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Effectivity of Bacterial Suspension *Bacillus thuringiensis Var Israelensis* in Killing *Aedes aegypti L.*
Mosquito Larvae

Tri Wahyuni Sukesi¹, Sulistyawati², Eva Hendrawati³, Surahma Asti⁴

Abstract:

Objective: Dengue hemorrhagic fever is a disease transmitted by the mosquito *Aedes aegypti*. Prevention of transmission of this disease one of which is the chemical control with temefos, but the use of sustainable temefos can cause insect resistance and environmental damage. Therefore we need an effective and safe alternative to larvicide, the suspension of *Bacillus thuringiensis var israelensis*. **Methods:** This study used a type of true experimental research design with posttest only control group design. Samples used in this study are the third instar larvae of *Aedes aegypti*. Concentrations used in this study were a 0.01%; Of 0.02%; Of 0.03%; 0:04% and 0.05% by the number of 25 larvae per treatment and 3 times replication. **Results:** Based on the Kruskal Walls obtained a value of $0.009 > 0.05$ means there is a stock mortality of larvae using a suspension of the bacterium *Bacillus thuringiensis*, temefos and distilled. Test Mann Whitney showed that among the positive control (temefos a 0.01%) and negative control (distilled water) there are differences in the number of larvae mortality significantly, the positive control treatment with no significant difference, and between negative control and treatments armpits there are differences in the number of deaths larvae significant. The LC_{50} value obtained was 0.010% and the LT_{50} value obtained was 2,683 hours. **Conclusions:** The suspension of bacteria *Bacillus thuringiensis* is effective in killing the larvae of *Aedes aegypti* with LC_{50} values of 0.0105 and LT_{50} values on the clock to 2.683.

Keyword: *Bacillus thuringiensis Var Israelensis*; *Aedes aegypti*; dengue hemorrhagic fever; effectivity

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Introduction

Dengue Fever is a disease that is always increasing. Since the first time in Surabaya and Jakarta in 1968. DHF incidence increased very rapidly in Indonesia and even in some area with Dengue Extraordinary Occurrence^[1] In 2010 the number of dengue cases nationally as many as 156,086 cases with the number of dengue fatalities of 1.358 people.^[2] Based on Health Profile in Indonesia revealed that

the morbidity rate of DBD in Yogyakarta is the 4th highest in Indonesia. The number of dengue fever cases in Indonesia has increased from 2009 to 2010. In 2009 cases were reported as 2,203 cases and in 2010 cases reported an increase of 5,121 cases. The increase in DHF cases is related to the high-risk factors of transmission in the community such as the free rate of larvae which is still below 95% that is 64.64% in 2011 and 71.8% in 2012 and in 2013 the

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free rate of larvae is 87.88 %.^[3]

Other factors that influence the increase of DHF cases are *Aedes aegypti* L. mosquito resistance against insecticides and larvicides used as chemical control devices. *Aedes aegypti* L. resistance of the temefos organophosphate compound which is often used as larvacide.⁴

Larvasid resistance occurs due to the use of insecticides and larvacide are too long, never rolling pesticides and dosage of applications in the field that was never done monitoring.

Their impact on insect resistance caused by chemical insecticides encourages created or insecticide discovery biology that is safe for the environment but can kill the larvae of *Aedes aegypti*. Other research finds toxicity of *Bacillus thuringiensis* to *Aedes aegypti* larva in Nganjuk City with LC_{50} 48 hour value equal to $3,53 \times 10^7$ cell/ml.^[5] *Bacillus thuringiensis* is a gram-positive bacteria that can form spores and proteins that are toxic to *Aedes aegypti* mosquito larvae.

These bacteria form toxins that when eaten by larvae then the toxin will enter through the digestive tract and then kill the larvae but not dangerous when consumed by humans.^[6] Bti can be used as an alternative way of controlling *Aedes aegypti* L. larvae. Biologically to reduce the risk of resistance. The Bti suspension used is various concentrations to be exposed to the *Ae Aegypti* L. larvae for 24 hours and will see the value of Lethal concentration 50 (LC_{50}) and Lethal time 50 (LT_{50}).

Methods and Materials ⁶

This study used a type of true experimental research design with posttest only control group design. In this design, the researchers compared the number of dead larvae between the uses of temefos with natural larvacides from *Bacillus thuringiensis var Israelensis* bacteria in various concentrations. The total *Aedes aegypti* larvae used in the study were 25 larvae of each glass. Observation of this research is done by direct observation by taking time, concentration that can kill and a number of dead test larvae. This study was conducted in August 2015. The material used in this study was the suspension of *Bacillus thuringiensis var Israelensis* bacteria diluted with the following dilution formula:

Description:

V1: initial solution volume

M1: initial solution concentration

V2: the dilution solution volume is diluted

M2: concentration of solution after dilution.

