A Study of Aedes aegypty Susceptibility Against Cypermetrin at Elementary Schools Yogyakarta

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ABSTRACT

Introduction: Dengue Hemorrhagic Fever is transmitted by *Aedes aegypti* mosquitoes. Yogyakarta is an endemic area of DHF. One of the efforts of eradicating DHF in Yogyakarta was fogging with cypermethrin insecticide. Cypermethrin had been used since 2008 with a frequency of 1.469 times. However, repeated and prolonged application of chemical insecticide in an ecosystem unity may lead to vector resistance against the particular insecticide. Monitoring and early detection for the vector susceptibility status against insecticide was necessary in potential locations of DHF transmission, such as in elementary school. Detection of vector susceptibility status against insecticide could be conducted with susceptibility test using 0.05% cypermethrin impregnated paper.

Objectives: To find out the status of *Aedes aegypti* susceptibility against cypermethrin insecticide and the relationship between the status of *Aedes aegypti* susceptibility with the frequency of cypermethrin application.

Methods: An analytical observational research was conducted using cross-sectional design. Female *Aedes aegypti* from mosquito eggs collected at 72 state Elementary Schools in 45 villages in Yogyakarta were evaluated. Detection of *Aedes aegypti* susceptibility against cypermethrin was conducted using WHO standard method, namely, susceptibility test, which used 0.05% cypermethrin *impregnated paper*.

Results: *Aedes aegypti* at elementary schools in Yogyakarta had been resistant against 0.05% cypermethrin, with an average mortality of 4.03%. No correlation between *Aedes aegypti* susceptibility in the elementary schools in Yogyakarta and the frequency of cypermethrin application. This might be attributable to the use of household insecticide and to the resistance history of the test animals.

Conclusions: The population of *Aedes aegypti*at the State Elementary Schools in Yogyakarta was found to be resistant against 0.05% cypermethrin. No relationship between *Aedes aegypti* susceptibility status and the frequency of cypermethrin application.

Keywords: *Aedes aegypti*, Cypermethrin, impregnated paper, Susceptibility Test,

INTISARI

Pendahuluan: Demam Berdarah Dengue adalah penyakit infeksi yang disebarkan oleh nyamuk *Aedes aegypti*. Kota Yogyakarta merupakan daerah endemis DBD. Salah satu cara penanggulangan DBD di kota Yogyakarta dengan pengasapan menggunakan insektisida sipermetrin. Penggunaan sipermetrin sudah dilakukan sejak tahun 2008 dengan frekuensi aplikasi sebanyak 1469 kali. Aplikasi insektisida kimia dalam waktu lama dan

berulang di satuan ekosistem dapat menimbulkan resistensi vector terhadap insektisida yang digunakan. Monitoring dan deteksi dini status kerentanan vector terhadap insektisida di kota Yogyakarta perlu dilakukan di lokasi yang berpotensi sebagai tempat penularan DBD diantaranya di sekolah dasar. Deteksi status kerentanan vector terhadap insektisida dapat dilakukan dengan *susceptibility test* menggunakan *impregnated paper* sipermetrin 0.05%.

Tujuan: Mengetahui status kerentanan nyamuk *Aedes aegypti* di Lingkungan SDN di Kota Yogyakarta terhadap insektisida sipermetrin dan mengetahui hubungan status kerentanannya dengan frekuensi aplikasi insektisida sipermetrin.

Metode: Penelitian ini merupakan penelitian observasional analitik dengan rancangan *crosssectional*. Subyek penelitian populasi nyamuk *Aedes aegypti* dari telur yang berasal dari 72 SDN di 45 kelurahan di Kota Yogyakarta. Deteksi status kerentanan nyamuk *Aedes aegypti* terhadap insektisida sipermetrin dilakukan dengan metode standar WHO *susceptibility test* menggunakan *impregnated paper* sipermetrin 0.05%.

Hasil: Hasil uji kerentanan menunjukkan nyamuk *Aedes aegypti* di lingkungan sekolah dasar di kota Yogyakarta sudah resisten terhadap insektisida sipermetrin 0.05% dengan angka kematian nyamuk rata-rata 4.03%. Tidak ada korelasi antara status kerentanan nyamuk *Aedes aegypti* dengan frekuensi aplikasi insektisida sipermetrin. Hal ini bisa disebabkan karena penggunaan insektisida rumah tangga yang digunakan oleh masyarakat dan karena adanya riwayat resistensi dari nyamuk uji.

