International Journal of Advances in Applied Sciences (IJAAS)
Vol. 14, No. 2, June 2025, pp. 291~299
ISSN: 2252-8814, DOI: 10.11591/ijaas.v14.i2.pp291-299

Susceptibility of Aedes aegypti to malathion and permethrin insecticides in Enrekang Regency: an experimental study

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Article Info

Article history:

Received Aug 1, 2024
Revised Feb 10, 2025
Accepted Mar 28, 2025

Keywords:

Aedes aegypti
Arbovirus
Insecticide resistance
Malathion
Permethrin

ABSTRACT

Insecticide resistance in Aedes mosquitoes can undermine arbovirus control efforts. Malathion and permethrin insecticides belong to the group of insecticides used for control and if used continuously will cause immunity of target mosquitoes. This study aims to assess the level of susceptibility of Aedes aegypti to insecticides commonly used in public health in the Enrekang Regency. The type of research used was experimental research. Female Aedes aegypti were collected from rearing results with a total sample size of 240 mosquitoes which were divided into 120 mosquitoes each in 4 treatments and 2 controls on malathion 0.8% and permethrin 0.25% insecticides. The results obtained from the research on insecticide susceptibility test results using malathion 0.8% in 60 minutes of exposure averaged 55% dead and exposure for 24 hours averaged 90% mosquito death, while permethrin 0.25% insecticide in 60 minutes of exposure averaged 90% dead mosquitoes and 24 hours exposure averaged 100% mosquito death, while for the control all live. The conclusion of the study was the susceptibility test of Aedes aegypti mosquitoes to malathion 0.8% insecticide in the category of moderate resistance while permethrin 0.25% insecticide in the category of susceptible.

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1. INTRODUCTION

The control of Aedes aegypti, the primary vector for dengue, zika, chikungunya, and yellow fever viruses, relies heavily on the use of chemical insecticides. Over the past few decades, the extensive and often indiscriminate use of these insecticides has led to the development of resistance in Aedes aegypti populations worldwide. Insecticide resistance poses a significant challenge to vector control programs and necessitates an understanding of the mechanisms, distribution, and management strategies for resistance. The emergence of insecticide resistance in Aedes aegypti is a result of genetic mutations that confer survival advantages to mosquitoes exposed to these chemicals [1]. In addition, an increase in mortality and morbidity caused by dengue virus infection has been noted over the past decade in contrast to the decline in other major infectious diseases [2].

Each arbovirus is transmitted by several mosquito species that differ in their vector capacity and geographical distribution. Aedes aegypti and Aedes albopictus mosquitoes are the main vectors for chikungunya, dengue, yellow fever, and zika which are prevalent in Southeast Asia and make these species the main vectors for the spread of infectious diseases [3]–[5]. Dengue fever is a disease caused by dengue virus (DENV) infection, transmitted by the Aedes mosquito. About 50% of the world's population is at risk of contracting dengue, making it one of the most important arbovirus diseases in the world [6], [7]. In the past five years, dengue hemorrhagic fever (DHF) cases in Indonesia have shown a significant increase. In 2023, Indonesia reported around 62,000 dengue cases with 475 deaths just from January to mid-April, almost tripling compared to the same period the previous year. In January 2023 alone, there were 25,218 cases, which was the highest monthly number in the last five years [8].

Reported levels of insecticide resistance in Aedes aegypti differ under varying geographic conditions and pelapo. The geographic distribution of insecticide resistance in Aedes aegypti varies. Factors leading to these differences such as insecticide use patterns, local ecology, and mosquito population genetics, thus monitoring and mapping resistance status is essential for implementing effective vector control strategies [9]. The development of resistance to commonly used insecticides has become a major problem in controlling mosquitoes as disease vectors [10], [11]. Control of Aedes vectors with insecticides using permethrin and malathion is a major concern with rapid insecticide resistance potentially reducing the effectiveness of insecticide-based control strategies [12].

