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Toxicity of *Litsea petiolata* Hook.f. essential oil against *Aedes aegypti* (Linn.), *Aedes albopictus* (Skuse), *Anopheles minimus* (Theobald) and *Culex quinquefasciatus* (Say)

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Abstract This study evaluated the oviposition deterrent, ovicidal, larvicidal, pupicidal, and adulticidal activities of essential oil (EO) from *Litsea petiolata* leaves against *Aedes aegypti*, *Aedes albopictus*, *Anopheles minimus* and *Culex quinquefasciatus* with double-choice, dipping, and contact assays. *Litsea petiolata* EO was tested at 1, 5, and 10% concentrations in ethanol and their efficiencies were compared with those of 1% w/w temephos and 10 % w/v cypermethrin. Oviposition deterrent was evaluated on gravid females. Larvicidal and pupicidal activities were tested on the fourth larvae and 2-day-old pupae. The adulticidal activity was tested against two-day-old adult females. Ten percent of *L. petiolata* EO exhibited the highest oviposition deterrent activity against gravid females and 100% repellency against *Ae. albopictus* and *An. minimus*, 97.0% against *Ae. aegypti* and 94.6% against *Cx. quinquefasciatus*. The oviposition activity index (OAI) against females of those four mosquito species ranged from -0.9 to -1.0. Ten percent of *L. petiolata* EO also exhibited the highest ovicidal activity against the eggs of the four mosquito species, with an inhibition rate ranging from 87.2 to 100%. Moreover, it also showed the highest larvicidal and puicidal activities against the larvae and pupae of the four mosquito species, with a 100% mortality rate at 10 and 60 min, respectively. The adulticidal activity was recorded at 1 and 24h. Ten percent of *L. petiolata* EO exhibited the highest toxicity to female adults of the four mosquito species, with 100% knockdown (1h) and mortality (24h) rates. On the other hand, 1%w/w temephos did not deter gravid females, and it was only slightly toxic to the eggs and larvae and non-toxic to the pupae. In the same manner, 10%w/v cypermethrin was less effective against the female adults of the four mosquito species than 10% *L.petiolata* EO. *L.petiolata* EO is a highly effective and eco-friendly alternative to synthetic insecticides.

Keywords: *Litsea petiolata*, *Aedes aegypti*, *Aedes albopictus*, *Anopheles minimus*, *Culex quinquefasciatus*, Essential oil

Introduction

Mosquitoes are serious vectors and a considerable threat to people's livelihood worldwide. They are a major cause of death for 700,000 hundred

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thousand people per year (WHO, 2020). Serious diseases vectored by mosquitoes include dengue, malaria, japanese encephalitis, filariasis, yellow fever, and zika virus. *Aedes aegypti* (*Ae. aegypti*) and *Aedes albopictus* (*Ae. albopictus*) mosquitoes, in particular are vectors of dengue widely distributed globally especially in tropical and subtropical areas (Paixao *et al*., 2018; Sharma *et al*., 2022). Areas infested with dengue vectors are habitats of about two-thirds of the world's population. Three-point nine billion people in more than 128 countries are at risk of contracting dengue. In 2019 an estimated 229 million cases and 56 million cases of dengue had been reported worldwide (Mustafa, 2015; Pompon *et al*., 2017; Demirak and Emel, 2022. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) is a globally distributed cosmopolitan mosquito, especially in tropical and subtropical areas (Chaiphongpachara *et al*., 2018). *Cx.quinquefasciatus* is a vector of lymphatic filariasis, West Nile fever, and Japanese encephalitis. Also, about 859 million people are vulnerable to lymphatic filariasis, and over 3 billion people are threatened by Japanese encephalitis worldwide (WHO, 2020). It causes annoyance, pain, and dermatitis (Muthukumaran *et al.*, 2015), and it is reported to cause lymphatic filariasis, a widely distributed tropical disease estimated to infect around 600 million people in the Southeast region of Asia in 2019 (Rai *et al*., 2019). On the other hand, *Anopheles minimus* (*An. minimus*) is a vector of malaria. Malaria is a severe disease and public health problem that causes many deaths of children and adults annually. A report stated that, in 2019, there were 214 million infected cases worldwide (Moxon *et al*., 2019). Most of the deaths were under 5-year-old children (Sonkong *et al*., 2015; WHO, 2016).

In recent years, many synthetic insecticides have not been used comprehensively in mosquito control programs: their harmful effects on human and non-target populations, their difficult-to-biodegrade nature, and the ever-increasing mosquito resistance to them (Forstinus *et al*., 2017). Thus, new, safer insecticides for controlling the mosquito population are urgently needed. (Demirak and Emel, 2022). Recently, plant extracts or phytochemicals as potential sources of mosquito control agents have attracted much attention from researchers (Singh *et al*., 2006; Arokiyaraj *et al*., 2015). Natural products are more desirable because they are safer for non-target organisms and biodegrade quickly. Essential oils are suitable potential controllers of mosquito vectors of diseases. Plant essential oils (EOs), the first generation of herbal pesticides, are known as green pesticides. They show anti-insect activities, including insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory, and (Tahghighi *et al.*, 2019). Many researchers have observed that some EOs from herbal plant sources had larvicidal and pupicidal activity (Chantawee and Soonwera, 2018), repellent activity (Wu *et al*., 2019), insecticidal activity (Dua *et al*., 2010), and ovicidal and oviposition-deterrent activity (Cotchakaew and Soonwera, 2018)

against mosquito vectors. This study focused on *Litsea petiolata* Hook.f. (*L. petiolata*), Lauraceae family. It is a native tree in the South and Northeast of Thailand. *L. petiolata* leaves and twigs odor like an edible insect called Mangdana (*Lethocerus indicus*: Hemiptera; Belostomatidae), which is used as a common flavoring agent in Thai food.

