

Analysis Resistance of Malathion and Cypermethrin Insecticide on *Aedes aegypti* (Linnaeus, 1762) from Kaliwungu Kudus and Kotagede Yogyakarta Using CDC Bottle Bioassay

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Abstract. *Aedes aegypti* is a primary vector of Dengue infection and is frequently found near to human dwellings. Indonesia is a tropical country with environmentally suitable for mosquito breeding. Insecticides are commonly used to control mosquito population, however long and continuous use it will create resistance. This study aimed to determine the resistance of *Aedes aegypti* populations from Kaliwungu District, Kudus Regency and Kotagede District, Yogyakarta City to malathion and cypermethrin using CDC Bottle Bioassay. Ovitrap were placed in 50 houses of each location, and the eggs were brought to the laboratory for rearing until adulthood. Fifteen of female mosquitoes were used as test for each concentration of malathion and cypermethrin. Results showed that *Ae. aegypti* mosquitoes from Kaliwungu and Kotagede were resistant to malathion at the diagnostic dose of 1x. However, at the 2x diagnostic test, mosquito from Kaliwungu showed tolerant, while mosquito from Kotagede was susceptible to malathion. The cypermethrin test showed that both mosquito samples were resistant and susceptible at the diagnostic dose of 2x and 5x respectively.

1 Introduction

Dengue Hemorrhagic Fever (DHF) is a viral infectious disease that remains a major health problem in various countries, including Indonesia. It is transmitted through the bite of *Aedes aegypti* mosquito [1], which is not only the main vector of DBD but also Zika and other arboviral diseases [2,3,4].

Dengue fever is the most common mosquito-borne disease in the world, with an estimated 3.9 million people at risk of infection and about 390 million cases are reported

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each year [5]. The virus that causes dengue infection belongs to the genus *Flavivirus* and family *Flaviviridae*, and it has four serotypes: DEN-1, DEN-2, DEN-3, and DEN-4 [6].

Aedes aegypti mosquito can rapidly grow in hot, humid, and high rainfall environments [7]. Indonesia is a tropical country with environmentally suitable for mosquito breeding, and is highly vulnerable to the spread of Mosquito-Borne Diseases [8]. To control the excessive growth of mosquito populations, various preventive measures were taken, one of which was by fogging or using insecticides. However, continuous use of insecticides can cause resistance in mosquitoes, which can make them tolerant to certain types of insecticides [3].

Therefore, this study aimed to analyze the resistance of *Ae. aegypti* mosquitoes from Kaliwungu District, Kudus Regency and Kotagede District, Yogyakarta City to malathion and cypermethrin insecticide by using CDC bottle bioassay.

2 Materials and Methods

2.1 Time and place of research

The research was done from February – July 2023 and the mosquito eggs collection was carried out at area in the Kaliwungu District, Kudus Regency and Kotagede District Yogyakarta City.

2.2 Ethical Clearance and funding

The research has been approved by Ethics Commission from Faculty of Medicine, Public Health and Nursing UGM, Ref. No.: KE/FK/0260/EC/2022, and funding by Final assignment Recognition Project UGM, No.: 3550/UN1.P.III/Dit.lit/PT.01.05/2022.

2.3 Materials

The materials used was *Ae. aegypti* mosquito egg's, malathion, cypermethrin insecticide, and acetone as an insecticide solvent. The mosquitoes used were F1, which was the result of reared the eggs.

The tools used were an aspirator for picking up mosquitoes, Wheaton Bottles with caps, micropipette and pipette tips, cages for *Ae. aegypti* breeding, timers, permanent markers for marking bottles, Pasteur pipettes, black plastic pots as containers for ovitraps, gloves, and data tables to record the results.

