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Breeding Site Preferences and Resistance Status of Aedes aegypti in Malang City

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Abstract

Dengue Hemorrhagic Fever (DHF) remains a significant health challenge in urban and semi-urban regions, including Malang City, Indonesia, where Aedes aegypti is the primary vector. This study aimed to identify the breeding site preferences of Ae. aegypti and assess its resistance to 0.8% malathion and 0.05% cypermethrin insecticides. Using a descriptive observational design, ovitraps were deployed in three districts to collect mosquito larvae and eggs, and interviews with residents provided additional data on breeding site preferences. Resistance tests followed WHO guidelines, with mortality rates analyzed after insecticide exposure. Results indicated that, Ae. aegypti larvae were predominantly found in bathroom water tanks (45%) and flower vases (35%). Resistance status revealed geographical variability: Ae. aegypti in District 1 were resistant to cypermethrin, while populations in Districts 2 and 3 were susceptible, with an average status of Ae. aegypti is tolerant to cypermethrin. For malathion, resistance was widespread, particularly in District 3, with an average status of Ae. aegypti to malathion is resistant. These findings suggest that the use of malathion for vector control in Malang is no longer effective, while cypermethrin remains viable under strict monitoring to prevent future resistance. This study underscores the need for targeted insecticide use and regular monitoring to optimize vector control strategies and minimize DHF transmission

Introduction

The incidence of Dengue Hemorrhagic Fever (DHF) continues to rise, especially in urban and semi-urban regions, establishing it as a significant global public health concern (Frank *et al.*, 2024; Lorenz *et al.*, 2020; Yang *et al.*, 2021). Dengue fever is a viral infection caused by the dengue virus and transmitted through mosquito bites (Paz-Bailey *et al.*, 2024). *Aedes (Ae.) aegypti* and *Ae. albopictus* are vectors of dengue fever. Morphologically, the two are very similar but can be distinguished by their morphology, especially in the dorsal thorax, called the scutum. The scutum of *Ae. aegypti* is black with two parallel white stripes in the middle of the dorsal side, flanked by two curved white lines (Azari-Hamidian *et al.*, 2024; Yamany *et al.*, 2024; Yamany & Abdel-Gaber, 2024). *Ae. albopictus* mosquitoes are more commonly found outdoors, in gardens, and in bushes, while *Ae. aegypti* prefers indoor habitats, often resting on hanging clothes (Seang-arwut *et al.*, 2023; Supriyono *et al.*, 2023)

The *Ae. aegypti* mosquito, as the primary vector for dengue fever, is largely attributed to its preference for blood feeding on humans indoors and its selective breeding habits (Bursali & Simsek, 2024; Facchinelli *et al.*, 2023). *Ae. aegypti* tends to lay eggs in indoor water-holding containers, such as water buckets, flower vases, or unclean bathtubs.

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Research by (Dalpadado *et al*., 2022; Mbanzulu *et al*., 2022) indicates that the mosquito's breeding preferences are heavily influenced by the availability of stagnant clean water, which provides an ideal environment for eggs and larvae to develop. If individuals fail to recognize the importance of maintaining cleanliness and monitoring such sites, the potential for increased cases of diseases transmitted by *Ae. aegypti* will rise. Therefore, preventive efforts through regular monitoring of mosquito breeding sites are crucial to reducing the risk of disease transmission (Kolimenakis *et al*., 2021).

Mapping the breeding sites of *Ae. aegypti* mosquitoes can be an effective vector control strategy to reduce dengue fever cases. Preventive efforts also include mosquito breeding site eradication through the program covering, draining, and burying promoted by government (Puluhulawa *et al*., 2023), and the use of chemical insecticides, which are considered more efficient in quickly killing mosquitoes. These efforts will be effective as long as the targeted mosquitoes have not developed resistance to the insecticides being used (Namias *et al*., 2021). The emergence of resistant insects was first reported in 1914 with *Quadraspidiotus pernicious*. Since the first mosquito resistance case was reported in 1947, over a hundred mosquito species have become resistant to one or more insecticides. The development of resistant mosquito strains is triggered by prolonged and ineffective exposure to chemical substances (Dahmana & Mediannikov, 2020). Resistance occurs when mosquitoes develop immune systems capable of countering frequently used insecticides (Suh *et al*., 2023). Studies also highlight the occurrence of cross-resistance, where resistance to one insecticide arises due to exposure to another (Moyes *et al*., 2021). Insecticide sources—such as agriculture, households, industries, healthcare, and others may all contribute to the emergence of resistance (Okeke *et al*., 2022).

