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The Extraction of Zingiber Officinale Rosc as a Natural Insecticide for Aedes Aegypti Larvae

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Abstract

Dengue fever is a health problem in Indonesia, including in Gorontalo Province, with an incidence rate of 101.53 per 100,000 population. One of the efforts to prevent the incidence of dengue fever is the use of natural insecticides, including red ginger. This study aims to assess the effectiveness of red ginger juice on the mortality of Aedes aegypti mosquito larvae. This research took place at the Laboratory of the Department of Public Health, the State University of Gorontalo, in 2021 using a True Experiment design. The mosquito larvae samples used were 500 larvae of Aedes aegypti instar IV mosquitoes taken from endemic areas of dengue fever in Gorontalo. Data were analyzed using the One Way ANOVA statistical test. Red ginger extract was given in five concentrations, namely 60%, 70%, 80%, 90%, and 100%. The results showed an effect of red ginger extract on the mortality of Aedes aegypti mosquito larvae at each treatment concentration given (p-Value = 0.000). The higher the concentration level given, the higher the larval mortality rate will also be. Red ginger is expected to be used by the community to prevent dengue fever.

Introduction

Dengue fever is an infectious disease caused by the Dengue virus, which belongs to the group B Arthropod-Borne Virus (Arboviroses) known as the genus flavivirus, family flaviviridae, and is transmitted through the Aedes Aegypti and Aedes Albopictus mosquitoes (Kemenkes, 2019). In 1960 this disease spread to many countries, including the World Health Organization South-East Asia and the Western Pacific region including Indonesia (WHO, 2011) (Gubler, 1998). It is because the ecological disturbances during and after World War II created ideal conditions for increasing the transmission of mosquito-borne diseases (Gubler, 1998). WHO noted that the region in Southeast Asia with the highest number of DHF cases was Indonesia in the DHF stratification by WHO, which indicated a large number of hospitalizations and deaths from DHF, especially in children (WHO, 2011).

Dengue fever is a serious global threat to humanity, especially in endemic areas in the tropics and subtropics, where about 40% of the world's population now lives in countries with a high risk of dengue transmission (Dehghani and Kassiri, 2021) (Sanyaolu et al., 2017). WHO describes cases of dengue fever worldwide annually, reaching 50-100 million, of which there are 250,000-500,000 cases of DHF with a mortality rate of about 24,000 people per year (WHO, 2011). Overall mortality from dengue fever worldwide is 0.5-2.0% which can reach 20% if the case has entered the dengue shock syndrome phase in the ICU/hospital (Guo et al., 2017). Dengue Hemorrhagic Fever (DHF) is still one of Indonesia's main public health problems (Kemenkes, 2019). Along with increasing mobility and population density, the number of sufferers, and the increasing area distribution. In Indonesia, dengue fever was first discovered in Surabaya in 1968. At that time, 58 were infected, and 24 of them died. The mortality rate (CFR) reached 41.3%. Nowadays,

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it has spread widely throughout Indonesia (Kemenkes, 2016).

DHF cases were established with a diagnosis consisting of clinical symptoms and laboratory results indicating a decrease in platelets <100,000/mm3 and plasma leakage characterized by an increase in hematocrit >20%. DHF cases in Indonesia from 1968 to 2015 tend to continue to increase (Kemenkes, 2016). DHF reported in 2019 were 138,127 cases. This number increased compared to 2018, which were 65,602 cases. Deaths due to DHF in 2019 also increased compared to 2018, from 467 to 919 deaths (Kemenkes, 2019). Based on data from the Ministry of Health in 2019, the Provinces of North Kalimantan, East Kalimantan, and Bali had the highest IRs among 34 other provinces, which were 239, 180.66, and 114.8 per 100,000 population, respectively. Gorontalo province ranks fourth with an incidence rate of 101.53 per 100,000 population. However, if you assess the Case Fatality Rate (CFR) of each region, it has a CFR of DHF of 1.88%, ranks second highest in Indonesia after Maluku Province, which has the highest CFR of 2.12% (Kemenkes, 2019).