2.4 Eggs collection

Three ovitraps for each house were installed in every 50 houses in Kaliwungu and Kotagede District. Ovitrap was made using cans painted with black color and then filled with water and placed filter paper inside for mosquitoes to lay their eggs. The installation of the ovitrap will not be exposed to direct sunlight and slightly damp, and checked every two days after installed. The filter papers were then dried, and transferred to the laboratory for reared. The filter paper that contains of *Ae. aegypti* mosquito eggs were then placed on trays that depend on the location of mosquito collection, and the eggs were hatched and grown until adults. Some of adult mosquitoes were transferred into a plastic container with a lid and then euthanized and identified.

2.5 Rearing and identification of mosquito

The rearing and bioassay for resistance status of *Ae. aegypti* was done in the Entomology Division at the Laboratory of Vector and Disease-carrying Animals, Center for Environmental Health and Disease Control Techniques, Yogyakarta. During rearing, the larvae and adult had been identified, then the adult mosquitoes were grouped based on the species and place of origin eggs collected. As many as 15 adult female mosquitoes were put into each paper cup, and each resistance test required 5 paper cups for both malathion and cypermethrin insecticides.

The identification of larvae is based on the comb teeth on the last segment of the abdomen and for the adult *Ae. aegypti*, firstly by observing the black and white color patterns on the thoracic abdomen and legs, as well as the distinctive pattern on the thoracic mesonotum. Adult mosquitoes were then collected and put in plastic containers with lids, after which they were euthanized. It is important to identify mosquitoes before conducted the bioassay because this is to ensure the *Ae. aegypti* from other genera and guarantee the accuracy of the resistance test results [9].

2.6 CDC Bottle Bioassay

In conducting the CDC bottle bioassay, the following procedures were followed. Firstly, there were 4 bottles for malathion test, 4 bottles for cypermethrin test, and 4 bottles as control was prepared. One hundred and forty adult female *Ae. aegypti* mosquitoes, aged 3-5 days were prepared and fed with sugar, then 15 mosquitoes were aspirated and put into each bottle of treatment and control by using an aspirator.

There were 12 bottles for malathion test (4 control, 4 diagnostic 1x; 4 diagnostic 2x) and 16 bottles for cypermethrin test (4 control, 4 diagnostic 1x; 4 for diagnostic 2x, and 4 bottles for diagnostic 5x). Thirdly, the timing started, and the bottles were observed at time from zero to count the number of live and dead mosquitoes. The observation of test was on the period time firstly in 0, 15, and 30 minutes. The period of 30 minutes is the diagnostic time for mosquito to get the first exposure from the insecticides.

The observation time could be started for each bottle, which the time was started when the first or last mosquitoes was transferred to the bottle with aspirator. A note was made on the observation sheet if any dead mosquitoes were found at time 0, then the observation was continued for the dead and live mosquitoes every 15 minutes until all the mosquitoes were died or for a maximum time of 2 hours. According to the procedure, the test time limit is 2 hours. Live mosquitoes are transferred to a paper cup, and can be used for molecular testing. Lastly, the observation results were recorded in the report. These procedures were followed to conduct the bioassay experiment in a standardized manner [9].

2.7 Interpretation and Analysis of Results

During the bioassay, all of the control and treatment mosquitoes were observed, and the dead mosquitoes were considered following the mortality criteria, that was mosquitoes cannot stand and weak. By gently the bottle while doing the observation and calculation will made easier the bioassay process. The percentage of dead mosquitoes at the diagnostic time data was presented, and it will be analyzed descriptively. CDC [2] stated that the dead mosquitoes per total mosquitoes in the bioassay test was an important value in the determining the level of resistance to an insecticide. The mortality of mosquitoes was calculated by using Equation (1):

$$\text{Mortality} = \frac{\text{Number of dead mosquitoes}}{\text{Total mosquitoes in bioassay}} \times 100\% \quad (1)$$

The corrected Abbot's formula was used if the mosquito mortality on the control same or more than 20% of the total mosquitos. Based Suwito *et al.* and Zufar [9,10] recommends that the interpretation of the mortality results as follows:

1. If the mortality was in the range of 98%–100% at the recommended diagnostic time, it indicates susceptibility
2. If the mortality was in the range of 80%–97% at the recommended diagnostic time, it suggests the possibility of resistance that needs to be confirmed,
3. If the mortality is less than 80% at the recommended diagnostic time, it suggests resistance.

