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Red Ginger Essential Oil (Zingiber officinale var. Rubrum) as a Biolarvicide in the Control of Aedes Aegypti Mosquitoes

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Abstract

Synthetic larvicides are often used to control Aedes aegypti mosquitoes, but they are detrimental to humans, the environment, and the occurrence of resistance. The purpose of the study was to determine the influence and effectiveness of red ginger essential oil (Zingiber officinale var. Rubrum) as the biolarvicide of Ae. Aegypti and the most effective concentration (LC50). A total of 25 instar 3 larvae with concentrations of 200, 400, 600, and 800 ppm and K+(temephos), K-(aquaade), four repetitions every 30 minutes of observation for 3 HSPs. The results of the study showed that red ginger essential oil affected the death of Ae. Aegypti larvae. There was a significant difference in mortality rates between concentrations (Kruskal-Wallis test, $p = 0.001$). Likewise, there was a moderate but significant correlation between concentration and mortality (Spearman correlation: $r = +0.503$, $p = 0.001$). The probit analysis, LC50 was 257.89 ppm, most effectively influencing mortality in Ae. Aegypti larvae based on LC50. Red ginger essential oil (Zingiber officinale var. Rubrum) is effective and effective as a bio-larvicide to control Ae. aegypti so that it can reduce dengue fever cases.

Introduction

Mosquitoes are one of the important insects in the world of health. Based on its classification, mosquitoes are included in the phylum Arthropoda, Order Diptera, Family Culicidae, with three tribes, namely Tribus Anophelini (*Anopheles*), Culicini Tribes (*Culex, Aedes, Mansonia*), and Toxorhynchitini Tribe (*Toxorhynchites*). In humans, mosquitoes are ectoparasites that act as vectors for various diseases, including malaria, filariasis, and dengue fever (Wahyuni, Makomulamin & Sari, 2021). According to the World Health Organization (WHO), vector-borne diseases account for more than 17% of all infectious diseases and cause more than 700,000 deaths annually worldwide (WHO, 2023b). *Aedes aegypti* (L) is one of the main vectors of transmission of viral diseases to humans, such as yellow fever, Zika virus fever, chikungunya, dengue fever, and other arboviruses (Scalvenzi *et al.*, 2019; Valle *et al.*, 2019). Dengue fever is one of the viral diseases transmitted by *Ae. Aegypti,* until now, is still a global public health threat (Darriet, 2016; da Botas *et al.*, 2017; Amelia-Yap *et al.*, 2018). According to WHO, there are about 390 million cases of dengue virus infection per year, with 96 million of them showing severe clinical manifestations and about 40,000 deaths each year. WHO also estimates that around 3.9 billion people in more than 129 countries are at risk of contracting the dengue virus (WHO, 2023a).

In Indonesia, dengue hemorrhagic fever (DHF) is still an infectious disease that has become an unresolved health problem, with the incidence rate still fluctuating. In 2020, there were 103,509 cases of dengue fever, with

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a mortality rate of 725 people, with a dengue IR of 38.15 per 100,000 population (Kemenkes RI, 2020). In 2022, there was an increase of 143,000 cases, the national dengue fever IR of 52 per 100,000 population, higher than the target set in the previous period (which was 49 per 100,000 population) (Kemenkes RI, 2023). The results of these statistics show that there are still challenges in controlling this disease, so it requires a concerted effort to overcome these health problems.

As dengue vectors, mosquitoes *Aedes* consist of two species, namely *Ae. Aegypti* and *Ae. Albopictus* with the characteristics of the body and limbs covered with scales, with silvery white stripes. *Ae. Aegypti* Females bite during the day and like human blood as their food (Rojas-Pinzón *et al.*, 2018). Water reservoirs such as bathtubs, containers, buckets, clay vases, buckets, cans, used tires, and others are places to lay eggs from *Ae. Aegypti* mosquito (da Botas *et al.*, 2017). So that in the rainy season, there will be an increase in the mosquito populations of *Ae. Aegypti,* which is accompanied by dengue fever cases. (Akollo *et al.*, 2020). Until now, the problem that still occurs, the right method for population control of mosquitoes has not been found for *Ae. Aegypti* both in the adult and larval stages, which is evidenced by dengue fever cases that are always present every year (Pandiyan *et al.,* 2019).

Ae. Aegypti mosquito control that has been done so far is the modification and manipulation of the environment where the patient is located, control chemically, biologically, physically, mechanically, 3M plus, and others, but has not been effective. However, chemical (synthetic) control is still a Popular and frequently used control, both for controlling mosquito populations of *Ae. Aegypti* and larvae (Govindarajan *et al.*, 2016), such as pyrethroid-based fumigation for the mosquito stages of *Ae. Aegypti* Adults and larvicides for larval stages (Galvão *et al.*, 2019). The use of synthetic compounds is carried out because it is considered more effective, can kill large and fast insects, and have lower production costs compared to natural materials.

