

The Effect of Mango (*Mangifera indica*) Peel Extract on *Aedes aegypti* Larvae Mortality

Sisi Ananda¹, Delima Engga Maretha^{2*}, Asnilawati³, Nurhaida Widiani⁴

^{1,2,3} Universitas Islam Negeri Raden Fatah Palembang, Indonesia

⁴ Universitas Islam Negeri Raden Intan Lampung, Indonesia

ARTICLE INFO

Article History

Received : 19-04-2023

Accepted : 16-06-2023

Published : 30-06-2023

Keywords:

Aedes aegypti Larvae; Dengue Fever Hemorrhagic; Mango Peel Extract; Mortality.

*Correspondence email:

delima.engga_uin@radenfatah.ac.id

ABSTRACT

Aedes aegypti mosquito can transmit the virus due to biting humans. This study aims to determine whether there is effectiveness in mango (*Mangifera indica*) peel extract to inhibit the growth of instar III *Aedes aegypti* larvae. This type of research is a true experimental study to look at the effect of mango (*Mangifera indica*) peel extract on the growth and development of *Aedes aegypti* larvae. The observation showed results with various concentrations that had different influences on the mortality of *Aedes aegypti* larvae. The higher the concentration, the greater the mortality rate in the larvae ($p=0,000$). Based on the results of the study showed that extracts from the peel of mangoes have different influences on each concentration. The extract from the peel of mangoes can kill the larvae of *Aedes aegypti*. Using probit regression analyses, it is known that the value of LC_{50} is 3.78% every minute.

Pengaruh Ekstrak Buah Mangga (*Mangifera indica*) Terhadap Kematian Larva *Aedes aegypti*

ABSTRAK: *Aedes aegypti* dapat menularkan virus akibat menggigit manusia. Penelitian ini bertujuan untuk mengetahui apakah ada efektivitas ekstrak kulit buah mangga (*Mangifera indica*) dalam menghambat pertumbuhan larva *Aedes aegypti* instar III. Jenis penelitian ini merupakan penelitian true eksperimental untuk melihat pengaruh ekstrak kulit mangga (*Mangifera indica*) terhadap pertumbuhan dan perkembangan larva *Aedes aegypti*. Pengamatan menunjukkan hasil dengan berbagai konsentrasi yang digunakan memiliki pengaruh yang berbeda terhadap kematian larva *Aedes aegypti*. Semakin tinggi konsentrasi yang digunakan maka semakin besar tingkat kematian yang terjadi pada larva ($p=0,000$). Berdasarkan hasil penelitian menunjukkan bahwa ekstrak kulit buah mangga memberikan pengaruh yang berbeda pada setiap konsentrasi. Kemampuan ekstrak kulit buah mangga dapat membunuh larva *Aedes aegypti*. Hasil analisis regresi probit diketahui nilai LC_{50} sebesar 3,78% setiap menit.

INTRODUCTION

The *Aedes aegypti* lives in tropical, sub-tropical climates and in areas with moderate temperatures (Nurdin et al., 2022). The *Aedes*

aegypti mosquito lives close to humans, who are more likely to suck human blood. Initially, the *Aedes aegypti* mosquito originated in Africa. It spread in subtropical and tropical

areas due to human migration and urbanization (Manikandan et al., 2023). Uniquely, only female *Aedes aegypti* mosquitoes spread the virus that causes this tropical disease because female *Aedes aegypti* mosquitoes need blood for egg production. In contrast, male mosquitoes only need glucose to live (Allman et al., 2020). However, not all female *Aedes aegypti* mosquitoes have the virus in their bodies. This virus can reproduce in the body of the *Aedes aegypti* mosquito when the mosquito bites a human infected with a certain virus (Gunawan et al., 2016; Miranda et al., 2022).

When the virus enters the body of the *Aedes aegypti* mosquito, it will infect the mid-stomach of the mosquito and spread to the salivary glands within 8-12 days (Saputra et al., 2022). During this time, mosquitoes *Aedes aegypti* can transmit the virus by biting humans (Captain-Esoah et al., 2020). Immature *Aedes aegypti* mosquitoes can generally be found indoors and in water-filled places, such as water storage areas. Adult *Aedes aegypti* mosquitoes can be found around housing and fly within a radius of 400 meters (Bhadauriya et al., 2020).

