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by Rina Priastini Susilowati

LARVACIDE EFFICATION TEST OF MAJA (*Aegle Marmelos*) LEAVES POWDER ON *Aedes aegypti*

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ABSTRACT *:* *Aedes aegypti* is a vector control that mostly done today is still not satisfactory, one of which is because of the use of chemical pesticides. Continuous use of chemical pesticides can interfere with health and cause resistance to mosquito species. Therefore, in this research to develop natural larvacide that is environmentally friendly and biodegradable (easy to decompose in environment). Indonesia is one tropical country rich in various herbs, one of which is the maja plant (*Aegle Marmelos*). The objective of this study was to observe the leaves of maja as a killing power of maja leaf powder with the dose of *Ae. aegypti* larvae mortality compared with temephos 1% powder treatment as chemical larvacide. This study was an experimental study using a completely randomized design with treatment consisting of positive control of temephos 1% , negative control (without exposure) and maja leaf powder as much as 6 concentrations (0.5%, 1%, 1.5%, 2 %; 2.5% and 3%) with 4 repetitions. This research was conducted in research laboratory of FK Ukrida with population of instar larvae III/IV of *Ae. aegypti* counted 25 larvae per replication. The results obtained LC_{50} value of 0.63% and LC_{90} of 2.43%. Based on One Way Anova test result, *Ae. aegypti* larvae mortality percentage on exposure temephos 1% reach 100% as in exposure of maja leaf powder concentration 2.5% and 3%. It can be concluded that effective maja leaf powder concentration killed *Ae. aegypti* larvae was 2.43%.

Keywords : maja leaves, larvacide, *Ae. aegypti*, LC_{50}

INTRODUCTION

Mosquitoes are very important insects, can cause some deadly diseases in humans such as malaria, encephalitis, yellow fever, dengue, dengue hemorrhagic fever (DBD), filariasis and arbovirus, especially *Aedes aegypti*.[1] *Ae. aegypti* is a major vector of dengue and dengue, spread over tropical and subtropical areas, endemic to more than 100 countries and threatens the health of 2.5 billion people.[2,3] Therefore it is important to control mosquitoes in order to prevent mosquito-borne diseases and improving public health.[4,5] Mosquito control programs using growing chemical insecticides widely used by society today such as organophosphates, organochlorides and carbamates have provoked resistance to mosquitoes and caused environmental damage and biological systems.[6-10] Therefore larvasides need to be developed naturally friendly, safe and inexpensive, one of which is the maja plant (*Aegle marmelos*).

Aegle marmelos (L) Correa known as Bael or Bilva, belonging to the family Rutaceae, has medicinal properties

and is widely used in India. In addition, *Aegle marmelos* is also found in India, Burma, Bangladesh, Thailand and Indo-China.[11,12] In Indonesia, the maja plant is known as maja, a shrub, consisting of leaves, roots, bark, seeds and fruit - houses. The skin of the green fruit is the size of a volleyball, has a hard shell skin, with leaves slightly wider, alternating and alternating, borne alone or in groups, consisting of 3-5 oval, pointed, shallow, 4-10 cm long, 2-5 cm wide, terminal with long leaf stalk.[13,14] Several studies have been conducted on *Aegle marmelos*, including some compounds such as alkaloids, terpenoids, phenylpropanoids, tannins, and carotenoids. While the leaves of *Aegle marmelos* contain γ-sitosterol, aegelin, lupeol, routine, marmesinin, β-sitosterol, flavones, glycosides, oisopentenyl halfordiol, marmeline and phenylethyl cinnamamides. Limonene (82.4%) is the main constituent of *Aegle marmelos* leaves and characteristic markers for the identification of *Aegle marmelos* oil samples.[14] In addition, the leaves also contain tannins which are bitter-tasting compounds, reacting with proteins, amino acids and alkaloids containing many hydroxyl groups and carboxyl groups to form strong bonding complexes with proteins and other macromolecules such that it tastes bitter and is not favored by insects that become pests in plants.[13] In addition the workings of saponins and alkaloids as abdominal toxins and inhibit the action of the enzyme cololesterase in larvae, the workings of flavonoids as respiratory toxins and polyphenols as abdominal toxins causing death of larvae. Research conducted by Dass (2014) using the three plants extract against *Cu. quinquefasciatus* larvae, reported that *Aegle marmelos* leaves are quite effective as vegetable larvacids.[15] Based on the above background, a study was conducted to see if maja leaf powder could kill *Ae. aegypti* larvae when compared temephos 1% with multilevel doses.

