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ORIGINAL ARTICLE

Resistance Test of Aedes aegypti Mosquito Larvae Against Organophosphate Insecticides at Medan Selayang

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Abstract: Aedes aegypti is the main vector in several diseases such as Dengue Hemorrhagic Fever (DHF).Various types of efforts to control Aedes aegypti were carried out to terminate the transmission chain by spraying (organizing) the organophosphate insecticide. The use of irrational insecticides causes an increase in esterase enzymes, giving rise to the resistance of Aedes aegypti to insecticides. The purpose of this study is to determine the resistance status of Aedes aegypti larvae to organophosphate insecticides in Medan Selayang. The type of research used in this study is descriptive research with cross-sectional methods, using 276 larvae of Aedes aegypti. The results of this study are mosquito larvae that are susceptible (sensitive) to organophosphate insecticides by 66.3%, while mosquito larvae that are tolerant (moderate resistance) of organophosphate insecticides are 33.7% and resistant (high resistance) mosquito larvae were not found. There is 33.7% of Aedes aegypti larvae are tolerant (moderate resistance) to organophosphate insecticides and resistant (high resistance) mosquito larvae were not found.

Keyword: Aedes aegypti, Insecticides, Organophosphates

INTRODUCTION

Aedes aegypti mosquito is the main vector in several diseases such as Dengue Hemorrhagic Fever (DHF), Yellow Fever, Chikungunya Fever, and Zika Fever. *Aedes aegypti* mosquito species has an important role related to the environmental health.[1,2] *Aedes aegypti* mosquitoes have diurnal properties, during the day the female mosquitoes suck blood for the maturation of their eggs so that endemic areas where the average community works during the day has the potential to become a place of transmission.[3]

The World Health Organization (WHO) classifies DHF as an infectious disease that continues to increase because the geographical spread of this disease continues to spread and the increasing number of affected populations. World Health Organization (WHO) has estimated that 50-100 million people have been infected every year in developing countries ranging from 1% -2.5% so that every 100 cases of DHF will get 1-3 people to die from DHF.[4]

Dengue Hemorrhagic Fever (DHF) is one of the vector infectious diseases that most often causes epidemics in an area in Indonesia. Indonesia is a country with the

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highest dengue cases in Southeast Asia since 1969-2009. In 2011, Indonesia recorded 24,352 cases with 196 deaths.[5,6,7]

Various efforts have been made to control the *Aedes aegypti* mosquito, but have not yet reached maximum results. Control efforts are primarily intended to break the chain of transmission, namely by eradicating adult mosquitoes by spraying (fogging) insecticides. Until now, fogging is still often found that is not on target, not sustainable, does not refer to information about vectors, and even incorrect doses. So things like this can trigger insecticide resistance in *Aedes aegypti* mosquitoes in some areas.[8]

Insecticides are divided into organic insecticides (organic insecticides derived from nature) and synthetic organic insecticides. Synthetic organic insecticides consist of organic chlorine groups (DDT, curtains, BHC, dieldrin, linden), organic phosphorus groups (parathion, malathion, temephos, diazinon, DDVP, fenitrothion, dipterex), organic nitrogen (dinitrophenol), sulfur (parathion, malathion, temephos, diazinon, DDVP, fenitrothion, dipterex) groups, organic nitrogen (dinitrophenol), sulfur (parathion, malathion, temephos, diazinon, DDVP, fenitrothion, dipterex) groups, organic nitrogen (dinitrophenol), sulfur groups (parathion, malathion, temephos, diazinon, DDVP, fenitrothion, dipterex), organic nitrogen (dinitrophenol), sulfur (parathion, malathion, temephos, diazinon, DDVP, fenitrothion, dipterex) group) and the thiocyanate group. The types of insecticides commonly used in vector control include organophosphate, carbamate, and pyrethroids. In controlling DHF, insecticides which are often used are organophosphate groups that work by inhibiting the enzyme cholinesterase, namely malathion, and temephos.[9]

