Agustin et al., *Green and Tropical Laboratory for Sustainability* (2024), 1(1): 39-47

Research Article

# *Aedes aegypti* mosquitoes in Malang Regency: What is the resistance status to malathion 0.8%?

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**Article history:**
Received: 5 July 2024
Revised: 10 August 2024
Accepted: 25 August 2024
Published: 31 August 2024

## ABSTRACT

**Background:** Dengue hemorrhagic fever (DHF) carried by the *Aedes aegypti* has become a health problem in Karangploso, Singosari, and Lawang Districts. The vector control program implemented by the government is fogging. Fogging contains an active material malathion which can hit adult mosquitoes. Excessive use of malathionic active ingredients can lead to vector resistance.

**Objectives:** The purpose of this study is to analyze the resistance status of *Ae. aegypti* to malathion 0.8% in Karangploso, Singosari, and Lawang Districts, as well as community behavior in controlling dengue disease.

**Methods**: Type of research is a descriptive observational study with *cross sectional* approach. Data was obtained through *susceptibility* tests in the laboratory and observations of public attitudes in controlling dengue disease through a *Google form* survey. The data was presented in tabular form and discussed descriptively. The results showed that the mortality percentage of *Ae. aegypti* mosquitoes in Karangploso District was 98%, Singosari 96%, and Lawang 98%, all of which were in the tolerant category.

**Results:** The results of observations to the community showed that the community already knew the most common breeding sites for *Ae. aegypti* larvae is bathub and the way people control dengue fever using several types of household insecticides, namely spray, burn, and anti-mosquito lotions.

**Conclusion:** The conclusion of this study is the resistance status of *Ae. aegypti* in the three sub-districts are classified as tolerant and the community already knows the most preferred breeding sites for the *Ae. aegypti*, but the way people control DHF is still using some household insecticides that may increase the status of *Ae. aegypti* becomes resistant.

**Keywords:** *Aedes aegypti*, DHF, malathion, resistance

**SDGs Relevance:** This research supports SDG 3: Good Health and Well-Being.

**Laboratory Affiliation:** This research was conducted at the Chemistry Laboratory, Campus 3, Universitas Muhammadiyah Malang.

## INTRODUCTION

The *Aedes aegypti* mosquito is a vector that causes the infectious disease Dengue Hemorrhagic Fever (DHF) which is still a health problem in the world today (Fakhriadi et al., 2015). This disease is caused by arbovirus which is one of the dengue viruses in the serotype of the flavivirus genus (Sudiharto et al., 2020). Based on the World Health Organization (WHO) (2015), as many as 40% of the world's human population is at risk of DHF, especially those in urban areas in the tropics and subtropics. East Java Province is one of the areas with the second highest cases of DHF with a total of 7,838 cases (Simatupang, Oktivaningrum, Pratiwi, and Gestafiana, 2019). The Incidence Rate (IR) of DHF in 2018 was 24 per 100,000 population, but increased again in 2019 with an IR value of 47 per 100,000 population (East Java Health Office, 2020). Malang Regency is one of the areas in East Java that has high DHF cases with an IR (Incidence Rate) reaching more than 30.0 in 2018 (East Java Provincial Health Office, 2019).

This figure is below the national target of Incidence Rate (IR) ≤ 49 per 100,000 population, but the DHF cases in Malang Regency that occurred in 2018 were 751 cases, increasing to 1,570 cases in 2019 (East Java Health Office, 2020). The number of cases of Dengue Hemorrhagic Fever (DHF) based on data from the



