The Toxicity of Seeds Extract of Annona squamosa L., Leaves Extract of Terminalia catappa L. and Leaves Extract of Acacia nilotica L. on The Mortality of *Aedes aegypti* L. Larvae

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Abstract—*Aedes aegypti* L. is a species of mosquito that carries the dengue virus causes dengue fever. The way to solve the disease is still focused on synthesis larvacides which have a negative impact on the environment, it needs to be replaced with natural larvicides that are environmentally friendly. *Annona squamosa* L. seeds extract contains *annonain* and *squomacin*. *Terminalia catappa* L. leaf extract contains *quinone, saponin, flavonoid* and *tannin*. *Acacia nilotica* L. leaf extract contains *terpenoid, saponin, flavonoid* and *tannin*. This research aims to determine The toxicity of seeds extract of *A. Squamosa*, leaves extract of *T. Catappa*, and Leaves extract of *A. nilotica* on the mortality of *Ae. aegypti*. The method used in this research was complete randomized design using four replications. Concentration used 5, 10, 50, 100, 150, 200 and 250 ppm for the seeds extract of *A. Squamosa*, 50, 100, 150, 200, 250, 300 and 350 ppm for the leaf extract of *T. catappa*, and 80, 120, 180, 240, 300, 350 and 400 ppm for the leaf extract of *A. nilotica*. The data were analyzed using probit analysis to determine the LC_{50}. The result showed LC_{50} is 109.43 ppm for *A. Squamosa* seeds extract, LC_{50} is 171.65 ppm for *T. catappa* and LC_{50} is 203.63 ppm for *A. nilotica*. This result showed the biggest toxicity is seeds extract of *A. Squamosa*.

Keywords— *Anonna squamosa. Acacia nilotica, Terminalia catappa, mortality, Aedes aegypti, toxicity*

INTRODUCTION

Aedes aegypti L. is a species of mosquito that carries the *dengue* virus that causes dengue fever. Dengue Hemorrhagic Fever (DHF) still health problem in Indonesia [1]. *Ae. aegypti* mosquitoes also carries *zika* virus that causes *microcephaly* [2]. Dengue fever and *microcephaly* became the biggest threat of the health from Indonesia population because the climatic conditions in Indonesia support for breeding of *Ae. Aegypti.*

Dengue fever in Indonesia increased to 48% in 2014 [3] and Researchers Eijkman Institute for Molecular Biology have found a positive *zika* virus infection in Jambi Province [4], so the government solve it which is to free distribute the synthesis larvicides to the public.

Eradication of mosquito larvae by using synthesis larvicides that harm to society because it can cause environmental pollution [5], and should be replace with natural larvacides from plants that environmentally friendly [6].

The plants that have potential as a source of natural larvicides is *Annona squamosa* L., *Terminalia catappa* L., and *Acacia nilotica* L. *A. squamosa* seed extract contains *annonain* and *squomacin* that can inhibit the electron transfer process [7], *T. catappa* leaf extract contains *quinone* that can disturb regulation of Ca^{2+} ions in the body [8], *A. nilotica* leaf extract contains *terpenoid* types 2.5 *sikloheksadiena* that can kill insects in low concentration [9]. Based on the considerations, this study aims to explore the potential of plants as natural larvicides to control larvae *Ae. Aegypti*.

MATERIALS AND METHOD

a. Tools and Materials

The materials used in this research are *A. squamosa* seeds from Kotakan village Situbondo-East Java, *T. catappa* leaves, and *A. nilotica* leaves from Baluran National Park Situbondo-East Java, Ethanol 96%, aquades and *Ae. aegypti larvae*.