The objective of this study was to evaluate the efficacy of *Litsea petiolata* EO against *Aedes aegypti*, *Aedes albopictus*, *Anopheles minimus* and *Culex quinquefasciatus* in terms of oviposition deterrent, ovicidal, larvicidal, pupicidal, and adulticidal activities.

Materials and methods

Mosquito rearing

Colonies of four mosquito species—*Aedes aegypti* (*Ae. aegypti*), *Aedes albopictus* (*Ae. albopictus*), *Anopheles minimus* (*An. minimus*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) were provided by the Entomological Laboratory, Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok. The laboratory colonies were kept under the following conditions: 27.0±3.3 ℃, 72.5±1.5%RH, and 12-h light and 12-h dark lighting periods. The eggs were hatched in a tray (28×35×4 cm^3) and filled with 2000 ml of drinking water. The tray held about 200 larvae. They fed on fish food (OPTIMUM®, 32% protein). Fourteen-day-old larvae developed into pupae, then 150 pupae were collected in a 250 ml plastic cup containing 200 ml of drinking water. The cup was transferred into a mosquito cage (30×30×30 cm^3). No food was fed to the pupae. Two-day-old pupae developed into adult mosquitoes. The adults fed on 5% sugar solution in drinking water soaked in cotton pads. When they were 5 days old, female mosquitoes were given blood as food for 60 min by an artificial membrane method (Chantawee and Soonwera, 2018). Three days afterward, the gravid mosquitoes laid eggs. An oviposition deterrent bioassay was performed on 2-day-old female adults that had been fed with the blood meal, while the eggs were used in an ovicidal bioassay. Larvae of the four mosquito species were collected. Fourth instar larvae were used in a larvicidal bioassay, and pupae were used in a pupicidal bioassay. Two-day-old adult female mosquitoes that had not been fed with blood were used in an adulticidal activity assay.

Plant materials

Fresh leaves of *Litsea petiolata* (*L. petiolata*) from 3-year-old trees were collected from Bankhai district, Rayong province (12° 40′ 48″ N and 101° 16′ 48″ E)

in the Eastern part of Thailand (Figure 1). The specimens were identified by a botanical taxonomist from King Mongkut's Institute of Technology Ladkrabang. Fresh leaves were rinsed with drinking water and cut into small pieces. One kilogram of leaves was placed in a flask (5L.), then added with 2,000 ml of drinking water and extracted for their EO by a water distillation method. The extraction process took about 5 hours. The EO obtained was diluted into 1, 5 and 10% solutions in ethyl alcohol. All EO solutions were stored at room temperature (27.5±1.5℃; 75.5±1.5 %RH) before being used in a test. Gas chromatography and gas chromatography-mass spectrometry were used to analyze the composition of the *L. petiolata* EO.

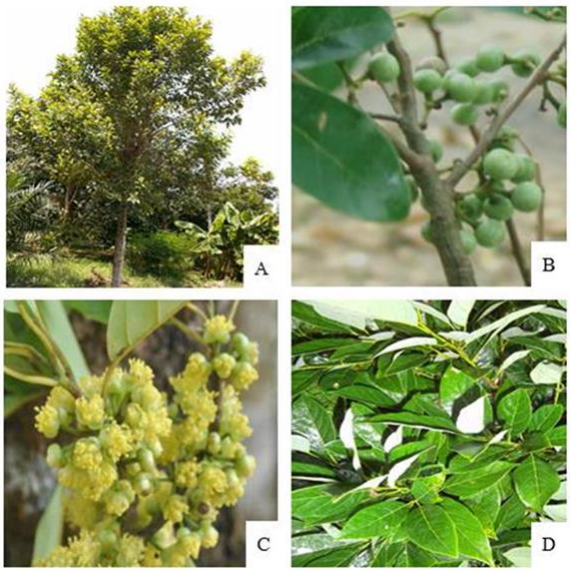

Figure 1. *Litsea petiolata* Hook.f. (F. Lauraceae) (A) tree, (B) fruits, (C) flowers and (D) leaves

GC-MS determination of oil components of L. petiolata

The composition of *L. petiolata* EO was analyzed by GC-MS are presented in Table 1. The analysis was carried out at the Scientific Instrument Center, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The GC-MS system was an agilent system. It was composed of a model 6890-N gas chromatographer, a model 5973-N mass spectrometer with 70 EV electron energy, a 7683 Auto-sampler, and a Chemstation data system. The GC column was an HP-5 ms fused silica capillary with 5% phenyl methylpolysiloxane coating and 0.25-μm film thickness—length of 30 m and an internal diameter of 0.25-mm. The initial

oven temperature was kept at 50 ˚C for 2 min, and then it was heated up to 200 ℃ at a rate of 10 ℃/min^{-1} and held there for 20 min. The injector temperature was maintained at 270 ℃. Each EO sample was diluted at 1:100 in ethyl alcohol, and 0.2 µl of the diluted sample was injected into the GC-MS system at a slit ratio of 1:100. The mobile gas was 99.9% helium. The flow rate was 1.0 ml per min. Spectra were scanned in a range of 30 to 500 m z^{-1}. Chemical components of EOs were analyzed and identified with Agilent software (version G1701DA D.00.00), NIST mass spectral search program for Wiley 7n.1, and NIST tandem mass spectral library v7.1.

