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Survival Analysis of Natural Insecticide Soursop Seed Extract in Aedes Aegypti Larvae Vector of Dengue Haemorrhagic Fever in Manado City

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Abstract- Survival Analysis of Natural Insecticide Seed Extract Soursop In vector Aedes aegypti larvae Dengue Haemorrhagic Fever in Manado City has done. Testing the assumption of homogeneity of variance test performed with SPSS Levene Test with the Levene's Test Sig> 0.05, which indicates the assumption of homogeneity range is met. Testing the normality assumption of the data was done by Kolmogorov Smirnov test. Testing is a tool of SPSS with the Sig (p-value)> 0.05 indicates the data met the assumption of normality. . One Way Anova Test results showed that the value of 230 879 Fcount and Sig F for 0000. From table-F with the statistics obtained Ftable of 4066. Because the value Fcount> F table and Sig F <0.05 indicates that there are differences in the level of real mosquito larvae mortality (significant) at different concentration levels. . The test results show MTTF or Mean Time To Failure (survival value) mosquito is the provision of 183 476 ppm concentration. Thus, the concentration of 183,476 ppm figure is a massive concentration of the most effective way to kill mosquito larvae. According to the criteria of toxicity by Australia Petroleum Energy Association, 183 476 concentration of ethanol extract of soursop seeds or (Mean (MTTF) = 183 476) at 24 hours of observation included in the criteria of Toxicity Medium (Moderately Toxic).

Keywords- *Survival Analysis, Natural Insecticides, Seeds Soursop, Aedes aegypti.*

I. INTRODUCTION

Mosquitoes including one of the types of insects that gained great attention in human health, because it has potential as a vector in the transmission of the disease [1]. Dengue Haemorrhagic Fever is an infectious disease caused by the dengue virus and transmitted by the mosquito Aedes aegypti and Aedes albopictus are characterized by sudden fever 2 to 7 days with no obvious cause, weak or lethargic, anxiety, heartburn, accompanied by signs of bleeding in the skin such as bleeding spots (petechie), hematoma (echymosis), or rash (purpura), sometimes bleeding, dysentery, vomiting of blood, decreased consciousness or shock (shock) [2].

Until now still not found drugs and an effective vaccine for dengue fever. Mosquito nest eradication (PSN) is a method of vector control as one of the efforts to prevent the transmission of dengue disease PSN campaign has encouraged the government in this case the Ministry of Health with the slogan 3M, which drain the water reservoirs on a regular basis, closing shelters water and bury the used goods can become mosquito breeding [3].

Manado City is an area endemic Dengue Hemorrhagic Fever (DHF) which in the year 2010 and an increase in cases. Data dengue hemorrhagic fever (DHF) in Manado City in 2010 as many as 998 cases with 25 deaths (CFR = 2.5%). When compared to January s / d April 2010, which is as many as 832 cases, the declining trend in the case of very sharp. However, the patterns of this disease are subject to change and may increase unexpectedly [4].

Control method is the fastest break the cycle of transmission is the use of larvacide and synthetic insecticides, but synthetic chemical can cause mosquito resistant properties. Some of the resistant cases were also reported in the world, such as mosquito resistance A. aegypti to organophosphat in Brazil [5].

How to control nature is to use the plant as biopesticides, as an environmentally friendly alternative to control, easy to apply and is not harmful to natural enemies and other beneficial insects. Insecticides from plants is more selective and safer, because it is easily biodegradable (degraded) in nature so it does not leave any residue on the soil, water and air [6].

One plant in the Annonaceae family that has studied the content of the active compound is Annona muricata Linn locally known as Soursop. Other studies mentioned Annonaceae plant family contains many compounds are suspected asetogenin larvasidal and ingredients are also asetogenin as insecticides, acaricides, antiparasitic and bactericidal [7].

Based on the above background, it is necessary to investigate the mean life (optimal concentration level) known as MTTF or Mean Time To Failure (MTTF) or the expected value where mosquito larvae mortality will be obtained with the use of natural insecticides soursop seeds

using larva of dengue mosquito Aedes aegypti as bioindicator.

