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Comparative study of larvicidal activity of commercial essential oils from aromatic rosemary, vanilla, and spearmint against the mosquito *Aedes aegypti*

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Abstract. *Laojun S, Chaiphongpachara T. 2020. Comparative study of larvicidal activity of commercial essential oils from aromatic rosemary, vanilla, and spearmint against the mosquito* Aedes aegypti. *Biodiversitas 21: 2383-2389.* Dengue fever is a dangerous mosquito-borne viral disease of humans. This disease is caused by dengue virus, a member of the genus Flavivirus of the family Flaviviridae. The *Aedes aegypti* mosquito is a principal vector for this virus. To control *Ae. aegypti* populations, there is extensive focus on larval eradication, because breeding sites are often close to human populations, especially man-made containers. The objective of this research was to evaluate the efficacy of commercial essential oils from rosemary (*Rosmarinus officinalis*), vanilla (*Vanilla planifolia*), and spearmint (*Mentha spicata*) with regard to larvicidal activity against *Ae. aegypti* larvae after 24 and 48-hours of exposure in the laboratory. The results showed highly efficacious larvicidal activity, with median lethal doses (LC_{50}) of 0.23, 0.10, and 0.12 ppm after 24-hour exposure to commercial rosemary, vanilla, and spearmint oil, respectively. The results of this study will be useful for the control of a common dengue vector and may replace the use of pesticides that may have broad environmental impacts.

Keywords: *Aedes aegypti*, essential oils, larvicidal activity, vector control, public health

INTRODUCTION

Mosquitoes are small flying insects in the family Culicidae. Several subfamilies exist with many species. Of particular interest are species in the subfamilies Anophelinae and Culicinae, which include mosquitoes in the genera *Aedes*, *Anopheles*, and *Culex*. Mosquitoes in these genera are vectors of human pathogens, including viruses (such as yellow fever virus, West Nile virus, Japanese encephalitis virus, dengue virus, chikungunya virus, and Zika virus), protozoa (such as *Plasmodium* parasites), and nematodes (such as *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*; Bond et al. 2014; Derua et al. 2017; Pratiwi et al. 2019; Souza-Neto et al. 2018; Tandina et al. 2018). Immature stages of mosquitoes, including larvae and pupae, live in the water before emerging from it as adults. Adult male and female mosquitoes feed on nectar from flowers and plant juices. However, females require blood to furnish nutrients for developing eggs (Service 2008). This requirement facilitates transmission of dangerous pathogens to humans and other animals when females bite to draw blood. Dengue fever is one dangerous mosquito-borne viral disease of humans. The disease is caused by dengue virus, a member of the genus Flavivirus of the family Flaviviridae. The virus occurs throughout the world, especially in tropical and sub-tropical regions.

The female *Aedes aegypti* mosquito is a principal vector (also called a primary vector) of dengue (Hasan et al. 2016). *Aedes aegypti*, the yellow fever mosquito, belongs to the subgenus *Stegomyia* within the subfamily Culicinae (Harbach 2019). *Aedes* species originated in Africa but are presently distributed throughout the world due to human-based transportation (Eskildsen et al. 2018). On the other hand, *Aedes albopictus*, the “Asian tiger mosquito,” is a secondary vector of dengue and originated in Southeast Asia (Goubert et al. 2016). The World Health Organization (WHO) reports that dengue fever is the largest human disease burden, with an estimated 390 million people in 128 countries infected every year (WHO 2020).

Reducing the number of *Aedes* mosquitoes is recognized as an effective means to reduce risks and control the outbreak of dengue fever (Ma et al. 2016; Weeratunga et al. 2016). For controlling *Ae. aegypti* populations, there is an intense focus on larvae eradication, because breeding sites are often close to human populations, especially man-made containers (Chaiphongpachara et al. 2018; Rohani et al. 2014). Currently, the insecticide that is widely used to control *Aedes* mosquito larvae is temephos (original trade name Abate), which is a non-systemic organophosphorus insecticide (George et al. 2015). However, dengue vector resistance to temephos has been reported in many countries (Hasmiwati and Supargiyono 2018; Hasmiwati et al. 2018), including Argentina (Albrieu et al. 2010), Brazil (Valle et al. 2019), Colombia (Grisales et al. 2013), Ecuador (Morales et al. 2019), and Guadeloupe and Saint Martin (Goindin et al. 2017). Therefore, other alternative products are needed to solve this problem. Synthetic chemical insecticides are highly effective for killing several insects, including mosquito vectors. However, these synthetic chemicals are not accepted by the community because they are highly toxic to humans and animals (Ndakidemi et al. 2016; Andila et al. 2018). In addition, these chemicals can accumulate in the environment (Nkya et al. 2013; Jayaraj et al. 2016). Natural products are alternative insecticides that are currently popular (Mossa et al. 2018). The advantage of natural products for mosquito control is elevated environmental safety and no toxicity to organisms (Govindarajan and Benelli 2016).