Table 1. Chemical composition of *Litsea petiolata* EO as identified by GC-MS

No.	Constituent	Percent of total	R.T. (min)
1	Benzene	0.17	7.27
2	1,8-Cineole	2.74	7.39
3	8-Nonen-2-one	0.15	8.23
4	2-Nonanone	54.69	8.48
5	2-Decanone	31.30	11.31
6	Undec-10-en-2-one	1.41	11.40
7	2-Undecanone	0.47	11.46
8	2-Dodecanol	0.04	11.72
9	2-methyl	0.03	12.58
10	1-Hepten-6-one	0.02	12.77
11	α -Cubebene	0.04	13.10
12	Butanal	6.81	13.18
13	1,3-Oxazin-2-one	2.09	14.00
Total		99.96	

Positive and negative controls

- Cypermethrin (Cyperguard10 EC®, 10% w/v) manufactured by Expert pest system Co. Ltd, 4/151 Borommaratchachonnani Road, Chimplee, Bangkok 10170, Thailand, functioned as a positive control.

- Temephos (SaiGPO-1®, 1.0% w/w) manufactured by 138 Government Pharmaceutical Organization, Rangsit -NakhonNayok Road, Pathumthani province, Thailand, functioned as another positive control.

- Drinking water (Crystal®) manufactured by Sermsuk Co. Ltd., 72 Phaholyothin Rd, Nakhon Sawan province, Thailand, functioned as a negative control.

Assays for determination of various activities of L. petiolata EO

Oviposition deterrence bioassay

The oviposition deterrence bioassay was a double-choice method. Each treatment was performed in five replicates. The outcomes were statistically analyzed by a paired t-test ($P<0.05$). It was performed on 15 gravid females in a mosquito cage (30x30x30cm) mentioned in the section "Mosquito rearing

method." Two 250-ml plastic cups were brought into the cage and filled with 99 ml of drinking water, then placed at opposite corners of the cage. The cups were switched their positions in each replicate of the experiment. The treatment cup was added with 1 ml of either 1 or 5 or 10% *L. petiolata* EO or 1 ml of drinking water with 0.01g of dissolved temephos. The non-treatment cup was added with 1 ml of drinking water. After 72 hours and under a stereomicroscope, the number of eggs laid in the treatment and non-treatment cups was counted and recorded. The results from the two types of cups were statistically analyzed and compared. The oviposition activity index (OIA), percentage effective repellency (ER%), and percentage effective attractant (EA%) were determined. The OAI was calculated by the following formula (Govindarajan *et al.*, 2018; Shaalan and Canyon, 2018; Soonwera and Phasomkusolsil, 2017):

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

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

ER% was calculated for the case of positively repellent and deterrent by the following formula: $ER\%= [UC-TC/UC] \times 100$,

On the other hand, EA% was calculated for the case of positive attractant by the following formula: $EA\%= [TC-UC/TC] \times 100$.

Ovicidal bioassay

The ovicidal bioassay was the same dipping method used by Cotchakaew and Soonwera (2019). This experiment was of a completely randomized design. Five replicates of each treatment were run, and the average results were compared to that produced by 1%w/w temephos. LT_{50} values (Lethal Time for 50% mortality) and LC_{50} (Lethal Concentration for 50% mortality) were calculated by probit analysis. A Duncan's Multiple Range Test was conducted on the mortality data with SPSS statistical software for Windows (version 16.0). Twenty-five eggs of each species of mosquitoes were placed in a 250-ml plastic cup containing 99 ml of drinking water and added with 1 ml of 1 or 5 or 10% of *L.petiolata* EO solution.In the case of temephos, 0.01g of temephos was dissolved in 100 ml of drinking water in a 250 ml plastic cup and used as a positive control, while 100 ml of drinking water in a 250 ml plastic cup was used as a negative control. After 48 hours, the hatched

larvae were counted. Five treatment replicates were run, and the results were compared to those produced by temephos and drinking water. The percentage egg inhibition rate was calculated by the following formula:

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

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

Larvicidal and pupicidal bioassay

The larvicidal and pupicidal bioassays followed the method of Soonwera and Phasomkusolsil (2017) and were of a completely randomized design. Five replicates of each treatment were run, and the average results were compared to that produced by temephos. The LT_{50} values (Lethal Time for 50% mortality) and LC_{50} (Lethal Concentration for 50% mortality) were calculated by probit analysis. A Duncan's Multiple Range Test was conducted on the mortality data with SPSS statistical software for Windows (version 16.0). In a 250-ml plastic cup, one milliliter of *L. petiolata* EO was added to 99 ml of drinking water at each concentration. Ten specimens of fourth instar larvae and 10 specimens of pupae were placed in such plastic cups. Larval mortality was recorded at 1, 5, 10, 15, 30, 60 min, and 24 h and pupae mortality at 15, 30 min, 1, 3, 6, 12, 24 and 48 h. Larvae were considered dead if they could not rise to the water surface or did not manifest a diving reaction. The mortality rates were recorded and calculated by the following formula:

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

where NT is the total number of dead larvae/pupae and NC is the total number of treated larvae/pupae.

