



Field Test of Clove Oil (*Syzigium aromaticum*) as Biolarvacide Against Mosquito Larvae

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ABSTRACT

Introduction: The vector control that should be carried out today is biological control made from natural ingredients to reduce the negative impacts of the use of chemicals. Therefore, this research aims to examine the ability of residual oil from clove leaves (*Syzigium aromaticum*) to kill the larvae of the mosquito through field tests.

Methods: This research uses experimental methods carried out in the field. This investigation is a follow-up investigation based on laboratory test results. This research uses test larvae obtained directly from the field without going through the breeding process in the laboratory. This research used the concentration of clove leaf (*Syzigium aromaticum*) residual oil that was adopted from laboratory test results, that is, a concentration of 0.006%; 0.007%; 0.008%; 0.009% and 0.01%. This research uses guidelines from the WHO Guidelines testing standards.

Results: The results of this research show that waste oil from clove leaves (*Syzigium aromaticum*) is capable of killing mosquito larvae in field tests. For *Aedes aegypti* larvae, the concentration that is effective in killing larvae starts from a concentration of 0.008%-0.01%, while in tests with *Culex* sp and *Anopheles* sp larvae, leaf waste oil Clove (*Syzigium aromaticum*) is effective in killing larvae at all concentrations (0.006-0.01%). The implications and significance of this research show that clove leaf (*Syzigium aromaticum*) waste oil has proven to be effective as a base for developing natural and economical larvicidal products and can support government programs to eradicate diseases such as dengue, malaria and chikungunya, which are caused by mosquito vectors

Conclusion: Waste oil from clove leaves (*Syzigium aromaticum*) can be used as an alternative ingredient to kill *Aedes aegypti*, *Culex* sp and *Anopheles* sp mosquito larvae in field tests.

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INTRODUCTION

The incidence of dengue has grown dramatically around the world in recent decades, with cases reported to WHO increasing from 505 430 cases in 2000 to 5.2 million in 2019. A vast majority of cases are asymptomatic or mild and self-managed, and hence the actual numbers of dengue cases are under-reported. Many cases are also misdiagnosed as other febrile illnesses (1).

The highest number of dengue cases was recorded in 2023, affecting over 80 countries in all regions of WHO. Since the beginning of 2023 ongoing transmission, combined with an unexpected spike in dengue cases, resulted in a historic high of over 6.5 million cases and more than 7300 dengue-related deaths reported. One modelling estimate indicates 390 million dengue virus infections per year of which 96 million manifest clinically (2).

Globally, 3.5 billion people are at risk of contracting dengue fever and 1.3 billion live in dengue-endemic areas in 10 countries (3). Another study on the prevalence of dengue estimates that 3.9 billion people are at risk of infection with dengue viruses (4). Multiple burdens of those viruses stimulated community efforts to control the diseases actively, focusing on vector control since antiviral medication has not been available yet (5).

Vector control, especially mosquito vectors, can be carried out in the egg, larva and adult stages (6)(7)(8). Chemical control is carried out more frequently than physical and mechanical control. This causes new problems for the environment and public health because chemicals used continuously over a long period of time can contaminate the environment, kill non-target organisms and endanger human health and cause resistance in mosquitoes (6)(7)(8).

The insecticide resistance data from the 11 countries were asymmetric both within the country and between the countries. Indonesia The first outbreak of dengue was recorded in 1968–1970 in Jakarta, Indonesia. Organophosphates were introduced in the 1970s and pyrethroids in the 1980s. *Ae. aegypti* as found to be resistant to multiple insecticides (3).

Although insecticides were once effective in controlling mosquito-borne diseases, the increasing trend of mosquito-borne diseases may indicate increased resistance or ineffectiveness of insecticides in controlling disease transmission (9).

Studies show that the use of natural insecticides can reduce the risk of insect resistance and is more sustainable for the environment (10). This is supported by research on the impact of using natural ingredients in vector control, which shows that various plant extracts can effectively kill mosquito larvae without causing side effects on non-target organisms (11).

Therefore, using natural control of mosquito vectors is a better option, especially in endemic areas, to reduce the negative impact of chemical insecticides and maintain ecosystem balance. It has been shown that the content of active compounds in natural larvicides/insecticides such as eugenol, flavonoids, saponins and other active ingredients can reduce the number of diseases caused by mosquito vectors. The reactions caused by plant essential oils in insects, especially in mosquitoes, can alter their biochemical, metabolic and physiological processes and are toxic (12).

Indonesia has many types of plants that are sources of insecticides that can be used to control disease vectors (13)(14). The use of clove oil can reduce dependence on chemical insecticides, reduce the risk of resistance and provide a safer solution for public health and ecosystem sustainability.

Several studies have demonstrated the effectiveness of clove oil under laboratory conditions, but field testing is needed to determine its effectiveness in natural settings and under a variety of conditions (15)(16). This research aims to examine the ability of residual oil from clove leaves (*Syzygium aromaticum*) to kill the larvae of the mosquito through field tests.

