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The effectiveness of arabica coffee (*Coffea arabica* L) grounds on mortality and growth of *Aedes aegypti* Larva

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Abstract
Background: Control of *Aedes aegypti* mosquito by using synthetic insecticides has potential effect to environmental pollution and increase the resistance of target vectors. It is needed to search the proper methods in controlling *Aedes aegypti* which is environmentally friendly.
Materials and Methods: This study used coffee grounds as inhibiting factor in mortality process and inhibiting the growth of *Aedes aegypti* larva. This study was an experimental design, conducted in June-August 2016 in the laboratory of Environmental Health, Polytechnic of Health Banda Aceh. The doses were 28/liter, 30g/liter, 31g/liter, 33g/liter, and 34g/liter. The mortality data were analyzed by ANOVA, followed by Least Significant Difference (LSD) test and probit test. Growth data were analyzed by using GI (Growth index) and RGI (Relative growth index).
Results and Discussion: The results showed that the grounds of arabica coffee was capable of causing the death of *Aedes aegypti* larva (p=0.00). The treatments with coffee ground also caused a decrease in GI and RGI values, which means that it inhibited the growth of larva.

Keywords: natural insecticide, mortality of larva, coffee grounds, *Aedes aegypti*

Introduction
Dengue fever (DF) is a vector-borne disease caused by four distinct serotypes of dengue virus (DEN 1-4). It is transmit among people through bite of female *Aedes* mosquito [1, 2].
In Indonesia, DF is one of the emerging diseases and remains a major and growing public health problem. The first reported DF cases were in 1968, in Surabaya and Jakarta and the number of its cases was increasing from year to year. In 1997 all provinces in Indonesia already reported the DF cases [3, 4]. Aceh is a province with DF as a public health problem as well and one of DF endemic province in Indonesia. The main preventive way to control DF cases by controling *Aedes aegypti*. The control can be conducted on adult mosquitoes or larva.[5]
Control of larva can be done by chemical, biological, and physical methods. One of the larvacides that can be used in chemical control is temephos, which is a type of organophosphate insecticide. Excessive use of temephos insecticides can cause water pollution and cause insect resistance to insecticides. Research on temephos and malathion 0.8% insecticides used over a long period of time caused *Aedes aegypti* resistance [5]. The use of natural insecticides is another alternative that is safer for the environment.
Coffee is one of beverages that comes from the processing and extraction of coffee bean seeds. In Aceh Province, drinking coffee is a habit and lifestyle, this is marked by the number of coffee shops, with a serving of filtered coffee or brewed coffee. Coffee that has been used produced coffee grounds which only been thrown away which can actually be used as natural larvicides which is safer for the environment.
Research used coffee grounds for the mortality test of *Aedes aegypti* had been conducted by Kardinan (2004) and found that coffee ground can kill *Aedes* larva [6]. The chemical compound in Robusta coffee are alkaloids, saponins, flavonoids and polyphenols [7]. Research-based arabica coffee conducted by Gunalan *et al*. found that coffee grounds contain tannins (special variety-A, while kumbakonam varieties do not contain tannins), alkaloids, flavonoids, koumarins, quinones, phenols, and essential oils. These compounds cause various reactions in the body of the larva so that it can interfere with the growth and development of larva [8].
Based on the background, a study will be carried out on the effectiveness of coffee grounds in

The process of mortality and growth inhibition of *Aedes aegypti* larva.

Materials and Methods

The design of this study was quasi experiment with post test only control group design. The experiment was conducted in the environmental health laboratorium, Department of Environmental Health, Banda Aceh Polytechnic of Health of The Ministry of Health, Indonesia, from June to August, 2016.

The determination of dosage was based on the results of Sato study, which found that the coffee ground concentration of 4.7gr/ 150 ml was the best concentration in inhibiting the development of *Aedes* mosquito eggs [9]. The calculation of concentration levels of Lethality Concentration (LC) 50-24 hours based on treatment in the sublethal toxicity test by Hubert,[10] who obtained a sequence of concentrations of 28gr/liter, 30gr/liter, 31gr/liter, 33gr/liter and 34gr/liter. The number of replications was calculated by the Federer formula: (t-1) (r-1) ≥15 and the repetition was 4 times.