Based on data from the Early Alert and Response System (SKDR) of the Gorontalo Provincial Health Office, the incidence of DHF ranks highest for diseases that often experience Extraordinary Events (KLB), which is 14 times in 2016. from 2017 to 2019. There were 151 cases with three people died in 2017, and in 2018 it increased to 244 cases with four people died. Data from the Gorontalo District Health Office as of February 2019, there were 217 cases, and one died from dengue fever (Dinkes Gorontalo, 2020). This vector-borne viral disease spreads very quickly and poses public health and economic challenges requiring various prevention and control techniques (Sabir et.al., 2021). In general, an epidemic of dengue fever can occur if there are mosquito vectors (usually Aedes aegypti), dengue virus, and a group of susceptible human hosts (CDC, 2009). Environmental factors such as rainfall, air temperature, and humidity also affect the spread of DHF (Widyorini et al., 2017). In addition, human mobility also brings the dengue virus into motion, allowing it to spread to a larger area. The distribution is more found in urban areas than in rural areas, including areas with quite a lot of standing water (Zhang et al., 2020). The spread of dengue fever is expected to increase due to factors such as the modern dynamics of climate change, globalization, socio-economics, as well as the evolution of the virus (Murray et al., 2013).

Nowadays, the dengue fever vaccine is still under development (Sanyaolu et al., 2017; Murray et.al, 2013). Some of the efforts by pursuing the habit of eradicating mosquito nests through the 3 M Plus movement. It is early detection of dengue cases, immediate handling, and the implementation of Aedes Aegypti vector surveillance and control. As well as promotional efforts involving all sectors through lectures and digital media (Muchukota et al., 2019; Murray et.al, 2013; Ahmad et al., 2021). Increasing public awareness of dengue fever must also continue to be improved. People who are indifferent to efforts to prevent dengue will have a more significant impact (Indawati dkk., 2021).

The use of insecticides in controlling disease vectors is one of the government's efforts to overcome vector-based diseases (Rivero et al., 2010). The use of insecticides in the health sector, especially those from chemicals, is still widely used in Indonesia. The various active ingredients of insecticides that are currently on the market will indirectly have an impact on health. Although in some cases, insecticides tend to cause a decrease in the number of vectors, a decrease in infectiousness, or a change in behavior, all of which will reduce the capacity of insect vectors, on the other hand, insecticides will also increase the resistance of disease vectors, so that vectors are increasingly resistant to the use of insecticides in eradicating mosquitoes larvae (Rivero et al., 2010; Nauen, 2007). Therefore, another approach is needed in efforts to reduce dengue vectors.

Some efforts that can be used as alternative efforts in controlling dengue vectors are natural ingredients, such as insecticides. Apart from being cheap, they are also friendly to the environment (Sharma et al., 2021). Some that are often used as mosquito repellent Aedes aegypti include basil (Ocimum Sanctum L), Zodia leaves (Evodia suaveolens Scheff), Bintaro leaves (Cerbera manghas), Lemongrass stalks (Cymbopogon citratus), Chenopodium ambrosioides, Conyza sumatrensis, Eucalyptus camaldulensis, Mentha spicata and others (Mathew, 2017; Kumar et al., 2017; Sasmitasari, 2022; Soonwera and Phasomkusolsil, 2016; Azeem et al., 2019). Another natural ingredient found to have an insecticidal effect on mosquitoes is red ginger (Hamada et al., 2018). The study only examined red ginger in the form of oil combined with Garlic Cloves (Allium sativum), but so far no research has been found on the repellent effect of red ginger in the form of extract. Red ginger is a natural ingredient that is easy to grow and found in Gorontalo. The squeeze method is also interesting to assess its effectiveness because it is so easy to apply in the community. So this study aims to assess how the insecticidal activity of red ginger juice as a repellent for Aedes aegypti mosquito larvae.

