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Susceptibility status of *Aedes aegypti* (Diptera: Culicidae) larvae against Temephos in Padang, West Sumatera, Indonesia

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Abstract
The Larvicide Temephos has been widely used since the 1980's to control the larvae of *Aedes aegypti* which is the main vector for Dengue Fever. The continuous usage of Temephos for a long period of time may cause resistance for mosquito larvae. The aim of this study was to monitoring the susceptibility status of the *Ae. aegypti* towards Temephos in five locations in Padang, West Sumatera, Indonesia. The Susceptibility Test was based by WHO's Bioassay method (temephos 0.0120 mg/L) towards the *Ae. aegypti* larvae. The *Ae. aegypti* mosquito was resistant at Kp. Koto and Gn. Pangilun populations, tolerant at Cp. Tangah population, and still susceptible at KDP. Karakah and Ka. Dalam populations. In general, the concentration proposed as the diagnostic concentration by WHO was no longer effective in controlling the larvae of *Ae. aegypti* in Padang. The concentration of Temephos needed to control the larvae of *Ae. aegypti* in Padang was1-3.3 times higher than WHO's diagnostic concentration (0.012 mg/L).

Keywords: monitoring, dengue fever, mosquito, pest management

1. Introduction
The main vector of dengue fever is the *Ae. aegypti* mosquito that is endemic to Indonesia and other tropical Asian countries. The monitoring of Dengue fever in West Sumatra Province, Indonesia was found that the case of this Fever increased gradually in last four years. In,2015 there has been 3,047 cases with an Incidence Rate (IR) as much as 62.87/100,000 resident with 19 death or Case Fatality Rate (CFR) as much as 0.62 percent [1]. However, the most of dengue fever cases were reported from Padang city, the capital city of West Sumatra [2, 1], with 660 cases of dengue fever that caused 6 deaths in 2014 and increase to 1,126 cases with 8 deaths in 2015 [2, 1].
The vectors control still the best available effort and strategy to control and prevent dengue transmission. In Indonesia, the ministry of health suggested using Larvicide Temephos which is also recommended by WHO to control *Ae. aegypti* in household clean water containers [3, 4]. The Larvicide Temephos tends to be overused in controlling the mosquitoes, although its concentrate increased every year since 1980, the rate of the dengue fever cases is still high until recently [5]. The continuous usage of Temephos has led to development resistance in the insect. The insecticide resistance in insect will happen in such of conditions when it used in high frequency and lower the susceptibility rate of the insect towards the insecticide [6]. The insecticide resistance, especially for Temephos, was reported from around of the world, such as Malaysia, Argentina, India, Thailand, Brazil and Cuba [7, 8, 9, 10, 11, 12].
The *Ae. aegypti* and *Ae. albopictus* mosquito has been reported to be resistant towards Temephos in Selangor, Malaysia in 2005 [7] and in Thailand [13]. *Ae. aegypti*is also resistant towards Temephos in several cities in Indonesia like Tasikmalaya, Banjarmasin, Palembang, Surabaya, Kendari, Bali, Bandung [14], Bogor, Tasikmalaya, Sumedang, Garut and Semarang [15].
The cases of dengue fever in Padang city was categorized as relatively serious cases compared to other cities in Indonesia. The resistance of mosquitoes to Temephos is the main concern to take new strategy controlling and preventing dengue transmission. So the aim of the present study was to evaluate the susceptibility status to Temephos in *Ae. aegypti* populations in five locations in Padang where are the most cases happen.

2. Method
2.1 Rearing and Larvae Identification
Ae. aegypti larvae were collected from the households in five locations in Padang with a purposive sampling method (Table 1). Then it was reared in Animal Physiology Laboratory, Department of Biology, Andalas University. The larvae were kept in plastic containers filled with clean water and then fed some grinded chicken feed (merk: charoen pokphand) until it is grown into the 3rd and early 4th instars [16]. The larvae were identified following [17].

