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EFFECTIVIY OF THE JACKFRUIT LEAVES INFUSION (*ARTOCARPUS HETEROPHYLLUS* L.) AS A LARVACIDE TO THE MORTALITY RATE OF *AEDES AEGYPTI* LARVA

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ABSTRACT

Larval stage control using temephos, a chemical larvicide powder, was not completely safe. WHO recommended to use biological controls. Jackfruit leaves which contain saponins, flavonoids, and tannins can be used as alternate. This study was conducted to determine the effectivity of jackfruit leaves (*Artocarpus heterophyllus* L.) infusion on the mortality of *Aedes aegypti* larvae using post test only control group design. Six hundreds of *Aedes aegypti* larvae were divided into 1 control group and 5 experiment groups that consist jackfruit leaves infusion concentrations of 4%, 8%, 12%, 16%, 20% with four times replication. The mortality of larvae was observed every 8 hours to 48 hours. The LC_{50} and LT_{50} were obtained by probit analysis were 3.842% and 2.150 hours. In the repeated Kruskal-Wallis test and Mann-Whitney, there were significant differences ($p<0.05$) in the mortality of *Aedes aegypti* larvae that died based on differences in the concentration of jackfruit leaf infusion. In the Spearman test, there were significant correlations ($p<0.05$) between the concentration of jackfruit leaves infusion and the mortality of *Aedes aegypti* larvae and the death rate of *Aedes aegypti* larvae. The results of the study are expected to be alternatives to chemical larvicides commonly used by the community.

Keywords: *Aedes aegypti*; *Artocarpus heterophyllus* L.; Jackfruit leaf infusion; Larvacide

INTRODUCTION

Dengue Haemorrhagic Fever (DHF) is one example of the Indonesian public health problems with a various number of sufferers and spreading areas. The disease that were caused by dengue virus is carried by *Aedes aegypti* mosquito as one of its main vectors.[1] Various methods have been used to prevent and overcome DHF, one of the example is by controlling *Aedes aegypti* mosquito vector through eradication of mosquito nests, larva, and the adult stage.[2]

The mosquito vector populations control that performed during the larva stage is easier compared to the other stages.[3] The eradication of the larva stage that commonly use *temephos* chemical larvicide powder with the trademark abate® apparently is not completely safe, because it can cause several side effects such as poisoning reaction, environmental pollution, and resistance.[4] Therefore, the World Health Organization (WHO) recommends seeking for a breakthrough, through biological or environmental control.[5]

One example of the biological control using the natural ingredients from plants is the utilization of jackfruit leaf plants (*Artocarpus heterophyllus L.*) which the extracts were proven to affect the mortality of larva *Culex sp.*[6] Based on the previous research, jackfruit plant leaves contain saponins, flavonoids, and tannins.[6] These three substances are predicted to have larvicidal effects. The chosen form of extract is infusions because it was easy to make and easier to applicate.

MATERIAL AND METHODS

This study is a true experimental laboratory study using a post-test only control group design held in the Basic Laboratory Faculty of Medicine,

Diponegoro University on May. The samples that used in the study were obtained from the larva breeders on Urip Sumoharjo Ungaran Street that met the inclusion and exclusion criteria. The inclusion criteria used in this study were healthy *Aedes aegypti* larvae that had reached instar III / IV and active. While the exclusion criteria are larvae that have turned into pupae or adult mosquitoes and larvae that die before being treated.

The study was conducted by analysing the results of observations in the control group and treatment group. Prior to the treatment, randomization was performed in all groups. The sample was divided into 6 groups, 5 treatment groups and 1 control group. Five treatment groups were given larvacides with various concentrations, namely 4%, 8%, 12%, 16%, and 20%. While the control group was not given larvacide as a comparison. Observations were carried out every 8 hours to 48 hours. The dead larvae were counted per time. Six hundred larvae were used in this study for each group was repeated four times, each container contained 25 larva. Subsequently, observations were performed to see the number of larvae that died every 8 hours to 48 hours.

This study uses the administration of jackfruit leaf infusion with various concentrations and time spans of observation as the independent variables. While for the dependent variable was *Aedes aegypti larvae* mortality determined by Lethal Concentration 50 (LC_{50}), Lethal Time (LT_{50}), and the speed of larval death (tail / hour).

The observations were processed using *probit* analysis to determine LC_{50} and LT_{50} values, and the repeated Kruskal-Wallis test followed by Mann-Whitney to assess differences between groups along with the Spearman correlation test to assess the correlation between concentration and the number of larvae that died in each observation hour and the speed of larval death.

RESULTS

After 8 hours of observation, larvae death was found at all concentrations of jackfruit leaf infusion. The average number of dead larvae increase along with the increasing concentration.