RESEARCH METHODS

This study was an experimental study using a complete randomized design (RAL) with treatment consisting of positive control of temephos 1%, negative control (no exposure) and maja leaf powder as much as 6 concentrations (0.5%, 1%, 1.5 %; 2%; 2.5% and 3%) with 4 repetitions.

This research was conducted in research laboratory of FK Ukrida with population of instar larvae III/IV *Ae. aegypti* of 25 larvae per replication. The study was conducted from June to September 2017.

The population used was instar larvae III *Ae. aegypti* obtained from the rearing of *Ae. aegypti* egg from Unit Kajian Pengendalian Hama Pemukiman (UKPHP) IPB. Based on WHO reference, the sample used was 25 larvae per replication, with repetition counted 4 times with 6 concentrations, positive control of temephos 1% and negative control (without exposure), resulting in total larvae of 800 larvae.

The powder used is maja leaf (*Aegle marmelos*). Dry maja leaves blended and then weighed with concentrations of 0.005 grams, 0.01 grams, 0.015 grams, 0.02 grams and then put into 100 ml aquadest each to obtain concentration of 0.5%, 1%, 1.5%, 2 %, 2.5% and 3%.

Ae. aegypti egg is placed into a plastic tub containing water for the maintenance of larvae. Eggs will hatch into larvae in 1-2 days. The larvae will develop 3-5 days from stage I larvae to stage III. During its development larvae are fed fish pellets. When the larvae have reached stage III, the larvae are transferred into plastic cups containing maja leaf powder (*Aegle marmelos*) with a titrated dose and temephos 1%, then observed the death of the number of *Ae. aegypti* larvae

in minutes 15, 30, 45, 60, 120, 180, 240, 300, 360, and 1440. After that a probit test is done by calculating LC_{50} and LC_{90}.

Data obtained from this study were calculated using one-way anova analysis and continued with the smallest real difference test (LSD) when there was a significant difference.

RESULTS AND DISCUSSION

In the study, there are some confounding variables that can affect the results of research such as media temperature and pH media. Therefore, the temperature of the media should be measured and controlled by placing the test media in the room so that the temperature is stable. While the pH of the media should be measured to determine the pH changes in the media due to the addition of maja leaf powder. The results of temperature and pH measurements during the study can be seen in Figure 1.

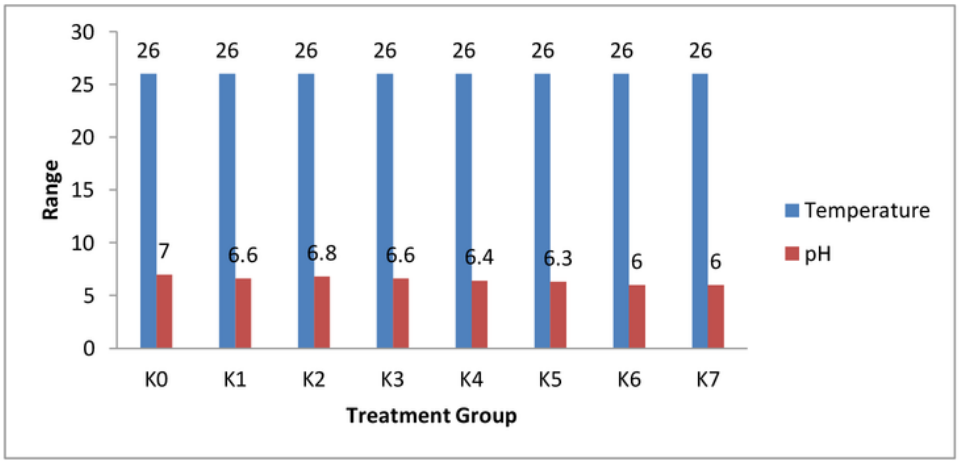
The result of temperature measurement from beginning to end of research during 24 hour observation, got temperature on stable test medium that is 26°C. It can be concluded that the temperature of each test medium does not affect the growth of the larvae because it is included in the optimum temperature criterion for the growth of *Ae. aegypti* larvae is 25-35°C.[16] If there is a difference in the number of deaths of *Ae. aegypti* larvae between the test media, then the difference is not caused by the temperature of the test media.

