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

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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 alternative larvicide and safe from bacteria 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 is the third instar larvae of Aedes aegypti. Concentrations used in this study was 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 2683.*

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

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 areas 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 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 3,53x107 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

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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

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 use 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:

$$V_1.M_1=V_2.M_2$$

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.

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 larva death			Average	Percentage
	Repetitior				
	I	II	III		
Konsentrasi 0,01%	25	25	25	25	100%
Konsentrasi 0,02%	25	25	25	25	100%
Konsentrasi 0,03%	25	25	24	25	100%
Konsentrasi 0,04%	25	25	25	25	100%
Konsentrasi 0,05%	25	25	24	24,67	98,68%
Kontrol positive	25	25	25	25	100%
Kontrol 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 means 0.009 <0.05 means that 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 LT_{50} (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 LC_{50} suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

	Repetition	**LC_{50} (%)**
LC_{50} *Bacillus thuringiensis var Israelensis* suspension	I	0.010
	II	0.010
	III	0.010
	Average	0.010

Based on regression analysis obtained average LC_{50} at concentration 0.010%. 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 LT_{50} suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

	Repetition	**LT_{50} (%)**
LT_{50} *Bacillus thuringiensis var Israelensis* suspension	I	2.764
	II	2.646
	III	2.639
	Average	2.683

Based on the regression analysis obtained average LT_{50} at the time (hours) to 26683. 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 26683. This means that at 2.683 hours the bacterial suspension of *Bacillus turingiensis 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 thuringensis 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 (Insectisidal 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]

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.

Acknowledgment

Authors would like to thank 1) Research and Development Institute of Ahmad Dahlan University (2) Ahmad Dahlan University, 3) Entomology Laboratory of Faculty of Public Health, Ahmad Dahlan University, 3) Research Team for their kind support and contribution to this research.

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ABSTRACT

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 alternative larvicide and safe from bacteria suspension of Bacillus thuringiensis var israelensis.
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~~*Research papers should be accompanied by an abstract, which will appear in front of the main body of the text. It should be written in complete sentences and should summarize the aims, methods, results and conclusions in less than 250 words. The abstract should be comprehensible to readers before they read the paper and abbreviations, citations and mathematical equations/notations should be avoided.*~~

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Keywords: *Bacillus thuringiensis Var Israelensis, Aedes aegypti, dengue hemorrhagic fever, effectivity* ~~Regression, data structure, prediction, simulation.~~

~~**Mathematics Subject Classification:** 62J12, 62G99~~

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1. INTRODUCTION

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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 areas 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]

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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}).

~~In the establishment of the prediction model, three stages are fundamental: possible selection of the variables, the estimation of the coefficients of the variables selected and the validation of the model. Ideally, this validation should be done on different observations. But in most practical situations, the selection of the variables, the estimation of the coefficients and the validation are done using the same sample. Indeed, it is often difficult to have separate samples for the various stages of modeling, because the dataset available to the researcher is frequently too small to use part of it to establish the~~

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regression model and the remaining for its validation. Sometimes, the number of predictors is higher than the number of observations.

The objective of this work is to bring some useful information for the users, especially those who do not have the possibility to validate the models from external data. In a more concrete way, we propose to examine the predictive value of a regression model by calculating a coefficient, similar to the multiple coefficient of determination, which we call coefficient of determination of prediction. It is denoted R_p^2 and is defined, for n_p new observations, as follows:

$$R_p^2 = 1 - \sum_{i=1}^{n_p} (y_i - \hat{y}_i)^2 \Big/ \sum_{i=1}^{n_p} (y_i - \bar{y})^2 .$$

In this relation, y_i indicates the actual value of the dependent variable for the new individual i ($i = 1, \ldots, n_p$). $\hat{y}_i$, is the predicted value for this individual given by the regression model, $\bar{y}$ is the arithmetic mean of n observations of the dependent variable in the sample which was used to establish the model.

2. GENERATION OF THE DATA

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 use 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:

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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.

The realization of this work supposes the availability of a great number of repetitions of samples responding to the same known theoretical model. In practice, as the theoretical model is unknown, we use the Monte-Carlo method based on the generation of the data by computer according to a fixed theoretical model.

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2.1. Theoretical model

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We consider the traditional theoretical model of multiple linear regressions as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\varepsilon},$$

where $\mathbf{y}$ is an $n \times 1$ vector observations of the dependent variables, $\mathbf{X}$ is the matrix $n \times k$ of k explanatory variable, $\boldsymbol{\varepsilon}$ the vector of n theoretical residuals and $\boldsymbol{\beta}$ the vector of the theoretical regression coefficients. It is supposed that the residuals are independent random variable of the same normal distribution of null mean and constant variance σ^2. The parameters to be simulated are $\mathbf{X}$, $\boldsymbol{\beta}$ and $\boldsymbol{\varepsilon}$, while the vector $\mathbf{y}$ is calculated by the model.