Community behavior also plays a significant role in the emergence of resistant species, particularly with the frequent use of spray insecticides. Various types of insecticides are available in Indonesia, such as Malathion, Chlorpyrifos, Cypermethrin, Permethrin, Carbaryl, and acetamiprid. Moreover, 0.8% Malathion and 0.05% Cypermethrin insecticides are the most used in Indonesia (Silalahi *et al*., 2022). Vector control efforts with insecticides are effective only if the targeted mosquitoes have not developed resistance to the insecticides. Research on breeding site preferences and the resistance status of *Ae. aegypti* mosquitoes to Cypermethrin and Malathion has not been conducted in Malang city, Indonesia, making this study crucial for providing recommendations to the government on appropriate insecticide usage. This study aimed to assess the breeding preferences of *Ae. aegypti* in Malang City and evaluate their resistance to 0.8% Malathion and 0.05% Cypermethrin.

Methods

This study uses a descriptive observational research design. The sampling technique used was simple random sampling. The samples consist of *Ae. aegypti* eggs and larvae obtained using ovitraps, distributed in three districts within Malang City, and for the preference of breeding sites, a citizen science based on the interview was used. *Ae. aegypti* eggs were hatched in plastic trays containing ±1000cc of clean water. The larvae that hatch are fed pellets daily. The larvae are maintained until they reach the third instar stage, approximately 2-3 days, after which the fourth instar larvae (pupae) are transferred to trays containing water inside mosquito cages, so that when the pupae transform into mosquitoes, they will be inside the cage (Permana *et al*., 2024). The next step is to acclimatize the mosquitoes until they are 3-7 days old, providing them with a 10% sugar solution as food (Nuryady *et al*., 2024).

In the resistance test, mosquitoes are divided into four tubes, each containing 20-25 female *Ae. aegypti* mosquitoes that are well-fed with sugar and 3-7 days old. The four tubes consist of three replicate tubes containing 0.8% Malathion insecticide and one control tube. The same procedure is performed for the 0.05% Cypermethrin treatment. The mosquitoes are exposed to the insecticide for 60 minutes (with mortality observations at 15, 30, 45, and 60 minutes). After being exposed, the mosquitoes are transferred to holding tubes without insecticide, left for 24 hours in a room with a room temperature around 22°C-25°C, and provided with a 10% glucose solution as food. Subsequently, an analysis is performed by counting the mosquito mortality and interpreting the results according to the WHO guidelines. Observations are made by counting the mosquito mortality and interpreting it according to the WHO guidelines. The criteria are: mortality <80% is resistant, mortality 80-98% is tolerant, and mortality 99-100% is susceptible. Testing must be repeated if mortality in the control group exceeds 20%. The mortality rate of the test mosquitoes is corrected using Abbott's formula (Qibtiyah *et al.*, 2022).

Result and Discussion

Malang City is known for its high incidence of dengue fever (Dengue Hemorrhagic Fever, DBD), with a mortality rate reaching 0.80% over the past decade. One of the factors that correlated with the high incidence of dengue fever is the abundance of the vectors, *Ae. aegypti* (Horta *et al.*, 2014). Based on the results of monitoring the breeding site preferences of *Ae. aegypti* mosquitoes in Malang City, bathroom water tanks are the most common places where *Ae. aegypti* larvae are found (Figure 1).

