

STATUS OF RESISTANCE OF AEDES SP. MOSQUITOES TO MALATHION 0.8% AT POLTEKKES BANJARMASIN, BANJARBARU

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ABSTRACT

Status of Resistance of *Aedes sp.* Mosquitoes to Malathion 0.8% at Poltekkes Banjarmasin, Banjarbaru. Dengue Hemorrhagic Fever (DHF) is a serious public health problem. Control of DHF is commonly carried out through the use of insecticides, particularly malathion. However, long-term and continuous use of insecticides may lead to the development of resistance in mosquito populations. This study aimed to assess the resistance status of *Aedes sp.* mosquitoes to malathion 0.8% at Poltekkes Banjarmasin in Banjarbaru. This research employed a descriptive observational design with a cross-sectional survey approach. Sampling of *Aedes sp.* mosquitoes was conducted within the campus area of Poltekkes Kemenkes Banjarmasin. Mosquito resistance was assessed using the WHO standard susceptibility test (WHO susceptibility test). Data analysis consisted of descriptive analysis to determine the level of mosquito resistance. The resistance test results showed that 100% of *Aedes sp.* mosquitoes in the test tubes experienced knockdown, with an LT_{50} of 18.3 minutes, an LT_{90} of 39.3 minutes, an LT_{95} of 47 minutes, and an LT_{99} of 56.8 minutes. Therefore, the resistance status of *Aedes sp.* mosquitoes at the Poltekkes Banjarmasin campus was classified as susceptible to malathion 0.8%. These findings contribute to locally specific insecticide resistance data, which can support dengue vector control strategies through the rational and sustainable use of insecticides.

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INTRODUCTION

Dengue Hemorrhagic Fever (DHF), also known as Dengue Fever, is a significant global public health problem, particularly in tropical regions. This disease is caused by the dengue virus (DENV) and is transmitted through the bite of female *Aedes sp.* mosquitoes ⁽¹⁾. In 2022, the number of DHF cases in Indonesia reached 143,000, of which 764 cases were reported in South Kalimantan Province ⁽²⁾. Banjarbaru is one of the cities with a relatively high number of cases, reaching 140 cases ⁽³⁾. In 2024, DHF cases in Banjarbaru increased to 283 cases ⁽⁴⁾. Various control strategies have been implemented to reduce the incidence of DHF in Indonesia, including biological, physical, and chemical control methods. One of the chemical control measures involves the use of insecticides through thermal fogging or cold fogging/ultra-low volume (ULV) applications ⁽⁵⁾. Malathion is one of the insecticides commonly used in thermal fogging operations ⁽⁶⁾. Although insecticide application is effective in reducing mosquito populations, long-term and continuous use may lead to the development of resistance in mosquitoes ⁽⁷⁾.

Several studies conducted in different regions of Indonesia have reported that *Aedes aegypti* mosquitoes have developed resistance to insecticides belonging to the pyrethroid, organophosphate, and carbamate groups^(8,9). Studies conducted in Pekanbaru City and Riau Province demonstrated that *Aedes aegypti* mosquitoes were resistant to malathion^(10,11). In Banjarmasin City, South Kalimantan Province, *Aedes aegypti* mosquitoes have been reported to be resistant to permethrin, deltamethrin, lambda-cyhalothrin, bendiocarb, and cyfluthrin⁽¹²⁾. In Banjarbaru City, South Kalimantan Province, *Aedes aegypti* mosquitoes have also been reported to be resistant to malathion, cypermethrin, lambda-cyhalothrin, and deltamethrin⁽¹³⁾. Most of these studies were conducted in residential areas, while data on mosquito resistance in campus environments remain very limited.

