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ORIGINAL ARTICLE

Comparative efficacy of commercial ylang-ylang (*Cananga odorata*) essential oils from India and Thailand against larval *Aedes aegypti* (L.) (Diptera: *Culicidae*)

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ABSTRACT

Objective: The objective of this study was to determine the comparative larvicidal efficacy of commercial ylang-ylang (*Cananga odorata*) essential oils from India and Thailand against the *Aedes aegypti* mosquito to be used as a guideline for *Aedes* mosquito control.
Materials and Methods: The bioassay for the larvicidal activity of commercial ylang-ylang essential oils in this experiment was modified from the World Health Organization standard protocols. The concentration ranges at 0.025, 0.050, 0.075, 0.100, 0.125, and 0.150 ppm in each treatment were used for testing, and four replicates were used per concentration. The larval mortality was observed and recorded 24- and 48-h after exposure.
Results: The results of this study clearly revealed that commercial ylang-ylang essential oils from India and Thailand were highly toxic to the larvae of the dengue vector *Ae. aegypti*, and Indian ylang-ylang had an LC_{50} value of 0.064 ppm, whereas Thai ylang-ylang had an LC_{50} value of 0.042 ppm after 24-h exposure.
Conclusion: This study revealed the efficacy of commercial Indian and Thai ylang-ylang essential oils as natural vector control for the larval stage of the dengue vector *Ae. aegypti*. Usually, natural larvicide products are not commonly found in the market due to complex production processes. The results of this research support the use of commercial essential oils to aid in further control of *Aedes* mosquito larvae populations in the community.

ARTICLE HISTORY

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KEYWORDS

Cananga odorata; essential oils; *Aedes aegypti*; dengue vector; ylang-ylang.

Introduction

Dengue fever is a dangerous mosquito-borne viral disease that causes 390 million dengue virus infections of people per year worldwide. About 3.9 billion of the world's population are at risk of infection in 128 countries. The primary vector of dengue fever is the mosquito *Aedes aegypti*, which transmits the virus to humans [1]. The incidence of dengue fever is often related to the density of the mosquito vector population in the area [2]. The control of this disease often focuses on the elimination of breeding sites for *Ae. aegypti* [3]. Female *Ae. aegypti* lays their eggs in small water containers near human houses because the immature stages of the mosquito life cycle are aquatic [4].

Some plant products, bioinsecticides, have been proven to be an option to eliminate *Aedes* larvae and replace chemical insecticides that are harmful to the environment [5]. The impacts of chemical insecticides on ecosystems and human health are a global concern [6]. Chemical insecticides can negatively affect different body systems (e.g., integumentary, gastrointestinal, neurological, respiratory, reproductive, and endocrine systems) and can have carcinogenic effects [7]. Besides, insecticide residues, which often appear in the environment, can contaminate the food and drinking water of humans and animals [8]. The temephos, an organophosphate larvicide, is the main product used to control *Aedes* larvae in water-storage containers [9]. This insecticide is known to be highly effective in destroying the larvae of dengue vectors and has low toxicity to humans and animals [10]. However, the widespread and long-term use of temephos affects the resistance of mosquitoes to this insecticide in many areas worldwide [11]. One way to solve the problem of increased mosquito resistance to this insecticide is to utilize other mosquito

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control methods [12,13]. Currently, alternative methods, such as organic insecticides that can replace synthetic insecticides, are still urgently needed [14,15].

Essential oils are natural products from plants, and the earlier research reports have revealed that some essential oils can kill mosquito larvae in the laboratory [16]. *Cananga odorata,* also known as ylang-ylang, is a tropical, fast-growing tree that is often used to produce essential oils due to its unique aroma, which is commonly applied to aromatherapy [17]. Besides, ylang-ylang has antibacterial, antifungal, amoebicidal, and cytotoxic properties [18]. Recently, the insecticidal activity of ylang-ylang essential oil has been studied and found to be highly effective on important insect vectors such as *Ae. aegypti*. Commercial ylang-ylang essential oil, which is generally sold in Thailand, came in two varieties that originate from either India or Thailand.

Therefore, this study was conducted to determine the comparative larvicidal efficacy of commercial ylang-ylang (*C. odorata*) essential oils from India and Thailand against the *Ae. aegypti* mosquito to be used as a guideline for *Aedes* mosquito control.