3 Results and Discussion

3.1 Identification of larvae and adult mosquitoes

Rearing was carried out before the bioassay, in which to prepare enough the adult mosquitoes during the test. This process also could validate the species before the test was carried out, and to ensure the valid results. Figure 1 showed the appearance of the same stadium of *Ae. aegypti* larva from Kotagede and Kaliwungu which was differ in size, but it showed the same characters and morphology as *Ae. aegypti* larva.

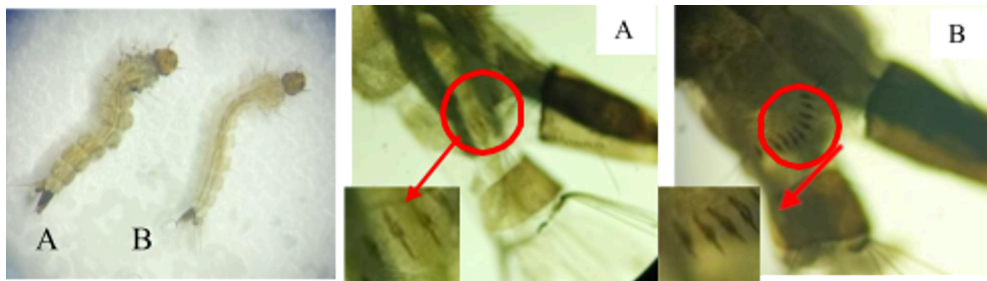


Fig. 1. Comparison of *Ae. aegypti* larvae from Kotagede (A) and Kaliwungu (B), and the presence of an anal comb in the VIII abdominal segment, without thickening, and the shape of the comb is branched and trident-like (RTA team work).

The larvae and adult mosquito observation were carried out according to the guide book for the Dengue Haemorrhagic Fever Entomology Survey and the Key to *Aedes* Mosquito Identification [9]. It was determined that there were several characters should be observed to ensure the larvae were *Ae. aegypti*. In Figure 1 showed features that were easy to identify *Ae. aegypti* larva, namely the anal comb or comb teeth, which were the teeth arranged in rows on the VIII of abdomen segment of the larvae.

Anal comb in *Ae. aegypti* has 5 to 19 teeth that were arranged in a row or sometimes two rows of irregular ones. Then the shape of the comb teeth was in the form of an indentation that clearly looks like a trident with small spines that follow. Observations could be seen clearly and easily identified using a stereo microscope or light microscope. Figure 1 also showed the suitability of the characteristics with the number of combs in the Kaliwungu specimen as many as 6 teeth and in the Kotagede specimen as many as 10 teeth. There is also an inset showing the suitability of the shape of the serrations according to Suwito *et al.* [9].

Adult mosquitoes were also observed to ensure and validate of adult *Ae. aegypti* for the bioassay. Figure 2 showed an adult mosquito specimen that could be identified based on the character by using the guide book from the Indonesian Ministry of Health [9,10]. The main character of adult *Ae. aegypti*, that it was a lyre-like pattern on the dorsal of thorax with two curved and two white straight lines, also on the mesepimeron with two separate white patches at the anterior of the legs, and on the femur of midleg there is a long white stripe.

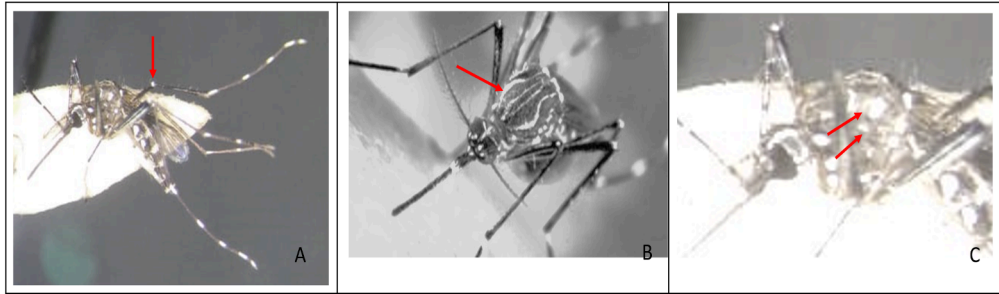


Fig. 2. Character of adult *Aedes aegypti* mosquitoes (RTA teamwork). **A.** white band on the femur of 2nd leg, **B.** lyre on dorsal thorax [11], **C.** White spot on mesepimeron

