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Research Article

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Mosquitoes' larval habitat characteristics and *Aedes aegypti* resistance status to malathion in Jember

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ABSTRACT

Water-holding containers serve as breeding sites for Aedes aegypti. The control of mosquito breeding sites and the use of household insecticides are some of the DHF control methods. The use of household insecticides over a long period can reduce the susceptibility of mosquitoes to these insecticides. This research aims to determine the mosquito's habitat characteristics and to determine the Aedes aegypti resistance status to malathion in Jember. A total of 61 houses were surveyed, and the various types of containers encountered at the sampling locations were recorded. Larvae and pupae found at the sampling location are brought and hatched in the laboratory. Aedes aegypti was tested for resistance status to malathion insecticide using a CDC bottle bioassay and biochemical nonspecific esterase enzyme activity. A total of 140 containers were found in this study, and 36.43% of them are Aedes aegypti larvae/pupae habitat. Types of containers used as mosquito larval habitat are bottles/glasses, bathtubs, used basins/buckets, water storage buckets, trash cans, refrigerator water containers, used cans, gutters, livestock drinking water containers, fish ponds, plant pots, used livestock cages, used toilets, used jugs, used animal feeders, and used aquariums. The mortality of Aedes aegypti reached 42.3% (resistant). Meanwhile, the results of biochemical tests showed an increase in the activity of the nonspecific esterase enzyme. This study concludes that Aedes aegypti from Jember has been resistant to malathion insecticide.

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INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is an important health problem in the world, including in Indonesia. A total of 474 (92.2%) districts/cities in Indonesia were infected with dengue fever in 2021 (Kementerian Kesehatan RI, 2022). Jember Regency is one of the endemic areas for Dengue

Hemorrhagic Fever (DHF) in East Java. In 2021, Jember Regency occupied the second-highest area with a dengue fever incidence rate reaching 447 sufferers (Dinas Kesehatan Provinsi Jawa Timur, 2022). DHF is a disease caused by the dengue virus (DENV 1 – DENV4) and is transmitted through the bite of female mosquitoes. The primary vector of dengue fever is *Aedes aegypti* and *Ae. albopictus* (Harapan et al., 2020). Distribution of mosquitoes *Aedes aegypti* covers almost all regions in the world, including the Jember Regency. Various efforts have been made to prevent the incidence of dengue fever. Integrated vector control is one of the programs implemented in Indonesia, namely vector control that takes into account human, vector, environmental, and host aspects. Mosquito Nest Eradication activities aim to prevent mosquito breeding. Some places used as breeding grounds for mosquitoes include bathroom pools, buckets, used tires, barrels, and so on (Triana et al., 2021). The more mosquito breeding sites available and there is no effort to eradicate mosquito nests, the higher the mosquito density in the area.

A high density of mosquito larvae in an area will generally be followed by the use of insecticides to eradicate adult mosquitoes. Many types of household insecticides are sold freely. However, unfortunately, public understanding regarding the rules for using insecticides still needs to be improved; the use of insecticides by the public is still excessive (Wigati & Susanti, 2012). One of the most popular insecticides in Indonesia is organophosphate. Organophosphates have been used in Indonesia since the 1970s in agriculture, plantations, and households, public hygiene, and medical settings (Siegfried & Scharf, 2001). The insecticide that has long been used in Indonesia is malathion. Excessive chemical control of mosquitoes over a long period can trigger a decrease in mosquito susceptibility to insecticides (Nihayah et al., 2023). The mechanism of mosquito susceptibility to insecticides can be through mosquito behavior or physiology. Physiological resistance can occur through increased enzyme activity, which plays a role in degrading mosquito insecticides. In this way, the insecticide that enters the mosquito's body will be degraded before it reaches the target gene. Another mechanism is through changes in the genetic composition of the insecticide target gene. The enzyme that plays a role in degrading organophosphorus insecticides is the Acetylcholinesterase (AchE) enzyme. The AchE enzyme degrades the excitatory neurotransmitter, thereby causing the cessation of nerve impulse transmission. Several studies have tested the susceptibility or resistance status of *Ae. aegypti* has been done a lot in Indonesia, but such data has not been found for the Jember Regency area (World Healt Organization, 2022). An example is *Ae. aegypti* from Bengkulu, which was declared resistant to the insecticides malathion and cypermethrin (Triana et al., 2019). This research aims to determine the mosquito's habitat characteristics and to determine the *Ae. aegypti* resistance status to malathion in Jember.

