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RESEARCH ARTICLE

The effect of varied concentration of *Pandanus amaryllifolius roxb* as a natural larvacide on the mortality of *Aedes aegypti* instar III mosquito larvae

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Abstract

Dengue Hemorrhagic Fever (DHF) is a disease caused by Dengue virus infection. The use of synthetic larvicides causes side effects on human health. Efforts are made to use natural insecticides to minimize contact with dangerous chemical substances. Fragrant pandan leaf plants have secondary metabolite compounds including alkaloids, saponins, flavonoids, tannins and polyphenols, these secondary metabolite compounds can kill larvae. This study aims to determine the effect of variations in the concentration of *Pandanus amaryllifolius Roxb.* extract on the death of *Aedes aegypti* Instar III larvae. This type of research is experimental, with a Control Group Post-Test Design. The level of influence of concentration variations is seen from the Lethal Concentration (LC50) and Lethal Time (LT50) values. The data obtained were analyzed using a probit regression test or probit analysis to determine the effect of variations in the concentration of *P. amaryllifolius Roxb.* extract on the death of *A. aegypti* Instar III larvae and expressed as Lethal Concentration (LC50) and Lethal Time (LT50) with a confidence level of 95.0%, the probit test obtained the results of LC50-6 hours was 7.770%, LC50-8 hours was 6.516%, LC50-24 hours was 4.409%, LT50-2% is 31,973 hours, LT50-4% is 24,745 hours, and LT50-6% is 16,129 hours, from the parametric tests carried out the results were not normal and homogeneous, then the Kruskal-Wallis test was continued and showed a P-Value 0,000 (<0,05). So it can be said that there is effect of variations in the concentration of *P. amaryllifolius Roxb.* extract on the mortality of third instar *A. aegypti* larvae.

Keywords: Fragrant Pandan Leaves, Natural Larvacide, *Aedes aegypti*, Larvae.

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a disease caused by infection with the Dengue virus. This virus is transmitted through the bite of the *Aedes aegypti* and *Aedes albopictus* mosquito vectors which are commonly found throughout the world, especially in tropical areas, such as in Indonesia. Data from the Ministry of Health of the Republic of Indonesia in 2021 shows that there were 73,518 cases of dengue fever and the number of deaths due to this incident was 0.9% or the equivalent of around

705 cases. Around 43.25%, equivalent to around 31,796 of the total dengue fever cases in Indonesia, mostly occurred in the 5 to 14 year age group (Kemenkes, 2023).

The use of synthetic chemical larvicides is currently still a way for people to limit contact with *A. aegypti* mosquitoes. The use of synthetic larvicide cannot be separated from further problems, one of which is side effects on human health, besides that it is quite expensive and causes drug resistance in mosquitoes. To reduce this impact, efforts are made to use natural insecticides to minimize contact with dangerous chemical substances (Mallick et al., 2016). One type of plant that can be used as a natural larvicide is *P. amaryllifolius Roxb* extract (Utari et al., 2017).

According to Megbie, 2021 it was found that ethanol extract of *P. amaryllifolius Roxb* for 24 hours at a concentration of 4000 ppm (0.4%) could kill 9% of third instar *A. albopictus* larvae, while at a concentration of 12,000 ppm (1.2%) it could kills third instar *A. albopictus* larvae by 98% . According to Putri, 2019 it was found that the results of giving fragrant pandan leaf extract for 24 hours against *A. aegypti*, a concentration of 5% could kill 75% of *A. aegypti* larvae, while a concentration of 10% could kill 85% of *A. aegypti* larvae, and for the concentration 15% can kill *A. aegypti* larvae by 100%.

Based on this description, this research aims to determine the effect of variations in the concentration of *P. amaryllifolius Roxb* extract on the death of *A. aegypti* larvae at the third instar stage. The novelty of this research is that it uses varying concentrations, namely concentrations of 2%, 4%, 6%, and uses different mosquito samples, namely using third instar *A. aegypti* larvae.