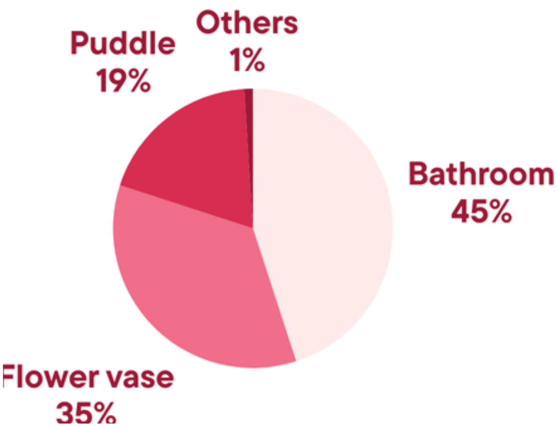

FIGURE 1. Breeding Site Percentage of *Ae. aegypti* in Malang City

The figure illustrates the distribution of *Ae.aegypti* breeding sites, highlighting that bathrooms account for the largest proportion (45%), followed by flower vases (35%), puddles (19%), and other sources (1%). This data aligns with recent research findings emphasizing that the bathroom water tank is a suitable breeding site for mosquitoes. Most bathroom water tanks in Indonesia are made of concrete, which presents a significant potential breeding site for Aedes mosquitoes due to their larger volume (Nurjana & Kurniawan, 2017; Simatupang & Wicharana, 2021). Additionally, these bathroom tanks are rarely cleaned at least once a week, and many are also less exposed to direct sunlight (Prasetyowati *et al.*, 2018). Furthermore, Malang City is also known as an educational hub, with numerous public and private universities, which are attracting a large number of students, and this is accompanied by an increasing number of boarding houses for students. A survey on the breeding sites of *Ae. aegypti* mosquitoes show that bathroom water tanks are still related to the environmental factors found in many boarding houses. The hygiene of boarding houses is generally lower compared to private residences, which results in the highest number of mosquito larvae being found in bathroom water tanks (Agus Nurjana *et al.*, 2023). Besides, flower vases also had a fairly high number of breeding preferences due to the stagnant water in these vases which provides an ideal breeding environment. The water is often not disturbed or changed frequently, creating a perfect habitat for mosquito larvae to thrive, especially in shaded or enclosed areas that limit sunlight exposure and reduce evaporation (Herath *et al.*, 2024). These results show that upcoming plans to control mosquito breeding should focus not only on making the environment cleaner but also on changing the habits of people who live or stay temporarily, like students or those who run boarding houses.

While identifying breeding sites provides an essential foundation for environmentally-based interventions, vector control efforts also rely heavily on the use of insecticides (Chahaya *et al.*, 2024). The success of such chemical control measures, however, depends greatly on the susceptibility of mosquitoes to the compounds being applied (Versari *et al.*, 2021). Therefore, the following section of this study assesses the resistance status of *Ae. aegypti* populations in three districts of Malang City to two commonly used insecticides: 0.05% cypermethrin and 0.8% malathion. To support this, ovitrap