Larva of *Aedes aegypti* was obtained from the results of rearing in the entomology laboratory of Bogor Agricultural Univesity (IPB), Indonesia. The sample size for each treatment and replication was 30 larva. The larva used for the test were instar III.

The test consists of a preliminary test to ensure that the dose range used has met the requirements for calculating the LC50 value. The preliminary test did not use repetition. The next test was a toxicity test to determine the effectiveness of the extract against the death of mosquito larva due to the administration of coffee grounds.

All test stages use five levels of extract concentration and one control

Tests on larval population growth by the method of Zhang *et al*. [11] The concentration of extract tested was three sublethal concentrations, namely concentrations below the LC50 value. The growth parameters used are GI (Growth Index) and RGI (Relative Growth Index) with the formula [11]:

$$GI = \frac{[n(I\ max)xI\ max + [n'(i)x(i-1)}{N\ x\ i\ max}$$

GI = grouwth index
I = number of stage
n (i max) = number of alive larva at the i max stage
n'(i) = number of dead larva at i stage
i max = highest stadium achieved by larva
N = total number of larva in the test group

$$RGI = \frac{GI\ treatment}{GI\ control} x\ 100\%$$

The larvas that used in the growth test were instar I. Data were obtained from the toxicity test and analysis using ANOVA and the Least Significant Difference (LSD) advanced test. The toxicity of coffee grounds against larva was presented with LC50 values obtained from probit analysis.

Results

1. Preliminary Test Due to the Treatment of Coffee Grounds

The results of measurements of room temperature during the test time, both the preliminary test, the main toxicity test, and the sublethal effect test, ranged from 28 °C and 30°C. The results of the preliminary test are presented in Table 1.

Table 1: The mortality of Aedes larva after the treatment of various levels of concentration for 24 hours

Concentration of coffee grounds (gr/liter water)	No. Of larva	No. of dead larva	Mortality (%)
0 (control)	30	0	0.00
28	30	2	6.67
30	30	6	20.00
31	30	11	36.67
33	30	14	46.67
34	30	23	76.67

Table 1. shows that larval mortality values increase with increasing concentration. This coffee grounds can cause 76.67% mortality of *Aedes aegypti* larva at of 34gr/liter of water. Coffee grounds extract concentration of 28gr/liter was able to kill 6.67% of *Aedes aegypti* larva. This data then used as a benchmark to determine the concentration used in the follow-up test. The dosage range used in the preliminary test was eligible for calculating the LC50 value. The data in table 1 shows that larval mortality was in the range 0-76.67%, so that the data can be used to estimate the LC50 value.

2. Advanced test for the coffee grounds toxicity

Further tests to see the toxicity of coffee grounds extract against *Aedes aegypti* larva in 4 replications is presented in Table 2.

Table 2: The toxicity of coffee grounds extract towards dead of *Aedes aegypti* larva

Concentration of coffee grounds (gr/l of water)	Total number of larva	No. of dead larva								Alive larva	Average	%
		R1		R2		R3		R4				
		N	%	n	%	n	%	N	%			
0(control)	30	0	0,00	0	0,00	0	0,00	0	0,00	0	0	0,00
28	30	5	16,67	4	13,33	4	13,33	2	6,67	15	4	12,50
30	30	9	30,00	5	16,67	9	30,00	10	33,33	33	8	27,50
31	30	12	40,00	9	30,00	11	36,67	10	33,33	42	11	35,00
33	30	13	43,33	11	36,67	13	43,33	11	36,67	48	12	40,00
34	30	21	70,00	20	66,67	22	73,33	20	66,67	83	21	69,17

R1 = Replication 1 R2 = Replication 2
R3 = Replication 1 R4 = Replication 4

Table 2 presents data on the number of *Aedes aegypti* larva which died in each treatment. The highest number of deaths was obtained from the results of the first treatment dose 34gr/liter of 70%, and the lowest number of deaths resulted

from the treatment of the second and third repetitions of 28gr/liter, amounting to 13.33%. The results of the probit analysis get the regression line equation: Y = 8.963x - 4.9721.