RESEARCH METHODS

The design of this study was experimental with a post-test only control group design. Surveys and larval sampling were carried out in Sumbersari Village, Jember Regency (8°09'55.9"S 113°43'35.4"E). This area has a height of ±124 meters above sea level with an area of 4.88 km^2. The average daily rainfall in the Sumbersari District in 2022 reached 13.08 mm/day. The highest average rainfall occurs in January (24 mm/day), and the lowest average rainfall occurs in May (7 mm/day) and October (4mm/day).

Sampling was carried out at 61 sampling points with a minimum distance between points of 100 m. Determination of the number of samples was carried out based on WHO calculations (Table 1) to determine the vulnerability status of mosquitoes (World Health Organization, 2011). Sumbersari District's house index data reached 31.1% with the number of buildings ranging from 5,000 – 10,000. Sampling was carried out in May-July 2023. At each sampling point, various types of containers were checked. Data on (i) type of container, (ii) container material, (iii) location (inside or outside the house), and (iv) color are recorded, as well as positive/negative larvae-pupae. Every larva and pupa found in the container was recorded and taken to the biology laboratory of Universitas Jember for rearing and identification. The F2 generation was used in this study. Larvae were fed fish pellets once a day, while adult mosquitoes were fed 10% sucrose and mouse blood. Adult mice were put into a cage and inserted into the mosquito cage. The mouse and its cage were left for 1 hour. Mouse blood feeding was given every 2 days.

Table 1. Number of Buildings Surveyed based on the House Index

Number of Buildings	House Index		
	>1%	>2%	>5%
100	95	78	45
200	155	105	51
300	189	117	54
400	211	124	55
500	225	129	56
1.000	258	138	57
2.000	277	143	58
5.000	290	147	59
10.000	294	148	59
Unlimited	299	149	59

(Source: World Health Organization, 2011)

The bioassay test was carried out following the mosquito susceptibility testing guidelines by the CDC (CDC, 2020). Female *Aedes aegypti* (F2) aged 3-5 days and full of 10% sucrose were tested against the insecticide malathion at a dose of 50 µg/bottle. For each experiment, 4 test bottles and 1 control bottle (acetone) were used, each filled with 25 test mosquitoes, so there were 125 mosquitoes in each test. The experiment was repeated 3 times. Each test bottle was given 1 ml of the malathion insecticide, 50 µg/bottle, and 1 ml of acetone in the control bottle. The bioassay test was carried out for 2 hours, and the knockdown mosquitoes were counted every 5 minutes. The percentage of knockdown mosquitoes in the first 30 minutes was used to determine the susceptibility status of mosquitoes to the insecticide malathion 50 µg/bottle. Analysis data for bioassay resistance based on mosquito mortality, if mosquito mortality reaches 98-100% in the susceptible category, 80-97% in the tolerant category, and <80% in the resistant category. After the test, the mosquitoes were then transferred into a recovery bottle and given 10% sucrose. After 24 hours, mosquito deaths were counted.

Biochemical tests were carried out on early-stage IV larvae instar from F2. A total of 24 test larvae and 4 resistant control larvae were tested in this study. Each larva was ground individually in 500 µl PBS solution. A total of 50 µl of homogenate was taken and put into a microplate and added with 50 µl of substrate (3mg α-Naphil acetate dissolved in 0.5 ml of acetone, 100 µl was taken and added with PBS until the volume reached 10 ml). The homogenate was then allowed to stand for 60 seconds. Next, 50 µl of coupling reagent was added (30 mg of fast blue plus 7 ml of 5% SDS and 3 ml of distilled water). The color change was allowed to proceed for 10minutes at room temperature. Next, 50 µl of 10% cetic acid solution was added. The homogenate was then read using a microplate reader with a wavelength of 450 nm. The value that appears from the reading results is the absorbance value (AV), which is used to determine the activity of the esterase enzyme. Analysis of data for biochemical test based on absorbance value. Larvae *of Aedes aegypti* included in the resistant category if the AV value > cut off AV + 6 SD.

FINDING AND DISCUSSION

A total of 140 containers were found, and 51 of them (36.43%) contained mosquito larvae or pupae, indicating potential breeding sites. Of the 61 houses surveyed, 34 (55.74%) were positive for Culicidae larvae or pupae. From these containers (Table 2), 1,927 adult mosquitoes were successfully reared, consisting of *Aedes aegypti* (67.67%), *Aedes albopictus* (10.74%), *Culex quinquefasciatus* (21.48%), and *Anopheles* sp. (0.1%). The most common container type was bathtubs (45%), with plastic being the most frequent material (56.43%) and white as the most common color (27.86%).