Essential oils are naturally occurring volatile substances extracted from plants (Geetha and Roy 2014; Utami 2016; Arifiyanto et al. 2017; Hasani et al. 2017). These oils are used for their anti-stress properties as well as aromatherapy (Ali et al. 2015). They are also major components of cosmeceuticals (Sarkic and Stappen 2018), and their use in medicine is currently of some interest (Ali et al. 2015). Volatile oils of some plants are used to treat skin diseases (Orchard and Van Vuuren 2017) and are also used as insect repellents or for insect control, e.g., for mosquitoes (Pavela 2015; Lee 2018). Essential oils are easy to obtain, commercially available, and represent sustainable plant products (Dhifi et al. 2016). Thus, practical applications for these oils have been repeatedly sought.

Rosmarinus officinalis (rosemary), *Vanilla planifolia* (vanilla), and *Mentha spicata* (spearmint) are plants grown partly to extract essential oils. The aromas of these oils are easily recognized by many people across the globe (de Sousa et al. 2015; Baqueiro-Peña and Guerrero-Beltrán 2017; Fiume et al. 2018). Previous studies have reported that rosemary, vanilla, and spearmint essential oils repel arthropods (Nerio et al. 2010; Koc et al. 2012; Francikowski et al. 2019). Rosemary and spearmint oils are also toxic to small insects, such as larvae of armyworms *Pseudaletia unipuncta* (Isman et al. 2008) and *Culex quinquefasciatus* (Susilowati 2018) for rosemary oil, and larvae of rice moth *Corcyra cephalonica* (Khani et al. 2012) for spearmint oil. *V. planifolia* shows antimicrobial activity against some pathogenic bacteria (Bilcu et al. 2014). It is unknown whether three essential oils be able to kill larvae of *Ae. aegypti*. Therefore, this study used commercial rosemary, vanilla, and spearmint essential oils and tested their larvicidal activity against *Ae. aegypti* in the laboratory. The results of this study will be useful for the control of a common dengue vector and may replace the use of pesticides that may have broad environmental impacts.

MATERIALS AND METHODS

Commercial essential oils

The three commercial aromatic essential oils including rosemary (*R. officinalis*), vanilla (*V. planifolia*), and spearmint (*M. spicata*) were purchased from Chemipan (Bangkok, Thailand). The product data indicate that all three natural oils are extracted by steam distillation from rosemary and vanilla flowers, and spearmint leaves. Oils were stored in amber glass bottles at room temperature (25-28°C) and away from sunlight.

Rearing of mosquito larvae

Laboratory-reared *Ae. aegypti* larvae were procured from the Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand. Larval mosquitoes were sent to the laboratory of the College of Allied Health Sciences, Suan Sunandha Rajabhat University, Thailand, and reared in a plastic tray containing filtered water until the late third larval stage. Mashed dog biscuits were used as larval food. Laboratory conditions were controlled at 25-28°C, 70-80% relative humidity, with a 12-hour light/12-hour dark cycle.

Larvicidal bioassay

The larvicidal bioassay was modified from the WHO method (WHO 2005). Aromatic rosemary, vanilla, and spearmint essential oils were prepared and diluted with filtered water containing 1 mL of absolute methanol (solvent) at concentrations of 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 ppm.

Twenty-five late third instar larvae were put into 250 mL beakers containing essential oil solutions. For the duration of testing, the larvae were not fed. For each concentration of the three oils, four replicates were used, for a total of 100 larvae for each oil and each concentration. Each oil and concentration test included a set of control larvae not exposed to essential oils. Twenty-four and 48 hours after treatment, larval mortality was recorded. *Aedes aegypti* larvae were considered dead when they did not move.