Method

This research is an experimental study using a True Experiment approach, where Aedes aegypti larvae are given direct treatment by inserting red ginger leaf juice. The treatment followed a Completely Randomized Design (CRD) approach because the experimental unit was homogeneous. The treatments were given in five different concentrations, namely 60%, 70%, 80%, 90%, and 100%. This research took time in February 2021. Mosquito larvae samples were collected in West Limboto District, Gorontalo Regency, a dengue fever endemic area. The larval selected was the fourth instar Aedes aegypti mosquito larvae, so the larvae used were homogeneous. The selection of the fourth instar larvae is due to the larger size, and the defense system is considered stronger. The experiment took place at the Laboratory of the Department of Public Health, State University of Gorontalo, and with a laboratory assistant's help.

The test was carried out by providing five containers as a place to put the red ginger extract. Each was labeled with a concentration, namely 60%, 70%, 80%, 90%, and 100%. The extract addition is adjusted to the concentration. Then, in each container were added in 20 fourth instar Aedes aegypti larvae. The containers were then covered with gauze, labeled, and observed for 24 hours. After 24 hours, the concentration of some mosquito larvae can die effectively. To get more accurate results, the experiment is repeated the next day. It was repeated five times for each concentration, so 500 Aedes aegypti mosquito larvae were needed. The experiment flow is shown in Image 1. The observations obtained were then recorded by the laboratory assistant through the observation sheet. Data were analyzed using One Way ANOVA with the help of the SPSS 26 computer application.

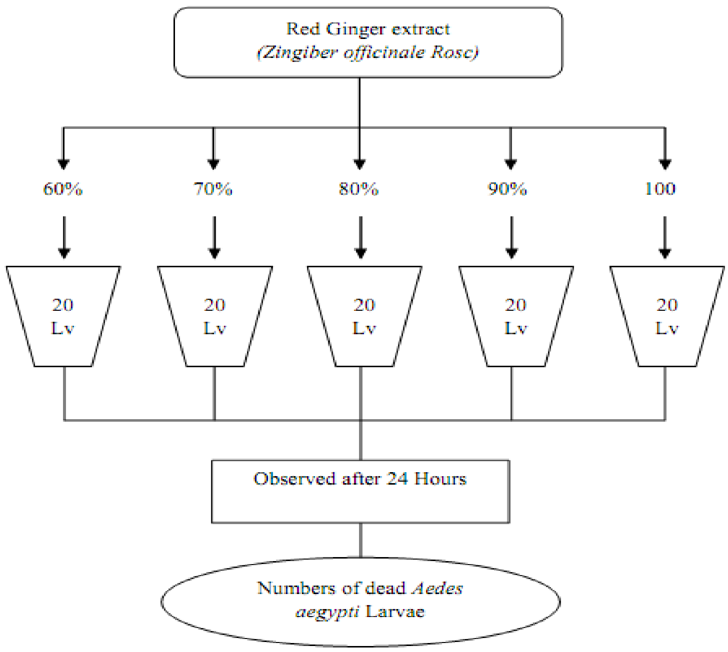

Image 1. Experiment Flow

Results And Discussions

The experimental data were analyzed using the Test of Normality to assess the distribution of the data. The analysis results obtained indicate that the data is normally distributed with p-value = 0.814 (> = 0.05). Since the data were normally distributed, the One-Way ANOVA test was then carried out. The results of the analysis are shown in table 1.

Table 1. Differences in Number of Larvae between Concentration Groups

Concentration	Mean	F	p-Value
60%	8.60	84.500	.000
70%	11.20		
80%	13.60		
90%	16.60		
100%	19.40		

Source: Primary Data, 2021

The analysis results showed a significant difference in the mortality of Aedes aegypti mosquito larvae between the five concentration groups with p-value = 0.000 < = 0.05 (95% CI). Then a follow-up test (Post hoc Games-Howell) was conducted to assess which groups had a different number of deaths in each group (Table 2).

