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Original Article

The Effect of Temephos to Mortality and Life Level of *Aedes aegypti* mosquitoes

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ABSTRACT

Background: Temephos is an insecticide active ingredient often used by a community as Dengue Hemorrhagic Fever (DHF) vector control in imago. The development of *Ae aegypti* is influenced by biotic and abiotic factors.
This study aimed at determining the effects of temephos concentration on mortality and a life level of *Ae aegypti* at some concentration tests.
The experimental design used in this study was Complete Random Design (CRD). This study involved temephos as the active ingredient and third instar Ae. Aegypti larvae originating from Pangandaran Regency. Data analysis used an ANOVA test with 5 treatments and 5 replications.
The results showed that there was any influence of concentration towards a number of mortality, a life level, morphology of larvae, pupae and imago, a number of males and females, and a number of eggs hatchability. Temephos concentration affected the mortality of third instar larvae of Ae. aegypti with temephos concentration of 0.019 ppm, 0.069 ppm, 0.105 ppm, and 0.140 ppm and affected a life level of Ae. aegypti with biological parameters.

INTRODUCTION

Temephos is one of the insecticide active ingredients for controlling vectors of Dengue Hemorrhagic Fever (DHF). Chemically, temephos is included as a type of organophosphate. Temephos solution can be used by mixing it with water. A study showed that this kind of chemical material can kill mosquito larvae and is not harmful to human health.[1] Another study revealed that temephos can kill mosquito larva quickly by absorbing more than 99% of temephos in a mosquito body for 24 hours.[2] Indonesia has some types of mosquitoes and about 23 species play a role as a vector-like genera of *Anopheles, Aedes, Culex, Armigeres,* and *Mansonia*.[3] One of these mosquito types, *Aedes aegypti*, is being a vector of DHF.[4] *Ae. aegypti* is included as a class of insect, ordo of Diptera, and family of Culicidae. *Ae. aegypti* lives in the environment with average rainfall is more than 500 mm per year, room temperature is 32-34 ^{0}C , water temperature is 25-30 ^{0}C, pH of water is 7, and humidity is about 70%.[5] These environmental conditions affect to lifecycle and development of *Ae. aegypti.*

A lifecycle of *Ae. aegypti* starts from egg, larva, pupae, and imago. This species is included as an insect that has complete metamorphosis. A study demonstrated that a lifecycle of *Ae. aegypti* can be broken using temephos.[6] Temephos is one of the most commonly used Larvicides in a community and well-known to control *Ae. aegypti* larva. Nowadays, the use of temephos provides a positive effect for the success in preventing DHF. Notwithstanding, as a negative effect, *Ae. aegypti* mosquitoes are becoming resistant to temephos.[7] A study in West Banjarmasin showed *Ae. aegypti* larva in Banjarmasin was resistant to temephos. This was shown from the concentration of temephos that was provided did not influence the mosquito lifecycle. [8] Another study found temephos influenced the morphology of *Ae. Aegypti* egg of Bogor strain. Concentrations consisted of 0.150 ppm; 0.280 ppm; 0.330 ppm; 0.384 ppm, and 0.433 ppm.[9]

This study used a high-concentration of temephos. Even though it was effective, the high-concentration of temephos could contaminate surface waters, affect human health, and become resistant to larva. There needs to examine by using lower concentration for measuring to what extent the influence of temephos towards a lifecycle of *Ae. aegypti* larva particularly Pangandaran strain.

MATERIALS AND METHOD

This was an experimental study using a Complete Random Design (CRD). *Ae. Aegypti* egg was from Pangandaran strain that was reared at the Health Research Laboratory of Lokalitbangkes Pangandaran. Mosquito eggs are hatched and bred at the Animal Physiology and Entomology in Sunan Gunung Djati State Islamic University of Bandung.

The examination was performed using larva instar III as many as 25 as individuals for each concentration that was repeated five times. Concentrations of temephos used for the tests consecutively were: 0.019 ppm, 0.069 ppm, 0.105 ppm, and 0.140 ppm. The observation was conducted during 1x24 hours with observations at hour 0, hour 1, and hour 24. After 24 hours, some following activities were performed to observe larva morphology, pupae, and imago that were still alive from concentrations of the test.

Data analysis used ANOVA to identify the influence of concentrations of temephos towards the mortality of *Ae. aegypti* larva. Furthermore, a Duncan test was performed to find significance value. Mosquito life level was calculated using the following formula:

$$SR = \frac{Nt}{No} x\ 100\ \%,$$

Whereas SR means *Ae. Aegypti* mosquitos that were still alive (%), Nt means the number of mosquitos that were still alive at the end of a study (individual), and No means number of larvas that were still alive at the beginning of a study (individual).

