

# Resistance Test for *Anopheles* spp. to Cypermethrin Insecticide Using Bottle Bioassay Test Method

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## ABSTRACT

The resistance of *Anopheles* spp to insecticides is the most important and dangerous problem that affects malaria control programs. One of the methods used to detect insecticide resistance is the bottle bioassay test method from Centers for Disease Control (CDC) which can be applied as part of a broader insecticide resistance monitoring program. This study aimed to determine the resistance of *Anopheles* spp. against the insecticide cypermethrin at the location of malaria cases in 2018 in Sabang City. The locations for resistance tests were carried out in Balohan, Batee Shok and Iboih areas. The resistance test of *Anopheles* spp. against cypermethrin insecticide was carried out using the CDC bottle bioassay method with 100 mosquitoes per location. The resistance data were analyzed using probit regression. The results of resistance testing against the insecticide cypermethrin showed that the mortality rate of *Anopheles* spp in three different locations, namely Balohan with a mortality rate of 3%, Iboih 98% and Batee Shok 99%. The LT<sub>50</sub> values of the Balohan were 101.47 minutes, Batee Shok 25.38 minutes, and Iboih 13.50 minutes, with resistance ratio values of 16.67, 1.82 and 1.02, respectively. It can be concluded that *Anopheles* spp. in Balohan is resistant to the insecticide cypermethrin, while *Anopheles* spp. found in Batee Shok and Iboih areas are classified as susceptible to the insecticide cypermethrin.

**Keywords:** *Anopheles* spp., insecticide cypermethrine, centers for disease control bottle bioassay test

## 1. INTRODUCTION

Malaria is a reemerging disease, a disease which came back to infect the masses transmitted by *Anopheles* spp. mosquito (vector borne disease) [1]. In Indonesia, malaria cases are still occurred in several provinces, among them in Aceh Province. Regions in Aceh Province where malaria cases could be found are West Aceh, Northwest Aceh, East Aceh, and Sabang City. In 2014, Sabang City has nationally been declared eliminating malaria, and in 2015 is within the list of malaria elimination maintenance stage [2]. This elimination program refers to regional malaria infection prevention program applied on a geographical area. With the elimination program in place, it does not mean imported malaria cases does not occur or if the vector ceases to exist. There were still 37 malaria cases reported caused by *Plasmodium knowlesi*, in 2018 spreaded over several region in Sabang city [2]. Thus, vector control efforts should still be implemented in

Sabang City.

*Anopheles* spp. mosquito control in Sabang City in the last two years used synthetic perythroid group insecticide, one of them is cypermethrin. The control method conducted was indoor residual spraying (IRS) and outdoor residual spraying (ORS) in several villages. Within nine villages chosen as the focus of mosquito control effort, only three villages were within target scope and receptive (conductive environment for mosquito growth) in Sabang City: Balohan with 969 houses as target, Batee Shok with 424 houses, and Iboih with 576 houses [2]. However, resistance evaluation of *Anopheles* spp. mosquito against insecticide in Sabang City has never been conducted before. Thus, a study in *Anopheles* spp. mosquito resistance against insecticide, especially cypermethrin is required as an effort to evaluate and monitor malaria control in Sabang City. According to Vatandoost [3], insecticide resistance in mosquitoes acting as vector for diseases, is a most important developing problem in many countries and

poses danger, and thus bioassay test to detect and determine insecticide resistance characteristics in a natural vector population of *Anopheles* spp. should be done as part of malaria control effort. Centers of Disease Control method of bottle bioassay test is a new method used to detect insecticide resistance. This method could be implemented as part of broader insecticide resistance monitoring program. The advantages of this method are more efficient, rapid, and easier to be performed compared to other resistance tests [4]. This research aimed to determine *Anopheles* spp. mosquito's resistance against cypermethrin insecticide.

## 2. MATERIALS AND METHODS

Samples used in this research were 375 *Anopheles* spp. mosquitoes collected in three locations in Sabang city: Balohan region, Bate Shook region, and Iboih region of Sabang city. Independent variable in this research was insecticide with Cypermethrin as its active compound, while dependent variable in this research was *Anopheles* spp. mosquitoes.

### 2.1. Research Procedure

Mosquitoes were collected by purposive sampling using animal bite trap method with cow as a bait, using aspirator from 17.30 to 07.00 GMT+7. Collected mosquitoes were stored in paper cup labeled by their collection location and would then be used for bottle bioassay test resistance test. Coordinate point was taken by using Global Positioning System (GPS) to mark mosquito collection sites.

### 2.2 Resistance Test Preparation

In this research, insecticide of pyrethroid group, cypermethrin in 12.5 µg dosage was used. To obtain the concentration of 12.5 µg/bottle, 13.2 mg cypermethrin insecticide was diluted in 1000 ml acetone and every 1 ml liquid contain 12.5 µg Cypermethrin insecticide which would then be used for this research's resistance test. 1 ml cypermethrin was added to the bottle then sealed. For control bottle, one drip of ethanol was used and then the bottle was sealed. The test bottles were placed horizontally and then flipped, then the bottle cap was slightly opened to let ethanol to evaporate. The bottles were then covered by fabric or carton to shield them from light.

