Larvacide effectiveness of Papaya leaf extract (*Carica papaya*) on the mortality of larvae vector of Dengue hemorrhagic fever caused by *Aedes aegypti*

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Abstract. Dengue Hemorrhagic Fever (DHF) is an infection caused by the dengue virus, which is still a public health problem in Indonesia. Chemical insecticides as larvicides used to control *Aedes aegypti* have created resistant populations. Thus, higher doses are needed, which, of course, have toxic effects on animals, humans, and the environment. To analyze the effectiveness of papaya leaf extract (*Carica papaya*) against the mortality of the third instar *Aedes aegypti* mosquito larvae. This study is a true experimental research design with a posttest-only control group design. In this study, the groups in this study were the larvae of the *Aedes aegypti* mosquito instar III / IV, papaya leaf ethanol extract, abate as a positive control group, and aquadest as a negative control. The results of the mean number of larvae deaths in each treatment group was zero (0) larvae in negative control group, positive control group (25 larvae), 5% concentration (9.5 larvae), 10% concentration (11.75 larvae), 15% concentration (12.75 larvae), a concentration of 20% (14.75 larvae), and a concentration of 25% (19.5 larvae). The results of Kruskal Wallis analysis showed that papaya leaf extract was effective as a larvicide for *Aedes aegypti* (*p* = 0.001). In the probit analysis, it was found that the LC50 of the extract against *Aedes aegypti* was 23%, while the LC99 was 55%. Papaya leaf extract is effective as a vegetable larvicide for 3rd / IV instar *Aedes aegypti* larvae.

Keywords: papaya leaf extract, Aedes aegypti, larvicide

1. Introduction
Dengue Hemorrhagic Fever (DHF) is an infection caused by the dengue virus. Dengue virus is found in tropical and sub-tropical areas, mostly in urban and suburban areas of the world. The tropical climate in Indonesia is very suitable for the growth and development of various diseases, especially diseases carried by vectors. The *Aedes aegypti* mosquito is a vector of Dengue Hemorrhagic Fever and has a significant role in disease transmission in Indonesia. Until now, Dengue Hemorrhagic Fever is one of the public health problems in Indonesia that can cause Extraordinary Events that can lead to death.[1]

According to a World Health Organization (WHO) report in 2008, the highest number of DHF sufferers is in the Western Pacific, Southeast Asia, and several countries in America. The number of cases was recorded at more than 1.2 million in 2008 and then increased to more than 3.2 million in 2015. Even in 2016, dengue outbreaks occurred in various parts of the world.[2]

According to the 2018 Kupang City Health Profile, the DHF morbidity rate in Kupang City in 2014-2018 fluctuated, wherein 2014 it was 26.60/100 000 of populations, increased in 2017 to 32.00/100 000 of population and then increased in 2018 to 56.00/100 000 population. In 2020 Sikka Regency was still the area with the highest number of cases, namely 1,292 cases or 38% of cases in NTT occurred in Sikka Regency, so that the status of Extraordinary Events was determined in the area. [4,5]

Mosquito vector control is urgently needed with the hope that it will impact reducing the population of the *Aedes aegypti* mosquito vector so that it is no longer significant as a disease
transmitter. Various vector control methods have been applied; such as chemical control using pesticides that are widely used in the form of malathion intended to eliminate adult mosquitoes and temephos, which are intended to kill larvae, then biological control is by utilizing larvae-eating animals and eradicating mosquito nests by the people themselves in their respective neighborhoods with 3M plus. The most often used control is chemical control because it is considered to work more effectively and the results can be seen quickly compared to biological control. Control is done by killing the larvae of the vector to break the chain of transmission by using abate (temephos). Abate (temephos) is a class of pesticides used to kill insects in the larval stage. However, the use of chemical larvicides used to control Aedes aegypti has created a population that is resistant to it, so higher doses are needed, which of course have toxic effects on animals, humans and the environment.[6,7,8,9]

Therefore, it is necessary to make an effort to obtain alternative larvicides, namely by using natural larvicides. Natural larvicides are larvicides made from plants that are toxic to insects at the larval stage. Natural larvicides are expected to have no side effects on the environment and humans and do not cause resistance to insects. One type of plant that has potential as a source of natural larvicides is papaya leaf (Carica papaya).[10]

