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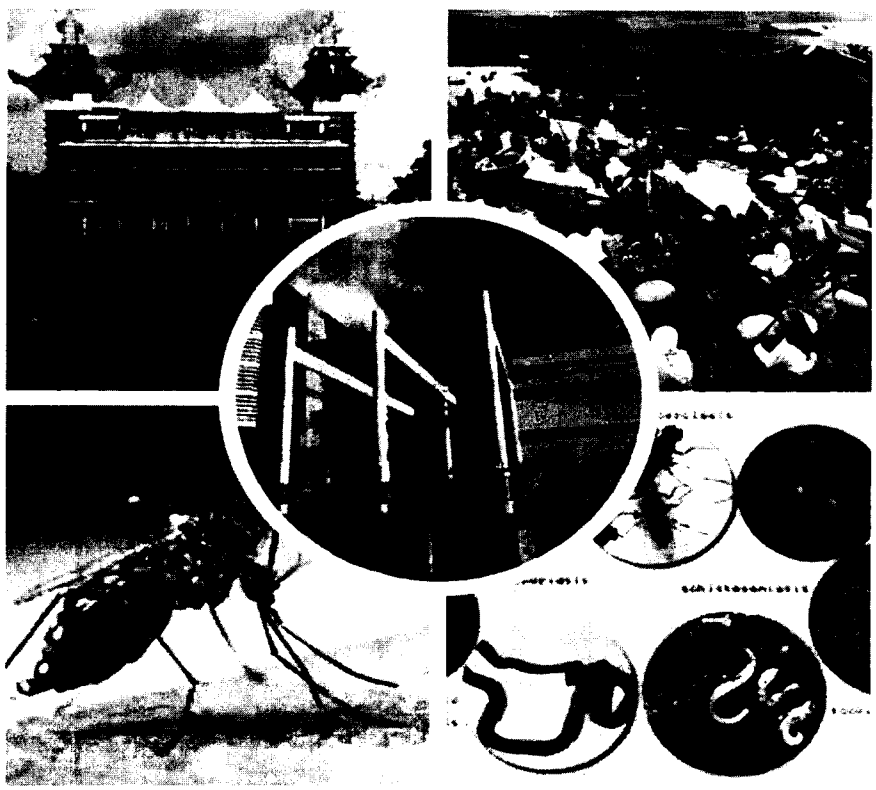
Development of Tropical Diseases Research Based on Wetland and Indonesian Local Wisdom

03-05th November 2017
Banjarmasin, South Kalimantan



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2017

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Theme :
"Development of Tropical Diseases Research Based on Wetland and Indonesian Local Wisdom"

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Banjarmasin, South Kalimantan.
03-05th November 2017

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BIOCHEMICAL TEST OF MAJA LEAVES POWDER (*Aegle marmelos*) AS A LARVACIDE AGAINST *Aedes aegypti*

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ABSTRAK : One effort to reduce dengue hemorrhagic fever is through the controlling of DBD vector larvae using larvasida. The larvacide used to control *Aedes aegypti* larvae is temephos 1%. The use of 1% temephos as a larvicide in Indonesia has been implemented since 1976. However, due to its continuous and long-term use, it can cause resistance to *Ae. aegypti* mosquito larvae. Therefore in this study used maja leaf powder (*Aegle marmelos*) as an alternative larvacide (natural larvacide). This research design uses postest only control croup design) with sample of *Ae. aegypti* instar III/IV larvae. Larvae mortality can be caused by resistance to voltage gated sodium channels due to exposure to contact toxic larvae or toxicity. Through the biochemical test can be seen the increased activity of acetylcholinesterase enzyme along with the increasing death of larvae. The treatment group was divided into positive control using temephos 1%, negatif control (without treatment), 0.5% leaf powder; 1%; 1.5%, 2%; 2.5% and 3%. Each treatment used 25 *Ae. aegypti* larvae. The results showed an increase in acetylcholinesterase activity in larvae exposed by 1% temephos and 3% maja leaf concentration. It can be concluded that there is correlation between high concentrations of maja leaf powder with acetylcholinesterase activity of *Ae. aegypti* larvae.