An organophosphate is an insecticide group that is commonly found in households in various regions in Indonesia, which is 30% of the total insecticide. Organophosphate insecticide works on the enzyme acetylcholinesterase which functions to control the hydrolysis of acetylcholine, a neurotransmitter produced in axon vesicles. Acetylcholine by the acetylcholinesterase enzyme will be hydrolyzed into choline and acetic acid after the impulses are passed on. When acetylcholinesterase is not present, the resulting acetylcholine will accumulate resulting in impaired impulse transmission which can cause a decrease in muscle coordination, convulsions and death.[10]

One mechanism of resistance occurs when there is an increase in the amount of the esterase enzyme. Insecticides that have ester bonds can be hydrolyzed by the esterase enzyme. Organophosphates are esters of phosphoric acid so metabolic mechanisms are considered as the main mechanism of resistance to organophosphates. Increased esterase enzymes are the most commonly known mechanism of metabolic resistance.[11,12]

METHODS

This type of research is descriptive research with a cross-sectional method. This research is about determining the status of resistance of *Aedes aegypti* larvae from endemic areas of dengue hemorrhagic fever, namely Medan Selayang District in Medan City against organophosphate insecticide by biochemical tests.

Aedes aegypti mosquito larvae samples were tested for 276 larvae (stage instar III-IV) collected using a reservoir containing clean water as a breeding place for mosquito larvae. Retrieval of *Aedes aegypti* mosquito larvae directly from the breeding place (houses in Medan Selayang District), aims to avoid mosquito larvae, not from the same broodstock, so they do not have the same resistance to the insecticide. The identification of *Aedes aegypti* mosquito larvae was carried out at the Laboratory of Parasitology at the Faculty of Medicine, University of Muhammadiyah North Sumatra.

Data collection techniques are based on the results of *Aedes aegypti* mosquito larvae

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testing on organophosphate groups with biochemical tests.

Susceptibility/bioassay tests were carried out on all mosquito larvae that had been collected using temephos 6.25 mg/L that had been previously dissolved with 249 ml of distilled water to obtain a concentration of 0.02 mg/L (according to WHO standards). Temephos is one of the organophosphate class insecticides. *Aedes aegypti* mosquito larvae were put into a beaker glass containing temephos 0.02 mg/L and allowed to stand for 1 hour. After 1 hour the larvae are soaked and allowed to stand, visible dead larvae and larvae that are still alive. Dead larvae are collected and counted how many dead larvae, as well as surviving larvae, are collected and counted. Dead larvae are considered to be susceptible (sensitive) larvae to organophosphate insecticides. To prove whether the surviving larvae are tolerant (moderate resistance) or resistant (high resistance), a biochemical test is carried out, namely the activity test of the acetylcholinesterase enzyme.

The acetylcholine esterase enzyme activity test was performed using QuantiChrom™ Acetilcholin Esterase Assay Kit. In the bioassay, the kit contains an assay buffer, reagent, and calibrator. Instar III-IV mosquito larvae are crushed individually to be made homogenate and dissolved with 0.5 ml of 0.1 M phosphate buffer saline (PBS) solution, pH = 7.5. Then centrifuged at 14,000 rpm for 5 minutes. The supernatant is then transferred to the microplate using a micro-pipette for inspection. The reagent was prepared by adding 200 ul of the assay buffer to 2 mg of the reagent, then diverted it to dissolve. Insert 190 ul of the reagent mixture into each well containing the supernatant on the microplate, tapping briefly to mix. Insert the microplate into the Elisa reader, read Optical Density (OD), or also called Absorbance Value (AV) at a wavelength of 412 nm at minute 2 and minute 10. AV value is read from the 10th minute OD. The level of the acetylcholine esterase enzyme was calculated using the following formula :

$$AchE\ Activity = \frac{OD_{10} - OD_2}{OD_{kal} - OD_{H_2O}} \times n \times 200\ ({}^{U}/_{L})$$

Information :

- OD2 and OD10 are absorbance values that are read at minute 2 and minute 10
- ODcal and ODH2O are OD at calibration and H2O is read at 10 minutes
- n is the dilution factor because it is not diluted then n = 1
- The number 200 is the Equivalent Activity of the calibrator under test conditions

Data analysis was performed by looking at the results of the Absorbance Value which will be determined quantitatively by ELISA Reader at a wavelength (λ) of 412 nm. Quantitative enzyme activity was then read with the Elisa reader at a wavelength (λ) of 412 nm. Resistance status can be seen from the results of the color intensity reading on the Elisa reader if the Absorbance Value (AV) <0.102 = vulnerable, AV 0.102 - 1.254 = tolerant and AV> 1.254 = resistant.