p-ISSN: xxxx-yyyy
e-ISSN: xxxx-yyyy

Central Statistics Agency of Malang Regency (2018) in Karangploso District there were 106 cases, Singosari District there were 173 cases, and Lawang District there were 168 cases since 2015-2017. The Malang Regency Government through the Health Office has a strategy to reduce the number of DHF, namely by means of 3 M (Draining, Covering, Burying), monitoring the number of free larvae (ABJ), and recognizing and handling DHF disease in households. Considering that DHF is caused by the bite of the *Ae. aegypti* mosquito, the government is implementing a frequently used vector control program, namely fogging. Fogging contains the active ingredient malathion which will kill adult mosquitoes (Sunaryo & Widiastuti, 2018). Malathion has been used in Indonesia as an active ingredient for controlling DHF vectors since the 1970s (Kawatu et al., 2019). This effort was made because it was considered the easiest and the results could be seen directly by the community (Hijroh et al., 2017). The use of insecticides containing the active ingredient malathion for a long period of time will cause cases of resistance to *Aedes aegypty* mosquitoes (Sutarto, 2018; Prasetyowati, 2016). This can cause failure of vector eradication because resistance occurs when the vector cannot be killed by a standard dose of insecticide, for example 0.8% malathion (WHO, 2016). *Ae. aegypti* resistance is inherited, so this problem becomes an obstacle to chemical control of dengue vectors (Karauwan et al., 2017).

The emergence of cases of resistance of *Ae. aegypti* mosquitoes to active insecticide ingredients has been found in almost every region of Indonesia (Ambarita, 2015). This is due to the ineffectiveness of control carried out by the government through fogging activities (Rahman, 2016). The ineffectiveness of this government effort is because *Ae. aegypti* mosquitoes are resistant to active insecticide ingredients (Sucipto, 2015). Resistant *Ae. aegypti* mosquitoes will produce resistant offspring, which will increase the number of resistant vectors in the population (Ikawati et al., 2015). Vector resistance testing aims to determine the susceptibility status of vector species to active insecticide ingredients that have been and will be used to control vectors in an area (Akollo et al., 2020). This is used as a basis for regulating the use of insecticides in vector control (Abdurrakhman, 2019). Previous research results showed that *Ae. aegypti* resistance occurs in various dengue fever endemic areas (Prasetyowati et al., 2016). Research on *Ae. aegypti* resistance to malathion was reported in several regions of Indonesia, namely Sumatra with a percentage of *Ae aegypti* mortality is 100%, Kalimantan with a percentage of <80%, Sulawesi 0%, Maluku 20%, and Java Island 14% (Ambarita et al., 2015; Safitri, 2011; Soenjono, 2011; Tasane, 2015; and Widiarti et al., 2011). Research on the resistance status of *Ae. aegypti* mosquitoes to malathion in East Java reported that resistance had occurred with a percentage value of mosquito mortality of 0% in Tegaldlimo, 0% in Purwoharjo, and 1% in Banyuwangi (Yudhana, 2017).

Case studies of *Ae. aegypti* mosquito resistance in Malang Regency, especially in Karangploso, Singosari, and Lawang Districts, have never been conducted, so this study is very important because it will be the basis for recommendations for the use of active insecticide ingredients to eradicate *Ae. aegypti* mosquitoes. This study aims to analyze the resistance status of *Aedes aegypti* mosquitoes to 0.8% malathion insecticide in Karangploso, Singosari, and Lawang Districts, Malang Regency.s