Materials and Methods

Essential oils

The commercial, cosmetic grade ylang-ylang (*C. odorata*) essential oils from India and Thailand were purchased from Chemipan Co. Ltd. The leaves were extracted into oil via steam distillation and packed into a brown glass container.

Larvae rearing

The second-instar larvae of *Ae. aegypti* mosquito (laboratory strain) were provided courtesy of the Department of Medical Sciences, Ministry of Public Health, Thailand. The second-instar mosquito larvae were reared in plastic trays of filtered water. The larvae were fed daily with ground dog biscuits until *Ae. aegypti* grew into late third-instar larvae, which were later used for testing. They were maintained in a laboratory at the College of Allied Health Sciences, Suan Sunandha Rajabhat University, Thailand, under the following conditions: at the room temperature (26°C ± 2°C), the relative humidity of 80% ± 10%, and under a photoperiod cycle of 12-h light and 12-h dark. These experiments were performed from January to August 2018.

Larvicidal assay

The bioassay for the larvicidal activity of commercial ylang-ylang essential oils from India and Thailand in this experiment was modified from the World Health Organization standard protocols [19]. The concentration ranges at 0.025, 0.050, 0.075, 0.100, 0.125, and 0.150 ppm in each treatment were used for testing, and four replicates were used per concentration. Batches of 25 late third-instar larvae were transferred into 250-ml beakers containing essential

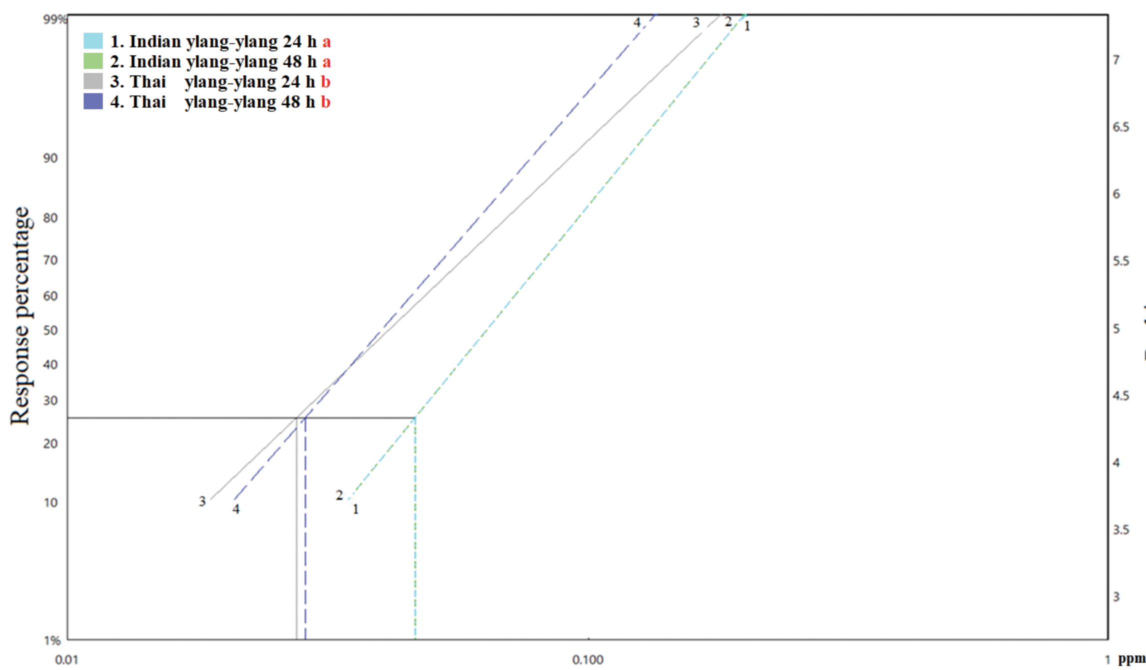

Figure 1. Graph showing the LC_{25} values of Indian and Thai ylang-ylang essential oils on *Ae. aegypti* mosquito larvae after 24- and 48-h exposure. Statistically, significant differences are indicated by different letters that appear at the end of the descriptive words in the top left corner.

Table 1. Mortality percentages of *Ae. aegypti* mosquito larvae for each concentration of Indian and Thai ylang-ylang essential oils after 24- and 48-h exposure.