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2.2. Controlled factors

The factors controlled for the theoretical models are the number of explanatory variables (k), the number of observations (n), the index of collinearity of the explanatory variables (IC), the index of decrease of the regression coefficients (Ib) and the theoretical coefficient of determination (R_0^2).

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$$\beta_i = c(Ib)^{i-1} \qquad (i = 1, 2, 3, 4, 5)$$

where β_i is the value of coefficient i, Ib the index of decrease of the regression coefficients and c a constant.

2.3. Methods of regression studied

On the one hand, we considered the classical method of least squares without variables selection and on the other hand, the *stepwise* selection method of variables is used. These methods were adopted, because they are among the most used methods, and are available in almost all statistical software.

The selection of variables is based on the *t* test of Student or *F* test of Snedecor for significance of the regression coefficients. We used the same level of significance for the introduction and the exclusion of a variable in the model. Two theoretical levels were retained: 0.15 and 0.05.

3. RESULTS

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 11. 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 larva death (Repetition) I	II	III	Average	Percentage
Konsentrasi 0,01%	25	25	25	25	100%
Konsentrasi 0,02%	25	25	25	25	100%
Konsentrasi 0,03%	25	25	24	25	100%
Konsentrasi 0,04%	25	25	25	25	100%
Konsentrasi 0,05%	25	25	24	24,67	98,68%
Kontrol positive	25	25	25	25	100%
Kontrol 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 means 0.009 <0.05 means that 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 LT_{50} (Lethal Time 50) which means the time required suspension *Bacillus thuringiensis var Israelensis* to be able to kill 50% larvae *Aedes aegypti.*

Table 22. Value of LC_{50} suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

	Repetition	LC_{50} (%)
***LC_{50} Bacillus thuringiensis var Israelensis* suspension**	I	0.010
	II	0.010
	III	0.010
	Average	0.010

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

Table 33. Value of LT_{50} suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

	Repetition	LT_{50} (%)
***LT_{50} Bacillus thuringiensis var Israelensis* suspension**	I	2.764
	II	2.646
	III	2.639
	Average	2.683

Based on the regression analysis obtained average LT_{50} at the time (hours) to 26683. This means that at 2.683 hours the suspension of *Bacillus thuringiensis var Israelensis* can kill 50% of *Aedes aegypti* larvae.

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3.1. Effects of the various factors on the coefficient R_p^2

The analysis of table 1 shows that coefficient R_p^2 is more often lower than the theoretical coefficient of determination. The ratio increases as the sample size increases, for a given value of k and R_0^2.

Table 1: Average observed values of R_p^2, expressed in proportion of R_0^2, according to k, n and R_0^2.

k	n	$R_0^2 = 0.20$	$R_0^2 = 0.40$	$R_0^2 = 0.60$	$R_0^2 = 0.80$
Complete model					
5	8	-14.39	-4.76	-1.54	0.06
10	200	0.82	0.93	0.97	0.99
30	50	-0.15	0.52	0.77	0.90
30	600	0.91	0.96	0.98	0.99
Model selected ($\alpha = 0.15$)					
5	8	-1.66	-0.31	0.26	0.65
5	100	0.79	0.92	0.96	0.98
10	17	-0.99	0.15	0.56	0.81
10	200	0.89	0.96	0.98	0.99
30	50	-0.40	0.44	0.75	0.90
30	600	0.93	0.98	1.00	1.00
Model selected ($\alpha = 0.05$)					
30	600	0.93	1.00	1.00	1.00

For known values of R_0^2 and k/n, the ratio R_p^2/R_0^2 depends little on the values of k and n. We also note that the ratio is weaker for the low values of R_0^2. Finally, the use of variables selection tends to increase the ratio.

3.2. Determination of the levels of factors combinations leading to a null predictive value

In order to obtain results easily usable in practice, we determined the validity limits of the equations for the purpose of prediction by being unaware of the effect of factors IC and Ib on the prediction. These limits are obtained by determining the levels of the ratio k/n leading to a zero value of R_p^2. These levels give on average the thresholds of combinations of factors from which the model led to predictions of quality lower than the prediction given by the arithmetic mean of the dependent variable of the sample.