Data analysis

Probit analysis was applied to determine the concentrations required to kill 25, 50, and 90% of the larvae (LC_{25}, LC_{50}, and LC_{90}, respectively) 24-hours post-exposure. Statistical analysis including probit analysis, 95% confidence intervals, and chi-square test using LdP Line software (http://www.ehabsoft.com/ldpline/). Analysis of variance (ANOVA) followed by Duncan's multiple range test was used to compare larval mortality among groups (24 and 48 hours each concentration) using R software. The statistical significance was set at $p < .05$.

RESULTS AND DISCUSSION

Results

In the present study, commercial pure rosemary (*R. officinalis*), vanilla (*V. planifolia*), and spearmint (*M. spicata*) essential oils showed significant larvicidal activity against *Ae. aegypti* larvae (Table 1). Overall, there was 100% larval mortality for all plant oils at concentrations ranging from 0.05-0.30 ppm. Percent mortality increased with the essential oil concentrations (Table 1).

Larvicidal activities of three oils against *Ae. aegypti* larvae after 24 and 48 hours of exposure were analyzed using probit analysis. After 24-hour exposure, vanilla oil was most potent (LC_{25} = 0.08 ppm, LC_{50} = 0.10 ppm, and LC_{90} = 0.15 ppm), followed by spearmint oil (LC_{25} = 0.11 ppm, LC_{50} = 0.12 ppm, and LC_{90} = 0.16 ppm) and rosemary oil (LC_{25} = 0.18 ppm, LC_{50} = 0.23 ppm, and LC_{90} = 0.35 ppm) (Table 2). For the 48-hour exposure, vanilla oil again was most potent (LC_{25} = 0.07 ppm, LC_{50} = 0.08 ppm, and LC_{90} = 0.12 ppm), followed by spearmint oil (LC25 = 0.10 ppm, LC50 = 0.12 ppm and LC90 = 0.15 ppm) and rosemary oil (LC_{25} = 0.15 ppm, LC_{50} = 0.21 ppm and LC_{90} = 0.41 ppm).

There were statistically significant among the oils with regard to LC_{25}, LC_{50}, and LC_{90} values (Figures 1-3). The efficacy of larvicidal activity of commercial aromatic oils on *Ae. aegypti* between 24 and 48 hours of exposure was significantly different for rosemary and vanilla oils, but not for spearmint oil (Figures 2 and 3).

Discussion

The control of *Aedes* spp. larvae is an important key to reducing the mosquito vector population and decreasing the incidence of dengue fever (Barrera et al. 2018; Roiz et al. 2018; Chaiphongpachara et al. 2019). Essential oils are a group of natural products that may have important larvicidal properties that are useful for the control of mosquito larvae in man-made containers (Dias and Moraes 2014). Our data showed that all three commercial aromatic essential oils were highly efficacious against *Ae. aegypti* larvae in the laboratory: rosemary (LC_{50} = 0.23 ppm at 24 hours), vanilla (LC_{50} = 0.10 ppm at 24 hours), and spearmint (LC_{50} = 0.12 ppm at 24 hours).

For the interpretation of natural product performance, Komalamisra et al. (2005) and Cheng et al. (2003) suggested that an LC_{50} < 50 mL/L (ppm) indicates high activity. The current results are consistent with previous reports on the LC_{50} of rosemary essential oil for mosquito larvae, including *Cx. quinquefasciatus* (30.60 µg/mL), *Culex tritaeniorhynchus* (115.38 µg/mL), and *Anopheles subpictus* (64.50 µg/mL) (Mahmoud et al. 2019). Govindarajan et al. (2012) studied larvicidal activity of spearmint essential oil on mosquito vectors and estimated LC_{50} values of 62.62 ppm for *Cx. quinquefasciatus*, 56.08 ppm for *Ae. aegypti*, and 49.71 ppm for *Anopheles stephensi.* Vanilla essential oil has not previously been tested for larvicidal activity, but there were reports on antimicrobial activity against some human pathogenic bacteria (Bilcu et al. 2014).

In general, the biological activity of essential oils is related to their major constituents (Dias and Moraes 2014). The major constituents of three essential oils are 1,8-cineole (52.8 %) for rosemary (El-Massry et al. 2008), aliphatic acids, and phenolic compounds for vanilla (Pérez-Silva et al. 2006), and carvone (48.60%) for spearmint. These chemicals are all reportedly toxic to insects (Tripathi et al. 2003; Tripathi et al. 2009; Pavela 2015; Bullangpoti et al. 2018). From the statistical analysis, vanilla oil proved to be most potent, followed by spearmint and rosemary. This result is consistent with historical literature reviews that found at equivalent concentrations, rosemary essential oil is less toxic than spearmint essential oil (Dias and Moraes 2014).