In the mosquito control of *Ae. Aegypti,* in particular, larvae usually use abate powder containing 1% temephos, which is sprinkled on water reservoirs in the house, such as bathtubs, drums, containers, and others. However, the use of temephos, which is used to kill larvae, can cause resistance from mosquitoes. There have been reported cases of larval resistance to *Ae. Aegypti* in several countries, including Brazil (Valley *et al.*, 2019): Mexico (Kandel *et al.*, 2019); Southeast Asia (Indonesia, Malaysia, Philippines, Thailand, Singapore, Laos, Myanmar) (Amelia-Yap *et al.*, 2018) and Indonesia (Akollo *et al.*, 2020). In addition, the sustainable use of temephos in the community will harm humans because it contains non-systemic organophosphate chemical compounds by inhibit the production of the enzyme acetylcholinesterase, which functions to change acetylcholine to its original state. When an excessive amount of acetylcholine will cause excessive stimulation of the nervous system and lead to the death of larvae of *Ae. Aegypti*. Therefore, temephos poses a significant risk if exposed to humans because organophosphate compounds can cause disorders of the nervous, respiratory, and cardiovascular systems that which can lead to death (WHO, 2005).

In connection with the above, new alternatives from plants are needed to control *Ae. Aegypti* mosquito. Biolarvicide is one of the environmentally friendly solutions to reduce the negative impact of the excessive use of synthetic chemical compounds (Rohmah *et al.,* 2020), because it is not so toxic that it is safe for humans & the environment, does not cause resistance, and decomposes naturally (Pandiyan *et al.,* 2020). From some previous studies on mosquito control of the *Ae. aegypti* It seems that they are trying to find alternatives by utilizing ingredients found in nature from plants that are safer for humans and the environment, available in large quantities, and easy to get. Based on the research, it is proven that various types of plants have the potential to be important biological resources for humans and can be used as bioinsecticides and biolarvicides.

One plant that is thought to be effective and potentially bio-larvicide is red ginger (*Zingiber officinale* Var. Rubrum) because it contains active compounds and essential oils. Red ginger is an annual plant that belongs to the Zingiberaceae family, the genus Zingiber, that originated in India and spreads almost all over the country, including the equator. This plant is often used in traditional medicine in several countries in Asia, such as for headaches, indigestion, nausea, and vomiting (Sivasothy *et al.*, 2011). Some studies state that red ginger has a wide range of pharmacological activities (Zhang *et al.*, 2022), antioxidant and anti-cancer (Ghasemzadeh *et al.,* 2016), and antibacterial (Sivasothy *et al.*, 2011). Other scientific evidence also proves that red ginger exhibits Immunomodulatory, Antihypertensive, Antihyperlipidemic, Antihyperuricemic, Antimicrobial, and Cytotoxic activity (Zhang *et al.*, 2022).

Effectiveness and how red ginger works as a mosquito biolarvicide of the *Ae. Aegypti is* caused by the active compounds contained in it, one of which is essential oil (EO). The essential oil content of red ginger is a Sesquiterpene compound consisting of three substances, namely: (Zingiberene, Zingiberol, Farnesene), which play a role in providing aroma, and Gingerol, Shogaol, and Paradol as compounds that give a spicy taste (Stoner, 2013). Zingiberene and zingiberol as contact venoms, act as receptors that activate anti-feeding signals in the insect's central nervous system (Moon *et al.*, 2020). Gingerol, as a stomach toxin, actively works to damage the outer membrane and cytoplasmic membrane so that the lysis of the larval cell membrane results in leakage (Syukur *et al.*, 2018)). Shogaol is effective in suppressing intestinal contractions and is an antitussive (Mao *et al.*, 2019).

Essential oils (EO) are a mixture of complex compounds that are volatile and are lipophilic, flavorful and flavorful, and liquid in form (Moon *et al.,* 2018). From several previous studies, it was found that essential oils act as an insect repellent. Da Botas explained that essential oils are plant volatile compounds that function as plant chemical defenses against insects, with the main mechanism of action being by inhibiting acetylcholine (da Botas *et al.*, 2017). Essential oil compounds are also toxic and can protect plants from pests, inhibit the growth of some types of insects, and act as mosquito larvicides that are safe against greenery (Govindarajan *et al.*, 2016; AlShebly *et al.*, 2017; Rezzoug *et al.*, 2019). The content of essential oil compounds has an anti-insect effect as an alternative to chemical compounds for insect control with repellent, feeding deterrent/antifeedant, toxicant, growth inhibitor, chemosterile, and attractant (Hikal *et al.,* 2017). Based on the explanation above, the content of essential oils in plants, especially red ginger, is likely to be effective and potentially used as a biolarvicide for mosquito control of the *Ae. aegypti.*