Keywords : biochemical test, larvacide, *Ae. aegypti*, maja leaves

INTRODUCTION

Dengue hemorrhagic fever (DHF) is a disease caused by dengue virus. DHF has spread to all provinces in Indonesia. Mode of transmission of dengue through *Ae. aegypti* mosquito bites which is the main vector of DBD in Indonesia. Until now there has been no specific drug that can be used for the treatment of dengue, while the response is highly dependent on vector control.[1]

Until now, insecticides are used for the control of *Ae. aegypti* mosquitoes, among others, pyrethroid groups, carbamates and organophosphates.[2,3] Because it is considered very effective, quickly know the results and without looking at the environmental impact. The more advanced technology, it is known

that insect vector is resistant to synthetic insecticides and the occurrence of environmental pollution and can kill non-target organism.[4] Therefore it is deemed necessary to look for an environmentally friendly, easy to obtain, easy to use and effective killer bioinsecticide to kill *Ae. aegypti* mosquitoes.

In general, experts try to overcome this problem by using products derived from plants to suppress or control the development of mosquitoes. It is well known that natural products derived from plants are effective, safe and widely used as biologically active compounds, especially in the field of infectious diseases.[5] Several studies have been conducted to utilize plant products as effective insecticides and larvacides to control various types of mosquitoes.[6-8]

Plant-based pesticides have no harmful effects on ecosystems, and secondary metabolites and synthetic derivatives provide an alternative to mosquito control. Thus, an innovative strategy for controlling vectors is to use phytochemicals as an alternative source of insecticidal and larvacide materials to fight against disease-causing vectors inevitably.[9]

One way of eradicating *Ae. aegypti* that can be done simply and does not cause negative impact to the environment is the eradication of larvae using natural chemical compounds or derived from plant active compounds. Therefore, it should be pursued the existence of alternative insecticides in the form of natural chemical compounds derived from plants and environmentally friendly such as maja (*Aegle marmelos*). In Indonesia known as mojo plant.

WHO (1980) has recommended the assessment of effectiveness of mosquito repellent with bioassay test (biological test), but now has been developed the detection method by using biochemical test such as activity of esterase enzyme such as acetylcholinesterase activity and glutathione S-transferase.[10,11]

This study aims to determine the effect of maja leaf powder (*Aegle marmelos*) as larvae of *Ae. aegypti* mosquitoes by calculating LC_{50} and LC_{90}, as well as acetylcholinesterase activity which can cause paralysis and even death in mosquitoes.

RESEARCH METHODS

The materials used in this study were maja leaves (*Aegle marmelos*) made powder, 1% temephos powder as positive control; instar larvae III/IV of *Ae. aegypti* which has been colonized at Research Laboratory FK Ukrida Jakarta. The tools used in this study include oven, pollinator, glass tube, glass stirrer, rotary drier, water bath, analytical balance, Buchner funnel, filter paper, flannel cloth, chamber and chamber cover, plastic tub.

Bioassay Test on *Ae. aegypti* Larvae

In each maja leaf powder, the toxicity test of *Ae. aegypti* larvae was tested. The test is done in a beaker. The instar larvae III/IV was obtained from the laboratory of the F1 generation crosses in the laboratory. Deuteronomy in both treatment and control testing four times and each replication containing 25 larvae.[12] Prepared solution with 0.5% maja leaf powder concentration; 1%; 1.5%; 2%; 2.5% and 3%. Mortality or death of the test larvae was observed during the 24 hour treatment period. The larvae are considered dead when it shows no signs of movement when touched using a needle. For the glasses control group only filled with water without the addition of maja leaf powder. The percentage of larvae mortality was calculated when control group mortality occurred in the range of 5-20% corrected by using the Abbott formula.[13]

The population used was instar larvae III/IV *Ae. aegypti* obtained from the rearing of *Ae. aegypti* eggs 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 g, 0.01 g, 0.015 g, 0.020 g, 0.025 g and 0.030 g and then put into 100 mL water 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/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

various concentrations increase the acidity of the test medium, but the pH is only in the range of 6-6.8 wherein the pH is *Ae. aegypti* larvae can live well in the range of 4.4 to 9.3.