World Health Organization (WHO) susceptibility test

The knockdown rate, mortality rate and susceptibility testing of mosquito females were carried out following the standard World Health Organization (WHO) protocol (WHO, 2018) contact method, with a completely randomized design. Five replicates of each treatment were run, and the average results were compared to that produced by 10% w/v cypermethrin, the positive control. Twenty-five female mosquitoes (2 days after emergence) unfed with blood meal were exposed to *L. petiolata* EO at 1 or 5 or 10% concentration in a treatment tube. Two ml of an EO solution were dropped and absorbed on a filter paper (the size of 12×15 cm, Whatman No1®) and put in the treatment tube (the size of 44 mm in diameter and 125 mm in length). After 1 h of exposure, the mosquitoes were transferred to the non-treatment tube (containing a filter paper but without *L. petiolata* EO). The knockdown rates were recorded at 1, 5, 10, 15, 30, and 60 min, and the mortality rates were recorded 24 h after

the exposure. The criterion for both knockdown and mortality was that no mosquito body parts (head, antenna, thorax, wings, legs, abdomen, and other appendages) moved.

Knockdown rate and mortality rate were calculated by the following formula: Knockdown rate (%) = [NT/NC] x 100,
Where NT is the total number of knocked down mosquitoes, and NC is the total number of treated adult mosquitoes.

KT_{50} (50% knockdown time) and LC_{50} (50% lethal concentration) were calculated by using probit analysis. The mortality data were analyzed by Duncan's Multiple Range Test with SPSS for Windows software (version 16.0). An agent's susceptibility criteria as classified by WHO are as follows: 98.00-100% mortality signifies susceptibility (S); 80.00-97.00% mortality signifies possibly resistant that needs confirmation (PR); and less than 80.00% signifies resistant (R).

Results

Oviposition deterrent bioassay

The results of the oviposition deterrent assay are presented in Table 2. The oviposition activity index (OAI) values of *L. petiolata* EO at 1, 5 and 10%conc. Against *Ae.aegypti*, *Ae.albopictus*, *An.minimus* and *Cx. quinquefasciatus* are presented in Figure 2. It can be observed that 10% of *L. petiolata* EO had a high ER% (percentage effective repellency) and a higher oviposition deterrent activity against all species of mosquitoes than 5 and 1% concentrations had. *L. petiolata* EO at all tested concentrations (1, 5 and 10%) exhibited effective oviposition deterrent activity against *An. minimus* females with the highest deterrent activity of 100% ER and -1.0 OAI. In addition, *L. petiolata* EO at all tested concentrations successfully prevented oviposition. When compared with temephos, *L. petiolata* EO showed a higher oviposition deterrent activity (24.9% EA (effective attractancy) and 0.1 OAI) against *An. minimus* females.

Against *Ae. albopictus* females, *L. petiolata* EO at all tested concentrations showed a high oviposition deterrent activity, not as high as it was against *An. minimus* but higher against all of the other tested species.

In addition, the EO at all tested concentrations also showed a high oviposition deterrent activity against *Ae. albopictus* females with %ER ranging from 75.0 to 100% and OAI ranging from -0.6 to -1.0, whereas temephos did not show an effective oviposition deterrent activity against *Ae. albopictus* (0.4%ER and -0.1OAI). *Ae. aegypti* females ranked third out of the four mosquito species tested in sensitivity to *L. petiolata* EO (in terms of oviposition deterrent activity), with ER ranging from 89.7 to 97.0% and OAI ranging from

-0.8 to -0.9. Temephos did not show an effective oviposition deterrent activity against *Ae. albopictus* females (0.03% ER and -0.01 OAI). *Cx. quinquefasciatus* females ranked the last out of all tested species in terms of their oviposition activity deterred by *L. petiolata* EO. *L. petiolata* EO at 5 and 10% showed % ER of 18.5 and 94.6% and OAI of -0.1 and -0.9, respectively, but 1% *L. petiolata* EO showed %EA of 52.3%. Similar to the EO at a low concentration, temephos did not show an effective oviposition deterrent activity against *Cx. quinquefasciatus* females (8.6% ER and -0.1OAI). To stress, *L. petiolata* EO at 5 and 10% deterred the ovipositioning of all four tested mosquito species, i.e., the number of eggs laid in the treatment cups was significantly lower than laid in the non-treatment cups, whereas temephos was not shown.

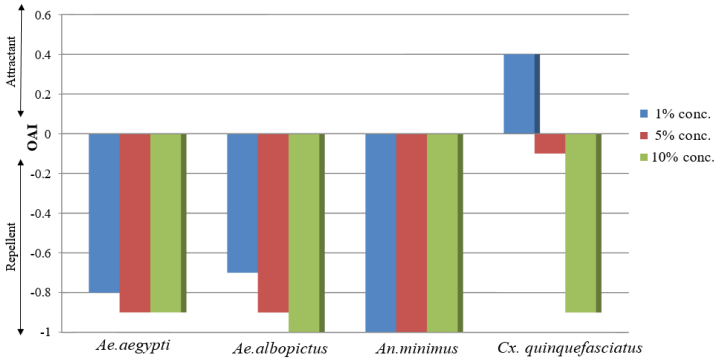

Figure 2. Ovipositon activity index (OAI) values of *L. petiolata* EO at three concentrations (1, 5 and 10%) against *Ae. aegypti*, *Ae. albopictus*, *An. minimus*, and *Cx. quinquefasciatus*