The *Aedes aegypti* mosquito is the first medium of the tropical disease dengue fever. When a person is bitten by an *Aedes aegypti* mosquito infected with the dengue virus, the symptoms of dengue fever will appear within 4-7 days afterward (Tallon et al., 2020). Dengue virus generally causes symptoms like a rash, high fever, and joint and muscle pain (Sampat, 2021).

However, other symptoms that can arise are pain behind the eyes, swollen lymph nodes, bone pain, nausea, vomiting, and diarrhea headache (Foller et al., 2017). Severe dengue fever can cause damage and rupture of blood vessels and can be life-threatening. To a less severe degree, dengue fever is handled only in the form of fluids to maintain balance in the body and anti-fever drugs. The morbidity rate of Dengue Hemorrhagic Fever (DHF) per 100,000 population in Central Java in the last five years was 59.2 in 2008: 57.9 in 2009, 56.8 in

2010; 15.3 on year 2011; and 19.29 in 2012 (Fidayanto et al., 2013).

Its spread occurs not only in urban areas but also in rural areas. Since 2007, 33 regencies/cities out of 35 regencies/cities in Central Java have been endemic areas for DHF. In 2008-2009, it spread to all districts/cities with a fairly high number of cases. In 2010-2011, all regions experienced a decline in dengue cases. Global environmental changes or Global Environmental Change (GEC), especially Global Warming, more or less played a role in the incidence of DHF (Santosa et al., 2023). Every season, especially from the dry to the rainy season, various health problems, including the most frequent occurrence, increase the incidence of dengue fever. Other risk factors for dengue infection include host immunity, population density, vector interactions, and host and viral virulence. Vector density also contributes to the dengue epidemic (Segun et al., 2020).

Indonesia is known as a mega-biodiversity country. This country has the second largest biological species globally, after Brazil. According to the World Conservation Monitoring Committee (1994) (Madduppa et al., 2022), Indonesia's natural wealth includes 27,500 flowering plant species or 10% of all plant species in the world. Therefore, Indonesia is a country that has high biodiversity. Biodiversity is the foundation of human life because everyone needs it to sustain life as a source of food, feed, industrial raw materials, pharmaceuticals, and medicines (Rahmah et al., 2020).

Mango plants are consumed not only for their nutritional value but also for their larvicidal benefits. As Allah SWT says in the Qur'an in the letter Meaning: (God) Who has made for you the earth as a stretch and who has made for you the earth a path, on it for you and sends down rain from the sky, so we grow with it various kinds of plants in Surah Taha: 53. *Mangifera indica* is a large evergreen tree in the family Anacardiaceae that grows 10-45 m tall, dome-shaped with

dense foliage, usually branching heavily from a sturdy trunk. (Omilani, 2021) The leaves are spirally arranged on the branches, linear, oblong, lanceolate, elliptical, and pointed at both ends; the blades are mostly about 25 cm long and 8 cm wide, sometimes much larger, reddish, and thin flaccid when first formed and fell off an aromatic odor when crushed. Inflorescence occurs on panicles consisting of about 3000 small flowers of whitish red or yellowish green (Moyib et al., 2021). The fruit is a well-known large drupe but exhibits great variation in shape and size. When ripe, it contains thick yellow flesh, a single seed, and a thick yellowish-red skin. The seeds are solitary, ovoid, or oval, encased in a hard, dense fibrous endocarp (Kumar & Maurya, 2022).

Mangifera indica is an herb commonly used in conventional medicine. *Mangifera indica*, or mango, has been an important herb in Ayurvedic and native medicine systems for over 4000 years (Jain et al., 2022). Mango belongs to the genus *Mangifera*, consisting of about 30 species of tropical fruit trees in the Anacardiaceae flowering plant family. According to Ayurveda, various medicinal properties are attributed to different parts mango tree (Mounika et al., 2021).