Campuses, as centers of knowledge development, are characterized by high levels of mobility and human activity, which may increase the potential for DHF transmission. To date, there has been no available data regarding the resistance status of *Aedes* sp. mosquitoes to malathion 0.8% in the Poltekkes Banjarmasin campus area located in Banjarbaru City. The availability of such data is essential as a basis for insecticide resistance surveillance and for evaluating the effectiveness of vector control measures in campus environments, particularly at Poltekkes Banjarmasin. Therefore, this study aimed to assess the resistance status of *Aedes* sp. mosquitoes to malathion 0.8% in the Poltekkes Banjarmasin campus area, Banjarbaru City, as a foundation for resistance surveillance and evaluation of dengue vector control effectiveness in the campus setting.

MATERIALS AND RESEARCH METHODS

This study employed a descriptive observational design with a cross-sectional survey approach. This design was selected because it is appropriate for determining the baseline status of vector mosquito resistance to insecticides within a specific area over a defined period. The sampling location for *Aedes* sp. mosquitoes was the campus area of Poltekkes Kemenkes Banjarmasin in Banjarbaru. The study was conducted starting in January 2025.

Samples of *Aedes* sp. mosquitoes were obtained by rearing *Aedes* sp. larvae until they developed into adult mosquitoes. A minimum of 150 *Aedes* sp. larvae were collected from the Poltekkes Banjarmasin campus environment. Larvae that successfully developed into adult mosquitoes were used for testing under sugar-fed conditions. Mosquito identification was performed up to the genus level (*Aedes* sp.), and no differentiation was made between *Aedes aegypti* and *Aedes albopictus*, which constituted one of the limitations of this study.

Mosquito resistance testing was conducted using the WHO standard susceptibility test method, which consisted of several stages, including preparation, collection of test mosquitoes, test implementation, data analysis, and interpretation of test results⁽¹⁴⁾. During the preparation stage, the researchers prepared the research equipment and materials as well as the larval sampling locations. The primary equipment and materials used in this study were WHO susceptibility test kit tubes and insecticide-impregnated papers containing malathion 0.8%.

During the collection of test mosquitoes, larvae collected from the field were reared until producing F0/F1/F2 generations to be used as test mosquitoes, with a total of 120–150 mosquitoes. Each treatment unit (four tubes) and control unit (two tubes) consisted of 20–25 mosquitoes. The mosquitoes used for testing were required to be sugar-fed and aged between 3 and 5 days.

In the testing procedure for *Aedes* sp. mosquitoes, six susceptibility test kit tubes were prepared. Insecticide-impregnated papers were placed into four tubes, with four sheets per tube, while the remaining two tubes were fitted with plain paper without any insecticide. Adult mosquitoes were introduced into each tube at a density of 25 mosquitoes per tube. The tubes were positioned vertically, and observations were conducted by recording the number of dead and knocked-down mosquitoes using the standardized recording forms provided.

Data analysis focused on determining the level of mosquito resistance using descriptive analysis. If the percentage of mortality in the control tubes ranged between 3% and 5%, mosquito mortality data were corrected using Abbott's formula, as shown in the equation below.

$$AI = \frac{A - B}{100 - B} \times 100$$

AI = % Test mosquito mortality (corrected)

A = % Test mosquito mortality

B = % Control mosquito mortality

B = % Test mosquito mortality (corrected)

The interpretation of the observation results was based on the criteria established by the World Health Organization (WHO), whereby a mosquito mortality rate of $\geq 98\%$ indicates that the mosquito population is still susceptible, a mortality rate of 90% to $<98\%$ indicates suspected resistance, and a mortality rate of $<90\%$ indicates resistance. These criteria are applied as the WHO diagnostic thresholds for determining the resistance status of mosquitoes to insecticides.

RESEARCH RESULTS AND DISCUSSION

Adult *Aedes sp.* mosquito samples used for the resistance test observations were derived from mosquito eggs collected using ovitraps. The collected eggs were subsequently reared in trays and fed with cat food until they developed into fourth-instar (instar IV) larvae. These larvae were identified macroscopically by observing the characteristic morphological features of *Aedes sp.* larvae, namely the presence of a short, stout, dark-colored siphon. This identification process was conducted to ensure that the test mosquitoes originated from the genus *Aedes sp.*, thereby allowing accurate interpretation of the observation results.

