

Research Article



Entomological Index and Insecticide Susceptibility Status In Dengue-Endemic Areas Yogyakarta

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ABSTRACT

Backgrounds: Dengue Hemorrhagic Fever (DHF) is a disease that receives special attention because of causing outbreaks in some countries, including Indonesia. The problems that often emerge on the DHF Control Program are the high vector density (*Aedes aegypti*) and the vector resistance resulting from continuous insecticide use. This study describes the entomological index and susceptibility status of *Aedes aegypti* against malathion and permethrin insecticides in Sorosutan, Umbulharjo, Yogyakarta.

Methods: This study used a descriptive observational method with a cross-sectional design. An entomological survey was done at 354 houses in Sorosutan, which was randomly selected. The sample of *Aedes aegypti* was obtained through the larval collection and ovitrap installation in 100 places. Susceptibility tests performed using the bioassay method with impregnated paper were based on the WHO standard. This study was conducted from December 2018 – February 2019. The data were analyzed using univariate analysis

Results: The entomological survey in Sorosutan showed the House Index value was 35.03%; Container Index was 16.68%; Breteau Index was 46.33; Density Figure was 5.00, and Larva Free Rate was 64.97%; meaning that Sorosutan had a high level of vector density. So, the risk of DHF transmission was high. The population of *Aedes aegypti* in Sorosutan has been tolerant against Malathion, with 87.83% mosquito mortality.

Conclusion: Sorosutan was an area with high DHF transmission risk. *Aedes aegypti* population in Sorosutan has been tolerant against Malathion.

Keywords: Entomological index; Susceptibility status; *Aedes aegypti*; Malathion

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a disease that receives concern globally because it continues to cause an outbreak in several countries. The incidence of DHF has increased rapidly worldwide in recent decades. It was estimated that as many as 390 million people per year were infected with DHF, of which 96 million people have clinical manifestations with a relatively high level of disease severity. In addition, as many as 3.9 billion people in 128 countries are at risk of being infected with the dengue virus (1).

In Indonesia, dengue has a moderately severe impact socially and economically. Since the discovery of the first case in 1968, there has been an increase in dengue cases and endemic areas of DHF. The high number of dengue cases in Indonesia is related to high population mobility, uncontrol urban growth, climate change, density changes, population distribution, low public awareness to maintain environmental sanitation, and other epidemiological factors that still require further research (2). DHF cases in Indonesia are spread across cities in Indonesia, including Jakarta, Surabaya, Medan, Bandung, and Yogyakarta (3,4).

Yogyakarta City is an endemic area of DHF in Daerah Istimewa Yogyakarta (DIY). The highest incidence of dengue fever in this city in 2017 was in Sorosutan village, with an Incidence Rate of 20.6 per 100,000 population (5). Sorosutan village has the highest population compared to 44 other villages in Yogyakarta City. With this condition, there is a potential for an increased risk of dengue transmission in the area.

Efforts to treat DHF so far are only based on the symptoms of the disease. However, in a situation where there is no specific dengue medicine, it is necessary to take effective countermeasures to reduce the incidence of dengue fever so that it does not continue to increase. Vector control is the primary prevention effort that has been and is still being carried out by the government to break the chain of transmission of dengue fever (6). In addition, monitoring *Aedes* sp larvae and the breeding place is also continuously carried out.

Vector density can be an indicator of the success of a DHF control program. The density of *Aedes* sp can be identified using the larva free rate (LFR), House Index (HI), Container Index (CI), Breteau Index (BI), and Density Figure (DF). The density Figure is a combination of HI, CI, and BI (7). Besides Mosquito Nest Eradication or PSN in Indonesia, chemical control is also carried out by applying fogging and household insecticides. Malathion is a chemical for fogging that has been used in the Dengue Hemorrhagic Fever eradication program in Indonesia since 1973. This insecticide is one of the safe and cheap ones widely used in households (8). The use of Malathion as a fogging solution in Yogyakarta has been substituted with cypermethrin since 2016. DHF control by fogging is only carried out if cases are found and transmission occurs in the area (9). Permethrin is one of the chemicals contained in over-the-counter household insecticides (10). The danger of using chemicals continuously can cause resistance to the dengue vector. Vectors that have been resistant when they reproduce will produce offspring that have resistant genes. The higher the frequency of individual resistant vectors, the rate of resistant development will be faster. Accordingly, the population will be dominated by resistant vectors. Insects such as *Aedes aegypti* can maintain and pass resistance traits to their offspring for a long time (11,12)

Resistance to Malathion has been reported in several areas in Indonesia, including Simbangkulon Village, Buaran District, West Kedungwuni Village, Kedungwuni District, and Karang Sari Village, Karang Anyar District, Pekalongan District (13). In the Special Region of Yogyakarta (DIY), *Aedes aegypti* resistance to Malathion was reported in Panggunharjo Village, Bantul Regency, Plosokuning Minomartani, and in several hamlets in Gamping District, Sleman District (14). Malathion as a fogging chemical has not been followed by evaluating sensitivity to *Aedes Aegypti* in the Sorosutan area. This study aimed to determine the entomological index and susceptibility status of *Aedes aegypti* to malathion insecticides in Sorosutan Village, Umbulharjo District, Yogyakarta City.

