



# The Role of Mango (*Mangifera indica*) Leaves as Biolarvicide on *Aedes aegypti* Larva Mortality

Chairunnisah J. Lamangantjo<sup>1,\*</sup>, Lindasari Pakaya<sup>1</sup>, Jusna Ahmad<sup>1</sup>, Mustamin Ibrahim<sup>1</sup>, Syam S Kumaji<sup>1</sup>, and Nurul Fajryani Usman<sup>1</sup>

Biology Department, Faculty of Mathematics and Natural Sciences, 96554. Province Gorontalo, Indonesia

Corresponding author. [chairunnisah@ung.ac.id](mailto:chairunnisah@ung.ac.id)

**Abstract.** This study aims to know the effect of *M. indica* leaf extract on mortality of *A. aegypti* larvae, know the difference in the impact of

*M. indica* leaf extract on the mortality of *A. aegypti* larvae, know the 24-hour LC50 value of *M. indica* leaf extract, know the content of secondary metabolites of *M. indica* leaf feeling. This study uses an experimental method with a completely randomized design of 6 treatments and four repetitions. Each treatment contains 10 *A. aegypti* larvae test animals. The research data were analyzed using ANAVA and Duncan's test and probit analysis. The results showed that the results of the ANAVA analysis test showed the effect of treatment on the mortality of *A. aegypti* larvae ( $p < 0.05$ ), and the Duncan test showed differences between treatments on mortality of *A. aegypti* larvae; the concentration with the highest mortality was 80%. Based on the LC50 (Lethal Concentration) test, 60.675% of *M. indica* leaf extract can kill 50% of *A. aegypti* larvae within 24 hours. *M. indica* leaf extract contains steroids, saponins, flavonoids, tannins, and alkaloids. Based on the results, it is known that *M. indica* leaf extract can be used as a biocide against *A. aegypti*.

**Keywords:** *Mangifera indica*, liolarvicide, larva mortality

## 1 Introduction

Gorontalo is an area with a tropical climate which causes the emergence of various tropical diseases, one of which is carried by mosquitoes. As a vector, mosquitoes play a role in transmitting several diseases including filariasis or elephantiasis which is transmitted by *Culex* sp mosquitoes infected with filarial worms, malaria which is caused by the bite of *Anopheles* mosquitoes which are infected with the plasmodium parasite and dengue hemorrhagic fever (DHF) which is caused by mosquito bites type *A. aegypti*.

Dengue hemorrhagic fever (DHF) is a disease caused by a virus dengue and is transmitted through mosquito bites *A. aegypti* female who are infected with the virus dengue in his body [1]. DHF is an infectious disease that is still endemic in Indonesia [2]. In Gorontalo, based on data from the Gorontalo Province Health Service (Dinkes), until January 2019, dengue fever reached 329 cases. This case

is quite high, this is caused by changes in housing which have an impact on the environment, such as sewers that are difficult to flow due to the large amount of rubbish strewn about, stagnant rainwater, thus increasing the mosquito population *A. aegypti*.

Eradicating dengue hemorrhagic fever is basically carried out using the method commonly used in eradicating infectious diseases, namely by controlling the mosquito population as a vector of transmission [3]. The most common method used by the public to control the Dengue Hemorrhagic Fever vector is to use insecticides which function as larvicides. However, the continued use of chemical larvicides can cause various new problems, including environmental pollution such as water pollution and also insect resistance to larvicides [4]. Therefore, natural larvicides originating from plants or known as biolarvicides are needed. One plant that has the potential to act as a biolarvicide is mango (*M. indica*). The part of the mango that can be used as a biolarvicide is the mango leaf.

The use of mango leaves as a biolarvicide has been carried out by several researchers. Mango leaves contain secondary metabolite compounds consisting of several compounds, namely flavonoids, saponins, gallic tannins, catecate tannins, quinones, steroids or triphenoids and mangiferin (C-glucoxanthones) [5], which can inhibit the nervous system and respiratory system and cause death in the larva. On the other hand, mango leaves can be used as a biolarvicide because mango leaves are relatively safe for humans and other organisms and the environment and are also easy to obtain.