Organophosphates (malathion and temephos) have been widely used in Indonesia to suppress mosquito vectors. Subsequently, pyrethroids (permethrin) came into use in the 1980s came into use in the 1980s and are used in the agricultural sector and the national mosquito control program in Indonesia [13], [14]. The extensive use of pyrethroids in recent decades has led to pyrethroid resistance in Aedes aegypti, which has posed a challenge to dengue vector control programs and affected the effectiveness of insecticide-based vector control strategies [15]. Monitoring resistance to commonly used insecticides is essential in the management of disease-monitoring resistance to commonly used insecticides is essential in the management of disease transmitting vectors and the spread of infectious diseases. However, the development of resistance to commonly used insecticides in mosquitoes has created serious obstacles in the control of Aedes aegypti under the threshold capable of transmitting DENV. Although several studies have been conducted domestically on the resistance of Aedes aegypti to insecticides, comprehensive research on the related mechanisms leading to resistance is lacking. This study evaluated the resistance status of commonly used insecticides in the Enrekang Regency. Therefore, this study was designed to determine the resistance status of Aedes aegypti populations to commonly used insecticides such as permethrin and malathion at various concentrations.

2. RESEARCH METHOD

The type of research used is experimental research with a descriptive approach so that the status of resistance of Aedes aegypti mosquitoes in Enrekang Regency to insecticides can be known. The samples of this study were female Aedes aegypti mosquitoes obtained from the rearing of mosquito larvae found in Enrekang Regency. The number of samples to be tested is 300 with three treatments, in one treatment of 20 mosquitoes. The susceptibility of adult Aedes aegypti mosquitoes to permethrin and malathion were evaluated using World Health Organization (WHO) standard mosquito bioassay protocols with WHO standard tubes and impregnated insecticides containing diagnostic doses. The reared mosquitoes were then analyzed in the laboratory of the Poltekkes Kemenkes Makassar to identify the type of mosquito and only Aedes aegypti which is the main vector of DHF in Enrekang Regency was taken.

The test mosquitoes were exposed for 1 hour to impregnated paper filters containing WHO-prescribed diagnostic doses (permethrin 0.25% and malathion 0.8%). Control experiments consisted of filter paper impregnated with a specific insecticide solvent. After exposure for 1 hour, mosquitoes were transferred to collection tubes and mosquito mortality was recorded 24 hours after exposure. The percentage mortality categories of the treatments used WHO guidelines: 98-100% mortality indicates susceptibility; 90-97% mortality indicates moderate likelihood of resistance; and less than 90% mortality indicates high resistance. Figure 1 shows the research flowchart.

Determination of LC50 susceptibility of Aedes aegypti mosquitoes to permethrin insecticide was calculated using the bottle bioassay method. A 1 milliliter solution of each diagnostic dose was added to a 250 ml wheaton bottle, spread evenly on the surface of the bottle, and allowed to dry overnight. Newly emerged, 3-4 days old, non-blood-fed female mosquitoes were introduced in groups of 20 into bottles coated with each dose of permethrin. The experiment was replicated four times. Mosquito mortality was recorded every 10 minutes up to 1 hour, Final mortality was recorded after 24 hours of exposure. Data obtained from observations in the field and the results of laboratory examinations in this study will be presented as follows laboratory examinations in this study will be presented in tables, and figures and analyzed descriptively.

