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Effectivity Test on *Bacillus thuringiensis* Isolate in Land Around Lampung University, Bandar Lampung, Indonesia

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Abstract: Until now, efforts to control mosquitoes as vectors still using chemical insecticides. The use of chemicals in continuing can cause resistance towards mosquitoes and cause environmental damage. One of the alternatives that can be utilized is a biological control, such as *Bacillus thuringiensis*. This research was conducted for three months in the Laboratory of Microbiology Department of Biology, Faculty of Mathematics and Natural Sciences, Lampung University. The results showed that isolates of *B. thuringiensis* from the ground under trees at Lampung University environment equally effective towards *Aedes aegypti* larval mortality. The study design was Completely Randomized Design (CRD) arranged as factorial. The density of *B. thuringiensis* isolates 2x107cell / ml Br (A) can cause mortality to larvae of *Aedes aegypti* high of 22.5% followed by the density of the density of 2x106 cells / ml by 7.5%.

Keywords: *Bacillus thuringiensis*, Aedes Aegypti, Mortality

1. Introduction

Bandar Lampung is one of the endemic areas of dengue hemorrhagic fever (DHF) in the case of a high enough in Lampung [1]. DHF is caused by *Arbovirus* transmitted through the bite of *Aedes aegypti* mosquito as the main vector and *Aedes albopictus* as a potential vector.

Until now vector control efforts are still using chemical insecticides. The use of chemicals to control mosquitoes in continuing can cause the vector mosquito resistance to insecticides chemistry and cause damage to the environment [2].

One alternative that can overcome dengue is to use biological control agents, such as *Bacillus thuringiensis.* The benefits of using biological control agents are: ecologically safe because the population is normal and does not accumulate in the food chain; some biological control can last longer in nature so that the control process can still continue to run; requires less cost; as well as biological control agents have many variations in controlling pests or disease vectors [5].

One of the characteristics of *B. thuringiensis* is containing the crystal protein in cells along with spores when the cells undergo sporulation [6]. The protein crystals in some strains of *B. thuringiensis* are toxic to Diptera members of both the larval or adult stage. *B. thuringiensis* can be isolated from a variety of habitats, including on the ground, dead insects, and leaves of some plants conifer. [4] Pakpahan states that isolates of *B. thuringiensis* of some ground at the Lampung University have toxicity towards Lepidoptera larvae. Based on this information we want to know how the effectiveness of *B. thuringiensis* towards larvae of *Aedes aegypti*. The purpose of this study was to examine the effectiveness of isolates of *B. thuringiesis* of ground at the Lampung University towards larvae of *Ae. aegypti*.

2. Materials and Method

This research was conducted for three months in the Laboratory of Microbiology Department of Biology, Faculty of Mathematics and Natural Sciences, Lampung University.

Materials used are isolates of *B. thuringiensis* from ground under Banyan Tree at the Lampung University, eggs *Ae. aegypti* obtained from Vector Disease Research Station (SPVP) Salatiga. The study design was Completely

Randomized Design (CRD) arranged as factorial. The first factor is the three isolates of B. thuringienis obtained from the three-point shade namely Br (A), Br (B) and Br (C). The second factor is the density of *B. thuringiensis* namely 2x104 cells / ml (K1); 2x105 cells / ml (K2); 2x106 cells / ml (K3); 2x107cell / ml (K4) and control (K0), replications performed 4 times

Steps:

(a). Prepare the suspension test following the method isolates of *B. thuringiensis* [7] as follows: breed the *B. thuringiensis* which is derived from the bacterium stock, and inoculated on NA slant medium at a temperature of 37ºC for 5 days. Breed the bacteria that lived 5 days suspended with a sterile saline solution. Early suspension called the stock suspension. Before being used in calculating the number of tests, the density of spores was determined of *B. thuringiensis*. Determination of the density of *B. thuringiensis* can be done by using this formula below:

$$V1\ N1 = V2N2 \quad (1)$$

V1 = Volume of stock solution are taken (ml)
N1 = Density of stock solution bacterium (cell/ml)
V2 = SolutionsVolume after added stock solution (ml)
N2 = Bacterial density desired (cell/ml)

The dilution results obtained are suspensions test that will be used is the density of *B. thuringiensis* $2x10^4$ cell/ml (K1); $2x10^5$ cell/ml (K2); $2x10^6$ cell/ml (K3) and $2x10^7$ cell/ml (K4).