METHOD

This research was a descriptive observational study. An entomological survey was conducted in 354 houses in Sorosutan Village. Mosquito samples were collected by collecting larvae and installing ovitraps in 100 purposively selected houses. House used for placing ovitrap should fulfill criterias: have a positive larval house, have 100 m distance previous ovitrap installed, and a house with poor lighting. Mosquito breeding was carried out at the Test Animal Maintenance Laboratory, Universita Ahmad Dahlan. Vulnerability testing was carried out using impregnated paper according to WHO standards. Impregnated paper with 5% malathion content was obtained from Universiti Sains Malaysia (USM), Penang, Malaysia, a WHO Collaborating Centre.

Mosquito for testing purposes was prepared by incubating *Aedes aegypti* eggs from ovitrap installation. The hatched larvae were reared by feeding chicken liver powder, and water was distorted every two days. Larval rearing was carried out until they became adults of the first generation (F1). Mosquitoes used as samples for susceptibility testing were *Aedes aegypti* adult females aged 3-5 days with sugar-water satiety conditions.

The susceptibility test was carried out on treatment tubes containing impregnated paper and control (without impregnated paper). Each tube was filled with 25 mosquitoes exposed to impregnated paper for one hour. Then, the mosquitoes were transferred to the storage holding, observed the number of mosquitoes that fainted, and the number of deaths recorded after 24 hours. The test was carried out three times on each 5% impregnated paper malathion. Vulnerability testing was carried out at the Entomology Laboratory of Universitas Ahmad Dahlan. Vulnerability status was obtained from the number of mosquito deaths which were then categorized as vulnerable (98% – 100%), tolerant (80% – 97%), and resistant (< 80%) (15). The research was conducted in December 2018 – February 2019.

RESULTS

Sorosutan Village entomological index

The entomological index was obtained from an entomological survey in 18 RWs in the Sorosutan Village, Yogyakarta.

Table 1. Sorosutan Village Entomology Index December 2018 – January 2019

No.	Entomology Index	Results
1	House Index (HI)	35.03%
2	Container Index (CI)	16.68%
3	Breteau Index (BI)	46.33
4	Density Figure (DF)	5.00
5	Larva Free Rate (LFR)	64.97%

Table 1 shows that the HI, CI, and BI values in Sorosutan Village are pretty high, namely the HI of 35.03%, CI of 16.68%, and BI of 46.33. The combination of the three indicators produces a DF value of 5.00. In addition, the larva-free rate (ABJ) obtained is relatively low, namely 64.97%.

Status of *Aedes aegypti* susceptibility to Malathion

The following are the susceptibility test results to the malathion insecticide carried out on the *Aedes aegypti* mosquito from the Sorosutan Village area. Table 2 shows that the population of *Aedes aegypti* in Sorosutan Village is tolerant to malathion insecticide with an average mortality of 87.83%.

Table 2. Vulnerability Status of *Aedes aegypti* Population in Sorosutan Village to Malathion and Permethrin Insecticides

Insecticide	Average Mosquito Mortality (%)	Vulnerability Status
Malathion	87.83	Tolerant

DISCUSSION

Based on the results of the entomological survey, the inspected houses and containers can be calculated values that are indicators of vector density and the risk of dengue transmission in the area, namely the House Index (HI), Container Index (CI), Breteau Index (BI), Density Figure (DF), and Larvae Free Rate (LFR). The HI value is 35.03%, and the CI is 16.68%. HI, values > 5% and CI > 10% indicate that the Sorosutan area has a high risk of DHF transmission. Meanwhile, based on the BI value obtained, 46.33, where the value is not more than 50, the transmission of DHF in the Sorosutan area is not included in the high category (16). Based on the DF value, which is 5, it was indicated that the risk of DHF transmission in the Sorosutan area is in the moderate category. The WHO stated that the HI value > 5% and/or BI > 20 for each region indicates that the region is sensitive to dengue fever. Accordingly, Sorosutan was categorized as a sensitive area to dengue fever (17).

