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The Comparison of Local to Commercial *Bacillus thuringiensis* Against *Aedes aegypti* Larvae

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Abstract
DHF as vector-borne disease become a problem in the world. The most effective way for suppressing the incidence is killing the vector, *Aedes aegypti*. One of the ways is using a natural insecticide, local *Bacillus thuringiensis* (Bt). The study aimed to determine the efficacy of local Bt against *Aedes aegypti* larvae. The study was conducted by using an experimental design with 1.250 3rd and 4th instar larvae *Aedes aegypti* as a sample. The study consist of 19 groups, 6 groups of local Bt from Curugdago, 6 groups of local Bt from Setiabudi, 6 groups of Bactivec® (commersial Bt), and a group without larvicide. Each group consist of 25 larvae included in the glass of media with concentrations of 0.04; 0.03; 0.02; 0.01; 0.008; and 0.005 ppm. An observation was made after 24 hours of exposure by counting the number of dead larvae. Data were analyzed using probit analysis to determine LC50, LC90, and LC99. The study was conducted in Unpad Faculty of Medicine Microbiology and Parasitology Laboratory. The result of the study showed that local Bt could kill *Aedes aegypti* larvae with LC50, LC90, LC99 is 0.043; 0.153; 0.436 for local Bt from Curugdago and 0.104; 0.21; 0.809 for local Bt from Setiabudi. That value is higher if compared with Bactivec®, therefore, it can be concluded that local Bt has larvicide effect against *Aedes aepgyti* larvae, however, there is not as effective as commercial Bt.

Key Words: *Aedes* aegypti, *Bacillus thuringiensis*, efficacy, larvicide

1. Introduction

DHF is a disease caused by a viral infection which is transmitted through *Aedes aegypti*. DHF is the most developing vector-borne disease in the world, increased up to 30 times in the last 50 years. There are 50 million people infected every year, hundreds of them are severe cases, and 20.000 between them cause death.

Vector controlling is the first choice to break the transmission of DHF, one of which is by controlling the larvae, the immature form of the vector. Chemical control is popular in the community, but it is very likely to cause resistance from vectors, pollution to the environment, and also to humans and institutions that are not the target (Kumar, Wahab, Mishra, Warikoo, 2012). Therefore, another alternative is needed to control the disease, one of which uses biological control, namely by using *Bacillus thuringiensis* (Bt).

Currently, commercial Bt products have been widely circulated, one in the race, namely Bactivec®. However, there has never been a study whether local Bt, in which bacteria in the soil in the Curugdago and Setiabudi regions, has a larvicidal effect or not. Therefore, this study was conducted to determine the efficacy of local *Bacillus thuringiensis* as a larvicidal tool for *Aedes aegypti* larvae.

Bt is a gram-positive bacterium that can synthesize crystalline inclusion of parapora that has high toxicity to mosquito larvae but has very low toxicity to organisms that are not the target, hence these bacteria are not harmful to the environment and humans (Ibrahim, Griko, Junker, Bulla, 2010) (Poopathi, Tyagi, 2006). Bt can be found widely in the world, and its main habitat is the soil (Argôlo-Filho, Loguercio, 2014) (Yuningsih, 2007).

2. Methods

The study was conducted at the FK Unpad Microbiology and Parasitology Laboratory in March-May 2017 and has obtained permission from the Faculty of Medicine, Padjadjaran University and a statement of ethical feasibility from the Health Research Ethics Committee, Faculty of Medicine, Padjadjaran University with numbers 327/UN6.C10/PN/2017.

The study used an experimental design with research samples were *Aedes aegypti* larvae obtained from the Laboratory of Parasitology FK Unpad with inclusion criteria in the form of active III and IV instars and exclusion criteria in the form of dead larvae or turned into pupae or adult mosquitoes before testing. Based on WHO 2005, the sample size in larvacide research was 25 larvae for each test group so that in this study a total of 1,250 larvae were needed for 2 repetitions (WHO, CDC, 2005).

The independent variables of this study are various concentrations of local *Bacillus thuringiensis* (Bt), while the dependent variable is the number of deaths of *Aedes aegypti* larvae (LC50, LC 90, and LC99).

Local Bt was obtained from FK Unpad Microbiology Laboratory in the form of liquid preparations. The local Bt was obtained from the soil in the Curugdago and Setiabudi regions. Then the local Bt of liquid preparations was taken to the Unpad Central Laboratory for the freeze-drying process so that the resulting powder preparations from Curugdago local Bt and Setiabudi local Bt weighing 0.241 g and 0.040 g respectively.