(b). Prepare the larvae of *Ae. aegypti* by entering the egg into a container which is already filled with water, maintained until the *instar III*

(c). Test the effectiveness of *B. thuringiensis* towards larvae of *Ae. aegypti* by entering a 5 ml test suspension into a glass beaker which already contains 20 ml of sterile water wells. Then enter the larvae that had been prepared as many as 10 heads, allow up to 24 hours after it was observed, as the controls are used sterile water wells.

The observation was done by calculating the mortality of larvae of *Ae. aegypti* after given of *B. thuringiensis*. Each treatment was conducted four repetitions. To determine the percentage of *Ae. aegypti's* larvae mortality after given of *B. thuringiensis* is calculated using the formula:

$$M = a/b \times 100\% \quad (2)$$

Note: M = Percentage of mortality
a = Number of dead *Ae. aegypti*'s larvae
b = Number of *Ae. aegypti*'s larvae used

This data research was analyzed using ANOVA (analysis of variants) at the 5% significance level and is there is any significance differences, the further test can be using Least Significance Difference (LSD) at the 5% significance level.

The observation of *B. thuringiensis* isolates from ground under trees at the Lampung University presented in the figure (1).

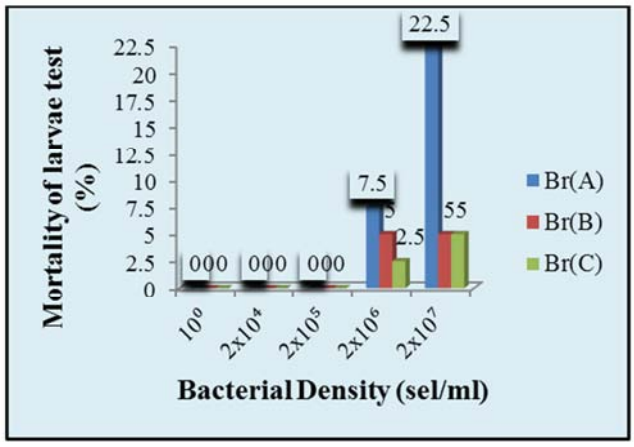

Figure 1. *The percentage of Ae. aegypti's larvae mortality after treatment with three isolates of B. thuringiensis on the observation 24 hours after testing.*

Note: Br (A) = location of the isolates of *B. thuringiensis* A; Br (B) = location of the isolates of *B. thuringiensis* B; Br (C) = location of the isolates of *B. thuringiensis* C

The highest density of *B. thuringiensis* (2x107cell / ml) in isolates Br (A) can cause the highest mortality of 22.5%, followed by the density of isolates of *B. thuringiensis* (2x106 cells / ml) with the amount of mortality of 7.5%. While isolates of *B. thuringiensis* with a density of 100 cells / ml, 2x104 cells / ml and 2x105 cells / ml, no consequences for the larvae.

The results of the effectiveness analysis of the isolates of *B. thuringiensis* Br (A), Br (B) and Br (C) to larvae of *Ae. aegypti* can be seen in table 1.

The results showed that isolates Br (A), Br (B) and Br (C) with a density (2x104 cells / ml) K1; (2x105 cells / ml) K2; 2x106 cells / ml) K3 and control, did not show different results. Br isolates (A) with a density (2x107cell / ml) K4 significantly different from other treatment may imply that isolates Br (A) with a density 2x107cell / ml is equally effective against larval mortality *Ae. aegypti.*

Table 1. *The average of Ae. aegypti's larvae mortality after being infected to isolates of B. thuringiensis Br (A), Br (B) and Br (C).*