RESULTS

Resistance testing of *Aedes aegypti* mosquito larvae against organophosphate insecticide was carried out on 276 *Aedes aegypti* mosquito larvae that had been collected previously.

From table 1, it can be seen from 276 *Aedes aegypti* mosquito larvae that were tested using temephos 0.02 mg/L, found 183 dead larvae and 93 live larvae. The larvae of the dead *Aedes aegypti* mosquitoes are larvae that are vulnerable (sensitive) to

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organophosphate insecticides. While the larvae that live are larvae that are resistant (high resistance) to organophosphate insecticides. To prove whether the surviving larvae are tolerant (moderate resistance) or resistant (high resistance), a biochemical test is carried out, namely the activity test of the acetylcholinesterase enzyme. The surviving larvae are then put into a measuring cup containing clean water for further testing of the activity of the acetylcholinesterase enzyme.

Table 1. Bioassay Test Results for *Aedes aegypti* Larvae

Jenis Larva	Jumlah	Persentase	Kerentanan
Larva hidup	93	33,7	Toleran/ Resisten
Larva mati	183	66,3	Rentan
Total	**276**	**100**	

The measurement results of the Absorbance Value (AV) of *Aedes aegypti* larvae in Medan Selayang District are as follows:

Table 2. Test Results for The Acetylcholinesterase Enzyme Activity in *Aedes aegypti* Larvae

Status Resistensi	Jumlah	Persentase
Rentan*	183	66,3
Toleran**	93	33,7
Resisten***	0	0
Total	**276**	**100**

*= sensitive; **= resistensi sedang; ***= resistensi tinggi

In table 2 it can be seen that the results of examinations carried out on 93 mosquito larvae of *Aedes aegypti* showed that all (100%) mosquito larvae were included in the group tolerant to organophosphate insecticides and none were found (0%) of mosquito larvae belonging to the organophosphate insecticide-resistant group.

The overall status of resistance of mosquito larvae examined is as follows:

Table 3. Status of Resistance of *Aedes aegypti* Larvae in Medan Selayang District

Status Resistensi	Jumlah	Persentase
Rentan*	183	66,3
Toleran**	93	33,7
Resisten***	0	0
Total	**276**	**100**

In table 3 it can be seen that the resistance status of *Aedes aegypti* larvae collected from several houses in Medan Selayang District, namely mosquito larvae that are susceptible to organophosphate insecticides is 183 (66.3%) larvae, whereas mosquito larvae that are tolerant to organophosphate insecticides are 83 (33.7%) larvae and larvae of resistant mosquitoes were not found.

DISCUSSIONS

This study used *Aedes aegypti* mosquito larvae collected from several houses in Medan Selayang Subdistrict, which is one of the areas endemic to dengue hemorrhagic fever in the city of Medan. Research on the status of resistance of *Aedes aegypti* larvae in the Medan Selayang Subdistrict has never been examined before. This can be used as baseline data for other researchers as a comparison by looking at the status of resistance of *Aedes aegypti* larvae against organophosphate insecticides. Based on the results of research conducted on 276 mosquito larvae, it is known that 33.7% of *Aedes aegypti* mosquito larvae are tolerant of organophosphate class insecticides.

