This file has been cleaned of potential threats.

If you confirm that the file is coming from a trusted source, you can send the following SHA-256 hash value to your admin for the original file.

559622efbdbe4d598496286ab58030302949e63689c42c16244c378447218af2

To view the reconstructed contents, please SCROLL DOWN to next page.

EVALUATING LARVICIDAL EFFICACY OF COMMERCIAL CLOVE OIL (SYZYGIUM AROMATICUM) AGAINST AEDES AEGYPTI FOR DENGUE CONTROL

Tanisorn Wanpetch[*], Sedthapong Laojun[**], Nantana Suwandittakul[***], Pongmada Damapong[****], Peerada Damapong[*****], Arina Abdulloh[******] & Tanawat Chaiphongpachara[*******]

[*,**,***,****,*****,******] *Department of Public Health and Health Promotion, College of Allied Health Sciences, Suan Sunandha Rajabhat University, Samut Songkhram, Thailand*

E-Mail: [*]s64122239064@ssru.ac.th,[**]sedthapong.la@ssru.ac.th,[***]nantana.su@ ssru.ac.th,[****]pongmada.da@ ssru.ac.th,[*****]peerada.da@ssru.ac.th, [******]arina.ab@ssru.ac.th,[*******]tanawat.ch@ssru.ac.th.

ABSTRACT

Essential oils, as natural products, offer a promising alternative for controlling the dengue vector population, specifically targeting the immature stages of *Aedes aegypti* that develop in water containers. Previous research has shown that clove oil is effective against a variety of insects, including rhinoceros beetles, red flour beetles, and mosquitoes. This study evaluated the larvicidal activity of commercial clove essential oil (*Syzygium aromaticum*) against *Aedes aegypti*, a critical vector of dengue, over various exposure durations: 12, 18, 24, and 48 hours. The LC_{25} values recorded at 12, 18, 24, and 48 hours post-exposure were 0.05, 0.05, 0.05, and 0.04 ppm, respectively. Similarly, LC_{50} values were 0.07, 0.06, 0.06, and 0.06 ppm, respectively, for the same time points. The LC_{90} values at these time intervals were 0.11, 0.10, 0.10, and 0.12 ppm, respectively. These results contribute to the existing body of knowledge by confirming the potential of clove essential oil as an alternative approach for controlling the dengue vector.

Keywords: Mosquito larvicidal activity, Clove oil, Aedes aegypti

INTRODUCTION

Plant-based alternatives are increasingly sought after as substitutes for chemical insecticides, gaining popularity for their reduced environmental impact [1]. These chemical insecticides play a critical role in mitigating vector-borne diseases [2]. However, the long-term ecological harm that synthetic chemicals cause—harming plants, animals, and human health—has raised concerns on a global scale [1]. In contrast to natural compounds, synthetic insecticides persist in the environment, leading to the accumulation of harmful residues in food and water sources. This contamination poses a risk to multiple generations, potentially compromising the health of entire communities.

Syzygium aromaticum, commonly known as clove, belongs to the Myrtaceae family and originates from the Maluku Islands in Indonesia. However, it is now cultivated in various regions worldwide. Previous research has shown that clove oil is effective against a variety of insects, including mosquitoes [3]. Mosquitoes represent the most significant vector globally, capable of transmitting diseases such as chikungunya, dengue fever, lymphatic filariasis, yellow fever, Zika, and malaria to humans, leading to millions of deaths annually. The incidence of these diseases, particularly dengue fever transmitted by *Aedes aegypti*, is on the rise, unlike other mosquito-borne diseases like lymphatic filariasis and malaria, which are declining. While clove oil has shown potential in controlling *Aedes* larvae [3], the specific

exposure durations required for maximum larval mortality remain unclear. Such details are crucial for assessing the efficacy of insecticides [4].

Therefore, this study employed commercial clove essential oil to evaluate its larvicidal effect on *Ae. aegypti*, the principal dengue vector, over various exposure periods: 12, 18, 24, and 48 hours. The findings aim to enrich the current understanding and affirm the potential of clove essential oil as a sustainable alternative for dengue vector control.