No	Treatment	The Average of Mortality ±SE		
		Br(A)	Br(B)	Br(C)
1	K0 (controll)	0.00 ± 0.0000^{a}	0.00 ± 0.0000^{a}	0.00 ± 0.0000^{a}
2	K1 ($2x10^4$ cell/ml)	0.00 ± 0.0000^{a}	0.00 ± 0.0000^{a}	0.00 ± 0.0000^{a}
3	K2 ($2x10^5$ cell/ml)	0.00 ± 0.0000^{a}	0.00 ± 0.0000^{a}	0.00 ± 0.0000^{a}
4	K3 ($2x10^6$ cell/ml)	0.75 ± 0.2500^{b}	0.50 ± 0.2885^{ab}	0.25 ± 0.2500^{ab}
5	K4 ($2x10^7$cell/ml)	2.25 ± 0.4785^{c}	0.50 ± 0.2885^{ab}	0.50 ± 0.2885^{ab}

Description: Subscribe with different signs indicated a significant difference ($P < 0.05$), and the same sign indicated no difference between of them

Differences in the ability of all three isolates of *B. thuringiensis* used in killing the larvae of the test can be influenced by differences in potential toxicity of each isolate. [8] The potential toxicity of a particular crystal toxin of *B. thuringiensis* can be influenced by the *proteolytic* activity that breaks *protoxin* become active toxin, the toxin affinity for the receptor on the cell membrane epithelium of insects, as well as

the damage to the cell membrane epithelium of insects

Besides other factors which may affect, among other strains of bacteria (specification toxin), insect species tested, the concentration of bacteria, instar larvae of mosquitoes, the period of exposure, the quality of water, the feeding behavior of mosquito larvae targets, as well as the amount of toxin (crystal) were inedible [9].

A factor of bacteria is a protein crystal structure that plays an important role for toxin activity. In one strain of *B. thuringiensis* have ties more easily broken down by enzymes produced by insects and size of protein molecules that make up the crystal and molecular structure of amino acid and carbohydrate content in the crystal [10].

B. thuringiensis contains of Cry protein has a specific toxic activity towards certain insects. Cry I proteins known to be toxic to Lepidoptera, being Cry II toxic against Lepidoptera and Diptera. Cry III known to kill Coleoptera, and Cry IV toxic to Diptera [11]; [12]. All the subspecies *B. thuringiensis* are known to produce large amounts of protein crystals. [13] "When the spores and crystals protein ingested by an insect sensitive there will be paralysis that resulted in the death of the host". The crystal proteins that form protoxin be dissolved into an active toxin in the digestive tract and can kill insects.

The density of bacteria also affects the effectiveness of the target insect mortality. [14] Gamma conducted a test using the density 1,51x108 *B. thuringiensis* cells / ml to larvae of *Ae. aegypti* is also only the third instar larvae cause mortality test 26.67%, the more spores formed in *B. thuringiensis* estimated more the crystal proteins or toxins that are released to kill the larvae of *Ae. aegypti*.

In this research, the highest bacterial density used is 2x107sel / ml can cause larval mortality highest test only 22.5%.

Another factor influencing the effectiveness of *B. thuringiensis* on mortality can also be determined by the period of infection, water quality and feeding behavior of the target insect. The longer the larvae infection to the toxin of *B. thuringiensis*, the more toxins accumulate in the body of the larvae. The quality of water includes; water temperature, water pH and oxygen content will affect the toxicity of *B. thuringiensis* isolates. Larvae feeding behavior will determine the amount of *B. thuringiensis* toxins are ingested by the larvae. In the treatment with high concentrations have more number of spores, resulting in the number of spores are ingested by the larvae test will also be greater than the number of treatments that fewer spores.

3. Conclusion

The results that can be concluded from this research are:

1. In general, *B. thuringiensis* isolate which is derived from ground under trees around Lampung University effective to mortality.
2. The density of *B. thuringiensis* isolate $2x10^7$sel/ml Br (A) can cause the highest mortality towards *Aedes aegypti*'s larva with 22,5% followed by bacterial density $2x10^6$ cell/ml.

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