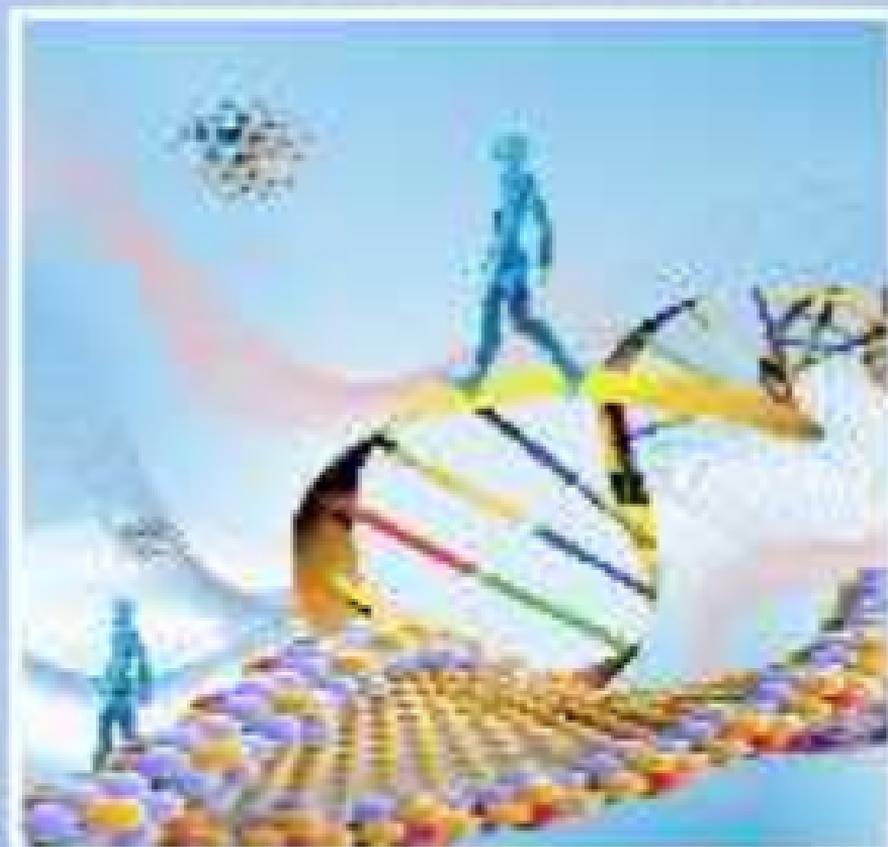




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Research article

The Efficacy of *Aedes aegypti* Natural Larvicide Gained from *Averrhoa bilimbi* and *Averrhoa carambola*

GIANINA ANGELIA SANTOSO¹, NATHANIA DISA ARIESTA ANDRIANI¹ AND
HEBERT ADRIANTO^{1*}

¹Medical School, Universitas Ciputra, Surabaya, Indonesia

*Correspondence : hebert.rubay@ciputra.ac.id

Abstract

Background: Dengue Hemorrhagic Fever (DHF) is an endemic disease transmitted by *Aedes aegypti* which is supposed to be demolished by using the Larvicidal activity. However, there are some *Ae. aegypti* larvae which are found resistant against the temephos in some provinces in Indonesia. Thus, this has been one of the biggest concern of The Ministry of Health Republic Indonesia. Apart from the resistance effect caused, temephos has been reported causing a water pollution which triggers the decreasing standard of human health and immune system. These factors have created a need for search of a new and natural larvicide, which one of it can be gained from *Averrhoa*. This research is aimed at comparing the effectiveness between the extract *Averrhoa bilimbi* and *Averrhoa carambola* towards the *Ae. aegypti* larvae's mortality.

Method: An experimental laboratory research involving the design of post test-only control group. Twenty five larvae *Ae. aegypti* third instar were carried out at 5 different concentrations (0%, 1%, 1.6%, 2.6%, 3.4%, and 4%). The larva demolition is counted within 24 hours. The mortality data is then analysed with the probit analysis.

Results: The extract of *Averrhoa bilimbi* and *Averrhoa carambola* can cause the mortality of the *Ae. aegypti* larvae. LC₉₉ 24 hours from *Averrhoa bilimbi* was recorded = 1.47% (1.20-2.04%), whereas LC₉₉ 24 hours was recorded from *Averrhoa carambola* = 8.96% (7.59-11.31%). The major loses appeared is the stretchy necks and injured appendices. The bioactive substances which are predicted to be the causes of the mortality in this research are saponin glycoside and flavonoid.

Conclusion: The extract of *Averrhoa bilimbi* produces the strongest and the most effective larvicide which will be potentially developed as a new larvicide.

Keywords: *Natural larvicide, Aedes aegypti, Averrhoa bilimbi, Averrhoa carambola*

INTRODUCTION

Dengue fever is one of the endemic disease in Indonesia that is caused by dengue virus which is transmitted through mosquito bite which is called *Aedes aegypti*. Based from Infodatin (Kemenkes RI, 2018; Kemekes RI, 2017) by The Minister of Health, Dengue Fever has been found in Indonesia, Surabaya for the exact place, since 1968. There were 58 infected people and 24 people among them died. So the mortality rate was 41.3%, and it was growing bigger and the disease has been spreading to lots of cities in Indonesia until now. The latest report from The health ministry by June 2017, there are 17.877 cases of Dengue fever. The incidence rate (IR) in 34 province in 2016 is 78.85 by 100.000 population, high above the national targeted IR which is only 49 by 100.000 population.

