

Natural Insecticide Spray for *Aedes sp.*, Made from Ethanol Extract of Purple Eggplant Fruit (*Solanum melongena L.*)

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by the Dengue virus. This virus is transmitted by *Aedes sp.* Many studies have been carried out on the use of plants as biological insecticides, one of which is purple eggplant (*Solanum melongena L.*). This study aims to determine whether *Solanum melongena L.* extract spray can be used as an insecticide for *Aedes sp.* This research was a true experimental study, with a sample of 25 *Aedes sp.* 2-5 days old. The treatment given was eggplant extract spray with a concentration of 80%, 60% and 40% then left for 24 hours, with 3 replications. The concentration of 80% ethanol extract caused 84% of the deaths of *Aedes sp.*, The concentration of 60% caused 68% of deaths, and a concentration of 40% caused 44% of deaths. The p-value of the probit test was 0.000 (*Solanum melongena L.* ethanol extract in spray form significantly caused the death of *Aedes sp.*) LC₅₀ and LC₉₀ values were at concentrations of 49.124% and 81.343%. Spray of *Solanum melongena L.* ethanol extract can be used as natural insecticide for *Aedes sp.*

Keywords: Ethanol extract, *Solanum melongena L.*, Natural insecticide, *Aedes sp.*, Spray

INTRODUCTION

DHF is an infectious disease caused by the dengue virus, which is transmitted through the main vectors, namely the mosquitoes *Aedes aegypti* and *Aedes albopictus*. DHF is spread throughout the tropics including Indonesia. The spread of this disease is influenced by rainfall, temperature, and urbanization⁽¹⁾. Before 1970, dengue outbreaks occurred in only 9 countries, but subsequently became endemic in more than 100 countries in Africa, America, the Eastern Mediterranean, Southeast Asia and the Western Pacific. The highest rates of DHF occur in America, Southeast Asia and the Western Pacific, with the number of cases > 1.2 million in 2008 > 2.3 million cases in 2010. In 2013 there were 2.35 million cases in America, and 37,687 cases were Severe DHF. From 1968 to 2009, WHO reported that Indonesia was the country with the highest DHF cases in Southeast Asia⁽²⁾.

In 2015, the Ministry of Health of the Republic of Indonesia noted that the number of dengue sufferers was 129,650 cases, with the number of deaths being 1,071 (morbidity = 50.75 per 100,000 population and mortality = 0.83%). This number is greater than in 2014, namely 100,347 cases, with morbidity = 39.80⁽¹⁾. In 2015, the number of dengue cases in NTB province was 1,340, then increased to 3,385 cases, with an increase of 152.61% in 2016. The most cases occurred in Sumbawa, East Lombok and Mataram City. DHF morbidity in NTB in 2016 was 69.10 per 100,000 population. This condition shows an increase compared to 2015, even exceeding the nationally defined limit of <40 / 100,000 population, with the number of cases dying = 32 people⁽³⁾.

Indonesia has high air humidity, and this condition triggers the breeding of *Aedes aegypti* and *Aedes albopictus*. *Aedes sp.* is a diurnal animal that starts sucking blood when the sun rises (08.00 - 12.00) until before sunset (15.00 - 17.00). *Aedes aegypti* prefers human blood and is often found indoors, while *Aedes albopictus* prefers animal blood and is usually found outside the home. Population of *Aedes sp.* greatly increased in the rainy season, due to the availability of breeding sites, namely puddles of rain water in used cans, used tires, pieces of bamboo, holes in trees, places to drink birds, etc.⁽⁴⁾.

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Vector control has been carried out both mechanically, biologically and chemically. Vector control is mechanically and biologically friendly to the environment. Mosquito repellent products generally contain high concentrations of synthetic chemicals, which can interfere with human health⁽⁵⁾, so that there are currently many studies conducted on the use of plants as biological insecticides to minimize negative effects on humans. Biological insecticides have proven potential for controlling vectors, both for eradicating adult larvae and mosquitoes. In addition, these insecticides are biodegradable so they do not pollute the environment, and are relatively safe for nature, humans and livestock, because the residues of these substances quickly disappear.

