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Susceptibility of *Aedes aegypti* Larvae against Temephos in Dengue Hemorrhagic Fever Endemic Area Tasikmalaya City

Kerentanan Larva Aedes aegypti terhadap Temefos pada Daerah Endemik Demam Berdarah Dengue di Kota Tasikmalaya.

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Abstrak. *Salah satu upaya pengendalian vektor nyamuk Aedes aegypti adalah menggunakan larvasida sintetis seperti temefos. Pemanfaatan temefos secara terus-menerus dan berulang meningkatkan risiko resistensi. Tujuan penelitian adalah untuk menentukan kerentanan larva Ae. aegypti terhadap temefos pada daerah endemis Demam Berdarah Dengue (DBD) di Kota Tasikmalaya. Penelitian ini adalah penelitian eksperimen dengan rancangan acak kelompok. Populasi adalah larva Ae. aegypti yang berada pada rumah tinggal di Kota Tasikmalaya. Jumlah sampel sebanyak 700 larva nyamuk Ae. aegypti yang diperoleh dari rumah tinggal di daerah endemis DBD dan telah dibiakkan hingga keturunan ketiga (F3). Kerentanan temefos diuji dengan metode Elliot dan Polson menggunakan konsentrasi diagnostik WHO sebesar 0,02 ppm. Hasil penelitian menunjukkan bahwa LC_{95} temefos adalah 0,00926 ppm di Kelurahan Sukamanah, 0,01015 ppm di Kelurahan Cikalang, 0,01137 ppm di Kelurahan Kersanagara, dan 0,02045 ppm di Kelurahan Tugujaya. Penelitian ini menyimpulkan bahwa larva Ae. aegypti dari Tugujaya terindikasi resisten terhadap temefos.*

Kata Kunci: Resistensi, *Aedes aegypti*, temefos, kerentanan.

Abstract. One of the effort for controlling *Aedes aegypti* as dengue vector is by using synthetic larvicide such as temephos. Continuous and repeating utilization of temephos may increase the risk of resistance. The objective of this study was to determine the susceptibility of *Ae. aegypti* larvae against temephos in endemic areas of dengue fever in Tasikmalaya. This was a true experimental study with a block-randomized design. The populations were *Ae. aegypti* larvae, which existed at household in Tasikmalaya City. There were 700 larvae of *Ae. aegypti*, which taken from households in each Dengue Hemorrhagic Fever endemic area, and have been bred to third generation (F3). Susceptibility of temephos was tested by Elliot and Polson methods using WHO diagnostic dose 0.02 ppm. Results showed that the LC_{95} of temephos were 0.00926 ppm in Village of Sukamanah, 0.01015 ppm in Village of Cikalang, 0.01137 ppm in Village of Kersanagara, and 0.02045 ppm in Village of Tugujaya. This research concludes that *Ae. aegypti* larvae from Tugujaya were indicated resistant to temephos.

Keywords*: Resistance, Aedes aegypti, temephos, susceptibility.*

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INTRODUCTION

Temephos is non-systemic organophosphate group; have been used in vector control program approximately since 1980, especially to be used at the elimination of *Ae. aegypti* larvae.[1,2,3] On the other hand, exposure to insecticides continuously and repeatedly over a period of 2-20 years can lead to the emergence of insecticide resistant insects.[4] Moreover, using synthetic insecticides long-term (continuously and repeatedly) can cause environmental pollution, and disturbing of the surrounding non-target organism ecosystem.[5]

Tasikmalaya City has been used temephos in Dengue Haemorrhagic Fever (DHF) endemic areas since 1997[6], but this effort has not been able to decline the number of DHF cases. Be proven in 2004 incidence rate (IR) reached 37.99 per 100,000 population, to 129,44 per 100,000 population in 2013.[6] Several Villages with the highest DHF number were Villages of Kawalu, Tawang, and Cihideung, have become endemic area since 1997.[6] In that villages, larval free index always lower than the Government's resolve by 95%[6]. Therefore, it was possible that the tolerance of temephos used in control of *Ae. aegypti* contributes to the increase DHF case in Tasikmalaya City.