Mortality of *Ae. aegypti* Larvae

In this study used graded dosage for maja leaf powder that has been tested in each treatment group. Death of *Ae. aegypti* larvae increases with increasing concentration. This proves that the higher the concentration the higher the number of larvae deaths (Figure 1).

From the graph above shows the increase in concentration given is directly proportional to the number of deaths of *Ae. aegypti* larvae, the average increase in *Ae. aegypti* larvae mortality is directly proportional to the increase in the concentration of maja leaf powder. Based on one-way Anova test, there was a significant difference between control group and treatment group of maja leaf powder with multilevel dosage. The test was continued by LSD test. The result showed that all treatment groups of maja leaf powder with multilevel doses had significant differences, except the 3% maja leaf powder group. This means that during the treatment period for 24 hours only treatment of maja leaf powder with dose of 3% which reach 100% death of *Ae. aegypti* larvae, same with the themepos 1% treatment. Based on LC_{50} bioinsecticide calculation of maja leaf powder on *Ae. aegypti* larvae is 0.63% and LC_{90} at a dose of 2.43%.

Acetylcholinesterase Activity of *Ae. aegypti* Larvae

Ae. aegypti larvae used in this study after death (observation hours 1, 6, 12, and 24 hours) tested the activity of acetylcholinesterase enzyme. The acetylcholinesterase enzyme activity test is necessary because there is a relationship between paralysis and knockdown conditions in *Ae. aegypti* with increased activity of acetylcholinesterease enzyme. The measurements of acetylcholinesterase activity are expressed in hydrolysed substrate per minute per mg of protein as shown in Figure 2.

In this study used various concentrations of maja leaf powder (*Aegle marmelos*) which have been tested in each group of larvae. The death of the test larvae increases with increasing concentration and time. This proves that the higher the concentration and the longer the exposure time, the higher the death of the larvae. This is in accordance with the proposed by Hoedojo and Sungkar (2008) that the efficacy of insecticide to kill insects is very dependent on the shape, the way into insect body, kinds of chemicals, concentration and amount (dose) of insecticide.[15]

Sastrodihardjo (1979) stated that this toxicity test is carried out by inserting the mosquito larvae into an extract solution with a certain concentration.[16] Thus the entire body of the larvae is exposed by toxic substances from maja leaf powder. Toxic compounds contained in maja leaves can enter through the body wall of larvae and through the mouth because larvae usually take food from their place of life. Insect body walls are part of the body of an insect that can absorb toxic substances in large quantities.

The mechanism of action of larvacides in killing larvae is larvacide entry through contact with the skin. Then applied directly through the integument of insects (cuticle), trachea or sensory glands and other organs related to the cuticle. The chemicals contained in the insecticide dissolve the fat or wax layer on the cuticle so that the active ingredients contained in the insecticide can penetrate the body of the insect.[17]

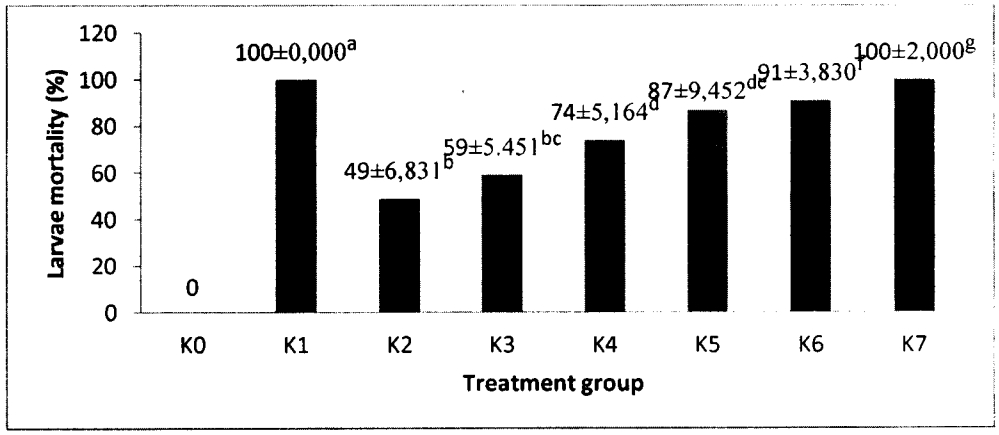

Figure 1. Average mortality graph of *Ae. aegypti* larvae treatment group of maja leaf powder with concentration of stratified observation 24 hours. Different letters indicate a significant difference ($p < 0.05$)