OBJECTIVE

To evaluate the larvicidal effect of commercial clove essential oil on *Aedes aegypti*, the primary vector of dengue, across various exposure periods: 12, 18, 24, and 48 hours.

METHODOLOGY

1.Population and Sample Group

The research was conducted on a population of 94 Grade 7 students Gifted English program in at the Demonstration School of Suan Sunandha Rajabhat University (SDSSRU) in Bangkok, Thailand. The school has two learning programs, namely Gifted English program (GEP) and English Program (EP). In the first semester of the academic year 2023, it was found that a significant number of students in grade 7 at SDSSRU struggled with their English Grammar and lack of motivation. The students enrolled in the EP have a higher level of language proficiency than those in the GEP. The sample group for this research comprised of 32 Grade 7 students in the GEP, who were selected using the Cluster Random Sampling technique.

2. Research Tools

The following items have been evaluated by experts:

1. A test in the past simple tense consisting of 30 multiple-choice questions.

2. Five lesson plans using the 2W3P model with a Kahoot game incorporated into the practice stage. This provides students with ample opportunities to apply the grammar rules they have learned.

3. Questionnaires that measure student satisfaction with the use of the 2W3P model and the Kahoot game.

3. Data Collection

In this research, the researcher spent five weeks collecting data. The process included the following steps:

1) Students took the Pre-test in Past Simple Tense.

2) Teacher started teaching grammar content using 5 lesson plans in the 2W3P model with Kahoot game.

3) Students took the Post-test in Past Simple Tense.

4) Students took the satisfaction questionnaire.

4. Data Analysis

The data analysis was performed using means, standard deviations, and t-tests.

4.1) The comparison of the Pre-test and Post-test Scores were analysed through means, standard deviations, and t-tests.

4.2) The overall satisfaction level was analysed through means, and standard deviation.

RESULTS

Table 1 presents the mortality rates of *Ae. aegypti* larvae exposed to various concentrations of clove essential oil at 12, 18, 24, and 48 hours post-exposure. It demonstrates an increase in larval mortality proportional to the concentration of clove essential oil used. No mortality was observed in the control group, where larvae were not exposed to the oil. The efficacy of clove oil's larvicidal activity against *Ae. aegypti* across different time periods is detailed in Table 2.

The LC_{25} values for clove essential oil at 12, 18, 24, and 48 hours post-exposure were consistently low, recorded at 0.05 ppm for the first three time points and slightly decreasing to 0.04 ppm at 48 hours. Similarly, the LC_{50} values remained stable at 0.07 ppm at 12 hours and adjusted to 0.06 ppm for the subsequent intervals. Notably, the LC_{90} values were 0.11 ppm at 12 hours, with a slight fluctuation observed in later measurements, concluding at 0.12 ppm by 48 hours. The statistical analysis indicated that the differences in larvicidal activity of clove oil against *Ae. aegypti* over the varying exposure times were not significant (Figure 1and 2). This suggests that extending the exposure duration does not necessarily enhance larval mortality.

Table 1 Mortality rates of *Ae. aegypti* larvae exposed to different concentrations of clove essential oil at various post-exposure times.

Concentrations (ppm)	**% of larval mortality (means ± SE) at different times**			
	12 hours	**18 hours**	**24 hours**	**48 hours**
0.05	15.00 ± 3.79	25.00 ± 9.98	26.00 ± 5.77	33.00 ± 7.55
0.10	92.00 ± 1.63	94.00 ± 2.00	97.00 ± 1.00	98.00 ± 1.15
0.15	97.00 ± 1.91	97.00 ± 1.91	99.00 ± 1.00	99.00 ± 1.00
0.20	99.00 ± 1.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
0.25	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 2 Larvicidal activity of clove oil against *Ae. aegypti* across various post-exposure periods