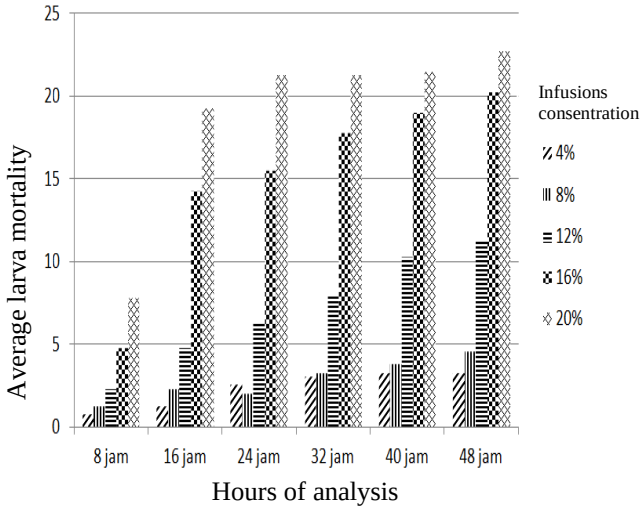

Figure 1. Average Larva Mortality

From the *probit* analysis it was found that the LC_{50} value or the concentration of jackfruit leaf infusion that can cause 50% of larvae death is 3.842%. Whereas the LT_{50} value or time needed for jackfruit leaf infusion to cause 50% death of larvae is 2,150 hours (Table 1).

Table 1. Analysis of LC_{50} and LT_{50} Scores

	95% Confidence Limits		
	Mean	Minimum	Maximum
LC_{50} (%)	3,842	3,340	4,254
LT_{50} (jam)	2,150	0,055	4,334

Table 2. Repeated Kruskal-Wallis Test Results

Hours of analysis	P
8 hours	0,001
16 hours	0,001
24 hours	0,000
32 hours	0,001
40 hours	0,001
48 hours	0,000

In this study, the difference between the number of dead *Aedes aegypti* larvae based on differences in the concentration of

jackfruit leaf infusion was assessed using the repeated Kruskal-Wallis tests (Table 2) and there was a significant difference ($p < 0.05$) between the study groups. The Mann-Whitney test between each concentration group and the hours of observation were performed to find out which group has the difference.

From the Mann-Whitney test, it is known that all p values were <0.05 except between the concentrations of 4% and 8% at all observation hours, and the concentrations of 8% and 12% at 8 hour of observation. This shows significant differences in the number of larval mortality between concentration groups every 8 hours of observation.

The Spearman correlation test was used to see the correlation between concentration and increases in the number of the dead larvae. This test was chosen because the data were not distributed normally. From the Spearman correlation test, it was found that $p < 0.05$, which means that increases on the infusion concentration were positively correlated with an increase in the number of larvae that died at each hour of observation and were strongly correlated (0.960-0.981).

The highest larval mortality rate in this study was on the concentration of 20% during 16-hour of observation, it was 4,813 larvae / hour and the lowest speed was at the concentration of 4% during 48-hour of observation, it was 0.271 larvae / hour (Table 3).

Table 3. Analysis of the Larva Mortality Rate

Hours of Analysis	The concentration of jackfruit leaves infusions				
	4%	8%	12%	16%	20%
8 hours	0,375	0,625	1,125	2,375	3,875
16 hours	0,313	0,563	1,188	3,563	4,813
24 hours	0,417	0,334	1,042	2,584	3,542
32 hours	0,375	0,406	1,000	2,219	2,656
40 hours	0,325	0,375	1,025	1,900	2,150
48 hours	0,271	0,375	0,938	1,688	1,896

Spearman correlation test was also performed to determine the relationship between concentration and larval mortality rate per hour (v). From the Spearman correlation test, it was found that $p < 0.05$ on the concentrations of 12%, 16%, and 20% (Table 4), which means an increase in infusion concentration was positively correlated with an increase in mortality rate and was strongly correlated (0.886-0.943).

Table 4. Spearman Correlation Test results between the larva mortality rate (v) and the increases of consentration

	Spearman Correlation Test		
	v 12%	v 16%	v 20%
Coefficient correlation	0,886	0,829	0,943
P value	0,019	0,042	0,005

DISCUSSION

DHF is one example of Indonesia's public health problems with a various number of sufferers and spreading area.[1] Various methods are used to prevent and control DHF, one of which is by controlling the *Aedes eagypti* mosquito vector at the larval stage using chemical larvaside *temephos*, with the trademark of abate. [2.4] Instar III / IV *Aedes aegypti* larvae were chosen as the subject because their anatomical structures were grown completely and clearly, consisting the head (chepal), the chest (thorax), and the abdomen (abdomen).[7]

The larvicide material chosen in this study were the jackfruit leaves in the form of infusion. The infusion concentrations used in this study were 4%, 8%, 12%, 16%, and 20%, according to the previous studies that used bay leaf infusions which contain saponin and tannin compounds against the *Aedes aegypti* larvae.[8] Observation to see the number of the dead larva was carried out for 48 hours according to the length of development of larval instar III / IV to pupae which lasted for 2-3 days. [9-11] The larvae were considered dead if it does not move, does not respond when stimulated, and does not swim to the surface of the water. In this study stimulation was given in the form of shaking the plastic containers

for larval media to assess whether the larvae still responds to stimuli or not.[20]