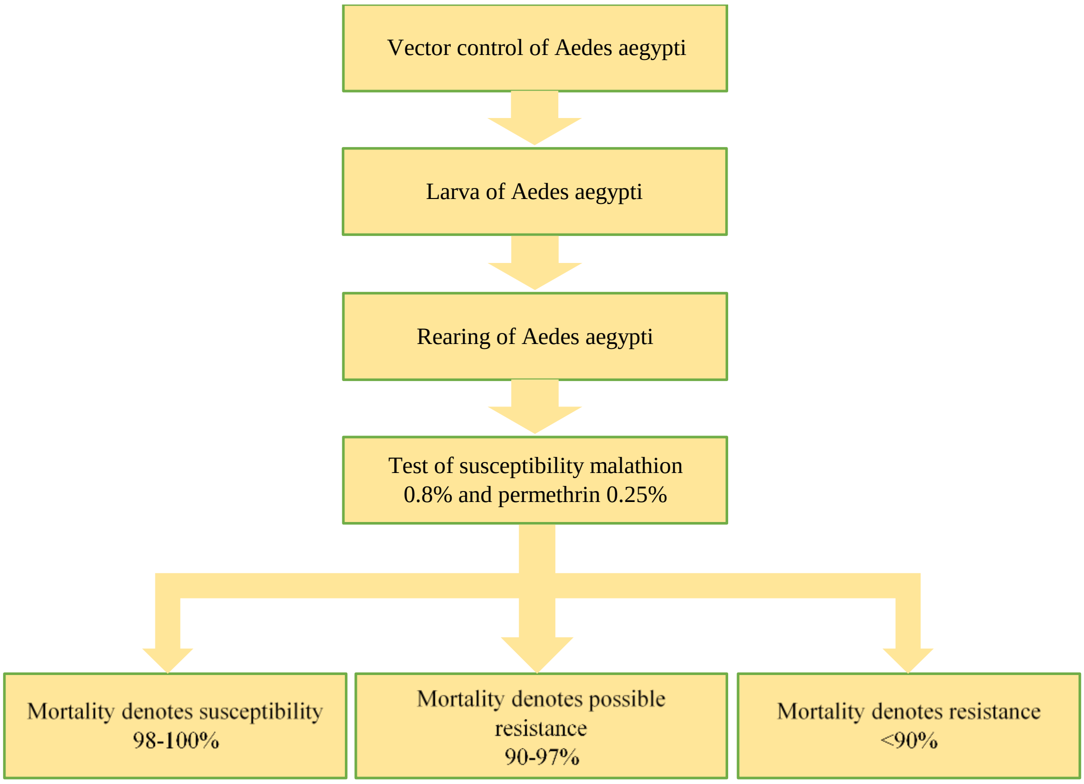

Figure 1. Research flowchart

3. RESULTS AND DISCUSSION

Figure 2 shows the mortality graph of the test mosquitoes using malathion 25% insecticide with 4 repetitions and 2 control groups. The second repetition showed the highest mosquito mortality of 60% and the third repetition showed the lowest mortality with 50% for an observation time of 1 hour. For the observation time of 24 hours, it can be seen that the fourth repetition of exposure to 25% malathion insecticide was able to kill mosquitoes by 95% and the first repetition was able to kill mosquitoes by 50%. was only able to kill 85% of mosquitoes. For the control group, no mosquitoes were killed with observation times of 1 and 24 hours.

Figure 3 shows the mortality graph of the test mosquitoes using 0.25% permethrin insecticide with 4 repetitions and 2 control groups. The first repetition showed the highest mosquito mortality of 95% and the third repetition showed the lowest mortality with 8,550% for an observation time of 1 hour. For the observation time of 24-hours, it can be seen that the fourth repetition of malathion 0.25% insecticide exposure was able to kill mosquitoes by 100% and the first repetition was only able to kill mosquitoes by 85%. For the control group, no mosquitoes were killed with observation times of 1 and 24 hours.

Table 1 shows the results of the Aedes aegypti mosquito susceptibility test for 24-hour exposure to an insecticide with malathion 0.8% at a temperature of 31 °C and humidity of 64%. In the first replicate, 17 Aedes aegypti mosquitoes died (85%). In the second replication, 18 mosquitoes died (90%). In the third replication, 18 mosquitoes died (90%) and in the fourth replication, 19 mosquitoes died (95%) so the average percentage of mosquito mortality for the use of malathion 0.8% insecticide was 90%. For control mosquitoes, mortality was not calculated using the Abbot formula, where if the control mortality rate is under 10%, it can be ignored and no correction is needed (Minister of Health Regulation No.50 of 2017).