The papaya plant is a tropical plant that is easy to grow in tropical areas such as Indonesia. Papaya leaves contain active ingredients such as papain enzymes, saponins, flavonoids, alkaloids and tannins, which can be used as larvicides to kill mosquito larvae. These compounds cause various reactions in the larval body to interfere with the growth and development of the larvae. Research conducted by La Taha and Nur Inang on the ability of papaya leaf extract to kill Aedes aegypti and Culex Sp. mosquito larvae in 2018 showed that papaya leaf extract (Carica papaya) as a larvicide was able to kill Aedes aegypti mosquito larvae in the third instar with the concentration of 15% and 20%. In addition, at the time of observation for 12 hours and it was able to kill the larvae of Culex sp mosquitoes in the third instar with a concentration of 20% at the time of observation for 12 hours.[11]

Another study was conducted by Jonathan Payangka et al. about the effect of papaya leaf extract on the mortality of Aedes aegypti mosquito larvae instar III in 2019. The results of this study showed that papaya leaf extract (Carica papaya) at several concentrations affected the mortality of Aedes aegypti larvae instar III with the lowest effect was at a dose of 0.5%, namely 4% and the highest at 2.5%, namely 85%.[12]

2. Materials and Methods

This research was true experimental with a post-test only control group design. The groups in this study were Aedes aegypti mosquito larvae instar III/IV, papaya leaf ethanol extract concentration, abate as a positive control group and aquades as a negative control.

This study used seven groups consisting of five treatment groups with papaya leaf extract, one negative control group which were only given water and one positive control group using abate. After being given treatment, the results of each group were analysed.

2.1 Preparing Papaya Leaf Extract

The papaya leaves used in this study were collected from the Kupang City area. Papaya leaves were collected and sorted then washed thoroughly using running water to clean papaya leaves from adhering dirt, after which they are dried by aerating. The dried papaya leaves are mashed using a blender until they become powder. Papaya leaf powder is then macerated by soaking it in 1 litre of 70% ethanol solvent, then stirring and closing it tightly. The marinade was allowed to stand for 3x24 hours but still stirred every day. After that, separate the pulp and filtrate by filtering it using filter paper to obtain a liquid extract of papaya leaves. The obtained filtrate was evaporated at 40°C to obtain papaya leaf extract.

2.2 Phytochemical Tests

2.2.1. Alkaloid test
Papaya leaf extract was put into test tubes A and B, then added 10 drops of Meyer's reagent and 10 drops of Wagner's reagent were added to tube B. The test is positive if a white to yellowish precipitate is form with Meyer's reagent and a reddish-brown precipitate is form with Wagner's reagent [12]

2.2.2 Saponin test
Papaya leaf extract was put into a test tube and then added 20 drops of hot water. The sample was shaken in a vertical direction for 10 seconds. The formation of a stable foam identified the presence of saponin compounds in the sample and with the addition of one drop of 1% hydrochloric acid the foam remained stable.

2.2.3 Flavonoid test
2 ml of papaya leaf extract was put into a test tube and added with 0.5 ml of concentrated hydrochloric acid (concentrated HCl) and magnesium metal (Mg). The presence of flavonoids is indicated by red, orange, and green colours depending on the structure of the flavonoids contained in the sample.

2.2.4 Tannin test
Papaya leaf extract was dissolved in 10 ml of distilled water and then filtered. The filtrate was added with 3 drops of FeCl₃. If the test solution forms a strong black green or blue-black colour means the test extract is positive for tannin compounds.

2.3 Treatment Solution Preparation
The treatment solution used in the study had a higher concentration than the Concentration to be used, which was 100% made by 100 grams of papaya leaf extract added with distilled water until the volume was 100 ml.

For the positive control group, the Concentration of abate (temephos) was used based on the effective dose of abate. The effective dose of abate is the dose of abate that can kill 100% of Aedes sp mosquito larvae, which starts from 1 gr/10L of water. The available abate preparations are 1 gr/10L of water, so the required concentration is 100ppm.[13,14]

2.4 Treatment Stage
Prepared 100 ml container as many as 28 pieces. The papaya leaf extract was added into the containers according to the concentration that has been distributed. Subsequently, distilled water was added into the container until it reached 100 ml. For each container, 25 Aedes aegypti larvae instar III/IV were added. Observations were made at the 24th hour for four times according to the number of repetitions.[15]

2.5 Data Analysis
Primary data was obtained from the observation of the number of Aedes aegypti mosquito larvae that died at 24 hours, both the positive control group, the negative control group and the papaya leaf extract group. Data is recorded and evaluated by ANOVA One Way followed by the post hoc test Least Significant Difference (LSD). The values obtained were considered significant at p ≤ 0.05.LC₅₀ calculations was used EPA Probit Analysis Software Program version 1.5. In the User's guide supplied by the EPA, it stated that this program could calculate the estimated value of LC/EC₅₀ with an interval of 95% confidence.