### 2.3. Dead Mosquito Criteria

Mosquito is considered dead when it is unable to stand anymore. To determine whether the mosquitoes were dead or not, test bottles were slowly rolled. If the mosquitoes were unable to move and fell, the mosquitoes would be regarded as dead. CDC bottle test

data interpretation was interpreted according to the protocol used. The vulnerability threshold was calculated on 30 minutes of diagnostic time for all tested insecticide with the following criteria: a) if the tested mosquito death was 98-100% it would be categorized as vulnerable, b) if the tested mosquito death was 80-97% it would be categorized as suspectedly resistant, if the tested mosquito death was <80%, it would be categorized as resistant [5]. Data of *Anopheles* spp. resistance against Cypermethrin insecticide in this research was analyzed by Probit Regression.

## 3. RESULTS AND DISCUSSION

### 3.1. *Anopheles* spp. Mosquito Morbidity after Resistance Test

Table 1 showed the result of *Anopheles* spp. mosquito resistance against cypermethrin insecticide in three test sites (Balohan, Batee Shok, and Iboih).

Based on the below Table 1, the highest  $LT_{50}$  value was observed in Balohan region with 101.47 minutes with resistance ratio value 16.67 times, showing lower mosquitoes morbidity (61%) compared to Batee Shok and Iboih region. This showed that *Anopheles* spp. mosquitoes in Balohan region was 16.67 times more resistant against Cypermethrin insecticide compared to mosquitoes in Batee Shok and Iboih region.

**Table 1.** *Anopheles* spp. mosquito resistance test against cypermethrin insecticide

Sampling site	Number of tested mosquitoes	$LT_{50}$ (SK95%) minute	RR (30 minutes)	Status
Balohan	100	101.47 (96.35-107.59)	16.67	Resistant
Batee Shok	100	25.38 (22.36-27.79)	1.82	Vulnerable
Iboih	100	13.50 (10.93-15.35)	1.02	Vulnerable

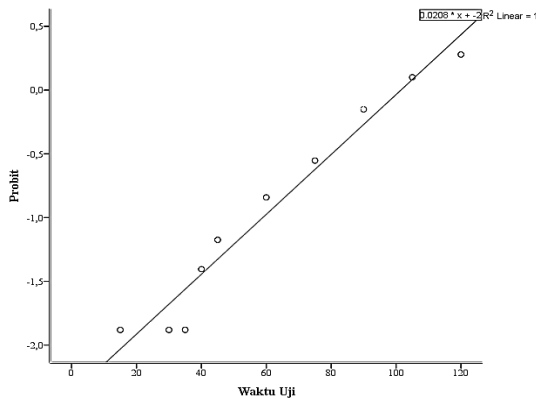
$LT_{50}$  : Lethal Time 50

RR : Resistance Ratio

The result obtained in this research was quitesimilar to what was reported by Isfanda *et al.* [6] on *Aedes aegypti* mosquito exposed to cypermethrin with highest  $LT_{50}$  on  $F_5$  generation larvae with 99.56 minutes. Susanti and Boesri [7] also reported morbidity of *An. aconitus* outside of houses exposed to cypermethrin 50 ml/Ha by thermal fogging only reached 73,6% within ( $LT_{50}$ ) 46.39 minutes period compared to 100 ml/Ha dosage with morbidity reaching 100% within ( $LT_{50}$ ) 11,74 minutes time period, and the  $LT_{50}$  value in *An. aconitus* mosquito was faster compared to *A. aegypti* (61.46 minutes) and *C. quinquefasciatus* (107.23 minutes).

The high resistance ratio in Balohan region might be caused by the high usage of insecticide in the region as well as land use pattern which potentially increase *Anopheles* spp resistance against insecticide. Based on

the map of land use in Balohan region (Figure 1), land is usually used for dwelling/housing, agricultural land, plantations, and fisheries which involve the use of insecticide and pesticide in everyday activities. Residents around Balohan also use insecticide containing net and other commercial home-use insecticide with perythroid active compound in aerosol form to avoid mosquito bites.



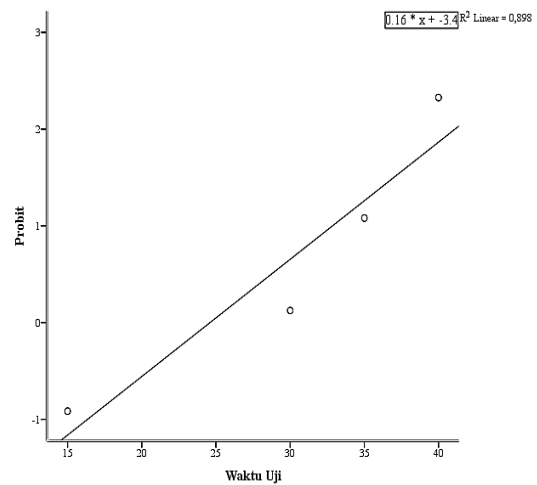
**Figure 1** The relationship of *Anopheles* spp. morbidity and resistance period against Cypermethrin insecticide for Balohan region.

