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Original Article

Posttest Only Evaluation of Garlic Extract as Aedes aegypti Larvicide

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ABSTRACT

Background: Dengue remains an important Aedes-borne disease, and Aedes aegypti is the primary vector in many endemic settings. The expansion of dengue risk, increasing insecticide resistance, and the need for environmentally acceptable vector-control options support the evaluation of plant-based larvicides. This study assessed the mortality of Aedes aegypti larvae exposed to garlic (Allium sativum) extract.

Methods: A true laboratory experiment with a posttest-only control group design was conducted at the Entomology Laboratory, Department of Environmental Health, Poltekkes Kemenkes Palu, in September 2023. Third-instar Aedes aegypti larvae were allocated to six groups: control (0 ppm) and garlic extract concentrations of 0.8, 0.9, 1.0, 1.1, and 1.2 ppm. Each group had four replications with 20 larvae per container (n = 480). Mortality was observed after 2, 4, 6, 12, and 24 hours and analyzed descriptively.

Results: No mortality occurred in the control group. Cumulative 24-hour mortality increased from 8/80 larvae (10.0%) at 0.8 ppm to 12/80 (15.0%) at 0.9 ppm, 13/80 (16.25%) at 1.0 ppm, 14/80 (17.5%) at 1.1 ppm, and 16/80 (20.0%) at 1.2 ppm.

Conclusion: Garlic extract produced a concentration-related increase in *Aedes aegypti* larval mortality, but concentrations below 2 ppm did not produce immediate high-level larval control within 24 hours. These low-concentration findings support further studies on sublethal effects, including delayed larval development, impaired pupation, and reduced adult emergence, alongside broader concentration ranges, lethal concentration analyses, and standard positive-control comparisons.

INTRODUCTION

Dengue is a mosquito-borne viral infection that continues to affect tropical and subtropical countries. The virus is transmitted mainly by infected female Aedes mosquitoes, especially Aedes aegypti, and can produce a wide clinical spectrum ranging from mild fever to severe dengue (Wilder-Smith et al., 2019; World Health Organization, 2025). Global analyses show that dengue transmission risk is shaped by urbanization, human mobility, vector distribution, and climate suitability (Kraemer et al., 2019; Messina et al., 2019; Ryan et al., 2019). In Indonesia, dengue remains a major public health concern, with national reports showing a marked rise in reported cases and deaths in 2024 compared with the previous year (Kementerian Kesehatan Republik Indonesia, 2024).

Vector control is a central component of dengue prevention because specific antiviral treatment is not available, and vaccines do not remove the need for environmental and entomological measures. Source reduction, larval control, surveillance, community participation, and careful insecticide management are recommended as integrated approaches for Aedes-borne disease control (Achee et al., 2019; World Health Organization, 2023). However, reliance on chemical insecticides can contribute to resistance, operational constraints, and environmental concerns. Reviews from Southeast Asia and Indonesia have documented insecticide resistance in Aedes mosquitoes, making alternative or complementary strategies increasingly important (Dusfour et al., 2019; Gan et al., 2021; Hamid et al., 2018; Kasman et al., 2025; Silalahi et al., 2022).

Botanical larvicides are being explored because plant metabolites may offer biodegradable, locally available options for mosquito control. In the local community context, garlic is readily available, relatively inexpensive, and widely recognized as a household staple, making it a practical candidate for early laboratory screening. Its long empirical use in traditional practice also supports scientific evaluation of whether its bioactive compounds have measurable activity against mosquito larvae. Reviews of plant-derived larvicides and nanoparticles indicate that phytochemicals such as phenolics, terpenoids, alkaloids, saponins, and sulfur-containing compounds may affect larval physiology, survival, and detoxification systems (Bharathithasan et al., 2024; Nawarathne & Dharmarathne, 2024; Vasantha-Srinivasan et al., 2024). Garlic (*Allium sativum*) contains organosulfur compounds, including allicin, alliin, diallyl sulfides, ajoene, and S-allyl-cysteine, which are associated with several biological activities (El-Saber Batiha et al., 2020; Shang et al., 2019). Several laboratory studies have reported larvicidal potential of garlic preparations against *Aedes* or other mosquito larvae, although potency varies by extraction method, concentration, exposure duration, and larval stage (Agesti et al., 2025; Assemie & Gemeda, 2023; Mulyono et al., 2021; Nasir et al., 2022; Putra & Delfita, 2021; Yearsi & Munawaroh, 2021).

The present study was conducted to determine the mortality level of third-instar Aedes aegypti larvae after exposure to garlic extract at concentrations of 0.8, 0.9, 1.0, 1.1, and 1.2 ppm. The findings are expected to contribute laboratory evidence on the potential and limitations of garlic extract as a botanical larvicide candidate.

METHODS

Study design and setting

This was a true experimental laboratory study using a posttest-only control-group design. The study was conducted in September 2023 at the Entomology Laboratory of the Department of Environmental Health, Poltekkes Kemenkes Palu, Central Sulawesi, Indonesia. The design was selected to compare larval mortality across different extract concentrations after exposure, while maintaining an untreated control group.