MATERIALS AND METHODS

Materials

The preparation of materials needed in this research includes *P. amaryllifolius Roxb*, 96% ethanol, distilled water, third instar *A. aegypti* larvae, 1% Abate, and labels.

Data Collection Procedure

Data was collected based on the results of experimental tests obtained from the number of third instar *A. aegypti* larvae that died during 24 hours of observation in each treatment group there was control and *P. amaryllifolius Roxb* extract.

1. Extract preparation method

P. amaryllifolius Roxb extract by powdering *P. amaryllifolius Roxb* then soaking with 96% ethanol, covering the soaking container with black plastic to reduce the possibility of direct exposure to the sun, let stand for 3 x 24 hours, stirring occasionally. After 3 x 24 hours the soaking results were filtered 4 times using filter paper and a funnel until a solution was obtained from *P. amaryllifolius Roxb*. The solution of *P. amaryllifolius Roxb* was then concentrated using a rotary evaporator at a temperature of 60°C to obtain a thick extract of *P. amaryllifolius Roxb*. The concentrated extract of *P. amaryllifolius Roxb* obtained will be made into several concentration variants using distilled water. Then the ethanol extract of *P. amaryllifolius Roxb* was diluted to varying concentrations of 2%, 4% and 6%.

2. Natural larvicide trial on *Aedes aegypti* larvae

Prepare 25 plastic cup, each of the 5 plastic cups was labeled positive control, negative control, 2%, 4% and 6% concentration, then add 25 third instar *A. aegypti* larvae, in each group the test was repeated 5 times, make observations and count the number of larvae that die over 6, 8, and 24 hours.

Data Analysis Procedure

The data obtained from the deaths of *A. aegypti* larvae were then analyzed. Probit analysis was carried out to determine the LC and LT values using SPSS. The effectiveness of *P. amaryllifolius Roxb* extract seen from data analysis, starting with finding out whether the data distribution is normal or not using the Shapiro Wilk Normality test. Followed by the Levene Homogeneity test to determine

whether the data variants are homogeneous or not. This research uses more than 2 treatment groups so that, if the results of the data distribution are normal and the variance is homogeneous then the parametric test is continued with the One way ANOVA test, but if these conditions are not met then the non-parametric test is continued with the Kruskal Wallis test.

RESULTS AND DISCUSSION

Results

Research has been carried out with the results of data obtained from treatment of *(P. amaryllifolius Roxb)* extract with concentrations of 2%, 4%, 6%, using 2 control groups, namely negative control using aquadest and positive control using Abate. The number of larval deaths was calculated for 24 hours, observations and data recording were carried out over a period of 6 hours, 8 hours, and 24 hours. This study aims to determine the effect of administering *(P. amaryllifolius Roxb)* extract on the death of third instar *A. aegypti* mosquito larvae.

Table 1. Larvicide test results for *Pandanus amaryllifolius Roxb.* extract

			Number of Death							
Treatment	Time	Amount of *Aedes aegypti*	Replication					Total Larval Mortality	Average Larval Mortality	Percent Death of Larvae
			1	2	3	4	5			
Control (-)	6 hours	25	0	0	0	0	0	0	0	0%
Aquadest	8 hours	25	0	0	0	0	0	0	0	0%
	24 hours	25	0	0	0	0	0	0	0	0%
Control (+)	6 hours	25	11	11	11	12	12	57	11.4	45.6%
Abate 1%	8 hours	25	18	18	19	19	18	92	18.5	73.6%
	24 hours	25	23	23	23	24	23	116	23.2	92.8%
Concentration 2%	6 hours	25	1	1	2	1	2	7	1.4	5.6%
	8 hours	25	3	4	3	2	3	15	3	12%
	24 hours	25	7	9	9	8	8	41	8.2	32.8%
Concentration 4%	6 hours	25	3	3	5	4	5	20	4	16%
	8 hours	25	7	8	7	7	9	39	7.6	30.4%
	24 hours	25	11	13	13	11	12	60	12	48%
Concentration 6%	6 hours	25	8	8	7	8	8	39	7.8	31.2%
	8 hours	25	10	10	11	11	12	54	10.8	43.2%
	24 hours	25	15	15	15	16	15	76	15.2	64.80%