## 2 Method

### 2.1 Place and Time of Research

This research was conducted at the Zoology Laboratory of the Faculty of Mathematics and Natural Sciences and the LPPT-UGM testing laboratory. This research was carried out from February to March 2023.

### 2.2 Research Design

The method used in the research is a laboratory experimental method. The research used was a Completely Randomized Design (CRD). Based on [6], it can be used as a reference for 6 treatments and 4 repetitions with the concentrations used being 50%, 60%, 70%, 80%, 0% (negative control) and 0+% (positive control) as controls with each -Each treatment used 10 third instar *Aedes aegypti* test larvae.

### 2.3 Research Procedures

**Preparation of mango leaf extract (*M. indica*)** *Mangifera indica* leaves were washed with water until clean. then air dried to reduce the water content contained in the leaves. After drying, let it dry, cut it into small pieces, then grind it using a mortar and pestle. After that, the fine *M. indica* leaves are squeezed and filtered using a sieve into a 100 ml beaker.

**Preparation of test animals** The III instar *A. aegypti* mosquito larvae were obtained from mosquito farms in Limboto and then kept for acclimatization in the laboratory environment for 2 days before carrying out the research.

**Application of *M. indica* leaf juice to *A. aegypti* mosquito larvae** Prepare 24 petri dishes then add 25 ml of mango (*Mangifera indica*) leaf juice in each concentration, namely, 50%, 60%, 70%, 80%. Each petri dish contained 10 *Aedes aegypti* larvae and then observed every 3 hours for 1 x 24 hours. At each observation time, the number of dead larvae from each petri dish was counted based on the criteria that dead larvae were no longer moving or did not respond to any stimulation.

## 2.4 Data Analysis

The data analysis used was ANOVA to determine the effect of treatment on the mortality of *A. aegypti* larvae, and continued with the Duncan test if there was a significant difference. Data analysis using SPSS 16.0 and Microsoft Excel applications. The statistical hypothesis in this research is as follows: H0: The juice of *M. indica* leaves has no effect on the mortality of *A. aegypti* larvae. H1: The juice of *M. indica* leaves affects the mortality of *A. aegypti* larvae.

If  $F_{count} > F_{table}$  then H0 is rejected and H1 is accepted so that the conclusion is that there is an influence of *M. indica* leaf juice. If  $F_{count} \leq F_{table}$  then H0 is accepted and H1 is rejected, so the conclusion is that there is no effect of *M. indica* leaf juice.

Probit analysis is used to determine the 24 hour LC50 value (Lethal Concentration 50%) The 24 hour LC50 aims to determine the optimum concentration that can kill 50% of *A. aegypti* larvae within 24 hours.

## 3 Result and Discussion

### 3.1 Result

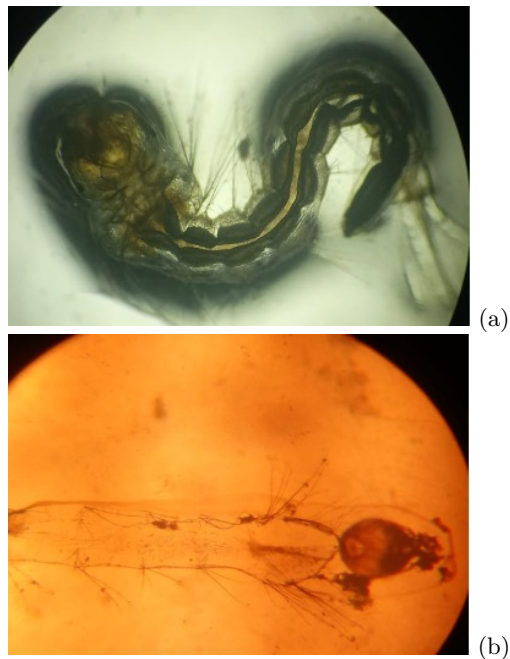
Mortality data for *A. aegypti* larvae after being fed *M. indica* leaves for 24 hours can be seen in table 1 below.