The results of the probit regression calculation of the effect of the coffee grounds concentration on probit were presented in Figure 1.

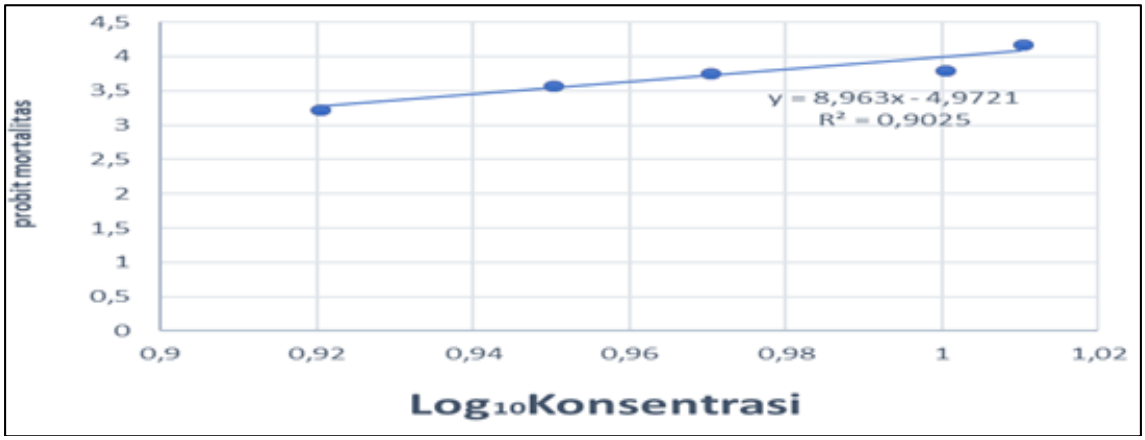

Fig 1: Log10 concentration of coffee grounds toward probit

Figure 1 shows that the probit 5 value (50% mortality) was found at the Log 10 concentration of 0.95. The results of the probit analysis found that the value of LC50 coffee grounds was 33.66 gr/liter of water. The results of the calculation of growth tests using the 1st instar larva are presented in Table 3.

Table 3: Growth relative index of Aedes aegypti larva after treatment

Coffee grounds dosage (gr)	iI		iII		iIII		iIV		No. Of alive larva	No. of dead larva	GI	RGI (%)
	A	D	A	D	A	D	A	D				
0	30	0	30	1	29	0	29	0	29	1	0.99	100
2.5	30	1	29	5	24	2	22	7	15	15	0.83	84
5	30	6	24	5	19	2	17	9	8	22	0.76	76
10	30	9	21	7	14	5	9	5	4	26	0.71	72

i: treatment **A:** Alive larva **D:** dead larva

Discussions

LC50 of coffee grounds against *Aedes aegypti* larva was 33.66gr/liter of water with a 24-hour of observation time. The LC50 of coffee grounds showed that the coffee grounds was toxic to *Aedes aegypti* larva especially the instar III. The results of this research found that coffee grounds contained secondary metabolites in the form of alkaloids, flavonoids, folifenol, saponins, triterpoid and tannins. These compounds synergize and cause mortality of *Aedes aegypti* larva. Alkaloid compounds, flavonoids, and tannins have the potential to be anti-eating, can inhibit parasympathetic nerves in the nervous system of insects, have a bitter and sharp taste and can cause stomach irritation when eaten. This compound is also able to reduce the activity of digestive enzymes and inhibit the absorption of food when consumed by insects (compounds that inhibit the eating process but do not kill directly) [12]. Another content of coffee extracted with ethanol is alkaloids, flavonoids and tannins [13, 14].

