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Efficacy of commercial botanical pure essential oils of garlic (*Allium sativum*) and anise (*Pimpinella anisum*) against larvae of the mosquito *Aedes aegypti*

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ABSTRACT

Aedes aegypti, also called the dengue or yellow fever mosquito, is a significant vector species of several viruses especially the dengue virus. Reducing *Ae. aegypti* population can directly reduce dengue outbreaks in the community. The research aim is to assess the efficacy of commercial botanical pure essential oils of garlic (*Allium sativum*) and anise (*Pimpinella anisum*) against *Ae. aegypti* larvae, the mortality of larvae was monitored after 24- and 48-h of exposure. The larvicidal activity of garlic oil after 24-h exposure was 0.005 ppm for LC_{25}, 0.006 ppm for LC_{50}, and 0.012 ppm for LC_{90}, and after 48 h exposure was 0.004 ppm for LC_{25}, 0.006 ppm for LC_{50}, and 0.014 ppm for LC_{90}. Meanwhile, the larvicidal activity of anise oil after 24-h exposure was 0.016 ppm for LC_{25}, 0.023 ppm for LC_{50}, and 0.043 ppm for LC_{90}, and after 48-h exposure was 0.014 ppm for LC_{25}, 0.020 ppm for LC_{50}, and 0.037 ppm for LC_{90}, whereas, *Aedes* larval mortalities increased with an increase in the concentration of garlic and anise essential oils after both 24- and 48-h exposure. The results of this study were clear evidence for the efficiency of commercial plant oil for dengue vector larval control, especially garlic essential oils.

1. INTRODUCTION

The organic essential oils, also called volatile liquids or ethereal oils, are natural volatile compounds obtained from parts of aromatic plants, for example, leaves, roots, stems, seeds, fruits, and flowers depending on the type of plant [1]. Botanical pure essential oils are easily available on the market and are widely used in many industries including in pharmaceutical, food applications, perfumery, sanitary, medicine, and cosmetic products [2]. Products developed from the essential oils of many plants have been recognized and popular for use in repelling adult mosquitoes including flowering plants in the Lamiaceae (also called Labiatae) family such as *Ocimum basilicum* (basil), *Mentha* spp. (mint), *Hyptis suaveolens* (hyptis), *Lavandula* spp. (lavender), *Salvia* spp. (sage), and *Thymus* spp. (thyme); plants in the Myrtaceae family such as *Eucalyptus* spp. (eucalyptus) and *Melaleuca* spp. (tea tree); and plants in the Poaceae family such as *Cymbopogon* spp. (citronella, lemongrass, and palmarosa) [3]. In addition, it was reported that the essential oils of various species of plants including camphor, thyme, lemon, cedarwood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum, sandalwood, cassia, cinnamon, East Indian lemongrass, bay, sweet basil, holy basil, and ginger can be used to control immature stages of many mosquito species that are vectors of dangerous infectious diseases [4,5].

The garlic plant (*Allium sativum*), belonging to the family Liliaceae, and anise plant (*Pimpinella anisum*), belonging to the family Apiaceae, are important components of many foods and contain important substances that are beneficial to human health [6,7]. The previous research has reported that the essential oils of both of these plants are very effective in controlling many insects including the mealworm beetle [8], the cotton leafworm [9], and *Culex* mosquitoes [10].

Mosquitoes are insect vectors and each mosquito species has specificity to different diseases [11]. *Aedes aegypti*, also called the yellow fever mosquito, is a significant vector species of several viruses, especially the dengue virus [11,12]. Reducing *Ae. aegypti* population can directly reduce dengue outbreaks in the community [13]. Although the use of synthetic chemical insecticides is a highly effective method to control insects, including mosquito vectors, it directly affects living organisms, which includes human and animal health [14]. In addition, these harmful substances can leave residue and accumulate for a long time in the environment [15]. Therefore, using natural insecticides, which are non-toxic to the environment, are a necessary community

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need [16,17]. Some essential oils have been observed to be potential candidates to aid in the control of *Ae. aegypti* mosquito populations.

Garlic and anise oils could possibly be implemented to control mosquito larvae, but there is no clear evidence for their effectiveness in mosquito control. Thus, the aim of the present study is to assess the effectiveness of commercial garlic (*A. sativum*) and anise (*P. anisum*) essential oils in killing *Ae. aegypti* larvae. The research results will confirm the efficacy of killing larvae of the dengue vector using garlic and anise and could become evidence for further use of essential oils to control mosquito vectors.

2. MATERIALS AND METHODS

2.1. Commercial Garlic and Anise Essential Oils

Commercial essential oils of *A. sativum* (garlic) and *P. anisum* (anise) from the Chemipan Corporation Company Limited in Thailand were used for testing to evaluate the efficacy of *Ae. aegypti* larvae removal in this research. Both plant oils are pure essential oils that have been extracted from seeds by a steam distillation method.