Widawati and Kusumastuti [8] reported that the use of direct home-use insecticide cannot kill some of adult female mosquito which may lay eggs and also unable to kill larvae around the house. Zulfikar *et al.* [9] stated that the use of deltamethrin insecticide within WHO recommended dosage (25 mg/m<sup>2</sup>) is ineffective to kill *Anopheles* spp. in house. Moreover, according to Cissel *et al.* [10] the use of insecticide in agricultural land, plantation, and fisheries (pond) may also influence the rising of *Anopheles* spp. mosquito resistance ratio against perythroid insecticide.

The LT<sub>50</sub> value in tested mosquito of Batee Shok region was 25.38 minutes with resistance ratio of 1.82 times, and mosquito morbidity reaching 99%. This means that on average *Anopheles* spp. mosquito in Batee Shok area were dead within 25.38 minutes and categorized as vulnerable against cypermethrin with lower resistance ratio compared to Balohan with 1.82 times, and even higher if compared to Iboih (1.02 times). The resistance level graph of *Anopheles* spp. against cypermethrin for Batee Shock region can be seen in Figure 2.

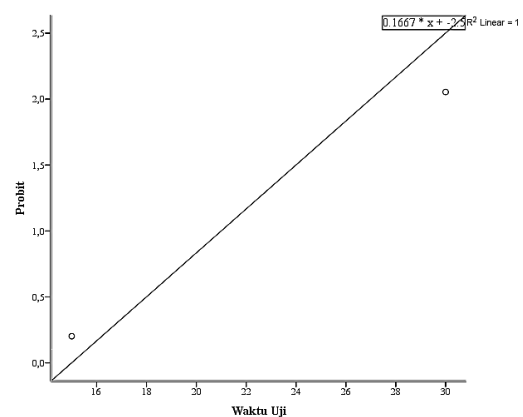
Moreover, the low resistance ratio of *Anopheles* spp. in Batee Shok region may also be related to land usage by the residence of the area. Based on observation, the land usage of Batee Shok region is dominated by agricultural land, open field, wild bush, jungle, forest, and a lake/dyke as well as limited resident dwelling. This showed that Batee Shok has a high potential for *Anopheles* spp. growing area and not often exposed to

insecticide and agricultural pesticide and fisheries (pond) compared to Balohan area.



**Figure 2** The relationship of *Anopheles* spp mosquito morbidity and cypermethrin insecticide resistance period for Batee Shock region.

The LT<sub>50</sub> value of tested mosquito in Iboih region was 13.50 minutes with 1.02 times resistance ratio and mosquito morbidity reaching 98 %. This means that on average *Anopheles* spp. mosquitos in Iboh region were dead within 13.50 minutes and considered as very vulnerable against cypermethrin insecticide. According to mortalitytime, *Anopheles* spp. mosquitoes from Iboih region had a faster mortality time compared to Balohan region, it was quite similar with *Anopheles* spp. obtained from Batee Shok area. The graphic of *Anopheles* spp. resistance level against cypermethrin insecticide for Iboih region is provided in Figure 3.



**Figure 3** The relationship between *Anopheles* spp. morbidity and resistance period against Cypermethrin insecticide in Iboih region.

The resistance level of *Anopheles* spp. mosquitoes in Batee Shok and Iboih region were considered as 1.82 and 1.02 times, respectively. Based on these, it could be said that *Anopheles* spp. mosquitoes in Balohan region

are proven to have exhibited resistance against cypermethrin insecticide. This is in line with the statement from Brogdon and Chan [11] who said if the morbidity of tested mosquito was <80% within 30 minutes period, the mosquito is categorized as resistant against insecticide.

#### 4. CONCLUSION

Based on the research result, it can be concluded that the *Anopheles* spp. mosquito insecticide resistance against cypermethrine on three malaria case location sites in 2018 in Sabang city, one of locations, Balohan region, was found to be resistant, while in Batee Shok and Iboih regions appeared to be vulnerable against cypermethrine insecticide. The resistance data of cypermethrine in *Anopheles* spp mosquito calculated by probit regression showed that highest  $LT_{50}$  was found in Balohan region (101.47 minutes) with resistance ration being 16.67 times and lower mosquito morbidity (61%) compared to Batee Shok and Iboih region. The  $LT_{50}$  value of tested mosquitoes in Batee Shok was 25.38 minutes with resistance ratio value being 1.82 times and mosquito morbidity reacing 99%. The  $LT_{50}$  value of tested mosquitoes in Iboih region is 13.50 minutes with resistance ratio value being 1.02 kali times and mosquito morbidity reaching 98%.

#### AUTHORS' CONTRIBUTIONS

MH, YF, and S conceptualized dan designed this research. The research was carried out by S, and MH, R, YF, TZH, and S drafted, revised, and finalized the manuscript. All authors read and approved the final manuscript.

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