Table 3 showed that there was an increase in the mortality of *Aedes aegypti* larva along with the increase dose of coffee grounds. The results showed that there was a linear relationship between the doses of coffee grounds and the mortality of *Aedes aegypti* larva. The probit regression equation was Y = 8.963x – 4.9721 with an effective probit of 33.66gr / liter of water. The close relationship was indicated by the R2 correlation value of 0.94. R2 value of 0.94 means the percentage of the effect of the coffee grounds variable on larval mortality was 94.0% and the remaining 6.0% was influenced by other variables. LC50 is a concentration that can cause the death of 50% of the test animal population [15]. The results showed that with a concentration of 34gr/liter of water able to kill 76.67% of the *Aedes aegypti* population, so that it could be concluded that coffee grounds is toxic to *Aedes aegypti* larva.

Based on Table 3, the GI values and RGI values decrease in line with the increasing concentration of extracts test. A high concentration of extract will cause a low GI value. At a dose of 5gr the GI value dropped down to 0.76 from 0.83. The decrease in GI value caused by this treatment was also followed by a decrease in the RGI value. This showed that the coffee ground was able to inhibit the growth of larva. The presence of coffee grounds causes the instar I to be unable to change the skin towards the instar II larva. At higher doses, cause the death of larva. This result was in accordance with Fidrianny *et al* (2016) study that the ethanol extract of arabica green coffee grounds has insecticidal activity indicated by LC50 values between 0.70-134.56µg /ml [15].

The ANOVA results obtained p value of 0,000 which showed that the coffee grounds treatment given had a significant effect on the mortality of *Aedes aegypti* larva. The LSD test found that the dose of 34gr/liter of water was the most effective dose of killing test larva of 69.17%, this value was significantly different from the value of the results of other doses (p <0.05). The treatment of coffee grounds with doses of 31gr/liter of water and 33gr/ liter of water was able to kill larva by 35% and 40%, but the LSD test showed that these two numbers did not differ significantly (p> 0.05).

Several other studies have been conducted to test the

effectiveness of the use of coffee grounds against larval death. Grounds of coffee left after drinking coffee is used for *Aedes aegypti* mortality [16, 14, 9].

The chemical compounds in Robusta coffee are alkaloids, saponins, flavonoids and polyphenols. Whereas Arabica coffee based on the research of Gunalan, *et al* (2012) contains: tannins, alkaloids, flavonoids, koumarins, quinones, phenols and essential oils [8]. These compounds cause various reactions in the larva's body so that they can disrupt the growth and development of larva [17]. Research others also get the results of the leftover coffee that has been roasted to produce chemicals that can reduce the life of Ae larva. Aegypti [16].

Saponins are bioactive compounds that act as toxin substances. Saponins are included in the contact poison class because they can enter through the body walls of the larva. This substance is also classified as a stomach poison that enters through the mouth. Saponins have detergent-like properties so they can increase the penetration of toxins because they can dissolve lipophilic substances in water. Saponins can also irritate the digestive tract mucosa. In addition, saponins also have a bitter taste that decreases the appetite of larva, then the larva will die by starvation [18].

Saponin and flavonoid compounds can inhibit the growth of brain hormones, namely hormone edicone, which with no development of these hormones can inhibit larval growth [19].

Conclusion & Suggestion

Coffee grounds can increase mortality and inhibit the growth of *Aedes aegypti* larva. There was a significant influence on the average mortality of *Aedes aegypti* larva from various doses of filtered coffee grounds.

The research conducted in this study is still laboratory scale, which has shown that coffee grounds can be used to control *Aedes aegypti* larva. Further research is needed to test whether this coffee grounds can be applied to control the *Aedes aegypti* population in field scale. In addition, other studies needed to be done on the effectiveness of this coffee grounds against other types of mosquito larva such as *Culex*, *Anopheles* and *Mansonia*.

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