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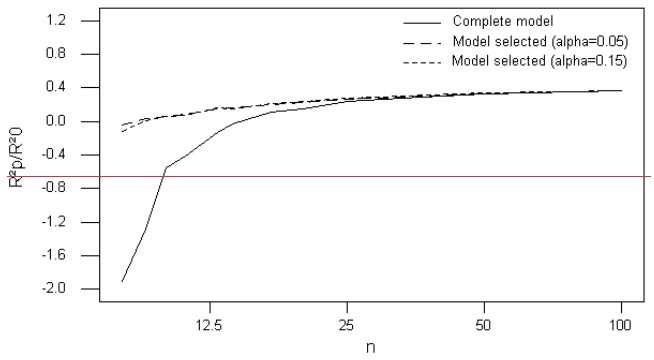

Figure 1. Evolution of the ratio R_p^2/R_0^2 according to the sample size on logarithmic scale in X-coordinate, for $k=5$, R_0^2=0.40.

From this table, we note that this size varies according to the method used to establish the model. It is higher for the complete models and decreases gradually with the intensity of the selection. It also decreases as the theoretical value R_0^2 increases.

4. DISCUSSION AND CONCLUSION

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 (aguades) 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.

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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 26683. This means that at 2.683 hours the bacterial suspension of *Bacillus turingiensis 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 thuringensis 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.[(Jusuf, 2010)~~10~~] While the ICP protein (Insectisidal 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.[(Zeiger, 1999)~~11~~]
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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Several authors documented criteria that assess the quality of a model. These criteria are based on the difference between the estimated model and the presumed known theoretical model. In the present study, the criterion used compares to new observations resulting from the same population as individuals of the sample, the variability of the errors of prediction, when the predictions are carried out by a regression equation and on the other hand when these predictions are equal to the arithmetic mean $\bar{y}$ of the dependent variable in the sample. It thus gives an idea of the improvement of the quality of prediction by taking into account the explanatory variables. It also informs about the validity limits of a prediction model.

The plan of simulation considers data of varied structures. In particular, we considered the case where all the explanatory variables available are indeed present in the theoretical model ($k \leq 5$) and the case where certain explanatory variables available are not present in the theoretical model. This approach makes it possible to be close to the situations often encountered in practice.

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Revisi 1

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

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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 alternative larvicide and safe from bacteria 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 is the third instar larvae of Aedes aegypti. Concentrations used in this study was 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 2683.*

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

Introduction

Dengue Fever is a disease that is always increasing. Since the first time in Surabaya and Jakarta in 1968(1). DHF incidence increased very rapidly in Indonesia and even in some areas 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 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](6)(7)

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(6)(8)(7)(9).

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 3,53x107 cell/ml.[5] Bacillus thuringiensis is a gram-positive bacteria that can form spores and proteins that are toxic to *Aedes aegypti* mosquito larvae(11)(12)(13)(14).

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

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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

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 use 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:

$$V_1.M_1=V_2.M_2$$

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.

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 larva death			Average	Percentage
	Repetitior				
	I	II	III		
Konsentrasi 0,01%	25	25	25	25	100%
Konsentrasi 0,02%	25	25	25	25	100%
Konsentrasi 0,03%	25	25	24	25	100%
Konsentrasi 0,04%	25	25	25	25	100%
Konsentrasi 0,05%	25	25	24	24,67	98,68%
Kontrol positive	25	25	25	25	100%
Kontrol 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 means 0.009 <0.05 means that 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 LT_{50} (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 LC_{50} suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

	Repetition	**LC_{50} (%)**
LC_{50} *Bacillus thuringiensis var Israelensis* suspension	I	0.010
	II	0.010
	III	0.010
	Average	0.010

Based on regression analysis obtained average LC_{50} at concentration 0.010%. 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 LT_{50} suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

	Repetition	**LT_{50} (%)**
LT_{50} *Bacillus thuringiensis var Israelensis* suspension	I	2.764
	II	2.646
	III	2.639
	Average	2.683

Based on the regression analysis obtained average LT_{50} at the time (hours) to 26683. 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.

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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 26683. This means that at 2.683 hours the bacterial suspension of *Bacillus turingiensis 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 thuringensis 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 (Insectisidal 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]

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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Authors would like to thank 1) Research and Development Institute of Ahmad Dahlan University (2) Ahmad Dahlan University, 3) Entomology Laboratory of Faculty of Public Health, Ahmad Dahlan University, 3) Research Team for their kind support and contribution to this research.

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

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ABSTRACT

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 alternative larvicide and safe from bacteria suspension of Bacillus thuringiensis var israelensis.

This study used a type of true experimental research design with posttest only control group design. Samples used in this study is the third instar larvae of Aedes aegypti. Concentrations used in this study was 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.

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.

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 2683.

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

Introduction

Dengue Fever is a disease that is always increasing. Since the first time in Surabaya and Jakarta in 1968(1)(2). DHF incidence increased very rapidly in Indonesia and even in some areas 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 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. (6)(7) 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(6)(8)(7)(9).