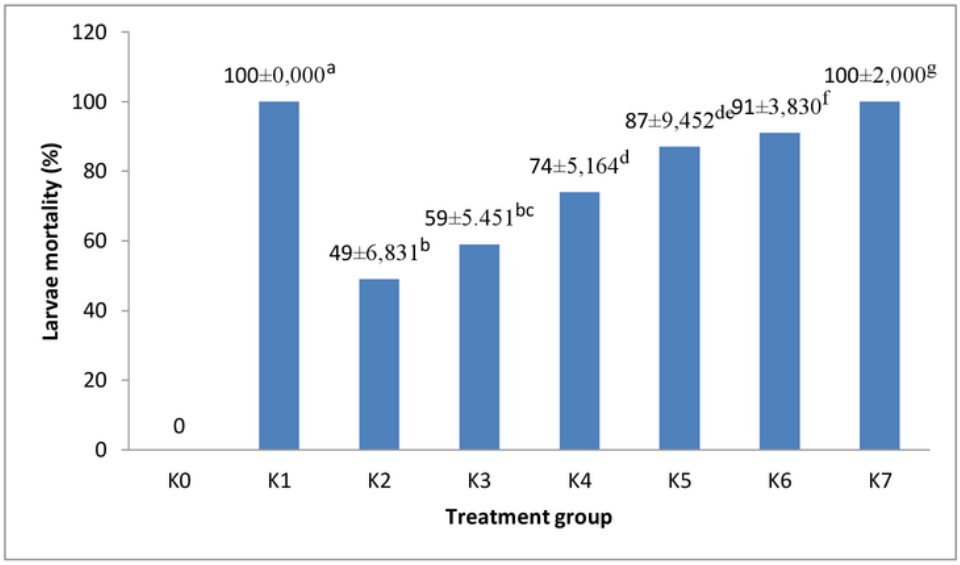
The pH measurement during observation 24 showed that the pH of the control group was 7.0, which is a normal water pH, whereas the addition of maja leaf powder on the test medium with various concentrations resulted in the addition of acidity of the pH of the test medium ie 6.0-6.8. However, this does not affect the death of *Ae. aegypti* larvae because of *Ae. aegypti* larvae can grow in the pH range from 6.0 to 7.0. If there is a difference in the number of deaths of *Ae. aegypti* larvae with various test media, then the difference is not caused by pH on test media.

To break the transmission chain by vector, one of the methods used is to kill the larvae using temephos 1%. Temephos 1% is a chemical larvacide group of organic phosphate compounds which, if used continuously, can cause resistance to mosquito species. In addition, this larvicide has a working mechanism by inhibiting cholinesterase enzyme, causing disruption to nerve activity due to accretion of acetycholine on nerve endings. Temephos binds to the cholinesterase enzyme and is destroyed resulting in continuous contractions, seizures, and eventually the larvae die.[17,18] It is therefore necessary to develop natural larvacides which are larvacides made from plants that have a toxic content to insects at the larval stage. It is hoped that the use of this natural larvacide, one of which maja (*Aegle marmelos*) has no adverse environmental, human, and insect resistance. The maja plant (*Aegle marmelos*) is one of the most important medicinal plants in India.[19,20] Maja leaves are considered to be one part of the plant with the highest bioactive compound content synthesized as secondary metabolites.[20,21] This research uses maja leaf powder because of some the compounds therein are thought to have a killing force against the *Ae. aegypti* larvae and expected this maja leaf powder can be used and made by housewife as an alternative temephos 1%. The study started with negative control, positive control using temephos 1% and maja leaf powder with concentration of stratified and then observed in minutes to 15, 30, 45, 60, and 1440. Death of *Ae. aegypti* larvae can be seen in Figures 2 and 3.

Figure 1. Mean temperature and pH of live medium of *Ae. aegypti* larvae during treatment

Figure 2. Average mortality graph of *Ae. aegypti* larvae treatment group of maja leaf powder with concentration of stratified observation 24 hours. Different letters indicate a significant difference ($p < 0.05$)