After two days, the trays containing *Aedes sp.* larvae were transferred to mosquito cages and maintained until they developed into adult mosquitoes. A total of 150 adult mosquitoes were used for the observations and were allocated into six tubes, consisting of four replicate tubes and two control tubes, with each tube containing 25 mosquitoes. Prior to treatment, all adult mosquitoes were maintained under sugar-fed conditions.

Table 1. Mosquito Mortality in the Control Tubes

Time	Control 1	Control 2	Mean	
			Number	(%)
10 minutes	0	1	0,5	2%
15 minutes	0	1	0,5	2%
20 minutes	1	1	1	4%
30 minutes	1	2	1	4%
40 minutes	1	2	1	4%
50 minutes	1	2	1	4%
60 minutes	1	2	1	4%
24 hours	1	2	1	4%

Because the percentage of mosquito mortality in the control group after 24 hours of observation ranged between 3% and 10%, the mortality percentage of the test mosquitoes was corrected using Abbott's formula ⁽¹⁴⁾.

The following are the observation data on the number of mosquitoes experiencing knockdown after exposure to malathion 0.8%, following correction using Abbott's formula.

Table 2. Mortality of *Aedes* sp. Mosquitoes After Exposure to Malathion 0.8%

Time	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	
					Number	(%)
10 minutes	5	6	4	5	5	18%
15 minutes	8	11	11	9	10	37%
20 minutes	10	21	16	11	15	56%
30 minutes	21	21	20	21	21	82%
40 minutes	24	22	23	22	23	91%
50 minutes	25	24	23	25	24	97%
60 minutes	25	25	25	25	25	100%
24 hours	25	25	25	25	25	100%

Lethal Time (LT) is defined as the duration required to kill a test population of organisms after exposure to a chemical agent. LT_{50} , LT_{90} , LT_{95} , and LT_{99} represent the time required to kill 50%, 90%, 95%, and 99% of the test population, respectively ⁽¹⁵⁾. These LT parameters are used to determine how rapidly a chemical agent exerts its effect on *Aedes* sp. mosquitoes.

Table 3. Estimated Lethal Time (LT) of *Aedes* sp. Mosquitoes Exposed to Malathion 0.8%

No.	Lethal Time	munite
1	LT_{50}	18,3
2	LT_{90}	39,3
3	LT_{95}	47,0
4	LT_{99}	56,8

Mosquito mortality in the control tubes ranged from 2% to 4%, indicating that the bioassay was valid and that correction using Abbott's formula was appropriate. Mortality of test mosquitoes exposed to malathion 0.8% reached 100% at 60 minutes and remained stable until the 24-hour observation period.

The LT estimates showed an LT_{50} of 18.3 minutes and an LT_{90} of 39.3 minutes, indicating that the *Aedes* sp. mosquito population responded rapidly to exposure to malathion 0.8%. The LT_{95} and LT_{99} values of 47.0 minutes and 56.8 minutes, respectively, demonstrate that nearly the entire *Aedes* sp. population was killed within less than one hour of exposure.

The results of this study indicate that the *Aedes* sp. population originating from Poltekkes Banjarmasin remains susceptible to malathion 0.8%. This finding is consistent with WHO criteria, which classify mosquito mortality of $\geq 98\%$ within 24 hours as susceptible ⁽¹⁴⁾. Several studies conducted in Asia have demonstrated that resistance in *Aedes* sp. to insecticides is heterogeneous across locations and over time. Reviews of the literature indicate considerable variation in the resistance status of *Aedes* sp. mosquitoes to organophosphate and pyrethroid insecticides across different countries ⁽¹⁶⁾. In some locations, *Aedes* sp. mosquitoes remain susceptible to certain organophosphates, whereas other areas show indications of resistance or tolerance ^(9-13, 17). Variations in resistance status among *Aedes* sp. populations across locations are often influenced by the history of insecticide use, intensity of previous insecticide exposure, changes in vector behavior, and local micro-environmental conditions ⁽¹⁸⁾. The findings of this study further support the notion that the resistance status of *Aedes* sp. mosquitoes is location-specific and strongly influenced by local insecticide use patterns.