All of the powder preparations from local Curugdago Bt and local Setiabudi Bt were mixed with aquabides of 2.4 ml and 4 ml respectively to produce a stock solution of 1% or 10,000 ppm. Then the stock solution is diluted to produce 10 ppm. Then taken in a row of 400 µl, 300 µl, 200 µl, 100 µl, 80 µl, and 50 µl with a micropipette then put into a plastic glass containing 100 ml aquabides and 25 *Aedes aegypti* larvae. The final concentration produced is 0.04 ppm; 0.03 ppm; 0.02 ppm; 0.01 ppm; 0.008 ppm; and 0.005 ppm. As a positive control, the six concentrations were made using Bactivec® which was commercial Bt and as a negative control. One plastic cup was only filled with 100 ml of aquabides and 25 *Aedes aegypti* larvae without local Bt or commercial Bt. Repetition was carried out 2 times, then larval death was observed after 24 hours of testing.

Then the data obtained is analyzed by probit with the Miller and Tainter method to determine LC50, LC90, and LC99. First of all, the percentage of dead larvae is converted to probit values using the probit transformation table, then log 10 concentration and probit values are made, then the percentage of larval mortality is 50%, 90%, and 99% transformed into probit values and substituted into the line equation curve regression, so that the values of LC50, LC90, and LC99 are obtained.

3. Results

Larvae mortality results after 24 hours of treatment can be seen in table 1.

Table 1. The Percentage of Larval Deaths

Treatment	Concentration (ppm)	Number of larval deaths after 24 hours in the test-		Average Number of Deaths	% Mortality
		I	II		
CRDG 2.01	0,04	15	15	15	60%
	0,03	9	3	6	24%
	0,02	7	11	9	36%
	0,01	0	1	0,5	2%
	0,008	1	0	0,5	2%
	0,005	1	2	1,5	6%
STBD 3.01	0,04	3	1	2	8%
	0,03	0	2	1	4%
	0,02	2	0	1	4%
	0,01	0	1	0,5	2%
	0,008	2	2	2	8%
	0,005	0	0	0	0%
Bactivec®	0,04	24	24	24	96%
	0,03	23	20	21,5	86%
	0,02	20	20	20	80%
	0,01	15	14	14,5	58%
	0,008	3	3	3	12%
	0,005	2	0	1	4%
Control -	0	0	0	0	0%

Furthermore, the data were analyzed using the probit analysis from Miller and the Tainter method, so that the log10 concentration curve and probit analysis were obtained, also the curve regression line equation is presented in Figure 1, Figure 2, and Figure 3. Then the probit larval mortality value was 50%, 90 %, and 99% is replaced by the line equation, so that LC50, LC90, and LC99 are obtained from each treatment presented in table 2.

Table 2. Probit Analysis Result

Treatment	Concentration of Death		
	LC50	LC90	LC99
Curugdago Local Bt	0,043	0,153	0,436
Setiabudi Local Bt	0,104	0,321	0,809
Bactivec®	0,013	0,029	0,056