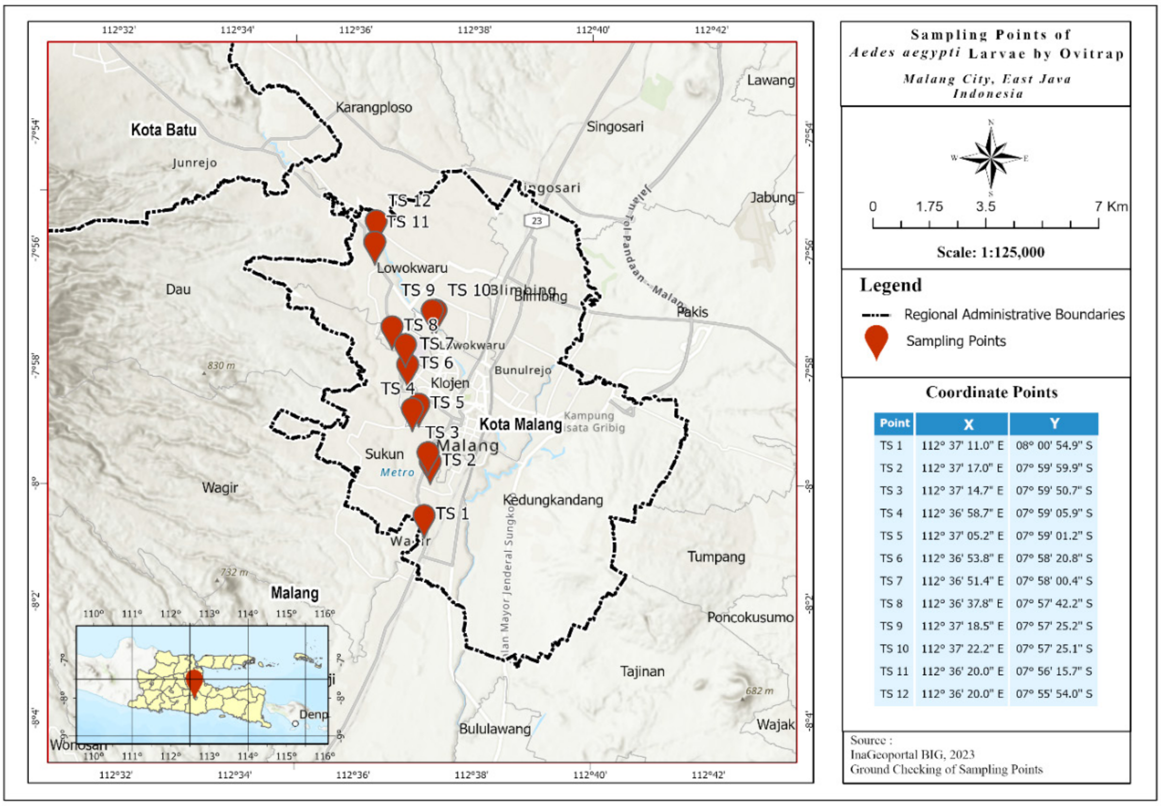

FIGURE 2. Sampling Point of Egg and Larvae of *Ae. aegypti* by Using Ovitrap

sampling was conducted in selected locations based on dengue fever case data, enabling a spatially informed evaluation of both breeding site prevalence and insecticide resistance levels. The distribution of ovitraps sampling for *Ae. aegypti* larvae and eggs were carried out based on dengue fever (DF) case data from the Health Department of Malang (Dinkes, 2019), which consists of three districts. The sampling points in each district varied, with a minimum of three sampling points in each district (Figure 2).

The result of resistance tests for *Ae. aegypti* to Cypermethrin 0.05% (Table 1) revealed differing susceptibilities across districts. In District 1, the mortality rate consistently ranged between 78% and 82%, classifying the population as resistant. In contrast, mosquitoes from Districts 2 and 3 achieved 100% mortality in all repetitions, indicating full susceptibility to the insecticide. This geographical variability might show that different areas have different amounts of fogging, different levels of city buildings, and different ways people use insecticides at home. District 1 is a place with more people and more businesses, so it might have more pressure on insects because people there use insecticides more often at home.

For 0.8% Malathion, the susceptibility results varied more significantly. In District 1, mosquitoes exhibited high mortality rates (94-100%), categorizing them as either susceptible or possibly resistant. In District 2, a similar trend was observed, with a mortality range from 94% to 100%. However, in District 3, the mortality rates were substantially lower (33-53%), confirming resistance. It indicates a concerning level of Malathion resistance in District 3, underscoring the potential need to reconsider its usage in this region (Table 2).

The resistance test results indicate that *Aedes aegypti* in Malang City exhibits a tolerant status to 0.05% cypermethrin. This finding aligns with several previous studies reporting similar outcomes in various regions of Indonesia, including Banyumas, West Lombok, Pekanbaru, Batam, Yogyakarta, and Bitung, where Aedes spp. populations were also categorized as tolerant to cypermethrin

TABLE 1. Resistance Test Results of *Ae. aegypti* Susceptibility to 0.05% Cypermethrin

Testing	Sample (n)	Time (hour)	District 1		District 2		District 3	
			% Death mosquito	Status	% Death mosquito	Status	% Death mosquito	Status
Control	23	1	4		8		0	
Repetition 1	23	1	78	Resistance	100	Susceptible	100	Susceptible
Repetition 2	23	1	82	Resistance	100	Susceptible	100	Susceptible
Repetition 3	23	1	82	Resistance	100	Susceptible	100	Susceptible

*Average mortality 93,6% indicate Tolerant

TABLE 2. Resistance Test Results of *Aedes aegypti* Susceptibility to Malathion 0.8%

Testing	Sample (n)	Time (hour)	District 1		District 2		District 3	
			% Death mosquito	Status	% Death mosquito	Status	% Death mosquito	Status
Control	15	1	0		0		0	
Repetition 1	15	1	100	Susceptible	94	Tolerant	53	Resistance
Repetition 2	15	1	94	Tolerant	100	Susceptible	33	Resistance
Repetition 3	15	1	94	Tolerant	100	Susceptible	40	Resistance

*Average mortality 78,7% indicate Resistance

(Adrianti *et al*., 2024; Handayani, 2023). Meanwhile, the average mortality rate for *Aedes aegypti* exposed to 0.8% malathion was 78.7%, which classifies *Ae. aegypti* in Malang City is resistant to malathion, based on WHO criteria. However, this result is primarily driven by the significantly lower susceptibility observed in District 3, while populations in Districts 1 and 2 remained within the tolerant or susceptible range. It highlights a potential localized resistance in District 3, which substantially influences the overall resistance status. This finding is consistent with previous studies conducted in North Toraja, West Lombok, Magetan, and Jakarta, which also reported malathion resistance among *Ae. aegypti* populations (Ishak & Ponno, 2018; Triana *et al*., 2020).