Table 1: *Ae. aegypti* larvae populations collecting locations in Padang

Locations	Population Code	Year Collected
Cupak Tangah	Cu Tangah	End of 2016
Kampung Dalam	Ka Dalam	End of 2016
Kapalo Koto	Ka Koto	End of 2016
Kubu Dalam Parak Karakah	KDP Karakah	Early 2017
Gunung Pangilun	Gu Pangilun	Early 2017

2.2. Susceptibility Test

A Temephos stock with a concentration of 6.25 mg/L was diluted with ethanol to obtain several concentrations. Furthermore, the concentrations of solutions were designed by the result and effect of WHO diagnostic concentration (0.0120 mg/L) to the larvae. It was used to determine the LC_{50} and LC_{95} in following conditions; if the larval mortality rate is<90% then need to increase in concentrations of Temephos, and if the larval mortality rate is >98% then decrease the concentrations. Based on this preliminary test, we determined the five treatment concentrations including the WHO diagnostic concentration: 0.0050 mg/L; 0.0075 mg/L; 0.0120 mg/L; 0.0200 mg/L and 0.0250 mg/L.

Susceptibility Test of the *Ae. aegypti* larvae follow the WHO Bioassay standard method. The Temephos solution with each concentration was poured into 300 mL test containers (plastic cups). Then 20 individuals of 3rd and early 4th instar larval stage were inserted into each plastic cups. The selected larvae were used in this test, and the small, deformed, and unhealthy larvae will remove. The mortality was recorded 24 hours [18]. The moribund or dying larvae (not able to swim into the water surface and not showing any diving reaction when interfered) were considered as dead larvae. Every treatment was repeated three times.

2.3. Data Analysis

The *Ae. aegypti* larval mortality rate on each population was evaluated by Probit Analysis to determine its $LC_{95,}$ and LC_{98} after 24 hours with Polo PC [19]. The Susceptibility test data were analyzed according to WHO [18]: resistant if mortality rate < 90%; tolerant if mortality rate 90-97%; Susceptible if mortality rate 98-100%.

3. Result and Discussion

The larval mortality rate in WHO diagnostic concentration (0.0120 mg/L) was varied among the populations. The highest mortality rate in this concentration was recognized in Ka Dalam and KDP Karakah populations with 100% and then followed by Cu Tangah population with 95%, Gu Pangilun population with 61.7% and Ka Koto population with 10% (Table 2).

Table 2: The susceptibility of *Ae. aegypti* against Temephos in various concentrations after 24 hours

Population Code	Percentage of *Ae. aegypti* larvae mortality rate in each concentration (%)						Susceptibility status*
	Control	0.0050 (mg/L)	0.0075 (mg/L)	0.0120(mg/L)	0.0200 (mg/L)	0.0250 (mg/L)	
Cu Tangah	0	18.30	36.60	95.00	-	-	Tolerant
Ka Dalam	0	55.00	63.30	100.00	-	-	Susceptible
Ka Koto	0	5.00	-	10.00	100.00	-	Resistant
KDP Karakah	0	5.00	52.00	100.00	-	-	Susceptible
Gu Pangilun	0	-	-	61.70	-	86.70	Resistant

(-) mark is not tested

(*)Susceptibility status towards 0.0120 mg/L (WHO diagnostic concentration)

The results obtained from the present study showed that each population of *Ae. aegypti* mosquito larvae have different reactions to the Temephos with a concentration of 0.0120 mg/L. The Ka Koto and Gu Pangilun populations were shown as resistant which has low mortality rate. In other hands, Cu Tangah population was exhibited tolerant and susceptible in Ka Dalam and KDP Karakah populations. The case of resistance in *Ae. aegypti* towards Temephos with 0,0120 mg/L concentration has also been reported in the previous study such as Surabaya (Indonesia) [20]; Brazil [21] and Malaysia [7].

The difference of susceptibility status among mosquito populations is caused by using various concentrations of Temephos in each location. While the resistance case in a population was possibly caused by the usage of Temephos in high dosage. According to a direct interview and information from Padang Health Office, Temephos (locally known as Abate) is used by local residents sporadically without any schedule. This also related to the practice of residents when the Dengue Fever cases increase, they used Temephos in uncontrolled high dosage. Based on Hudson [22], the rate of insecticide affectivity depends by the selection pressure in each individual insect in fighting the toxin of Temephos, such as how long, how frequent, and how much insecticide used.

