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Effectiveness of Combination Concentration of Lime Leaves Extract and Galangal Rhizome on Mortality of Aedes Aegypti Larvae

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Abstract: ***Dengue Hemorrhagic Fever (DHF) is a contagious disease and often appears to be Extraordinary Event as it spreads very fast and can cause death. Efforts to prevent DHF can be done by breaking up the life cycle of chemical vectors by utilizing lime leaves (citrus aurantifolia) and galangal rhizomes (Alpinagalanga) which have the potential as natural larvicides because they contain chemical compounds consisting of limonoids, flavonoids and phenols. The City of Kupang is a yearly endemic area of dengue where the case of the disease has increased in the last three years. This study is aimed at determining the effectiveness of the combination of the concentration of lime leaf extract and galangal rhizome on the mortality of Aedesaegypti larvae. This type of research is a pure experiment using a completely Randomized Design as the research design. There were three treatments of concentration used, namely 0% concentration, LC_{50} combination of lime leaf extract and galangal rhizome, LC_{90} combination of lime leaf extract and galangal rhizome with a number of repetitions three times. The results of the study, the combination of LC_{50} of lime leaf extract and galangal rhizome was able to cut off the life cycle of 19 larvae (76%), while the combination of LC_{90} of lime leaf extract and galangal rhizome was able to completely cut off the larval life cycle of 25 larvae (100%). The result of the One-Way Anova Test analysis on the treatment of a combination of LC_{50} and LC_{90}extracts of lime leaves and galangal rhizomes to Aedes Aegypti larvae mortality during 24 hours obtained sig (p) = 0.00 (p <0.05) showed that the combination of LC_{50} extract lime leaves and galangal rhizome and LC_{90}combination of lime leaf extract and galangal rhizome have the effect of treatment on the number of larvae deaths of Aedesaegypti instar III and IV. The Least Significant Difference Test (LSD) analysis of the three treatment concentrations of Aedesaegypti larvae mortality showed a difference in treatment because a sig (p) = 0.00 (p <0.05) was obtained.***

Keywords: Combination of Concentration of lime LeafExtract, Combination of Galangal Rhizome Extract Concentration, LC_{50}, LC_{90}

1. Introduction

Dengue Hemorrhagic Fever (DHF) is a contagious disease and often appears to be an Extraordinary Event (EE) as it spreads very quickly and can lead to a fatal death. DHF is a disease caused by dengue virus that is classified as Arthropod-Borne Virus, genus Flavivirus, and family Flaviviridaealbopictus. DHF can appear throughout the year and canattach all age groups. The disease is closely related to environmental situations and people's behavior (Ministry of Health Republic of Indonesia, 2015).

DHF cases in the last four years in Indonesia are as follows, in 2015, the number of DHF sufferers were 129,650 cases with the number of deaths of 1,071 people (CFR: 0.83% and IR: 50.75 / 100,000 population) (Ministry of Health Republic of Indonesia, 2016). In 2016,thecases were 201,885 with the number of deaths 1,585 people (CFR: 0.79% and IR: 77.96 / 100,000 population) (Ministry of Health Republic of Indonesia, 2017). In 2017, cases reduced to 59,047 with 444 deaths (CFR: 0.75% and IR: 22.54 / 100,000 population)) (Ministry of Health Republic of Indonesia, 2018). In 2018, the numbers of dengue cases reached 65,602 cases with the number of deaths were 462 people (CFR: 0.70% and IR: 24.73 / 100,000 population).

In 2014-2018 the spread of dengue cases in NTT Province fluctuated, in 2014 there were 487 cases (10 / 100,000 population), in 2015 there were 665 cases (13 / 100,000 population), in 2016 there were 1,213 cases (23.3 / 100,000 population) , in 2017 there were 542 cases (10.3 / 100,000 population), and in 2018 there were 1,333 cases with 12 deaths (Ministry of Health of the Republic of Indonesia, 2018).

Plants that can be used as biolarvasides in *Aedesaegypti* larvae are galangal and lime leaves which are thought to contain flavonoid, limonoid, phenol compounds that function as larvasides in the control of DHF vectors (Noshirma and Willa, 2016).

2. Research Methodology

This study used larvae of *Aedesaegypti* instar III and IV. The characteristics of *Aedesaegypti* instar III mosquito larvae were 4-5 mm in size or 3-4 days after hatching eggs, chest hairs were clearly visible and the mouthpiece of blackish brown breathing, while IV instar larvae have characteristics of 5-6 mm in size, already had a head, chest and stomach.

There were two types of tests used in this study- the preliminary test and the main test. Preliminary test was aimed to determine $LC_{50}$and LC_{90} extracts of lime leaf and galangal rhizomes against the mortality of *Aedesaegypti* larvae. The concentration used in the preliminary test for the LC_{50}determination of lime leaf extract was 630 ppm, 640 ppm, 650 ppm, 660 ppm and 670 ppm, while the concentration to find out the LC_{90} of lime leaf extract was 1080 ppm, 1090 ppm, 1100 ppm, 1110 ppm and 1110 ppm. and 1120 ppm. The concentrations used in the preliminary test for LC_{50} determination of galangal rhizome extract were 20 ppm, 25 ppm, 30 ppm, 35 ppm and 40 ppm, while the concentrations for identifyingLC_{90} galangal rhizome extract were 80 ppm, 85 ppm, 90 ppm, 95 ppm and 100 ppm ppm.

The main test was the combination of LC_{50} extract of lime leaf and galangal rhizome and LC_{90} extract of lime leaves and galangal rhizomes against the mortality of Aedesaegypti larvae.