The minister of health determined that Dengue fever control in Indonesia is prioritized on vector control by using organophosphate consist of chemical insecticides to eradicating mosquito breeding. Chemical vector control using larvicides and insecticides is still the main solution since 1980. The most widely used larvicide to control *Aedes aegypti* larvae is temephos. Temephos is one of the most commonly used chemical for dengue vector control and it has been documented since 1965 to be put in ponds, marshes, and swamps at a dosage of 0.1-0.5 kg/ha. Temephos inhibits an enzyme vital to the normal function of the nervous system and resulting died. By treating stagnant water with temephos, the mosquito larvae are killed before they become adults capable of reproduction (Putra *et al.*, 2016)

However, the frequent use of larvicide using chemical substance may lead to the development of mosquito's resistance. Several researchers have stated that resistance *Ae. aegypti* larvae are found in their country, as Mulyatno *et al* in Surabaya-Indonesia, Singh *et al* and Tikar *et al* in India, Grisales *et al* in San Jose de Cucuta-Colombia, and Komalamisra *et al* in Thailand (Mulyatno *et al.*, 2012; Singh *et al.*, 2014; Tikar *et al.*, 2008; Grisales *et al.*, 2013; Komalamisra *et al.*, 2011). Based from all of these reports, there is a newly interest in the use of botanicals larvicides which more effective, proven that giving no harmful effects on environment especially to the living things, and it must be easily biodegradable.

The star fruit or carambola (*Averrhoa carambola*) is originated from Asia. That star-shaped fruit is an Oxalidaceae family that has a sweet and sour flavor. Star fruits is consumed as fresh fruit and juice. Traditionally, star fruit is widely used as a home remedy to overcome thus hangovers and sunburns. The leaves can be used to treat stomach ulcers. Each fruit only contains of 30 calories plus lots of fiber that helps people to lose some weight. Based from Journal of Biological Sciences (2005), It has been suggested that fruits of some *A. carambola* are relatively more resistant to infestation of a variety of larvae and insects, indicating the presence of compound(s) that could inhibit infestation by some types of invertebrates. As seen in Journal of Pharmacognosy and Phytochemistry, the fruit has lots of beneficial phytoconstituents that can be used as larvicides such as, *saponins, alkaloids, and flavonoids*. Those compounds have been studied that can affect the nervous system, digestion, and breathing in larvae.

The capability of *Averrhoa carambola* in killing larvae has been analyzed by Wasilah SZ using concentration with 1.5%, 2%, 2.5%, 3%, 3.5% and 1 negative control of tap water and 1 positive control using 0.01% temephos. The morality larvae calculated after 24 hours and the results showed that star fruit extract can kill *Aedes aegypti* larvae with the value of probit test is 3.035%. For that, further research on larvicides activity by comparing *Averrhoa carambola* with *Averrhoa bilimbi* with some concentrations 0%, 1%, 1.6%, 2.6%, 3.4%, and 4%. The aims of this study are to determine LC₉₉ of both *Averrhoa carambola* and *Averrhoa bilimbi* and conclude which is the most effective to kill mosquito larvae of *Ae. aegypti* and its best concentration.

METHODS

This research is an experimental laboratory research involving the design of post test only control group. This research is divided into two treatment groups. The first group is the group that is given the juice of *Averrhoa bilimbi* with different concentration. The second group is the group given the juice of *Averrhoa carambola* with different concentration. The study was held in September 2018. The place of research was carried out at the Bio medic Laboratory, Medical School, Universitas Ciputra.

The population in this study were all maintenance larvae of *Ae. aegypti* 3rd instar. Larvae *Ae. aegypti* 3rd instar was chosen because the size was big enough, easy to identify, and would not become a pupa during the study. The sample size used was 25 larvae each treatment at each concentration and been repeated four times.

Research steps include: making a test solution which is done by squeezing. *A. bilimbi* and *A. carambola* are washed clean with running water. Then cut into pieces to get more and smoother concentrate results. The next step is pieces of *A. bilimbi* and *A. carambola* blended to get a rough solution. The results of the blender are filtered using a filter to separate the solution from the pulp. The result of this filtrate is called the primary solution (baku induk). From the primary solution, 5 concentrations were made, namely 0%, 1%, 1.6%, 2.6%, 3.4%, and 4%. Concentration obtained through dilution with aquadest in a certain volume. The steps of this research works includes: preparing 40 plastic cups, 20 cups used for the treatment containing various concentration of *A. bilimbi* juice and 20 cups for treatment containing various concentration of *A. carambola* juice. Add the juice solution of *A. bilimbi* and *A. carambola* for each concentration that has been made. Put mosquito larvae into the cup, each with 25 larvae *Ae. aegypti* third instar, record the number of larvae that died after being exposed after 24 hours of treatment.

