Resistance Status of *Aedes aegypti* Larvae to Temephos in Dengue Hemorrhagic Fever Endemic Areas

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Abstract

Dengue fever every year always present throughout the district in the Republic of Indonesia, often even a tremendous incident. Vector control is also not getting optimal results. One way of vector control is done by using larvae-type temephos. The use of temephos at first indeed proved effective in lethal *Aedes aegypti* larvae. However, continuous use of temephos feared to cause resistance to *Ae.aegypti* larvae. This study aims to determine the resistance of *Ae.aegypti* larvae to temephos in Banyumas and Kebumen District, Central of Java Province. This research was an experimental study with postest only with control group design by conducting a survey to DHF endemic areas. *Ae.aegypti* egg sample was taken by installing ovitrap in Kelurahan Teluk, South Purwokerto Sub-district of Banyumas District and Jatinegara Village, Sempor Sub-District, Kebumen District. After that the rearing of egg (grown) to become larvae in the laboratory. After the larvae reach instar III, the larvae are then tested for resistance to temephos. The results showed that *Ae.aegypti* larvae in Teluk Village, South Purwokerto District, Banyumas Regency and Jatinegara Village, Sempor District, Kebumen Regency were still susceptible to temephos. The government and the public can still use temephos to control the DHF vectors by the terms and conditions applicable. It is recommended to the health department conduct periodic evaluation to know the effectiveness of insecticides used, especially in endemic areas
***Keywords**: DHF ; Aedes aegypti; larvae; resistance status*

1. Introduction

Diseases transmitted through vectors are still endemic diseases that can cause outbreaks or extraordinary events and cause public health problems, so it is necessary to control the spread of vectors. Vector control efforts are efforts made to suppress, reduce, or reduce population density/vector density to the lowest possible level so as not to endanger human life. Diseases in Indonesia that are transmitted through mosquitoes are endemic in certain areas, such as Dengue Hemorrhagic Fever (DHF) which is transmitted through the bite of *Aedes aegypti* and Aedes albopictus mosquitos.

The number of DHF cases always fluctuates every year. Data from the Ministry of Health of the Republic of Indonesia states that DHF cases nationally tend to fluctuate. In 2014 the number of cases in 34 provinces was 71,668 people, with the death toll of 641 people. In 2013 the number of DHF cases in Indonesia was 112,511 people, and the number of deaths was 871. (Kemenkes RI, 2014).

The incidence rate (IR) of Dengue Hemorrhagic Fever per 100,000 population in Central Java in the last five years were as follows: in 2011 it was 15.27, in 2012 it was 19.29, in 2013 it was 45.52, in 2014 it was 32.95, and in 2014 it was 32.95. 2015 amounted to 34.87. In 2007 out of 35 regencies/cities in Central Java, 33 regencies/cities were endemic areas of DHF, and in 2008-2009 it had spread to all regencies/cities, in 2010-2011 in all regions there was a decrease in dengue cases and an increase in years 2013-2015.

The case illustrates that there are still many problems related to the handling of dengue cases. DHF case prevention by eliminating the cause of the disease (dengue virus), isolating patients, preventing mosquito bites, and vector control. Until now, no effective drugs and vaccines have been found for dengue fever, so efforts to prevent dengue fever are more focused on breaking the chain of vector development.

Various efforts to control the dengue vector have been implemented so as not to cause fatal problems. Various controls have been implemented both physically, chemically, and biologically. Physical control is generally known as Mosquito Nest Control and 3M (draining, closing, and burying or destroying)

draining the bath or toilet tub, closing water reservoirs (buckets, drums, jars), and bury or destroy used goods such as cans, tires, plastic, and others. (Dirjen PPM & PLP, 2001).

Chemical control has been carried out, among others, by fogging to control adult mosquitoes and larviciding to control mosquito larvae. Larviciding using 1% abate formulation at a dose of 1 gram/10 liters of water can effectively kill larvae for 8-12 weeks. Abate (temephos) is a pesticide used to kill insects at the larval stage and is usually used in the form of sand granules.

The use of abate (temephos) in Indonesia has been going on since 1976. Four years later, in 1980, abate (temephos) was established as part of the mass eradication program of *Aedes aegypti* in Indonesia. It can be said that abate (temephos) has been used for more than 30 years in Indonesia (Felix, 2008). However, the use of abate can cause various problems such as resistance and environmental pollution. (Merty Dwi K., et al, 2014). Reports of resistance of *Aedes aegypti* larvae to abate (temephos) have been found in several countries such as Brazil, Bolivia, Argentina, Cuba, the Caribbean, and Thailand (Felix, 2008).