Simpulan: Populasi nyamuk *Aedes aegypti* di lingkungan Sekolah Dasar Negeri di Kota Yogyakarta sudah resisten terhadap insektisida Sipermetrin 0.05%. Tidak ada hubungan status kerentanan nyamuk *Aedes aegypti* di lingkungan sekolah dasar negeri di Kota Yogyakarta dengan frekuensi aplikasi insektisida sipermetrin.

Kata kunci: *Aedes aegypti*, Insektisida Sipermetrin, *impregnated paper*, Uji kerentanan, Sekolah Dasar

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) remains a main health problem, particularly in tropical and sub-tropical countries. Dengue virus transmitted to human being by means of female *Aedes aegypti* bites1. Control over DHF vector by means of fogging with cypermethrin insecticide had become a main option in the efforts of controlling DHF in Yogyakarta. The insecticide had been used since 2008 with an application frequency of 1.469 times2.

Prolonged and repeated application of insecticide in an ecosystem unit may lead to insect resistance against the insecticide. Resistance is inheritable and becomes a substantial obstacle in controlling vector by using chemical insecticide3. A research on the resistance of *Aedes aegypti* against cypermethrin had been conducted by Widiarti (2011) in Yogyakarta. The research showed that *Aedes aegypti* mosquitoes had been resistant in Umbulharjo Sub district and tolerant in Gondokusuman and Tegalrejo Sub-districts, Yogyakarta4.

Early detection for the susceptibility status of DHF vector against insecticide had been conducted in elementary schools because it was proved that *Aedes aegypti* mosquitoes in some sub-districts in Yogyakarta had been resistant against cypermethrin insecticide. There are many elementary schools in Yogyakarta, distributed in 45 villages and many of them are located near to settlement areas and therefore highly susceptible to insecticidal exposure from home and government-initiated usages. Data

on vector resistance against insecticide are necessary to be considered in designing DHF vector control programs.

MATERIALS AND METHODS

This stydy a descriptive analytical research with cross-sectional design was used to evaluate *Aedes aegypti* susceptibility against 0.05% cypermethrin. The subjects of the study Female *Aedes aegypti* mosquitoes (F2) collected from 72 State Elementary Schools in 45 Villages in Yogyakarta.

Installation of Ovitrap: Filter paper is installed around the inner part of black-painted glass, which was then filled up to three-fourth level to make sure that some parts of the filter paper were immersed. Ovitrap was placed in the favorite area of the mosquitoes. Ovitraps were installed inside and outside the school complexes. Each school was installed with 10 ovitrap for 6 days. Then, impregnated paper was collected from the glass, dried under room temperature, and then stored in labeled plastic bag5.

Colonization and maintenance of *Aedes aegypti* mosquitoes in the Laboratory. Eggs were put into water-containing plastic trays. After the eggs hatch, the larvae were fed with chicken liver and water. The feeding was replaced every two days. The mosquitoes were fed with syrup and blood. Then, ovitrap was installed within the cage6.

Susceptibility test: The mosquitoes from colonization in the laboratory and WHO standard tubes were prepared. Each of the tube was installed with 0.05% cypermethrin impregnated paper (*insecticide impregnated paper*) in circular way. Then, 15-25 female mosquitoes were introduced to the tubes and exposed to the insecticide for 9 hour. A control tube was given green label and non-impregnated paper. Room temperature and humidity were recorded during the test procedure. After one hour of contact with the insecticide, the mosquitoes were remove into a storage tube/*paper cup*. Mosquito mortality was determined/observed after 24 hours of storage. During the observation, the number of dead, knocked down and living mosquitoes were recorded. During the storage period, the mosquitoes were fed with syrup and humidity was kept by placing a wet cotton patch at the end of the paper cup. When the mosquito mortality in the control tube was more than 20%, the test was said to fail, and the results were unusable, and needed to be repeated7.

