

The Effectiveness of *Carica papaya L.* Sap and *Piper betle L.* in Control of Mosquito Larvae *Aedes Aegypti* Growth

Nuh Huda and Nur Chabibah

Nursing Department STIKES Hang Tuah Surabaya, Jl. Gadung No.1, Surabaya Indonesia

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Abstract: Natural insecticides can be used to infect *Aedes Aegypti* larvae. Natural insecticides have advantage of containing compounds that are not harmful for human and do not cause resistance, e.g. *Carica papaya L.* sap and *Piper betle L.* The research purpose was to analyze the effectiveness of *Carica papaya L.* sap and *Piper betle L.* against *Aedes aegypti* larvae death. The research was done at Entomology Laboratory of East Java Health Department. Instar III larvae *Aedes Aegypti* put in tube 250 mL, each tube was contains 25 larvae. 1 gram of *Carica papaya L.* sap and *Piper betle L.* powder put in each tube, abate as much as 0.01 gram put in tube another as positive control and last tube only contain *Aedes Aegypti* larvae as negative control. Then counted and observed the number of *Aedes aegypti* larvae death every days. Research design was using true experiment with randomized post-test only control design. Data were analysed by using one sample Kolmogorov Smirnov Test. The results showed that *Piper betle L.* and *Carica papaya L.* sap can use to control *Aedes Aegypti* larvae's growth.

1 BACKGROUND

Several diseases are associated to the mosquito-human interaction (El-sheikh, Al-fifi, & Alabboud, 2016), e.g. Dengue Haemorrhagic Fever (DBD). Dengue Haemorrhagic Fever is infectious diseases caused by dengue virus with clinical manifestations of fever, muscle pain or joint pain accompanied by leukopenia, rash, lymphadenopathy, thrombocytopenia and haemorrhagic dieses (Aru, 2009). Dengue Haemorrhagic Fever symptoms are sudden fever, lasting 2 – 7 days, redness, headache, back and stomach (Sucipto, 2011). Dengue eradication efforts can be done by breaking the spread of vector *Aedes Aegypti* larvae using insecticides (Wahyuni, 2015).

Aedes Aegypti mosquito is the main vector of haemorrhagic fever transmission (Marques & Kaplan, 2015). *Aedes Aegypti* proliferates in temporary containers and around house such as drums, jars, tanks, flower vase, bottles, tin cans, etc (Subramaniam, Kovendan, Murugan, & Walton, 2012). *Aedes Aegypti* uses human blood and clean water storage for life cycle and breeding process (Marques & Kaplan, 2015). Eradication of *Aedes Aegypti* mosquitoes is very difficult, *Aedes aegypti*

mosquito eggs can survive in latent periods without water for several months in the environment (Halstead, 2008). In the rainy season *Aedes Aegypti* mosquito in optimal condition for seedling and larval development (Rajasekaran & Duraikannan, 2012). Larval stage from *Aedes aegypti* mosquito is attractive target for pesticides because they breed and easy to kill in the water (Jacob, 2016).

Boundless use of conventional pesticides in the water source is harm to people and their environment. Repellents such as vaporizers, diethyltoluimide, and herbs are widely used in the country to combat *Aedes Aegypti* mosquito. These repellents are harmful to human health, and their use should be avoided and discouraged. Although symptoms disappear shortly after withdrawal, those who do not suffer acute toxicity symptoms and continue to use these repellents for long run may suffer neurotoxic and immunotoxin hazard (Jacob, 2016). In Indonesia, during This time the larvae of *Aedes Aegypti* only has one exterminator is themepos 1% (abate) (Wahyuni, 2015). In the long time exposure of single type insecticide will cause resistance, resurgence of pest species, environmental pollution, toxic hazards to humans and other non-target organism (Sarwar, 2009).

Resistance Phenomena cause occurrence of dengue haemorrhagic fever because larvae is resistance to themepos 1% (abate) will continue to grow into adult mosquitoes that will transmit dengue virus to humans. This is also increase incidence of Dengue Haemorrhagic Fever (Wahyuni, 2015). Based on data from East Java Provincial Health Office until June 2013, there were 11,207 dengue incidence with Incident Rate (IR) 29.25 and CFR 0.88% (99 people). In Surabaya the incidence rate is 1.504 cases with CFR 0.4% (6 persons) (East Java Provincial Health Office, 2013). Based on date from Surabaya health department; in 2013 there are 2.207 cases of dengue haemorrhagic fever, in 2014, dengue haemorrhagic fever fell dramatically to 816 case, in 2015 also decreased to 640 cases, but in 2016 experienced a significant increase up to 938 case (Surabaya health Department, 2013).