Ovicidal bioassay

The percentage hatching-inhibition rates of *L. petiolata* EO at 1, 5, and 10% against *Ae. aegypti*, *Ae. albopictus*, *An. minimus*, and *Cx. quinquefasciatus* eggs are presented in Table 3. *L. petiolata* EO at all tested concentrations exhibited a 100 % inhibition rate against *Cx. quinquefasciatus* eggs. In addition, 5 and 10% *L. petiolata* EO were highly toxic to *Ae. albopictus* and *An. minimus* eggs with a 100% inhibition rate, while the EO at 1% was toxic against *Ae. albopictus* and *An. minimus* eggs at 4.5 and 69.7% hatching-inhibition rate, respectively. However, *L. petiolata* EO at 1, 5 and 10% showed moderate toxicity against *Ae.aegypti* eggs with inhibition rates of 74.4, 79.2 and 87.2%, respectively. In contrast to the EO, temephos showed low toxicity to the eggs of all mosquito species tested, with inhibition rates ranging from 6.2 to 9.5%. Not surprisingly, the drinking water (negative control) showed no toxicity against mosquito eggs.

Table 2. Oviposition deterrent activities (OAI) values of *L. petiolata* EO at 1%, 5% and 10% concentrations and 1*w/w* temephos against *Ae. aegypti*, *Ae.albopictus*, *An.minimus* and *Cx. quinquefasciatus*

Mosqutioes species	Treatment	Conc. (%)	Number of eggs ±SD		OAI**	ER%	EA%	No. of eggs laid per female (in treatment cup)
			Treatment cup	Non-treatment cup				
Ae. aegypti	*L. petiolata* EO	1	75.0 ±20.9*	731.0 ±105.5	-0.8	89.7	-	5.0
		5	49.2 ±17.5*	819.6 ±65.7	-0.9	94.0	-	3.3
		10	31.6 ±6.9*	1060.4 ±150	-0.9	97.0	-	2.1
	temephos	1*w/w*	249.3 ±32.1	256.1 ±46.6	-0.01	0.03	-	17.3
Ae. albopictus	*L. petiolata* EO	1	148.6 ±128.6*	595.8 ±122.0	-0.6	75.0	-	9.9
		5	9.8 ±6.7*	553.8 ±160.2	-0.9	98.2	-	0.6
		10	0.0 ±0.0*	599.4 ±107.05	-1.0	100	-	0.0
	temephos	1*w/w*	308.3 ±83.58	455.4 ±171.98	-0.1	0.4	-	33.2
An. minimus	*L. petiolata* EO	1	0.0 ±0.0*	533.6 ±95.7	-1.0	100	-	0.0
		5	0.0 ±0.0*	737.2 ±175.8	-1.0	100	-	0.0
		10	0.0 ±0.0*	610.2 ±124.2	-1.0	100	-	0.0
	temephos	1*w/w*	395.3 ±172.3	297.1 ±129.31	0.1	-	24.9	26.3
Cx. quinquefasciatus	*L. petiolata* EO	1	974.0 ±72.4*	464.2 ±97.9	0.4	-	52.3	64.9
		5	646.8 ±72.3*	793.6 ±64.4	-0.1	18.5	-	43.1
		10	62.2 ±14.2*	1161.4 ±192.0	-0.9	94.6	-	4.2
	temephos	1*w/w*	243.1 ±31.1	266.0 ±31.0	-0.1	8.6	-	16.2

* Significant difference between treatment and non-treatment cups by paired t-test ($P < 0.05$)

** The OAI ranges from -1 to +1; positive index value (+) indicates that the test solution was an attractant; negative index value (–) indicates that the test solution was a deterrent; and 0 indicates a neutral response, OAI = Oviposition Active Index; ER = Effective Repellency; EA = Effective Attractancy

Table 3. Ovicidal activities of *L. petiolata* EO at 1%, 5% and 10% concentrations and 1w/w temephos against *Ae. aegypti*, *Ae. albopictus*, *An. minimus* and *Cx. quinquefasciatus* eggs

Mosquito Species	Inhibition rate (%)				
	1% cone	5% conc.	10% conc.	1*w*/*w* Temephos	drinking water
Ae. aegypti	74.4±5.3[c/1]	79.2±8.4[c]	87.2±8.9[b]	9.5±4.4[a]	0.0
Ae. albopictus	4.5±2.2[b]	100[a]	100[a]	9.4±10.4[b]	0.0
An. minimus	69.7±13.1[b]	100[a]	100[a]	6.2±4.6[c]	0.0
Cx. quinquefasciatus	100[a]	100[a]	100[a]	6.3±1.4[b]	0.0

[1]Means in each row followed by the same letter are not significantly different ($p<0.05$, by one-way ANOVA and Duncan's multiple range test)

Larvicidal and pupicidal bioassay

The larvicidal activities of *L. petiolata* EO at 1, 5 and 10% concentration against fourth instar larvae of *Ae.aegypti*, *Ae.albopictus*, *An.minimus* and *Cx. quinquefasciatus* are presented in Table 4. *L. petiolata* EO at 1, 5 and 10% concentrations showed higher toxicity against fourth instar larvae at 10 min of exposure than at 5 min. At 10 min, *L. petiolata* EO at all tested concentrations was highly toxic to *An. minimus* larvae with 100% mortality, LC_{50} value of 0.5%, and LT_{50} values ranging from 2.4 to 9.1 min; the LC_{50} against the larvae of *Cx. quinquefasciatus* was 0.7%, with a mortality rate ranging from 93.6 to 100%, and the LT_{50} values ranging from 4.2 to 5.4 min; the LC_{50} against the larvae of *Ae. aegypti* was 1.8%, with mortality ranging from 30.4 to 100%; the LT_{50} values ranged from 3.6 to 15.4 min; lastly, the LC_{50} against the larvae of *Ae. albopictus* was 2.8% with a mortality rate ranging from 0 to 100%; the LT_{50} values ranged from 2.4 to 27.1 min. Temephos showed slight toxicity against the larvae of *Ae. aegypti*, *Ae. albopictus*, *An. minimus* and *Cx. quinquefasciatus* within 10 min of exposure. *L. petiolata* EO at all tested concentrations were able to control the larvae of all tested mosquito species, while temephos was not controlled.