3.2 Bioassay

Table 1. Mortality of *Ae. aegypti* Mosquito from Kaliwungu, Kudus to Malathion

Time (Minute)	Mortality of <i>Ae. aegypti</i> (1x Dose)				Mortality of <i>Ae. aegypti</i> (2x Dose)			
	TreatmentGroup		Control Group		TreatmentGroup		Control Group	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
0	0	0%	0	0%	0	0%	0	0%
15	0	0%	0	0%	7	10,94%	0	0%
30	0	0%	0	0%	52	86,67%	0	0%
35	0	0%	0	0%	60	100%	0	0%
40	0	0%	0	0%				
45	0	0%	0	0%				
60	1	1,67%	0	0%				
75	2	3,28%	0	0%				
90	3	4,84%	0	0%				
105	7	10,77%	0	0%				
120	12	17,91%	0	0%				
Total Number of Mosquitoes	60		15		60		15	

Bioassay was proposed to determine the resistance status of *Ae. aegypti* against malathion [1 1] and cypermethrin, that was carried out on mosquitoes originating from Kudus and Kotagede based on the diagnostic dose. Mosquito mortality was calculated on control and treatment test bottles of malathion and cypermethrin at the diagnostic time, which was at 30 minutes after insecticides exposure (Table 1).

The resistance status of *Ae. aegypti* originating from Kaliwungu, Kudus used a total of 150 mosquitoes, where 60 mosquitoes were used in the test with a 1x malathion diagnostic

dose and 60 were used in the 2x malathion diagnostic dose, the rest 30 mosquitoes were used as tcontrol, with 15 mosquitoes of each group test. In the resistance test of the two diagnostic doses, observation was started at 0 minutes showed that there were no mosquitoes died in control and treatment bottles. It indicated that the mosquito mortality was 0%. The observation was then followed at the 15 and 30 minutes after exposure. The 30th minutes of test is the diagnostic time for all insecticide test, at this time the effect of 1x diagnostic dose to mosquito mortality was observed.

Furthermore, the mosquito resistance status test by using the 2x diagnostic dose was done and the percentage of mosquito mortality was recorded, and it showed that on the treatment bottle was 86.67%, while in the control bottle was 0%. The continued observation was done on the 35th minutes to 2 hours after exposure.

It was showed that the mortality at the 2x diagnostic dose had reached 100% or all the mosquito of the treatment bottles was died. In contrast the mortality in all treatment bottles by using 1x diagnostic dose was still 0%. After 2 hours of observation, the mosquito mortality in the treatment by using 1x diagnostic dose was 17.91%. There was no mosquito mortality in the control bottles, this indicated that the calculation of mosquito mortality in the test bottles did not require correction using the Abbott formula.

Table 1 shows that the *Ae. aegypti* mosquito from Kaliwungu was resistant to 1x dose of Malathion, which was indicated by a mosquito mortality rate of 0% at 30 minutes of observation. Mosquitoes showed tolerance leading to resistance to 2x dose of malathion with mortality 86.67% in the 30 minutes.