RESULTS AND DISCUSSIONS

The results of susceptibility test were analyzed in a descriptive way based on WHO criteria (1960). Percent mosquito mortality was determined using the following formula:

$$\% \text{ mortality} = \frac{\text{Total number of dead mosquitoes}}{\text{Total sample size}} \times 100\%$$

A similar calculation should be made in order to obtain a value for the control mortality. If the control mortality is above 20%, the tests must be discarded. When control mortality is greater than 5% but less than 20%, then the observed mortality has to be corrected using Abbots formula, as follows:

$$\text{ABBOT´S} = \frac{\text{\% observed mortality} - \text{\% control mosquito mortality}}{100 - \text{\% control mosquito mortality}} \times 100\%$$

The results of susceptibility test were obtained by calculating mosquito mortality rate in both test and control groups after being exposed to 0.05% cypermethrin for 60 minutes. Test mosquito mortality rate was calculated/ observed after 24 hours of storage. Results of Susceptibility Test are presented in Table.1

Table 1. Percent mortality and status of *Aedes aegypti* susceptibility after being exposed to 0.05% cypermethrin under an observation for 24 hours.

No. Of Elementary Schools	No. Of test mosquitoes	No. Of Mortality		Test mosquito mortality (%)	Susceptibility status
		Test group	Control group		
26	45	0	0	0	Resistant
18	45	1	0	2,22	Resistant
18	45	2	0	4,44	Resistant
5	45	3	0	6,66	Resistant
2	45	4	0	8,88	Resistant
1	45	7	0	16,6	Resistant
1	45	14	0	31,1	Resistant
1	45	30	0	66,7	Resistant

The data presented in Table 1 shows a difference in the mortality rate *Aedes aegypti* mosquitoes. The highest mortality rate (66,7%). According to WHO criteria (1998), a mortality rate of 99-100% is categorized as *Susceptible*; a mortality rate of 80-98% is categorized as *Tolerant* and a mortality rate of <80% is categorized as *Resistant*. Based on the criteria, population of *Aedes aegypti* mosquitoes within the Elementary School complexes in Yogyakarta was resistant against 0.05% cypermethrin, with an average mortality rate of 4.03%. Distribution of susceptibility status and mortality rate of test mosquitoes is presented in the following Figure 1.