Mortality data from the effect of administering *P. amaryllifolius Roxb* extract in the negative control, positive control, concentrations of 2%, 4%, and 6% to 25 third instar *A. aegypti* larvae with observations for 6 hours, 8 hours, and 24 hours. In each treatment group, repetition was carried out 5 times. So the results obtained at 6 hours of observation were 0% in control (-), 45.6% in control (+), 5.6% in concentration 2%, 16% in concentration 4%, and 31.2% in concentration 6%. At 6 hour observation it was 0% in control (-), 73.6% in control (+), 12% at 2% concentration, 30.4% at 4% concentration, and 43.2% at 6% concentration. At 24 hour observation it was 0% in control (-), 92.8% in control (+), 32.8% at 2% concentration, 48% at 4% concentration, and 64.80% at 6% concentration.

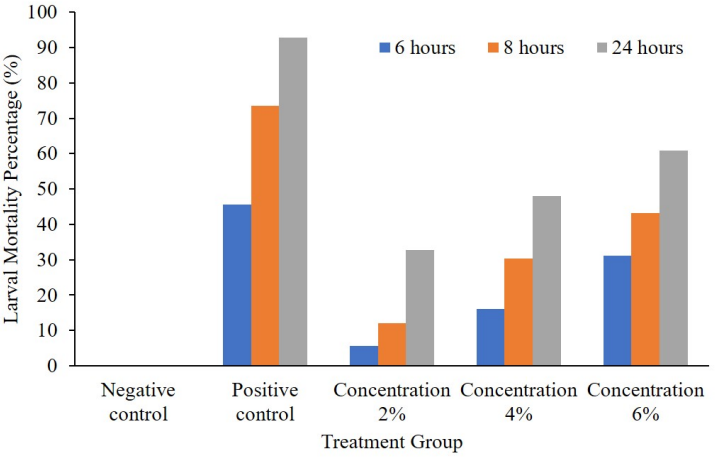

Figure 1. Percentage of death larvae (%)

Table 2. Results of probit analysis

Treatment	Mortality	Concentration	Level Trust	Lower Lower	Limit On
LC_{50} 6 hours time	50	7.770	95.0	6.756	9.845
LC_{50} 8 hours time	50	6.516	95.0	5.743	7.952
LC_{50} 24 hour time	50	4.409	95.0	3.674	5.329
LT_{50} concentration 2%	50	31.973	95.0	27.026	41.336
LT_{50} concentration 4%	50	24.745	95.0	20.516	33.015
LT_{50} concentration 6%	50	16.129	95.0	12.599	21.269

Table 3. Normality test result

Variable (group)	% death	P-Value	Information
Negative control	0%		
Positive control	92.8%	0.000	Not significant
Concentration 2%	32,8%	0.314	Significant
Concentration 4%	48%	0,119	Significant
Concentration 6%	64.80%	0.000	Not significant

Based on the results of the normality test using Shapiro-Wilk, the values obtained are as shown in table If the data is > 0.05, it can be assumed that the data is significant, but if the data is < 0.05, it is assumed that the data is not significant (Iskandar et al., 2017).

Table 4. Homogenity test result

Variable (group)	% death	P-Value	Information
Negative control	0%		
Positive control	70%	0.300	Homogeneous
Concentration 2%	16.8%	0.649	Homogeneous
Concentration 4%	31.73%	0.944	Homogeneous
Concentration 6%	45.06%	0.253	Homogeneous

Based on the results of the homogeneity test using Levene, the values obtained as shown in the table are obtained. If the results obtained are P-Value > 0.05, it can be assumed that the data is homogeneous (Kasma et al., 2018).