2.2. Rearing of Mosquitoes

The second stage and early third stage *Ae. aegypti* larvae were obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. Obtained larvae of *Ae. aegypti* were placed in a tray of filtered water (length 14 × width 11 × depth 7 inches). The experiment was conducted in 2018 at the biological laboratory of the College of Allied Health Sciences, Suan Sunandha Rajabhat University, Thailand. The laboratory conditions included a 12 h light and 12 h dark cycle at 25–28°C and relative humidity levels at 70–80%. Ground dog kibble was used for larval food and given only once a day to prevent spoilage. After about 2 days, larvae developed into late third-stage larvae of *Ae. aegypti*, which were then used for larvicidal testing in the next step.

2.3. Mosquito Larvicidal Tests

The garlic and anise essential oils were diluted with filtered water in 250 mL glass beakers. The serial dilutions of the tested essential oils for this testing were at concentrations of 0.005, 0.012, 0.025, 0.037, and 0.050 ppm. The concentration range of the larvicidal test was conducted in accordance with recommendations from the World Health Organization. After the essential oil solutions were prepared, 25 late third-stage larvae were put in each 250 ml beaker containing the diluted essential oil for each of the previously mentioned concentrations. Afterward, *Aedes* larvae were observed and counted. Larval mortality was calculated 24- and 48-h after treatment, where the death of larvae was noted by their inactivity. Experiments for each concentration were performed in four replicates and one control group (beakers filled with water that contained no essential oils).

2.4. Statistical Analysis

Larval mortality data were used in log-probit analyses to obtain the 50% and 90% lethal concentrations (LC_{50} and LC_{90}), and Chi-square values. The log-probit analysis and lethal concentration graphing in this research were conducted using the program LdP Line which can be downloaded at http://www.ehabsoft.com/ldpline/. For statistical comparisons of the mortality of the mosquito larvae among essential oils, the analysis of variance (ANOVA) was used followed by the Duncan's multiple range test (DMR) in R statistical software package as a free program. $p< 0.05$ was accepted to be statistically significant in this study.

3. RESULTS

To examine the efficacy of commercial botanical pure essential oils of garlic (*Allium sativum*) and anise (*Pimpinella anisum*) against *Ae. aegypti* larvae, the mortality of larvae was monitored after 24- and 48-h of exposure. The study results of larval mortality after 24- and 48-h exposure in the five different concentrations (0.005, 0.012, 0.025, 0.037, and 0.050 ppm) of garlic and anise essential oils are presented as percentages in Table 1. *Aedes* larval mortalities increased with an increase in the concentration of garlic and anise essential oils after both 24- and 48-h exposure. In this experiment, no larvae died in the control groups.

Table 2 shows the results of probit analysis which reveal larvicidal efficacy of garlic and anise oils against *Ae. aegypti*. The garlic essential oil was more effective than the anise oil. The larvicidal activity of garlic oil after 24-h exposure was 0.005 ppm for LC_{25}, 0.006 ppm for LC_{50}, and 0.012 ppm for LC_{90}, and after 48 h exposure was 0.004 ppm for LC_{25}, 0.006 ppm for LC_{50}, and 0.014 ppm for LC_{90}. Meanwhile, the larvicidal activity of anise oil after 24-h exposure was 0.016 ppm for LC_{25}, 0.023 ppm for LC_{50}, and 0.043 ppm for LC_{90}, and after 48-h exposure was 0.014 ppm for LC_{25}, 0.020 ppm for LC_{50}, and 0.037 ppm for LC_{90}.

Statistical analyses showed that the larvicidal efficacy between the 24- and 48-h exposure periods of essential oils was not different except for the LC_{90} value for anise oil, as shown in Figures 1-3, respectively. In addition, analyses revealed the difference of larvicidal efficacy between garlic and anise oils against *Ae. aegypti*.

4. DISCUSSION

Natural products are an important alternative that is recognized by the community as being environmentally friendly and many studies

Table 1: Mortality of mosquito larvae after 24- and 48-h exposure to garlic and anise oils in each concentration.

Concentrations (ppm)	% mortality (means±SE)			
	Garlic essential oils		Anise essential oils	
	24 h	48 h	24 h	48 h
0.005	25.00±1.91	28.00±1.63	3.00±3.00	4.00±4.00
0.012	93.00±3.42	100.00±0	7.00±3.00	7.00±3.00
0.025	99.00±1.00	99.00±1.00	35.00±9.57	49.00±11.47
0.037	100.00±0	100.00±0	96.00±2.83	100.00±0
0.050	100.00±0	100.00±0	100.00±0	100.00±0
Control	00.00±0	00.00±0	00.00±0	00.00±0

ppm: parts per million, 10^{-6}, %: Percentage, SE: Standard error, h: Hours

Table 2: LC_{25}, LC_{50} and LC_{90} (ppm) of garlic and anise oils on *Aedes* mosquito larvae.

