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Bio Larvacide Efficacy and Residual Effect on Aedes Aegypti Larvae's Elimination

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ABSTRACT

Introduction: *Dengue Hemorrhagic Fever (DHF) has always become a phenomenon throughout Indonesia every year while vector control conducted has not yet obtained optimal results. As such, it is necessary to search for alternatives vector control to minimize DHF cases.*

Objective: *Biolarvacide (Bacillus thuringiensis var israelensis) can kill genus of mosquito larvae and has received government's approval usage in Indonesia. The preliminary study indicated 1 drop in 2.5 liters of water was able to kill the larvae of aedes aegypti less than 80% indicating inaccuracy dosage yet making it is necessary to do research on the efficacy and residual effect of Biolarvacide before it is widely disseminated for public use. The purpose of this study is to determine the effective dosage of biolarvacide and its residual effects in killing the larvae of aedes aegypti.*

Methods: *This research was an experiment at the entomology laboratory in Pangandaran, West Java. Biolarvacide efficacy test was conducted at various dosages (1 drop, 2 drops, and 3 drops) in 2.5 liters of clean water occupied by 25 larvae of aedes aegypti. Efficacy test was performed during 48 hours. Once the effective dosage was found, the residual effect test was performed at 1^{st} to 7^{th} weeks to count the number of aedes aegypti larvae bodies. The data were analyzed by using The Mann-Whitney U test.*

Result: *The efficacy test results showed that the effective biolarvacide dosage to kill Aedes aegypti is 2 drops per 2.5 liters of clean water. The test results of biolarvacide residual effect to Aedes aegypti larvae elimination in the 1^{st} to 4^{th} week was 100%, 72.8% in the 5^{th} week, 7.2% in the 6^{th} week and 2.4% in the 7^{th} week which were less effective if compared to abate residual which is always fixed at 100% the whole weeks.*

Conclusion: *The residual effect of biolarvacide can effectively control Aedes aegypti larvae up to four weeks at a dosage of 2 drops per 2.5 liters of clean water. However, the residual effect of biolarvacide is still less powerful than the abate.*

Keywords: *Dengue Hemorrhagic Fever (DHF), Biolarvacide, residual, Aedes aegypti.*

INTRODUCTION

DHF cases in Indonesia are still difficult to control even though various vector control efforts have been implemented physically, chemically and biologically. Control by chemical means has been done frequently, among others, by fogging to control adult mosquitoes and larvaciding to control mosquito larvae. Larvaciding using a 1% Abate formulation with a dosage of 1 g / 10 liters of water can effectively kill the larvae for 8-12

weeks. However, the use of Abate can cause problems such as resistance (Anggraeni, et.al, 2013).

One of the alternative controls is biological control using biolarvacide, namely biolarvacide with the active ingredient of bacteria Bacillus thuringiensis var israelensis (Bti). Larvacide Bti is a high-energy larvacidal and is not harmful to the environment. If the mosquito larvae swallow Bti, then Bti will release endotoxin which can damage the digestive tract of larvae so that the larva will die (Dewi, 2012).

Use of biolarvacide to control the spread of mosquitoes shows a high mortality rate of 24-48 hours. Biolarvacide is used by dropping or spraying aerially and terrestrially in mosquito breeding places. Biolarvasides can remain active in the mosquito breeding environment for 7 days to 60 days depending on the type of reservoir, water conditions, and species' target.

The recommended dosage of biolarvacide is 1 drop for 2.5 liters of clean water. But from the results of preliminary research, it was revealed that with a dosage of 1 drop for 2.5 liters of clean water, the power to kill Aedes aegypti larvae only reached < 80% indicating the dosage was not effective yet. Therefore, it is necessary to conduct a research to determine the efficacy and residual effects of Biolarvacide to eliminate aedes aegypti larvae.

The aim of this research is to know the efficacy (killing power) and effectiveness of biolarvacide residue on aedes aegypti larvae mortality when compared with abate.

MATERIALS AND METHODS

The independent variables of the study were dosages of biolarvacide and abate and the dependent variable was the number of death bodies of Aedes aegypti larvae. Control variables were aedes aegypti larvae's age, contact time, water type, water volume and sunlight condition, abate, and biolarvacide dosage. Intervening variables were water temperature and water pH.

The type of research is a quasi experiment in the laboratory. Aedes aegypti larvae used were instar 3. Efficacy test was conducted at 1 drop per 2.5 liters; 2 drops per 2.5 liters and 3 drops per 2.5 liters of clean water. Aedes aegypti larvae's samples were put into each treatment and control container consisting of 25 larvae each container with 5 times replication and observed during 48 hours. The residual effect test was performed by using the effective efficacy dosage test result that was 2 drops per 2.5 liters of clean water. Biolarvacide dropped with a dosage of 2 drops into 2.5 liters of the water container. Abate is put into containers with a dosage of 0.25 grams in 2.5 liters of water. Observation of residual effect was performed for seven weeks. *Kruskal-Wallis Test* was used to test the hypothesis if biolarvacide has a different killing power in eliminating the mosquito's larvae in each week.