## METHODS

The study used a cross-sectional approach. This type of research is descriptive observational, where researchers determine the resistance status of *Ae. aegypti* mosquitoes in three sub-districts, namely Karangploso, Singosari, and Lawang, Malang Regency to 0.8% Malathion. The study was conducted at the Chemistry Laboratory of the Universitas Muhammadiyah Malang. The study was conducted from July 22, 2020 to December 23, 2020. The research population used was all *Ae. aegypti* mosquitoes taken from egg surveys in three sub-districts, namely Karangploso, Singosari, and Lawang for testing mosquito resistance. Meanwhile, all humans living in the three sub-districts were respondents for supporting data on community behavior. The sampling technique used in the study was a random sampling technique, namely *Ae. aegypti* mosquito eggs were taken from three sub-districts in Malang Regency, namely Karangploso, Singosari, and Lawang using ovitraps that were distributed, then rearing would be carried out in the laboratory. The sampling technique used for the community behavior survey was random sampling, namely the survey was conducted at the same time as taking *Ae. aegypti* mosquito eggs. The research sample was 60 adult female *Ae. aegypti* mosquitoes aged 3-5 days, the first generation (F1) obtained from the results of mosquito egg rearing in the Chemistry laboratory of the University of Muhammadiyah Malang, taken from egg surveys in three sub-districts, namely Karangploso, Singosari, and Lawang, which were tested for resistance to 0.8% malathion and humans living in Karangploso, Singosari, and Lawang sub-districts who filled out the google form questionnaire to obtain community behavior data. If mosquito mortality in the control is >10%, it is considered a failure and must be repeated. If it is less than 10%, the ABBOTS formula correction factor is used. The results of the susceptibility test are grouped with the following mosquito mortality criteria: 1) Mortality ≥ 98% vulnerable category; 2) Mortality 90-97% tolerant category; and 3) Mortality < 90% resistant category.

Sampling of *Ae. aegypti* mosquito eggs was carried out using ovitraps placed in randomly selected villages in Karangploso, Singosari, and Lawang Districts. The ovitraps were placed in dark places inside the house. The ovitraps were taken after there were mosquito eggs in them. Data collection for community behavior surveys was carried out using a questionnaire via Google Form. Data for the community behavior survey were taken at the same time as taking samples of *Ae. aegypti* mosquito eggs.

The tools used in the mosquito resistance test study are ovitrap, pipette, 20 ml Erlenmeyer flask, cotton, stick, egg storage container, egg rearing container until it becomes a pupa, pupa rearing container until it becomes an adult mosquito aged 3-5 days, mosquito cage, susceptibility test tube (treatment tube containing malathion-impregnated paper, control tube containing whatman paper without insecticide, and holding/storage tube), and aspirator. The materials used in this study were mosquito eggs that were reared until they became adult mosquitoes aged 3-5 days, water, food for larvae until they become pupae (poultry pellets), and 10% sucrose solution. Meanwhile, the tool used to collect data on community behavior surveys was a smartphone that had been equipped with a google form link as a medium to obtain survey results.

Landing Collection of *Aedes aegypti* Mosquito Eggs was carried out through the following steps. *Ae. aegypti* were obtained by placing ovitraps in the house with a simple random sampling technique in Karangploso, Singosari, and Lawang Districts, namely taking eggs randomly that are members of the population and have the same chance and are freely selected as research samples,

Rearing (breeding) mosquito eggs was carried out in the mosquito room of the Chemistry Laboratory of the University of Muhammadiyah Malang by hatching the eggs in a plastic tray filled with water. Eggs that have become larvae are fed poultry pellets every day until they become pupae (Widiarti et al., 2011). The larvae that have become pupae are transferred to a mosquito cage and given a 10% sucrose solution (Wahyuningsih, 2015 and Nuryady et al., 2017) which is placed in a 20 ml Erlenmeyer flask equipped with a stick wrapped in cotton as food for adult mosquitoes. Adult mosquitoes aged 3-5 days were tested using the susceptibility method. Adult mosquitoes from rearing were identified with a Portable LCD Digital Microscope 3.6 Mp. Identification is done by observing the legs, scutum, and antennae.