The results of the pupicidal activity assay of *L. petiolata* EO against pupae of *Ae. aegypti*, *Ae. albopictus*, *An. minimus* and *Cx. quinquefasciatus* are presented in Table 5. *L. petiolata* EO at all tested concentrations (1, 5, and 10%) were more toxic to the pupae when they were exposed to each of them for 60 min than for 30 min. At 60 min, *L. petiolata* EO at all tested concentrations was highly toxic to *Cx. quinquefasciatus* pupae with a mortality rate ranging from 4.0 to 100%, LC_{50} of 2.1%, and an LT_{50} value ranging from 18.1 to 180.3 min; the LC_{50} against the pupae of *An. minimus* and *Ae. albopictus* was 2.8%, with a mortality rate ranging from 0 to 100% and an LT_{50} ranging from 0 to 6.3 min; the LC_{50} against the pupae of *Ae. aegypti* was 3.2%, with a mortality rate ranging from 7.2 to 100% and an LT_{50} ranging from 18.1 to 138.2 min. In contrast, temephos was not an effective larvicide against the pupae of *Ae. aegypti*, *Ae. albopictus*, *An. minimus*, and *Cx. quinquefasciatus*.

Table 4. LT_{50}, LC_{50}, and Mortality rate of *L. petiolata* EO at 1%, 5% and 10% concentrations against larvae of *Ae. aegypti*, *Ae.albopictus*, *An.minimus* and *Cx. quinquefasciatus* at 5 and 10 min

Mosquito species	Treatment	Conc. (%)	Mortality (%) ±SD		LT_{50} (min)	Confidence limit 95%		LC_{50} (%) at 10 min	x^2
			5 min	10 min		LCL	UCL		
Ae. aegypti	*L. petiolata* EO	1	1.6 ±3.6[c]	30.4 ±5.4[c]	15.4	0.2	0.2	1.8	65.4
		5	87.2 ±7.2[b]	99.2 ±1.8[b]	4.8	0.06	0.1		153.4
		10	98.4 ±2.2[a]	100[a]	3.6	0.04	0.09		150.5
	temephos	1*w/w*	0.0 ±0.0	0.0 ±0.0	ns	-	-	-	-
Ae. albopictus	*L. petiolata* EO	1	0 ±0.0[b]	0 ±0.0[b]	27.1	0.4	0.5	2.8	51
		5	100[a]	100[a]	2.4	-0.1	0.1		178.9
		10	100[a]	100[a]	2.4	-0.1	0.1		178.9
	temephos	1*w/w*	0.0 ±0.0	0.0 ±0.0	ns	-	-	-	-
An. minimus	*L. petiolata* EO	1	0 ±0.0[b]	100	9.1	0.07	0.2	0.5	159.4
		5	100[a]	100	2.4	-0.1	0.1		178.9
		10	100[a]	100	2.4	-0.1	0.1		178.9
	temephos	1*w/w*	0.0 ±0.0	0.0 ±0.0	ns[1/]	-	-	-	-
Cx. quinquefasciatus	*L. petiolata* EO	1	89.6 ±5.4[c]	93.6 ±5.4[b]	5.4	0.06	0.1	0.7	182.4
		5	95.2 ±4.4[b]	100[a]	4.8	0.05	0.1		190.2
		10	99.2 ±1.8[a]	100[a]	4.2	0.04	0.1		196.3
	temephos	1*w/w*	0.0 ±0.0	0.0 ±0.0	ns[1/]	-	-	-	-

All values were based on five replications; UCL= upper confidence limit; LCL = lower confidence limit; X^2 = chi square; LC_{50} = 50% lethal concentration.
[1/]ns = not computed by Probit analysis

Table 5. LT_{50}, LC_{50}, and Mortality rate of *L. petiolata* EO at 1%, 5% and 10% concentrations against pupae of *Ae. aegypti*, *Ae.albopictus*, *An.minimus* and *Cx. quinquefasciatus* at 30 min and 60 min

Mosquito species	Treatment	Conc. (%)	Mortality (%) ±SD		LT_{50} (min)	Confidence limit 95%		LC_{50} (%) at 60 min	x^2
			30 min	60 min		LCL	UCL		
Ae. aegypti	*L. petiolata* EO	1	4.0 ±0.0	7.2 ±3.3	138.2	96.1	336.3	3.2	3.5
		5	52.0 ±5.7	89.6 ±9.2	30.3	24.0	36.5		25.6
		10	100	100	18.1	6.3	24.4		0.03
	temephos	1*w/w*	0	0	ns [1/]	-	-	ns	-
Ae. albopictus	*L. petiolata* EO	1	0	0	ns	-	-	2.8	-
		5	100	100	18.1	6.3	24.4		0.1
		10	100	100	18.1	6.3	24.4		0.1
	temephos	1*w/w*	0	0	ns	-	-	ns	-
An. minimus	*L. petiolata* EO	1	0	0	ns	-	-	2.8	-
		5	100	100	18.1	6.3	24.4		0.1
		10	100	100	18.1	6.3	24.4		0.1
	temephos	1*w/w*	0	0	ns	-	-	ns	-
Cx. quinquefasciatus	*L. petiolata* EO	1	3.2 ±5.2	4.0 ±4.9	180.3	121.3	376.1	2.1	22.6
		5	89.6 ±6.1	100	24.2	6.2	24.4		3.9
		10	100	100	18.1	6.3	24.4		0.03
	temephos	1*w/w*	0	0	ns	-	-	ns	-