3. Results
Phytochemical tests were carried out to determine the secondary metabolites that were successfully withdrawn by the solvent used. The results of the phytochemical test are shown in table 1.

From table 2 above, it was notable that the treatment group with the highest number of mortality of Aedes aegypti larvae instar III/IV was K2, namely the positive control group that was given abate with the number of dead larvae of 100% (25) and the negative control group that used aqauades found no mortality of the larvae. For the treatment group using papaya leaf extract, the highest mortality in K7 was 68%. At the same time, the group with the lowest mortality was K3 which was
16%. From the data above, it could be assumed that for each treatment group, there was an increase in the number of larval mortalities along with the increase in the Concentration of the treatment.

**Table 1. Phytochemical Test Results.**

| Secondary metabolite | Result |
|----------------------|--------|
| Alkaloids            | +      |
| Saponins             | +      |
| Tannins              | +      |
| Flavonoids           | +      |

Note:
Alkaloids: The test is considered to be positive if a white to yellowish precipitate is formed with Meyer reagent and a reddish-brown precipitate with Wagner reagent.
Saponins: The test is said to be positive if a stable foam is formed which identifies the presence of saponin compounds in the sample and with the addition of one drop of 1% hydrochloric acid the foam remains stable.
Tannins: The presence of flavonoids is indicated by red, orange and green colors depending on the structure of the flavonoids contained in the sample.
Flavonoids: The test solution formed a strong black green or blue black color, then the test extract was positive for tannin compounds.

**Table 2. Number of dead larvae after 12 hours of treatment.**

| Replication | Groups | K1 | K2 | K3 | K4 | K5 | K6 | K7 |
|-------------|--------|----|----|----|----|----|----|----|
| I           |        | 0  | 25 | 5  | 5  | 8  | 10 | 18 |
| II          |        | 0  | 25 | 3  | 7  | 7  | 12 | 16 |
| III         |        | 0  | 25 | 4  | 6  | 10 | 11 | 17 |
| IV          |        | 0  | 25 | 4  | 7  | 9  | 14 | 17 |
| Sum         |        | 0  | 100| 16 | 25 | 34 | 47 | 68 |
| Mean        |        | 0  | 25 | 4  | 6.25| 8.5| 11.75| 17 |
| Percentage  |        | 0  | 100| 16 | 25 | 34 | 47 | 68 |

*Note:*
K1 = Negative Control Group (water)
K2 = Positive Control Group (abate)
K3 = 5% Papaya Leaf Extract Group
K4 = 10% Papaya Leaf Extract Group
K5 = 15% Papaya Leaf Extract Group
K6 = 20% Papaya Leaf Extract Group
K7 = 25% Papaya Leaf Extract Group

From table 3, it was found that the treatment group with the highest number of deaths of *Aedes aegypti* larvae instar III/IV was K2, namely the positive control group that was given abate with the number of larvae that died 100% (25) and the negative control group that used distilled watershow no mortality of the larvae. For the treatment group using papaya leaf extract, the highest mortality in K7 was 78%. While the group with the lowest mortality was K3 which was 38%. From the data above, it can also be found that for each treatment group, there was an increase in the number of larval deaths along with the increase in the concentration of the treatment.

Based on table 4, it is shown that the normality test shows that the significance value is 0.104 ($p > 0.05$), it can be concluded that the data is normally distributed. From table 5, it can be seen that
the p-value of the data is <0.05 therefore, H0 is rejected and H1 is accepted. It can be concluded that papaya leaf extract is effective as a larvicide on Aedes aegypti mosquito larvae.

From table 6, it can be seen that the p-value of the data is p<0.05, and each group is significantly different compared to the negative control group, namely the group using distilled water.

| Table 3. Number of dead larvae after 24 hours of treatment. |
|---|---|---|---|---|---|---|---|
| Replication | Groups | |
| | K1 | K2 | K3 | K4 | K5 | K6 | K7 |
| I | 0 | 25 | 10 | 11 | 13 | 16 | 19 |
| II | 0 | 25 | 9 | 12 | 14 | 19 | 20 |
| III | 0 | 25 | 11 | 12 | 14 | 15 | 20 |
| IV | 0 | 25 | 8 | 11 | 12 | 14 | 19 |
| Sum | 0 | 100 | 38 | 47 | 51 | 59 | 78 |
| Mean | 0 | 25 | 9.5 | 11.75 | 12.75 | 14.75 | 19.5 |
| Percentage | 0 | 100 | 38 | 47 | 51 | 59 | 78 |