The power to kill biological insecticides comes from the toxic substances they contain. These substances can be contact poisons, respiratory toxins and stomach poisons in soft-bodied animals⁽⁵⁾. Biological compounds that have functions as insecticides include saponins, tannins, flavonoids, alkaloids, steroids and essential oils⁽⁶⁾.

Purple eggplant contains an active substance that can be used as an insecticide, because it contains steroid alkaloids (solasodine, solanine, solanidine), saponins, flavonoids, tannins, coumarin. This fruit skin is rich in anthocyanin and chlorogenic acid⁽⁷⁾. Saleh (2015) reported that purple eggplant ethanol extract contained flavonoids, tannins, glycosides, steroids and alkaloids. Purple eggplant has better antioxidant activity than 5 other eggplant varieties because it contains high levels of total flavonoids⁽⁸⁾. The total flavonoid content of purple eggplant ethanol extract was $29.35 \pm 0.09\%$ b / b Equivalent of Quercetin⁽⁹⁾. Purple eggplant also contains alkaloids = 0.99 ± 0.0 mg / 100 g, tannin = 11.34 ± 0.48 mg / 100 g, and saponin = 11.63 ± 0.29 mg / 100 g (11). Kandita et al. (2015) reported that leunca fruit had an insecticidal effect on *Aedes aegypti* with a concentration of 80%. Purple eggplant is one genus with leunca fruit, so it is logical to do research on the utilization of purple eggplant fruit ethanol extract as an insecticide for *Aedes sp.*, Because purple eggplant fruit is easier to obtain in NTB and has high flavonoids⁽¹⁰⁾.

MATERIALS AND METHOD

The design of this study was Post-Test Only Control Group Design, with 3 treatments and 1 negative control, namely T0: Negative Control (Aquadest); T1:

Extract with a concentration of 80%; T2: Extract with a concentration of 60%; T3: Extract with a concentration of 40%. The population of this study were *Aedes sp.* adults obtained from egg breeding. The sample were 25 *Aedes sp.* adults 2-5 days for each group, referring to WHO guidelines, using insecticides in the form of spray, with 3 repetitions⁽¹¹⁾. The sample was chosen by purposive sampling technique. The research tools and materials were knives, trays, ovens, blenders, analytic balance, glass jars (simplicia containers), funnels, filter paper, extract storage, aluminum foil, rotary evaporator. The tools used for preparation and maintenance of mosquitoes were black buckets, filter paper, cages, containers (eggs and mosquito larvae), drops of pipettes, aspirators. The tools used for dilution of the test solution were beaker glass, measuring cup, stirring rod, mosquito cage (test barrel) measuring 30 x 30 x 30 cm³, sprayer, label, cotton, paper cup, aspirator, tweezers, tray, basin, observation sheet, purple eggplant fruit, 96% ethanol, water, sugar solution, fish pellets, *Aedes sp.* adult.

The preparation stage was preparing the eggs of *Aedes sp.*; making ovitrap using black bucket 3; insert clean water up to $\frac{3}{4}$ bucket; put filter paper on the bucket wall; put the ovitrap in dark places and leave it for 5-7 days. Filter paper containing eggs was removed and then dried.

The maintenance phase of *Aedes sp.* was soaking filter paper in a container filled with water; leave it for 1-2 days until the eggs hatch, then become larvae. Larvae develop from stage I to IV within 5 days. The larvae turn into pupae; then transferred to the cup. Each cup contains 30 pupae, then the cup was moved into the barrel, each barrel contains 2 cups. During its development period, pupae which had become adult mosquitoes were fed with a 10% sugar solution on cotton.

Making ethanol extract of purple eggplant fruit was washing purple eggplant, then slicing it thinly, then putting it in the oven at 40°C for 24 hours. The dried fruit is mashed using a fine mesh, then dissolved in 96% ethanol with a ratio of 1:5, then taken 200 grams then added to 1000 ml of 96% ethanol. The maceration container is closed and then stored for 3 x 24 hours in a place protected from direct sunlight, then stirred every 5 minutes. After leaving, then filtered, then the extract is separated from the pulp. Ethanol was evaporated using a rotary evaporator at 40°C, so that 100% extract was obtained⁽⁸⁾.