This result aligns with a study in the Mamuju region, Sulawesi, which shows a larva-free rate of 62.7% (July – August 2015) (18). The larva-free rate nationally for 2016-2019 showed 67.6%, 46.6%, 31.5% and 79.2%, respectively. This shows that areas in Indonesia are at risk of dengue transmission. From 2010 to 2019, there was an increase in the area of dengue cases in Indonesia. In 2019, 93.58% of the area in Indonesia was infected with dengue fever. While in DIY during 2019, the incidence rate was more than 49 per 100,000 population (85.9 per 100,000 population) (19).

Poor entomological indicators lead to a high risk of dengue transmission. This is because knowledge, behavior, and community participation to be directly involved in Mosquito Nest Eradication (PSN) activities are still inadequate (20,21). This can be seen from the results of the larva-free rate (LFR) in Sorosutan of 64.97%, which was still far from the national target according to the Indonesian Ministry of Health (95%). The low larva-free rate indicates that the PSN program in Sorosutan was still lacking. This is in line with research that states a relationship between PSN's actions and the presence of larvae at Pulang Pisau Port with a p-value of 0.000. Respondents who have bad actions have a 3.89 times greater risk of larvae than respondents who have good actions (22). Some studies have shown a relationship between mosquito density and high dengue cases (23,24).

Community participation is needed to implement vector control mostly influenced by the community's knowledge, attitudes, and behavior (21). The implementation of vector control can be carried out through environmental management called integrated vector management

(IVM). IVM combines mosquito breeding site elimination and chemicals by administering larvicides at mosquito breeding sites and fogging to kill adult mosquitoes that are implemented simultaneously (23). IVM was proven by a study in Makassar that reduced the incidence of dengue (24).

Based on the susceptibility test to malathion insecticide, it was found that the population of *Aedes aegypti* in the Sorosutan village area was in the tolerant category. Malathion is an organophosphate insecticide. The way these insecticide works is by inhibiting the enzyme cholinesterase (AChE) in nerve cells. This enzyme works to hydrolyze acetylcholine into acetate, and choline—acetylcholine functions as a neurotransmitter in the synaptic cleft. When the organophosphate is sprayed on the target organism, the AChE enzyme cannot work normally. Organophosphates phosphorylate the enzyme into a stable component form. So that acetylcholine cannot be decomposed in the postsynaptic (25). The research results in Plosokuning Yogyakarta showed that *Aedes aegypti* in the area was already resistant, increasing the esterase enzyme activity. Esterase enzymes detoxify organophosphates, carbamates, and pyrethroid synthesis by hydrolyzing ester bonds and binding pesticides to the active esterase site (26).

Resistance can arise due to the use of chemicals continuously for a long time and be given to the next generations of these mosquitoes. We found that in the household in Sorosutan, most of them applied the insecticide daily and continuously. *Aedes aegypti* is able to be given the resistant traits to their offspring for a long time (11). A study in the United States showed that the population of *Ae. albopictus* in Florida and New Jersey were found to be resistant to Malathion. The study also found significant differences in the activity of detoxification enzymes which suggest a metabolic-based resistance mechanism. Malathion-resistant populations have increased esterase and glutathione S-transferase enzymes (27). The presence of esterase enzyme activity in the mosquito population will resist other insecticides with ester groups. Therefore, in areas with mosquito populations resistant to Malathion, it is advisable to choose an active ingredient not from the organophosphate group and has a different mechanism of action from Malathion (13).

The results of this study are in line with research conducted by Sukmawati that the *Aedes aegypti* population originating from Makassar City is tolerant to 5% malathion with an average mortality of 87% (28). Tolerance status can become resistant to Malathion if the insecticide is used continuously without rotation. The area reported to be resistant to Malathion is Panggungharjo Village, Bantul, with a 0% mortality percentage (100% resistance) (14). In Tomohon City with 0% mosquito mortality (29). In Semarang, resistance was reported at Tanjung Emas Port with mosquito mortality in the buffer area of 13% and perimeter of 20% (30). In Pekalongan District, Central Java, the mortality of the test mosquitoes ranged from 13.75-25% (14). East Jakarta has 53% mosquito mortality, West Jakarta 75%, and South Jakarta 53% (31). In Banten province, including South Tangerang, mosquito mortality was 73.9%, Serang was 46.9%, and Cilegon was 60.2% (32). In Ambon City, Maluku Province, resistance was reported in the Yos Sudarso Port area and the Nusantara Fisheries Port with 20% mosquito mortality, at Pattimura Airport with 16% mosquito mortality in the perimeter area and 20% in the buffer area (33).