RESULTS

The test of temephos concentration on larva instar III shows the number of larvae that remained alive until imago from 125 individuals with five repetitions for 24 hours. Table 1 demonstrated the number of all interventions for *Ae. aegypti* mosquitos after exposed to temephos concentration.

Table 1. The influence of providing temephos towards number of *Ae. aegypti* mosquitos

Concentration (ppm)	Baseline Data	Mosquito Development Phase			Number		Genital Ratio
		larva	Pupae	Imago	♀	♂	♀ : ♂
Control	125	125	110	98	45	53	1:1
0.019	125	46	38	6	5	1	5:1
0.069	125	24	5	2	2	0	-
0.105	125	20	8	2	2	0	-
0.140	125	16	5	2	2	0	-

The results of *one way* ANOVA showed that the p-value was 0.01 ($p<0.05$). It means that there was any significant influence of providing temephos concentration towards *Ae. Aegypti* larva. Furthermore, the results of the following tests for each concentration demonstrated that there was any influence towards the decrease of tested larva numbers. The mortality level differed for each concentration provided. Environmental parameters demonstrated that an average temperature was 27^0C, the room temperature was 28^0C, pH was 6.68, and humidity was 85%.

Mosquitos that were still alive after exposed to temephos, then they were observed from larva to imago in terms of the change of their morphology particularly the length of larva and pupae. Table 2 demonstrated that for each provided concentration, there was any difference in size and the length of larva and pupae compared to the control group. In the phase of amigo, the length of wings and abdomen for male and female mosquitos were observed. The observations were conducted for each provided concentration (Table 3).

Table 2 The Influence of temephos towards the length of larva and pupae of *Ae. aegypti* mosquito (mm)

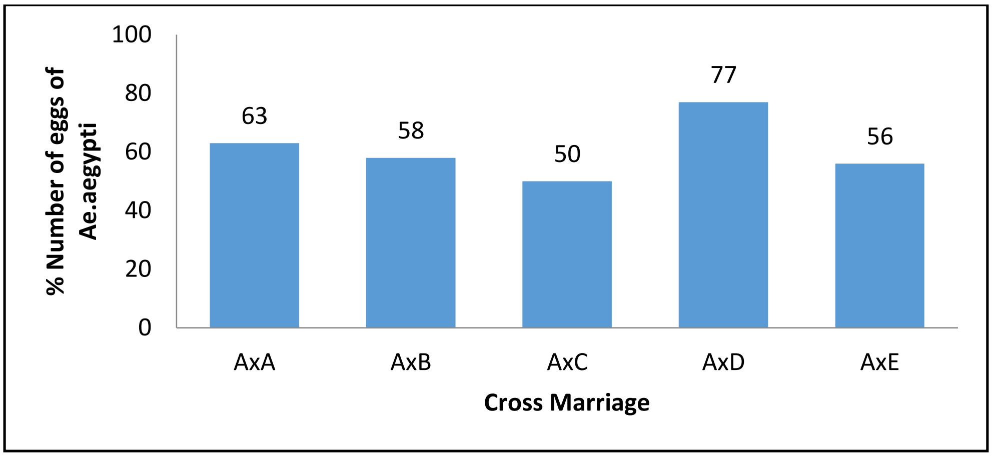
Growth phase	Concentration (ppm)				
	0.019	0.069	0.105	0.140	Control
Larva	3.3	3.2	3.3	3.5	3
Pupae	2.5	2.7	2.6	2.8	2.3

Table 3 The influence of temephos towards the length of wings and abdomen of imago (mm)

Genital Ratio	Concentration (ppm)									
	0.019		0.069		0.105		0.140		Control	
	W	A	W	A	W	A	W	A	W	A
♂	1.2	1.3	-	-	-	-	-	-	1	1,1
♀	1.4	1.6	1.7	1.9	1.5	1.9	1.9	1.3	1.5	1.5