Table 2 shows the results of the resistance test of Aedes aegipty mosquitoes for 24-hour exposure to 0.25% permethrin insecticide with a temperature of 31 °C and humidity of 74%. In the first treatment, 20 Aedes aegipty mosquitoes died with a percentage of 100%. In the second replication, 20 mosquitoes died (100%). In the third replication, 20 mosquitoes died (100%) and in the fourth replication, 20 mosquitoes died (100%) so the average percentage of mosquito mortality for the use of 0.25% permethrin insecticide was 100%. For control mosquitoes, mortality was not calculated using the Abbot formula, where if the control mortality rate is under 10%, it can be ignored and no correction is needed.

In Table 3, the average results obtained from the susceptibility test in the Enrekang Regency area using impregnated paper permethrin 0.25% were obtained at 100% with a susceptible category where the percentage of mosquito deaths ≥98% with a temperature of 31 °C and humidity of 74%. Malathion 0.8% insecticide obtained an average mortality of 90% with a moderate resistant category at a temperature of 31 °C and humidity of 74%. These resistance status classifications follow the standards set by the Minister of Health Regulation No.50 of 2017.

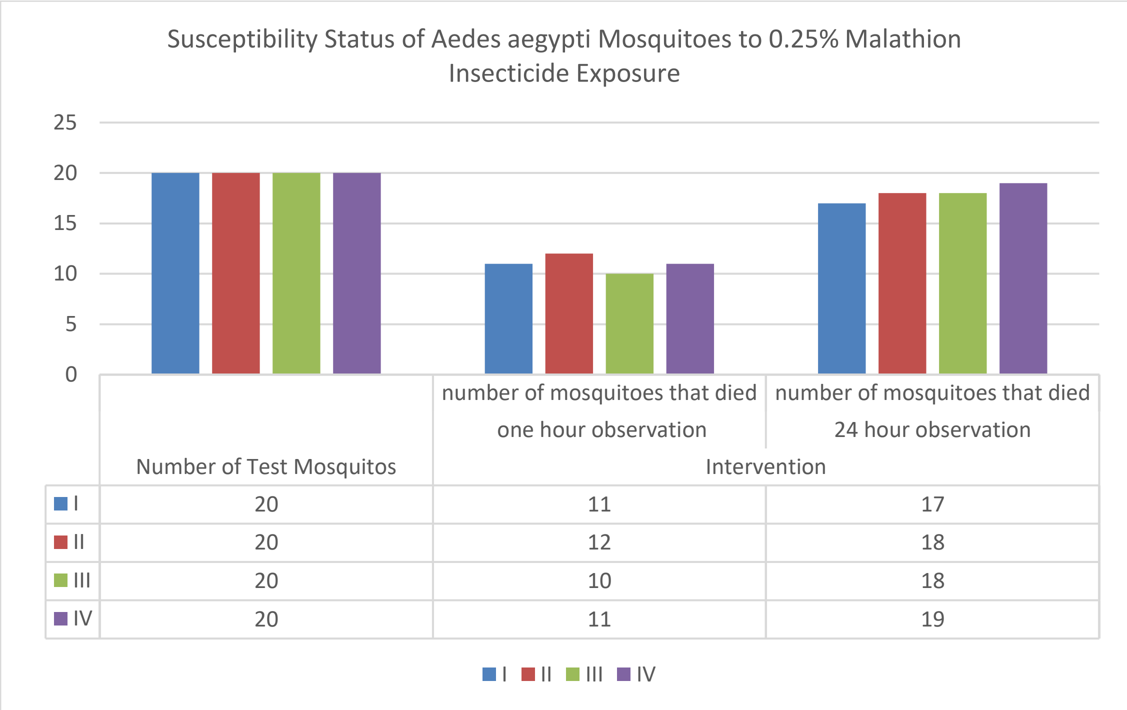

Figure 2. Susceptibility status of Aedes aegypti mosquitoes to 0.25% malathion insecticide exposure