Testing the resistance of *Ae. aegypti* mosquitoes using the susceptibility method according to the World Health Organization (WHO) (2016) standards. Mosquitoes were placed in 4 different tubes (WHO, 2016) each containing 15 adult female mosquitoes aged 3-5 days (Pradani et al., 2018) that had been fed 10% sucrose (Wahyuningsih, 2015 and Nuryady et al., 2017). The four tubes used consisted of 3 tubes containing 0.8% malathion impregnated paper and 1 control tube containing whatman paper without insecticide. Mosquitoes were exposed to 0.8% malathion insecticide for 60 minutes, then transferred to a holding tube (storage) without 0.8% malathion insecticide for 24 hours. The holding tube is placed in a box covered with a wet towel to maintain temperature and humidity (Ambarita, 2015; Widiastuti 2016). After 24 hours, observations were made by counting the number of dead mosquitoes (WHO, 2016). The data collection technique was carried out by field observation to collect *Ae. aegypti* mosquito eggs and then filtered in the Chemistry laboratory of the University of Muhammadiyah Malang. Adult female *Ae. aegypti* mosquitoes aged 3-5 days were tested using the Susceptibility method. *Ae. aegypti* mosquito resistance test data were obtained by calculating the percentage of mosquito deaths after 24 hours. The analysis was carried out by calculating the number of mosquitoes that died after exposure to 0.8% malathion for 1 hour and 24 hours in the holding tube. The data on the percentage of mosquito deaths after 24 hours that had been obtained determined their resistance status based on WHO criteria. The susceptibility criteria are determined as follows: 1) It is said to be susceptible if mortality is between ≥ 98%, 2) It is said to be tolerant if mortality is between 90-97%, 3) It is said to be resistant if mortality is <90% (WHO, 2016). Data analysis was carried out by comparing the percentage of *Ae. aegypti* mosquito mortality with the resistance criteria according to WHO.

## RESULTS

The research was conducted in three areas of Malang Regency, namely Karangploso, Singosari, and Lawang Districts. The results of the research that has been conducted on the resistance of *Ae. aegypti* mosquitoes to 0.8% malathion can be seen based on the results of the resistance status of *Ae. aegypti* mosquitoes, data on the use of active insecticide ingredients for fogging in Malang Regency by the Malang Regency Health Office, and a survey of the community. The results of the survey include a breeding site survey and a survey of the types of household insecticide use. Based on the results of the *Ae. aegypti* mosquito resistance status test, the distribution of *Ae. aegypti* mosquitoes will then be mapped with susceptible, tolerant, or resistant status. The results of the *Ae. aegypti* mosquito breeding site survey in Karangploso, Singosari, and Lawang Districts can be seen in Table 1. Based on the results of the breeding site survey from 50 respondents, it shows that the place where *Ae. aegypti* mosquito eggs are most often found is the bathtub with a percentage value of 38%. Clean water puddles occupy the second highest percentage after bathtubs with a percentage value of 34%. Examples of clean water puddles include dispenser water reservoirs, AC water reservoirs, and bird drinking places (Hendri, 2016, Kawatu, 2019, and Syaidah, 2019). The breeding place for *Ae. aegypti* mosquitoes with the fewest mosquito larvae is a flower vase with a percentage value of 28%.

**Table 1.** Results of a survey of breeding sites for *Aedes aegypti* mosquitoes in Karangploso, Singosari, and Lawang Districts

| No | Breeding place | % Mosquito larvae |
|---|---|---|
| 1 | Bathtub | 38 |
| 2 | Clean water reservoir | 34 |
| 3 | Flower vase | 28 |

The results of the *Ae. aegypti* mosquito resistance test in Karangploso, Singosari, and Lawang Districts can be seen in Table 2. The results of the *Ae. aegypti* mosquito resistance test in Karangploso, Singosari, and Lawang Districts show that *Ae. aegypti* mosquitoes in the three districts that were the research sites were classified as tolerant to malathion 0.8%. Singosari District has the lowest percentage value of *Ae. aegypti* mosquito mortality to malathion 0.8% with a percentage value of 96%. The percentage of *Ae. aegypti* mosquito mortality in control tubes in all sub-districts was 0%.