The main strength of using commercially aromatic rosemary, vanilla, and spearmint essential oils all plant-derived natural products to control *Ae. aegypti* in the community is their wide availability and relatively low cost. Usually, the habitat of immature *Aedes* mosquitoes is artificial containers with clean water that are located around houses (Getachew et al. 2015; Islam et al. 2019; Kahamba et al. 2020). The results from this study indicate that all three essential oils might be useful in the field to control dengue vector mosquitoes. However, successfully controlling *Aedes* species larvae will require strong cooperation of the people in the area (Achee et al. 2015; Chaiphongpachara and Moolrat 2017; Flores and O'Neill 2018).

Table 1. Mosquito larval mortality after exposure to three oils in each concentration

Concentrations (ppm)	% Mortality (means ± S.E.) after exposure to pure essential oils					
	Rosemary		Vanilla		Spearmint	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
0.05	0	3 ± 1.91	0	3 ± 1.91	0	0
0.10	0	4 ± 2.83	55 ± 7.72	80 ± 9.93	14 ± 6.83	24 ± 5.16
0.15	0	6 ± 1.15	92 ± 1.63	98 ± 1.15	87 ± 1.91	87 ± 1.91
0.20	62 ± 8.87	63 ± 9.29	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
0.25	60 ± 3.65	63 ± 5.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
0.30	69 ± 3.00	74 ± 3.83	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
Control	0	0	0	0	0	0

Note: % : percentage and S.E. : standard error

Table 2. Larvicidal potency of three oils against laboratory-reared *Aedes aegypti* larvae after 24- and 48-hour exposure

Essential oils	Period of exposure									
	24 hour after exposure					48 hour after exposure				
	LC_{25} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± S.E.	χ^2	LC_{25} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± S.E.	χ^2
Rosemary	0.18	0.23	0.35	6.72 ± 0.58	50.15	0.15	0.21	0.41	4.55 ± 0.40	71.05
Vanilla	0.08	0.10	0.15	7.63 ± 0.81	6.22	0.07	0.08	0.12	8.49 ± 0.75	1.05
Spearmint	0.11	0.12	0.16	12.74 ± 1.16	0.17	0.10	0.12	0.15	10.56 ± 1.00	2.57

Note: LC_{25}: concentration that kills 25% of mosquito larvae; LC_{50}: concentration that kills 50% of mosquito larvae; LC_{90}: concentration that kills 90% of mosquito larvae; ppm: parts per million; S.E.: standard error; $\chi 2$: chi-Square