Table 2. Comparison of Each Group

Concentration		p-Value	95% Confidence Interval	
		Lower	Upper	
60%	70%	.024	-4.83	-.37
	80%	.001	-7.49	-2.51
	90%	.000	-10.49	-5.51
	100%	.000	-13.07	-8.53
70%	60%	.024	.37	4.83
	80%	.036	-4.63	-.17
	90%	.000	-7.63	-3.17
	100%	.000	-10.09	-6.31
80%	60%	.001	2.51	7.49
	70%	.036	.17	4.63
	90%	.020	-5.49	-.51
	100%	.000	-8.07	-3.53
90%	60%	.000	5.51	10.49
	70%	.000	3.17	7.63
	80%	.020	.51	5.49
	100%	.018	-5.07	-.53
100%	60%	.000	8.53	13.07
	70%	.000	6.31	10.09
	80%	.000	3.53	8.07
	90%	.018	.53	5.07

Source: Primary Data, 2021

The results of further test analysis showed that all groups had a significant difference in the number of Aedes aegypti larvae mortality compared to other groups with p-value < = 0.05. The average difference in larval mortality is shown in Image 2.

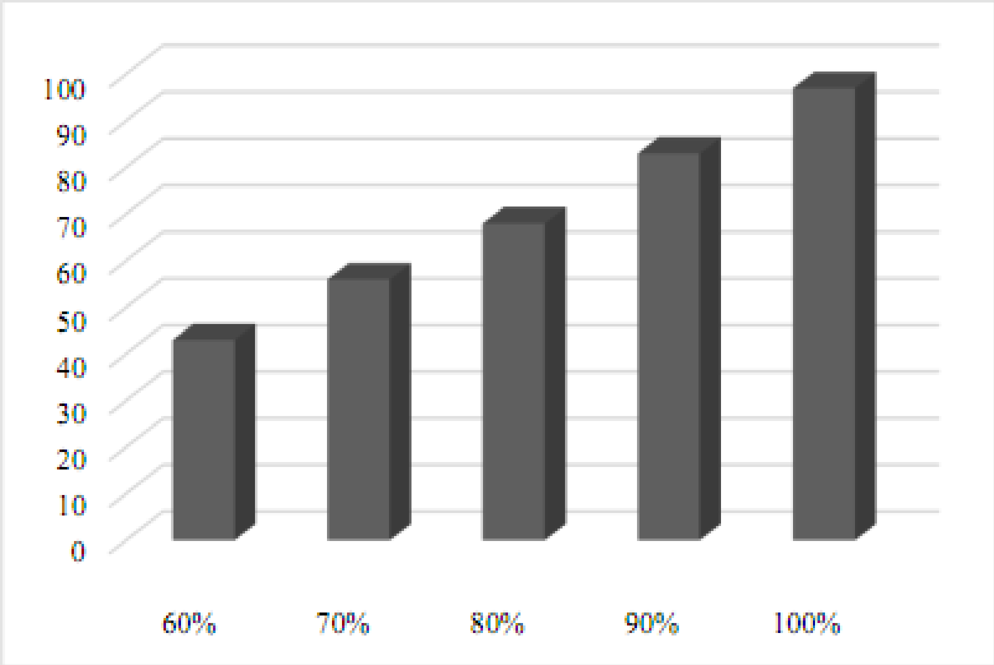

Image 2. Percentage Average Mortality Rate of *Aedes Aegypti* Larvae

At a concentration of 60%, the average larval mortality reached 43% after 24 hours. At 70%, it reached 56% after 24 hours. At 80%, it reached 68%. At the ginger concentration of 90%, it reached 83%. At 100% concentration, it reached 97%. The results showed that the higher the concentration of red ginger extract, the higher the mortality rate of the Aedes aegypti mosquito larvae. The Aedes aegypti mosquito larvae's highest mortality was found at a concentration of 100% and the lowest at 60%. The same results after five repetitions, with the 24 hours duration for each repetition.