Concentrations (ppm)	% of larval mortality (means ± SE)			
	Indian ylang-ylang		Thai ylang-ylang	
	24 h	48 h	24 h	48 h
0.025	2.00 ± 1.15	2.00 ± 1.15	16.00 ± 4.00	16.00 ± 4.00
0.050	23.00 ± 5.26	23.00 ± 5.26	71.00 ± 5.97	74.00 ± 3.83
0.075	78.00 ± 6.83	78.00 ± 6.83	83.00 ± 1.00	83.00 ± 1.00
0.100	80.00 ± 6.73	80.00 ± 6.73	96.00 ± 2.83	99.00 ± 1.00
0.125	96.00 ± 4.00	96.00 ± 4.00	97.00 ± 3.00	99.25 ± 0.75
0.150	98.00 ± 2.00	98.00 ± 2.00	99.00 ± 1.00	100.00 ± 0
Control	0	0	0	0

% = percentage, SE = standard error.

Table 2. Larvicidal activity of Indian and Thai ylang-ylang essential oils against *Ae. aegypti*.

Essential oils	Time after exposure	LC_{25} (UCL-LCL) (ppm)	LC_{50} (UCL-LCL) (ppm)	LC_{90} (UCL-LCL) (ppm)	Slope ± SE	χ^2
Indian ylang-ylang	24 h	0.046 (0.028–0.052)	0.064 (0.046–0.080)	0.120 (0.107–0.198)	4.748 ± 0.376	23.392
	48 h	0.047 (0.028–0.052)	0.064 (0.047–0.080)	0.120 (0.107–0.198)	4.748 ± 0.377	23.392
Thai ylang-ylang	24 h	0.028 (0.017–0.033)	0.042 (0.030–0.051)	0.093 (0.078–0.139)	3.691 ± 0.300	13.667
	48 h	0.028 (0.025–0.031)	0.041 (0.036–0.044)	0.078 (0.072–0.087)	4.469 ± 0.324	8.642

LC = lethal concentration, UCL = upper confidence limit, LCL = lower confidence limit, SE = standard error, χ^2 = Chi-square.

oils and diluted in filtered water with 1-ml absolute methanol. Larval mortality was observed and recorded 24- and 48-h after exposure. Larvae were confirmed to be dead when there was no sign of movement. Control groups for each concentration were put in filtered water with absolute methanol, containing no essential oils.

Statistical analysis

Data from the replicates of each treatment were used for Probit analysis to calculate the lethal concentration values (LC_{50} and LC_{90}). Besides, other statistics were included, such as 95% confidence intervals of upper confidence limit (UCL) and lower confidence limit (LCL) and the Chi-square values. These analyses were performed using the LdP Line software (http://www.ehabsoft.com/ldpline/), which was developed by Ehab Mostafa Bakr, whereas the differences in larval mortality among groups were checked by the analysis of variance and the Duncan's test using R software, which was significant at $p < 0.05$.

Results and Discussion

The percentages of larval mortality for *Ae. aegypti* after 24- and 48-h exposure to Indian and Thai ylang-ylang essential oils in each concentration were examined and are shown in Table 1. In all of the tests, dead mosquito larva was not found in the control groups (Table 1).

The larvicidal efficacy of pure Indian ylang-ylang essential oil against *Ae. aegypti* was identified at 0.046 ppm of LC_{25}, 0.064 ppm of LC_{50}, and 0.120 ppm of LC_{90} after 24-h exposure and at 0.047 ppm of LC_{25}, 0.064 ppm of LC_{50}, and 0.120 ppm of LC_{90} after 48-h exposure. The toxicity of pure Thai ylang-ylang essential oil was exhibited at 0.028 ppm of LC_{25}, 0.042 ppm of LC_{50}, and 0.093 ppm of LC_{90}after 24-h exposure and at 0.028 ppm of LC_{25}, 0.041 ppm of LC_{50}, and 0.078 ppm of LC_{90}after 48-h exposure (Table 2).

At present, essential oils have been recognized as critical weapons against mosquito vectors; for example, some essential oils can repel adult mosquitoes [20,21], and some essential oils can kill larval mosquitoes in water containers [16, 22].