II. Data Analysis Methods

Analysis of variance (completely randomized design). To test the comparison group of more than two such groups of a concentration of 10 ppm, 100 ppm, 500 ppm and 1000 ppm, used ANOVA analysis tools or Analysis of Variance. ANOVA is equivalent to a completely randomized design (CRD or Completely Randomize Design). Before testing the One Way or Two-Way ANOVA, first testing the fulfillment of assumptions. There are two assumptions that must be met in One Way or Two-Way ANOVA, namely: (1) the assumption of homogeneity of variance and (2) the assumption of normality of data. The first assumption, homogeneity of the various tests performed by Levene Test. Testing tool is a program SPSS. If the value of Sig (p-value)> 0.05, indicating the assumption of homogeneity range is met, otherwise if Sig (p-value) <0.05, then the assumption of homogeneity of diversity are not met. Fulfillment of these assumptions indicate that the range (variation) of the inter-group treatment is the same.The second assumption is the assumption of normality of data, done by Kolmogorov Smirnov test. Testing tool is a program SPSS. If the value of Sig (p-value)> 0.05 indicates the data met the assumption of normality. To fulfill the assumptions of normality indicated that the data used in this study were normally distributed, so it is worth using the Parametric Analysis of One-Way or Two-Way ANOVA.

Mortality patterns Aedes aegypti mosquito larvae. In previous analyzes have concluded that the difference in mortality rates on different types of mosquito larvae concentrations ranging from 10 ppm to 1000 ppm. However, to determine the extent of the pattern (shape) from mosquito larvae mortality, it needs more in-depth analysis tools namely Survival Analysis.

III. Results And Discussion

Comparison of Mortality Aegepty Aedes Mosquito Larvae. The analysis tools are Analysis of Variance (ANOVA) with type Completely Randomized Design (CRD) or the equivalent of one-way ANOVA. The data in Table 1 gives the number of deaths and the mortality rate Aegepty Aedes mosquito larvae at four levels, namely the concentration of 1000 ppm, 500 ppm, 100 ppm, and 10 ppm. The data used is the data rate of death (mortality) in the form of a percentage score from 0% to 100%. Number 0% said from 25 mosquitoes, no one died, while the 100% state of 25 overall dead mosquitoes.

TABLE I. Description Average Value and Variations Each Concentration

Concentration	Average	Variation
10 ppm	28.00	4.00
100 ppm	66.67	6.11
500 ppm	97.33	2.31
1000 ppm	100.00	0.00

Graphically presented as follows:

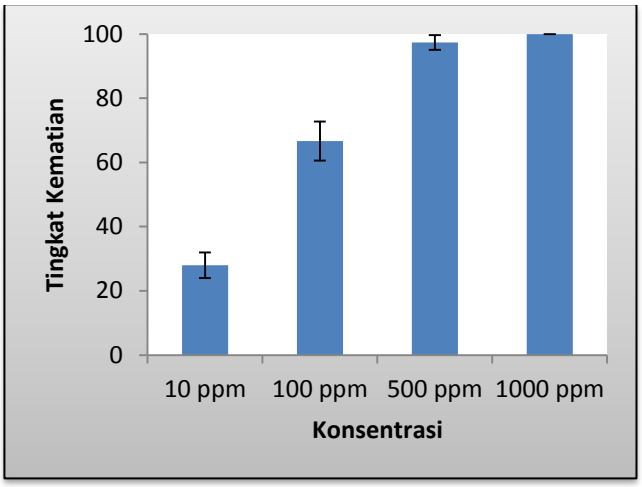

Figure 1. Description Average Value and Variations Each Concentration

In the picture above, high bar chart states average each concentration, while the line that runs vertically on the average value of each declared distribution or concentration data variation (standard deviation values). From the table and figure above appears there are differences in the mortality rate of mosquitoes at different concentration levels from 10 ppm to 1000 ppm, but it appears that the concentration of 500 ppm and 1000 ppm looks almost the same. To determine whether there are differences in the mortality rate of mosquitoes real (significant) on all four tested concentrations of the One Way ANOVA or equivalent Completely Randomized Design (Completely Randomize Design).