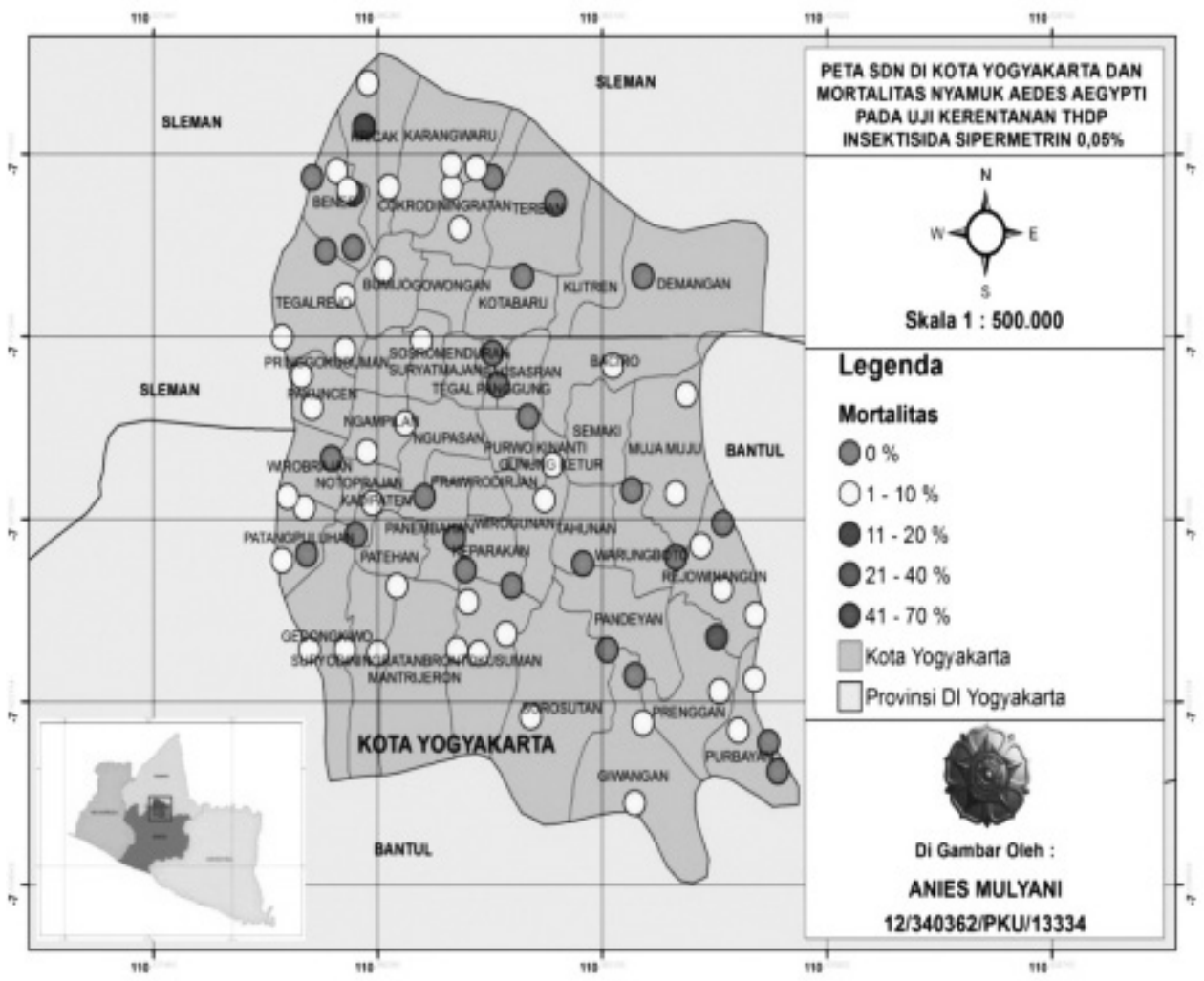

Figure 1. Distribution of susceptibility status and *Aedes aegypti* mortality rate at Elementary Schools in Yogyakarta

The figure shows the distribution of susceptibility status and *Aedes aegypti* mortality rate at the State Elementary Schools in Yogyakarta against cypermethrin insecticide. *Aedes aegypti* mosquitoes were found to be resistant at all of the villages in Yogyakarta, including at State Elementary Schools located in the villages with the low frequency of insecticidal application.

Prolonged and repeated insecticidal application in an ecosystem unity may lead to insect resistance against the insecticide. Therefore, it is important to determine the relationship between susceptibility status of *Aedes aegypti* at the elementary school complexes in Yogyakarta and the frequency of insecticidal application used for vector control program. Average mortality of *Aedes aegypti* after being exposed to cypermethrin at State Elementary School and Frequency of Cypermethrin Application are presented in Table 2.

Table 2. Average mortality of *Aedes aegypti* mosquitoes against cypermethrin at Elementary Schools and Frequency of Cypermethrin Application at each village in Yogyakarta

No	No. Of School	Villages	Average mosquito mortality (%)	Frequency of cypermethrin application	Susceptibility Status
1	2	Notoprajan	3,33	41	Resistant
2	1	Sorosutan	2,22	66	Resistant
3	1	Muja muju	4,44	33	Resistant
4	1	Warungboto	0	35	Resistant
5	2	Tahunan	0	40	Resistant
6	2	Pandeyan	1,11	40	Resistant
7	2	Giwangan	3,33	38	Resistant
8	4	Rejowinangun	2,77	35	Resistant
9	3	Prenggan	23,71	46	Resistant
10	3	Purbayan	2,96	37	Resistant
11	1	Gunung ketur	4,44	16	Resistant
12	1	Purwokinanti	0	26	Resistant
13	2	Terban	0	29	Resistant
14	1	Kotabaru	0	6	Resistant
15	1	Klitren	0	34	Resistant
16	1	Demangan	0	29	Resistant
17	1	Baciro	2,22	46	Resistant
18	3	Tegal panggung	0	29	Resistant
19	1	Wirogunan	2,22	34	Resistant
20	3	Keparakan	2,22	26	Resistant
21	3	Brontokusuman	2,96	45	Resistant
22	1	Ngupasan	6,66	24	Resistant
23	2	Suryodiningratan	4,44	42	Resistant
24	2	Pakuncen	4,44	42	Resistant
25	2	Wirobrajan	4,44	46	Resistant
26	1	Patangpuluhan	4,44	38	Resistant
27	2	Gedongkiwo	2,22	50	Resistant
28	2	Panembahan	0	18	Resistant
29	4	Cokrodiningratan	4,44	20	Resistant
30	3	Bumijo	2,22	31	Resistant
31	1	Karangwaru	2,22	33	Resistant
32	3	Bener	2,22	13	Resistant
33	3	Kricak	19,99	34	Resistant
34	2	Tegalrejo	2,22	35	Resistant
35	2	Pringgokusuman	3,33	53	Resistant
36	2	Patehan	3,33	14	Resistant
37	1	Kadipaten	4,44	7	Resistant

The data were then tested for correlation by using statistical test of Rank Sperman, with a significance rate of 95%. The results showed a p value of 0,285 > 0.05; it means no relationship was found between the susceptibility status of *Aedes aegypti* at the Elementary Schools and the frequency of cypermethrin application in Yogyakarta.