Making variations in extract concentration was: making a solution of purple eggplant ethanol extract with a concentration of 80%, 60%, and 40% from the solution of 100% purple eggplant ethanol extract using a dilution formula:

$$\text{The 1 time spray weight} = \frac{(A - B) + (B - C) + (C - D) *}{3 \text{ replicants} \times 10 \text{ sprays}}$$

The amount of spray needed is calculated by the formula:

$$\text{Amount of spray} = \frac{\text{Standard does (0.70 grams) **}}{\text{The 1 time spray weight}}$$

Note:

*The difference between each test must be <0.20 grams

**Standard dosage used at UPKV/USM Malaysia⁽¹⁰⁾.

The testing stage was to prepare 4 barrels of square test (30 cm³) and 4 spray bottles containing a solution of ethanol extract of purple eggplant fruit. Prepare 10%

sugar solution for each barrel. Move 25 *Aedes sp.* to each barrel. Spray the extract solution with a certain concentration into each barrel, on the barrel wall. Barrel 1 is sprayed using aquadest, a maximum of 10 sprays; 2-4 test barrels were sprayed using extracts with concentrations of 80%, 60% and 40%; then let stand for 24 hours, then the number of dead mosquitoes was calculated and recorded. If the number of mosquito deaths in the negative control group was <5%, it could be ignored; but if > 80%, then the test must be repeated. If the mortality of mosquitoes in the negative control group was 5% -80%, then the percentage of mosquito deaths in each dose was calculated using the Abbot formula:

$$\frac{\text{Percentage of deaths in the treatment group} - \text{Percentage of deaths in the control group}}{100\% - \text{Percentage of deaths in the control group}} \times 100$$

The data analysis stage was using a probit test with a confidence level of 95%, to prove the effect of giving purple eggplant ethanol extract and Lethal Concentration value (LC₅₀ and LC₉₀).

FINDINGS

Insecticide test results of purple eggplant fruit extract against *Aedes sp.* can be seen in table 1.

Table 1: Death of *Aedes sp.*

Extract concentration	Number of <i>Aedes sp.</i>	<i>Aedes sp.</i> Deaths (replication)			Mean	Percentage
		1	2	3		
80%	25	22	21	21	84%	
60%	25	17	18	16	68%	
40%	25	11	11	10	44%	
Negative control	25	0	0	0	0%	

Table 1 shows that the number of deaths of *Aedes sp.* the highest was at a concentration of 80%, followed by a concentration of 60%, 40% and control with the number of deaths = 0.

The relationship between the percentage of mosquito deaths with extract concentrations is shown in Figure 1.

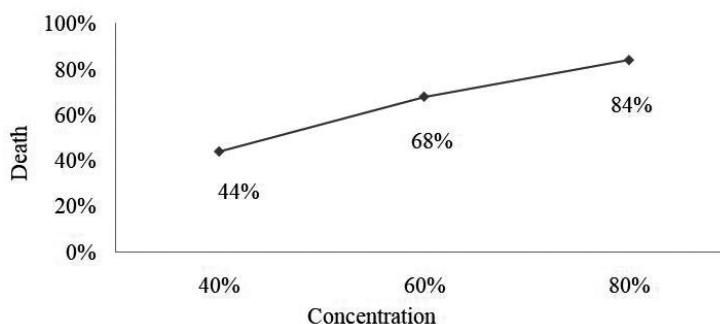


Figure 1: Relationship between the percentage of mosquito deaths with the concentration of purple eggplant extract

Figure 1 shows that the percentage of mosquito deaths was directly proportional to concentration.

Effect of purple eggplant extract on the death of *Aedes sp.* can be seen in tables 2 and 3.

Table 2: Parameter estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% CI	
						Lower	Upper
PROBIT	Concentration	0.040	0.004	9.457	0.000	0.032	0.048

Table 2 shows the p-value of the Probit test was 0.000 (purple eggplant extract caused the death of *Aedes sp.* significantly).