The results of this study clearly showed that commercial ylang-ylang (*C. odorata*) essential oils from India and Thailand were highly toxic to the larvae of the dengue vector *Ae. aegypti* (Indian ylang-ylang had an LC_{50} value of 0.064 ppm, whereas Thai ylang-ylang had an LC_{50} value of 0.042 ppm after 24-h exposure). Ylang-ylang essential

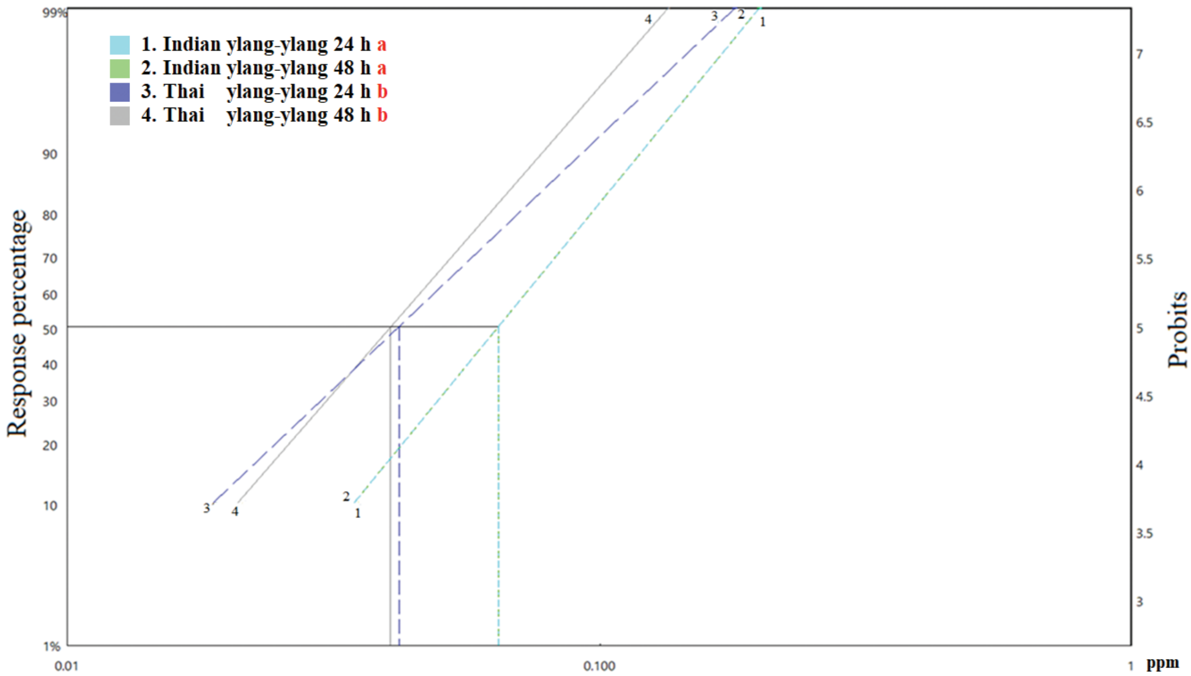

Figure 2. Graph showing the LC_{50} values of Indian and Thai ylang-ylang essential oils on *Ae. aegypti* mosquito larvae after 24- and 48- h exposure. Statistically, significant differences are indicated by different letters that appear at the end of the descriptive words in the top left corner.