Increasing dengue haemorrhagic fever case requires needed appropriate solution such as natural Insecticide. The use of herbal product from plants is one of the main alternative to control the growth of *Aedes Aegypti* mosquito (Wulandari, 2012). Plants are rich sources for alternative agents to control of *Aedes Aegypti* mosquitoes, because they have bioactive chemicals, specific target-insects and eco-friendly, less toxic, delay the development of resistance and are easily biodegradable (Jacob, 2016). Major emphasis on the use of natural plant based product as larvacides because the constitute a rich source of bioactive chemical (Wahyuni, 2015). The advantage of natural insecticide can eradicate *Aedes Aegypti* larvae, not to cause resistance and harmless for humans, as like as *Carica Papaya L.* sap and *Piper betle L.* powder.

Carica papaya L., is species from the genus *Carica* and the plant family is *Caricaceae*. The extract of seed and leaf have were investigated larvasidal to *Aedes Aegypti* (Wahyuni, 2015). *Carica Papaya L.* sap including protease enzymes (protein decomposition) (Wahyuni, 2015). *Carica Papaya L.* sap easily decomposes in nature, has good heat resistance, can damage and break down larvae skin protein, destroys amino acid that necessary to development of larvae *Aedes Aegypti* for their growth (Wulandari, 2012). *Piper betle L.* contains Saponins and Flavonoids. Saponins and Flavonoids can decrease the surface tension of the mucous membrane tractus of the larval digestive through the lipid destruction system in the digestive tract region so that the tract wall becomes corrosive (destroyed) in the larvae (Devi & Bora, 2017). Based on the background above, the purpose of the research was to determine the effectiveness of *Carica papaya L.* and *Piper betle*

L. extract to control the growth of *Aedes Aegypti* larvae.

2 METHODS

2.1 Study Design

Methodology or the research uses true experiment. This research was conducted at the Entomology Laboratory of Health Service of East Java, addressed at Jl. Ahmad Yani No. 118 Surabaya. This research use tube with size 200 mL, scales, *Aedes Aegypti* larvae instar III, temepos (abate) powder, *Carica Papaya L.* sap, and *Piper betle L.* powder.

2.2 Study Population, Sampling, and Procedure

Sampling Technique using non probability sampling with purposive sampling approach. The dependent variable was *Aedes Aegypti* larvae and independent variable are variable are *Carica Papaya L.* sap, *Piper betle L.* powder, and abate. Research instrument was an observation sheet to find out the number of *Aedes Aegypti* larvae that died from being treated by using *Carica papaya L.* sap and *Piper betle L.* powder.

Aedes Aegypti larva obtained from the Entomology Laboratory of Health Service of East Java. The inclusion criteria of *Aedes Aegypti* larvae were larva in healthy condition, can move agile and reach Instar III, can adapt to room temperature 26-28°C. *Piper betle L.* powder obtained from t *Piper betle L.* leaf that dried after washing clean. Dried *Piper betle L.* leaf ground using mortal and sifted by using sieve 10/20 mesh. *Carica papaya L.* sap powder obtained from *Carica Papaya L.* fruit taked sap then dried by oven. Dried *Carica Papaya L.* Sap ground using mortal and sifted by using sieve 10/20 mesh.

2.3 Intervention

Each tube was filled with 100 mL of clean water. 25 *Aedes Aegypti* larvae are added to the tube. After adapt (approximately 10 minutes) the powder is added according to the respective group. The 1st group was negative control, without added anything. The 2sd group *Carica papaya L.* sap added 1 gram, the 3th group added 1 g *Piper betle L.* powder and the 4th group added abate powder 0,01 mg.

2.4 Data Analysis

The data analysed by using Kolmogorov–Smirnov Test.

3 RESULTS

Based on the results observations and calculations on larvae mortality and Bioassay test there was no death in control larvae, that the observed data on the whole group can be used for research. The data larvae mortality can be seen Table 1.