The negative impact caused by chemical insecticides, among others, in the form of resistance to Aedes sp larvae in several areas, has prompted researchers to conduct research on *Aedes sp* larvae resistance to temephos.

Abate is a larvicide containing the active ingredient temephos which has also been registered as a larvicide in Indonesia as stated in the Minister of Health Regulation No. 374 of 2010 which states that the insecticides used to control the mosquito larvae/lattice of dengue vectors are Temephos, Pyriproxyfen, and Bacillus thuringiensis var israelensis.

Based on the description above, the researcher intends to conduct a study entitled " Resistance Status of "*Aedes aegypti* Larvae to Temephos in Dengue Hemorrhagic Fever Endemic Areas".

2. Material and Methode

This research was an experimental study with postest only with control group design by conducting a survey to DHF endemic areas (districts of Banyumas and Kebumen, Central Java Province). The time of the study was carried out from August to November 2017.

The research sample were eggs *Aedes aegypti* mosquito obtained by installing ovitrap in dengue-endemic areas in Banyumas Regency (Teluk Village, South Purwokerto District) and Kebumen Regency (Jatinegara Village, Sempor District). Larvae were obtained by incubating eggs (rearing) which was carried out in the Entomology Laboratory of the Research and Development Center P2B2 Ciamis. The numbers of mosquito larvae used in the study were 400 items from each district. The total numbers of mosquito larvae were 800.

In this study, the subjects were divided into two groups: the intervention group (receive temephos intervention at several different doses) and the control group (receive no intervention). The test initially used 25 larvae per cup for each treatment. The treatments (doses) given are temephos at 0,1 g/l. The replication group has as many as 4 groups. The time exposure was 24 hours. After 24 hours, the dead larvae were counted for each treatment.

Primary data collection was conducted by counting the number of dead *Ae.aegypti* larvae in the intervention group and control group. The results obtained were presented in the form of larval mortality percentage calculated by the following formula:

$$\frac{\textit{The number of dead larvae}}{\textit{The total number of larvae}} \times 100\%$$

The analysis was presented in the form of a percentage of larval mortality and resistance categories based on WHO guidelines. The category of resistance status of *Ae.aegypti* toward temephos is as follow:

a. Susceptible/sensitive, if the larvae death was 98-100%.
b. Indicates resistance and requires further investigation, if the larvae death was 90-97%.
c. Resistant, if the larvae death was < 90%.

3. Result and Discussion

a. Installation of ovitrap at the research site

Installation of ovitrap aims to get *Aedes aegypti* mosquito eggs. Installation of ovitrap in Kebumen Regency was carried out on Saturday, September 30, 2017 in Jatinegara Village, Sempor District, Kebumen Regency. Ovitrap was taken on Saturday, October 7, 2017.

The installation of ovitrap in Banyumas Regency was carried out on Saturday, October 21, 2017 in Teluk

Village, South Purwokerto, Banyumas Regency. Ovitrap collection in Banyumas Regency was carried out on Friday, October 27, 2017. After obtaining the eggs, then the eggs were drained and then taken to the Ciamis P2B2 Research and Development Center in Pangandaran to test the resistance of *Aedes aegypti* larvae to temephos.

b. Rearing in the laboratory

Ovistrip, which has been sent to the P2B2 Ciamis Research and Development Laboratory, is then reared to become *Aedes aegypti* larvae. The ovistrip immersion was carried out using a tray filled with clean, chlorine-free water and then the tray was placed in the egg hatchery in the laboratory. After the eggs hatch into larvae, during the rearing process the larvae are given food in the form of previously mashed fish pellets. After the sufficient number and age of the larvae have reached the 3rd instar, the *Aedes aegypti* larvae are ready to be used for testing resistance to temephos. If the number of larvae is not sufficient, then the larvae can be reared until they turn into adults and then the adult mosquitoes are reared to lay eggs and then the eggs are hatched into larvae.

c. Implementation of resistance test

Resistance test of *Aedes aegypti* larvae against temephos is to prepare 5 pieces of 200 ml beaker glass consisting of 1 control and 4 treatments. Then prepare 1 beacker glass and fill it with 200 ml of distilled water which will be used as control and 4 other beacker glasses which are given temephos solution at a dose of 0.1 g/l. In each treatment and control, 25 larvae of *Aedes aegypti* instar 3 were filled per treatment. After 1 hour, the larvae were removed using a sieve, and then the larvae were washed with clean water. After washing, the larvae were transferred to a beacker glass containing distilled water and fed fish pellets. The number of larval deaths was counted at the 5th hour and observed again at the 24th hour.