Mango is one of the most popular tropical fruits. Mangiferin, as a polyphenol antioxidant and glucosyl xanthone, has strong antioxidant activity, anti-lipid peroxidation, immunomodulation, cardiotoxic, hypotensive, wound healing, anti-degenerative and antidiabetic activity (Wang et al., 2020). Various parts of the plant are used as dental medicine, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative, and diuretic, as well as to treat diarrhea, dysentery, anemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, vaginal discharge, bleeding, and piles (Raut et al., 2021). All parts are used to treat abscesses, broken horns, mad dog or wolf bites, tumors, snake bites, stings, datura poisoning, heatstroke, miscarriage, anthrax, abrasions, sores in the mouth, tympanitis, colic,

diarrhea, glossitis, indigestion, bacillosis, bloody dysentery, liver disorders, excessive urination, tetanus and asthma (Stoilova et al., 2005).

METHOD

Aedes aegypti larva larval mortality. The data was obtained from treating *Aedes aegypti* larvae by giving fruit peel extract mango (*Mangifera indica*) 3 treatments with each different concentration. *Aedes aegypti* larvae were observed within 24 hours with every one-hour spraying using a 50ml spray bottle to give mango skin extract (*Mangifera indica*) to *Aedes aegypti* larvae. Then after 24 hours, you can observe how many larvae experience mortality.

Analysis technique

The analysis of the data used in this study is quantitative data to determine growth and development using the Analysis of Variance (ANOVA) test using the software application, namely SPSS. Data transformation can be carried out if the data is not normally distributed and the data variance is not homogeneous. If the results of the transformation data are successful, then the one-way ANOVA test is continued. If the analysis results show a significant difference, then analyze the data using the Post Hoc test (advanced test) LSD to find the differences between the pairs of groups. The degree of significance used is $\alpha = 0.05$ (a significant difference if $p < 0.05$) (Putri et al., 2023).

RESULTS AND DISCUSSION

The results of giving mango (*Mangifera indica*) peel extract with each dose of 10 ml, 30 ml, and 50 ml once every 1 hour for 24 hours can affect the mortality of *Aedes aegypti* larvae. Here is the average of the measurements of larvae mortality.

Table 1. *Aedes aegypti* larvae mortality

<i>Aedes aegypti</i> larvae mortality every 1 hour For 24 hours				
Concentration	P1	P2	P3	Average
Control	0	0	0	0

<i>Aedes aegypti</i> larvae mortality every 1 hour For 24 hours				
Concentration	P1	P2	P3	Average
P1 (10ml)	2	4	3	3
P2 (30ml)	3	4	4	3.6
P3 (50ml)	5	5	5	5
Average				4.1

Based on the results, data revealed that 100% of *Ae. aegypti* larvae mortality from the highest concentration (50 mL) was observed for *Mangifera indica* skin extract (Table 1 and Figure 1). There are significant differences in larvae mortality between concentrations in the plant extracts ($F= 169.00$, $p=0.00$; Table 2). The result shows that *Mangifera indica* peel extract showed the highest toxicity from the lowest concentration. No mortality was recorded for the control experiment.

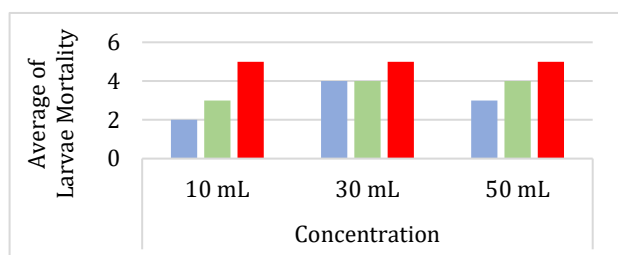


Figure 1. Mortality of *Aedes aegypti*

As shown in Table 2 and Figure 1, there is a positive correlation between plant extracts and larvae mortality. The methanolic extracts tested, *Mangifera indica*, showed the strongest larvicidal effects with the highest concentration, 50 mL. Although variation between concentrations is not quite different, it describes the average number of larvae deaths in each treatment group. In this study, the negative control was distilled water not added to mango peel extract. After obtaining the research data, then the research data was tested using Probit Analysis with a 95% confidence level to obtain the LC_{50} value using the SPSS Statistics 21.0 program. Based on Probit Analysis, a large estimate of concentration was obtained, which resulted in the death of larvae at LC_{50} values at a concentration of 3.78%, meaning that the concentration could kill larvae half of the population of larvae. These results are presented in Table 4.