The tools used are grinders, paper label, oven, rotary evaporator, stem funnels, bottles, scales, glass beaker, stir bar, aluminum foil, gauze, bathtub, pipettes, beakers, jars, refrigerators, microscopes, cameras, cover glass and glass objects.

b. Manufacture Extract

Extract manufacture begins with the select *A. squamosal, T. catappa*, and *A. nilotica*, then dry the materials for 7 days and put into oven for 2-3 hours, after which the grinder into powder and maceration ethanol 96% with ratio 1: 4, after maceration for 3 days then filtered using a is with filter paper in buchner funnel, and the result is inserted in Rotary Evaporator with the temperature of 50ºC and 90 rotations per minute (rpm) to separate ethanol and extract. Recently save extract in 100 ml glass beaker and kept in the refrigerator.

c. Toxicity test

Toxicity test is done by filling seven glasses 200 mL with different serial extract concentration and enter 20 larvae use a pipette into each solution concentration. The method used in this research was complete randomized design using four replications in each test extracts. Concentration used 5, 10, 50, 100, 150, 200 and 250 ppm for the seeds extract of *A. squamosa*, 50, 100, 150, 200, 250, 300 and 350 ppm for the leaf extract of *T. catappa*, and 80 , 120, 180, 240, 300, 350 and 400 ppm for the leaf extract of *A. nilotica*, negative control use aquades mixed with tween 80, and the positive control use abate 100 ppm.

d. Data analysis

Result were presented as mean ± S.D for Mortality of Ae. Aegypti larvae from each extract. LC_{50} were presented by probit analysis on statistical computer software program, Minitab 16 for windows. The value of LC_{50} were presented value toxicity.

RESULT AND DISCUSSION

Toxicity Result

Table 1 *Ae aegypti* larvae mortality after treatment of the extract for 24 hours

A. squamosa extract		*T. catappa* extract		*A. nilotica* extract	
Treatment (ppm)	Mortality (%) Mean±SD	Treatment (ppm)	Mortality (%) Mean±SD	Treatment (ppm)	Mortality (%) Mean±SD
K-	0±0,00	K-	0±0,00	K-	0±0,00
5	1,25±2,50	50	5±0,00	80	5±4,08
10	6,25±2,50	100	23,75±2,50	120	31,25±2,50
50	18,75±4,79	150	40±10,80	180	45±4,08
100	38,75±2,50	200	61,25±4,79	240	63,75±4,79
150	58,75±2,50	250	71,25±7,50	300	68,75±7,50
200	80±4,08	300	90±4,08	350	81,25±2,50
250	100±0,00	350	98,75±2,50	400	95±4,08
K+	100±0,00	K+	100±0,00	K+	100±0,00

Description:
K_- : negatif control
K_+ : positif control

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From Table 1 it is known that the lowest concentration of the three extracts can kill larvae of *Ae. aegypti* is *A. squamosa* seeds extract, it has lowest mortality at a concentration 5 ppm with a mean mortality is 1.25%, and the highest mortality at a concentration 250 ppm with a mean mortality is 100%.

Table 2 LC_{50} *A. squamosa* seeds extract, *T. catappa* leaves extract, and *A. nilotica* leaves extract to mortality of Ae. aegypti for 24 Hours

Extract	LC50 (ppm)	Lower	Upper
A. squamosal extract	109.43	97.36	120.73
T. catappa extract	171.65	158.47	183.60
A. nilotica extract	203.63	186.74	218.99

From table Table 2 the concentration of *A. squamosa* seed extract that needed to obtain 50% mortality of larvae for 24 hours is 109.43 ppm. The concentration of *T.catappa* leaves extract that needed to obtain 50% mortality is 171.65 ppm. The concentration of *A. nilotica* leaves extract that needed to obtain 50% mortality is 203.63 ppm. *A. squamosa* seed extract has LC_{50} smaller than the *T.catappa* leaves extract and *A. nilotica* leaves extract, it indicates that *A. squamosa* seed extract is highest toxicity, the smaller the LC_{50} value, the greater toxic effects in a substance to kill the target animal [10].