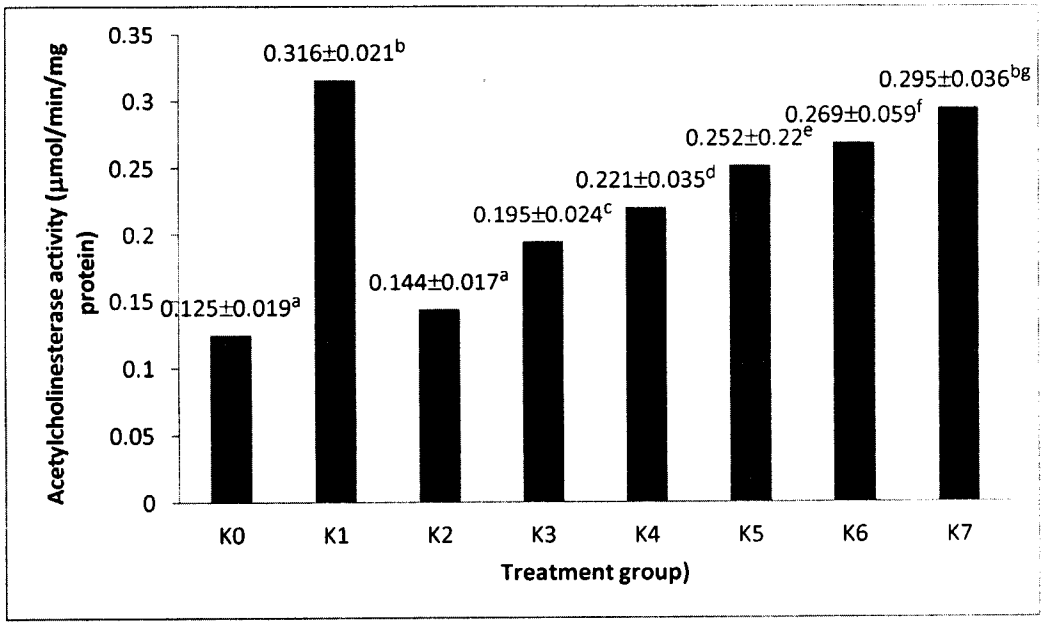

Figure 2. Activity of acetylcholinesterase enzyme *Ae. aegypti* larvae between treatment groups after 24 hours of observation. Different letters indicate a significant difference ($p < 0.05$)

The way larvacide works can also enter the body of the larva through the mouth (through the food eaten). Larvae die due to toxins that enter through the food then in the mosquito body cells will inhibit cell metabolism that inhibits the transport of electrons in the mitochondria so that the formation of energy in the cell does not occur and the cell cannot move.

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, phenylethyl cinnamamides and limonene (82.4%) are the main constituents of *Aegle marmelos* leaf.[18] In addition, the leaves also contain tannin which is a bitter-tasting compound, reacts with proteins, amino acids and alkaloids that contain many hydroxyl and carboxyl groups to form strong bonding complexes with proteins and other macromolecules so that it tastes bitter and is not favored by insects that become pests on plants.[19] Cania (2013) states that alkaloids and saponins have a way of working as a stomach poison and inhibit the action of cholinesterase enzymes in larvae. Flavonoids act as a respiratory toxin that causes the death of larvae. Tannin can reduce the ability to digest food by reducing the activity of digestive enzymes (proteases and amylases).[20]

