mosquitoes by stretching the arm into the cage for three minutes. If at least 2 mosquitoes landed or hovered over the arm, it was considered that the mosquito reacted to the test.

First, 0.1 mL of the essential oil was dropped into the skin and left for 5 minutes until the test substance dried, then the arms were inserted into te cage. Within 3 minutes if there were 2 or more mosquitoes which tried to bite, it was considered not able to repel mosquitoes. But if there is no mosquito bite within 3 minutes, it was considered be able to repel mosquitoes. If within 3 minutes

and no mosquito has bitten, then the arms were lifted out of the cage and rested for 15 minutes. The time between operations of the repellents was recorded as protection time. For comparison, the percentage of mosquito bites was calculated for each test by using the following formula : % Biting = B 250 × 100, where B is the total number of bites at the end of the test.

Results

The repellency results for the three essential oils repellents and DEET 10% w/w as a positive control against *Ae. albopictus* under laboratory conditions are shown in Table 1. The three essential oils at 10% concentration provided the best repellency and 15 min of protection, allowing only 2.6 - 6.3 % biting rate. In contrast, DEET 10% w/w exhibited 45 min protection time, allowing 3.2 % biting rate, followed by essential oils at 5 and 10% concentrations which provided 15 min protection time, allowing between 3.6 and 10.3 % biting rate. DEET 5% w/w exhibited 30 min protection time and 3.0% biting rate. The efficacies of the 5 and 10% essential oils were not significantly different. The essential oils at 1% concentration provided the least efficacy: 3 min protection time and from 1.6 to 7.9 % biting rate, whereas 1% DEET provided 15 min protection time and 2.6% biting rate. In summary, essential oils at 5% concentration was the best performer because their strengths were two times weaker than the 10% ones but their efficacies were identical to the oils at 10% concentration.

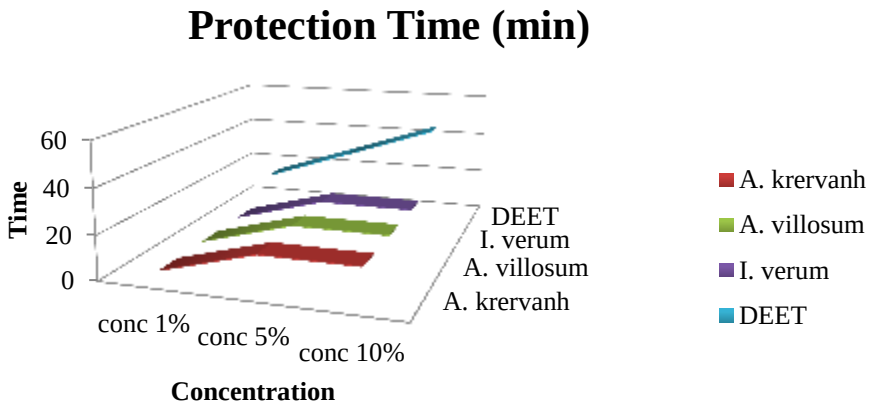

Figure 1. Repellent activity of essential oils at 1,5 and 10 % conc. and DEET against *Ae. albopictus*

Table 1. Repellent activities of essential oils at 1,5 and 10 % concentrations and DEET against *Ae. albopictus*

Treatment	concentration 1%		concentration 5%		concentration 10%	
	(Protection Time) ±SD (min)	(%biting) ±SD	(Protection Time) ±SD (min)	(%biting) ±SD	(Protection Time) ±SD (min)	(%biting) ±SD
A. krervanh	3.0 ±0.0$^{b1/}$	7.9 ±3.7^{a}	15.0 ±0.0^{b}	6.6 ±4.7 A^{b}	15.0 ±0.0^{b}	2.6 ±2.6^{a}
A. villosum	3.0 ±0.0^{b}	1.6 ±0.6^{b}	15.0 ±0.0^{b}	3.8 ±5.0^{b}	15.0 ±0.0^{b}	2.9 ±2.7^{a}
I. verum	3.0 ±0.0^{b}	2.6 ±0.5^{b}	15.0 ±0.0^{b}	10.3 ±5.7^{a}	15.0 ±0.0^{b}	6.3 ±4.4^{a}
DEET	15.0 ±0.0^{a}	2.6 ±1.4^{b}	30.0 ±0.0^{a}	3.0 ±1.5^{b}	45.0 ±0.0^{a}	3.2 ±1.0^{a}
CV%	18.7%	54.9%	26.2%	76.4%	28.6%	78.7%

1/ Mean mortality in each column followed by the different letter are significantly diffent (P> 0.05) by ANOVA and DMRT

Discussion

Our study clearly revealed that the three essential oils from Thai herbs provided the best repellency at 5% concentration against *Aedes albopictus* with protection time of more than 15 min. The effective repellencies of 5 and 10% essential oils were not significantly different. These three essential oils are not the only essential oils that exhibited mosquito repellency, several other essential oils inclusive citronella, lavender, peppermint, and lemongrass oil have already been commercially marketed as mosquito repellants. As previously reported (Chellappandiana *et al*., 2018) their repellent activities was the same as the synergistic effects of several derived oils from various medicinal and non-medicinal plant sources. According to research findings, essential oils provide protection in a short time usually in less than 2 h.

These results are similar with the findings of other researchers which reported efficacy of some EOs against dengue mosquito vector (*Ae. aegypti* and *Ae. albopictus*) (Govindarajan *et al*., 2016a, b, d). Phukerd and Soonwera (2014) reported that the 5% and 10% EOs from *Amomum biflorum*, *Curcuma zedoaria* and *Zingiber zerumbet* showed repellent activity against *Ae. aegypti* females with protection time ranging from 3 to 17 min and the biting rate ranging from 0.9 to 4.1%. In addition, 10% EOs from *C. zedoaria* and *I. verum* also showed adulticidal activity against *Ae. albopictus* females with mortality rate which ranged from 40 to 95.2% (Cotchakaew and Soonwera, 2018). Another research reported the repellency and adulticidal activities of EOs from *I. verum* and

Zinginer nimmonii against *Ae. aegypti* females (Govindarajan *et al*., 2016c; Voris *et al*., 2018)

Some essential oil have strong repellent activities against mosquito vectors of dengue. Since all chemicals are potentially harmful if exposure to them is above the safety level, it is important to quantify their toxicity levels in the same way as the toxicity of synthetic chemicals are quantified to ensure that essential oils are safe for human health and the environment. An accretion of any chemical fixatives or stabilizers like vanillin, while can improve the length of essential oils protection, necessitates a toxicity screening each time its new formulation is produced to assure safety at the recommended concentration. DEET is a synthetic chemical that has been used worldwide for repelling mosquitos. However, there are a considerable number of reports on its toxicity against the skin as well as the nervous and immune systems (Chellappandiana *et al*., 2018). Moreover, usage of synthetic chemicals is liable to development of insect resistance and will make the control of mosquito vectors to become more problematic. Plant essential oils have been recommended as new alternative agents for controlling mosquitoes because they have very low toxicity to the environment, animals and humans. Although the mosquito repellent effects of EOs do not normally last as long as a chemical one that can protect from mosquito bite for up to six hour. as reported by Navayan *et al*. (2017), essential oils are far safer to human skin and safe to the environment. These 3 essential oils from Thai herbs showed a potential for use as a repellent agent against *Ae.albopictus* mosquitoes.

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