Resistance to a type of insecticide is generally based on three mechanisms: a decrease in target site sensitivity, changes in the cuticle layer that can reduce the penetration of

insecticidal compounds into the insect's body, and an increase in detoxification enzymes. The mechanism of resistance can be determined by carrying out biochemical tests or molecular tests on vectors. Biochemical test to see the activity of enzymes that detoxify insecticides (metabolic resistance) as the basis for the occurrence of insect resistance to insecticides. Meanwhile, molecular tests were carried out to detect insect resistance to insecticides by finding the point mutation of the voltage-gated sodium channel (VGSC) gene as the target site of the insecticide workplace. The research results in Palembang showed that based on biochemical tests, there is no increase in the level of insecticide detoxifying enzymes that neutralize synthetic pyrethroid insecticides. Resistance is suspected due to a decrease in the sensitivity of the target site. This is evidenced by molecular tests showing mutations in the VGSC gene in *Aedes aegypti* (34).

The Yogyakarta City Government has substituted Malathion as an active insecticide ingredient for fogging with cypermethrin since 2016. This step was an effort to prevent further resistance from occurring. However, the use of cypermethrin without *Aedes* susceptibility testing is not appropriate because resistance has been reported in the Yogyakarta city area to cypermethrin and permethrin in 2010 (35). Several regions have stopped using malathion insecticide because resistance has occurred; after 5 - 10 years of retesting, the results still show resistance to the insecticide (36). Cypermethrin is a synthetic pyrethroid insecticide like permethrin. Permethrin is a third-generation pyrethroid, and cypermethrin is a fourth-generation pyrethroid. In areas where resistance to permethrin is found, replacing (rotate) with other insecticides that work differently from pyrethroids is recommended. This method can prevent or reduce permethrin resistance to a higher level and not spread to other areas in Indonesia (37). If cypermethrin is used continuously, it can pose a threat of resistance in the future. Efforts that can be made to reduce the potential for insecticide resistance in an area are by rotating the active ingredients of the insecticides used. Changes in the type and workings of insecticides for vector control must be carried out within a maximum of 2 – 3 years or 4 – 6 times of application. However, the change can be accelerated according to monitoring the vulnerability status (8).

In addition to organophosphate and pyrethroid insecticides, carbamate insecticides can also be used for vector control by misting (8). However, the mechanism of toxicity of carbamate insecticides is the same as that of organophosphates, namely by inhibiting the action of the acetylcholinesterase enzyme so that it undergoes carbamylation. Carbamates work by binding to the enzyme acetylcholinesterase, which functions to hydrolyze acetylcholine. The binding of the acetylcholinesterase enzyme causes a buildup of acetylcholine which causes the nerves to be continuously stimulated, causing tremors and other uncontrolled movements (9). This means that carbamate insecticides will not be effective when used on *Aedes aegypti* populations resistant to organophosphates due to increased acetylcholinesterase enzymes. Testing the sensitivity of the acetylcholinesterase enzyme in the biochemical microassay method using propoxur (a carbamate insecticide) to help detect the activity of detoxification enzymes involved in the resistance mechanism of mosquito larvae temephos (an organophosphate insecticide). A study showed that decreased acetylcholinesterase sensitivity also indicated the emergence of resistance to organophosphates and carbamates in the *Aedes albopictus* population from Selangor Malaysia (38).

As part of an effort to reduce the incidence of Dengue Hemorrhagic Fever, insecticides (chemical control) should be an option to control mosquito vectors. Environmental-based

control with PSN is the main activity that must always be carried out to suppress vector populations. The use of insecticides cannot continue to be a foundation because it has an unfavorable impact on the environment. Therefore, environmental-based prevention and control efforts are the main focus, and the community's active role is needed.

CONCLUSION

Sorosutan village, Yogyakarta City, has a high risk of transmission of Dengue Hemorrhagic Fever (DHF). The population of *Aedes aegypti* in the Sorosutan Village area was tolerant to malathion insecticides, with mosquito mortality of 87.83%. Mosquito nests by being independent larvae monitor in their respective neighborhoods and consistently applying 3M were the three Ms stands for Menutup, Menguras, and Mengubur, meaning covering water containers, cleaning water containers, and burying discarded containers to avoid DHF. The main focus of dengue control is on environmental management by eliminating the breeding place for *Aedes* sp.

Authors' contribution

FDA prepared the manuscript draft and research funding. AA was responsible for data collection and reviewed the manuscript.

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Conflict of interest

There is no conflict of interest in this research.

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