The results showed that red ginger has repellent activity against the larvae of the Aedes aegypti mosquito at various concentrations. The highest larval mortality rate was found at 100% concentration. The results obtained are in line with the results of research on the insecticidal activity of oil from garlic clove (Allium sativum) and ginger rhizome (Zingiber officinale), which have an insecticidal activity that can significantly lengthen larva and pupa duration. In addition, it also reduces the percentage of hatchability of deposited eggs (Hamada et al., 2018). Other studies have also shown that ginger can also be used as a repellent against vectors of a disease, such as cockroaches (Azhari et al., 2017).

Ginger (genus Zingiber) is widely used as a spice and medicinal herb worldwide and is the main ingredient in traditional local beverages such as herbs drink in Southeast Asia (Yanagawa et al., 2021). Ginger has many benefits in everyday life, including antibacterial, antiviral, and antifungal activities. The content of ginger can suppress the growth of phytopathogenic growth. So it can be used as a new alternative to synthetic fungicides and bactericides (Abdullahi et al., 2020; Yanagawa et al., 2021). Other studies have shown that red ginger has an anti-inflammatory effect by preventing changes in levels of several inflammatory cytokines/biomarkers and inhibiting hippocampus and prefrontal cortex acetylcholinesterase (AChE) and adenosine deaminase (ADA) activity (an important enzyme relevant in the management/prevention of neurodegenerative diseases) (Akinyemi and Adeniyi, 2018).

Red Ginger (Zingiber officinale Rosc) leaf juice contains active compounds which are toxic or toxic to insects so that it can cause death in Aedes aegypti larvae. The active compounds in red ginger will react with the larval cell membrane and damage it, causing lysis and disrupting the permeability of the plasma membrane. This results in leakage of the cytoplasmic membrane due to the breakdown of phospholipid molecules due to H+ ions from ginger compounds, one of which is gingerol (Anwar et al., 2018).

Active compounds such as essential oils in red ginger leaf juice are toxic or toxic to the larvae of the Aedes aegypti mosquito. In terms of nutrition, ginger contains calories, carbohydrates, fiber, protein, sodium, iron, potassium, magnesium, phosphorus, zinc, folate, vitamin C, vitamin B6, vitamin A, riboflavin, and niacin (Shahrajabian et al., 2019). Some of the active chemical compounds in ginger have pharmacological effects on health, including essential oils containing the active substances zingiberene, kamfena, lemonin, borneol, shogaol, cineol, fellandren, zingiberol, gingerol, zingeron, and kaemferol (Munda et al., 2018) (Ghasemzadeh et al., 2010). Red ginger contains 47.95% essential oil and 25.20% atsiri oil (dos-Santos Reis et al., 2020).

The kaempferol content in ginger can enter the respiratory system of the larvae and damage the mitochondria. Mitochondrial damage inhibits the electron transport process so that the energy metabolism process is disrupted and the formation of adenosine triphosphate (ATP) is reduced. Decreased ATP production in the larval body results in a weak larval body. Another active compound contained in red ginger is zingiberene. This compound results in the release of material in the cell and interferes with the nutrient transport process by the cell, damage to the cytoplasmic membrane will result in other compounds contained in red ginger more freely penetrating the larva's body. The entry of other compounds into the larval body freely as a result of damage to the membrane tissue will result in the disruption of physiological functions in the larval body (Srikandi dkk., 2020).

Conclusion

Red ginger has repellant activity against Aedes aegypti mosquito larvae. The higher the concentration of red ginger juice used, the higher the larval mortality rate for 24 hours. Red ginger can be used as a natural insecticide to prevent the spread of DHF, and it is hoped that it can be applied in the community, especially in DHF-endemic areas. Red ginger is very easy to obtain, besides it is so cheap and is not toxic to the surrounding environment.

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