RESULTS AND DISCUSSIONS

Environmental Conditions (Water Temperature and pH)

Environmental conditions analyzed include water temperature and pH of water at containers or experimental sites. The water used for experiments (larval life) is water extracted from dug wells, clear colored, odorless and chlorine-free. Water temperature and water pH measurements were performed at each treatment. The water temperature averaged 27.4^{0}C, while the pH of water averaged 8.7. The temperature at the site of the study is optimal for the growth of mosquito larvae as the preferred temperature of Aedes aegypti mosquitoes to breed is 25^{0}C – 27^{0}C. At the temperatures of less than 16^{0}C and higher than 32^{0}C, the mosquitoes will experience growth disorders. (Kemenkes RI, 2013).

As the pH can be affected by the water source used, the water used by researchers at the time of the study was water from the dug wells that did not contain chlorine. The alkaline pH of the water may be influenced by the presence of biolarvacide dropped into the water.

Efficacy of Bio larvacide to Elimination of Aedes Aegypti Larvae

The result of efficacy test or killing power of biolarvacide to aedes aegypti larvae mortality revealed 1 drop biolarvacide per 2.5 liters of clean water can kill 65.6% larva, 2 drops per 2.5 liters can kill 96% larva and dosage 3 drops per 2.5 liters can kill 99.2%. This indicates that the effective dosage of biolarvacide is a dosage of 2 drops per 2.5 liters of clean water and a dosage of 3 drops per 2.5 liters of clean water. On the basis of the results of the efficacy test, the researchers then used a dosage of 2 drops per 2.5 liters of clean water as a dosage to be used in the test phase of the residual effect of biolarvacide on the elimination of aedes aegypti larvae.

The residual effect of Biolarvacide to the elimination of Aedes aegypti's larvae

The effects of residual biolarvacide to aedes aegypti's larvae was performed for seven weeks. The dosage of biolarvacide used was 2 drops per 2.5 liters clean water. The death of aedes aegypti larvae in the controlled group at each treatment was < 5% making the calculation of mortality rate in the treatment group not to use the Abbot's formula as it is applied if the rates in the control group are 5% - 20%.

The mortality rate of aedes aegypti's larvae due to the residual effect of biolarvacide is observable in Table 1.

Table 1. Mortality rates of Aedes aegypti larvae due to residual effects of biolarvacide

No.	Weeks	Number of Larva Tested	Mortality Rates	
			Numbers	Percentage
1.	I	25	25	100
2.	II	25	25	100
3.	III	25	25	100
4.	IV	25	25	100
5.	V	25	18	72.8
6.	VI	25	2	7.2
7.	VII	25	1	2.4

The mortality rates as shown in Table 1 was statistically analyzed by using Kruskal-Wallis Test. Kruskal-Wallis test results obtained the p-value = 0.001 < α 0.05 indicating the alternate hypothesis acceptance meaning there is a difference in mortality rates of Aedes aegypti larvae due to the residual effect of Biolarvacide at the weeks of I, II, III, IV, V, VI and VII.

The ability of biolarvacide to completely eliminate Aedes aegypti larvae can last up to week IV, while week V, VI, and VII the effectiveness has decreased where Aedes aegypti larvae mortality reaches only < 80%. Biolarvasides may remain active in water or mosquito breeding sites for 7 to 60 days depending on the type of reservoir, water conditions, and targetted species (Anggraeni, et.al, 2013).

The residual effect of Abate on Aedes aegypti larvae's mortality

The effect of residual abate on aedes aegypti larvae's mortality was done for seven weeks. The abate dosage used is 0.25 grams per 2.5 liters clean water. Bodies of Aedes aegypti larvae due to residual effects of abate can be seen in Table 3 where the effect of residual abate on the weeks I to VII is always constant at 100%.

Table 2. Mortality rates of Aedes aegypti's larvae due to residual effects of Abate

No.	Weeks	Number of Larva Tested	Mortality Rates	
			Numbers	Percentage
1.	I	25	25	100
2.	II	25	25	100
3.	III	25	25	100
4.	IV	25	25	100
5.	V	25	25	100
6.	VI	25	25	100
7.	VII	25	25	100

Abate residue was proved still to have a stronger deadly effect on aedes aegypti mosquito larvae. Abate (temephos) is one of the organic phosphate compound pesticides. It works by blocking cholinesterase enzymes, both in vertebrates and invertebrates, causing disruption to nerve activity due to acetylcholine accumulation on the nerve ends. The function of the cholinesterase enzyme is

to hydrolyze acetylcholine into choline and vinegar that when the enzyme is inhibited the acetylcholine hydrolysis does not occur so making the muscle will remain contracted for a long time resulting in spasticity. After the contraction period is complete, acetylcholine will be destroyed by acetylcholinesterase enzyme into choline, lactate, and water. When acetylcholine is not immediately destroyed then the muscle will remain contracted for a long time so that there will be spastic muscles on mosquito larvae or convulsion continuously and finally the larvae of the mosquito will die. So just like other organophosphate compounds, abate is also characterized as an anti cholinesterase (Dwi K. et al, 2015).