Table 2. Mortality of *Ae. aegypti* Mosquito from Kotagede, Yogyakarta to Malathion

Time (Minute)	Mortality of <i>Ae. aegypti</i> (1x Dose)				Mortality of <i>Ae. aegypti</i> (2x Dose)			
	Treatment Group		Control Group		Treatment Group		Control Group	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
0	0	0%	0	0%	0	0%	0	0%
15	0	0%	0	0%	50	83%	0	0%
30	0	0%	0	0%	60	100%	0	0%
35	0	0%	0	0%				
40	1	1%	0	0%				
45	1	1%	0	0%				
60	1	1%	0	0%				
75	2	2%	0	0%				
90	4	5%	0	0%				
105	5	6%	0	0%				
120	7	9%	0	0%				
Total Number of Mosquitoes	60		15		60		15	

In Table 2 showed that *Ae. aegypti* mosquito from Kotagede was resistant to malathion at the 1x dose, which was indicated by a mosquito mortality rate of 0% at the 30th minute of observation. Mosquitoes showed susceptibility to malathion at 2x dose with a mortality rate of 100% at 30 minutes (at diagnostic time). In this test, the control mosquitoes did not show any mortality, so no analysis and correction with Abbott's formula was needed.

Based on the results of the bioassay, it can be said *Ae. aegypti* mosquitoes in both regions (Kaliwungu Kudus and Kotagede Yogyakarta) showed resistance to 1x dose of

Malathion (Table 3). Mosquitoes from Kaliwungu were still tolerant to the 2x dose (87% mortality), while mosquitoes from Kotagede showed susceptibility at the same dose (Table 3). These results of the research in contrast showed differences with previous research by Triana *et al.* [12] which conducted in Bengkulu. It was showed that *Ae. aegypti* was still tolerant to the 1x dose of malathion. The result study by Phillaberta [13] had similar results to this research, which showed that in Pogung and Sendowo Yogyakarta *Ae. aegypti* showed resistance to 1x dose of malathion.

Table 3. Interpretation of the results of the Malathion bioassay on *Ae. aegypti* mortality

Location	Malathion Diagnostic Dose	Mortality	Result Interpretation
Kaliwungu, Kudus	1x	0%	Resistant
	2x	87%	Tolerant, develop to Resistant
Kotagede, Yogyakarta	1x		Resistant
	2x		Susceptible

In Table 4 showed that the *Ae. aegypti* mosquito from Kaliwungu was resistant to Malathion at the 1x dose, which was indicated by mortality rate of 0% at the 30th minute of observation. Mosquitoes showed resistance to alpha-cypermethrin at twice the dose with a mortality rate of 72% at 30 minutes. Mosquitoes show susceptibility to alpha-cypermethrin at 5x dose with a mortality rate of 100% at 30 minutes. In this test, the control mosquitoes did not show any mortality, so no analysis and correction with Abbott's formula was needed.

Table 4. Mortality of *Ae. aegypti* Mosquito from Kaliwungu, Kudus to Alpha-cypermethrin

Time (Minute)	Mortality of <i>Ae. aegypti</i> (1x dose)				Mortality of <i>Ae. aegypti</i> (2x dose)				Mortality of <i>Ae. aegypti</i> (5x dose)			
	Treatment Group (n) (%)		Control group (n) (%)		Treatment Group (n) (%)		Control group (n) (%)		Treatment group (n) (%)		Control group (n) (%)	
0	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
15	0	0%	0	0%	7	12%	0	0%	44	69%	0	0%
30	0	0%	0	0%	43	72%	0	0%	60	100%	0	0%
35	2	3%	0	0%	58	97%	0	0%				
40	3	5%	0	0%	60	100%	0	0%				
45	3	5%	0	0%								
60	4	7%	0	0%								
75	5	8%	0	0%								
90	7	12%	0	0%								
105	8	13%	0	0%								
120	8	13%	0	0%								
Total of Mosquitoes	60		15		60		15		60		15	

Aedes aegypti mosquito from Kotagede showed resistance to alpha-cypermethrin at the 1x and 2x diagnostic dose (Table 5-6), which was indicated by the mortality rate of 0% at

the 30th minute of observation. Mosquitoes showed resistance to alpha-cypermethrin at the 2x diagnostic dose with a mortality rate of 55% at 30 minutes, whereas at 5x diagnostic dose indicated that mosquitoes are susceptible to alpha-cypermethrin with a mortality rate of 98% at 30 minutes. In this test, the control mosquitoes did not show any mortality, so there was no analysis, and correction with Abbott's formula was needed.

