Toxicity of Granule from Sugar Apple (*Annona squamosa*. L) Fruit Extract on The Mortality *Aedes aegypti* Larvae

Dini Aisyafahmi [1)], Dwi Wahyuni [1)]
[1]Faculty of Teacher Training and Education, University of Jember
email: dwi.wahyuni@fkip.unej.ac.id

Abstract

Aedes aegypti L. *is mosquito belong to the order diptera that widely distributed in the tropics. Female Aedes aegypti* L. *is the primary vector for the spread of dengue fever. Dengue fever has increased from year to year. Efforts to eradicate the disease done by government mainly using chemical insecticides which their residues can endanger non-target organisms. Eco-friendly Aedes aegypti* L. *larvae's control is needed to suppress the populations. One that can be achieve using natural larvacide from sugar apple (Annona squamosa* L.*). The aim of this research was to find out the LC_{50} and toxicty effect of granule from sugar apple extract (Annona squamosa L.) on Aedes aegypti* L. *larvae's mortality. The concentration used in this research were 1, 5, 25, 50, 75, 100, 125, 150 ppm, control 0 In the form of tween 80, positive control abate and negative control aquades. The result shows that LC_{50} granule extract of apple sugar (Annona squamosa* L.*) was 8.25 ppm.*

Keywords: *Aedes aegypti* L.*, Annona squamosa* L.*, granule, toxicity, LC_{50}*

1. INTRODUCTION

Aedes aegypti L. female mosquito is an insect of the order diptera that spread widely in the tropical area, especially in Indonesia. Aedes aegypti L. mosquito is a dengue virus vector for dengue disease [15]. The emerging rate of dengue fever has been increasing simultaneously from year to year. Indonesia is the second largest country with high numbers of people and death rates due to dengue fever [16]. In East Java, the morbidity or IR rate is 24.07 per 100,000 population with total deaths of 107 individuals.

Several efforts have been made by the government to overcome *Aedes aegypti* L. by fumigation (fogging), 3M methods (drain, cover, and hoard), as well as giving temephos or abate. Fogging are performed in two cycles, the first cycle is to kill the larva and the second cycle to eradicate the remaining mosquito that survived first cycle. All those eradication efforts are becoming more and more less effective. Based on the resistance test in Semarang City, Aedes aegypti larvae has been resistance to 0.25% cypermetrin and 0.8% of the organisms (group of organophosphates) [18]. Whereas 3M action only focus on water reservoirs. The use of temephos/abate can harm non-target organisms and is not environmentally friendly.

The negative effects of synthetic insecticide can be decreased by safe and environmentally friendly materials. Plants are potential for this purpose. One of them is sugar apple (*Annona squmosa* L.) or Srikaya by local. This particular plant bears biolarvacide activity [6]. The young fruit of *Annona squamosa* L contains acetogenin (annonin, annonacin, asymicin, squamosin, and cohibinsin), flavonoids (quercitrin, isoquercitin, myricetin-3-O-galactoside, and rotenone), tannins, saponins, steroid, fitosteroid, cyclic ketone, sterpenoid, borneol, verbenone, alkaloids, volatile compounds, glycosides, and phenolic compounds. Known

annonain is known to act as a dissolved poison or contact poison [11].

Each active ingredients will interact with eachother so it is highly possible to enhance the probability rate of mortality. The formula applied also held significant addition, different formulas bears different effectiveness. For example the binding substances in the formulation is determined-partly- by the particle size and the solubility of the active ingredients and other additives used [8]. This research was expected to provide new benefits and knowledge about the utilization of sugar apple (*Annona squamosa* L.) fruit as natural larvacide.

2. RESEARCH METHOD

The tool used in this research was incubator, rotary evaporator, analytical scales, refrigerator, blender, measuring cup, erlenmeyer, petridisk, spatula, beaker glass, oven, tray, funnel, strainer, glass extract, microscope, object glass, dropper drops and sieves. The materials used in this study include sugar apple fruit (*Annona squamosa* L.) aged approximately 3-7 weeks obtained from Pasirian Lumajang, *Aedes aegypti* mosquito L., Ethanol 70%, maltodextrin, sterile aquadest as a negative control, abate as positive control, tween 80 as control 0, aluminum foil, filter paper, label paper, and paper towel.