Note:
K1 = Negative Control Group (water)
K2 = Positive Control Group (abate)
K3 = 5% Papaya Leaf Extract Group
K4 = 10% Papaya Leaf Extract Group
K5 = 15% Papaya Leaf Extract Group
K6 = 20% Papaya Leaf Extract Group
K7 = 25% Papaya Leaf Extract Group

| Table 4. Kolmogorov-Smirnov Normality Test Results. |
|---|---|---|
| P value | Interpretation |
| Number of Larvae Mortality based on Extract Concentration | 0.104* | Normal distribution |

*p > 0.05 = Normal Distributed Data

| Table 5. Kruskal Wallis Test Results. |
|---|---|---|
| Extract concentration | Mortality Mean | P |
| Negative Control | 0 | |
| Positive Control | 25 | |
| 5% Concentration | 9.5 | 0.001* |
| 10% Concentration | 11.75 | |
| 15% Concentration | 12.75 | |
| 20% Concentration | 14.75 | |
| 25% Concentration | 19.5 | |

*p < 0.05

| Table 6. Mann Whitney Test Results. |
|---|---|---|
| Extract Concentration | Mortality Mean | P |
| Number of Larva | K1 VS K2 | |
| 0.008* |
Based on table 7, the results of the probit analysis show that the LC\textsubscript{50} value is 23%, which means that a papaya leaf extract concentration of 23% is needed to kill 50% of the larval population. Then from the results of the probit analysis, the LC\textsubscript{99} value was 55%, which means that a papaya leaf extract concentration of 55% was needed to kill 99% of the larval population.

**4. Discussions**

From the normality test results using the Kolmogorov-Smirnov, it was found that the data were normally distributed. However, in the homogeneity test using the Levene test, it was found that the data were not homogeneous. Because one of the one-way ANOVA bivariate test requirements is not fulfilled, the Kruskal Wallis alternative test is carried out. From the results of the Kruskal Wallis test, it was found that the p-value of the data was <0.05, where there was a significant difference between the Concentration of papaya leaf extract and the number of larval deaths. Then the Mann Whitney test was used to compare the negative control group, namely those using distilled water with the positive control group and the papaya leaf extract group. The results showed that the p-value of the data was <0.05, and each group was significantly different compared to the negative control group, namely the negative control group, which uses distilled water.

Lethal concentration measures the toxicity of a type of insecticide, which is determined based on the number of deaths of *Aedes aegypti* larvae at each Concentration.[24] The LC\textsubscript{50} value is the concentration of the test solution that causes 50% death in test animals, while the LC\textsubscript{99} value is the concentration of the test solution that causes death 99% mortality in test animals. The results of the probit analysis in this study showed an LC\textsubscript{50} value of 23%. This shows that it takes a concentration of papaya leaf extract with a value of 23% to kill 50% of the larval population. While the LC\textsubscript{99} value is 55%, which means that it takes a concentration of papaya leaf extract with a value of 55% to kill 99% of the larval population. The lower the lethal concentration value of a substance indicates that the substance has larvicidal solid activity. This is because these substances need much lower concentrations to kill test animals at the same time.[24]

Based on the results of data analysis, it could be suggested that papaya leaf extract has a significant effect on the death of *Aedes aegypti* mosquito larvae that can be seen from current observations. Dead larvae have characteristics that do not move when touched using a pipette, larvae body is a white or pale yellow and elongated body shape.[25] This is due to the larvicidal effect of papaya leaf extract (*Carica papaya*), which influences the content of secondary metabolites in it. It can be seen from the results of phytochemical tests conducted that papaya leaf extract contains secondary metabolites such as alkaloids, flavonoids, saponins and tannins.

In this study, we increased the dose of papaya leaf extract to 25%, where at an increase of 25%, an increase in the number of larval deaths was found. The description of the results when compared with previous studies that used the highest dose of 20%, it can be concluded that the higher the dose of papaya leaf extract, the higher the number of larval deaths.
5. Conclusions
The results of the probit analysis in this study showed LC90 value of 23%. This demonstrated that it takes a concentration of 23% to kill 50% of the larval population. While the LC99 value is 55%, which means that it takes a concentration of 55% papaya leaf extract to kill 99% of the larval population. The lower the lethal concentration value of a substance indicates that the substance has strong larvicidal activity. This is because these substances need much lower concentrations to kill test animals at the same time. Therefore, papaya leaf extract has indicated effective as a larvicide towards the mortality of larvae vector of dengue hemorrhagic fever Aedes aegypti.

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