The larvae were placed in beaker glass as intervention and non-intervention groups. Each treatment group consisted of 25 larvae with three replications. Extraction of lime leaves and galangal rhizomes were macerated using ethanol 95% liquid. Environmental conditions such as temperature, water pH, type of water and time of observation were always controlled to avoid biases from the research objectives.

The average number of larvae mortality was calculated based on the number of larvae that died in three repetitions compared to the number of repetitions of treatment or the percentage of larvae deaths was calculated based on the average number of larvae deaths in three repetitions compared to the total test larvae of each treatment multiplied by 100%. Death of larvae was marked by not moving, the larvae were sinking to the bottom of the container and no respond when stimulated. Statistical tests were performed using the one way ANNOVA method, linear regression and the Least Significant Difference test using the SPSS computer program version 16.0

3. Results and Discussion

The preliminary test of lime leaf extract to mortality of $_{Aedesaegypti}$ larvae for 24 hours obtained LC_{50} of 650 ppm with an average number of deaths of 13 larvae (52%) and LC_{90} of 1100 ppm with an average number of deaths of 23 larvae (92%), whereas LC_{50} galangal rhizome extract was 30 ppm with an average number of deaths of 13 larvae (52%) and LC_{90} of 90 ppm with an average number of deaths of 23 larvae (92%).

The main test of LC50 combination of lime leaf extract and galangal rhizome to the mortality of *Aedesaegypti* mosquito larvae obtained an average number of deaths of 19 larvae (76%), while the combination of LC_{90} of lime leaf extract and galangal rhizome could kill all25 tested larvae (100 %).

Table 1: The average number of death of instar III and IV larvae of Aedesaegypti larvae in the treatment of LC_{50} combination of lime leaf extract and galangal rhizome for 24 hours.

Concentration (ppm)	Total Tested Larvae	Replication			Total Larvae Death	Average	%
		I	II	III			
30 and 650	25	20	18	19	57	19	76
Control	25	0	0	0	0	0	0

Table 2: The average number of deaths of instar III and IV larvae of *Aedesaegypti* in the treatment of LC_{90} combination of lime leaf extract and galangal rhizome for 24 hours.

Concentration (ppm)	Total Tested Larvae	Replication			TotalLarva Death	Average	%
		I	II	III			
90 and 1100	25	25	25	25	75	25	100
Control	25	0	0	0	0	0	0

From the Tables 1 and 2 show that LC_{50} combination of lime leaf extract and galangal rhizome and LC_{90}combination of lime leaf extract and galangal rhizome can increase the average or percentage of the death of *Aedesaegypti* larvae in 24 hours. In the preliminary LC_{50}test of lime leaf extract and galangal rhizome each treatment concentration killed 13 larvae (52%), while in the main test the LC_{50} combination of the two biological was able to increase the average number of deaths to 19 larvae (76%). From the preliminary LC_{90} test results of the two extracts were able to kill 23 larvae (92%), while in the main test the LC_{90} combination of both biodiversity was able to increase the average number of deaths to 25 larvae (100%).

This indicates that by increasing the concentration it will also increase the number of larval deaths because the compounds of limonoids, flavonoids and phenols work synergistically as larvasides in cutting off the life cycle of larvae. In the control group, no deaths were found because all the confounding variables were minimized, such as ambient temperature, water pH and observation time. From the one way anova test, the value of sig (p) = 0,000 (p <0.05) has shown that the combination of LC50 extracts of lime leaves and galangal rhizomes and the combination of LC90 extracts of lime leaves and galangal rhizomes have demonstrated a treatment effect on the number of deaths of larvae. Mosquito *Aedesaegypti* instar III and IV with a coefficient of determination of 98.8%. The Least Significant Difference Test analysis results obtained sig (p) = 0.00, showing that there were differences in treatment between the control group, the combination of LC_{50} and LC_{90} extracts of lime leaves and galangal rhizomes against the mortality of *Aedesaegypti* larvae.

Limonoida is an analogue compound of juvenile hormone in insects that functions as a growth regulator of cuticle larvae and it is toxic stomach that enters digestion through immersion concentration of extracts that are consumed and absorbed through the intestinal wall, and then circulates with the blood to disrupt the larva's metabolism so that energy deficiency occurs (Prijadi, 2014). Lime leaves tested was positive containing limonoid compounds because the qualitative test results by Thin Layer Chromatography has demonstrated a yellowish green color on the TLC plate.

Flavonoids are compounds affecting the work of the respiratory system, which enter the larval body through the siphon, causing interference in the nervous system and respiratory system, thus the larvae do not breathe and eventually die (Syamsul and Purwanto 2014). The galangal rhizome was found containing flavonoid compounds as the qualitative test results by Thin Layer Chromatography has demonstrated a greenish-yellow color on the TLC plate.

Phenol is an inhibiting compound in the formation of cell walls (Abdullah, et al. 2015). The galangal rhizome was tested positive containing phenol compounds as the results of the qualitative Thin Layer Chromatography test has demonstrated a blackish blue color on the TLC plate.

4. Conclusions and Suggestions

From the results of the study it can be concluded that the combination of LC_{50} extract of lime leaves and galangal rhizome can kill 19 larvae (76%), while the combination of LC_{90}extract of lime leaves and galangal rhizome can kill all

the 25 tested larvae (100%). The increase in the average or percentage of the number of larvae deaths is directly proportion to the increase in the concentration of a combination of lime leaf extract and galangal rhizome, thus the combination of these two biological concentrations is effectively against the mortality of larvae of *Aedesaegypti* instar III and IV. Further research is suggested to identify these two biolarvasides quantitatively in order to obtain the right active ingredients in composing the formula of anti-larvae or anti-mosquito products in breaking the life cycle of Dengue Hemorrhagic Fever vector.

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