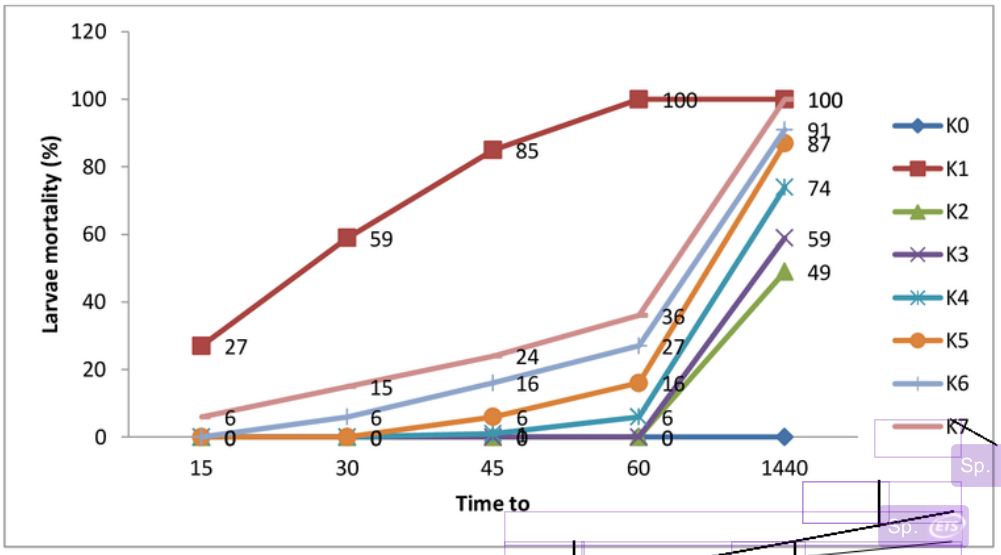


Figure 3. Average mortality graph of *Ae. aegypti* larvae group of powdered leaf treatment then with concentration of stratified based on time of observation

concentration of 2.43%. Thus it can be said that the effective concentration of maja leaf powder to kill *Ae. aegypti* larvae is 2.43%. Unlike the case with temephos 1% in the 60th minute alone is very effective at all in killing *Ae. aegypti* larvae. The dose of temephos used is 1%, which is the effective dose corresponding to WHO.

The data were analyzed using one-way Anova with p value <0.05, meaning there was significant difference between the number of dead larvae between the treatment groups. The test was continued with the smallest significant difference test and the result was a significant difference between the temephos 1% & with the 0.5% leaf powder group; 1%; 1.5%; 2% and 2.5%, but not significant with 3% leaf powder group.

Based on percentage mortality of *Ae. aegypti* larvae it can be tested probit to obtain the optimal dose of maja leaf powder in the form of lethal concentration value. The value of LC_{50} treatment of maja leaf powder was 0.63% and LC_{90} was 2.43%.

CONCLUSION

Mortality of *Ae. aegypti* larvae on temephos 1% and 3% maja leaf powder is 100%. The effective dose of maja leaf powder (*Aegle marmelos*) was 2.43%.

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Ae. aegypti egg is placed into a plastic tub containing water for the maintenance of larvae. Eggs will hatch into larvae in 1-2 days. The larvae will develop 3-5 days from stage I larvae to stage III/IV. During its development larvae are fed fish pellets. When the larvae have reached stage III/IV, the larvae are transferred into plastic cups containing maja leaf powder (*Aegle marmelos*) with a graded dose and temephos 1%, and then observed the death of the number of *Ae. aegypti* larvae in minutes 15, 30, 45, 60, 120, 180, 240, 300, 360, and 1440. After that a probit test is done by calculating LC_{50} and LC_{90}.

Acetylcholinesterase Activity Test (AChE)

The analysis of acetylcholinesterase activity was performed according to Ellman *et al.* (1961) method performed on *Ae. aegypti* larvae.[14] Into a test tube containing 1.95 mL of a 0.1 M potassium phosphate buffer pH 7.5; added 200 µL homogenate *Ae. aegypti*, 150 µL DTNB 0.0011 M in phosphate buffer and 100 µL test solution in water insulin and alkylaryl polyglycol ether 400 mg/L 0.1% emulsifier. In the control mixture the insecticidal suspension is replaced by buffer solution. After being perfectly shaken and left for 10 minutes, further into each test tube 100 µL of acetylcholine iodide 0.0105 M in phosphate buffer. Tests were performed at concentrations of maja leaf powder with multilevel doses. The blank mixture contains the same components as the test mixture, except the homogenate of the enzyme source is replaced by phosphate buffer. The reaction was allowed for 30 minutes, and then the permeability of the solution in each tube was measured at a 412 nm wavelength using a UV-Vis spectrophotometer. The activity of acetylcholinesterase is expressed in a hydrolysis-substrate molar per minute per mg of protein.

Data Analysis

The data obtained were analyzed using one-way Anova test and continued with 5% LSD test. Before analyzing the data is changed to a percentage form if it shows abnormal distribution because there are data above 70% and below 30% then it must be transformed to obtain normal distribution, then used ArcsinVpersen. The linear regression equation and the probit test are used to determine LC_{50} and LC_{90}. The software used for data analysis is SPSS 23.0 for Windows.

RESULTS AND DISCUSSION

Temperature Measurement

At the time of preliminary research, the results of initial temperature measurements to the end of the temperature can be stable at 25°C-26°C. This condition is the temperature ranges that *Ae. aegypti* larvae can use to live well.

Media pH measurement

The results of initial and final pH measurements of maja leaf powder at

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Missing "," You may need to place a comma after this word.

Proofread This part of the sentence contains a grammatical error or misspelled word that ma your meaning unclear.

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Verb This verb may be incorrect. Proofread the sentence to make sure you have used the corr form of the verb.

S/V This subject and verb may not agree. Proofread the sentence to make sure the subject agr with the verb.

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