Table 2. Anova Calculation Results

	Some of Squares	Df	Mean Square	f	Sig
Between Groups	42.26	3	14.08	169	.00
Within Groups	.667	8	.083	0	
Total	42.92	11			

The Analysis of Variance (ANOVA) results show the value of $F= 169.000$, $p= 0,000$. Therefore significant differences between groups. So it can be said that mango peel extract has a different effect based on its concentration. Table 2. above shows that the effect of mango peel extract (*Mangifera indica*) on the mortality of *Aedes aegypti* larvae after being treated increased mortality in each group with a dose of 50 ml and p -value = 0.000, which means that there is a significant difference significantly between groups experienced mortality. LSD Post Hoc Test shows that all treatment concentrations are very influential or significantly different. Mango peel extract significantly affects the mortality of *Aedes aegypti*.

Table 4. Probit test results

Lethal concentration (LC)	Concentration (%)
50	3,78%

Table 4. shows the results of the probit analysis of the LC_{50} value obtained at a concentration of 3.78 %. The lower LC_{50} value for a substance means that the substance has higher activity in killing experimental animals because these substances require lower concentrations to kill experimental animals in the same period (Fe, 2019).

Based on the process of extracting peel of the mango (*Mangifera indica*) carried out the direct maceration method by extracting the peel of the mango directly with 96% ethanol. Maceration was chosen because the process is quite easy, and the equipment is simple (Pal & Jadeja, 2020). This maceration

used as much as 250 grams, and the maceration process was carried out for 2x24 hours. The total solvent 96% ethanol used was 2.5 L. Ethanol is more efficient in cell wall degradation, so more polyphenols will be extracted. Furthermore, flavonoids were found to be higher in the use of ethanol in the extraction process. This study used 96% ethanol because the mortality test of water larvae is very influential because air is a good medium for growth for *Aedes aegypti* larvae, namely to help nutrients enter the organism using 96% ethanol which only contains 4% water; it can reduce contamination of the extract. The maceration filtrate was filtered using filter paper and then concentrated using an evaporator at a fixed temperature of 50°C.

At the time of the study, using a concentration of 10ml with five *Aedes aegypti* larvae, the movement of the larvae became faster and more active than before. The mortality of *Aedes aegypti* larvae began to be seen on the 8th spraying showing one larva died. On the 12th spraying, the number of larval deaths increased by one, then one mortality increased again on the 18th. The number of larvae that remained alive for 24 hours of every 1 hour of spraying was two. Meanwhile, three larvae experienced mortality.

Furthermore, the second was carried out where four larvae died; in the third, the number of larvae that experienced mortality was four. At a concentration of 30ml, larval death began to be shown on the 2nd spraying. Then in the next spraying, namely the 8th and 9th, two larvae experienced mortality. At the 12th spraying, one larva experienced mortality. The numbers of dead larvae were three.

Furthermore, in the second iteration, four larvae died during the test. In the third iteration, the numbers of larvae that died were five. Spraying was done every 1 hour for 24 hours.

The 50ml concentration was the treatment test that used the most extracts, indicating a very accurate significance. The

results obtained are that all repetition tests show the same results. The death of one larva began on the 2nd spraying. On the 6th spraying, there was one larva experiencing mortality. On the 9th, 11th, and 13th spraying, there were three larvae experiencing mortality. The numbers of larvae that experienced mortality were five. In the 2nd and 3rd iterations, the larvae that died were all the larvae tested, namely five in each iteration. Spraying was done every 1 hour for 24 hours.

The absence of larval movements characterizes dead larvae by being stimulated by the movement of water and sticks. In addition, the physical characteristics of the larvae look pale and larger; this is due to the osmosis process, namely the movement of solvent molecules from a dilute solution to a more concentrated solution where the test solution is more dilute into the larval body through a semipermeable membrane, due to this movement, the volume of concentrated systems that are more concentrated in the body of the larvae will swell, and the larval body pigments dissolve so that the body of the dead larvae looks large and pale (W. F. Zuharah et al., 2014).