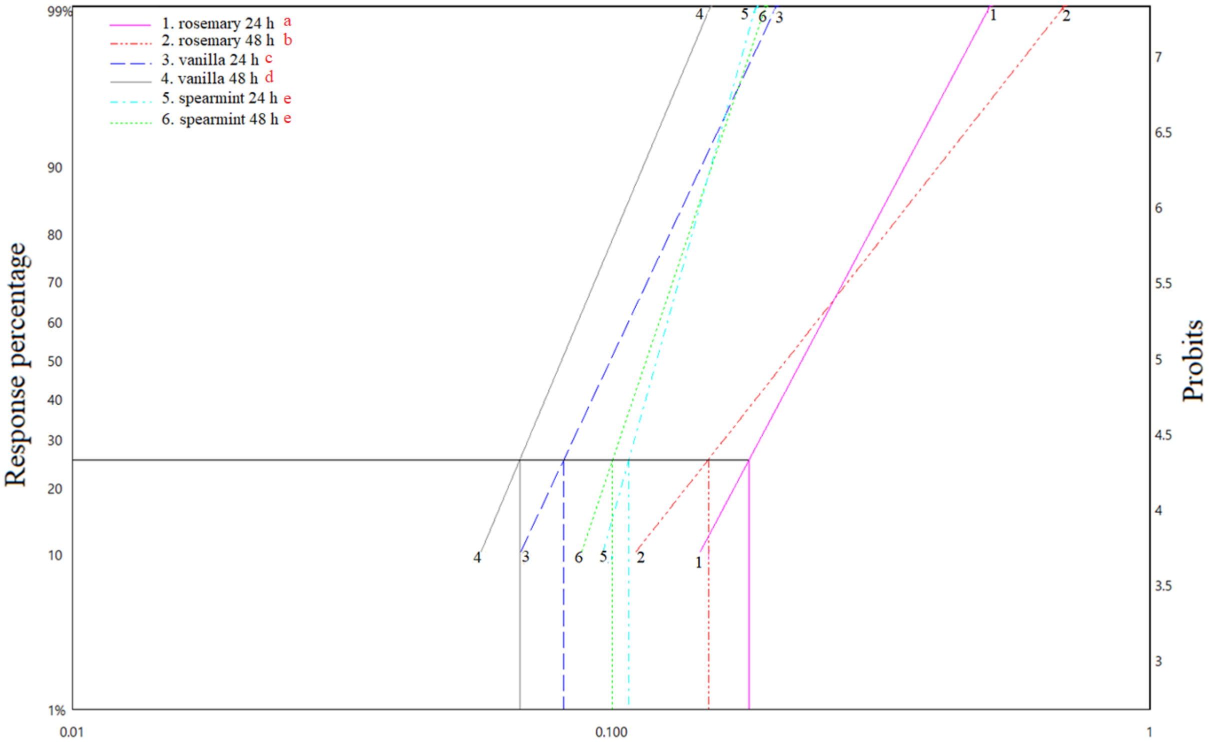

Figure 1. The graph shows the LC_{25} concentrations for *Aedes aegypti* larvae at 24 and 48 hours after exposure to commercial rosemary, vanilla, and spearmint essential oils. Significant differences are indicated by different red letters that appear at the end of the descriptions in the upper left corner

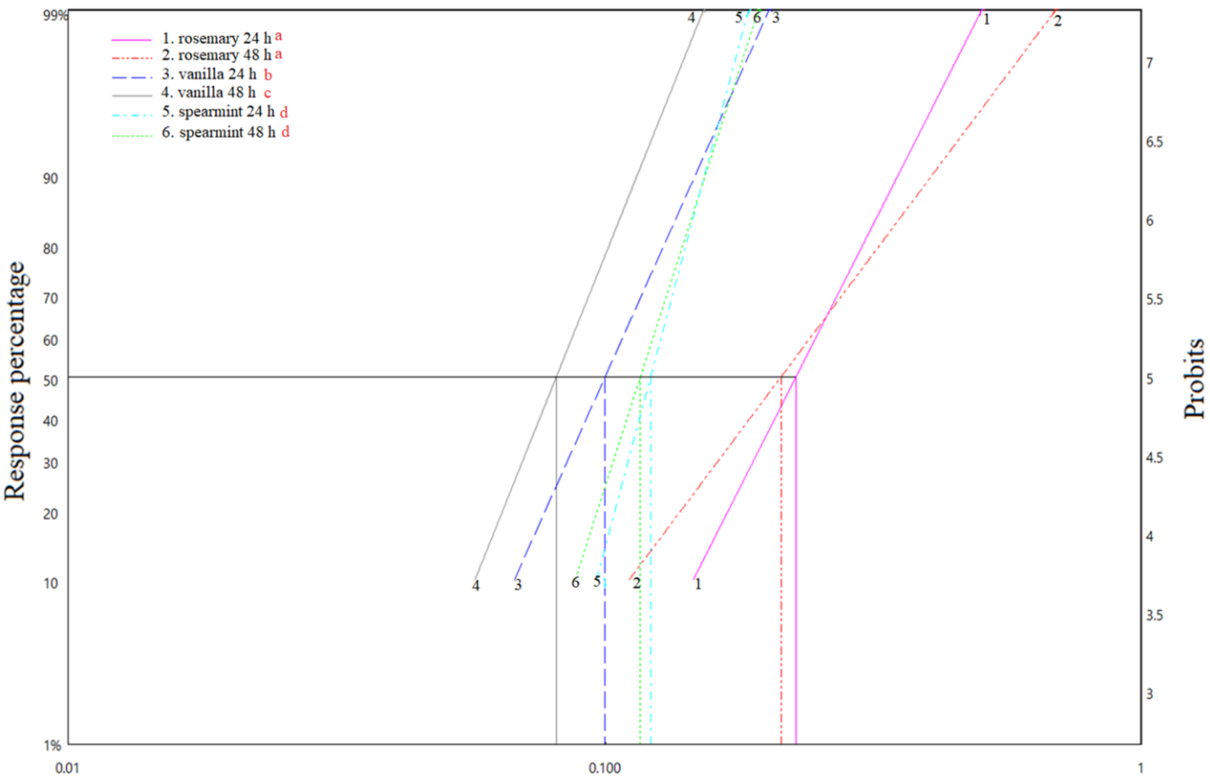

Figure 2. The graph shows LC_{50} calculations of *Aedes aegypti* larvae 24 and 48 hours after exposure to commercial rosemary, vanilla, and spearmint essential oils. Significant differences are indicated by different red letters that appear at the end of the descriptions in the upper left corner