The result about normality test base on One Sample – Kolmogorov – Smirnov Test. From the table know that all variant data are normal distribution, the distribution has no variance for this variable, and can use One – sample Kolmogorov – Smirnov – Test, seen from the sign value of 0.05 (table 2).

The Differences of Samples After Treated to Know Multiple Comparison by One Sample – Kolmogorov – Smirnov Test presented that there is difference significant, as we know that the value is under 0.05 level, from the mean different colom have star, so it mean have difference (table 3).

The Effectiveness of *Carica papaya L.* sap and *Piper Betle L* in Control of Mosquito Larvae *Aedes Aegypti* Growth showed that in subset one, three groups *Carica papaya L.* sap, *Piper betle L.*, and abate had not significant differences. Giving *Carica papaya L.* sap, *Piper betle L.*, and abate has same effect to mosquito *Aedes aegypti* larvae. But if compare with control there are has significant differences (table 4).

4 DISCUSSION

This study was designed to provide an overview the effectiveness of *Carica Papaya L.* sap and *Piper betle L.* with *Aedes Aegypti* larvae deaths. According to table 1 and figure 1, the average larvae mortality rate after 1 day observation was 10 larvae (with percentage of death category 40%), and time of 2 days was 23 (with 92% mortality percentage). Percentage results at 2 days observation time can be interpreted that with 92% percentage category included in tolerant category (tolerant because of larvae death are 80-98%).

Tube 2 is the tube which added by *Carica papaya L.* sap. *Aedes aegypti* larvae on tube 2 movement after being given a lively *Carica Papaya L.* sap dust, swam

to take food at the bottom of the container and rise to the surface. Water on a tube that is fed with brownish yellow and turbid. This occurs in the bottom deposit of the container derived from *Carica papaya L.* sap dust. Death of *Aedes Aegypti* larvae is caused by *Carica papaya L.* sap exposure. *Carica Papaya L.* sap contains papain, a proteolytic enzyme that can decompose larvae skin protein. The enzyme has good heat resistance. *Carica Papaya L.* sap compounds enter through the larvae skin membrane, the number of compounds entering *Carica papaya L.* sap causes damage to skin cells. The destruction of skin cell membranes causes loss of skin membrane permeability so that other free-toxic compounds enter the body of the larvae. The large number of toxic compounds that enter causes the protein in the larval skin membrane to be damaged so that the skin as a protective body is disrupted, and causing death.

From the other research “extract of papaya leaf and seeds are effective in killing mosquito larvae *Aedes Aegypti*, indicated by the percentage of larval mortality, the observation to 48 h, the highest larval mortality in LC50: 54 ppm and LC90 : 111 ppm” (Wahyuni, 2015).

In other than, in tube 3, The larvae were very active to eat but up to 60 minutes observation did not show significant mortality. But after 24 hours or 1 day there are 15 larvae that experience death (60%), and after 2 days 25 larvae were death (100%). *Aedes Aegypti* larvae deaths were observed because they have been exposed by *Piper betle L.*

Piper betle L. contains natural toxic such as saponin, flavonoid and triterpenoid. Saponin is a compound that has the activity of binding sterol free in the digestive system, so with the decrease in the number of free sterols will affect the process of skin turnover in insects (Devi & Bora, 2017). Saponin is a toxin have properties polar, soluble in water, caused haemolysis in the blood vessels of *Aedes Aegypti* larva when enter in the body, inhibit metamorphosis process and formation of the larval skin thus resulting the death of *Aedes Aegypti* larva. Saponins can also decrease the activity of protease enzymes in the digestive tract and interfere with food absorption. If in the process of food absorption is disturbed then the nutrients obtained only slightly so that cause death (Hidayati, 2013).

Saponins have a bitter taste and can cause irritation to the stomach. The saponins compound enters the gastrointestinal tract of the larvae due to the active phase larvae for feeding. The inherited saponins entering the gastrointestinal tract of the larvae, especially can inhibit the absorption of digestive enzymes and cause larval cell damage to the

Table 1: The data of number *Aedes Aegypti* larval deaths after treatment.

Times	Control	<i>Carica papaya L.</i>	<i>Piper betle L.</i>	Abate
0	25	25	25	25
1	25	15	10	0
2	25	2	0	0
3	25	0	0	0
4	25	0	0	0
5	25	0	0	0

Table 2: Normality test base on one sample Kolmogorov – Smirnov Test.