Before testing the One Way ANOVA, first testing the fulfillment of assumptions. There are two assumptions that must be met in the One Way ANOVA, namely (1) the assumption of homogeneity of variance and (2) the assumption of normality of data. The test results are presented in the following table:

TABLE II. TESTING HOMOGENEITY AND NORMALITY ASSUMPTION CONCENTRATION DATA

Assumptions	Testing	Sig
Homogeneity	Levene's Test	0.094
Normality	Kolmogorov-Smirnov	0.328

From table 2 looks Levene's Test Sig> 0.05, which indicates the assumption of homogeneity range is met. That is diversity (variation) of the four groups of concentrations (10 ppm, 100 ppm, 500 ppm and 1000 ppm) is the same. Seen the value of the Kolmogorov-Smirnov Test> 0.05, indicating that the data used in this study were normally distributed, so it is worth using the Parametric Analysis of One-Way ANOVA.

Further testing of One-way ANOVA. Treat (treatment) or otherwise significant concentrations (significantly different) if the value Fcount > F table or Sig F (P-value) <0.05 (5% error rate).

TABLE III. ONE-WAY ANOVA RESULTS CONCENTRATION

Source of variation	Number of Squares	Free degrees	Central squares	F Count	Sig F
Concentration	10158.7	3	3386.222	230.879	0.000
Error	117.3	8	14.667		
Total	10276.0	11			

The test results in Table 3 shows that the value of 230 879 Fhitung and Sig F for 0000. From table-F with the statistics obtained Ftable of 4066. Because the value Fhitung> F table and Sig F <0.05 indicates that there are differences in the level of real mosquito larvae mortality (significant) at different concentration levels.

To determine the concentration which gives the highest mortality rates used further test (post hoc test) the Tukey test (or the least significant difference test / honestly significance difference). If the concentrations were the same notation (a subset of the same), there are similarities between the concentrations indicated, otherwise if concentrations were different notations (yan different subset), indicating there is a difference between concentration. These test results are presented in full:

TABLE IV. ADVANCED TEST USING TUKEY TEST (BNT) CONCENTRATION DATA

Concentration	Average	Notation
10 ppm	28.00	A
100 ppm	66.67	B
500 ppm	97.33	C
1000 ppm	100.00	C

In table 4 shows that by giving a concentration of 10 ppm, will provide the lowest mosquito larvae mortality rate that is equal to 28.00%. With the increase in the concentration to 100 ppm, will provide mosquito larvae mortality better (different notation) when compared to concentrations of 10 ppm, which is at 66.67%. Meanwhile, with the increase being 500-1000 ppm concentration, will provide mosquito larvae mortality better (different notation) when compared to a concentration of 100 ppm, respectively 97.33% for the concentration of 500 ppm, and 100% for a concentration of 1000 ppm. However, it can be concluded that the provision of the concentration of 500 ppm and 1000 ppm will provide mosquito larvae mortality rates were the same (same notation).

IV. MORTALITY PATTERNS AEDES MOSQUITO LARVAE AEGEPTY

In previous analyzes have concluded that the difference in mortality rates on different types of mosquito larvae concentrations ranging from 10 ppm to 1000 ppm. However, to determine the extent of the pattern (shape) from mosquito larvae mortality, it needs more in-depth analysis tools namely Survival Analysis.