Abate powder (temephos) sprinkled into water containers will create a layer on the container wall that will last approximately 3 months with the condition that during cleaning the container, the inner wall is not brushed. However, if the container wall is brushed it will result in erasing the Abate layer that will not be effective as larvacide any longer (Dwi Nugroho & Arif, 2011).

Comparison of Residual Effects of Biolarvacide and Abate

The residual effect of biolarvacide and abate on aedes aegypti larvae's mortality during the seven weeks of the study showed different results as shown in Figure 1. In weeks I to IV the percentage of aedes aegypti larvae mortality tested with biolarvacide and abate remained the same effectiveness as both kill mosquito up to 100%. However, the residual effect of biolarvacide on aedes aegypti larvae's mortality gradually decreased at week V into 72.8%, week VI into 7.2% and week VII into 2.4%. This was the main difference with The residual effect of abate on aedes aegypti larvae death that remained constant at 100% on weeks I to VII.

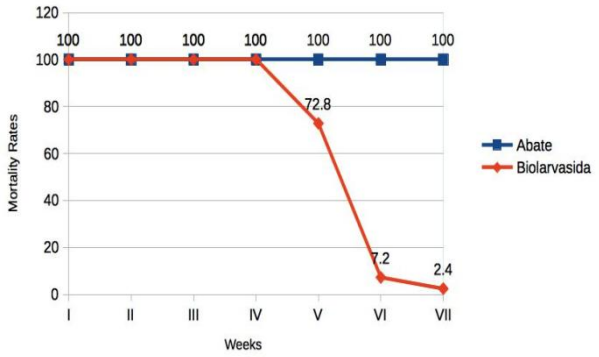

Figure 1. Percentage of Aedes aegypti larvae's mortality with Biolarvaside and Abate residues

The killing power of biolarvacide against aedes aegypti larvae is only effective until 4th weeks while Abate is still effective or still able to kill all Aedes aegypti larvae until the 7th week. This way, it is concluded that in terms of killing power, abate larvicide is still more effective than biolarvacide.

The effectiveness of biolarvacide residues against aedes aegypti larvae is only about four weeks or shorter than that of abate. This can be caused by several factors such as organism factors, water quality, and pH. Other organism found in the container may become bacteria or predatory competitor for Bacillus thuringiensis israelensis (Bti) so that the number of Bti is reduced and leads to a decreased effectiveness in killing aedes aegypti larvae. Water quality that tends to decrease compared to the earlier application in the 1st week due to increased pollutants and organic substance can lead to decreased effectiveness of Bti in eliminating aedes aegypti's larvae. The pH factor can also affect the effectiveness of Bti as the optimal pH for Bti to kill the larvae is in pH 5 - 7 (Dewi, 2012).

In contrast to biolarvacide, abate tends to have a stronger and longer lasting residual effect compared to biolarvacide. Abate residues are able to kill Aedes aegypti larvae for up to three months. Biolarvacide and abate both have some advantages, among others, harmless to humans, birds, fish and other domesticated animals, can be used in clean water without changing the taste,

color and odor of treated water (Dwi Nugroho & Arif, 2011).

CONCLUSION

Based on the results of research and discussion it is concluded that:

1. Efficacy or killing power of biolarvacide to aedes aegypti larvae's mortality during 48 hours of testing is that 1 drop per 2.5 liters of clean water can eliminate 65.6% larvae, 2 drops per 2.5 liters eliminate 96% larvae and 3 drops per 2.5 liters eliminate 99.2% larvae.
2. The effective biolarvacide dosage to kill aedes aegypti larvae is 2 drops and 3 drops per 2.5 liters of clean water as the killing power reaches ≥ 90%.
3. The residual effect of biolarvacide on aedes aegypti's larvae mortality in week I to IV was 100%, week V was 72.8%, week VI was 7.2% and week VII was 2.4%.
4. The effect of residual abate on aedes aegypti larvae mortality in weeks I to VII always remains constant at 100%.
5. The effect of residual abate on Aedes aegypti larvae mortality is stronger than biolarvacide. Abate residues in weeks I to VII can kill all aedes aegypti mosquito larvae. Biolarvacide residues can kill 100% Aedes aegypti larvae in weeks 1 through IV only, whereas after weeks V to VII the residual effect of biolarvacide is lower than 80%.

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