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Young and Old Sugar Apple (Annona squamosa Linn) Leaf Extracts As an Aedes aegypti Larva Insecticide

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Abstract: Vector-borne diseases are still a health problem in some tropical countries. One vector-borne disease is Dengue Hemorrhagic Fever (DHF). DHF never decreases and even tends to continue to increase, and many cause deaths in children, 90% of them attack children under 15 years. DHF cases in 2018 amounted to 65,602 cases, with 467 deaths (CFR = 0.71%). The behavior of holding water in various places such as tubs and tendons indirectly creates a breeding place for Aedes aegypti mosquitoes. The use of chemicals as insecticides can cause the death of non-target animals, environmental pollution, and the occurrence of vector resistance to insecticides. Therefore it is necessary to do other methods including the use of vegetable insecticides. Sugar apple leaves can be used to kill Aedes aegypti larvae. Sugar apple leaves will obtain throughout the year. The purpose of this study is to know the ability of young and old sugar apple leaves to kill Aedes aegypti larvae. This type of research is a posttest only control group design. The study sample was part of the Aedes aegypti larvae on the final instar III. The experiment to kill Aedes aegypti larvae use two types of sugar apple leaf extract, namely young and old sugar apple leaves with nine treatments four replications. Each treatment consisted of 25 larvae. The research results show There was no significant difference in the number of dead larvae using either extracts from young sugar apple leaves or old sugar apple leaves. LC90 of sugar apple leaf extract was between 0.05632 to 0.08324% and the effective residual age at LC90 (0.06568%) with the death of Aedes aegypti larvae was 92% over 24 hours (1 day). **Keywords**: sugar apple; Aedes aegypti

INTRODUCTION

Vector-borne diseases are still a health problem in some tropical countries. One vector-borne disease is Dengue Hemorrhagic Fever (DHF). DHF never decreases and even tends to continue to increase, and many cause deaths in children, 90% of them attack children under 15 years (Candra, 2009). The mosquito species that can be a DHF vector is Aedes aegypti. Since it first discovered in Surabaya and Jakarta in 1968, the number of dengue cases continues to spread to all regions in Indonesia. DHF cases in 2018 amounted to 65,602 cases, with 467 deaths (CFR = 0.71%)(Data and Information Center, 2015).

Residents usually store water in various places such as tubs and tendons. It estimated that each house has 5 to 6 reservoirs. The behavior of holding water is very dependent on the culture and water needs of the community. In areas where the piping system already exists, people still tend to store water as a backup if at any time the water does not flow. This habit indirectly creates a breeding place for Aedes

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aegypti mosquitoes. The results of the study showed that of the 268 containers examined; there were 82 containers (30.6%) containing *Aedes aegypti* larvae. The value of the Container Index (CI) is 30.6, and the Breteau Index (BI) is 82 (Yudhastuti et al., 2005).

Until now, vector control is more focused on the use of chemicals because they are considered more effective, easy, and fast. However, the use of chemicals can cause the death of non-target animals, environmental pollution, and the occurrence of vector resistance to insecticides. Chemical DHF control includes using Temephos (Abate®) as larvacide and malathion as adulticides. Temephos usually used in water reservoirs because of their very low toxicity to humans. The use of 1 mg/liter Temephos can control the Aedes aegypti larvae in breeding sites for 3 to 3.5 months (Kurniawan et al., 2019). The use of Temephos 1% at a dose of 1 gr / 10 liters does not guarantee the permanent removal of larvae in breeding sites because people generally do not like the smell caused by larvicides. The use of Temephos must also be routine. Vector control by chemical means requires funds that are not small and harm the environment (Hasibuan, 2004). In three subdistricts (Tambaksari, Gubeng, and Sawahan), the mortality rates were under 80%, indicating possible resistance to Temephos (Mulyatno et al., 2012). Therefore it is necessary to do other methods including the use of vegetable insecticides.

In general, plant insecticides interpreted as insecticide whose essential ingredients come from plants. Because it made from natural materials, this type of pesticide is biodegradable, so it does not pollute the environment and is relatively safe for humans and livestock. Vegetable insecticides are hit and run if applied to kill pests at that time, and after the period executed, the residue will quickly decompose in nature. The power to kill plant-based insecticides is because there are toxic substances which are stomach poisons and contact poisons in soft-bodied animals. The use of vegetable insecticides is intended not to abandon and prohibit the use of synthetic pesticides, but only an alternative method with the aim that users do not depend solely on synthetic insecticides. Another goal is to minimize the use of synthetic insecticides so that the environmental damage caused can reduce. Besides that, the use of plant-based pesticides will improve agro-industrial development, especially rural industries, new business growth, and environmental sustainability.