Resistancy in mosquito was emerge to describe of any durability of insecticide that continues to be widely used and explain the mosquito condition in which they were no longer killed by the standard dose of an insecticide [14]. Insecticide resistance is a global problem because it can affect efforts to control disease vectors by health authorities and the generally public. According to WHO [5], disease vectors such as mosquitoes and other insects cause more than one million deaths each year and pose a threat to global public health.

Table 5. Mortality of *Ae. aegypti* Mosquito from Kotagede, Yogyakarta to Alpha-cypermethrin

Time (Minute)	Mortality of <i>Ae. aegypti</i> (1x Dose)				Mortality of <i>Ae. aegypti</i> (2x Dose)				Mortality of <i>Ae. aegypti</i> (5x Dose)			
	Treatment group		Control group		Treatment group		Control group		Treatment group		Control group	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
0	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
15	0	0%	0	0%	1	2%	0	0%	47	68%	0	0%
30	0	0%	0	0%	33	55%	0	0%	59	98%	0	0%
35	2	3%	0	0%	49	82%	0	0%	60	100%	0	0%
40	3	5%	0	0%	57	95%	0	0%				
45	3	5%	0	0%	59	98%	0	0%				
60	4	7%	0	0%	60	100%	0	0%				
75	5	8%	0	0%								
90	7	12%	0	0%								
105	8	13%	0	0%								
120	8	13%	0	0%								
Total of Mosquitoes	60		15		60		15		60		15	

Table 6. Interpretation of the results of the alpha-cypermethrin bioassay on *Ae. Aegypti* Mortality

Location	Diagnostic Dose	Mortality (%)	Interpretation
	1x	0%	Resistant
Kaliwungu, Kudus	2x	72%	Resistant
	5x	100%	Susceptible
	1x	0%	Resistant
Kotagede, Yogyakarta	2x	55%	Resistant
	5x	98%	Susceptible

Resistance mechanisms in younger mosquitoes can show a higher resistance phenotype [15,16]. Mosquitoes have an enzyme called glutathione-S-transferase which is transcribed by the GSTe2 and GSTe7 genes. This enzyme plays a role in cypermethrin and other insecticides metabolism to prevent toxic nerve damage. In resistant mosquitoes, the amount of enzyme increases so that cypermethrin cannot have a deadly effect. At the 1x diagnostic dose of malathion, *Ae. aegypti* from Kaliwungu Kudus and Kotagede Yogyakarta, both

showed resistant. Moreover, *Ae aegypti* from both locations showed resistant at the 1x and 2x dose of alpha-Cypermethrin.

Mosquito is a wild and adaptable organism in any of environment. They reproduce rapidly under suitable conditions and will spread quickly. During reproductive cycle, the mutation, adaptation that from many of breeding sites may produce high genetic variation, and this condition may enable it to be resistant to insecticides [14] and environmental changes. These results indicated that to control the mosquito vector population in the locations should be rotated.

IVCC [14] suggested that there were 4 strategies to control and maintain the effectiveness of insecticide-based vector control to the mosquito population. This strategy of the Insecticide Resistance Management (IRM) was rotated, by switching of different modes of insecticides action, secondly by mosaics, that was by using of insecticides with different modes of action in neighboring locations. Third, by combine the formulation two or more insecticides with different mode of action, and finally, expose the vector population with the combination of two insecticides classes with different modes of action, for example by using the pyrethroid Long Lasting Insecticides Nets (LLNs) combined with a non-pyrethroid Indoor Residual Spray (IRS) [14].

3.3 Environmental Parameters

Environmental parameters in these two areas were different, and it could affect the presence of flora and fauna in the area. Therefore, it was important to understand the differences in environmental parameters between Kotagede Yogyakarta and Kaliwungu Kudus to protect and preserve the environment and biodiversity in each region.