Times	**LC_{25} (UL-LL) (ppm)**	**LC_{50} (UL-LL) (ppm)**	**LC_{90} (UL-LL) (ppm)**	**Slope ± SE**	**χ^2**
12 hours	0.05 (0.03-0.06)	0.07 (0.05-0.09)	0.11 (0.09-0.17)	6.43±0.54	12.57
18 hours	0.05 (0.04-0.05)	0.06 (0.06-0.07)	0.10 (0.09-0.11)	6.12±0.56	7.11
24 hours	0.05 (0.04-0.05)	0.06 (0.06-0.07)	0.10 (0.09-0.10)	6.57±0.51	3.35
48 hours	0.04 (0.04-0.05)	0.06 (0.06-0.10)	0.12 (0.10-0.15)	4.71±0.71	0.05

Note: LC_{25}= lethal concentration that killed 25% of exposed mosquito larvae; LC_{50}= lethal concentration that killed 50% of exposed mosquito larvae; LC_{90}= lethal concentration that killed 50% of exposed mosquito larvae; UL= upper limit; LL= lower limit; SE= standard error; χ^2= chi-square.

The larvicidal activity of clove oil against *Ae. aegypti* demonstrated an LC_{50} value of 0.06 ppm within one day. This LC_{50} value for pure clove oil signifies a high degree of larvicidal efficiency, aligning with the World Health Organization's criteria, which classify substances

with an LC_{50} less than 50 ppm (ml/L) as highly active [5]. Furthermore, achieving 100% larval mortality at concentrations below 100 ml/L in this study underscores the significant toxicity of clove oil against *Ae. aegypti* larvae [6]. These findings are consistent with previous research that evaluated the efficacy of clove essential oil on *Ae. aegypti* and confirmed its high effectiveness. Costa et al. [7] identified eugenol as a major component contributing to larval mortality. Eugenol, a principal active ingredient in clove oil, is known to be toxic to a wide range of insects, including mosquitoes.

Figure 1

Graph of the 50% lethal concentration (LC_{50}) values of clove oil against *Ae. aegypti* larvae at 12, 18, 24, and 48 hours post-exposure. Statistically significant differences between time points are denoted by distinct red letters displayed at the end of description phrases in the top right corner.

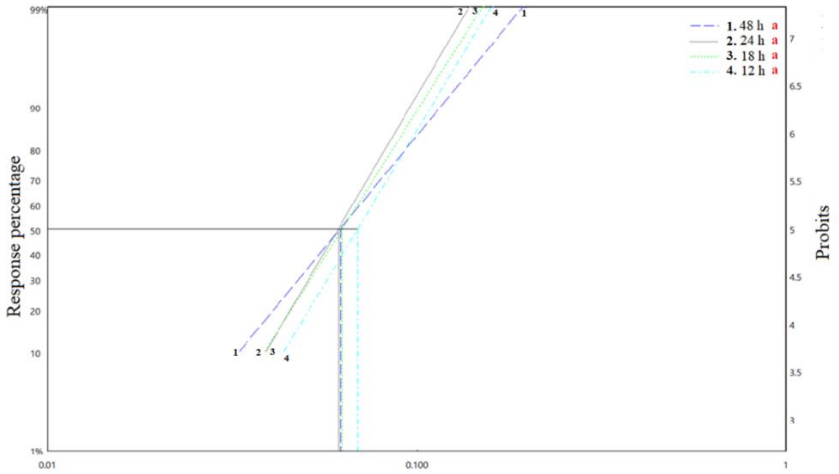
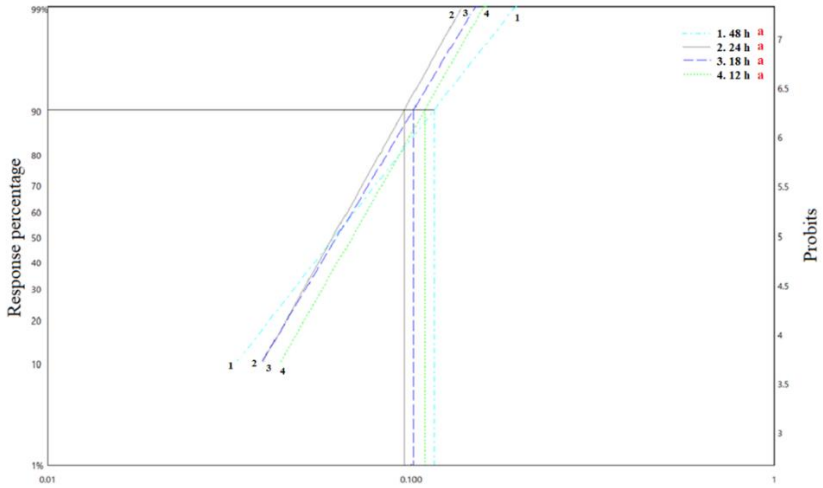