In Indonesia, which is a tropical country, a variety of plants can grow well, and many of them are sugar apple (Annona squamosa Linn). In the form of extracts, sugar apple is used as antifungal and antioxidant activities (Kalidindi et al., 2015), inhibiting the growth of Escherichia coli bacteria and Staphylococcus aureus (Tansil et al., 2016), antihyperlipidemic in humans (Rofida et al., 2015), and to control leaf-eating pest on soybean (Indiati, 2014). Sugar apple also used as an insecticide. Annona squamosa seed extract can be used to control Aedes albopictus and Culex quinquefasciatus (Ravaomanarivo et al., 2014).

All parts of sugar apple plants can be used as insecticides (Mondal et al., 2018). Sugar apple which contains active ingredients as vegetable insecticides are raw fruit, leaves, and roots. The active ingredient works as a contact and stomach poison and is as an insecticide, repellent, and antifeedant. Sugar apple seed extract has been used to kill Aedes aegypti larvae (Wahyuni et al., 2015). Sugar apple seeds solution with liquid electric method had used as an insecticide against the mortality of Aedes aegypti (Novasari et al., 2017). But the use of sugar apple seeds cannot be obtained throughout the year. Sugar apple seeds are only available at certain times, from January to March.

In a plant, there are usually leaves. There are parts of leaves that are still young and pieces of leaves that are old. A comparison between young leaves and young leaves of plants is not only done to determine the effect of the active compound content but also on its activity. The results of the study of Felicia et al. (2016) note that old avocado leaves have higher antioxidant activity than young avocado leaves. The difference in the age of leaves of each plant is not always the same between young leaves and old leaves. However, the presence of higher levels of active compounds can provide equal or more significant activity between immature and mature leaves (Mulangsri, 2018).

In sugar apple plants, the leaves can also use as an insecticide. The advantage is that sugar apple leaves will obtain throughout the year. It can be used to kill Aedes aegypti larvae (Purwaningsih et al., 2015). There are young leaves and old leaves on the sugar apple tree. Furthermore, it is necessary to know the ability of young and old sugar apple leaves to kill Aedes aegypti larvae.

MATERIALS AND METHOD

This type of research is a posttest only control group design. The study population was Aedes aegypti larvae on the final instar III. The study sample was part of the Aedes aegypti larvae on the final instar III. The experiment to kill Aedes aegypti larvae using two types of sugar apple leaf extract, namely young and old sugar apple leaves. Tests were carried out as many as nine treatments, both for attempting to kill larvae using young sugar apple leaves or using old sugar apple leaves. Each treatment carried out four replications. According to WHO standards for bioassay tests, each treatment consisted of 25 larvae (WHO, 2005).

Sugar apple leaf extract is made by maceration, which is soaked in ethanol solvent 70% for one night, and then the solution is taken. This treatment is carried out for three times to ensure that the leaves have extract properly. After that, the solvent evaporated with a rotary evaporator (RV10 IKA); the concentrated sugar apple leaf extract obtained.

Research procedures include carrying out preliminary experiments, determining LC50 and LC90, and determining the residual age. Initial tests were conducted to determine the types of sugar apple leaves and concentrations that could kill Aedes aegypti larvae. The determination of LC50 and LC90 was carried out to evaluate the effectiveness of larvacide toxicity through a bioassay test. Determination of residual age was carried out to determine the length of time the residue was still able to kill Aedes aegypti larvae. Data obtained by counting the number of larvae that died during the trial period. Descriptive data analysis is presented in the form of tables, while analytic data analysis is done using a t-test, ANOVA, and probit analysis.

RESULTS AND DISCUSSION

Preliminary and Follow-upTest

All treatment groups for both young and old sugar apple leaf extracts in the preliminary test showed the death of Aedes aegypti larvae. The higher the concentration of sugar apple leaf extracts given, the number of Aedes aegypti larvae that died. Aedes aegypti larvae did not die in the control and new groups. This proves that the death of Aedes aegypti larvae in the treatment group was caused by sugar apple leaf extract, not by the ethanol 70% or distilled water used in this study.

Table 1. Preliminary Test

Concentration	Death of Larvae			
	Young Sugar Apple Leaf		Old Sugar Apple Leaf	
	Average	(%)	Average	(%)
0.01563	2.3	9.2	2.6	10.4
0.03125	9	36	10.6	42.4
0.0625	21.7	86.8	22.4	89.6
0.125	24.3	97.2	24.7	98.8
0.25	25	100	25	100
0.5	25	100	25	100
1	25	100	25	100
Control (0.5 ml ethanol 70%+aquades)	0	0	0	0
Blank (aquades)	0	0	0	0

Table 2. Normality and Paired T Test (CI 95%)

	Normality Test P	Paired T Test P
0.01563	0.707	0.519
0.03125	2.500	0.067
0.0625	2.121	0.101
0.125	0.707	0.519

The results of statistical analysis for all extract concentrations using Paired T-Test showed p-value > 0.05. That means that there is no significant difference between young and old sugar apple leaf extracts at all concentrations tested to kill Aedes aegypti larvae. This shows that the content of active compounds found in young and old sugar apple leaves is the same.