Table 2. Type of Container

Parameter	Category	Positive larvae/pupae	Negative larvae/pupae	Total
Type	Used bottles/glasses	0 (0%)	3 (100%)	3 (2.14%)
	Bathtub	23 (36.51%)	40 (63.49%)	63 (45%)
	Used basin/ bucket	6 (46.15%)	7 (53.85%)	13 (9.29%)
	Water storage bucket	11 (44%)	14 (56%)	25 (17.8%)
	Rubbish bin	0 (0%)	1 (100%)	1 (0.71%)
	Refrigerator water reservoir	0 (0%)	2 (100%)	2 (1.43%)
	Used cans	1 (33.33%)	2 (66.67%)	3 (2.14%)
	Drain	0 (0%)	6 (100%)	6 (4.29%)
	Collecting drinking water for livestock	2 (22.22%)	7 (7.78%)	9 (6.43%)
	Fish pond	1 (25%)	3 (75%)	4 (2.86%)
	Plant pot	3 (75%)	1 (25%)	4 (2.86%)
	Former livestock pen	1 (50%)	1 (50%)	2 (1.43%)
	Used toilet	1 (100%)	0 (0%)	1 (0.71%)
	Used jug	2 (100%)	0 (0%)	2 (1.43%)
	Animal feed place	0 (0%)	1 (100%)	1 (0.71%)
	Used aquarium	0 (0%)	1 (100%)	1 (0.71%)
Material	Glass	0 (0%)	4 (100%)	4 (2.86%)
	Semen	8 (22.86%)	27 (77.14%)	35 (25%)
	Nothing	2 (40%)	3 (60%)	5 (3.57%)
	Plastic	31 (39.24%)	48 (60.76%)	79 (56.43%)
	Land	1 (33.33%)	2 (66%)	3 (2.14%)
	Iron	1 (100%)	0 (0%)	1 (0.71%)
	Ceramics	8 (61.54%)	5 (38.46%)	13 (9.29%)
Color	Clear	1 (10%)	9 (90%)	10 (7.14%)
	Chocolate	6 (42.86%)	8 (57.14%)	14 (10%)
	Black	6 (20.69%)	23 (79.31%)	29 (20.71%)
	Grey	5 (41.67%)	7 (58.33%)	12 (8.57%)
	Green	7 (50%)	7 (50%)	14 (10%)
	White	18 (46.15%)	21 (53.85%)	39 (27.86%)
	Blue	2 (20%)	8 (80%)	10 (7.14%)
	Cream	1 (100%)	0 (0%)	1 (0.71%)
	Red	2 (33.33%)	4 (66.67%)	6 (4.29%)
	Orange	2 (66.67%)	1 (33.33%)	3 (2.14%)
	Yellow	1 (50%)	1 (50%)	2 (143%)
Container location	Inside the house	20 (36.36%)	35 (63.64%)	55 (39.29%)
	Outside the house	31 (36.47%)	54 (63.53%)	85 (60.71%)

In the results of this study, the number of positive containers was 36.43% with bathtubs being the most frequently found container (45%). Another type of container that was also frequently found in this research was a water storage bucket. The types of containers that were positive for larvae/pupae in this study were bathtub, used basin/bucket, water storage bucket, used cans, collecting drinking water for livestock, fishpond, plant pot, former livestock pen, used toilet, and jug (Figure 1). These containers were used for mosquitoes laying eggs. Based on the CDC bottle bioassay, *Ae. Aegypti* from Sumbersari Village, Jember Regency have resistance with a death percentage reaching 42.33 % (Table 3). The results of the nonspecific esterase enzyme activity test also showed similar results, 58.33% of the test mosquitoes had increased nonspecific esterase enzyme activity (Table 4).