Study material and experimental units

The study population consisted of Aedes aegypti larvae. The experimental units were third-instar Aedes aegypti larvae because this stage is sufficiently developed for laboratory handling and mortality observation. Each test container received 20 larvae. Six exposure groups were prepared: control without garlic extract (0 ppm) and garlic extract concentrations of 0.8, 0.9, 1.0, 1.1, and 1.2 ppm. Each group was repeated 4 times, yielding 24 containers and 480 larvae overall.

Preparation of garlic extract

Garlic material was cleaned, dried, and blended into powder. A total of 500 g of powdered material was macerated with 96% ethanol for 3 x 24 hours. The macerate was filtered, and the filtrate was concentrated using a rotary evaporator to obtain a thicker extract. The extract was stored under cool conditions until preparation of test concentrations. The tested concentrations were prepared immediately before the larval exposure procedure.

Larval exposure and outcome measurement

Larvae were placed in prepared containers according to concentration group and replication. Mortality was observed after 2, 4, 6, 12, and 24 hours of exposure. Larvae were considered dead when they did not move after gentle stimulation and remained immobile during observation. The primary outcome was the number and percentage of dead larvae at 24 hours. Time-pattern mortality at earlier observation points was also recorded.

Data analysis

Data were summarized descriptively using frequencies and percentages. Mortality percentage was calculated as the number of dead larvae divided by the number of exposed larvae in each group, multiplied by 100. Because the purpose of this report was to describe the mortality level across concentrations, inferential testing and lethal concentration modeling were not performed. Control mortality was examined to assess the acceptability of the test condition.

RESULTS

The control group showed no larval mortality during 24 hours of observation, indicating that the larvae remained viable under the laboratory conditions. Mortality increased as garlic extract concentration increased. The highest cumulative 24-hour mortality was observed at 1.2 ppm, with 16 of 80 larvae dead (20.0%). The lowest mortality was observed at 0.8 ppm, with 8 of 80 larvae dead (10.0%).

Table 1. Cumulative mortality of Aedes aegypti larvae by garlic extract concentration and observation time

Conc.	n	2 h	4 h	6 h	12 h	24 h
Control (0 ppm)	80	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
0.8 ppm	80	1 (1.25)	2 (2.5)	5 (6.25)	8 (10.0)	8 (10.0)
0.9 ppm	80	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	12 (15.0)
1.0 ppm	80	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	13 (16.25)
1.1 ppm	80	0 (0.0)	0 (0.0)	0 (0.0)	4 (5.0)	14 (17.5)
1.2 ppm	80	2 (2.5)	2 (2.5)	5 (6.25)	8 (10.0)	16 (20.0)

Source: Primary Data, 2025

Values are presented as the number of dead larvae (percentage). Percentages are based on 80 larvae per group. Conc. = concentration.

At the end of the 24-hour observation period, garlic extract at 0.8 ppm caused 10.0% mortality, while 0.9 ppm caused 15.0% mortality. Mortality increased slightly at 1.0 ppm (16.25%) and 1.1 ppm (17.5%). The 1.2 ppm concentration produced the highest mortality at 20.0%. These results indicate a positive concentration-response pattern, although the increase was modest within the tested concentration range.

Table 2. Twenty-four-hour mortality by replication and concentration

Conc.	R1	R2	R3	R4	Dead/n	Mortality (%)
Control (0 ppm)	0	0	0	0	0/80	0.0
0.8 ppm	0	5	3	0	8/80	10.0
0.9 ppm	4	0	4	4	12/80	15.0
1.0 ppm	2	2	4	5	13/80	16.25
1.1 ppm	2	4	2	6	14/80	17.5
1.2 ppm	5	3	3	5	16/80	20.0

Source: Primary Data, 2025

Each replication used 20 larvae. Total mortality represents cumulative mortality after 24 hours. R = replication.

DISCUSSION

This study found that garlic extract caused mortality in Aedes aegypti larvae, and mortality increased with higher concentrations. The absence of mortality in the control group supports the conclusion that the observed mortality was related to extract exposure rather than handling or poor larval condition. Nevertheless, mortality remained low to moderate across all tested concentrations, ranging from 10.0% at 0.8 ppm to 20.0% at 1.2 ppm after 24 hours.

The concentration-response pattern is consistent with previous garlic and botanical larvicide studies, which show that mortality generally increases as exposure concentration or duration increases. Mulyono et al. (2021) reported that garlic extract produced larval mortality that rose with concentration in Aedes aegypti. Yearsi and Munawaroh (2021) also reported garlic extract activity against Aedes aegypti larvae, while Agesti et al. (2025) emphasized garlic extract as a potential larvicidal material. Nasir et al. (2022) found that Allium sativum-based preparations could produce dose- and time-dependent toxicity against Aedes aegypti larvae, particularly when formulated as green synthesized silver nanoparticles. Putra and Delfita (2021) Similarly, the larvicidal activity of fermented garlic against Aedes larvae was demonstrated.