If the larval mortality rate in the control group is > 20%, the study is considered a failure and the test must be repeated. If the mortality rate in the control group is between 5% - 20%, it must be corrected by the Abbot formula.

d. Resistance Test Results

The larvae used for this study were the rearing *Aedes aegypti* mosquito larvae. The resistance test of *Aedes aegypti* larvae against temephos was carried out using 3 instar *Aedes aegypti* mosquito larvae. The number of *Aedes aegypti* mosquito larvae used for resistance testing was 25 per treatment and in the control group. The concentration of temephos used was 0.1 g/l. Replication was used 4 times.

Resistance test was carried out by counting the number of dead larvae at 24 hours. During the resistance test, after the first hour of exposure, it was found that 100% of the test mosquitoes were knocked down or fainted. At the 24 hour observation, the number of dead *Aedes aegypti* mosquito larvae was as follows:

Table 3.1. Results of Resistance Test of *Aedes aegypti* Mosquito Larvae to Temephos in Teluk Village, South Purwokerto District, Banyumas Regency

Treatment	Death of *Aedes aegypti* Larvae in each replication				%
	R_1	R_2	R_3	R_4	
Temephos 0,1 gr/ l	25	25	25	25	100 %
Control	0	0	0	0	0%

Table 3.2. Results of Resistance Test of *Aedes aegypti* Mosquito Larvae to Temephos in Jatinegara Village, Sempor District, Kebumen Regency

Treatment	Death of *Aedes aegypti* Larvae in each replication				%
	R_1	R_2	R_3	R_4	
Temephos 0,1 gr/ l	25	25	25	25	100 %
Control	0	0	0	0	0%

Based on table 3.1 and table 3.2, the resistance status of *Aedes aegypti* larvae to temephos in the two different research locations (Banyumas and Kebumen districts) can be stated as vulnerable because based on resistance tests that have been carried out, the percentage of test larvae mortality is 100%. Based on the WHO standard, the larvae status is said to be vulnerable if the mortality percentage of the test larvae is between 98% -100%.

The results of the resistance test that had been carried out for 24 hours with a dose of 0.1 g/l showed that the percentage of mortality of *Aedes aegypti* larvae obtained in Teluk Village, South Purwokerto District, Banyumas Regency against abate was 100%. According to Suwasono, 1991 temephos is a pesticide belonging to the class of organic phosphate compounds. This group has a way of working by inhibiting the cholinesterase enzyme, causing nerve activity due to the

accumulation of acetylcholine at the end of the condition. The function of the cholinesterase enzyme is to hydrolyze acetylcholine into choline and vinegar. So that if the enzyme is inhibited, the hydrolysis of acetylcholine cannot occur so that the muscles will contract for a long time, spasms will occur (Arif Dwi Nugroho, 2013, p.44).

The results of the resistance test of *Aedes aegypti* larvae obtained in Teluk Village, South Purwokerto District, Banyumas Regency, and also in Jatinegara Village, Sempor District, Kebumen Regency are still susceptible to the larvacide temephos. This may occur because the application of temephos larvicides for larval control in the study area is still not often carried out, it can also be because many *Aedes aegypti* larvae control efforts are carried out physically through the mosquito nest eradication program by draining, closing water reservoirs and burying containers. - containers or containers that have the potential to become breeding nests. Thus, temephos larvicide can still be used in the dengue vector control program in Banyumas and Kebumen Regencies.

This result is different from previous research that has been conducted in Sekumpul Village, Banjar Regency, South Kalimantan Province where *Aedes aegypti* larvae are already tolerant of temephos.

Although the results of the resistance test at the research site are still susceptible to temephos, if the use of temephos is used continuously for a long time without being replaced with other larvicides, the possibility of resistance to temephos will be even greater.

4. Conclusion

Based on the results and discussion, the following conclusions can be drawn:

a. The number of DHF cases in Banyumas and Kebumen regency in the last three years (2014-2016) was relatively high, so efforts to control the dengue vector must be carried out.
b. The results of the resistance test of *Aedes aegypti* mosquito larvae to temephos in Teluk Village, South Purwokerto District, Banyumas Regency stated that Aedes *aegypti* mosquito larvae were still susceptible to temephos.
c. The results of the resistance test of *Aedes aegypti* mosquito larvae to temephos in Jatinegara Village, Sempor District, Kebumen Regency stated that Aedes aegypti mosquito larvae were still susceptible to temephos

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