From a biological perspective, the observed susceptibility suggests that detoxification mechanisms commonly associated with organophosphate resistance have not yet become dominant or have not reached a level sufficient to reduce post-exposure mortality. Nevertheless, the evolution of resistance is a gradual process. Early shifts are often first detected in kinetic parameters, such as increases in LT_{50} and LT_{90} , before manifesting as reduced mortality within 24 hours. In this context, Lethal Time metrics can serve as an early

warning system for the development of tolerance to specific insecticides, enabling vector control programs to adjust their strategies before a measurable decline in field efficacy occurs⁽¹⁹⁾.

This study has several strengths. First, the use of four replicates increases the reliability of mortality proportion estimates at each time point⁽²⁰⁾. Second, the low mortality observed in the control groups minimizes correction bias and supports the validity of the bioassay results⁽²¹⁾. Third, the inclusion of LT parameters provides insight into the speed of insecticidal action, which may assist policymakers in determining optimal exposure durations and evaluating the continued suitability of insecticide formulations used in effective *Aedes* sp. vector control programs⁽²²⁾.

However, this study also has several limitations. First, the malathion concentration of 0.8% represents a local operational setting that may not be identical to internationally recommended diagnostic doses⁽²³⁾. Second, mosquito eggs collected using ovitraps and subsequently reared under laboratory conditions may exhibit characteristics that differ from in situ wild populations exposed directly to diverse environmental pressures⁽²⁴⁾. Third, mosquito identification was conducted only to the genus level (*Aedes* sp.) without differentiation between *Aedes aegypti* and *Aedes albopictus*, despite the possibility that these two species may exhibit different susceptibility profiles to malathion 0.8%. Fourth, no confirmation of resistance mechanisms was performed through biochemical or molecular assays.

At the level of vector control programs, principles of mosquito resistance management must be integrated. Rotating insecticide classes based on local evidence remains a primary strategy to slow the accumulation of resistance alleles. The use of malathion should be complemented with non-chemical interventions, such as source reduction (3M Plus), habitat management, container cleaning, improved solid waste and drainage management, and behavioral education⁽²⁵⁾. The integration of these strategies aims to reduce single-agent selection pressure while enhancing the sustainability of control programs. Larval and adult mosquito surveillance can serve as early indicators of intervention effectiveness and help determine appropriate timing for insecticide rotation. Through a dynamic, locally informed, data-driven approach, vector control programs can maintain long-term efficacy and reduce the risk of intervention failure due to insecticide resistance.

CONCLUSIONS AND RECOMMENDATIONS

The results of this study indicate that the *Aedes* sp. mosquito population at the Poltekkes Kemenkes Banjarmasin campus in Banjarbaru City remains susceptible to malathion 0.8%. Corrected mosquito mortality after 24 hours reached 100%, with relatively rapid Lethal Time values ($LT_{50} \approx 18.3$ minutes; $LT_{90} \approx 39.3$ minutes; $LT_{95} \approx 47.0$ minutes; $LT_{99} \approx 56.8$ minutes). These findings indicate that malathion 0.8% remains suitable for use as one of the dengue vector control strategies in the Poltekkes Kemenkes Banjarmasin campus environment.

To support the sustainability of dengue vector control at the local level, routine insecticide resistance surveillance should be conducted periodically, at least once per year. Future research should incorporate spatial approaches using GIS-based mapping to guide prioritization of intervention locations and timing. In addition, further testing using insecticides from different chemical classes, as well as the inclusion of biochemical and molecular assays, is recommended to enable early detection of emerging resistance.

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