The data used to test survival analysis is the data in the table 4 present number of deaths and the mortality rate Aegepty Aedes mosquito larvae at four levels, namely concentration of 1000 ppm, 500 ppm, 100 ppm, and 10 ppm. The data used is the data the number of deaths (mortality) in the form of numbers from 0 to 25. Number 0 states of 25 mosquitoes, no one died, while 25 states figure of 25 dead mosquitoes whole. The data used was obtained overall from 25 mosquitoes in each repetition (there were 3 replications) to obtain 75 mosquitoes as a whole. Table 5 below parameter estimates presented survival analysis models:

TABLE V. SURVIVAL ANALYSIS MODEL PARAMETER ESTIMATION

Parameter Estimates	Standard	95.0%	Normal	CI
Parameter	Estimate	Error	Lower	Upper
Shape	0.886843	0.0704492	0.758977	1.03625
Scale	172.954	24.2031	131.466	227.534

Density function of opportunities Weibull distribution is

$$f(y) = \frac{\lambda y^{\lambda-1}}{\theta^{\lambda}} \exp\left\{-\left(\frac{y}{\theta}\right)^{\lambda}\right\} \quad (1)$$

With y is the concentration (in ppm) causing mosquito larvae mortality, λ is the shape parameter (shape) and θ is the distribution scale parameter (scale). To obtain survival analysis model to the data rate of mosquito larvae mortality as follows:

$$f(y) = \frac{0.88 y^{0.88-1}}{172.95^{0.88}} \exp\left\{-\left(\frac{y}{172.95}\right)^{0.88}\right\} \quad (2)$$

In the graph, the curve of survival analysis are presented as follows

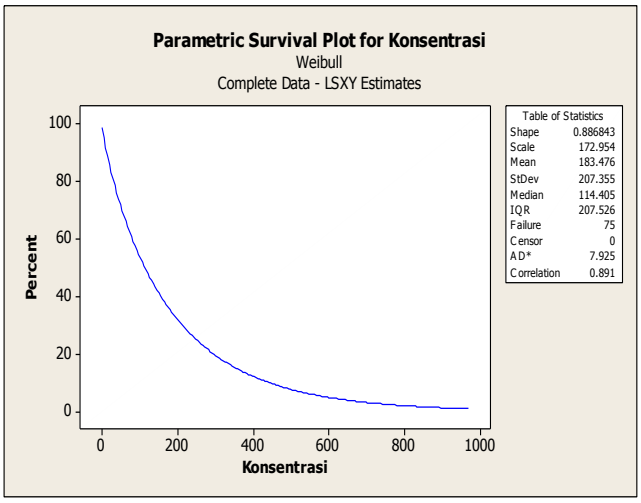

Figure 2. Survival Curve Analysis

TABLE VI. CURVES SURVIVAL ANALYSIS NUMERISASI

Table of Percentiles	Standard	95.0%	Normal	CI
Percent	Percentile	Error	Lower	Upper
0.1	0.0716784	.0502262	.0181526	0.283034
1	0.966491	0.481686	0.363890	2.56700
2	2.12382	0.930131	0.900185	5.01075
3	3.37425	1.35886	1.53245	7.42967
4	4.69440	1.77337	2.23887	9.84310
5	6.07295	2.17674	3.00817	12.2602
6	7.50328	2.57100	3.83342	14.6864
7	8.98109	2.95761	4.70994	17.1255
8	10.5035	3.33766	5.63442	19.5802
9	12.0684	3.71203	6.60441	22.0528
10	13.6743	4.08142	7.61806	24.5452
20	31.8710	7.60097	19.9706	50.8628
30	54.0847	10.9962	36.3088	80.5631
40	81.0922	14.4657	57.1659	115.033
50	114.405	18.2199	83.7306	156.317
60	156.718	22.5837	118.156	207.864
70	213.222	28.1890	164.551	276.290
80	295.784	36.5803	232.116	376.916
90	442.958	53.2732	349.938	560.704
91	465.878	56.1089	367.923	589.914
92	491.653	59.3743	388.029	622.951
93	521.060	63.1975	410.817	660.887
94	555.242	67.7699	437.109	705.303
95	595.980	73.3944	468.174	758.676
96	646.271	80.5904	506.138	825.201
97	711.762	90.3593	554.975	912.843
98	805.227	105.026	623.586	1039.78
99	967.837	132.361	740.273	1265.36
99.9	1528.84	240.553	1123.13	2081.10

Mosquito larvae mortality was 0.1%. Then, with a concentration of 0.96 ppm level, will result in mosquito larval mortality was 1.0%. With the interpretation of the same, to the provision of 1528.84 ppm concentration will produce mosquito larvae mortality was 99.9%. 1528.84 ppm concentration point is the level of the provision of the highest concentrations would reach the conclusion that the mosquito larvae will die as a whole (approaching 100%).