Based on the analysis of variance, the difference in the average number of Aedes aegypti larvae deaths was due to the effect of the concentration level of sugar apple leaf extract.

The results of statistical analysis using the Anova Test showed a p-value <0.05. That means that there are significant differences between the various concentrations of sugar apple leaf extract tested to kill Aedes aegypti larvae. Further analysis using the LSD method found that the level of sugar apple leaf extract of 0.0586% and 0.0676% showed the number of deaths of the same Aedes aegypti larvae. Thus it can be said that the concentration of 0.0586% has a sufficient level in influencing the mortality of Aedes aegypti larvae.

Table 3. Follow-up Test

Concentration	Death of Larvae Average	%
0.0136[a]	1	4
0.0226[b]	5.25	21
0.0316[b]	11.25	45
0.0406[c]	15.75	63
0.0496[cd]	18	72
0.0586[cd]	20.75	83
0.0676[c]	24	96
Control (0.5 ml ethanol 70%+aquades)	0	0
Blank (aquades)	0	0
Anova test	0.000	

LC_{50} and LC_{90} Tests

The lowest average of dead Aedes aegypti larvae occurred at sugar apple leaf extract concentration of 0.0136%, which is one larva (4%) and the highest average of dead Aedes aegypti larvae occurred in sugar apple leaf extract 0.0676%, which is 24 larvae (96%). So we can say that all concentrations of sugar apple leaf extract used in the LC50 and LC90 tests can cause death in Aedes aegypti larvae. The higher level of sugar apple leaf extract used will cause more Aedes aegypti larvae to die.

Table 4. LC_{50} and LC_{90} Tests

Death of Larvae (%)	Concentration	CI 95%
50	0.03418	0.03418 < LC< 0.03823
90	0.06568	0.05632 < LC < 0.08324

Residual Age Test

In the residual life test, old sugar apple leaf extract used at LC90 concentration of 0.06568% with the assumption that the level was considered very useful to kill Aedes aegypti larvae.

Table 5. Residual Age Test

Concentration of Extract (%)	Number of Larvae	Death of Larvae (%) 1	2	3	4	5	6	7	8
0.06568 (LC_{90})	25	96	86	74	61	43	26	2.2	0
Control	25	0	0	0	0	0	0	0	0
Blank	25	0	0	0	0	0	0	0	0

Based on the results of the residual life test obtained by larval mortality at two days (24 hours) remaining age of 92% with 24 hours contact time. This means that the old sugar apple leaf extract with a concentration of 0.06568% has a useful residue of more than 24 hours (1 day), although for the next residual age the larval mortality has decreased. This is because plant-based insecticides are "hit and run," if applied they will kill pests at that time and after their pests killed the residue will quickly decompose in nature.

Table 4 shows that LC50 and LC90 were 0.03418% and 0.06568%, respectively. The LC50 score in this study was slightly higher compared to the survey conducted by Purwaningsih et al. (2005) in the amount of 0.00902%.

In another study, it was found that LC50 from betel leaf extract was 910.38 or 0.09104% and LC50 from Annona squamosa seed extract was 20.62 ppm or 0.00206% (Wahyuni et al., 2015). This shows that the toxicity of sugar apple seed extract is still better when compared to sugar apple leaf extract in this study (0.03148%). However, the toxicity of sugar apple leaf extract is better than betel leaf extract.

Sugar apple leaves contain annonain which are effective active ingredients as vegetable insecticides, work as stomach poisons and contact poisons against insects. The toxic effects of a sugar apple seed or leaf extract contact can see from the clinical symptoms that arise in the larvae, namely the movement becomes slow, the body shrinks and eventually dies. These symptoms indicate that the larva is running out of energy (ATP) (Wardhana et al., 2005). Known annonain is known to act as a dissolved poison or contact poison. It can inhibit electron transfer by blocking the bond between NADH and ubiquinone in the electron transfer chain in the cell respiration process, causing the metabolic energy formation process to be inhibited (Aisyafahmi et al., 2017).

CONCLUSION

There was no significant difference in the number of dead larvae using either extracts from young sugar apple leaves or old sugar apple leaves. LC90 of sugar apple leaf extract was between 0.05632 to 0.08324% and the sufficient residual age at LC90 (0.06568%) with the death of Aedes aegypti larvae was 92% over 24 hours (1 day).

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