DISCUSSION

Symptoms of *Ae. aegypti* larvae poisoned by *A. Squamosa* seeds extract, *T. catappa* leaves extract, and *A. nilotica* leaves extract seen from the activity of larvae and color of larvae. Activities larvae poisoned is weak, larvae rarely appeared in the waters and larvae in the bottom waters with the body does not move [11]. Color of larvae after treatment is transparent, pale and his body became flabby, color changes to be transparent and flaccid due to hydrophobic and lipophilic cuticle so extracts nonpolar compounds easily penetrate the cuticle to dissolve grease or wax layer of the cuticle [12]. Symptoms of toxicity experienced by the larvae *Ae.aegypti* caused the toxic compounds in the extracts attack *Ae.aegypti* larvae body as a contact poison, stomach poison, and respiratory poison.

Toxic compounds that works as a contact poison are *annonaine, squamocin, quinone, tannins* and *saponin*. Toxic compounds attack the skin of *Ae. aegypti* larvae then dissolve grease or wax layer of the cuticle because it is polar, so toxic compounds easily penetrates the cuticle and into the larval body, active substances that enter the body larvae will be carried to blood flow in circulatory system and will be spreading into the larvae body [13]. The circulatory system larvae *Ae. aegypti* is an open circulatory system with liquid circulation called hemolymph. Hemolymph is an interstitial fluid that serves to distribute nutriens in the body. Hemolymph heart pump through the vessels into the sinuses, the spaces are filled with fluid where the materials exchanged between hemolymph and cells [14], so the toxic compound in the circulation system also be circulated into the body cells of larvae, then it's will cause chemical reactions in the metabolic process of the body that can lead to death [15].

Quinone that enters the body larvae also attack muscle cells, it's inhibit the Ca^{2+} regulation. Contraction of skeletal muscle begins with the action potential that triggers the release of Ca^{2+} from the sarcoplasmic reticulum, then the ions Ca^{2+} binding to troponin which causes the opening of a binding site for myosin on the actin, myosin is attached to actin and escape, thus attracting filaments thinner towards the middle of the sarcomere which indicates contracted muscles and ATP give strength to the launch of the filament [15], when Ca^{2+} inhibited the larval muscle can't contraction, it's cause the larvae to become paralyzed.

annonaine, squamocin, quinone, *tannin* and *saponin* work as a stomach poison. stomach poison compounds enter through the mouth and then towards the front of the *stomodeum, mesenteron* and *proktodeum*. The poison will cause indigestion in *mesentron*, especially on peritrophic membrane, so that the poison penetrate the body cavity that contains the hemolymph [16]. Hemolymph with poison will spread into the tissue, when there is an exchange of substances in hemolymph with body cells, the poison will inhibit the metabolic processes in the body, particularly in the cellular respiration in electron transfer process that is at sites I. Inhibition of ATP (adenosine triphosphate) to block the way between electrons from NADH with *ubiquinon* in the chain of electron transfer in cellular respiration process which results in the formation process metabolic energy is inhibited [17].

Toxic compound that works as a respiratory poison are *terpenoid* and *flavonoid*. Mechanism of *terpenoid* and *flavonoid* poisoned larvae that is when the larvae *Ae. Aegypti* lost the ability to close spiracles due to contact and stomach poison. Toxic compound that enter through the respiratory system is circulated to the whole body [14]. Toxic compounds that enter into the respiratory system directly be brought into the cells of the body and will disturb the process of cell respiration, toxic compounds that affects the respiratory system of insects inhibit the respiratory enzyme inhibition of electron transport in particular attack NADH [11]. Inhibition of electron transport system is characterized by paralysis and death, it is because the toxic compounds attack NpNH electron transport process and NADH [11].

CONCLUSION

Based on the results of research and data analysis can be concluded that the LC_{50} for 24 hours *A. squamosa* seeds extract on mortality of *Ae. Aegypti* larvae is 109.43, LC_{50} *T. catappa* leaves extract is 171.653 ppm, and LC_{50} *A. nilotica* leaves extract is 203.628 ppm. LC_{50} of *A. squamosa* seeds extract is smallest, it's showed that the toxicity of *A. squamosa* seeds extract is greater than the toxicity of *T. catappa leaves* extract and toxicity of *A. nilotica* leaves extract.

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