In this study, the results of the descriptive analysis showed that there were larvae that died at concentrations of 4%, 8%, 12%, 16%, and 20% with an average that continued to increase along with increasing concentration and hours of observation. While in the control group contained aquades there were no dead larvae found. This shows that the confounding variables such as temperature, humidity, pH, and rainfall in this study were able to be controlled.[11] When compared to the temephos which causes 100% of larval death since 3 hours of observation, jackfruit leaf infusion is less effective as a larvicide. This occurs because the temephos is a compound of organophosphate group synthesis that acts as an inhibitor of acetylcholinesterase. In mosquito larvae, organophosphate compounds cause larvae to experience tremors / shaking and uncontrolled movements.[12,13]

Lethal Concentration (LC_{50}) values obtained from the statistical analysis showed that the toxicity of jackfruit leaf infusion against *Aedes aegypti* larvae was large, thus the number of larval deaths were increased.[14] Low larvae LC_{50} values indicate that the effectiveness of larvicides was good because despite the raw material amount was small but the larvicidal power was high.[15]

The low Lethal Time (LT_{50}) value shows that the larvicide material contained in the jackfruit leaf infusion is poisonous, thus the rate of infection becomes faster. This happens due to a higher level of toxin that being exposed with tested larvae, therefore the required time to kill 50% of the test larva gets faster.

This study was conducted by observing the disparity between the number of died *Aedes aegypti* larva based on differences in the concentration of the jackfruit leaf infusion, a significant difference was obtained ($p < 0.05$) between the study groups in the repeated Kruskal-Wallis test based on the results and analysis of the data. Likewise in the Mann-Whitney test, significant differences of larva mortality between each concentration group for every 8 hours of observation was reported. This shows that each concentration of jackfruit leaf infusion affects the number of *Aedes aegypti* larva's death. According to the qualitative phytochemical test result, the arising effect is likely to occur due to the presence of active substances such as saponins, flavonoids, and tannins on the jackfruit leaves.[6] Based on the literature, jackfruit leaves that were extracted using ethanol and potassium hydroxide solvents contain saponins dominantly.[16] In the digestive system, saponins can reduce the activity of enzymes and absorption of food and act as stomach poisoning.[17–19] Flavonoids inhibits the eating process on insects and causes toxicicity.[20] Tannins play a role in reducing the ability of insects to digest food by lessening the activity of the digestive enzymes (proteases and amylases) and interfering the intestinal protein activity, consequently causing a decrease in growth and nutritional disorders.[17,19]

The results of *Spearman* correlation test showed that p is less than 0.05, which means an increase in the infusion concentration is positively and strongly correlated with an increase in the number of the dead larva (0.914-0.990). If the concentration used in the treatment is higher, then the number of dead larvae will increase.[21] This happens since higher concentration of solution will make the amount of active substances that cause metabolic, respiratory, and digestive disorders in the insect's body also gets greater.[6]

From the analysis of the larva death rate, it was reported a difference between the speeds depending on the concentration. This is consistent with previous research that stated the efficacy of insecticides to kill insects depends on the concentration or amount (dose) of insecticides.[22]

The Spearman correlation test showed that p was less than 0.05 at concentrations of 12%, 16%, and 20%, which means that an increase in the infusion concentration was positively and strongly correlated with an increase on larva mortality rate (0.886-0.943). The higher concentration of the solution will affect the death rate of targeted

organisms due to a greater accumulation of toxins caused by these insecticides.[21] Therefore, positive correlations were only obtained at concentrations of 12%, 16%, and 20%.

This study found that the infusion of jackfruit leaves affect the death of *Aedes aegypti* larva. This is caused by the presence of saponin, flavonoid, and tannin content in jackfruit leaves as they were obtained in qualitative phytochemical tests. Weaknesses and limitations in this study were the color changes in infused water and no quantitative phytochemical tests performed to see the dominant components and the components of each substance. Thus, jackfruit leaf infusion can be considered as an alternative to the regular chemical larvicides that were commonly used by the community.

CONCLUSION

The value of LC_{50} or concentration of jackfruit leaf infusion (*Artocarpus heterophyllus* L.) that will cause 50% death of *Aedes aegypti* larvae is 3.842%. Whereas the LT_{50} value or the time needed for infusion of jackfruit leaves (*Artocarpus heterophyllus L.*) to cause 50% death of Aedes aegypti larva is 2,150 hours. This study found a significant difference in the number of death on *Aedes aegypti* larvae based on differences in the concentration of jackfruit leaf infusion (*Artocarpus heterophyllus L.*) and a significant difference in the number of *Aedes aegypti* larvae that died per time (tail / hour) based on differences in the concentration of jackfruit leaf infusion (*Artocarpus heterophyllus L.*)

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