The occurrence of resistance to insecticides in insects is influenced by several factors. Genetic factors are genes that encode the formation of the esterase enzyme, which can cause insect resistance to insecticides. Biological factors include biotics (the existence of monogamous or polygamous marriages, the change of generations and at the end of the

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development of each generation of natural insects), the behavior of insects such as migration, isolation, monophagy or polyphagy and the ability of insects to protect against danger or behavioral changes. Operational factors, including chemicals used in vector control and insecticide applications in the form of application methods, frequency and duration of use.[9]

Resistance can occur with a mechanism of decreased sensitivity to the nervous system and the activity of the enzyme acetylcholinesterase in the insect's body. The existence of resistance can also be caused by cross-resistance with other insecticides with the same target site, namely acetylcholinesterase. Resistance occurs due to the ability of insects to modify the cuticle or lining of the digestive tract so as to prevent/slow the absorption of insecticides. In addition, there is the ability of insects to avoid the deadly effects of insecticides by changing behavior in response to insecticide spraying.[11,12]

Organophosphate insecticide works on the enzyme acetylcholinesterase which functions to control the hydrolysis of acetylcholine, a neurotransmitter produced in axon vesicles. Acetylcholine by the acetylcholinesterase enzyme will be hydrolyzed into choline and acetic acid after the impulses are passed on. When acetylcholinesterase is not present, the resulting acetylcholine will accumulate resulting in impulse transmission interruption which can cause decreased coordination of muscles, convulsions, and death. If there is a mechanism of the decreased sensitivity of the enzyme acetylcholinesterase then there is also a decrease in the sensitivity of insects to insecticides so that later insects will become resistant to insecticides.[10]

Based on research on-resistance of *Aedes aegypti* to organophosphate insecticides, it states that the use of organophosphate insecticides for a long time and with sublethal doses will induce resistance to the active ingredients.[13] In general, the mechanism of resistance is based on physiology and genetics through the thickening of the cuticle, metabolic mechanism, and changes in the target side. From the whole mechanism, the metabolic mechanism is the main mechanism of *Aedes aegypti* resistance to organophosphate. Where there is an increase in the activity of the esterase enzyme which will hydrolyze the organophosphate before reaching the target side of acetylcholinesterase.

Based on other studies of the status of *Aedes aegypti* resistance to organophosphates in three DKI Jakarta area, it shows that the susceptibility status of *Aedes aegypti* in all study areas has been resistant to organophosphate insecticides. The same resistance status with different location and time of collection with previous studies shows that the condition of mosquitoes is increasingly developing their habitat and more and more individuals are resistant to organophosphate insecticides.[14]

In this study it was found that about 33.7% of mosquito larvae have shown to be tolerant of organophosphate insecticides, so it needs to be prevented from spreading. Ways that can be done to overcome the problem of *Aedes aegypti* mosquito larvae that are tolerant of organophosphate insecticides so that they do not become resistant, it requires controlling the use of insecticides in a directed and controlled manner. Periodically it can be done by replacing organophosphate insecticides with other insecticides that do not contain ester groups such as pyrethroid and biopesticides. The use of Bacillus thuringiensis israeliensis (Bti) as a bacterial compound is also reported to be effective in controlling larvae and as a

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control for dengue hemorrhagic vector (DHF) for both larvae and adult mosquitoes is a good alternative for the use of chemical insecticides reduced or eliminated.[8,16]

The common method is to eradicate the habitat (nest) of mosquitoes through the simultaneous movement of draining a tub of water, closing potential places to breed nests, burying used goods that can hold water. Abatization water reservoirs such as bathtubs, ponds, water pots have been carried out. Conceptually the movement to eradicate mosquito nests once a week is sufficient to cut the life cycle of the mosquito.[13,16]

Control using natural predators *Aedes aegypti* larvae such as betta fish (*Ctenops vittatus*), lead fish (*Panchax panchax*) and other ways that can reduce the number of mosquitoes that bite humans.[8,13]

Individual physical-mechanical control can be carried out using repellent, using long-sleeved clothing and long pants, also by installing mosquito nets at bedtime and anti-mosquito gauze.[8]

CONCLUSIONS

From the results of research and analysis of resistance testing of *Aedes aegypti* larvae against organophosphate insecticides in Medan Selayang District, it was concluded that 66.3% of *Aedes aegypti* mosquito larvae are susceptible to organophosphate insecticides, 33.7% of *Aedes aegyti* mosquito larvae tolerant of organophosphate insecticides, and there were no (0%) *Aedes aegypti* mosquito larvae that were resistant to the organophosphate group insecticide.