**Table 1.** Larval mortality *A. aegypti* for 24 hours after being given leaf juice *M. indicataes*

Concentration	Mortality	Average Mortality	Percentage (%)
P- (Negative control)	0	0a	0%
P+ (Positive control)	40	10b	100%
P1 (50%)	10	2,5c	2,5%
P2 (60%)	21	52,5d	52,5%
P3 (70%)	37	92,5b	92,5%

The average value followed by different letters shows a significant difference based on the Duncan test at the level ( $p \leq 0.05$ )

After analyzing the mortality data for *A. aegypti* larvae, it was discovered that the larvae experienced an average of 10 deaths or mortality or 100% mortality in P+ (positive control). In P- (negative control), there was no death of *A. aegypti* larvae. Treatments P1, P2 and P3 showed an average death of 2.5 *A. aegypti* individuals, 52.5 individuals and 8 individuals, while treatment P4 showed an average mortality of 92.5 individuals. This average mortality is based on giving *M. indica* leaf flavoring within 24 hours, as shown in Table 1. Anova analysis shows a significant difference ( $p \leq 0.05$ ), which means that there is an influence of leaf pressing on the mortality of *A. aegypti* larvae shown in figure 1a and 1b.



**Fig. 1.** Larva *A. aegypti* before (a) and after (b) giving *M. indica* leaf juice. Arrows show differences in the integument system after treatment

Next, Duncan's further test was carried out to determine the differences in the effect of each treatment. The Duncan test carried out is based on the confidence value  $\alpha = 0.05$ . Based on Table 1, it is known that the P- (distilled water) treatment was significantly different from all treatments. The 50% treatment was significantly different from the P- control treatment (aquades), 60%, 70%, 80%, and P+ control (abate). The 60% treatment was significantly different from the 70%, 80%, P- (aquades) treatment, and P+ (abate) control. The 70%

treatment was significantly different from the P- (aquades), 80%, and P+ (abate) control. While the 80% treatment and P+ (abate) control were not significantly different, they were significantly different from the P- (distilled water), 50%, 60%, and 70% treatments. It was found that each of them was significantly different ( $p < 0.05$ ) or there was a difference in the treatment of *M. indica* leaf extract on the mortality of *A. aegypti* larvae.

The probit analysis carried out to determine LC50 for 24 hours can be seen in Table 2 below.

**Table 2.** 24 Hour LC50 value of *A. aegypti* larvae

Lethal Concentration	Concentration		
	Estimation	Lower limit	Upper limit
LC50	60,678	25,220	68,916

From Table 2, the 24 hour LC50 value of larvae *A. aegypti* of 60.678%, meaning to kill 50% of the larvae *A. aegypti* within 24 hours the concentration of leaf juice is needed *M. indicata* amounting to 60.678%.

**Table 3.** The content of secondary metabolites noticed in the leaves *M. indica*

Secondary Metabolites	Result
Quantitative Steroids	189,92 µg/g
Total Saponins	0,29% b/v
Total Tannin Equivalent Tannic Acid (Quantitative)	4,55% b/v
Total Alkaloids	33,41 mg/L
Total Flavonoids	1,05% b/v

### 3.2 Discussion

Based on the research results, it was found that leaf juice *M. indicata* influence on larval mortality *A. aegypti* with significant differences between treatments. In the negative control treatment there were no deaths or mortality because this treatment only contained larvae and distilled water, the control position contained leaf felt. *M. indicata* shows 100% mortality. Treatments P1 (50%), P2 (60%), P3 (70%), P4 (80%) respectively showed the lowest to highest average mortality rates. The results showed that there was an increase in larval mortality *A. aegypti* along with increasing concentration, which means leaf concentration *M. indicata* on mortality *A. aegypti*. It is known that the higher the leaf concentration *M. indicata* used, the number of dead larvae increases. This is based on increasing mortality *A. aegypti* at its highest concentration after 24 hours.

