

The Susceptibility of *Aedes aegypti* in Dengue Endemic Areas, Tegal, Central Java Indonesia

Kerentanan Aedes aegypti di Wilayah Endemis Dengue, Tegal, Jawa Tengah, Indonesia

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ABSTRACT

Tegal district is a dengue-endemic area. One of the strategies to control *Ae. aegypti* is the use of insecticides. The determination of insecticide resistance in a dengue-endemic area is useful for supporting policies for *Ae. aegypti* control program. The aim of this study is to determine the susceptibility of *Ae. aegypti* in Tegal district, Central Java. *Aedes aegypti* larvae were collected from June to July 2018. Susceptibility bioassay of *Ae. aegypti* larvae against temephos and *Ae. aegypti* female against permethrin were conducted refers to the WHO protocol. The susceptibility of *Ae. aegypti* was interpreted based on WHO protocol as well. The mortality of *Ae. aegypti* larvae were at 0.025 ppm by 90%. The LC₅₀ at 0.0005 ppm, and LC₉₉ at 1.1037 ppm, respectively. The mortality rate of *Ae. aegypti* against permethrin was 26%. The LT₅₀ at 6611.636 minutes, and LT₉₉ at 5958807.272 minutes, respectively. The susceptibility of *Ae. aegypti* larvae were possible resistant but adult *Ae. aegypti* was resistant.

Keywords: *Aedes aegypti*, permethrin, susceptibility, temephos

ABSTRAK

Kabupaten Tegal merupakan daerah endemis dengue. Salah satu strategi mengontrol *Ae. aegypti* adalah penggunaan insektisida. Penentuan resistensi insektisida di wilayah endemis dengue berguna untuk mendukung kebijakan program pengendalian *Ae. aegypti*. Penelitian ini bertujuan untuk menentukan kerentanan *Ae. aegypti* di Kabupaten Tegal, Jawa Tengah. Larva *Ae. aegypti* dikoleksi mulai bulan Juni sampai Juli 2018. Uji kerentanan larva *Ae. aegypti* terhadap temefos dan *Ae. aegypti* dewasa terhadap permetrin dilakukan dengan mengacu pada protokol WHO. Kerentanan *Ae. aegypti* diinterpretasikan berdasarkan standar WHO. Kematian larva *Ae. aegypti* berada pada konsentrasi 0,025 ppm sebesar 90%. Nilai LC₅₀ pada konsentrasi 0,0005 ppm dan LC₉₉ pada 1,1037 ppm. Kematian *Ae. aegypti* dewasa terhadap permetrin adalah 26%. Nilai LT₅₀ pada 6611,636 menit dan LT₉₉ pada 5958807,272 menit. Kerentanan larva *Ae. aegypti* adalah terduga resisten, tetapi *Ae. aegypti* dewasa adalah resisten.

Kata kunci: *Aedes aegypti*, permetrin, kerentanan, temefos

INTRODUCTION

Dengue fever (DF) is transmitted by *Aedes aegypti* and caused by Dengue Virus. In Indonesia, the incidence rate of Dengue Haemorrhagic Fever (DHF) was 77.96 cases per 100,000 people in 2016.¹ DHF cases has been reported in various regions of Indonesia throughout the country, including Tegal district. In 2017, the number of DHF cases in Tegal was 261 cases with a morbidity rate of 18.2 per 100,000 population. In Tegal district, DF cases still occur and the cases tend to fluctuate annually.² Nowadays, Tegal district is an endemic area for DHF.²

One of the strategies to control *Ae. aegypti* is the use of insecticides,³ because of quick action and high efficiency.⁴ Temephos, an organophosphate group, is currently still recommended as an insecticide for killing *Ae. aegypti* larvae.⁵ In the adult stage, permethrin, a pyrethroid group, is chosen to control *Ae. aegypti* due to its low toxicity against mammals and low price.⁶ However, if insecticides are used continuously for a long time, this may create an insecticidal-resistant generation of *Ae. aegypti*.⁷