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* (10)(11)(12). Other research finds toxicity of Bacillus thuringiensis to *Aedes aegypti* larva in

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Nganjuk City with LC_{50} 48 hour value equal to 3,53x107 cell/ml.[5](10). Bacillus thuringiensis is a gram-positive bacteria that can form spores and proteins that are toxic to *Aedes aegypti* mosquito larvae(14)(15)(16)(17).

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})(10)(14).

Methods and Materials

This study used a type of true experimental research design with posttest only control group design(19). In this design, the researchers compared the number of dead larvae between the use 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(10). 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:

$$V_1.M_1=V_2.M_2$$

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(20).

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.

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 larva death			Average	Percentage
	Repetitio				
	I	II	III		
Konsentrasi 0,01%	25	25	25	25	100%
Konsentrasi 0,02%	25	25	25	25	100%
Konsentrasi 0,03%	25	25	24	25	100%
Konsentrasi 0,04%	25	25	25	25	100%
Konsentrasi 0,05%	25	25	24	24,67	98,68%
Kontrol positive	25	25	25	25	100%
Kontrol 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 means 0.009 <0.05 means that 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 LT_{50} (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 LC_{50} suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

LC_{50} *Bacillus thuringiensis var Israelensis* suspension	**Repetition**	**LC_{50} (%)**
	I	0.010
	II	0.010
	III	0.010
	Average	0.010

Based on regression analysis obtained average LC_{50} at concentration 0.010%. 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 LT_{50} suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

LT_{50} *Bacillus thuringiensis var Israelensis* suspension	**Repetition**	**LT_{50} (%)**
	I	2.764
	II	2.646
	III	2.639
	Average	2.683

Based on the regression analysis obtained average LT_{50} at the time (hours) to 26683. 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.

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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 26683. This means that at 2.683 hours the bacterial suspension of *Bacillus turingiensis 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 thuringensis 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 (Insectisidal 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]

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.

Acknowledgment

Authors would like to thank 1) Research and Development Institute of Ahmad Dahlan University (2) Ahmad Dahlan University, 3) Entomology Laboratory of Faculty of Public Health, Ahmad Dahlan University, 3) Research Team for their kind support and contribution to this research.

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Covering Letter

To,

The Editor

Sub: Submission of Manuscript for publication

Dear Sir,

We intend to publish an article entitled **" Effectivity of Bacterial Suspension *Bacillus thuringiensis Var Israelensis* in Killing *Aedes aegypti L.* Mosquito Larva "** in your esteemed journal as an Original Article.

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2 Eva Hendrawati		13 Januari 2018
3 Sulistyawati		13 Januari 2018

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Abstract Page

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Abstract:

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Context:

Aims: DHF was 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 alternative larvicide and safe from bacteria suspension of Bacillus thuringiensis var israelensis.

Design:
This study used a type of true experimental research design with posttest only control group design.

Methods and Material:
Samples used in this study was the third instar larvae of Aedes aegypti. Concentrations used in this study was 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:
Kruskal Walls test 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. 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 2683.

Key-words: Bacillus thuringiensis var Israelensis**, A**edes aegypti**,** DHF, effectivity

Key Messages:

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Text

Introduction:

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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 areas 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 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 3,53x107 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}).

Subjects and Methods:

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 use 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:

$$V_1.M_1=V_2.M_2$$

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.

Results:

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 larva death			Average	Percentage
	Repetitior				
	I	II	III		
Konsentrasi 0,01%	25	25	25	25	100%
Konsentrasi 0,02%	25	25	25	25	100%
Konsentrasi 0,03%	25	25	24	25	100%
Konsentrasi 0,04%	25	25	25	25	100%
Konsentrasi 0,05%	25	25	24	24,67	98,68%
Kontrol positive	25	25	25	25	100%
Kontrol 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 means 0.009 <0.05 means that 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 LT_{50} (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 LC_{50} suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

	Repetition	LC_{50} (%)
LC_{50} *Bacillus thuringiensis var Israelensis* suspension	I	0.010
	II	0.010
	III	0.010
	Average	0.010

Based on regression analysis obtained average LC_{50} at concentration 0.010%. 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 LT_{50} suspension *Bacillus thuringiensis var Israelensis* in killing *Aedes aegypti* larvae

	Repetition	LT_{50} (%)
LT_{50} *Bacillus thuringiensis var Israelensis* suspension	I	2.764
	II	2.646
	III	2.639
	Average	2.683

Based on the regression analysis obtained average LT_{50} at the time (hours) to 26683. 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 26683. This means that at 2.683 hours the bacterial suspension of *Bacillus turingiensis 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 thuringensis 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 (Insectisidal 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]

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