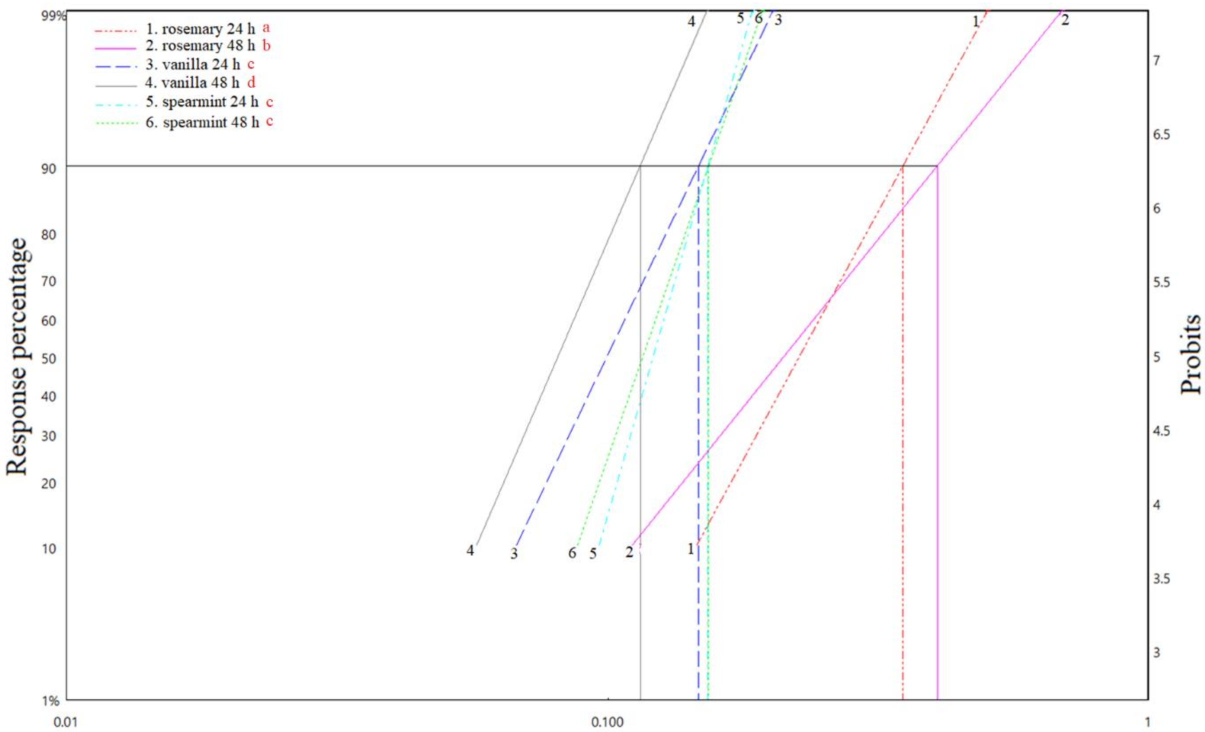

Figure 3. The graph shows the LC_{90} calculations for *Aedes aegypti* larvae 24 and 48 hours after exposure to commercial rosemary, vanilla, and spearmint essential oils. Significant differences are indicated by different red letters that appear at the end of the descriptions in the upper left corner

Aedes aegypti mosquito is the main vector of dengue fever, which is a major public health problem throughout the world. Controlling populations of this mosquito is done by destroying larval breeding sites. Natural products are an interesting option because they are not toxic to non-target organisms. The findings of this study demonstrate that commercially available aromatic rosemary, spearmint, and-especially-vanilla essential oils can eliminate *Aedes* spp. larvae. High-quality commercial essential oil products are readily available, easy to use, and relatively inexpensive. They are highly concentrated and pure. More studies are needed to test these oils in the field to determine whether they can be used effectively for controlling *Aedes* species in communities.

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