Research [7] shows the highest death rate for Culex Sp mosquitoes at a concentration of 3% and the lowest at a concentration of 1% given *Ananas comosus*

skin extract. In line with research conducted [8], Zodia (*Evodia suaveolens*) leaf extract resulted in 10% mortality at a concentration of 0.04 gr/ml and 80% mortality at a concentration of 0.25 gr/m. By increasing the concentration, the amount of active compounds such as flavonoids and alkaloids in the extract also increases. This causes the accumulation of active compounds in *A. aegypti* larvae to become greater, which in turn causes an increase in the number of dead larvae [9].

The results of tests on the mortality of *A. aegypti* larvae after administration of *M. indica* leaf juice with different concentrations, experienced mortality which was indicated by when touched they did not move and appeared to turn white. Mortal or dead *A. aegypti* larvae will move when touched, be at the bottom, and will turn pale [10]. Figure 1 shows the differences in the integument layer of *A. aegypti* larvae. In line with research [11] showing differences in the integument of *A. aegypti* larvae before treatment, the integument layer still appeared to consist of two layers (Figure 1a, shown by an arrow) whereas after treatment, namely administration of *M. indica* leaf juice, only one layer of integument remained (Figure 1b) indicated by arrows).

In the research conducted, it was found that the 24-hour LC50 value of *A. aegypti* larvae was 60.678%, meaning that to kill 50% of *A. aegypti* larvae within 24 hours, a concentration of *M. indica* leaf juice was needed of 60.678%. Compared with previous studies, with mango skin extract [2] and mango seeds [12] the results of this study showed lower potency. This indicates that mango peel and mango seed extracts are more effective in killing *A. aegypti* larvae compared to the *M. indica* leaf extract used in this study.

The death of *A. aegypti* larvae is caused by secondary metabolites found in *M. indica* leaf juice. In Table 3, based on phytochemical analysis, *M. indica* leaf juice contains steroids, saponins, tannins, alkaloids and flavonoids. Steroids are large molecules that have a chemical structure almost the same as triterpenoids. Steroids can bind free sterols in the digestive tract, where sterols work as precursors of the hormone ecdysone (skin molting). By decreasing sterols, it can affect the skin molting process in insects, making it difficult for larvae to develop to the next stage, namely pupae [13].

Saponins have the ability to damage membranes. Saponin compounds can disrupt the lipid layer of the epicuticle and the protein layer of the endocuticle, making it easier for toxic compounds to enter the body [13]. Saponin works by damaging the larval cell membrane, destroying the wax layer which functions as a protective body for the larvae, and disrupting the larval methanolysis process [14].

Tannin is a secondary metabolite compound that is thought to have larvicidal properties. Tannin works by reducing the activity of protease and amylase enzymes so that the larvae's ability to digest food decreases [14]. Yunita et al [15] added that tannin has a bitter taste which causes *A. aegypti* larvae not to want to eat so that the larvae will starve and eventually die. Flavonoids attack several vital organs and nerves in insects, resulting in weakening of the nerves, especially in important organs such as respiration, which ultimately causes the

death of the insect. Its function is as a respiratory inhibitor, namely inhibiting or reducing the rate of chemical reactions in the respiratory process. In addition, flavonoids also interfere with energy mechanisms in mitochondria by inhibiting the electron transport system [8].

## 4 Conclusion

Based on the results, it can be concluded that the feeding of *M. indica* leaves influences the mortality of *A. aegypti* larvae. A concentration of 60.678% is known to kill 50% of *A. aegypti* larvae within 24 hours. The leaves of *M. indica* contain steroids, saponins, tannins, alkaloids and flavonoids.

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