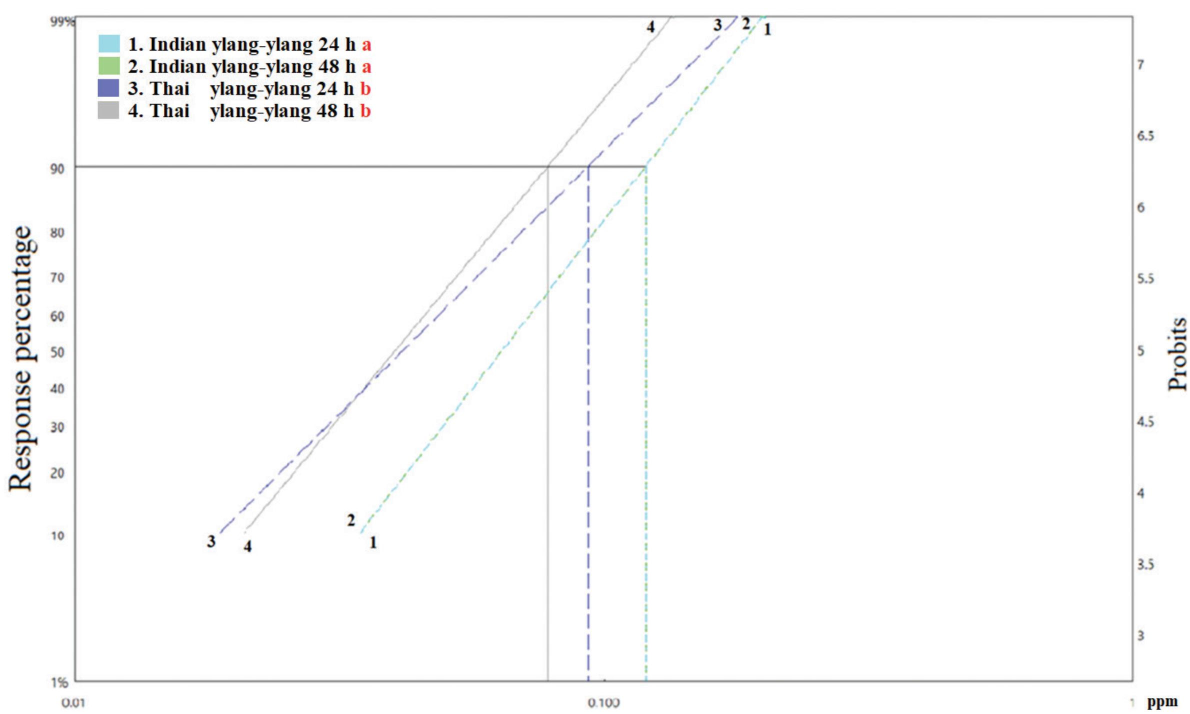

Figure 3. Graph showing the LC_{90} values of Indian and Thai ylang-ylang essential oils on *Ae. aegypti* mosquito larvae after 24- and 48-h exposure. Statistically, significant differences are indicated by different letters that appear at the end of the descriptive words in the top left corner.

oils are highly toxic to mosquito larvae, according to the study of Cheng et al. [23], which indicates that plant essential oils with LC_{50} < 50 ml/L (ppm) were highly effective. This result was in line with that of Vera et al. [24] on ylang-ylang essential oil concerning larvicidal activity against *Ae. aegypti* larvae and found that this essential oil had a strong effect on mosquito larvae control. The previous analyses by gas chromatography–mass spectrometry reported that the major compounds of this plant are *p*-methylanisole, methyl benzoate, benzyl benzoate, benzyl acetate, and geranyl acetate [18], which have several compounds that are reported to be toxic to insects, especially methyl benzoate [25, 26] and geranyl acetate [27].

Besides, these results of this study show that commercial ylang-ylang essential oils are more effective in killing mosquito larvae than have been reported in the results of previous research. The LC_{50} value for this study was LC_{50} = < 1 ppm versus LC_{50} < 70 ppm in a study of the insecticidal activity of two essential oils used in perfumery (ylang-ylang and frankincense) and LC_{50} = 9.77 ppm in the study of Phasomkusolsil and Soonwera [28]. This result occurs because commercial oil products are an essential component of cosmetics, so they are highly concentrated and pure grade without any dilution.

The results showed that the effects of commercial ylang-ylang essential oil from India were statistically different and more efficient than the effects of ylang-ylang obtained from Thailand ($p < 0.05$, Fig. 1-2). These results demonstrate that the composition of compound in an essential oil varies by geographic location of origin [16]. The previous research has studied the composition of essential oils contained in ylang-ylang leaves from Australia [29] and Cameroon [30] and found some differences in compound composition, including the absence of sabinene [18].

Conclusion

Essential oils are natural, plant-derived products that are environmental-friendly. The advantages of essential oils are that they are readily available, convenient to use, and often inexpensive. This study revealed the efficacy of commercial Indian and Thai ylang-ylang crucial oils as natural vector control for the larval stage of the dengue vector *Ae. aegypti*. Usually, natural larvicide products are not commonly found in the market due to complex production processes. The results of this research support the use of commercial essential oils to aid in further control of *Aedes* mosquito larvae populations in the community.

Acknowledgment

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Conflict of interest

The authors declare that they have no conflicts of interest.

Authors' contribution

Tanawat designed the study and supervised and provided suggestions throughout the experiment. Sedthapong assisted in the test in essential oils against larval *Ae. aegypti* (L.). All the authors contributed to writing and reviewing the manuscript and approved the final manuscript.

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