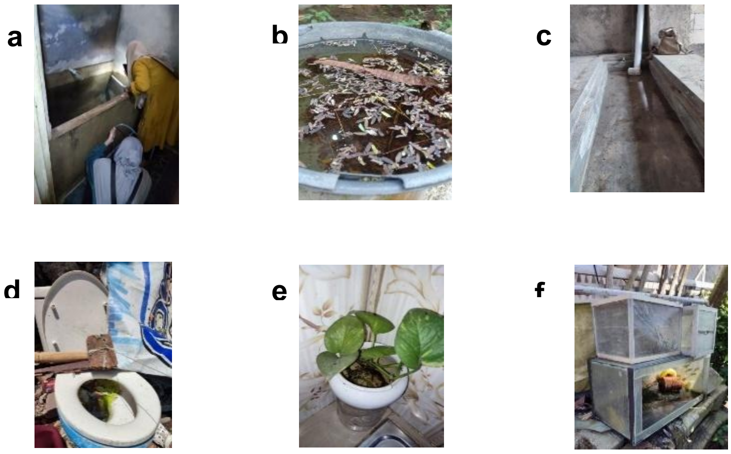

Figure 1. Some of the containers identified that were used as larval habitat: a Bathtub; b Used basin/bucket; c Drain; d Used toilet; e Plant pot; f Used aquarium.

Table 3. Resistance Status *of Ae.aegypti* Against Malathion Insecticide in Jember based on CDC Bottle Bioassay

Insecticide	Generation	∑ Mosquitoes	Mortality (%)		Resistance Status	LT50	LT95
			30 min	2 hours			
Malathion (50 µg/bottle)	F1	375	42.33	100	RR	32.1	51.9

Mortality: 98-100% = susceptible 80 – 97 % = tolerant <80% = resistant RR = resistant

Table 4. Larval Resistance Status of *Ae.aegypti* based on the Activity of the Nonspecific Esterase Enzyme (substrate α-naphthyl acetate) in Jember

Location	Frequency (%) AV Non-specific Esterase Enzyme Activity		
	Susceptible (AV< 0.487)	Tolerant (AV = 0.487 - 0.575)	Resistant (AV > 0.575)
Sumbersari	37.5	4.17	58.33
Susceptible strain	100	0	0
Resistant strain	0	25	75

Aedes aegypti is one of the main vectors of dengue fever (Harapan et al., 2020). The results of the resistance status test show*ed Ae.aegypti* has been resistant to the malathion insecticide at a dose of 50 µg/bottle. The mosquito susceptibility *of Ae. aegypti* is important to consider when controlling mosquitoes that cause dengue fever. Mosquitoes that are resistant to insecticides will be difficult to control chemically. On the other hand, the use of insecticides as mosquito control is still widely used by Indonesian people (Atikasari & Sulistyorini, 2019; Setiyaningsih et al., 2018). Malathion insecticide has been used as a mosquito control agent since the 1970s (>50 years) in Indonesia. Apart from the research location, *Ae. aegypti* has also been known to be resistant to malathion insecticides in parts of Bengkulu City (Triana et al., 2019), Ambon City (Akollo et al., 2020), Bengkulu (Sudiharto et al., 2020), and Pekanbaru (Elva et al., 2023). Meanwhile, the city of Padang (Sartika et al., 2020) is still vulnerable.

The physiological mechanism of resistance in mosquitoes can be through reducing the sensitivity of insecticide target sites, through metabolic detoxification of insecticides, or it can also be through delaying insecticide penetration in the cuticle (World Health Organization, 2022). In this study, there was an increase in the metabolic detoxification activity of insecticides. A total of 58.33% of test mosquitoes experienced an increase in esterase enzyme activity. Esterase enzyme activity is often related to insect resistance to carbamate and organophosphate insecticides (World Health Organization, 2022).

Organophosphate class insecticides target the acetylcholinesterase (AChE) enzyme in their mechanism of action. The AChE enzyme plays a role in hydrolyzing acetylcholine (ACh) into acetic acid and choline, stopping synaptic signal transmission mediated by ACh at neurotransmitter junctions. Organophosphates will bind and phosphorylate the nucleophilic serine in the active site of the enzyme esterase so that acetylcholine (ACh) cannot be hydrolyzed. This causes the concentration of ACh to increase excessively at the synapses, resulting in cholinergic hyperstimulus in the central and peripheral nervous systems. Some of the effects of increased ACh include respiratory failure, seizures, epileptics, and long-term brain damage (Aroniadou-Anderjaska et al., 2023).