Essential oils	Time (hours)	LC_{25} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± SE	χ^2
Garlic	24	0.005	0.006	0.012	5.031±0.520	5.495
	48	0.004	0.006	0.014	3.933±0.623	20.840
Anise	24	0.016	0.023	0.043	4.698±0.294	63.351
	48	0.014	0.020	0.037	4.857±0.367	134.592

LC_{25}: Lethal concentration that kills 25% of exposed *Aedes* larvae, LC_{50}: Lethal concentration that kills 50% of exposed *Aedes* larvae, LC_{90}: Lethal concentration that kills 90% of exposed *Aedes* larvae, ppm: Parts per million, SE: Standard error, χ^2: Chi-square

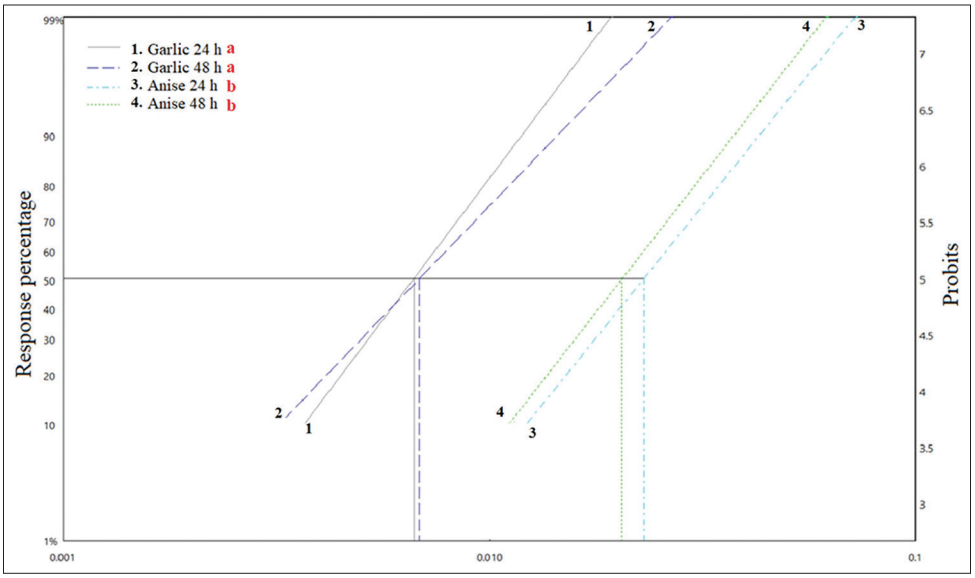

Figure 1: Relative toxicity based on LC_{25} values of pure garlic and anise essential oils on *Ae. aegypti* larvae after 24- and 48-h exposure. The different red letters that appear at the end of description words indicate statistically significant differences between groups ($P < 0.05$) (in the top left corner)

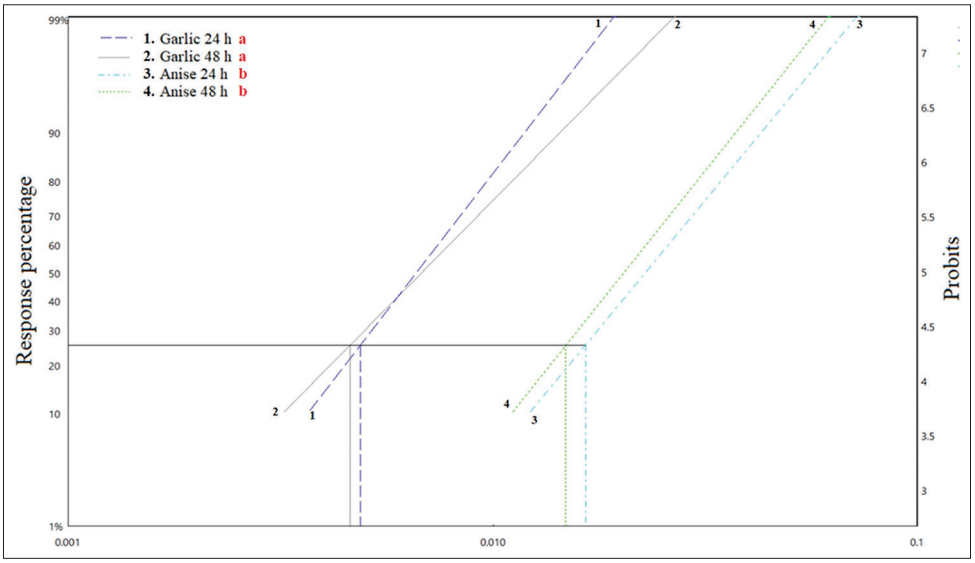

Figure 2: Relative toxicity based on LC_{50} values of pure garlic and anise essential oils on *Ae. aegypti* larvae after 24- and 48-h exposure. The different red letters that appear at the end of description words indicate statistically significant differences between groups ($P < 0.05$) (in the top left corner)