Table 5. Kruskal-wallis test result

Variable (group)	P-Value	Information
Negative control Positive control Concentration 2% Concentration 4% Concentration 6%	0.000	Significant

Based on the results of the Kruskal-Wallis statistical test, it is known that the effect of variations in the concentration of fragrant pandan leaf extract shows a significant value of p < 0.05. Therefore, it can be concluded that there is an influence of variations in the concentration of *P. amaryllifolius Roxb.* extract on the mortality of third instar *A. aegypti* larvae. So the results show that H1 is accepted and H0 is rejected (Garcia et al., 2018).

Discussion

Pandanus amaryllifolius Roxb is a type of monocotyledonous plant from the Pandanaceae family. In Indonesia, most *P. amaryllifolius Roxb* are used as food coloring, room freshener, food fragrance, medicine and also as a raw material for handicrafts. According to the results of the determination carried out by UPT Materia Medica Batu in Okta, 2018. In the research, old pandan leaves were used because the antioxidant content, especially flavonoids, is highest in old leaves. Based on research conducted by Aisyah, 2015 the *P. amaryllifolius Roxb* plant has secondary metabolite compounds including alkaloids, saponins, flavonoids, tannins and polyphenols. The tannins in larvicide act as poison in the larvae's digestion. Apart from that, the bitter taste of tannins can make larvae refuse food and lead to hunger and death (Purnamasari et al., 2017). Meanwhile, the flavonoid compounds in larvicides act as toxins in the larvae's respiration. This compound works by entering the larva's body through the respiratory system, which will then cause weakness in the nerves, as well as damage to the respiratory system which will result in the larva not being able to breathe and causing the larva to die. And saponins can damage cell membranes and disrupt the metabolic processes of larvae, which can cause death of larvae.

This research is a pure experimental research (true experimental research) with a control group design with a post-test (control group post-test design) to group and provide treatment to samples of third instar *A. aegypti* larvae. This research uses *A. aegypti* mosquito larvae as research subjects. *A. aegypti* mosquito larvae were treated with various concentrations of *P. amaryllifolius Roxb* extract consisting of concentrations of 2%, 4% and 6% for 6 hours, 8 hours and 24 hours. These results have been presented in tabular form.

CONCLUSIONS

From the results of research regarding the influence of variation concentration of *Pandanus amaryllifolius Roxb.* extract as a natural larvicide on the mortality of third instar *Aedes aegypti* mosquito larvae can be concluded that: *P. amaryllifolius Roxb* extract has an effect as a larvicide experience to mortality larvae mosquito *A. aegypti* instar III which is proven with mark Sig. 0.003 (< 0.05). The concentration of *P. amaryllifolius Roxb* extract has an effect on the death of *A. aegypti* larvae with an LC value of 50 -6 hours at this concentration. 7.770%, LC 50 -8 hours on concentration 6.516%, and at LC 50 -24 hours on concentration 4.409%. The timing of *P. amaryllifolius Roxb* extract has an effect to death larvae *A. aegypti* obtained mark LT 50 - 2% is 31,973 hours, LT 50 - 4% is 24,745, and LT 50 - 6% is 16,129 hours. As for suggestion Which given by researcher, that is need

done study more carry on about test influence variation concentration *P. amaryllifolius Roxb* extract as a natural larvicide against the mortality of *Aedes aegypti* mosquito larvae. In future research, plant parts such as flowers/roots from *P. amaryllifolius Roxb* can be used.

Author contributions

Dira Deviyani: Conceptualization, writing draft, writing review dan editing; Yauwan Tobing Lukiyono, Rahayu Anggraini, Ary Andini: Data curation, formal analysis.

Conflict of Interest

There is no conflict of interest in this study.

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Data availability

We thank all respondents involved in this research project.

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