Another alternative for controlling dengue fever is through controlling mosquito vectors by breaking their life cycle. Life cycle *of Ae.aegypti* consists of egg – larva – pupa – and adult. The egg, larva, and pupa stages require water media for growth, and adult mosquitoes live in terrestrial habitats (Arévalo-Cortés et al., 2022). The availability of water is important to control mosquitoes. A total of 16 types of water containers were found in this study with bathtubs (45%) and water storage buckets (17.86%) being the most common types found. This type of container tends to be controlled so that it becomes a habitat *of Ae. aegypti* can be omitted. Bathtubs, water buckets, trash cans, water containers, refrigerators, livestock drinking water containers, and animal feed areas have close interaction with the community.

Habitats commonly used for mosquito breeding sites are used tires, clay pots, containers, flower pots, and plant holes (Kahamba et al., 2020). The six containers had the highest number of positive mosquito larvae and pupae in this research were a bathtub, a used basin/bucket, a water storage bucket, a plant pot, a collecting drinking water for livestock, and a used jug. People use buckets to collect rainwater or clean water used for washing dishes. This type of bucket is often uncontrolled, so the potential for it to become a breeding site for mosquitoes is also high. Bathubs are the most frequently found containers (45%). Bathtubs are one of the important needs for storing water in Indonesian society. The water in the bathtub has the potential to be used as a breeding site for mosquitoes, especially if it's not cleaned regularly. The frequency of draining the bathtub is related to the presence of *Aedes* sp mosquito larvae (Mulyani et al., 2022). When draining the bathtub, you must also brush the walls of the bathtub using a brush. Brushing the walls of water reservoirs using soap can cause mosquito eggs to be damaged so that they cannot hatch into larvae and can reduce the decline in the larval population in the environment (Daulay et al., 2024). This is related to the characteristics of *Aedes* mosquitoes' eggs, which require a substrate to stick to. The *Aedes* sp mosquitoes will lay their eggs on a damp surface (such as the wall of a bathtub) just above the surface of the water where they breed (David et al., 2021). Thus, if you drain the tub without brushing the walls, it is possible that the eggs will still stick to the surface of the container and will hatch after being exposed to appropriate water. In terms of container location, there is no difference in the number of positive larva/pupa containers between inside and outside the house. The development *of Ae. aegypti* does not depend on the season (Kahamba et al., 2020). Several types of containers are capable of storing water in the long term. Community awareness and involvement are needed in breaking the life cycle *of Ae. aegypti*. The Mosquito Nest Eradication (MNE) activity, launched by the Ministry of Health of the Republic of Indonesia, is one method of breaking the mosquito cycle. Mosquito Nest Eradication activities include the 3M Plus activities (Draining water reservoirs, closing water reservoirs, and Recycling used objects). Factors that influence PSN behavior include age, education, knowledge, attitudes, support from health workers, and support from village health workers (Sutriyawan et al., 2022).

Based on the results of this research, the chemical method of preventing Dengue Hemorrhagic Fever using the insecticide malathion at a dose of 50 µg/bottle is no longer effective. Further studies are needed to review the vulnerability status of *Ae.aegypti* against other insecticides. *Aedes aegypti* in Aceh, Kapuas, North Banjarmasin, Samarinda, and Polewali are highly resistant to Deltamethrin. *Ae.aegypti* from Kapuas are also highly resistant to permethrin, while *Ae.aegypti* from northern Banjarmasin have very high resistance (Silalahi et al., 2022). Other methods that can be used to control *Ae.aegypti* are through biological control, for example, using Bacillus thuringiensis bacteria, which have been proven to significantly reduce the larval density index (Larval Density Index, LDI) (Sulistiawati et al., 2023). Apart from that, the ovitrap can also be used as an egg trap. Ovitrap containing tap water has proven effective for use as a mosquito egg trap (Nihayah & Purwatiningsih, 2023). The use of the ovitrap tends to be easy for the public to apply, so it has the potential to be used as an alternative mosquito vector control (Sasmita et al., 2021).

CONCLUSION

The habitat of the Culicidae mosquito larvae in this study was in the bathtub, a used basin/bucket, a water storage bucket, used cans, a collecting drinking water for livestock, a fish pond, a plant pot, a former livestock pen, a used toilet, and a used jug. A*edes aegypti* from Sumbersari District, Jember, was resistant to the insecticide Malathion 50 µg/bottle. An increase in the activity of the nonspecific esterase enzyme was proven in this study, so the resistance mechanism is through a physiological mechanism, through metabolic detoxification of insecticides. Various types of larval and pupa-positive containers were found quite high in this research; therefore, the public is advised to be more active in controlling mosquito vectors, especially by destroying places that have the potential to become mosquito breeding sites.

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