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The resistance status of *Aedes aegypti* larvae to Temephos in Depok, Sleman, Yogyakarta

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ABSTRACT

There are still many cases of dengue hemorrhagic fever and tend to increase over time. One strategy to reduce the increase in cases of dengue infection is to eradicate *Aedes aegypti* as a vector using insecticides. The use of insecticides for a long time can cause resistance. The purpose of this study was to determine the resistance status of *Aedes aegypti* larvae against temephos in Depok, Sleman. This quasi experimental test was carried out on 3rd instar larvae of *aedes aegypti* from RW 9 and 10 Minomartani, Depok, Sleman. The treatment group was exposed 0.02 ppm temephos for 24 hours. The analysis was presented in the form of percentage of larval mortality and resistance categories based on WHO guidelines. The mortality percentage of *Aedes aegypti* larvae in RW 9 was 100%, while the mortality from RW 10 was 97%. *Aedes aegypti* larvae in Depok district, Sleman are still susceptible to temephos at a dose of 0.02 ppm.

ABSTRAK

Kasus demam berdarah dengue masih banyak dan cenderung meningkat dari waktu ke waktu. Salah satu strategi untuk mengurangi peningkatan kasus infeksi dengue adalah dengan pemberantasan *Aedes aegypti* sebagai vektor dengan menggunakan insektisida. Penggunaan insektisida dalam waktu lama dapat menimbulkan resistensi. Tujuan penelitian ini adalah untuk mengetahui status ketahanan larva *Aedes aegypti* terhadap temephos di Depok, Sleman. Uji coba semu ini dilakukan pada larva *Aedes aegypti* instar 3 di RW 9 dan 10 Minomartani, Depok, Sleman. Kelompok perlakuan dipapar temephos 0,02 ppm selama 24 jam. Analisis disajikan dalam bentuk persentase kematian larva dan kategori resistensi berdasarkan pedoman WHO. Persentase kematian jentik nyamuk *Aedes aegypti* di RW 9 adalah 100%, sedangkan kematian di RW 10 adalah 97%. Larva *Aedes aegypti* di Kecamatan Depok Kabupaten Sleman masih rentan terhadap temefos dengan dosis 0,02 ppm.

INTRODUCTION

The burden of dengue infection is increasing day by day. In the last 2 decades, there has been an 8 times increase in cases in the world with 70% of cases in Asian countries.[1] The number of cases of dengue infection in Indonesia until December 2020 was recorded at 95,893 with a total of 661 deaths.[2] One of the endemic areas for dengue infection in Yogyakarta is Depok district, Sleman.[3]

Dengue infection is an infection caused by the dengue virus which consists of 4 serotypes, namely DENV-1, DENV-2, DENV-3 and DENV-4 and transmitted from human to human through the bite of the *Aedes aegypti* mosquito. Increased mobility and urbanization causes dengue infection can spread to many parts of the world.[4] Breeding grounds for the *Aedes aegypti* mosquito are usually in places that can collect water such as discarded buckets, flower pots, or even bath

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water container. Dengue infection cases also increase during the rainy season because the temperature and humidity are suitable for *Aedes aegypti* to breed.[5]

Dengue infection control case is necessary to prevent and control the vector of dengue. The control strategy consists of 3 methods, these are physical control, biological control, and chemical control methods.[6] One of the method is chemical control. It is the use of insecticides either from chemicals or plants.

Insecticides inhibit the development of *Aedes aegypti* by targeting 2 stages of the life cycle, namely pre-adult and adult. Insecticides that target the adult mosquito life cycle are the organophosphate (malathion) and pyrethroid (cypermethrine) groups which are administered by fogging. While the target of the pre-adult life cycle of *Aedes aegypti* is using organophosphate (temephos) and pyriproxifen, which are used by sprinkling these larvicides in water reservoirs where mosquitoes like to breed.[7]

The use of insecticides has been used for a long time in Indonesia to control the *Aedes aegypti*. The continuous use of insecticides can cause resistance in *Aedes aegypti* populations. It has been reported in several countries across the world that there has been insecticide resistance to *Aedes aegypti*, both at the larval stage and adult mosquitoes.[8] So the aim of this study was to determine the resistance status of *Aedes aegypti* larvae to temephos in Depok district, Sleman.

MATERIALS AND METHODS

Subjects

This quasi-experimental research was conducted in June 2015. The subjects of this study were 3rd instar *Aedes aegypti* larvae obtained from RW 9 and 10 Minomartani sub-districts, Depok, Sleman, Yogyakarta, Indonesia and have been bred in the Parasitology Laboratory, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada. Larvae were bred in plastic cups 9.25 x 15.5 cm containing water that did not contain chlorine or organic contaminants with an average temperature of 25 C. Larvae were given chicken liver as food ad libitum.

Treatments

One hundred and twenty five instar III larvae from each RW were divided into 5 groups, these were the treatment group, the control group, and the replication group as many as 3 groups. A total of 25 larvae were placed in a small plastic cup 9.25x12 cm with a total water volume of 250 mL. The treatment group and the replication group received a dose of 0.02 ppm temephos which was dissolved first using 70% alcohol, while the control group was given 70% alcohol. After 24 hours, the dead larvae were counted for each treatment. Larvae that drown, did not move after being touched with a stick, discolored, tremor, or uncoordinated were classified as dead.