recognize essential oils as a potential effective alternative [18, 19]. This research clearly demonstrated that commercial pure garlic (*A. sativum*) and anise (*P. anisum*) essential oils are highly effective against *Ae. aegypti* larvae (LC_{50}: Garlic = 0.006 ppm and anise = 0.020 ppm at after 24-h exposure). Our results were compared with the criteria of Cheng *et al*.[20] which describes that if the LC_{50}<50 ml/L (or ppm), means that it has a highly efficacy against the larvae of mosquitoes. This result was consistent with the previous biological research, which found that garlic and anise essential oils are toxic to mosquito species including *Culex restuans* (LC_{50}: garlic = 2.7 ppm) and *Cx. pipiens* (LC_{50}: Garlic = 7.5 ppm and anise = 28.7 ppm) [10,21]. According to Öz *et al*.'s study [10], anethole has been identified as a major component of anise essential oil (94.48%). Anethole is an organic compound found in several plants and is reportedly toxic to insects [22]. Regarding garlic essential oil, the previous research of Muturi *et al*. [21] found that a major component was diallyl disulfide (49.13%). Diallyl disulfide is an organosulfur compound found commonly in onions, garlic, and a few other plants in the genus *Allium*. This substance is reportedly toxicity to mosquito larvae [23].

During our evaluation of the efficacy of both garlic and anise essential oils in the killing larvae of *Ae. aegypti*, we found that garlic essential oil has a stronger effect than anise essential oil. This result is in line with the previous research [10,21]. Statistical analyses show that the efficiency of garlic and anise essential oils for *Aedes* larval removal between 24- and 48-h post-exposure was not different, except concerning anise essential oil (which had different value for LC_{9O}). Both commercial garlic and anise essential oils have high larvicidal activity at 24- and 48-h after exposure. Commercial essential oils are often used as cosmetic components, which require pure oils without any dilution. This may account for the strength of these oils in larvicidal activity.

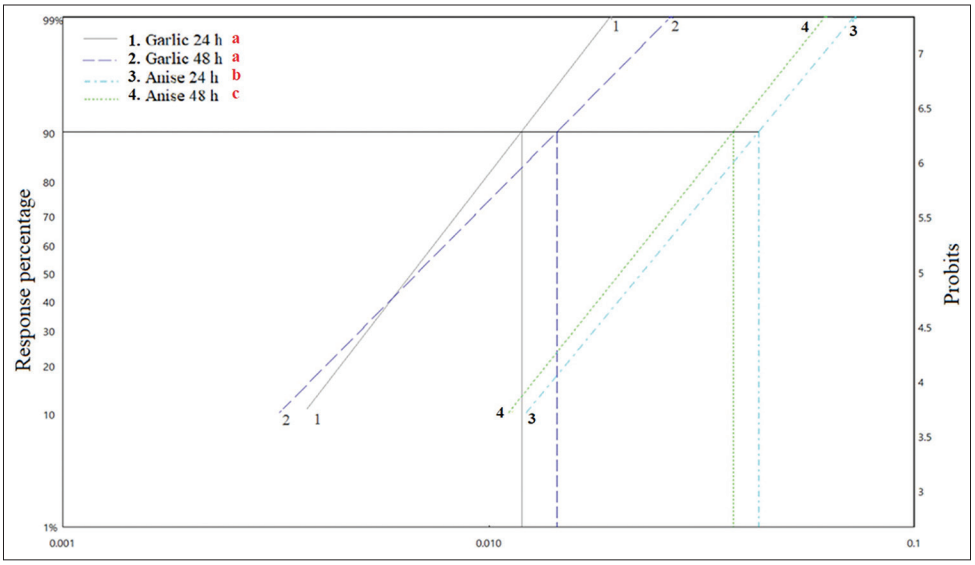

Figure 3: Relative toxicity based on LC_{90} values of pure garlic and anise essential oils on *Ae. aegypti* larvae after 24- and 48-h exposure. The different red letters that appear at the end of description words indicate statistically significant differences between groups ($P < 0.05$) (in the top left corner)

5. CONCLUSION

This study supports the use of natural products particularly botanical essential oils as alternative methods for controlling the larvae of *Ae. aegypti*, a dengue vector, in water containers around the houses. Nowadays, essential oils are natural products that are readily available and good quality because they are important components of common cosmeceutical products. The results of this study were clear evidence for the efficiency of commercial plant oil for dengue vector larval control, especially garlic essential oils. This research paves the way for further use of essential oils as natural mosquito vector controls.

6. ACKNOWLEDGMENTS

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7. CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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