The procedures of this study included the selection of sugar apple fruit (*Annona squamosa* L.), the extract of srikaya fruit (*Annona squamosa* L.) by maceration method using ethanol 70%, granule extract of srikaya fruit (*Annona squamosa* L.). The concentrations used in the difference test were 1, 5, 25, 50, 75, 100, 125, 150 ppm. Treatment control used abate, tween 80 and aquadest. Probit analysis was use to determine the lethal concentration (LC_{50}), the calculation carried out using Mini tab 14. The effect of granule from fruit extract to the mortality of larvae measured using Anova test analysis.

3. RESULT AND DISCUSSION

The result of probit analysis showed that the value of LC_{50} to the larvae mortality is shown in Table 1. Whereas the mean mortality of Aedes aegypti L. larvae can be seen in Figure 1.

Table 1 The value of LC_{50} within 24 hours time exposure.

***Lethal Concentration* (LC)**	**Granule concentration (ppm)**	**Under limit**	**Upper limit**
LC_{50}	8,25	5,71	10,97

Based on probit analysis in Table 1 it can be seen that 8.25 ppm of granule form sugar apple fruit extract are able to kill 50% of larvae test within 24 hours of time exposure. The upper and under limit is the highest and lowest concentration of granules which can kill 50% test larvae within 24 hours.

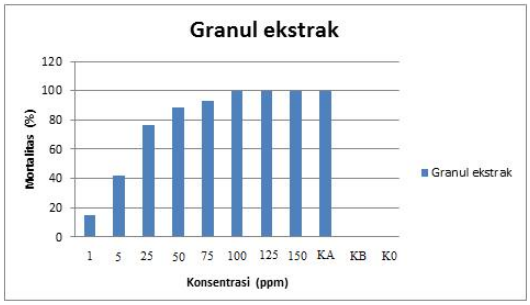

Figure 1. The relationship between mean mortality (%) of *Aedes aegypti* L. larvae with serial concentration of granule.

Figure 1 showed the increase in mortality of *Aedes aegypti* L. larvae in line with the higher concentration. The average mortality of larvae was lowest at 1 ppm concentration with mean mortality of 15% while the highest concentration was 100 to 150 ppm with mean mortality of 100%.

The result of toxicity test using Anova statistic can be seen in Table 2.

Table 2. Anova result.

ANOVA

	Sum of Square	df.	Mean square	F	Sig.
Between group	18655.2	7	2665.03	13.600	0.00
Within group	3135.4	6	0		
Total	21790.6	23	195.96		

Based on Table 2. Anova test results obtained significance value of 0.00 ($p < 0.05$). The results showed that there was significant effect of the granule on Aedes aegypti L larvae mortality. It can be seen that there is significant effect of granule from sugar apple (*Annona squamosa* L.) fruit extract granule to *Aedes aegypti* L. mosquito larvae mortality.

Toxicity testing of botanical insecticide from sugar apple fruit (*Annona squamosa* L.) in this study is in the form of granules. The advantage of the granule formula is that there is a process of preventing the separation of active ingredients contained in the fruit. In addition, in case of storage, granule is more effective as it is more resistant to air. The more maltodextrin added, the higher the polyphenol content [2]. Maltodextrin is able to protect the release of nutritional components, protecting important compounds due to high temperatures, and having the ability to form a strong binding force to the compound [10]. The active ingredients and additives will affect to each other, just as the effectiveness of the binding substances in the formula is influenced by the particle size and its solubility [8].

Sugar-apple plants contain polyphenolic compounds such as flavonoids, saponins, tannins, alkaloids [6]. It also contain substances from acetogenin groups such as annonain and squamosin. Group of acetogenin are able to inhibit ATP synthesis in mitochondria. Annonain and squamosine are able to inhibit electron transfer in site I by blocking the bond between NADH and ubiquinon in the electron transfer chain in the cell respiration process, causing the metabolic energy formation process to be inhibited [7].