The lower mortality in the present study compared with several earlier reports may be explained by differences in concentration scale, extraction method, extract stability, larval strain, exposure period, and the absence of formulation enhancement. Garlic contains biologically active organosulfur compounds, but several of these are unstable and may degrade under processing, storage, and solvent conditions (El-Saber Batiha et al., 2020; Shang et al., 2019). Plant-based larvicides often show variable potency because their phytochemical content depends on plant part, maturity, geographic source, extraction solvent, and preparation technique (Bharathithasan et al., 2024; Nawarathne & Dharmarathne, 2024). Evidence from studies on Allium sativum and other plant extracts indicates that methanol, ethanol, aqueous, and nanoparticle-based preparations can exhibit varying levels of larvicidal activity (Assemie & Gemeda, 2023; Nasir et al., 2022).

Mechanistically, the larvicidal effect of garlic extract is plausibly related to the reactivity and membrane affinity of its organosulfur compounds. Allicin is a reactive thiosulfinate that can interact with sulfhydryl-containing proteins; in mosquito larvae, this may disturb enzyme systems involved in digestion, detoxification, and oxidative balance. Diallyl sulfides and other lipophilic sulfur compounds may also pass through the larval cuticle or cell membranes and increase membrane permeability. After larvae ingest treated water, the midgut epithelium is likely an important target, as disruption of epithelial integrity can interfere with nutrient absorption, ion regulation, and energy metabolism. These physiological disturbances may reduce feeding and movement, weaken detoxification responses, and eventually lead to immobility and death. Because the present study did not include histopathological or biochemical assays, this mechanism should be interpreted as a biologically plausible explanation supported by garlic phytochemistry and plant-larvicide enzyme-inhibition literature rather than as a directly measured pathway in this experiment (El-Saber Batiha et al., 2020; Shang et al., 2019; Vasantha-Srinivasan et al., 2024).

The findings should also be interpreted within the broader context of dengue vector control. Insecticide resistance has been documented in Aedes populations in Indonesia and Southeast Asia, strengthening the need for integrated control strategies and locally evaluated alternatives (Gan et al., 2021; Hamid et al., 2018; Kasman et al., 2025; Silalahi et al., 2022). However, botanical larvicides should not be viewed as direct replacements for established vector-control measures without standardized bioassays, lethal concentration estimates, safety evaluation, formulation studies, and field validation. The experience of integrated programs, including Wolbachia-based and surveillance-supported approaches, shows that vector control requires continuous entomological monitoring rather than reliance on a single intervention (Tantowijoyo et al., 2022; World Health Organization, 2023).

From a public health perspective, the present laboratory findings suggest that garlic extract has measurable biological activity, but the 0.8-1.2 ppm range is not yet suitable for immediate operational larval control. The highest concentration produced only 20.0% mortality within 24 hours, indicating that future studies should test wider and higher concentration ranges, include a positive control such as an established larvicide, measure water quality conditions, quantify active phytochemical content, and calculate LC50 and LC90 values. At the same time, the measurable mortality at concentrations below 2 ppm suggests that low-dose garlic extract may be useful for investigating sublethal endpoints, such as delayed larval development, reduced feeding activity, impaired pupation, decreased adult emergence, or weakened adult fitness. Studies on enzyme inhibition and larval detoxification pathways may also clarify the mechanism of action of plant-derived compounds against *Aedes aegypti* (Vasantha-Srinivasan et al., 2024).

Study Limitations

This study has limitations. The analysis was descriptive and did not estimate lethal concentrations. The study used laboratory larvae and did not assess field populations or household water conditions. Phytochemical screening was not performed, so the concentration of the active compound in the extract was not quantified. The study also did not include a standard positive control for larvicide. Despite these limitations, the study provides useful preliminary evidence for refining garlic-based larvicide research in the local laboratory setting.

CONCLUSION

Garlic (*Allium sativum*) extract increased the mortality of third-instar *Aedes aegypti* larvae in a concentration-related pattern. No mortality occurred in the control group, while 24-hour mortality in treatment groups ranged from 10.0% at 0.8 ppm to 20.0% at 1.2 ppm. Although these concentrations below 2 ppm did not produce immediate high-level larval control, the findings open an important research direction for evaluating low-dose and sublethal effects, including delayed larval development, impaired pupation, reduced adult emergence, and decreased adult fitness. Future research should employ standardized bioassay procedures, broader concentration ranges, positive controls, phytochemical analysis, and LC50/LC90 estimates to assess the practical potential of garlic extract for dengue vector control.

AUTHOR'S CONTRIBUTION STATEMENT: Tjitrowati Djaafar: formulated the research concept, supervised laboratory work, interpreted the findings, and led the manuscript preparation. Amsal: contributed to larval preparation, laboratory procedures, and data recording. A. Bungawati: coordinated extract preparation, replication procedures, and laboratory quality control. Gusman: contributed to data verification, descriptive analysis, final editing, and approval of the revised version.

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