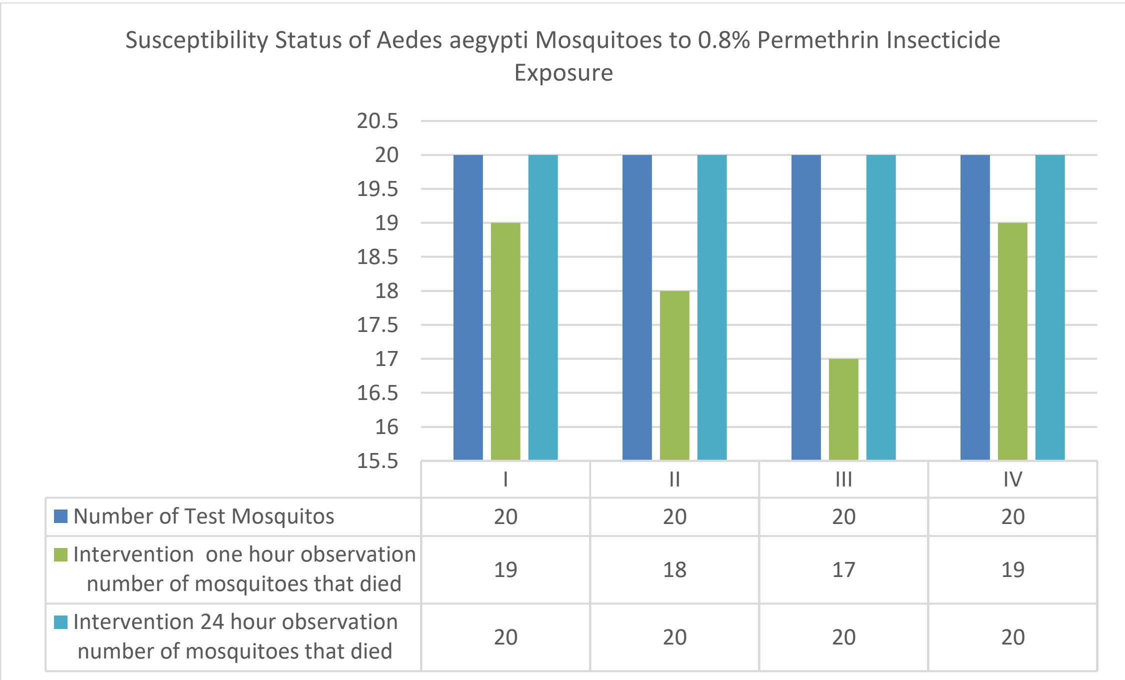

Figure 3. Susceptibility status of Aedes aegypti mosquitoes to 0.25% permethrin insecticide exposure

Table 1. Susceptibility status of Aedes aegypti mosquitoes to 0.25% malathion insecticide exposure

Replication	Number of tests mosquitoes	Intervention				Number of tests mosquitoes	Control			
		Observation 1 hour		Observation 24 hour			Observation 1 hour		Observation 24 hour	
		Number of mosquitos that died	% Died	Number of mosquitos that died	% Died		Number of mosquitos that died	% Died	Number of mosquitos that died	% Died
I	20	11	55	17	85	20	0	0	0	0
II	20	12	60	18	80	20	0	0	0	0
III	20	10	50	18	90					
IV	20	11	55	19	85					
Mean		11	55	18	90		0	0	0	0
Temperature		31 °C		31 °C			31 °C		31 °C	
Humidity		74%		74%			74%		74%	

Table 2. Susceptibility status of Aedes aegypti mosquitoes to 0.25% permethrin insecticide exposure