Table 3: Chi-square test

		Chi-Square	df	Sig.
PROBIT	Pearson Goodness-of-Fit Test	5.327	10	0.868

Table 3 shows that Pearson goodness-of-fit-test was 0.868 (> 0.500), so it was interpreted that the relationship between extract concentration and mosquito mortality was directly proportional.

The values of LC_{50} and LC_{90} are presented in table 4.

Table 4: Nilai LC_{50} dan LC_{90}

	LC	95% CI		
		Estimate	Lower	Upper
PROBIT	50	49.124	44.431	53.474
	90	81.343	75.029	90.208

Table 4 shows that extract concentrations caused 50% of the deaths of *Aedes sp.* was 49.124%, while the concentration of extract which caused 90% of deaths was 81.343%.

DISCUSSION

Eggplant powder was extracted by maceration method using 96% ethanol. The maceration method is chosen because it is a simple method of retrieval. The use of 96% ethanol aims to attract active substances namely flavonoids, glycalcaloid, saponins and tannins which are thought to have an insecticidal effect on *Aedes sp.* 96% ethanol is semipolar so it can dissolve polar and non-polar chemicals⁽⁶⁾.

Aedes sp. in this study was 2-5 days old. The age of mosquitoes is a factor that greatly influences the resistance of mosquitoes to exposure to chemical compounds, so

the age of mosquitoes is important. Referring to WHO insecticide test guidelines, 2-5 days is the best age, with good and productive body resistance⁽⁶⁾.

The results showed that the higher the concentration of purple eggplant extract given, the higher the percentage of death of *Aedes sp.* with LC_{50} and LC_{90} being 49.124% and 81.343%, respectively. Similar reports were submitted by Kandita et al. (2015) that leunca fruit extract (one genus with purple eggplant) has an insecticidal effect on *Aedes aegypti* with a concentration of 80%⁽¹⁰⁾.

Musdalifah (2016) reported that the average mortality of mosquitoes given lime peel extract with a concentration of 15% was 25%, with a concentration of 30% was 45% mortality, and with a concentration of 60% was 62% death⁽⁶⁾. These results are similar to the results of this study, that the percentage of mosquito deaths is directly proportional to the concentration of extracts of biological materials used. The higher the concentration used, the higher the active ingredient in the extract. The toxic power caused by purple eggplant extract is caused by the presence of active ingredients contained in the extract. Some bioactive compounds that are thought to be contained in purple eggplant extract include flavonoids, saponins, glycalcaloid and tannins which have proven to be contact poison and respiratory toxins in insects, especially *Aedes sp.*⁽⁶⁾.

As a phenol, flavonoids attack the nerves in some vital organs of insects, causing nerve weakness, such as respiratory problems and death. As terpenoids,

saponins can bind to free sterols in the digestive tract of mosquitoes, where sterols are precursors of the hormone ecdysone, so that if the number of free sterols decreases, there will be disruption of moulting of insects. Saponins can also destroy red blood grains and are toxic to cold blooded animals (6). Glikokalkaloid functions as a contact poison for *Aedes sp.* The ability of the glycosalkaloid to bind the 3 β -hydroxy sterol membrane can interfere with membrane function. This substance also inhibits the action of the acetylcholinesterase enzyme, giving rise to the accumulation of acetylcholine in the nervous system, which in turn causes digestive defects, nervous disorders and death⁽¹⁰⁾.

Utilization of biological compounds is relatively safe for the environment and humans, because it is easily biodegradable so that the residue quickly disappears. In general, the function and effectiveness of insecticides is directly proportional, meaning that the higher the dose of insecticide, the higher the chance to control insects. Even though there are no studies that directly explain the effects of using natural insecticides on human health, its application in the environment must remain under control. For now, the use of natural insecticides is an alternative to controlling insects in households safely, and helps minimize the risk of environmental damage.

CONCLUSION

Based on the results of the study it can be concluded that purple eggplant fruit extract is effective as an insecticide for *Aedes sp.*

Conflict of Interest: This research is free from conflict of interest

Ethical Clearance: This study has been equipped with a certificate of ethical clearance from the health research ethics committee

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