In research (Alexandre et al., 2017), mango peel extract (*Mangifera indica*) can effectively kill *Aedes aegypti* larvae because mango peel contains flavonoids and saponins. By extracting it using the maceration method, using 96% ethanol as a solvent to obtain the content of flavonoids and saponins, the mango skin is thought to have a larvicidal effect on *Aedes aegypti* larvae. Similarly, the mango plant is also reported several times for its activities against dengue vectors (Mada et al., 2012). Alwala et al., (2010) identified the repellent properties of oils of *M. indica* due to its hydrocarbon compounds against the *An. gambiae*. Methanolic extracts of *M. indica* showed no toxicity, while the aqueous and acetone extracts showed their bioactivity against the dengue vector *Ae. aegypti*, revealing that the plant has toxic activities

which may change with solvent selection (Das et al., 2007). Figueiredo et al. (2008) claim that the methanolic extracts of *M. indica* leave against *Ae. aegypti* was found to be effective against the fourth instar larvae, and the efficacy increased with extended exposure. *M. indica* stems bark extracts were analyzed, and the presence of flavonoids, alkaloids, phytosterols, saponins, tannins, and cardiac glycosides were revealed, which are also reported for their activities against different vector pests and pathogens (Belinato et al., 2013; Kamiabi et al., 2013).

This study was conducted with four treatments, one negative control, and three concentration treatments (10ml, 30ml, 50ml). Each concentration was tested by spraying on *Aedes aegypti* larvae every 1 hour for 24 hours, with three iterations in each treatment. With the positive control results, the *Aedes aegypti* larvae remained alive within 24 hours. Ethanol 96% used in the manufacture of mango peel extract is a solvent that is more selective and less toxic than other solvents. Ethanol 96% is semi-polar, so it can dissolve polar and non-polar chemicals. Mango peel extract is thought to contain flavonoids that have larvicidal properties. Flavonoids attack the nerves in several vital organs of insects, causing a weakening of the nerves, such as breathing and causing death (Nugroho, 2011; Swandi, 2020). Mango skin contains flavonoids, saponins, and tannins, which insects do not like. The content of secondary metabolites such as flavonoids can affect the nervous system and respiratory system in larvae, causing death in larvae, saponins as stomach poisons, and inhibiting the work of cholinesterase enzymes in larvae (Cania & Setyaningrum, 2013; Aji & Wahyuni, 2021). Meanwhile, tannins can reduce the ability to digest food by reducing the activity of digestive enzymes such as proteases and amylase (Ahdiyah & Purwani, 2015).

According to (Yu et al., 2011), flavonoids work as respiratory inhibitors. Inhibitors are substances that inhibit or reduce the rate of a chemical reaction.

Flavonoids interfere with energy metabolism in the mitochondria by inhibiting the electron transport system. Flavonoids have a way of working by entering the larval body through the respiratory system, which will then cause withering of the nerves and damage to the respiratory system and cause the larvae to be unable to breathe and eventually die. In addition, some saponins act as stomach poisons. Extract mango peel is thought to contain Alkaloids (Breitinger et al., 2021). According to (Yun et al., 2021), alkaloids are in the form of salts. Hence, they can degrade cell membranes by inhibiting the action of the acetylcholine esterase enzyme, which causes the larvae to spasm and die. According to (Jeong et al., 2022), alkaloids bind to the enzyme acetylcholine esterase, which is found in the synaptic area between nerve and muscle cells, so that the activity of the enzyme is inhibited and cannot hydrolyze acetylcholine.

Meanwhile, when transmitting impulses, acetylcholine forms a complex with its receptors on the postsynaptic membrane (muscle cell membrane). When acetylcholine esterase is inhibited, the acetylcholine receptor complex persists and continuously affects the postsynaptic membrane. Thus, stimulation of the muscle cell membrane continues. As a result, the muscle continues receiving the stimulus, and there is prolonged muscle contraction or spasm. Suspected to contain saponins. Saponins can bind to proteins and lipids that cause cell membranes which cause changes in the structure of cell membranes so that one of the membranes is damaged, so there is a hemostatic difference so that osmosis has an impact on cell lysis (Luo et al., 2020).