Based on the above formula, the concentration used in the larvicidal test is 0.01%; 0.02%; 0.03%; 0.04% and 0.05% respectively. The number of larvae used in this study was as many as 25 larvae. Furthermore, the researchers made a test media solution with various concentrations with each concentration of 100 ml. After the preparation of the test media solution is completed, the researcher inserts test larvae of 25 larvae of each glass. In this study, researchers used *Aedes aegypti* larvae stage III. The reason researchers use stage III larvae is that instar III size is large enough so easy to identify. After the larvae put into the test medium, then the researchers observe and count the number of test larvae mortality up to 24 hours.

If after 24 hours 50% the test larvae have not died then the researcher can increase the observation time up to 48 hours and so on up to a maximum of 96 hours because if more than 96 hours of death the larvae can be caused by other factors. Also, it is feared that the larvae have changed into pupa so that the research must be re-adventured.

This study was conducted in 3 replications. The temperature used in the study was 27 ° C. The data recorded by the researchers was larval mortality on each test media for 24 hours at 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 hours observation. After the researcher got data of *Aedes aegypti* larvae death, then researcher perform data analysis. In this research there is two analysis, that is linear regression analysis and probit regression analysis.

Ethical clearance: The study was approved by ethics committee of Ahmad Dahlan University, Yogyakarta.

Result

Table 1 shows the number and percentage of deaths of *Aedes aegypti* larvae treated by adding *Bacillus thuringiensis var Israelensis* concentration of 0.01%; 0.02%; 0.03%; 0.04% and 0.05%.

Table 1- Number and percentage of *Aedes aegypti* larvae that died after suspension of *Bacillus thuringiensis var Israelensis* for 24 hours in actual test

| Treatment group | Total cumulative of larvae death | | | Average | Percentage |
|---------------------|----------------------------------|----|-----|---------|------------|
| | Repetition | | | | |
| | I | II | III | | |
| Concentration 0,01% | 25 | 25 | 25 | 25 | 100% |
| Concentration 0,02% | 25 | 25 | 25 | 25 | 100% |
| Concentration 0,03% | 25 | 25 | 24 | 25 | 100% |
| Concentration 0,04% | 25 | 25 | 25 | 25 | 100% |
| Concentration 0,05% | 25 | 25 | 24 | 24,67 | 98,68% |
| Control positive | 25 | 25 | 25 | 25 | 100% |
| Control negative | 0 | 0 | 0 | 0 | 0% |

On the hour to 6 shows that the percentage of *Aedes aegypti* larvae mortality was highest at concentrations of 0.01%, 0.02% concentration, the concentration of 0.03% and a concentration of 0.04% with a percentage of 100% mortality. The percentage of death at concentration 0.05% with the number of larval mortality is 98.68%. Observations made on positive controls showed 100% mortality percentage, and negative controls showed a 0% mortality percentage.

After knowing the number of *Aedes aegypti* larvae death after 24 hours observation then the researcher did data analysis. The first data analysis is to look at the normality of the data. Based on the normality test, p value $0.000 < 0.05$ is obtained, which means that the data is not normally distributed. Homogeneity of data seen Levene statistical test its significance value is $0.000 < 0.05$ this means data variants are not equal or not homogeneous. Due to abnormal data distribution and non-homogeneous variation, the nonparametric statistical method used is the Kruskal Walls test.

The Kruskal Walls test aims to see the difference in mortality of test larvae between giving positive control, negative control and various bacterial suspension concentrations of *Bacillus thuringiensis var Israelensis*. Based on Kruskal Walls test, the value $0.009 < 0.05$ means t_{11} there is a difference of the cumulative number of *Aedes aegypti* larvae deaths using *Bacillus thuringiensis var Israelensis* suspension.

Mann Whitney test aims to see whether there is a difference between each treatment. Based on

Mann Whitney test result known that between negative control with positive control there is a significant difference because of the value obtained $0.025 < 0.05$. For negative control (aquades) have significant difference with all treatment concentration (concentration 0.01%; 0.02%; 0.03%; 0.04% and 0.05% concentration). As for the positive control (0.01% temefos) did not have a significant difference in larval mortality with all *Bacillus thuringiensis var Israelensis* suspension concentrations.

After performing regression analysis, researchers conducted a probit analysis to determine the value of LC50 and LT50 values. LC50 (Lethal Concentration 50) which means *Bacillus thuringiensis var Israelensis* suspension concentration which can kill 50% *Aedes aegypti* larvae. While LT₅₀ (Lethal Time 50) which means the time required suspension *Bacillus thuringiensis var Israelensis* to be able to kill 50% larvae *Aedes aegypti*.

Table 2. Value of LC₅₀ suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

| LC ₅₀ <i>Bacillus thuringiensis var Israelensis</i> suspension | Repetition | LC ₅₀ (%) |
|---|------------|----------------------|
| | I | 0.010 |
| | II | 0.010 |
| | III | 0.010 |
| | Average | 0.010 |

Based on regression analysis obtained average LC₅₀ at concentration 0.012%. This means that at a concentration of 0.010% *Bacillus thuringiensis var Israelensis* suspension can kill 50% of *Aedes aegypti* larvae.