All values were based on five replications; UCL= upper confidence limit; LCL = lower confidence limit; X^2 = chi square; LC_{50} = 50% lethal concentration.
[1/]ns = not computed by Probit analysis.

World Health Organization (WHO) susceptibility test

The knockdown rate (KT_{50}), mortality rate, susceptibility status, and lethal concentration (LC_{50}) of *L. petiolata* EO against females of *Ae. aegypti*, *Ae. albopictus*, *An. minimus* and *Cx. quinquefasciatus* are presented in Table 6. The mortality rate increased with increased EO concentration. Ten percent *of L. petiolata* EO was more toxic to females of the four mosquito species, with a 100% mortality rate than 5 % was (see Table 6). Against *An. minimus* females, 10 and 5 percent *L. petiolata* EO exhibited a mortality rate of 100% and a respective KT_{50} of 0.7 and 17.3 min after 1 h of exposure. Their LC_{50} was 2.9%, and the WHO susceptibility status of *An. minimus* against the 10% *L. petiolata* EO was susceptible. These results (KT_{50} of 0.7 min) were better than 10% w/v cypermethrin (KT_{50} of 3.7 min). Against *An. minimus* females, 1% *L. petiolata* EO and 1%w/v cypermethrin did not show an effective knockdown rate or mortality rate. Against *Ae. aegypti* females, *L. petiolata* EO at 1 and 5% were not fully effective, with a KT_{50} at 1h ranging from 7.9 to 46.5 and a mortality rate at 24 h ranging from 10.4 to 61.2%. In contrast, 10% of *L. petiolata* EO showed the highest mortality rate, at 100%, against *Ae. aegypti* females with a KT_{50} of 1.8 min and an LC_{50} of 4.1 %. Its WHO susceptibility status was 'susceptible.'Against *Cx. quinquefasciatus* females, 10% *L. petiolata* EO provided the highest mortality rate of 100% at 24 h, a KT_{50} of 2.9 min at 1 h, and an LC_{50} of 5.8 % at 24 h. The susceptibility status of *Cx. quinquefasciatus* against 10% *L. petiolata* EO was "susceptible." In addition, 1 and 5% *L. petiolata* EO provided a mortality rate of 2.4 and 28.0% at 1 h and KT_{50} values of 110.1 and 8.7 min, respectively. On the other hand, 10%w/v cypermethrin (KT_{50} of 1.3 min) was better than10% *L. petiolata* EO (KT_{50} of 2.9 min) against *Cx. quinquefasciatus* females. On the other hand, against *Ae. albopictus* females, 10% of *L. petiolata* EO exhibited the highest mortality rate of 100% at 24 h, KT_{50} of 0.7 min at 1 h, and LC_{50} of 6.1 % at 24 h. The susceptibility status of *Ae. albopictus* against 10% *L. petiolata* EO was "susceptible." However, 5% conc of *L. petiolata* EO did not show a very high mortality rate at 1 h (44%), and 1% of *L. petiolata* EO was not effective against *Ae. albopictus* females. Ten percent (w/v) Cypermethrin (KT_{50} of 5.3 min) was less effective than 10%*L. petiolata* EO (KT_{50} of 0.7 min) against *Cx. quinquefasciatus* females.

Table 6. KT_{50}, LC_{50} value, mortality rate and susceptibility status of *L. petiolata* EO concentrations and cypermethrin against *Ae. aegypti*, *Ae. albopictus*, *An.minimus* and *Cx. quinquefasciatus*

Treatment	Conc (%)	*Ae. aegypti*				*Ae. albopictus*				*An. minimus*				*Cx. quinquefasciatus*			
		KT_{50} (min)	% Mortality at 24 h	Susceptibility status	LC_{50} (%) at 24 h	KT_{50} (min)	% Mortality at 24 h	Susceptibility status	LC_{50} (%) at 24 h	KT_{50} (min)	% Mortality at 24 h	Susceptibility status	LC_{50} (%) at 24 h	KT_{50} (min)	% Mortality at 24 h	Susceptibility status	LC_{50} (%) at 24 h
L. petiolata EO	1	46.5	10.4±2.2[c]	R		ns	0[c]	R		ns	0[b]	R		110.1	2.4±5.2[c]	R	
	5	7.9	61.6±7.8[b]	R	4.1	5.1	44.0±8.6[b]	R	6.1	17.3	100[a]	S	2.9	8.7	28.0±12.5[b]	R	5.8
	10	1.8	100[a]	S		0.7	100[a]	S		0.7	100[a]	S		2.9	100[a]	S	
cypermethrin	1	4.2	90.0±5.3[a]	RS		ns	0[c]	RS		ns	0[b]	RS		4.1	92.0±4.0[a]	RS	
	5	3	100[a]	S	0.6	17.2	100[a]	S	2.6	10.2	100[a]	S	2.9	2.4	100[a]	S	0.6
	10	2.9	100[a]	S		5.3	100[a]	S		3.7	100[a]	S		1.3	100[a]	S	

KT_{50}, 50**%** knockdown time; LC_{50}, 50**%** lethal concentration**.** Mean **%** mortality followed by the same letter in the same column is not significantly different **(**one-way ANOVA and Duncan's multiple range test**).** *S*, Susceptibility is defined as 98-100**%** mortality; *RS*, Resistance suspected is defined as 80-97**%** mortality, *R*, Resistance is defined as <80**%** mortality**.** [1/]ns = not computed by Probit analysis.