**Table 2.** Results of the *Aedes aegypti* mosquito resistance test in Karangploso, Singosari, and Lawang Districts

| Region (District) | Treatment | ∑ Mosquito mortality | % Mosquito mortality | Average percentage of mosquito mortality (%) | Status | Average percentage of mosquito mortality to malathion in three sub-districts (%) | Status |
|---|---|---|---|---|---|---|---|
| Karangploso | 1 | 14 | 93% | 97% | Tolerant | 96% | Tolerant |
| | 2 | 15 | 100% | | | | |
| | 3 | 15 | 100% | | | | |
| | Control | 0 | 0% | 0% | Normal | | |
| Singosari | 1 | 14 | 93% | 95% | Tolerant | | |
| | 2 | 14 | 93% | | | | |
| | 3 | 15 | 100% | | | | |
| | Control | 0 | 0% | 0% | Normal | | |
| Lawang | 1 | 15 | 100% | 97% | Tolerant | | |
| | 2 | 14 | 93% | | | | |
| | 3 | 15 | 100% | | | | |
| | Control | 0 | 0% | 0% | Normal | | |

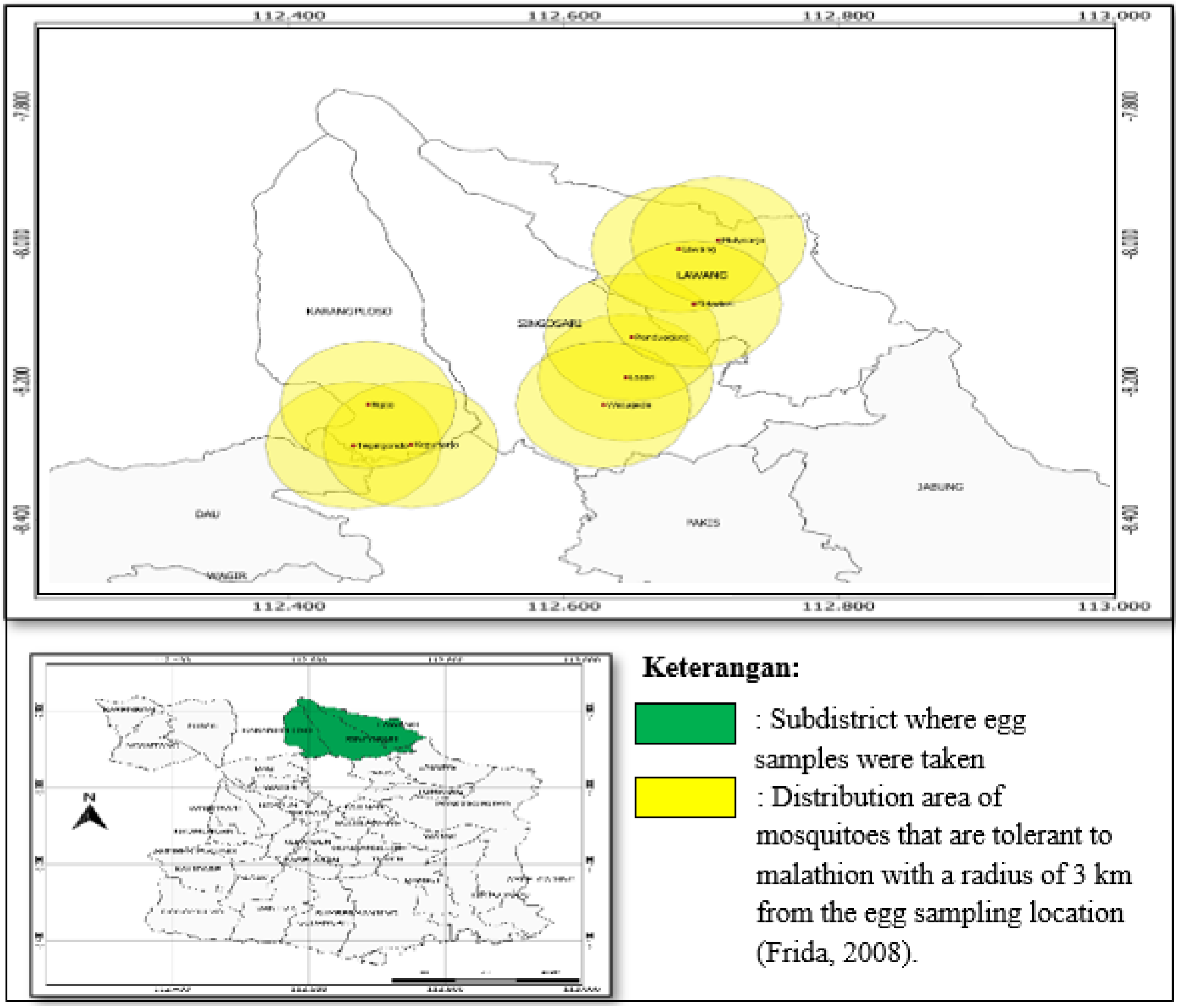


**Figure 1.** Map of the distribution of *Aedes aegypti* mosquitoes that are tolerant to 0.8% malathion in Karangploso, Singosari, and Lawang Districts

The resistance status of *Ae. aegypti* mosquitoes from Karangploso, Singosari, and Lawang Districts has been known and the behavior of the community in overcoming the spread of *Ae. aegypti* mosquitoes has been explained, so the resistance status can be mapped through the distribution map of the status of *Ae. aegypti* mosquitoes that are tolerant to 0.8% malathion in three Districts located in Malang Regency. Each district is represented by three points of mosquito egg sampling locations, including 7°55'00.5"S 112°35'38.6"E, 7°53'58.0"S 112°35'54.9"E, 7°54'59.0"S 112°36'42.1"E (Karangploso District), 7°52'14.8"S 112°40'46.4"E, 7°53'16.7"S 112°40'39.8"E, 7°53'58.3"S 112°40'14.9"E (Singosari District), 7°51'24.3"S 112°41'55.1"E, 7°49'60.0"S 112°41'38.4"E, 7°49'46.6"S 112°42'22.1"E (Lawang