Table 3. Value of LT₅₀ suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

| LT ₅₀ <i>Bacillus thuringiensis var Israelensis</i> suspension | Repetition | LT ₅₀ (%) |
|---|------------|----------------------|
| | I | 2.764 |
| | II | 2.646 |
| | III | 2.639 |
| | Average | 2.683 |

Based on the regression analysis obtained average LT₅₀ at the time (hours) to 2.6683. This means that at

2.683 hours the suspension of *Bacillus thuringiensis* var *Israelensis* can kill 50% of *Aedes aegypti* larvae.

Discussion

Based on the 24-hour observation, it was found that the concentration that caused the death of test larvae (*Aedes aegypti*) was the entire concentration of *Bacillus thuringiensis* (concentration (0.01%, 0.02%, 0.03%, 0.04% and 0.05%) of all *Bacillus thuringiensis* suspensions used almost all of them capable of killing 100% test larvae (25 larvae), but there are also concentrations that kill the entire test larvae with a percentage of 98.68% of 0.05% concentration. This is equivalent to death in positive controls (temefos 0.01%) where deaths were 100%, while for the negative control (aquades) showed no death at all in the test larvae (0% death). This indicates that the suspension of *Bacillus thuringiensis* var *Israelensis* bacteria is effective in killing *Aedes aegypti* larvae. The high mortality rate of test larvae caused by the ability of toxins in the form of protein crystals produced *Bacillus thuringiensis* bacteria that enter into the digestion system of *Aedes aegypti* larvae and then kill them.

Based on Kruskal Walls test, there were differences between cumulative number of *Aedes aegypti* larvae deaths between *Bacillus thuringiensis* bacteria suspension, positive control (0.01% temefos) and negative control (aquades) with significance value $0.009 < 0.05$.

In Mann Whitney test the results obtained were between positive control (temefos 0.01%) and negative control (aquades) there were significant differences in *Aedes aegypti* larvae mortality, whereas between positive control (temefos 0.01%) and *Bacillus thuringiensis* bacterial suspension had no difference significant concerning the death of *Aedes aegypti* larvae. For negative control (aquades) has a significant difference with *Bacillus thuringiensis* bacterial suspension.

Based on probit analysis obtained the average value of LC_{50} from bacterial suspension concentration of *Bacillus thuringiensis* is 0.010%. This means that the suspension concentration of *Bacillus thuringiensis* bacteria can kill 50% of the total test larvae at a concentration of 0.010%. The value of LC_{50} in temefos itself is at a concentration of 0.0177% meaning that the concentration of 0.0177% temefos can kill 50% of test larvae. It can be said that the ability of *Bacillus thuringiensis* bacteria suspension in killing *Aedes aegypti* larvae is better than temefos because the LC_{50} suspension value of *Bacillus thuringiensis* bacteria is smaller than the LC_{50} temefos.^[7] The

lower LC_{50} value of a natural larvasida the better is the effectiveness of the larvaside because the small amount of feedstock can produce high larvacidal power. Also the larvacides are environmentally friendly because they do not produce residues in the environment.⁸

Based on the results of probit analysis for Lethal Time, the LT_{50} value for the first test is 2,764 hours, the second repetition is 2,646 hours, and the third test is 2,639 hours. So we get the average value of LT_{50} at clock to 2.6683. This means that at 2.683 hours the bacterial suspension of *Bacillus thuringiensis* var *Israelensis* can kill 50% of the test larvae.

Research on the utilization of bacterial suspension of *Bacillus thuringiensis* var *Israelensis* as larvacide and bioinsecticide has been done one of them by Anggraeni et al (2013) who saw the killing power of endotoxin crystal extract of *Bacillus thuringiensis israelensis* to some type of mosquito that is *Aedes aegypti*, *An. aconitus* and *Culex quinquefasciatus* with the results of research in the form of LC_{50} value of *Aedes aegypti* mosquito with LC_{50} 0,06 ppm.⁹

Bacillus thuringiensis has a protein toxin that can be used for various things; such proteins include parasporin. Parasporin is a Cry protein that has cytosidal ability against cancerous cells.¹⁰ While the ICP protein (Insecticidal Crystal Protein) is a Cry protein that is produced which is an antiser. Each Cry protein has specific toxicity to specific target insects and may also have some targeted insects^{11, 12}.

Conclusion

Bacillus thuringiensis bacterial suspension is effective in killing *Aedes aegypti* larvae with Lethal Concentration 50 (LC_{50}) at concentration 0.010% and for Lethal Time 50 (LT_{50}) value is at 2.683 hours.

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Conflict of interest: None

Authors's contribution:

Data gathering and idea owner of this study: Sukesni TW, Hendrawati E, Sulistyawati

Study design: Sukesni TW, Hendrawati E

Data gathering: Sukesni TW, Hendrawati E, Sulistyawati, Mulasari SA.

Writing and submitting manuscript: Sukesni TW

Editing and approval of final draft: Sukesni TW, Hendrawati E, Sulistyawati, Mulasari, SA.

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