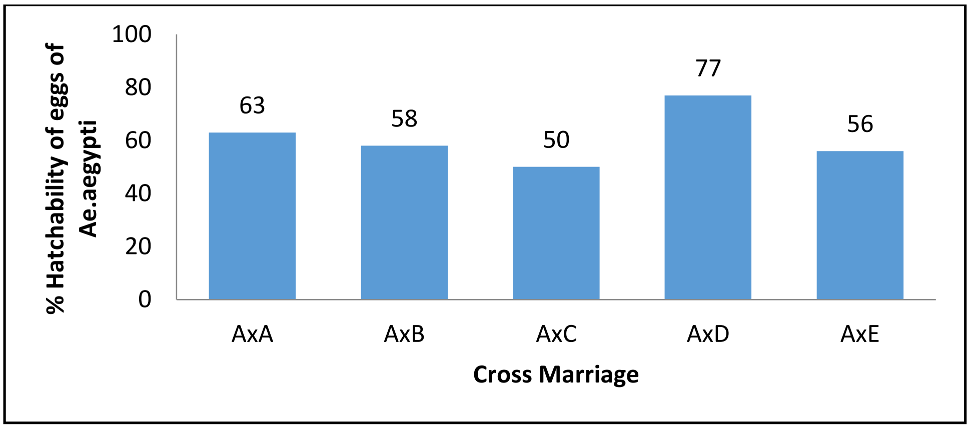
Remark: A= abdomen, W= wings, - = not available

The final results of observations for the life level *of Ae. Aegypti* was the number of eggs resulted from the cross marriage between each concentration. The number of eggs obtained from one gonotrophic cycle on *Ae. aegypti* exposed to temephos concentration is shown in Figure 1 and the percentage of hatchability is shown in Figure 2.



Remark: A= 0%, B= 0.019 ppm, C= 0.069 ppm, D= 0.105 ppm, E= 0.140 ppm. (A= ♂, B,C,D,E = ♀)

Figure 1 The Influence of temephos concentration towards the number of hatched eggs of *Ae. aegypti*



Remark: A= 0%, B= 0.019 ppm, C= 0.069 ppm, D= 0.105 ppm, E= 0.140 ppm.
A= ♂, B,C,D,E = ♀

Figure 2 Percentage of hatchability of *Ae. Aegypti*' egg

DISCUSSION

Temephos is an insecticide that can kill *Ae. aegypti.* The use of temephos to kill *Ae. aegypti* larva is still effective in reducing the population of mosquitos by breaking the lifecycle of mosquitos on the larva phase. The purpose of using a chemical substance like temephos is to damage a nervous system of *Ae. aegypti* larva if it is swallowed. The latest study demonstrated that larva was resistant to temephos in some regions like Tasikmalaya City[10] and Banten Province[11]. However, temephos was still effective in Sukabumi City[12]. This condition was very harmful because of *Ae. Aegypti larva* would be difficult to be controlled and would increase risk factors of DHF transmission. Resistance to temephos would be inherited in offspring[13].

In a study explained that temephos could inhibit the enzyme in a low dose[14]. The use of temephos as a larvicide active ingredient accounts for larva mortality. This is because temephos attacks the larva's nervous system. Temephos inhibits an enzyme of cholinesterase which causes a nervous system disorder due to the accumulation of acetylcholine at the nerve endings[14]. By using abate with temephos as an active ingredient, the activity of cholinesterase will be blocked. It causes muscle spasms to occur continuously and the death of insects.

All tested concentrations caused mortality by ≥60%. The percentage of mortality tended to be higher by increasing concentration. The highest mortality (87%) occurred in a concentration of 0.140 ppm. It differed from a study conducted in Banjarbaru that showed the highest mortality occurred in a concentration of 0.025 ppm namely 99%[15]. This was due to the difference in terms of the vulnerability of larva in each region towards temephos.

The percentage of the survived larva was 2-5 %. It means that these larvae had been resistant to temephos. A study in West Banjarmasin showed that LC_{99} during 24 hours was 0.0243 ppm with the percentage of the survived larva was 4% and they were categorized as resistant.[8] Besides, another study in South Kalimantan demonstrated that the lowest concentration was 0.005 ppm and percentage of larva mortality was 39%. In contrast, the highest concentration was 0.030 ppm with the percentage of larva mortality was 100%.[11] if it was compared to this study, *Ae. aegypti* could defend to the higher concentration because there was any continuous exposure from larvicide particularly temephos. The long-term use of larvicide would change gen and bring to be resistant to temephos and other larvicides[16].

The influence of temephos towards life level of *Ae. aegypti* indicated that the highest percentage to survive in the control group was 78%. This differed from the intervention group. By providing a concentration of 0.069 ppm, 0.105 ppm, and 0.140 ppm, the percentage of life level was ranging about 2-5%. This showed that providing temephos concentration influenced the life level of *Ae. Aegypti*. It means that only 2% of *Ae. aegypti* could survive from temephos exposure until an imago phase.