METHOD

Make used clove leaf oil

The process of manufacturing waste oil from clove leaves (*Syzygium aromaticum*) is carried out by a steam distillation (distillation) process using 20 kg of dried clove leaves dropped on the ground and then heated on a stove for 8 hours to produce oil.

Collection of Mosquito larvae

The larvae to be used are third instar larvae and considering the difficulties in carrying out the breeding/rearing process, the larvae collection process is carried out from the breeding habitat around the laboratory to facilitate the transfer process in order to that they are not used. Many WHO guidelines and scientific journals use

stage 3 larvae as test larvae because they have consistently shown toxic responses in bioassays, are able to metabolize foreign compounds, and have stable physiology, whereas stage 1 and 2 larvae are still very sensitive because they can die from mild stress. The fourth instar has begun to pupate, and its feeding activity has decreased.

Biolarvacide tests

This test was carried out at the field test. This test uses 5 concentrations of waste oil solution from clove leaves (*Syzgium aromaticum*), or 0.006%; 0.007%; 0.008%; Concentrations of 0.009% and 0.01%. Next, add 25 third instar mosquito larvae to each test bowl. This observation was carried out for 24 hours to determine the level of larval mortality with 4 repetitions. Observation and laboratory testing methods for biolarvacides refer to the WHO guidelines for larvicide standards (17).

Statistical analysis

Larval test results are broken down according to larval death criteria as follows (17): 1) Mortality >98% indicates a susceptible species, 2) 80-98% mortality indicates a tolerant species, 3) Mortality < 80% indicates a resistant species.

Ethical Approval

This study was conducted following ethical guidelines for mosquito vector research. All stages and procedures involving mosquito larvae following the Guidelines for laboratory and field testing of mosquito larvicides in the 2005 World Health Organization (WHO).

RESULTS

The results of the tests of the mortality rate of *Aedes aegypti*, *Culex* sp and *Anopheles* sp larvae during 24 hours of observation are visible in the table below.

Table 1. Percentage of death of *Aedes aegypti* larvae in various concentrations of clove leaf waste oil (*Syzgium aromaticum*) for 24 hours (Field test)

Concentration (%)	Number of Test (Larvae)	Repetitions		Larval Mortality Rate (Larvae)	Larval Mortality Percentage (%)
		1	2		
0	25	0	0	0	0
0,006	25	12	23	17,5	70
0,007	25	13	25	19	76
0,008	25	17	23	20	80
0,009	25	14	25	19,5	78
0,01	25	23	24	23,5	94

Based on the table above, it is known that the average mortality of *Aedes aegypti* larvae was highest at a concentration of 0,01% was 23,5 larvae (94%) and the lowest mortality rate was at a concentration of 0,006% was 17,5 larvae (70%). These results show that the higher the concentration of clove leaf waste oil (*Syzgium aromaticum*), the higher the average number of mortality of *Aedes aegypti* mosquito larvae during 24-hour observations.

These results indicate that *Aedes aegypti* larvae from field tests using waste oil from clove leaves (*Syzgium aromaticum*) are effective at concentrations of 0.008% to 0.01%.

Table 2. Percentage of death of *Culex* sp larvae in various concentrations of clove leaf waste oil (*Syzigium aroamticum*) for 24 hours (Field test)

Concentration (%)	Number of Test (Larvae)	Repitations		Larval Mortality Rate (Larvae)	Larval Mortality Percentage (%)
		1	2		
0	25	0	0	0	0
0,006	25	23	25	24	96
0,007	25	25	25	25	100
0,008	25	25	25	25	100
0,009	25	25	25	25	100
0,01	25	25	25	25	100

Based on the previous table, it is known that the average mortality rate of *Culex* sp larvae at a concentration of 0,006% was 24 larvae (96%) while for a concentration of 0,07%; At concentrations of 0,08%, 0,09%, and 0,01%, all larvae tested experienced 100% mortality within 24 hours.

For *Culex* sp larvae, field test results showed that clove leaf (*Syzigium aromaticum*) waste oil was effective in killing larvae at all concentrations (0,006%-0,01%).

Table 3. Percentage of death of *Anopheles* sp larvae in various concentrations of clove leaf waste oil (*Syzigium aroamticum*) for 24 hours (Field test)

Concentration (%)	Number of Test (Larvae)	Repitations		Larval Mortality Rate (Larvae)	Larval Mortality Percentage (%)
		1	2		
0	25	0	0	0	0
0,006	25	25	25	25	100
0,007	25	25	24	24,5	98
0,008	25	25	25	25	100
0,009	25	24	25	25	100
0,01	25	25	25	25	100

Based on the previous table, it is known that the average number of mortality of *Anopheles* sp larvae at a concentration of 0,007% was 24.5 Larvae (98%) while for a concentration of 0,06%; At concentrations of 0,08%; 0,09% and 0,01%, all larvae tested experienced 100% mortality within 24 hours. For *Anopheles* sp larvae, it is effective in mortality larvae at all concentrations (0,006%-0,01%).