Research about *Ae. aegypti's* susceptibility of *Ae. aegypti* in East Jakarta, West Jakarta, and South Jakarta showed there has been increased resistance status to temephos 0.02 ppm.[7] Similarly in Brazil, reported that from 14 cities of the research samples, 78.57% cities have been resistant *Ae. aegypti* to temephos, with the interval RR_{95} 6.6 to 252.7.[8]

Tasikmalaya City as one of the shelters of dengue transmission in West Java has set temephos to use as an effort to control *Ae. aegypti* in DHF endemic areas, through the program *"Selective Abate"*.[6] But, this effort has not been able to decline the number of DHF cases. In 2011, dengue cases reached 428 cases with three people die, increased in 2012 to 694 cases with five people die. Three Villages of the 10 Villages with the highest DHF number were Villages of Kawalu, Tawang, and Cihideung, have become endemic area since 1997. Since then, the local Government always does counselling and *abatisasi* (the appliances of temephos) vector in that area.[3]

In 2013, the Larvae Free Index in the town of Tasikmalaya reaches 92%, lower than the Government's resolve by 95%.[6] In fact, various efforts have been made by governments and local communities to suppress larval populations. We assumed that the tolerance of temephos used in control of *Ae. aegypti* contribute to the presence of larvae. This obstructs vector control on aquatic stage, and cause the number of dengue cases continues to increase.

Tasikmalaya City has never held study about susceptibility of *Ae. aegypti* to temephos. Therefore, it is important to know the *susceptibility* of *Ae. aegypti* larvae as early awareness in vector control efforts in Tasikmalaya. The objective of this study was to determine the *Ae. aegypti* susceptibility against temephos in endemic areas of dengue in Tasikmalaya. The information was expected to provide information to Health Officers about temephos evaluation.

MATERIALS AND METHODS

The experiment was conducted in May 2014 through two stages, consisted of the collection of samples of eggs, larvae, and pupae *Ae. aegypti* in the Villages Sukamanah, Cikalang, Kersanagara, and Tugujaya of Tasikmalaya City, and the susceptibility test of *Ae. aegypti* to temephos in the Laboratory of Entomology, Unit of Research and Development For Arthropod-Borne Disease Ciamis.

This was an experimental study with a block-randomized design. The population of *Ae. aegypti* larvae were within houses of dengue fever endemic area in Tasikmalaya. A total of 700 larvae of *Ae. aegypti*, were obtained from houses in each endemic area and have been bred to third generation (F3). The susceptibility tests against *Ae. aegypti* were obtained from four Villages as a sample, and from the Laboratory for comparison. The main material used was ABATE 1 SG Kimia Farma's production.

Survey of *Ae. aegypti* in settlements

Survey entomology in residential areas aimed at the collection of *Ae. aegypti* larvae and pupae samples. Purposive sampling technique used in residential area. A first stage was determined in the residential areas, which were four Villages, which have stated as endemic DHF by Tasikmalaya's Health Department. At each Village were selected three hamlets that include two regular settlements and one household. The survey carried out by observing the presence of larvae in water reservoirs or container according to procedure by Fuadzy and Hendri[9]:

- first checked bathtub or toilet, jars, drums, vase, birdbath, cans or plastic, old tires and other water reservoirs.
- used a flashlight to see larvae in the water
- if it does not appear, wait ± 30-60 second; the larvae will emerge to the surface to breathe air.

All of the *Ae. aegypti larvae* that have been found were brought to the laboratory.

Maintenance *Ae. aegypti* in Insectarium

Ae. aegypti were reared in Insectarium consist of sampled from the field and from laboratory. *Ae. aegypti* larvae samples acquired from the field, maintained in the laboratory until the third generation (F3). While *Ae. aegypti* larvae strain of Laboratory, obtained by hatching eggs 24 generation (F24) from insectarium. Procedures of larval rearing following performed by Chowanadisai and modification rearing by Uthai[9,10]. Approximately 1000 Larvae put into a container containing 1,500 mL. Then feed by using dog food for 0.5 mg. Larvae reared at room temperature 25 ± 10 degree celsius and humidity of 70 ± 2% RH. The larvae were used for susceptibility testing is larval instar III and IV or 4 days old.

Identification of *Aedes* larvae using a microscope and *Aedes* key guide books made by the Ministry of Health. The identification was carried out to distinguish *Ae. aegypti* and *Ae. albopictus*; the identification can be seen in the form of comb scales that are located on the eighth abdominal. *Ae. aegypti* has a serrated comb scale, whereas *Ae. albopictus* has a scale of comb hair.[11]

Susceptibility test

Susceptibility test *Ae. aegypti* was conducted using procedures Elliot and Polson.[11,12] six concentration and one control were used, 0.004; 0.005; 0.01; 0.02; 0.03; and 0.04 ppm; and also 0 as control. The number of replications is calculated by the Federer formula,

$$(r - 1)(t - 1) \geq 15 \quad (1)$$

$$(r - 1)(6-1) \geq 15$$

$$r \geq 4$$

r = replication

t = treatment

Based on the above calculation, we have to do 4 replication with the number of treatment of six concentrations and one control, so a total number are 28 observations, then the number of larvae required is 700 individual.