Chemical compounds contained in sugar-apple fruit (*Annona squamosa* L.) are contact-toxin, it enters into the insect body trough cuticle, carried away by the bloodstream and eventually it will be scattered throughout the insect body [12]. Annonain and squamosin will penetrate into the insect body through parts covered by thin cuticles, such as intercellular membranes, lining of the joints at the base of the embelan and the chemoreceptors present on the tarsus. Usually annonain and squamosin diffuse from the outermost layer of the cuticle then through the deeper layer to the hemolymph, following the flow of hemolymph and will be spread throughout the body of the larvae. The entry of active compounds from sugar-apple fruit (*Annona squamosa* L.) into the body of *Aedes aegypti* L. larvae through the cuticle in both directions is vertically and horizontally. The entry of the secondary metabolite compounds vertically through the outer portion of the cuticle to the hemolymph, while horizontally it enters the cuticle layer and then enters the body through the trachea directly related to the larval tissue [5].

Group of acetogenin has working mechanism as stomach poison that can work through mesenterone (middle gastrointestinal tract), the active compound will be absorbed by the mesenterone wall composed of epithelial cells consisting of lipids and lipophilic proteins. These epithelial cells will be damaged and even induce cell death. As a result of cell death in the mesenterone (middle gastrointestinal tract) can cause a delay in larval feeding activities so larvae do not appetite and eventually die [13].

Essential oils contained in the sugar-apple fruit (*Annona squamosa* L.) have an odor that is respiratory toxin to insects. The aroma contained in essential oils will inhibit cellular respiration in the body and cause certain enzymes to become inactive. The essential oil will enter into the insect's respiratory system into the insects body cells

which can lead to activity disruption within the mitochondria [13].

Saponin is an active compound that turn into foam when shaken. Saponin is stomach poison for the larvae. The action mechanism of saponins is that it can reduce the surface tension of the mucous membrane of digestive tract. This corrosive effect will make larvae lost appetite [14].

Flavonoid is a respiratory toxin for *Aedes aegypti* L. larvae. It enters the insect body work to reduce the ability of larvae to close the the spiracle at the time of diving. This reducing ability will make the spiracles accessible to water and hence prevent larvae for doing respiration. The larvae will eventually die due to lack of oxygen. In normal circumstances the mosquito spiracle will closed and open at the time of air change [12]. In an insect that has an open trachea system, air enters the body of the insect through the spiracles, then passes through the trachea to tracheol and oxygen and then enters the body cells by diffusion, CO2 will leave the body in the same way. [12].

The tannin compound is a contact-poison for *Aedes aegypti* L larvae. The tannin compound is suspected to decrease the activity of digestive enzymes such as amylase and protease, so that the absorption of proteins can be disrupted and result in death because the presence of impaired nutrient absorption and decreasing the growth rate in larvae [11].

The symptoms of poisoning due to the administration of sugar-apple granule (*Annona squamosa* L.) can be seen through morphological observation on larva body before and after treatment. Before being treated the *Aedes aegypti* L. larvae are active, the body color is blackish brown, siphon (respirator), caput, and the digestive tract look normal. After treatment, the body of *Aedes aegypti* L. larvae became pale, siphon also showing pale shade of color and the digestive tract color changed to blackish, larval body size shorter than normal size.

The death of mosquito larvae begin with blackening of the gastrointestinal tract and eventually the digestive tract is detached. Stomach poison or gastric poison is an insecticide that kills by entering the digestive tract through the food eaten. Insecticides entering the digestive organs will be absorbed by the intestinal wall and then transfered to target sites that can be deadly according to the type of active ingredient contained in the granule [17]. Microscopic examination showed the damage of gastrointestinal tract. The active compound will also attack the exoskeleton structure of *Aedes aegyti* L. larvae which composed of chitin. Exoskeleton damage can be attributed to the degradation of chitin which is the main exoskeleton polymer. [9]

4. CONCLUSION

The value of LC_{50} of sugar-apple granule (*Annona squamosa* L.) fruit extract to Aedes aegypti L. larvae mortality is 8.25 ppm within 24 hours time exsposure. And there is significant effect of the granule to the mortality of *Aedes aegypti* L. larvae.