Figure 2

Graph of the 90% lethal concentration (LC_{90}) values of clove oil against *Ae. aegypti* larvae at 12, 18, 24, and 48 hours post-exposure. Statistically significant differences between time points are denoted by distinct red letters displayed at the end of description phrases in the top right corner.



Investigating mosquito larvae mortality at various time increments (including 12, 18, 24, and 48 hours post-exposure) revealed no statistically significant differences. This outcome diverges from previous research, which indicated that *Anopheles* larval mortality increased with longer exposure times [8]. The distinctive efficacy observed in this research may be

attributed to the use of undiluted, pure clove essential oil, which ensures potent effects at all observed times.

CONCLUSION AND FUTURE WORK

Our research has demonstrated the significant efficacy of commercial clove essential oil (*S. aromaticum*) as an alternative method for controlling dengue vectors, which is crucial for public health. These essential oils, being both cost-effective and eco-friendly, can be easily obtained from markets and utilized immediately. Their advantages include affordability, availability, and minimal environmental impact. Nonetheless, the practical application of these oils for community-wide mosquito vector population reduction necessitates collective action. Future studies should aim to develop these oils into user-friendly products, such as in tablet form, to facilitate easy application.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the Suan Sunandha Rajabhat University Language Institute (SSRULI) and the College of Allied Health Science, Suan Sunandha Rajabhat University, Thailand, for their support of our research. Additionally, we extend our thanks to Associate Professor Dr. Tanawat Chaiphongpachara, my advisor, for his invaluable guidance throughout this research.

REFERENCES

[1] Zoubiri, S., & Baaliouamer, A. (2014), Potentiality of plants as source of insecticide principles, *Journal of Saudi Chemical Society*, Vol. 18, No. 6, Pp. 925–938.

[2] Chaiphongpachara, T., Laojun, S., & Wassanasompong, W. (2020), Screening seven commercial essential herb oils for larvicidal activity against the mosquito *Aedes aegypti* (Linnaeus), a vector of the dengue virus. Suan Sunandha Rajabhat University. *Journal of Applied Pharmaceutical Science*, Vol. 10, No. 7, Pp. 43–50.

[3] Fayemiwo, K., Adeleke, M., Okoro, O., Awojide, S., & Awoniyi, I. (2014), Larvicidal efficacies and chemical composition of essential oils of *Pinus sylvestris* and *Syzygium aromaticum* against mosquitoes, *Asian Pacific Journal of Tropical Biomedicine*, Vol. 4, No. 1, Pp. 30–34.

[4] World Health Organization (2005), Guidelines for laboratory and field testing of mosquito larvicides. World Heal Organ. Published online.

[5] Cheng, S., Chang, T., Chang, S., Tsai, K., & Chen, W. (2003), Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae, *Bioresource Technology*, Vol. 89, No. 1, Pp. 99–102.

[6] Dias C., & Moraes, D. (2014), Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: Review, *Parasitology Research*, Vol. 113, No. 2, Pp. 565–592.

[7] Costa, J., Rodrigues, F., Angélico, E., et al. (2005), Chemical-biological study of the essential oils of Hyptis martiusii, Lippia sidóides and Syzigium aromaticum against larvae of *Aedes aegypti* and *Culex quinquefasciatus*, *Revista Brasileira de Farmacognosia*, Vol. 15, No. 4, Pp. 304–309.

[8] Thomas A., Mazigo HD., Manjurano A., Morona D., & Kweka E. (2017), Evaluation of active ingredients and larvicidal activity of clove and cinnamon essential oils against *Anopheles gambiae* (sensu lato), *Parasites and Vectors*, Vol. 10, No. 1.