Susceptibility test performed against *Ae. aegypti* F3 samples from the field, and *Ae. aegypti* F24 from Laboratory. Tests carried out by the same procedure. Before the test, *Ae. aegypti* were fasted for 1 day.

The stages of susceptibility test is started by taking 25 individuals fourth instar of *Ae. aegypti* larvae, and inserted into each container containing a dose treatment with aquades volume of 100 mL. For control, 25 *Ae. aegypti* larvae were inserted into the container filled with 2 cc of ethanol with 100 mL aquadest. After larvae contacted with temephos for 1 hour, the larvae were transferred to a 50 mL glass of aquadest for 1 minute washing. After that, *Ae. aegypti* larvae were transferred into a container filled with 500 mL of aquadest to be maintained, while observation and recording of the condition of the larvae performed in 15 minutes, 30 minutes, 60 minutes, 2 hours, 3 hours, 4 hours, and 5 hours. Determination of mortality of larvae is the submerged larvae and larvae that did not move again as long as 1 minute.

Determination of Susceptibility

Total mortality of larvae can be further analyzed when mortality in the control <5%. If the mortality of larvae in the control between 5% to 20%, then the data is corrected using Abbot[12] formula, based on the mortality of larvae in the treatment and control, mortality (A1):

$$A1 = \frac{\text{treatment}(\%) - \text{control}(\%)}{100 - \text{control}(\%)} \text{x} 100 \quad (2)$$

If the mortality of larvae in the control > 20% of the study should be repeated. Larval mortality data were analyzed after 5 hours through probit calculation to determine the effective dose to approach 50 and 95 Lethal Concentration (LC_{50} and LC_{95}) using software applications open source POLO PC. After obtaining the results of calculation of the LC, the calculation Resistance Ratio (RR)[19] using the following equation:

$$RR = \frac{LC_{95} \text{ larvae observed}}{LC_{95} \text{ larvae comparison}} \quad (3)$$

One-way ANOVA analysis used to determine the effect of temephos to *Ae. aegypti larvae* mortality. Based on WHO standards, tentative dose diagnostic to detect the resistance of *Ae. aegypti* to temephos is 0.02 ppm. If LC_{95} is less than 0.02 ppm, it expressed as susceptible, but if LC_{95} above 0.02 ppm, expressed resistent[12].

This research has been approved for ethical clearance from the National Institute of Health Research and Development no. LB.02.01 / 5.2 / KE.619 / 2013 dated December 24, 2013.

RESULT

The susceptibility of the two larvae of *Aedes aegypti* group; Strain Sukamanah, Cikalang, Kersanagara, and Tugujaya, as field samples; and laboratory strains were used as a comparison sample in determining the Resistance Ratio (RR) (Table 1).

Table 1 illustrates that *Ae. aegypti larvae* from Village of Tugujaya have been indicated resistant with LC_{95} 0.02045, and 2.55 times the resistance of *Ae. aegypti larvae from* Laboratory. Based on the analysis of variance is known that in this study conducted in four Villages, susceptibility

Table 1. Susceptibility of *Aedes aegypti* against Temephos and Variance Analysis in Four Villages Tasikmalaya City 2014.

Villages	LC_{95} 95%CI (ppm)	RR_{95}	Slope	Status	*P-Value*
Cikalang	0.01015 0.00851 – 0.01326	1.27	y = 16.75 + 5.07 Log c	Susceptible	0.000
Kersanagara	0.01137 0.00899 – 0.01642	1.42	y = 13.02 + 3.28 Log c	Susceptible	0.000
Tugujaya	0.02045 0.01410 – 0.03845	2.55	y= 9.76 + 1.84 Log c	Resistance	0.000
Sukamanah	0.00926 0.00787 – 0.01163	1.16	y = 14.10 + 3.67 Log c	Susceptible	0.000
Laboratory	0.00801				

testing revealed that various concentrations temephos effect on mortality of *Ae. aegypti* (p-value = 0.000).

The study also collected data on the status of entomology in five Villages in Tasikmalaya. Entomological survey was conducted to determine the density of *Ae. aegypti* through indicators such as the number of household observed and existed larvae (positive), the number of container observed and existed larvae (positive), and the Larvae Free Index, as presented in Table 2.

Based on Table 2 shows that the Village of Tugujaya is DHF endemic area with lower Larvae Free Index (77.36%), the numbers of positive larvae were 60 households and 66 containers. As for the Village of Kersanagara is DHF endemic area with highest Larvae Free Index (81.63%), the number of positive larvae as many as 45 homes and 54 containers.