Data analysis

The results obtained were presented in the form of larval mortality percentage calculated by the following formula[9]:

$$\frac{\textit{the number of dead larvae}}{\textit{the total number of larvae per treatment group}} \text{ x } 100\%$$

If the mortality rate is less than 3% in the control, then the percentage of mortality does not need to be corrected. If mortality in the control group is more than 10%, the study needs to be repeated. If the mortality in controls is between 3-10%, then the percentage of mortality needs to be corrected using the Abbott formula[10] as follows:

$$\frac{\textit{treatment group mortality-control group mortality}}{\textit{100-control group mortality}} \text{ x } 100\%$$

Resistance status was interpreted using the categories defined by WHO[9] as follows: 98-100% mortality is

considered susceptible, <98% mortality indicates resistance and requires further investigation, 90-97% mortality requires at least 2 additional tests such as a bio-test with the same population or molecular test. If at least 2 additional tests show mortality below 98%, then it can be concluded that they are resistant. Mortality <90% is declared resistant without the need for additional testing.

RESULTS

The mortality percentage of third instar larvae of *Aedes aegypti* from RW 9 that were given exposure to temephos at a dose of 0.02 ppm for 24 hours can be seen in TABLE 1.

TABLE 1. Percentage of mortality of third instar larvae of *Aedes aegypti* RW 9 given 0.02 ppm temephos for 24 hours

	Mortality	
	n	%
Treatment	25	100
Replication I	25	100
Replication II	25	100
Replication III	25	100
Means of treatment group	25	100
Control	0	0

The mortality percentage for the control group was 0%, so it did not need to be corrected using the Abbott formula. The mean percentage of mortality in the *Aedes aegypti* larvae group from RW 9 which was given 0.02 ppm temephos was 100%, so based on the WHO category it was included in the susceptible category. These results indicate that temephos is still one of the most effective larvicidal options to kill *Aedes aegypti* larvae in RW 9. The choice of 0.02 ppm temephos dose is based on WHO[11] guidelines to determine the susceptibility status of mosquito larvae to insecticides.

The results of the percentage of mortality of third instar larvae of *Aedes aegypti* from RW 10 that were given exposure to temephos at a dose of 0.02 ppm for 24 hours are presented in TABLE 2. The percentage of larval mortality in the control group was 0%, so the mortality in the treatment group did not need to be corrected using the Abbott formula. The mean percentage of larval mortality in the treatment group was 97%. These results indicate that the third instar larvae of *Aedes aegypti* from RW 10 cannot be said to be susceptible or resistant to temephos. It is necessary to carry out 2 additional tests such as biological tests and molecular tests to confirm the presence of resistant genes. If the results of the 2 additional tests show that the mortality percentage of larvae is less than 98%, it can be said that the larvae have experienced resistance.

TABLE 2. Percentage of mortality of third instar larvae of *Aedes aegypti* RW 10 given 0.02 ppm temephos for 24 hours

	Mortality	
	n	%
Treatment	24	96
Replication I	23	92
Replication II	25	100
Replication III	25	100
Mean of treatment group	24.25	97
Control	0	0

DISCUSSION

The larval stage used to test the resistance of *Aedes aegypti* larvae to insecticides is in accordance with WHO guidelines[11] on the susceptibility status test for mosquito larvae, using third instar larvae. The characteristic of *Aedes aegypti* larvae when it is at rest, the head will be at the bottom and breathe using a siphon which is positioned on the water surface. The larvae will experience 4 times molting and the larvae that are formed at each stage are called instar larvae 1 to 4. The larval stage lasts approximately 6-8 days depending on temperature, food availability and larvae density. The length of the 3rd instar larva is about 3-5 mm.[12]

The resistance status of *Aedes aegypti* larvae obtained from RW 9 was categorized as susceptible. In this area, people rarely use temephos as a larvicide and community larvae surveillance or 'jumantik' did not actively encourage people to take preventive strategy to break the life cycle of the *Aedes aegypti* mosquito. So that in this area temephos can still be used as an alternative to eradicate *Aedes aegypti* larvae.

The resistance status of *Aedes aegypti* larvae from RW 10 cannot be said to be categorized as susceptible or resistant. The decrease ability of temephos to kill *Aedes aegypti* larvae is probably due to the use of a sublethal dose so that the larvae can adapt to that dose. The inhabitant of RW 10 often uses temephos as larvicide by dissolving 1 gram of temephos for 30 L of water. Meanwhile, according to WHO[5] recommendations, 1 gram of temephos is used for 10 L of water. This caused *Aedes aegypti* larvae in RW 10 receive a lower dose.

The resistance mechanism is broadly divided into 2, that is metabolic and target site insensitivity.[13] These mechanisms can occur simultaneously or independently. Metabolic resistance occurs due to overexpression of detoxification enzymes caused by mutations or amplifications of regulatory gene regions such as CCEAE3A and CCEAE6A[14] or a change in the amino acid sequence of the enzyme so that metabolic activity occurs more rapidly. The detoxification enzymes that play a role in this resistance are esterases, glutathione S-transferases (GSTs), monooxyigenase and carboxylcholinesterases (CCEs). The increase in A4 esterase activity was reported to be associated with temephos resistance.[15] The presence of polymorphism in the GST2 gene was also associated with *Aedes aegypti* resistance to temephos.[16] Meanwhile, resistance due to the insensitivity of the target site was caused by modification of the DNA coding the insecticide target molecule so that the target could not bind to the insecticide. The mutation that usually occurs in this mechanism is the acetylcholinesterase (ace-1) gene.

CONCLUSION

Aedes aegypti larvae in Depok district, Sleman are still susceptible to temephos at a dose of 0.02 ppm. Insecticide resistance tests need to be carried out regularly so that the life cycle of *Aedes aegypti* can be interrupted and can reduce cases of dengue fever.

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