REFERENCES

1. Hadi UK, Soviana S. Ektoparasit: Pengenalan, Identifikasi dan Pengendaliannya. Bogor (ID): IPB Press. 2010.
2. Djunaedi D. Demam Berdarah Dengue (DBD) Epidemiologi, Imunopatologi, Patogenesis, Diagnosis dan Penatalaksanaanya. Malang : UMM Press. 2006.
3. CDC. Dengue home page entomology & ecology. 2014 (diunduh 29 November 2014). Tersedia dari: http:// www. cdc. gov/ dengue/ entomology Ecology/ m_lifecycle.html.
4. Kemenkes RI. DBD di Indonesia tahun 1968−2009. *Buletin Jendela Epidemiologi*. 2010;2: 1−14.
5. Permatasari W.A. et al. Uji Resistensi Nyamuk *Aedes aegypti* terhadap Insektisida Transflutrin 25% dan Metoflutrin 3,5%. 2015.
6. World Health Organization. Dengue outbreak in the maldives. 2011. Available from: http:// www. searo. who. int/ entity/ emerging diseases/ links/ dengue_outbreak_maldives_2011/en/.
7. Kementrian Kesehatan Republik Indonesia; Dit PPBB, Ditjen PP dan PL, Subdirektorat Pengendalian Arbovirus. Informasi umum demam berdarah *dengue.* Jakarta; 2011. Availablefrom: http:// www. pppl. depkes. go. id/ _asset/ _download/ INFORMASI_UMUM_DBD_2011.pdf
8. Kementerian Kesehatan RI. Demam Berdarah Dengue. *Buletin Jendela Epidemiologi*. 2010;2:48.
9. Pradani FY, Ipa M, Marina R, Yuliasih Y. Penentuan Status Resistensi *Aedes aegypti* dengan Metode Susceptibility di Kota Cimahi terhadap Cypermethrin. Jurnal Vektora. 2011;3 (1): 35-43
10. Lesmana SD. Resistensi *Aedes aegypti* terhadap Insektisida

E-ISSN: 2528-410X

Golongan Organofosfat. Jurnal Ilmu Kesehatan. 2010;4(1):10-13.

11. Firmanta Y. Deteksi Resistensi Nyamuk *Aedes aegypti* yang Berasal Dari Daerah Endemis Dan Non Endemis *Dengue* di Kota Jambi Berdasarkan Aktivitas Enzim Esterase Non Spesifik Terhadap Insektisida Golongan Piretroid. 2008.
12. McCaffery A, Nauen R. Prevention and Management of Insecticide Resistance in Vectors and Pests of Public Health Importance. 2006. http://www.irac-online.org/documents/vectormanual.pdf, diakses pada tanggal 19 September 2007
13. Suyanto. Hubungan Pengetahuan dan Sikap dengan Praktek Pengendalian Nyamuk *Aedes aegypti* di Kelurahan Sangkrah Kecamatan Pasar Kliwon Kota Surakarta.Jurnal Kesehatan. 2011; 4 (1): 1-13
14. Prasetyowati H, Hendri J, Wahono T. Status Resistensi *Aedes aegypti* (Linn.) terhadap Organofosfat di Tiga Kotamadya DKI Jakarta. 2016:23-3
15. Widiastuti D, Sunaryo. Aktivitas Enzim Esterase Pada Populasi Nyamuk *Aedes aegypti* Terhadap Malation di Tiga Kabupaten di Provinsi Daerah Istimewa Yogyakarta. Jurnal Ekologi Kesehatan.Yogyakarta. 2018;16 (3):150-157.
16. Supartha, IW. Pengendalian Terpadu Vektor Virus Demam Berdarah Dengue, *Aedes aegypti* (Linn .) dan *Aedes albopictus* (Skuse) (Diptera: Culicidae). Makalah disampaikan dalam Seminar DiesUnud 2008. 2008;(September):1-18.