District). The area that is the distribution point of *Ae. aegypti* mosquitoes that are tolerant to 0.8% malathion is marked in yellow. While the white part is not included in the area with the status of Ae. aegypti mosquitoes that are tolerant to 0.8% malathion. The resistance status of *Ae. aegypti* mosquitoes in the three districts can be seen through the resistance status distribution map in Figure 1.

Based on the resistance status distribution map of *Ae. aegypti* mosquitoes, the yellow area is a place with *Ae. aegypti* mosquitoes that are likely to be tolerant to 0.8% malathion. The yellow area is a 3 km radius from the *Ae. aegypti* mosquito egg sampling point that was tested for resistance to 0.8% malathion. The white areas are not areas with *Ae. aegypti* mosquito status tolerant to 0.8% malathion.

## DISCUSSION

*Ae. aegypti* mosquitoes were obtained from 3 sub-districts in Malang Regency, namely Karangploso, Singosari, and Lawang Sub-districts. The results of the landing collection were *Ae. aegypti* mosquito eggs which were reared in the Laboratory until they became adult female mosquitoes aged 3-5 days. Mosquito eggs were obtained by placing ovitraps in designated locations, namely dark and humid places. The eggs that had become adult female mosquitoes aged 3-5 days were then tested for susceptibility (Tasane, 2015). Susceptibility resistance tests were carried out to determine the resistance status of mosquitoes by looking at the percentage of mosquito deaths in the holding tube (Agustin et al., 2017). The mosquitoes used were *Ae. aegypti* females that have been identified and have been fed 10% sucrose (Wahyuningsih, 2015). This aims to ensure that the tested *Ae. aegypti* mosquitoes do not die from starvation, but their deaths are caused by exposure to insecticides (malathion).