The results of susceptibility test in this study showed that population of *Aedes aegypti* mosquitoes collected from State Elementary Schools in Yogyakarta had been resistant against cypermethrin insecticide and no relationship was found between the frequency of cypermethrin application and susceptibility status of *Aedes aegypti* at Elementary Schools in Yogyakarta.

According to Georghiou (1993), the development of vector resistance against insecticide is influenced by multiple factors, including genetic factor, biological factor, and operational factor. The genetic factor depends upon the existence of resistant gen that is able to code the formation of certain enzymes in the mosquitoes' body. Biological factor includes insect behavior, number of generation and generation velocity, since all resistant gene can spread quickly to all populations. Adaptability toward natural pressures such as insecticidal application and high-speed regeneration rapidly produced resistant vector insects. Operational factor includes insecticide, application method, application frequency, dosages, and duration of application.

According to IRAC (2011)8, the primary mechanism that plays a role in the development of resistance/modification of susceptibility status of insects against insecticide includes: Increase in toxicant metabolism (insecticide) in the insects' body with *monooxygenase*, *esterase* and *glutathione-Stransferase*enzymes; Change in the sensitivity of target side resistance in the insects' body; Decrease in the penetration of toxicant (insecticide). Insects are able to modify chemical compositions and thicken the cuticles to reduce penetration of toxic substances in their body.

According to Martin et al (2009)9, there are two primary mechanisms of vector resistance development against synthetic pyrethroid insecticide, including cypermethrin, namely increase in the detoxification enzyme (metabolic resistance) and modification of target side sensitivity. Three enzymes related to insecticide detoxification include *cytochrome P450 monooxygenases* (P450s), *glutathione S-transferases* (GSTs) and *carboxy/ cholinesterases* (CCEs).

Target Side Resistance refers to resistance mechanism resulting from the decrease of target side sensitivity. There are three main targets of insecticide: 1) *Ligant gate ion channels target side* for *cycludien* and *phenyl pyrazole.* 2) *Voltage gate sodium channels target side* for DDT and pyrethroid. Target side resistance for pyrethroid is known as *knock down resistance* (Kdr) gene. 3) *Acetyl cholin esterase* is the target for organophosphate and carbamat group10.

A research that showed gene mutation resulting in the modification of sensitivity of the four target sides in the insects' body was conducted by Widiarti et al (2012)11. The study found mutation in codon 1014 from leusin (TTA) to *kdr*-w type phenylalanine (TTT), *VGSC* gene in *Aedes aegypti* in Simongan Village, Semarang; it was found to have a relationship with resistance against insecticide of pyrethroid group.

Since resistance is inherited, the existence of mosquito eggs and larvae at the elementary school complex could contribute to the resistance of test mosquito population. In

nature, frequency of susceptible individual allele is greater than that of resistant individual allele. Because of continuous selection, the number of susceptible mosquitoes keeps decreasing. Resistant mosquitoes bring together and generate resistant offspring. From a generation to another, proportion of resistant mosquitoes in a population keeps increasing and eventually dominates the population as a whole. In this case, the efforts of controlling the vector by using chemical insecticide become useless12.

The use of household insecticide can also trigger resistance of *Aedes aegypti* population within the elementary school complexes in Yogyakarta, since in their daily life, many society members around the school complex used household insecticide to get rid of the mosquitoes. It is known that most of household insecticide formulated from aerosol or other formula use pyrethroid insecticide13.

CONCLUSION

Population of *Aedes aegypti* mosquito at the state elementary school in Yogyakarta was found to be resistant against 0.05% cypermethrin. No correlation between the susceptibility status of *Aedes aegypti* mosquito and the frequency of cypermethrin applied as a part of vector control program. Suggested Insecticides sipermetrin discontinued its use for vector control of dengue in the city of Yogyakarta, as proved resistant, and look for a replacement alternative insecticides. Reduce the use of insecticides in the dengue vector control and eradication of mosquito breeding developed in a natural way.

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