DISCUSSION

Based on the concentration recommended by the WHO; 0.02 ppm, *Ae. aegypti* obtained from Tugujaya indicated a resistance to temephos. Potential resistance is 2.55 times of *Ae. aegypti* larvae reared in the laboratory. Resistance happens in three areas in Surabaya too, namely Tambaksari, Gubeng, and Sawahan with potential resistance 5.6; 5.6; and 8.5 times of *Ae. aegypti* laboratory strains.[13] Similarly, in Phnom Penh, Cambodia, with LC_{95} reached 0.030 to 0.038 ppm.[12] In contrast with research was performed in Sub-Distric Sidorejo, Salatiga City showed that *Ae. aegypti* were obtained from endemic, sporadic, and potential area of Dengue Haemorrhagic Fever are still susceptible to temephos, therefore temephos can be applied in community as an effort to control larvae.[14]

The researchers explained that *Ae. aegypti* larvae could become resistance against synthetic insecticide from organophosphate group strongly influenced by genetic factors, physiological, and operational mechanisms.[15,16] This occured due to the gene mutation-R as the controlling resistance, including esterase gene II and III are set esterase neutralize temephos insecticide. Physiological mechanisms can occur in two ways; thickening larval cuticle layer resulting in increasingly lower lipid layer to reduce the rate of penetration of toxins into hemocoel, and a decrease in the sensitivity of the enzyme acetylcholinesterase bond as a target (target sites) with temephos in insects. Operational mechanisms include the type of insecticide used and the method of application of insecticides in the community.[7,8,15,16]

Ae. aegypti have been resistant to experience a selection biochemical tolerance to neutralize exposure to temephos. The resistant population will increase compared to vulnerable populations. There will be visible trend of rising density of *Ae. aegypti* in the community, although efforts like the application of larvicidal temephos in the water container have been done.

The survey of entomology in the Village of Tugujaya found *Ae. aegypti* larvae at 60 household, and 66 containers. The existence of larvae and pupae are more commonly found in the bathtub. This shows that the community of Tugujaya using the bathtub as a reservoirs of freshwater for daily use. In accordance with citizens in Ramanathapuram-India, stating that *Ae. aegypti* larvae preference breeding site the form of water containers in the bathroom which was made from ceramics or cements. And also reported that a lot of household using that water containers for daily use.[17]

The Larvae Free Index in Tugujaya reached 77.36%, lower than the national target of 95%. This case indicates that the potential emergence of new cases of dengue fever. Commonly increasing cases of dengue fever can influenced by several components such as herd immunity, population density, mosquito-human interaction, virus strain, and climate, which affects mosquito biology and mosquito-virus interactions.[18,19] Components that have been identified in the study area is the existence of healthy humans and

Table 2. Indicator of Entomology for *Aedes aegypti Larvae* at DHF Endemic Areas in Tasikmalaya 2014.

Villages	Number of households		*Larvae Free Index*	Number of Container		*Container Index*
	Observed	Positive		Observed	Positive	
Cikalang	270	60	77.78	849	63	7.42
Kersanagara	245	45	81.63	736	54	7.34
Tugujaya	265	60	77.36	841	66	7.85
Sukamanah	283	64	77.39	886	75	8.47

Ae. aegypti. This means that if in these regions exist patient who carry the dengue virus, the local transmission could potentially occured. Based on the report of Tasikmalaya's Health Department, compared to the whole trend, Tasikmalaya dengue cases has decreased, Village of Tugujaya has increased of dengue cases number in the last five years. This happens because program of *"Selective Abate"* is not optimal.[6]

Based on these descriptions, it is known that resistance that occurs to larvae of *Ae. aegypti* caused by temephos. This has an impact on the level of larval density, and the trend of increasing number of dengue cases. Therefore, Tasikmalaya's Health Department should conduct a strategy to anticipate an increase in resistance of *Ae. aegypti* by means of insecticide rotation management. Some researchs explain that insecticide rotation management to four group of insecticides; organochlorines, organophosphates, carbamates, and pyrethroids could prevent or delaying the evolution of resistance.[20,21] Experiments carried out to the *Culex fatigan* who have resistant genes (gene-R) for the three types of insecticides (temephos, propoksur, and permethrin) showed that not develop into mosquitoes resistant, if rotation was done.[22]

CONCLUSION

Based on the result that has been described, it can be concluded that *Aedes aegypti* larvae obtained from the Village of Tugujaya is relatively resistant to temephos, whilst in the Villages Cikalang, Kersanagara, and Sukamanah still susceptible.

Tasikmalaya's Health Department needs to replace type of larvicides in order to control populations of *Ae. aegypti*, such as *Bacillus thuringiensis Israelis* (BTI) or by using Insect Growth Regulator.

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