This alkaloid content serves as a stomach poison and contact poison. Alkaloids in the form of salt so that it can degrade the cell membrane of the digestive tract to enter into and damage the cells and can disrupt the work system of larvae nerves by inhibiting the action of acetylcholinesterase enzyme. Where this enzyme cannot perform its duties in the body especially continuing the delivery of orders to the digestive tract of larvae (midgut) so that its movement cannot be controlled. The occurrence of color changes in the body of the larvae becomes more transparent and the movement of the larval body that slows when stimulated touch and always bend the body is also caused by alkaloid compounds.[21,22]

Tannin is the most content after alkaloids. Tannin is a polyphenol compound that can form complex compounds with proteins. Tannins cannot be digested in the stomach and have the binding power with proteins, carbohydrates, vitamins, and minerals.[23] According to Yunita *et al.* (2009), tannins may interfere with insects in digesting food because tannins will bind proteins in the digestive system that the insects need for growth so that the *Ae. aegypti* digestion process is predicted becomes disturbed due to the tannin substance.[22]

Saponins can inhibit the action of enzymes that cause a decrease in digestion and protein use. The properties of this saponin are foamed in water, have good detergent properties, are toxic to cold-blooded animals, have hemolytic activity, are not toxic to warm-blooded animals have anti-exudative properties and have anti-inflammatory properties. In addition, saponins have the ability to damage the membrane.[22] Saponins contain glycosides in plants that are soap-like and water-soluble. Saponins can decrease the activity of digestive enzymes and food absorption. Flavonoids are plant defense compounds that can be inhibiting insects and are also toxic. The workings of these compounds are as stomach poisoning or stomach poison which can lead to disruption of the digestive system of *Ae. aegypti* larvae, so that the larvae fail to grow and eventually die.[16]

Saponin in maja leaf powder allegedly can reduce the surface tension of the mucous membrane tractus digestivus larvae so that the gastrointestinal wall becomes corrosive. Chemical content of

saponins and flavonoids have potential as *Ae. aegypti* larvacides, saponin is a bitter-tasting compound that can cause allergies and often cause irritation to mucous membranes, saponins can destroy red blood grains through the reaction of hemolysis, is toxic to animals.[24]

The cause of weakness in the insect nerve can also be caused by the active ingredient flavonoids found in maja leaf powder, where its function is to inhibit the action of enzyme acetylcholinesterase. Acetylcholine formed by the central nervous system serves to deliver impulses from nerve cells to muscle cells. After the impulse is delivered, the process is stopped by the acetylcholinesterase enzyme that breaks acetylcholine into acetyl co-A and choline. The presence of flavonoids will inhibit the operation of this enzyme resulting in accumulation of acetylcholine which will cause disturbance in the system of impulse delivery to the muscles that can result in muscle spasms; paralysis occurs and ends in death. In addition, it can also disrupt the flow of Na^+ (sodium) in nerve cells and neurotransmitters (chemical transmitters) in synapses.[25]

Furthermore, Winslow (2002) explains that compounds that are nervous toxins can prolong the flow of Na^+ ions into the membrane by slowing down or blocking the channel closure.[25] If the active compound in the maja leaf powder slows the channel closure, the nerves in the depolarization state are long enough, so that Na^+ ions will enter the membrane. This will cause symptoms of seizures and tremors. Active compounds that are neural toxins are also able to block the closure of the channel, this condition will cause membrane excess Na^+ ion which eventually the nerve becomes inactive. This nerve inactivity is because the nerves are too positive and difficult to repolarize (back to the original state). Symptoms to be generated are paralysis or event death.[25]

Maja leaf powder (*Aegle marmelos*) has very useful larvacide properties to control mosquito larvae in place of breeding (in water). Further research is needed to explain its activity against the various stages of the type of mosquito and also identify the active ingredients in the maja leaf powder that serves as a larvacide.

CONCLUSION

The effective dose of maja leaf powder against *Ae. aegypti* larvae death is 2.43%. Paralysis or death of *Ae. aegypti* larvae is associated with increased activity of acetylcholinesterase induced by chemicals contained in maja leaf powder as a larvacide.

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