TABLE VII. MEAN TIME TO FAILURE (MTTF)

Characteristics of Distribution				
	Standard	95.0% Normal CI		
Mean(MTTF)	Estimate	Error	Lower	Upper
	183.476	22.5943	144.131	233.562

Table 7 above presents the mean life (optimal concentration level) known as MTTF or Mean Time To Failure (MTTF) or the expected value where mosquito larvae mortality will be obtained. The test results demonstrate the value of the mosquito life expectancy is the provision of 183 476 ppm concentration. Thus, the concentration of 183,476 ppm figure is a massive concentration of the most effective way to kill mosquito larvae.

According to the criteria of toxicity by Australia Petroleum Energy Association [8] 183. 476 ppm concentrations of extract of soursop seeds or (Mean (MTTF) = 183 476) at 24 hours of observation included in the criteria of Toxicity Medium (Moderately Toxic).

V. CONCLUSION

The test results assuming homogeneity variance test done with SPSS Levene Test with Levene's Test Sig> 0.05, which indicates the assumption of homogeneity range is met. Testing the normality assumption of the data was done by Kolmogorov Smirnov test. Testing is a tool of SPSS with the Sig (p-value)> 0.05 indicates the data met the assumption of normality. One Way Anova test results showed that the value of 230. 879 Fcount and Sig F for 0000. From table-F with the statistics obtained Ftable of 4.066. Because the value Fcount> F table and Sig F <0.05 indicates that there are differences in the level of real mosquito larvae mortality (significant) at different concentration levels. The test results show MTTF or Mean Time To Failure (survival value) mosquito is the provision of 183. 476 ppm concentration. Thus, the concentration of 183.476 ppm figure is a massive concentration of the most effective way to kill mosquito larvae. According to the criteria of toxicity by Australia Petroleum Energy Association (1994) in Ratningsih (2008) 183.476 concentration of extract of soursop seeds or (Mean (MTTF) = 183. 476) at 24 hours of observation included in the criteria of Toxicity Medium (Moderately Toxic).

REFERENCES

[1] Stocker, Uwe and Rene de Jong. , 2005. *Preventative Measures against Dengue Fever*. www.expat.or.id / medical / dengue.html.

[2] Indrawan. *Identify and Prevent Dengue Fever*. Pioner Jaya, London, 2001.

[3] *Department of Health. Development of Dengue Cases in Indonesia.* http://www.depkes.go.id. June 10, 2008.

[4] Mayor of Manado Circular No.4 of 2011, *On Poverty And Pemberantasa Dengue Haemorrhagic Fever (DHF).*

[5] Araujo MG, Costa IC, Dantas B, Maia S, Freitas BC. , 2006. *Effect of Stalk and Leaf Extracts From Euphorbiaceae Species On Aedes aegypti (Diptera, Culicidae) Larvae*. Rev. Inst. Med. Trop. S.Paulo. 48 (4): 211-214.

[6] Adebowale KO, CO Adedire. , 2006. *Chemical composition and insecticidal properties of the underutilized Jatropha curcas seed oil*. African J. Biotech. 5 (10): 901-906.

[7] Alali, F.Q., X.X. Liu and J.L. Mc. Laughlin. 1999. *Annonaceous Acetogenins*: Recent Progress. J. Nat. Prod. 62: 504-540

[8] Ratningsih N. , 2008. *Toxicity test molasses on respiration carp (Cyprinus carpio Linn.)* Journal Vol.6 No. Biotika. June 1. It. 22-33.