Data processing is done by: editing is correcting the data obtained from the observations by repeating the calculation of observations in each treatment to calculate the number of mortality larvae coding is giving the code for each sample and treatment tabulating that is compiling data into table form entry that is entering data in the computer then the data is entered into the SPSS program and in the probit analysis.

RESULT

This research was conducted in Bio medic laboratory of Medical School of Universitas Ciputra for several days. The making of star fruit juice was done in that laboratory that it took about 2 hours. This research started with *Ae. aegypti* egg rearing obtain from The Health Department of East Java . The observations were doing with 4 replications with each glasses consist concentration of 0%, 1%, 1.6%,

2.6%, 3.4%, and 4%, with positive control of 0.01% temephos and the tap water as negative control. Each glasses filled with 25 3rd instar larvae of *Aedes aegypti*. After 24 hours of observing, counting, and writing the amount of *Ae. aegypti* larvae which exposed in the juice of *Averrhoa bilimbi* and *Averrhoa carambola* per hour. (Wasilah SZ, 2017)

The results of the probit analysis are presented in Table 1. It explains that the concentration of *Averrhoa bilimbi* fruit juice to kill 99% of *Ae. aegypti* larvae. is 1.47% and *Averrhoa carambola* fruit is 8.96%.

Table 1. The result of PROBIT analysis

Fruit	LC₉₉ (%)	Interval (%)
<i>Averrhoa bilimbi</i>	1.47	1.20-2.04
<i>Averrhoa carambola</i>	8.96	7.59-11.31

DISCUSSION

Based from the result of this study it was found that the *Averrhoa* had a potential as larvicidal *Ae. aegypti*. This was proven by 50% population of larva died after being exposed 24 hours by *Averrhoa* juice larvicide, while the control group (aquadest) larval population was remained 100%. This study found that the higher the concentration of solution, the higher the larvae's mortality. The larvae that died in this study were proven by larvae at the bottom of the glass and not moving to the surface even though they were given a flashlight. Living larvae that are given a flashlight will move away from the light. Besides that, dead larvae are also touched with the stirring rod and it still not moving. Morphologically, the body of the dead larvae is white body color and longer body size than living larvae. From the results of the probit analysis, it was found that *Averrhoa bilimbi* was more effective and had faster larvicidal activity than *Averrhoa carambola* because the LC value is smaller.

Amalia's research found that the juice of noni leaves (*Morinda citrifolia*) demonstrated a toxic effect to *Ae. aegypti* larvae with $LC_{90} = 14,897\%$ (Amalia, 2018). The LC figure is higher than the results of this study, where LC_{99} *Averrhoa bilimbi* is only 1.47% and LC_{99} *Averrhoa carambola* of 8.96% which can cause larval death by 99%. Based on the LC value, *Averrhoa*'s distillation is more effective than *Morinda citrifolia*. Likewise with Manyullei et al found sweet orange peel juice (*Citrus aurantium*) had an LC_{95} of 2.81%. Manyullei et al., 2015 Compared with research from Manyullei et al then *Averrhoa bilimbi* has a more effective larvicidal ability with *C. aurantium*.⁸ This is because from the probit analysis found the interval from LC_{99} *Averrhoa bilimbi* is 1.20-2.04%. The juice of *Averrhoa carambola* is not effective because it has a higher LC_{99} than *Citrus Aurantium*. *Citrus aurantium* with LC_{95} of 2.81% can cause mortality up to 95% in larvae 3rd instar *Ae. aegypti*. (Hitesh, K., & Tejpal, A. 2016; Sina et al., 2016)

Some plants with larvicidal activity are attributed to its potential larvicidal metabolites compounds such as saponins, phytosterols, phenols, flavonoids and tannins. (Hamidah and Adrianto H, 2017) This study also found that there was a significant morphological damage to *Ae. aegypti* larvae after being exposed to these natural larvicidal, which the neck is elongated, the lateral hair is damaged, and the severe damage of gut. It is estimated that the chemicals that play this role are saponins, glycosides, and flavonoids. Vinayaka et al. (Vinayaka et al. 2010) mentions tannins, alkaloids, steroids, and glycosides in fruits and leaves of *Capsicum frutescens* are larvicidal substances because it can block the work of some metabolic enzymes. Mahyoub reported that saponins and terpenes in ethanol extract were 70% of *H. atra* sea cucumbers, causes neck elongation. (Mahyoub et al. 2016) Lateral fur was also damaged, similar as the study conducted by Aisah et al research showed the same results with concentration of 0.3% of yam bean extract (*Pachyrrhizus Erosus*)(Aisah et al. 2013). Mosquito's body damage occurred because of its body wall structure which can absorb large amounts of toxic substances and cause lateral hair to detach.

CONCLUSIONS

The study showed that the juice of *Averrhoa bilimbi* and *Averrhoa carambola* are highly recommended to be the future natural larvicide because its effectiveness to control the vector by killing the third instar of larvae *Ae. aegypti*. By comparing these two species of fruits, the most effective is the juice of *Averrhoa bilimbi*.

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