In Table 7 represents the range of environmental parameters in Kotagede Yogyakarta and Kaliwungu Kudus during the ovitrap for larvae collection. The results showed that the air temperature ranges from 29.0-36.0°C in Kaliwungu and 28.1-36.0°C in Kotagede. As Reiskind and Zarrabi [17] said temperature played an important role in the survival and development of *Aedes* and the range optimum temperature was 25°C-30°C. The ability to hatch, survival, and development temperatures reached and exceeded 40°C.

Table 7. Environmental Parameters in Kaliwungu Kudus and Kotagede Yogyakarta during the larvae collection

Environmental Parameters (range)	Kaliwungu	Kotagede
	Kudus, Central Java	Yogyakarta, DIY
Air Temperature (°C)	29,0-36,0	28,1-36,0
Humidity (%)	55,0-63,0	51,0-72,3
Water Temperature (°C)	28,0-32,0	22,5-29,7
pH of Water	6,4-7,8	6,4-8,4

Yasuoka and Levins [18] explained that the optimal temperature for mosquito survival was 20-25°C, and the optimum temperature for mosquitoes to lay eggs was around 25°C. Air temperature in the range of 31-35°C could reduce egg production, then low humidity would interfere with the metabolic processes of the larvae [19]. The air temperature at both locations was higher than the optimum temperature for the growth of *Aedes* spp. larvae, it could be assumed that in both locations the ability of eggs to hatch and larval development into adult will decrease and be hampered.

In addition to air temperature, air humidity also plays an important role in the growth of *Aedes* larvae. Gandahusada *et al.* [20] explained that *Aedes* spp. prefers a place with

humidity around 81.5-89.5%, because it is suitable for its growth. Air humidity in Kaliwungu was measured in the range of 51.0-72.3%, while in Kotagede, it was a range of 55.0-63.0%. This indicates that the air humidity in both locations during collection was under the optimal value for the growth of *Aedes* larvae.

Furthermore, the water temperature was also an important factor in the growth of *Aedes* larvae. In the study, Barry and Juliano [21] explained that water temperature was one of the factors that affect larval growth, the optimal temperature for the growth of *Aedes* spp. larvae was between 27-30 °C. Water temperature observations in Kaliwungu had a range of 28.0-32.0 °C, while those in Kotagede had a range of 22.5-29.7°C. The results at both locations indicated that the water temperature was still optimal for *Aedes* larvae growth.

Water pH was also a factor that needs to be considered in the growth of *Aedes* larvae. Normal water pH was a good condition for larval growth, while acidic pH would affect larval feed availability due to the disruption of plankton as a larval feed source. Good water pH for larval growth was 6–8 [22]. The results of water pH measurements in Kaliwungu were in the range of 6.4-7.8, while those in Kotagede were in the range of 6.4-8.4. The results of pH measurements at both locations indicated that the pH of the water was still appropriate for larval growth so that these conditions could support the growth of *Ae. aegypti*.

4 Conclusions and suggestions

4.1 Conclusion

Aedes aegypti from Kaliwungu and Kotagede showed resistance to malathion insecticide at 1× diagnostic dose and tolerant at 2× diagnostic dose. Whereas, at diagnostic dose 2x and 5× showed resistant and susceptible status to alpha-cypermethrin respectively. The water temperature and pH factors optimally supported the development of *Aedes* larvae.

4.2 Suggestions

It is necessary to rotate the use of insecticides in Kaliwungu, Kudus and Kotagede, Yogyakarta. It is necessary to carry out a careful risk assessment before deciding to use a higher dose and consider factors such as the level of exposure, the potential impact on the environment, and the safety of using insecticides on humans and other animals. In addition, more prevention and control efforts need to be made. holistic approach, such as keeping the environment clean and adopting a healthy lifestyle, so as to reduce the risk of spreading vector-borne diseases. Further research is needed on matters affecting the resistance mechanism of *Ae. aegypti* to insecticides, such as the frequency of use of insecticides, people's behavior in using insecticides, and others.

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