5. REFERENCES

Chavez – Giner, B.I dan Cannas, A. 2001. *Tannin: The Chemical Structural of Hydrolysable Tannins*. Cornert University.

Fiana, Risa Meutia, et.al. 2016. Pengaruh Konsentrasi Maltodekstrin Terhadap Mutu Minuman Instan Dari Teh Kombucha. *Jurnal Teknologi Pertanian Andalas*. Vol.20 No.2 September 2016 ISSN 1410-1920.

Harbone, J. B. 1996. *Metode Fitokimia Terbitan ke II*. Bandung: ITB.

Istiana, 2012. Resistance Status of Aedes aegypti Larvae to Temephos In West Banjarmasin. *Epidemiology and Zoonosis Journal*. Vol. 4(2); 53-58.

Jumar. 2000. *Entomologi Serangga*. Jakarta: PT. Rineka Cipta.

Kardinan, A. 2000. *Pestisida Nabati, Ramuan Dan Aplikasi*. Jakarta: PT. Penebar Swadaya.

Marianti. 2014. Pengaruh granul ekstrak daun sirih (Piper batle Linn) terhadap Mortalitas Larva Nyamuk Aedes aegypti L.https://www.scribd.com/doc/25234949/marianti-01-211-6443 (31 Mei 2017).

Parikh, 2005. *Handook of Pharmaceutical Granulation Technology Second Edition*. London: Taylor and Francis Group.

Pujiyanto, S., E. Kusdiyantini, dan M. Hadi. 2008. Isolasi dan Seleksi Bakteri Kitinolitik Isolat Lokal yang Berpotensi untuk Mengendalikan Larva Nyamuk *Aedes aegyti L. Biodiversitas.* Vol. 9(1).

Ramadhia, Muflihah. et.al. 2012. Pembuatan Tepung Lidah Buaya (Aloe vera L.) Dengan Metode Foam-Mat Drying. *Jurnal Teknologi Pertanian.* Vol. 13 No. 2 Agustus 2012

Saha, Rajsekhar. 2011. Pharmacognosy and pharmacology of *Annona squamosa:* A review. *International Journal of Pharmacy &Life Sciences*. ISSN 0976-7126.

Naria, Eva. 2009. *Insektisida Nabati Untuk Rumah Tangga*. Medan: Universitas Sumatra Utara.

Sastrodihardjo. 1984. Penghantar Entomologi Terapan. Bandung: Penerbit ITB.

Wardani, Ratih Sari. 2010. Pengaruh Konsentrasi Ekstrak Daun Tembelekan (*Lantana camara*) terhadap Kematian Larva *Aedes aegypti* L. *Journal Public Health*. Vol 6 (2).

Wati. 2014. Mengkaji Model Pengendalian Populasi *Aedes Aegypti* Dengan Sterile Insect Tehnique (SIT) dan Kombinasinya Dengan Insektisida. *Jurnal Of Natural Science*, Vol. 3 (1) March 2014. ISSN 2338-0950.

WHO. 2004. *Prevention and Control of Dengue and Dengue Haemorrhagic Fever*.

Widarto, H. 2009. Uji Aktivitas Minyak Atsiri Kulit Durian (*Durio zibethinus* Murr) sebagai Obat Nyamuk Elektrik Terhadap Nyamuk *Aedes aegypti* L. Skripsi. Surakarta: Universitas Muhammadiyah Surakarta.

Widiastuti, Dyah. 2015. Status Resistensi Vektor Demam Berdarah Dengue (*Aedes aegypti*) Terhadap Malathion 0,8% dan Permethrin 0,25% di Provinsi Jawa Tengah. Jurnal Ekologi Kesehatan. Vol. 13 No 2.