These resistance patterns should not be interpreted in isolation from ecological behaviors that contribute to their development. One of the key factors influencing the persistence of resistant mosquito populations is the location and nature of their breeding habitats (Herath *et al*., 2024). The observed breeding site preferences, predominantly bathroom water tanks and flower vases, indicate a strong tendency of *Ae. aegypti* to utilize indoor habitats for reproduction. These sites are largely protected from external insecticidal interventions such as fogging, which commonly targets outdoor environments. This ecological behavior may reduce the overall effectiveness of fogging programs and, more importantly, create microenvironments where mosquitoes can survive repeated public health interventions (Lee *et al*., 2020).

However, the variation in insecticide resistance across districts may still be partially explained by the patterns of fogging activity and the types of insecticides used (Dusfour *et al*., 2019). In Indonesia, malathion has been the standard compound for fogging operations for decades, including in Malang (Silalahi *et al*., 2022). It is plausible that District 3, which exhibited the highest level of malathion resistance, has experienced more frequent malathion-based fogging due to higher dengue case reports. Despite the indoor breeding tendencies, adult mosquitoes—once emerged—may still be exposed to fogging if they rest near entryways, windows, or semi-outdoor spaces. Repeated malathion exposure in such scenarios could drive resistance accumulation in that area (Silalahi *et al*., 2024).

In contrast, resistance to cypermethrin was only observed in District 1. This area is known to have higher population density and commercial activity, which may increase the frequency of household insecticide use, including over-the-counter sprays that often contain pyrethroids like cypermethrin. Such unsupervised use can contribute to sublethal exposures, promoting tolerance or resistance. The cumulative effect of breeding in protected indoor containers and inconsistent insecticide exposure—whether via fogging or household use—creates a complex ecological and chemical landscape that fosters resistance in specific zones, despite uniform breeding preferences across the city (Guedes *et al*., 2017).

Insecticide resistance in Aedes mosquitoes, particularly *Aedes aegypti*, is predominantly driven by an interplay of target-site mutations and metabolic detoxification mechanisms. Knockdown resistance (*kdr*) mutations within the voltage-gated sodium channel gene, especially V1016I, F1534C, and V410L (Barrera Illanes *et al*., 2022; Pareja-Loaiza *et al*., 2020). These mutations often co-occur and form tri-locus haplotypes that confer additive or even synergistic resistance effects, with their increasing frequencies documented across diverse geographic regions including the Americas, Africa, and Asia (Akhir *et al*., 2022). Concurrently, metabolic resistance mechanisms, primarily mediated through overexpression and gene amplification of cytochrome P450 monooxygenases, glutathione S-transferases, and esterases, significantly contribute to resistance, particularly against organophosphates such as malathion (Soumalia Issa *et al*., 2024). This metabolic resistance often coexists with target-site mutations, suggesting a multifactorial resistance architecture wherein both mechanisms are essential to fully explain phenotypic resistance.

Moreover, the possibility of cross-resistance between insecticide classes cannot be ruled out. Long-term use of a single insecticide class may select for generalist resistance mechanisms, reducing susceptibility across multiple compounds. It is particularly concerning given the reliance on both malathion and cypermethrin in local vector control programs (Bass & Nauen, 2023).

From a public health policy perspective, these findings emphasize the urgent need for integrated vector management strategies. It is crucial to promote routine insecticide resistance surveillance, diversify the types of insecticides used, and rotate chemical classes to prevent the buildup of resistance (Cahyati & Siyam, 2019). Additionally, public education on proper insecticide use and environmental management should be strengthened, particularly in high-risk zones like Districts 1 and 3. In the long term, establishing entomological surveillance systems that include molecular diagnostics for resistance markers can help authorities track the evolution of resistance and respond with timely interventions. Combining these efforts with behavioral change campaigns targeting breeding site reduction will offer a more sustainable and resilient approach to dengue vector control in Malang City.

Conclusion

This study highlights that *Ae. aegypti* in Malang City primarily breed in bathroom water tanks and flower vases, indicating the need to target these sites in control efforts. Resistance to 0.8% malathion is widespread, especially in District 3, making its use for vector control ineffective. Conversely, 0.05% cypermethrin remains effective in most areas but requires strict monitoring to prevent resistance. These findings emphasize the importance of region-specific vector control strategies and the rotation of insecticides to maintain their efficacy and reduce the risk of resistance development.

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