Various studies showed that *Ae. aegypti* population has been resistant against these insecticide groups in Indonesia. A study in Padang of West Sumatra reported that *Ae. aegypti* larval resistance occurs in the Ka Koto and Gu Pangilun populations.⁸ Resistance to permethrin in *Ae. aegypti* was reported in Banjarmasin populations with low mortality due to mutation in the voltage-gated sodium channel gene.⁹ *Aedes aegypti* has been resistant against malathion, temephos, cypermethrin, deltamethrin, lambda-cyhalothrin, and tolerant against alpha-cypermethrin in Tegal municipality.¹⁰

DHF cases are still reported, although temephos and cypermethrin of pyrethroid group have long been applied to control *Ae. aegypti*.² Also, insecticide resistance has become a global concern, and a challenge faced by policymakers in vector control.¹ Meanwhile, insecticide resistance may affect the effectiveness of vector control program.

Detection and monitoring of insecticide resistance in a DHF endemic area is useful for supporting vector control policy. Currently, limited data has been reported regarding the susceptibility of *Ae. aegypti* against temephos and permethrin in Tegal district of central Java Indonesia. Therefore, the aim of this study is to determine the susceptibility of *Ae. aegypti* against temephos and permethrin.

METHODS

Aedes aegypti Samples

Aedes aegypti larvae were collected in field surveillance site from 100 houses by systematic sampling in Pangkah village, Tegal district from June to July 2018. Larvae were reared into adults in insectarium at the Department of Parasitology, Faculty of Medicine, University of Indonesia. The larvae were fed with commercial larval food. After eclosion, the adult females were fed the blood through an artificial membrane feeding, as Costa-da-Silva had previously demonstrated with some modifications¹¹ and then cotton pads with 10% sugar solution were used to feed the adult females. F3 generation of larvae were used for temephos bioassay and F3 generation of adult females were used for permethrin bioassay. This study was approved by the Ethics Committee of Faculty of Medicine UI with a reference number; 0633/UN2.F1/ETIK/2018.

Insecticide Bioassay

Temephos bioassay to *Ae. aegypti* larvae were carried out according to the World Health Organization (WHO) protocol.¹² We used four temephos concentrations (0.05, 0.025, 0.0125, 0.00625 ppm) to estimate the lethal concentration LC₅₀, and the LC₉₉. The concentration determination was based on an approach to the WHO resistance cut-off value (the LC₉₉ = 0.02 ppm). To obtain these bioassay solution concentrations, we weighed commercial temephos powder (Abate). Briefly, 1 mg temephos powder was diluted in 200 ml water to produce 0.05 ppm bioassay solution. Following this, we formulated other

concentrations of bioassay solution under the same conditions: 0.5 mg for 0.025 ppm, 0.25 mg for 0.0125 ppm, and 0.125 mg for 0.00625 ppm, respectively. For each concentration, four replicates were used and each replicate consists of twenty-five larvae. The control solution was 200 ml distilled water. As the control larvae, we used *Ae. aegypti* larvae from insectarium of the Department of Parasitology, Faculty of Medicine, University of Indonesia. The larvae mortality was counted at the end of a 24 hours exposure. If there was a mortality of 5-20% on the control larvae, the result should be corrected by the Abbot formula.¹²

Permethrin Bioassays against *Ae. aegypti* were conducted using WHO test tubes containing permethrin (0.25%) insecticide papers obtained from the University of Sains Malaysia's Vector Control Research Unit. Briefly, we performed four repetitions. One repetition consists of four test tubes (red tube) and one tube as control (yellow tube). As the control mosquitoes, we used *Ae. aegypti* from the Department of Parasitology insectarium, Faculty of Medicine, University of Indonesia. In each tube, a total of 25 individuals *Ae. aegypti* was needed. Twenty-five individuals with a minimum of 4 days old and feeding-sugar female *Ae. aegypti* were introduced into a green tube without insecticide paper for 30 minutes. *Aedes aegypti* were then placed into a red tube with permethrin 0.25% paper and into a yellow tube as a control for 60 minutes. *Ae. aegypti* were then transferred into paper cups