DISCUSSION

Based on the results of field research, it is known that clove leaf waste oil (*Syzigium aromaticum*) has the ability to act as a biolarvicide against *Aedes aegypti*, *Culex* sp and *Anopheles* sp mosquito larvae. The test results showed an increase in mortality (mortality) of *Aedes aegypti*, *Culex* sp and *Anopheles* sp mosquito larvae as the concentration increased.

The results of this research are consistent with other research regarding the ability of clove plants (*Syzigium aromaticum*) to kill *Aedes aegypti*. Eugenol extracted from clove oil was able to kill *Anopheles stephensi* larvae with an LC₅₀ (57.49 ppm) and an LC₉₀ (93.14 ppm) (18). Clove (*Syzigium aromaticum*) essential oil has an LC₅₀ (17.527 µg/ml) against *Anopheles gambia* (19). In addition, the essential oil of clove leaves (*Syzigium aromaticum*) has an effect on the mortality of *Anopheles aconitus* larvae with an LC₅₀ (54.145 ppm) (20). Apart from this, other research has also revealed that *Syzigium aromatic* essential oil can kill 86.96% of *Anopheles stephensi* larvae (18).

This effectiveness comes from the residual oil content of clove leaves (*Syzigium aromaticum*), which contains natural ingredients such as eugenol and other active compounds that are toxic to insects through mechanisms that alter the nervous system and metabolism of the larvae. Apart from that, eugenol compounds also damage the cuticle, disturbing the osmotic balance in the body and causing respiratory function to be impaired until the larvae eventually die (2)(21).

The mortality rate of *Culex* sp and *Anopheles* sp mosquito larvae is, on average, greater than 98%. According to the 2005 WHO standards, the larval mortality rate is > 98%, indicating that the larvicide is susceptible to *Culex* sp and *Anopheles* sp mosquito larvae. Unlike *Aedes aegypti* mosquito larvae, the average mortality rate is less than 80%, indicating that the tested larvicide is resistant to *Aedes aegypti* larvae.

The results of this study demonstrate that *Aedes aegypti* larvae are more resistant to larvicides than *Culex* sp and *Anopheles* sp larvae. This is due to differences in mosquito resistance levels, which can be influenced by genetic, biological, physiological and ecological environmental factors (22).

In mosquito vector control programs, especially in urban areas (23), *Aedes aegypti* is used most frequently and mainly in the testing phase to allow larvicide resistance to develop (24). The cuticle of *Aedes aegypti* is thicker and richer in lipids, which causes inhibition of the absorption of larvicidal agents (25). Apart from that, the habitat of the *Aedes aegypti* mosquito is clean water and contains a minimum amount of contaminants and microorganisms, unlike *Culex* sp and *Anopheles* sp mosquitoes, whose habitats tend to be dirty and contain organic matter and microorganisms(22)(24).

This research may provide alternatives and solutions for government and health services to carry out natural and environmentally friendly control of mosquito vectors using clove leaf (*syzygium aromaticum*) waste oil.

Limitations and Cautions

Limitations of the research: This research cannot control all the environmental factors that influence the life of mosquito larvae, such as temperature, humidity, pH and organic matter in water, and has not studied the toxic effects on non-target organisms, such as aquatic biota.

Recommendations for Future Research

Recommendations for future research: It is hoped that future research can control environmental conditions and that there will be studies exploring toxic effects on non-target organisms.

CONCLUSION

This field evaluation demonstrates that residual clove-leaf oil (*Syzygium aromaticum*), a by-product of clove processing, exhibits strong larvicidal activity against *Culex* sp. and *Anopheles* sp., and moderate efficacy against *Aedes aegypti* larvae. These findings suggest that residual clove oil could serve as a sustainable, low-cost alternative to synthetic larvicides in integrated vector management programs, particularly in areas where resistance to conventional chemical larvicides is widespread.

However, variations in efficacy among mosquito species and the absence of non-target safety data highlight the need for further studies on dose optimization, formulation improvement, and ecotoxicological impact. Scaling up field trials under diverse ecological conditions will be essential to confirm the operational potential of clove oil-based larvicides as part of environmentally friendly vector control strategies.

AUTHOR'S CONTRIBUTION STATEMENT

B.B. was responsible for conceptualizing the study, designing the research methodology, analyzing the data, and drafting the initial manuscript. S.J. contributed to field data collection, supervision during the study, and critical review of the manuscript. H. assisted in laboratory analysis, data curation, and preparation of visual materials. P. participated in fieldwork, resources management, and preliminary data interpretation. R.R. contributed to project administration, validation of findings, and manuscript editing. M. provided additional resources, technical guidance, and oversight of the research process. All authors contributed to the interpretation of the results, critically reviewed the manuscript for important intellectual content, approved the final version for publication, and agreed to be accountable for all aspects of the work.

CONFLICTS OF INTEREST

The authors declare that there are no financial, commercial, or personal relationships that could be construed as potential conflicts of interest in the conduct and publication of this research.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors declare that they did not use Generative AI or AI-Assisted Technologies during the writing of this manuscript.

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