Discussion

The experimental results demonstrated that *L. petiolata* EO at 10% concentration is an effective oviposition deterrent, ovicidal, larvicidal, pupicidal, and adulticidal agent against *Ae. aegypti, Ae. albopictus, An. minimus* and *Cx. quinquefasciatus* mosquitoes. Ten percent of *L. petiolata* EO exhibited a high effective repellency against gravid females of all tested mosquito species and a high inhibition rate against the eggs. These results are supported by the finding from Phukerd and Soonwera (2014) that 10% of *L. petiolata* EO exhibited a high repellent activity against females of *Cx. quinquefasciatus* and *Ae.aegypti*. Muangmoon *et al.* (2019) also reported that *L. petiolata* EO showed a repellency activity against *Ae. aegypti* females. Along with the same trend, Uniyal *et al.* (2016) reported that 100 mg/L of *L. cubeta* EO showed a high oviposition deterrent activity against *Ae. aegypti* females with 87.17% effective repellency. Regarding ovicidal activity, our results agree well with those from Phasomkusolil and Soonwera (2012) and Cotchakaew and Soonwera (2019). They reported that essential oils from *the Cananga odorata* flower, *Curcuma zedoaria* rhizome, and *Ocimum basilicum* leaves were highly toxic to the eggs of *Ae. aegypti, Ae. albopictus, An. dirus, An. minimus,* and *Cx.quinquefasciatus,* with EC_{50} < 1.0 %. Wang *et al.* (2016) reported that *L. cubeta* EO was highly toxic to the eggs of *Callosobruchus chinensis* (Bruchidae: Coleoptera), with an LC_{50} of 3.78 µl/L. In addition, 10% *L. petiolata* EO was highly toxic to the larvae, pupae, and adults of all tested mosquito species. This results are supported by findings from Muangmoon *et al.* (2018) that *L. petiolata* EO exhibited high toxicity to the larvae (LD_{50} of 27.7 mg/L) and adult (LD_{50} of 2.4 µg/mg) of *Ae. aegypti*. Along the same line, Sinthusiri and Soonwera (2013) reported that 5 and 10% of *L. petiolata* EO exhibited high toxicity against *Musca domestica* adults (KT_{50} ranking of 16.7 to 22.8 min). While Dai *et al*. (2020) also reported that 100 µg/mL of *L. umbellate* and *L. iteodaphne* EOs was highly toxic to the larvae mortality of *Cx. quinquefasciatus* 100% (LC_{50} of 54.17 and 23.78 µg/mL) In addition, several *Litsea* EOs have shown good efficacy in repelling various types of mosquitoes compared to synthetic insecticides.

Identified by GC-MS, thirteen monoterpenes were found in *L. petiolata* EO, mainly 2-Nonanone (54.69%), 2-Decanone (31.30%), Butanal (6.81%), 1,8-Cineole (2.74%), and 1,3-Oxazin-2-one (2.09%). The percentage of 2-Nonanone) in the chemical profile that we obtained was different from that reported by Thongthip *et al.* (2017) and Muangmoon *et al.* (2018). The major composition differences may be affected by several factors such as harvesting time, soil structure and fertilizer, and other environmental factors. Monoterpenes from *L. petiolata* EO are volatile and lipophilic with low molecular weight, so they can penetrate through insect cuticles and enter their

tracheae system, causing mortality (El-Wakeil, 2013). Moreover, monoterpenoids from plant EOs are much less toxic to mammals and show only short persistence in the environment (Ebadollahi, 2011; El-Wakeil., 2013) than synthetic insecticides like temephos, an organophosphate or cypermethrin, a pyrethroid. These synthetic insecticides have been extensively used as mosquito control agents (Naqqash *et al*., 2016). Unfortunately, extended and repeated applications of synthetic insecticide have led to serious problems for humans, animals and the environment. For example, they are highly toxic to fish and other aquatic animals; they cause pruritus, numbness and difficulty breathing in humans; and they are possible human carcinogens (Sisay *et al*., 2019). To make matters worse, the usage of chemicals is liable to the development of insect resistance (Mouhamadou *et al*., 2019). Newer generations of insects will be harder to control. To remedy these issues, we suggest using a natural product like *L. petiolata* EO for controlling mosquitoes. It is a preferable and safer alternative to using chemical insecticides.

Essential oil from *L. petiolata* was effective at controlling four mosquito species at all four stages of their life cycle, showing a full potential for development into a highly effective and eco-friendly mosquito-controlling agent. Moreover, it was tested to be more effective than cypermethrin and temephos, widely used synthetic chemicals, in terms of effectiveness against mosquitoes at all stages of their life cycle (*Ae. aegypti, Ae.albopictus, An .minimus* and *Cx. quinquefasciatus)*.

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