N		D0	D1	D2	D3	D4	D5
		4	4	4	4	4	3
Normal parameter2	Mean	25.00	12.50	6.75	6.25	6.25	8.33
	Std. Deviation	.000	10.408	12.203	12.500	12.500	14.434
Most Extreme Differences	Absolute		.155	.401	.441	.441	.385
	Positive		.155	.401	.441	.441	.385
	Negative		-.155	-.290	-.309	-.309	-.282
Kolmogorov – Smirnov Z			.310	.803	.883	.883	.667
Asymp Sig (2-tailed)			1.000	.539	.417	.417	.766

Table 3: The Differences of samples after treated to know multiple comparison by one Sample – Kolmogorov – Smirnov Test.

Y tukey HSD						
95% Confidence Interval						
(I)X	(J)X	Mean Difference (I-J)	Std. Error	Sign.	Lower Bound	Upper Bound
C	C.P	18.000*	5.229	.013	3.36	32.64
	P.B	18.000*	5.229	.013	3.36	32.64
	A	20.833*	5.229	.004	6.20	35.47
C.P	C	-18.000*	5.229	.013	-32.64	-3.36
	P.B	.000	5.229	1.000	-14.64	14.64
	A	2.833	5.229	.948	-11.80	17.47
P.B	C	-18.000*	5.229	.013	-32.64	-3.36
	C.P	.000	5.229	1.000	-14.64	14.64
	A	2.833	5.229	.948	-11.80	17.47
A	C	-20.833*	5.229	.004	-35.47	-6.20
	C.P	-2.833	5.229	.948	-17.47	11.80
	P.B	-2.833	5.229	.948	-17.47	11.80

*. The mean difference is significant at the 0.05 level.

digestive system to become corrosive.

Flavonoids from *Piper betle L.* attack work system as stomach poison so *Aedes Aegypti* larva fail to recognize food stimulus, so for over time larva will death caused by starvation. Triterpenoid or triterpenes is acute toxic compound when applied topically and/ or incorporated into water. It is cause reduced feeding and increased mortality. Water colour on the tube added *Piper betle L.* green and there are many piles of betel leaf powder. To facilitate the observation, the water is filtered and lighted using a flashlight. *Aedes Aegypti* larvae movement after being given betel leaf powder is the

movement of larvae began quickly because of stress after exposure *Piper betle L.*, then the movement began to slow down as more and more toxic compounds are ingested and into the body of the larvae. Movement slow larvae when given a touch and rolled his body showed the larvae begin to experience the stage of paralysis to eventually cause death. Movement slow larvae when given a touch and rolled his body showed the larvae begin to experience the stage of paralysis to eventually cause death.

According to Table 2, the result was presented about normality test by using One Sample –

Kolmogorov – Smirnov Test. From the table 2, all variant data are normal distribution seen from the sign value of 0.05. The difference significant from 3 sample (*Carica papaya L. sap*, *Piper betle L.*, and Abate) is significant. The value is under 0.05 level and the mean different colom have star, so it's mean have difference.

According to Table 1 *Piper battle L.* more effective to reduce *Aedes Aegypti* larvae but base on Table 4, in subset one, three groups *Carica papaya L. sap*, *Piper betle L.*, and abate had not significant differences. Giving *Carica papaya L. sap*, *Piper betle L.*, and abate has same effect to mosquito *Aedes Aegypti* larvae. But if compare with control there are has significant differences. From the table 4 using abate, *Carica papaya L. sap* and *Piper betle L.* effective to reduce *Aedes aegypti* larvae. But using abate in the long time exposure is dangerous for the human and environment, so *Carica papaya L. sap* and *Piper betle L.* can use as an alternative material to reduce *Aedes Aegypti* larvae, with harmless and eco-friendly.

5 CONCLUSIONS

Based on the results obtained that *Carica Papaya L.*, *sap* and *Piper Betle L.*, can be used to minimize the growth of *Aedes Aegypti* larvae. *Piper betle L.*, is more effective than *Carica Papaya L. sap* to reduce larvae *Aedes Aegypti*. Within 2 days the number of *Aedes Aegypti* larvae that died caused by *Piper Bitle L.* powder more than *Carica Papaya L. sap*. For further research it is necessary to research the effectiveness of dosage of *Piper betle L.*, powder.

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