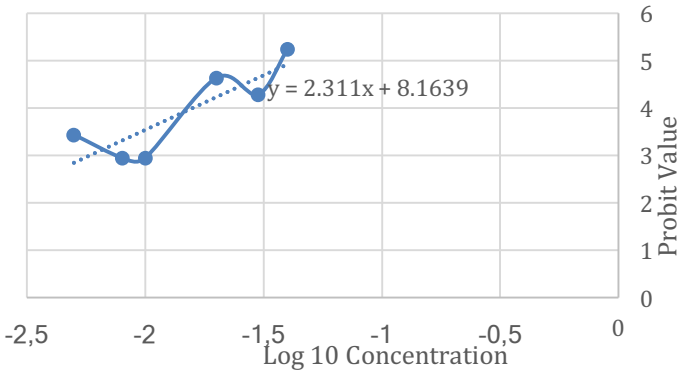

Figure 1. Log10 curve concentration and probit value of Curugdago local Bt

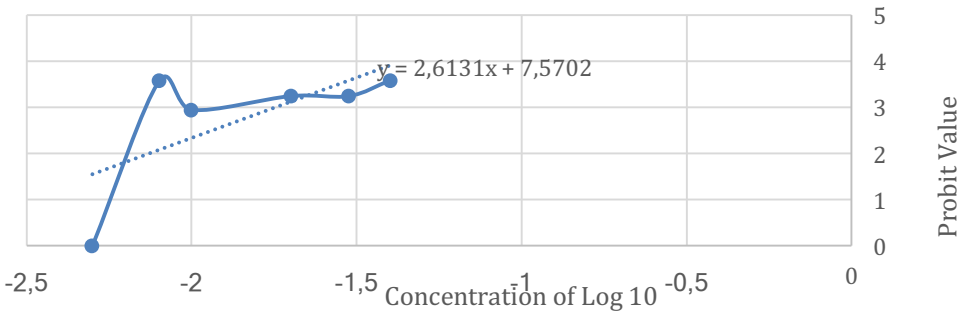

Figure 2. Concentration curve of Log10 and probit value of Setiabudi local Bt

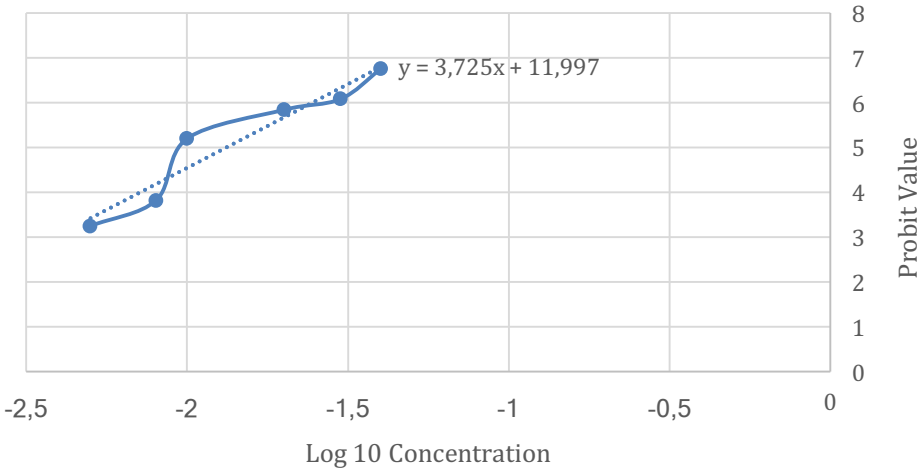

Figure 3. Log10 curve of Probit Bactivec® Concentration and Value

4. Discussion

Bacillus thuringiensis (Bt) is mainly found in the soil, in this study Bt was isolated from the soil in the Curugdago and Setiabudi regions. Bt liquid preparations underwent freeze-drying in advance to obtain Bt in powder preparations, because based on research conducted by Yusnita et al. Bt powder preparations are more potent than liquid preparations. It is because one of the stages is the deposition process with centrifugation at 4000 rpm for 15 minutes which result in partial rupture of the bacterial cell wall so that the cell contents, including paraspora crystal inclusions, can exit the cell (Anggraeni, Rahardianingtyas, Wianto, 2015).

When the crystalline inclusion of the paraspora is ingested and dissolved in the lumen of the alkaline midgut larvae, toxin activation occurs by proteases from which protoxin has been changed to the toxin. Then the fragment of the toxin binds to caderin receptors in midgut epithelial cells, forms an oligomeric structure, and binds to aminopeptidase receptors. After binding to secondary receptors, the structure can enter the membrane, forming pores and consequently lyse the cells. These conditions suitable for colonization, germination, and spore replication in hemolymph, causing septicemia, and death of larvae as the insect target (Palma et al. 2014) (Sanahuja, Banakar, Twyman, Capell, Christou, 2011).

Negative control in this study shows 0% mortality, so it can be concluded that the larval conditions are in good condition and can encourage other factors that cause larval death in the treatment group.

Curugdago and Setiabudi local Bt concentrations amounted to 0.043 ppm respectively; 0.153 ppm; 0.436 ppm and 0.104 ppm; 0.321 ppm; 0.809 ppm is capable of causing larval mortality of up to 50%, 90%, 99% after 24 hours exposure to *Aedes aegypti* larvae. Whereas Bactivec® requires a lower concentration, which is 0.013 ppm; 0.029 ppm; 0.056 ppm to kill 50%, 90%, 99% of the population of *Aedes aegypti* larvae. This shows that in this study, Bactivec® works more effectively with LC50, LC90, LC99 which is smaller compared to local Bt,

presumably this is caused by the reaction of the killing ability in the same larval midgut where the ability to kill local Bt requires a longer period of time than Bactivec®.[15] Besides that, differences in subspecies are important factors in determining the effectiveness of Bt, the Bactivec® is *Bacillus thuringiensis israelensis* while the local Bt subspecies are unknown. Therefore, further research is expected to be able to examine local Bt at the molecular level to identify its subspecies.

The results of this study are in accordance with Blondine Ch.P's study on the effectiveness of Vectobac 12 AS (BT H-14) and *Bacillus thuringiensis* H-14 which shows that local Bt has lower effectiveness than commercial Bt (Blondine, 2004).

The limitation of this study lies in the size of the different larvae when used due to the difficulty of finding *Aedes aegypti* larvae in one phase on a large scale at one time.

5. Conclusion

From this study it can be concluded that local *Bacillus thuringiensis* has a larvicidal effect on *Aedes aegypti*, although not as effective as commercial *Bacillus thuringiensis*, with LC50, LC90, LC99 which is 0.043 ppm; 0.153 ppm; 0,436 ppm for Curugdago local Bt and 0,104 ppm; 0.321 ppm; 0.809 ppm for the Setiabudi local Bt.

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