The results of the *Ae. aegypti* mosquito breeding site survey in 3 sub-districts show that the most *Ae. aegypti* larvae were found in the bathtub. *Ae. aegypti* mosquito eggs can hatch into larvae due to the supporting breeding media factor for the development of eggs (Sabila et al., 2013). The bathtub is the most supportive mosquito breeding place among the other two places (puddles of clean water in the house and flower vases) because people generally have bathtubs with dark or cemented base colors. This is in accordance with Agustina (2019) and Rahayu (2013) that *Ae. aegypti* mosquito larvae are often found in bathtubs with dark or cemented bases. This is also in accordance with the opinion of Wahyuni (2018) that *Ae. aegypti* mosquitoes prefer to lay their eggs in dark places and are not exposed to direct sunlight. A bathtub with dark conditions is a supporting factor for microorganisms to grow well. These microorganisms are food for larvae. Thus, the larvae get enough food for their development (Agustina, Abdullah, and Arianto, 2019). The depth of the bathtub also causes the optimal temperature (26-27ºC) for mosquito breeding and will not significantly change the pH (optimal pH 7.2-8.1) when the bathtub is contaminated by other materials, such as soap (Agustin, 2017; Agustina, 2019.)

The most preferred mosquito breeding place after the bathtub is a puddle of clean water. This is in accordance with the opinion of Syaidah et al. (2019) that *Ae. aegypti* mosquitoes are more interested in laying their eggs in puddles of clean water that do not directly touch the ground, such as used cans, water dispensers, and animal drinking places. In addition, flower vases are also a preferred breeding place for *Ae. aegypti* mosquitoes. This is because flower vases are places that are filled with water and do not directly touch the ground. *Ae. aegypti* can breed in places with little stagnant water, such as flower vases and their eggs are very resistant to drought (Nurjana & Kurniawan, 2017).

*Ae. aegypti* mosquitoes from Karangploso District with a percentage value of 98%, Singosari 96%, and Lawang 98%, all three of which are still in the tolerant category. In accordance with WHO (2016) that *Ae. aegypti* mosquitoes are said to be tolerant to insecticides (malathion) when the percentage of death is 90-97%. Malathion is one of the insecticides that is still often used in vector control (Tasane, 2015). Malathion has high toxicity when compared to other insecticides (Widiastuti 7 Ikawati, 2016). The way malathion works is by disrupting the nervous system in target insects by inhibiting cholinesterase which causes insects to become paralyzed and die (Kawatu et al., 2019). Malathion insecticide works by inhibiting the cholinesterase enzyme, which can cause disruption of nerve activity because acetylcholine accumulates until it finally becomes choline and acetic acid (Pambudi et al., 2018). Acetylcholine functions as a mediator between nerves and muscles that can facilitate the transmission of impulses that stimulate muscles to contract for a long time and can eventually cause seizures (Anam et al., 2019). Inhibition of the cholinesterase enzyme causes acetylcholine hydrolysis to not occur so that the transmission of electrical impulses that stimulate muscles to contract continuously causes seizures and even death in *Ae. aegypti* mosquitoes (Ridha & Nisa, 2011). Another factor that can affect the death of *Ae.* aegypti mosquitoes is temperature (optimal 25ºC-30ºC) (Anggraini & Cahyati, 2017). Mosquitoes that die during testing can also be caused by less than optimal temperatures during testing. Several repetitions in taking mosquito samples from the cages were carried out in this study because excessive pressure when using the respirator caused the mosquitoes to die before being tested.

The mortality of adult female *Ae. aegypti* mosquitoes in three sub-districts was 97%. This is different from the research of Sucipto et al. (2015) in Tangerang City, Rahman (2016) in Bantul Regency, Yogyakarta, and Akollo et al. (2020) in Ambon City regarding the resistance status of *Ae. aegypti* mosquitoes to malathion, it was found that generally *Ae. aegypti* mosquitoes are resistant to malathion, but there are still a small number that are tolerant. This is due to the high level of malathion toxicity to insects, comparable to the data obtained that *Ae. aegypti* mosquitoes tested on 0.8% malathion are not resistant to malathion, as evidenced by the high percentage of mosquito mortality.