Replication	Number of test mosquitoes	Intervention				Number of test mosquitoes	Control			
		Observation 1 hour		Observation 24 hour			Observation 1 hour		Observation 24 hour	
		Number of mosquitos that died	% Died	Number of mosquitos that died	% Died		Number of mosquitos that died	% Died	Number of mosquitos that died	% Died
I	20	19	5	20	0	20	0		0	
II	20	18	0	20	0	20	0		0	
III	20	17	5	20	0					
IV	20	18	0	20\	0					
	Mean	18	0	18	0	100	0		0	
	Temperature	31 °C		31 °C			31 °C		31 °C	
	Humidity	74%		74%			74%		74%	

Table 3. Category of resistance status of Aedes aegypti mosquitoes in the Enrekang Regency area

Insecticide (%)	Average Mortality (%)	Category	Standard
Malathion 0.8	90	Moderate resistant	Minister of Health Regulation No.50 of 2017
Permethrin 0.25	100	Vulnerable	

Resistance testing is a test to detect the presence of vector resistance to the insecticide used. Environmental risk control efforts are one of the efforts to prevent the spread of potentially epidemic diseases by breaking the vector breeding chain, especially in Enrekang Regency. The results of research in the Enrekang Regency of Aedes aegypti mosquitoes in the 0.25% permethrin insecticide test are still vulnerable, where the average mortality rate is 100% and is categorized as vulnerable.

In the resistance test, the use of malathion 0.8% insecticide is moderately resistant where an average percentage of 90% is obtained and this shows a moderate resistant category. In this study, measurements were taken on environmental conditions which in this study, measurements were made on environmental conditions including room temperature, and room humidity at the time the mosquitoes were initially exposed to 0.25% permethrin and 0.8% malathion insecticides. At the end of observation in the holding tube for 24-hours, a temperature of 31 °C and humidity of 74% were obtained. This is intended to determine changes-striking changes in environmental variables.

WHO standards indicate that the temperature of the resistant testing media ranges from 20-30 °C and the optimum humidity for resistant testing ranges from 70-90%. Room temperature and humidity did not affect the mortality of Aedes aegypti mosquitoes exposed for 1-hour to malathion in susceptibility test tubes and those held for 24-hours. For control mosquitoes that died, no correction mortality was calculated using Abbot's formula, where if the control mortality rate is under 10%, it can be ignored and no correction is needed. If the control mortality rate is greater than 10%, the observed mortality must be corrected using Abbot's formula.

Observations made with permethrin 0.25% insecticide contact for 1-hour in resistance testing of Aedes aegypti mosquitoes in Enrekang Regency obtained an average of 18 mosquitoes (90%) that died from the first replication to the fourth replication for 60 minutes. Meanwhile, in malathion 0.8% there were 11 (55%) total Aedes aegypti mosquitoes that died. Measurement of room temperature, and room humidity, in the implementation of the study showed results that still support the life of Aedes aegypti mosquitoes. In the 24-hour test using malathion, 0.8% insecticide on Aedes aegypti mosquitoes in Enrekang Regency, an average of 18 mosquito deaths was obtained with a percentage of 90% from the first to the fourth replication. In the 24-hour test using permethrin insecticide on Aedes aegypti mosquitoes obtained an average of

20 deaths with a percentage of 100% this is categorized as a vulnerable status. The moderate resistance status of malathion 0.8% insecticide against Aedes aegypti mosquitoes is one of the problems of changing the immune level in Aedes aegypti mosquitoes in Enrekang Regency. Moderate resistant status is categorized by mosquito mortality of 90-<98%. (Minister of Health Regulation No.50 of 2017). Using a malathion dose of 0.8% makes mosquitoes in the area tolerant or said to be moderately resistant to insecticides due to the use of insecticides for a long time so that the mosquito Aedes aegypti mosquitoes can form immune and immune to malathion insecticide 0.8%. These results are in accordance with research by Hamdan *et al* and Rodrigues *et al*, which states that Aedes aegypti after exposure to malathion, permethrin, and temephos insecticides for 32 generations has occurred resistance to permethrin [16], [17].