with absorbed cotton of 10% sugar solution and stored at room temperature.¹³

Aedes aegypti mortality was counted at 1, 6, 12, 24 hours period, respectively. The formula used to determine *Ae. aegypti* mortality rate was a total number of dead mosquitoes/total sample size at each exposure time x 100%. The susceptibility of *Ae. aegypti* was interpreted based on WHO protocol (\geq 98% mortality: susceptible, 90–97% mortality: possible resistance; $<$ 90% mortality: resistance)¹³, where the cut-off value for larvae was 0.025 ppm. Probit analysis was performed to calculate the LC₅₀ and LC₉₉ values, and the LT₅₀ and the LT₉₉ value using SPSS 20 (IBM, USA).

RESULTS

The temephos bioassay results for larvae are presented in Figure 1. The mortality of *Ae. aegypti* larvae were at a concentration over 0.025 ppm, as indicated by the average mortality rate at 0.025 ppm was 90%. Probit analysis result was $y = 0.701x + 7,296$. Based on this equation, the estimated Probit for LC₅₀ value was obtained 0.0005 ppm and LC₉₉ value was obtained 1.104 (Figure 2).

The permethrin bioassay results are presented in Figure 3. *Aedes aegypti* population from Pangkah showed mortalities less than 90%. Probit analysis result was $y = 0.787x - 3.008$. Based on this equation, the estimated Probit for LT₅₀ value was obtained 6611,636 minutes and LT₉₉ values were obtained 5958807,272 minutes (Figure 4).