According to Kristinawati (2013), the low level of resistance of *Ae. aegypti* mosquitoes (tolerant) indicates that the area can still use malathion insecticide as a vector control for Dengue Hemorrhagic Fever (DHF). However, continuous use of malathion can increase the potential for resistance of *Ae. aegypti* mosquitoes to malathion. Therefore, rotation of insecticide use is needed to avoid increasing mosquito resistance (Sunaryo, 2018). The results of other studies have also shown that the implementation of insecticide application rotation causes *Ae. aegypti* mosquitoes to still be susceptible to malathion even though they have been used for more than 32 years (Agustin et al., 2017).

Based on the results of research that has been conducted in three sub-districts of Malang Regency, the community still needs to implement 3 M as a form of environmental concern and an alternative to controlling dengue fever vectors even though currently *Ae. aegypti* mosquitoes in Karangploso, Singosari, and Lawang Sub-districts are not yet classified as resistant. This is in accordance with the opinion of Palgunadi and Rahayu (2011) that the 3 M activity (Draining, Covering, Burying) is an important factor in controlling mosquitoes that cause dengue fever. Rotation of insecticide use with various types of active ingredients can also be an alternative to control *Ae. aegypti* mosquitoes (Abdurrakhman, 2019) because the resistance status of *Ae. aegypti* mosquitoes in the three sub-districts is classified as tolerant, but can increase to resistance if there is no rotation of insecticide use. The community still needs to reduce dependence on household insecticides so that *Ae. aegypti* mosquitoes do not become resistant to other active ingredients (Hendri et al., 2016). According to Frida (2008), the range of mosquitoes reaches 400 m – 3 km. Based on the results of the analysis of the resistance status of *Ae. aegypti* mosquitoes which are classified as tolerant, it is possible that the distribution map of *Ae. aegypti* mosquitoes that are tolerant to 0.8% malathion in Karangploso, Singosari, and Lawang Sub-districts as in Figure 4.2 is ±3 km from the place where *Ae. aegypti* mosquito eggs are collected. The status of *Ae. aegypti* that are not yet resistant due to the use of malathion insecticide in the three sub-districts is carried out by meeting the established operational standards. Based on information from residents in the three areas, the government rarely or never conducts fogging with a frequency of more than 2 times a year. The status of *Ae. aegypti* mosquitoes in three areas of Malang Regency, namely Karangploso, Singosari, and Lawang Sub-districts which are classified as tolerant to malathion means that the mosquitoes are not yet immune because they cannot increase their immune system against exposure to active insecticide ingredients (Prasetyowati et al., 2016) which ultimately insecticides containing the active ingredient malathion can still be applied in fogging activities while still paying attention to the rotation of insecticide use.

## CONCLUSION

Based on the results of the research and discussion that has been done, the resistance status of *Ae. aegypti* mosquitoes to 0.8% malathion in Karangploso, Singosari, and Lawang Districts is tolerant. Suggestions for further research are that the method of transferring mosquitoes from the cage to the treatment tube using a respirator (suction) must be done correctly so that the death of mosquitoes in the test tube can be ascertained due to exposure to active insecticide ingredients, not death due to excessive air pressure due to the suction method. In addition, research on the resistance status of *Ae. aegypti* mosquitoes to 0.8% malathion or other active ingredients needs to be carried out periodically at least once every 3 years.

**Author Contributions:** J.U.A writing – original draft; S. Z. . review and editing; M.M. N. conceptualization, methodology, data curation; T. I. P. review and editing

**Conflicts of Interest:** The authors declare no conflicts of interest.

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