The vulnerable status that occurs in 0.25% permethrin against Aedes aegypti mosquitoes in the Enrekang Regency area is still vulnerable. This proves that there was no previous use of permethrin insecticide in the area. Therefore, Aedes aegypti mosquitoes in Enrekang Regency are not accustomed to this insecticide. On the other hand, this insecticide attacks the mosquito's nervous system, making it easy to kill. The existence of moderate resistant status to 5% malathion exposure in Makassar City is due to the continuous use of insecticides each year for dengue fever vector control without insecticide rotation. This is not in accordance with the guidelines for the use of insecticides that changes in the type and mode of action of insecticides for vector control should be carried out at a maximum time period of 2-3 years or 4-6 applications [17]-[19].

Two main mechanisms of pyrethroid resistance have been identified in Aedes mosquitoes: increased detoxification via cytochrome P450 monooxygenase and mutations in voltage-sensitive sodium channel (Vssc) (i.e., knockdown resistance (KDR)). These mechanisms of resistance are described as follows. Both mechanisms may occur, but the relative importance of each mechanism in different populations. The use of insecticides in some areas is locally specific so the results can vary by location. Differences in the results of the mosquito vector susceptibility test between districts can also be caused by the species, vector behavior, and the duration and frequency of insecticide use in each region so the frequency of contact is also different [20]–[23].

Differences in insecticide sensitivity can be influenced by the frequency of insecticide use. If the same insecticide is applied, there are individuals that are able to live and form resistant strains. The solution to this problem is to increase the dose or amount of spraying or replace it with other more toxic chemicals. The use of insecticides continuously and over a long period of time results in fewer and fewer susceptible insects, so the remaining insects are immune insects. In an insect population, there are insect members that are immune or resistant to insecticides. This resistance will be passed on to their offspring, resulting in resistance throughout the population [24]–[26].

This study has several limitations that should be considered. First, the resistance testing of Aedes aegypti to malathion and permethrin was conducted under controlled laboratory conditions, which may not fully reflect the resistance levels in more complex natural environments. Second, this study focused solely on two insecticides, thus not providing a comprehensive overview of mosquito resistance to other commonly used insecticides. Additionally, genetic and environmental factors that may influence mosquito resistance were not analyzed in depth, highlighting the need for further research using molecular approaches to identify the underlying resistance mechanisms. Lastly, the study's geographical scope was limited to Enrekang Regency, meaning the results may not be generalizable to other regions with different ecological conditions.

Given these limitations, future research should include field-based studies to validate laboratory findings and assess real-world resistance patterns. Expanding the scope to other insecticides will provide a more comprehensive understanding of Aedes aegypti resistance profiles, aiding in the development of more effective vector control strategies. Furthermore, molecular and genetic studies are necessary to identify specific resistance genes and their evolutionary dynamics, which could help predict resistance trends and improve management strategies. Lastly, incorporating environmental and climatic factors into future studies will offer deeper insights into the ecological drivers of resistance, enabling the development of region-specific mosquito control programs.

4. CONCLUSION

This study indicated resistance to malathion and permethrin in Aedes aegypti populations in Enrekang Regency. This finding was confirmed by the results of the resistance to mortality test of Aedes aegypti. The test results also showed that the use of permethrin as an adult insecticide was effective in controlling vector populations. Importantly, this study demonstrates the need for continuous monitoring of resistance to commonly used insecticides and their underlying mechanisms.

ACKNOWLEDGEMENTS

The authors would like to thank Poltekkes Kemenkes Makassar for the research grant that has supported our research.

FUNDING INFORMATION

This research was funded by the Poltekkes Kemenkes Makassar through the University Flagship Applied Research Grant Scheme.

AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

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C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

INFORMED CONSENT

This study has obtained permission from the Enrekang Regency Government. All informed consents are based on guidelines from the Ministry of Health on mosquito-based research.

ETHICAL APPROVAL

This study has complied with the research code of ethics, ensured that all procedures were carried out in accordance with established ethical standards and received approval from the relevant ethics committee with number 401/KEPK-PTKMKS/V/2019.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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