A few factors that correlate to the rate of resistance developing in mosquitoes can be the high frequency of insecticide usage and long exposures towards it, which causes death to susceptible individuals while the resistant ones prevail. Those are the most influential factors in accelerating the rate of resistance developing in mosquitoes in endemic sites of Dengue Fever [23]. There are three physiological mechanisms that can happen in a resistant's insects body which is the increase of insecticide detoxification because of certain enzymes, a decrease of sensitivity in insecticide's target cells, and a rate decrease in insecticide's penetration site in the integument [24]. Resistant mosquitoes in Padang especially in the Gu Pangilun population is suspected to already undergo that process. The Gu Pangilun population was collected from an endemic Dengue Fever site and according to a report by Padang Health Office, has the highest number of Dengue Fever Case in Padang, which reached 32 cases in 2016 [25].

The mode of entry from the Temephos toxin in *Ae. aegypti* larvae are from contact at its spiracle, a body segment between abdomen and mouth. Temephos exposure in 3rd instars larva accumulates the highest in muscle and spreads to its entire body by hemolymph. Generally, the effect of Temephos towards larvae starts with a tremor. The tremor that happens causes larvae to need larger energy intake, thus it gets drained with energy which ends with paralysis and death. The paralysis process in larvae happens due to an accumulation of *acetylcholine* on its nerve cells. *Acetylcholine* is a neurotransmitter on larvae's neurological system. The accumulation of *acetylcholine* in nerve cells is caused by Temephos which inhibits the *acetylcholinesterase* enzyme and

prevents it from hydrolyzing *acetylcholine* [24].
Based on the explanation on the mechanisms of insecticide resistance, it is suspected that there has been an insecticide detoxification caused by enzymes in the mosquitoes' body. Hence, a follow up biochemical experiment is needed.

Table 3: Lethal Concentration ($LC_{90\ dan\ 98}$) and concentration ratio of *Aedes aegypti* larvae towards Temephos on several populations collected in Padang after 24 hours

Population Code	LC_{90} (mg/L)	LC_{98}(mg/L)	Concentration Ratio*
Cu Tangah	0.0115	0.0136	1.33
Ka Dalam	0.0097	0.0125	1.04
Ka Koto	0.0189	0.0216	1.80
KDP Karakah	>0.0120$^{\alpha}$	>0.0120$^{\alpha}$	>1
Gu Pangilun	0.0335	0.0400	3.33

* Concentration Ratio: LC98 compared with WHO diagnostic concentration (2016) 0.0120 mg/L
A Probit analysis unavailable due to mortality over 95%

Susceptibility Status criteria compiled by WHO [18] determines resistant if mortality rate < 90%; tolerant if mortality rate 90-97%; Susceptible if mortality rate 98-100%. These criteria will meet if the concentrations are according to Table 3, where all fifth of the population would be tolerant towards Temephos with 0.0097-0.0335 mg/L concentration and will be susceptible towards concentrations of 0.0125-0.0400 or 1.04-3.33 higher than the WHO recommended concentration which is 0.0120 mg/L.
The highest Temephos concentration needed is on Gu Pangilun population which needed a concentration 3.3 times higher than the WHO diagnostic concentration. This supports the hypothesis that the steep number of Dengue Fever case in Gu Pangilun collection site is caused by the inability to control the mosquitoes with Temephos (Abate) with its designated concentration thus the risk of spreading Dengue Fever gets higher, moreover, it is a densely packed residential area.
The development of Temephos resistance towards *Ae. aegypti* will cause many problems in controlling this Dengue Fever vector mosquito [26]. Therefore, a monitoring in mosquito susceptibility towards insecticide needs to be done periodically in Padang to slow down the resistance rate and prevent the further spread of Dengue Fever. Resident awareness is also very much needed to lessen the breeding site of this mosquito. Socialization and monitoring by health officers is a crucial step in controlling insecticide use in Padang

4. Conclusion
The susceptibility status of *Ae. aegypti* larvae towards Temephos with the diagnostic concentration of WHO (0.012 mg/L) is still susceptible at Ka Dalam and KDP Karakah populations, tolerant at Cu Tangah population and resistant at Ka Koto and Gu Pangilun populations. The effective usage of Temephos in Padang is 0.0120-0.0400 mg/L with a ratio 1-3.3 times higher than the WHO diagnostic concentration.

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