Resistance can occur due to the long-term use of larvacide which stimulates mosquitos to develop an adaptation mechanism. *Ae. aegypti* mosquitos can survive from temephos exposure by keeping their energy.[4] In addition, they are able to adapt to not optimal environmental conditions. This showed a flexible trait of *Ae. aegypti* mosquitos to adapt to their environment (*plastisitas fenotif*).

A measurement of morphology on larva and pupae that survive after being exposed to temephos during 24 hours was done by measuring the length of larva and pupae. The length of larva and pupae in the control group was shorter than those in the intervention group. It indicated an adaptation process of larva and pupae living in a bad environment. There was any relationship between the length of larva and pupae and the success to survive until being imago. Wings and abdomen of imago that are not exposed to temephos were shorter than the exposed group. It demonstrated the adaption mechanism by changing their body sizes bigger. Notwithstanding, another study revealed that larva morphology exposed to temephos tended to change that was shown by seta hair loss, shriveled abdomen and blackened parts of the body[1].

The genital ratio due to exposure to temephos concentration was significant compared to the control group. Table 1 showed that in the concentration of 0.019 ppm, the number of female mosquitoes (5 mosquitos) was more than the number of male mosquitoes (1 mosquito). Similarly, in the concentration of 0.069 ppm, 0.105 ppm, and 0.140 ppm, the number of female mosquitoes (2 mosquitos) was more than the number of male mosquitoes (0 mosquito). The number of female mosquitos was more than the number of male mosquitos in each concentration of temephos. These results were in line with a previous study that revealed the number of female mosquitos was more among larva exposed to temephos than that of unexposed group.[14] a lifetime of female mosquitos exposed to temephos was longer than the unexposed group. On the other hand, in the control group, the number of male mosquitos was more than the number of female mosquitos. The results of this study were in line with previous studies that found mosquitoes that hatch from pupae into adults are mostly male[18].

Obtained mosquito eggs were from the cross marriage between each concentration and the control group. This test aimed at identifying the influence of temephos concentration towards quality of ovum among female mosquitos from each tested concentration. The number of eggs resulted by the control group was higher than that of the intervention group. It means that temephos exposure influenced the quality of ovum. Abate exposure contains temephos that can inhibit oviposition in a gonotrophic cycle.[14] Therefore, providing temephos that enters into ovum has a poison effect and accumulates in a follicle by which it causes less fertile on female mosquitos.[4]

The percentage of egg hatchability in the intervention group was higher than that of in the control group. It means that providing temephos could influence the egg hatchability of mosquitos. This result contradicts with other studies that stated temephos could decrease egg fertility. A penetration of temephos into ovum causes a poison effect for the growth of egg cells by which it brings to the decrease of fertility [19]. Egg hatchability after exposure to temephos tends to decrease. On a concentration of 0.105 ppm, there was the highest increase of egg hatchability among all provided concentrations. This condition was due to some factors, even though environmental factors like water temperature, room temperature, and humidity were ignored because they had the same conditions. The cause of differences was due to egg conditions in which not all fertile eggs could survive in the appropriate environmental conditions.

Environmental factors play an important role in the lifecycle of mosquitos. A study conducted in Trinidad demonstrated that high temperature would change a resistance status of insecticide from each population of *Ae. aegypti* with various types of insecticide use [20]. *Ae. aegypti* with strain Pangandaran had specific environmental conditions like temperature ranging from 25°C to 26°C and relative humidity ranging from 86% to 90%. Meanwhile, the temperature at the research site was a bit higher (28^0C). This condition supported *Ae. aegypti* mosquitos to change their physiologies and morphologies to survive.

CONCLUSION

Providing temephos concentrations influenced towards a life level of *Ae. aegypti* mosquitos with strain Pangandaran. The higher the temephos concentration, the bigger number of larva mortality. The influence of temephos towards the development of mosquito morphology could be seen by measuring the difference in the length of larva and pupae between the exposed and unexposed group to temephos. Larva and pupae in the intervention group were longer than in the control group. Imago in the intervention group had wings and abdomen longer than that of in the control group. Egg hatchability from mosquitos that survived after being exposed to temephos was higher than in the unexposed group.

There was any difference in the proportion of mosquito's sex that survived. In the intervention group, imago was dominated by females whereas, in the control group, imago was dominated by males.

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