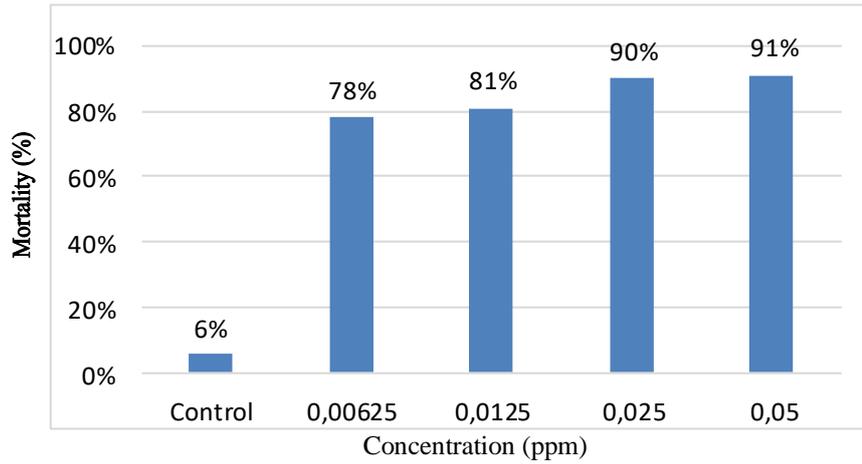


Figure 1. Mortality of *Ae. aegypti* Larvae Against Temephos

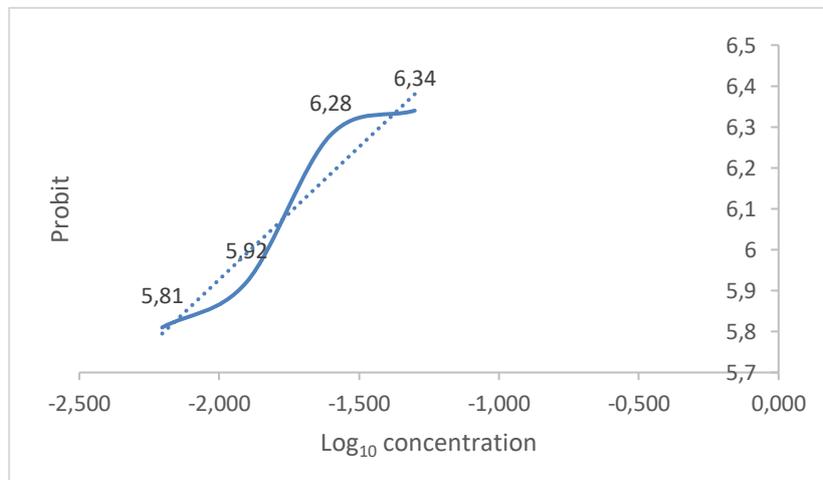


Figure 2. Scatter Graphic of *Ae. aegypti* Larvae Against Temephos

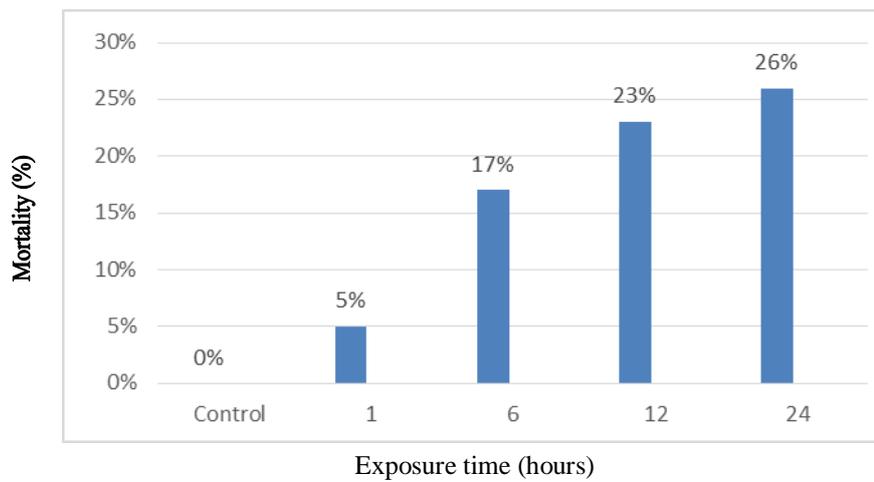


Figure 3. Mortality of *Ae. aegypti* Against Permethrin 0.25%

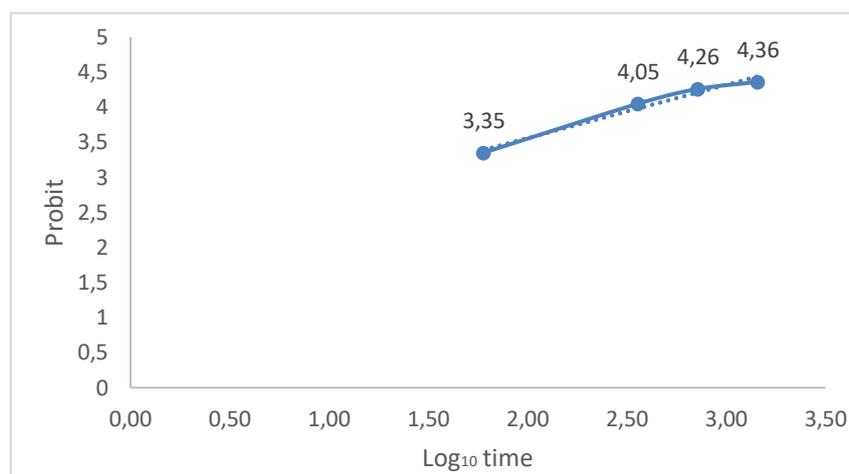


Figure 4. Scatter Graphic of *Ae. aegypti* Larvae Against Permethrin

DISCUSSION

As a result, *Ae. aegypti* larvae were classified as possible resistant since the larvae mortality rate was 90% at 0.025 ppm, according to WHO criteria.¹⁴ Temephos resistance can occur due to changes in the work of enzymes in the larval body. These changes make it difficult for larvicides to bind to targets or to interfere in the larval body.¹⁵ Furthermore, one of the factors contributing to *Ae. aegypti* larvae resistance is varying the application of temephos with various frequencies of implementation.¹⁶ Since the use of temephos in Mexico varies between urban and rural applications, the larval eradication program fails and risks causing resistance to *Ae. aegypti* larvae.¹⁷