According to Emam et al. (2022), the more concentrated the concentration of the solution, the more toxic substances contained in the mango skin extract (*Mangifera indica*), so the more toxins are consumed, and the mortality of the larvae is higher. According to Zuharah et al. (2021), the percentage of larval mortality in each treatment increased with increasing

concentration. This effect indicates that mango peel extract (*Mangifera indica*) has potential as a larvicide. Larvae can be said to be dead. It can be seen from their morphological conditions if the larvae do not move anymore, they settle to the bottom of the water, the larvae body is soft, and the color changes from darker to pale and slightly transparent. Suppose physiologically, the death of the larvae is caused by some of the content contained in the mango peel extract. In that case, it is suspected that it contains flavonoids which are insecticidal chemical compounds that attack parts of the nerves in several vital organs, causing nerve weakness, such as breathing to death. It is suspected that saponins function as stomach poisons and inhibit the action of the cholinesterase enzyme in larvae. It is suspected that they contain tannins, according to Pharm (2012); it is caused by tannins, which can reduce the ability to digest food by reducing the activity of digestive enzymes such as proteases and simulations. These toxic compounds enter the larva's body through the mouth as stomach poison that can kill.

Larvicides are substances used to break the mosquito life cycle. Chemical control efforts are generally effective enough to break the life cycle of mosquitoes as vectors. However, synthetic larvicides can cause the death of non-target flora and fauna. Synthetic larvicides can also cause environmental pollution. So larvicides can be interpreted as killing immature insects or caterpillars (larvae) (Pujiastuti et al., 2020). Mosquito eradication using larvicides is the best method to prevent the spread of mosquitoes. Parameters of the larvicidal activity of a chemical compound can be seen from the mortality of the larvae. Natural larvicides are defined as pesticides whose basic ingredients come from plants. Natural larvicides are relatively easy to make with limited skills and knowledge. Because it is made from natural ingredients, this insecticide is easily decomposed because the residue is easily lost (Hutchinson & Hutchinson, 2021).

Natural larvicides are hit and run; when applied, they will kill the pest at that time, and after the pest is killed, it will quickly disappear in nature. Using natural larvicides has rapid degradation or decomposition by sunlight, air, moisture, and other natural components, reducing the risk of soil and water contamination. In addition, natural larvicides generally have low toxicity in mammals because these properties make it possible for natural larvicides to be applied to human life.

Natural larvicides are made from plants that are toxic to insects at the larval stage. Natural larvicides derived from plants are good materials to be developed because they have the potential as controllers. The larvicidal potential of all the plant extracts tested was promising against *Aedes* species of both the laboratory and field strains. Still, the laboratory strains appeared more susceptible than the field strains (Manh et al., 2020). The resistance level was increased in field strain due to the pre-existing resistance level towards the insecticides, gene frequencies, and resistance mechanism evolved in inheritance. It was also reported the laboratory strain of *Ae. aegypti* as more susceptible than the field strains towards five essential oils containing pyrethroids because the study area was introduced with synthetic organophosphates, which led to the increase in the tolerance level of the *Ae. aegypti* field strain (Kirar & Sehwat, 2022).

The LC₅₀ values shown by the *M. indica* in this study against dengue vectors have sketched the potential of larvicidal activities. This study finally proposed new alternative potential biopesticides from local flora, which are easily available with low technology and can easily be integrated into the ongoing mosquito management programs. With this, it can reduce the cost of mosquito management rather than using conventional chemical control, which is more expensive than the biological control comprising plant extracts and is more effective and target-specific (Sarma et al., 2019). This study concluded that *M. indica*

can be one of the new potential biopesticides. These results also emphasized the need for further research and investigation to find out the bioactive compounds of *M. indica* and their activities against other vector pests. This may help in the enhancement of the bioactivity of their phytochemicals and the replacement of synthetic insecticides in the future.

CONCLUSIONS AND SUGGESTIONS

Mango (*Mangifera indica*) peel extract can affect the death of *Aedes aegypti* larvae. Mango (*Mangifera indica*) peel extract at 50 ml had a more significant effect on larval mortality with $p = 0.000$. Mango peel extract affected the mortality of *Aedes aegypti* larvae in the 3rd treatment group with a dose of 50 ml given treatment every 1 hour for 24 hours, seen by the number of larvae experiencing mortality.

The suggestions from this research are that the observed dose-dependent effect and the optimized treatment protocol underline the importance of continued investigation into the use of natural extracts for effective vector control. These findings open avenues for innovative and sustainable solutions in the realm of public health and disease prevention.

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