For the permethrin 0.25% bioassay, this result revealed that the Pangkah population was resistant to permethrin. Various studies regarding the permethrin resistant mechanism have been widely reported. Resistance against permethrin could be due to decreased sensitivity to sodium channels¹⁸ and increased metabolic detoxification mainly through the P450 enzyme.¹⁵ In Jakarta, *Ae. aegypti* population, the key mechanism of permethrin resistance through the V1016G gene mutation which plays a role in decreasing sodium channel sensitivity.³ Meanwhile, resistance is mainly

associated with mutations in Cys 1,534 gene which play a role in the sodium channel in Jamaican *Ae. aegypti* population.¹⁹

This study revealed different results compared to other similar studies. These could be due to differences in the study's geographical conditions. Tegal district is known to have a tropical climate, with temperatures ranging from 24 to 28°C. Glunt *et al* reported that the ambient temperature influences on the efficacy and toxicity of insecticides used in public health such as applications for *Anopheles* spp.²⁰ Temperature changes are known to affect permethrin effectiveness. Permethrin perform effectively within a temperature range of 30-32°C and perform poorly in a temperature range of 32-34°C.²¹

Pyrethroid and organophosphate resistance studies have been carried out worldwide including Indonesia since most dengue-endemic countries are currently using pyrethroids and organophosphates in their vector control programs. Similar to this study, *Ae. aegypti* larvae of Padang strain are possible resistant against temephos, where the mortality is 86.7%.²² The LC₅₀ value (0.0005 ppm) obtained in this study was lower than the LC₅₀ value obtained from the Bekasi strain (0.001 ppm), but the LC₉₉ value (1.104 ppm) was higher than the LC₉₉ value of the Bekasi

strain (0.018 ppm) reported by Sinaga *et al.*²³ However, based on the probit analysis, the LC₉₉ value exceeds 0.02 ppm, precisely at 1,104 ppm. These results indicate that there has been resistant of *Ae. aegypti* larvae against temephos. Ikawati *et al* revealed that insecticide resistance to *Ae. aegypti* may be due to cross-resistance in the same group of insecticides and long-term use of the same group of insecticides.²⁴

After the discovery of DDT resistance in mosquitoes in 1976, insecticide resistance has become a major issue in vector control. Moreover, if this issue is not addressed immediately, long-term resistance is likely to occur and the vector control system may fail while insecticides are very useful as vector control weapons.¹⁸ As mentioned previously, temephos and cypermethrin have long been used for controlling *Ae. aegypti* in Tegal district. Based on this fact, it seems urgent to immediately modify the insecticide control method that can be combined with other approaches such as the biological control method.

Biological control could be carried out using organisms that prey, parasitize, or compete with target species with the aim to reduce population. Biological control against *Ae. aegypti* can use *Gambusia affinis* fish, *Toxorhynchites* mosquitoes larvae, or cecopods especially mesocyclops and macrocyclops species.²⁵ Also, the use of plant extracts could be an alternative to kill *Ae. aegypti* larvae²⁶ and the adult stage.²⁷

CONCLUSION

In Pangkah village, Tegal, *Ae. aegypti* larvae is possible resistant against temephos, while adult *Ae. aegypti* has been resistant to permethrin 0.25%.

RECOMMENDATION

Insecticides have been confirmed resistance should be replaced or rotated with the susceptible insecticides for application in the field.

AUTHOR CONTRIBUTION

In this article, NEF and HW are the main contributors who conduct conceptualisation, investigation, methodology, validation, supervision. IK, BFA, HA, VAA, RW, RS, and LS as co-contributors who conduct field studies, data curation, formal analysis and writing-review and editing.

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