Red ginger (*Z.Officinale*) from several previous studies, besides being used for medicinal purposes, is also used for insect control. Such as research conducted by Ghahfarokhi about the Potential of essential oils *Zingiber officinalis* and *Eucalyptus globulus*, as a flea exterminator (*Rhipicephalus bursa*). Essential Oil Manifestation *Z. officinalis* and *E. globulus* have lethal activity against fleas (Madreseh-Ghahfarokhi *et al.*, 2019). Next, Boekoesoe and Ahmad's research using *Zingiber Officinale Rosc.* as a natural insecticide against larvae of *Ae. Aegypti* by using concentrations of 60%, 70%, 80%, 90%, and 100%. From the results of his research, it was found that the *Z.officinale Rosc.* Effectively kills larvae of *Ae. Aegypti* at 100% concentration with 97% larval mortality (Boekoesoe & Ahmad, 2022). This time, the author also conducted research on red ginger (*Zingiber officinale* Var. Rubrum) as a biolarvicide against *Ae. Aegypti,* however, no longer uses red ginger extract as done by Boekoesoe and Ahmad, but uses red ginger Essential Oil (EO) compounds using much lower concentrations of 200 ppm, 400 ppm, 600 ppm, and 800 ppm (mgL^{-1}), which refers to the WHO Standards (WHO, 2005).

Research on this topic is interesting because of the essential oil compounds isolated from red ginger (*Z. Officinale* Var. Rubrum) as a biolarvicide in mosquito control of the *Ae. Aegypti* has not been explored before. The question that needs to be answered is whether red ginger essential oil can be utilized as a biolarvicide in controlling *Ae. Aegypti* mosquito larval phase. The main objective of this study is to determine the influence and effectiveness of Red Ginger Essential Oil (*Z. Officinale* Var. Rubrum) in killing *Ae. Aegypti* larvae and know the Most Effective Concentration (LC_{50}) as a biolarvicide in *Ae. Aegypti* mosquito control.

Method

The research was conducted at the Natural Ingredients Pharmaceutical Laboratory of the Riau College of Pharmacy (STIFAR), Pekanbaru, in May–June 2022. This study examines the use of red ginger essential oil as a biolarvicide in the control of *Ae. Aegypti* mosquitoes without ignoring the factors that affect the life of *Ae. Aegypti*, namely temperature and air humidity. The research design used was the Complete Random Design Method (RAL) with four concentrations, namely 200, 400, 600, and 800 ppm, K (+)Temephos, K (-) aquatics, which were carried out four times. The ingredients used were 2500 grams of red ginger rhizome, 8 liters of Aquades, red ginger essential oil, 1 liter of N-Hexane, 50 ml of DMSO, 1 liter of Ethanol, 1 pack of Abate (Temephos) Powder, Na2SO4, Helium, 450 larvae *of Ae aegypti* instar three. Meanwhile, the tools used include analytical scales, blenders, separation funnels, micropipettes, distillation tools, stopwatches, cups, skewers, thermometers, large basins, and aquariums.

The preparation of test larvae is carried out in two ways: 1) first, by breeding *Ae. Aegypti* mosquito larvae. To get larvae by providing a medium (black basin) then fill it with clean water, and place it in a cool place and protected place from direct sunlight, as a place for mosquitoes to lay *Ae. Aegypti's* eggs. Then the eggs are left to wait for a few days so that they hatch into larvae. After becoming larvae, they are kept in an aquarium with a temperature range of 24.2-24.40C with a relative humidity of 67-70%. During the rearing period, the larvae are fed on coconut water feed (Fontana *et al.*, 2020) until it became the third instar larva used as a test larva. 2) The second method is to collect larvae in water reservoirs in residential environments. The three instar larvae used are healthy, actively moving larvae. The test larvae for each concentration consisted of 25 larvae of *Ae. Aegypti* with four (4) repetitions, plus 25 larvae for positive control and negative control, so that the total test larvae amounted to 450 acres of larvae.

The process of making essential oils begins by providing as many as 2500 grams of red ginger rhizomes, and then cleaned with running water, followed by thin slicing and drying in a place protected from direct sunlight, so that 400 g of dry powder is obtained. Then the process of making essential oils is carried out using the Water Distillation Method, using aqueducts as a solvent. The powder is stored in a distilled flask and then heated at 100^{o}C for 6 hours. After the distillation process is complete, the essential oils are separated from the solvent using an N-Hexane solution with a separate funnel. This is done by filling a separate funnel with 500 ml of distillate product and 50 ml of N-Hexane, followed by a perfect shake and letting it sit, and then the essential oils are separated. The extracted essential oils are put in a 10 ml vial and wrapped in aluminum foil and plastic wrap. Meanwhile, the essential oil content obtained is influenced by geographical conditions, environmental factors, agroclimatic conditions of plants, and the method of manufacture used (Pandiyan *et al.,* 2019), as well as rainfall and altitude of an area (topography) (Suryati *et al.*, 2022).

The preparation of the red ginger essential oil test solution uses DMSO as a solvent. The parent solution is made by weighing 200 µg of red ginger essential oil, which is put in a 20 ml measuring cup. DMSO is added so that a parent solution with a concentration of 10000 ppm is obtained. Furthermore, the parent solution was separated into concentrations of 200 ppm, 400 ppm, 600 ppm, and 800 ppm which were achieved by pipetting 0.4 ml, 0.8 ml, 1.2 ml, and 1.6 ml of the parent solution into each test cup, followed by the addition of 20 ml of aqueduct and homogenization. The red ginger bio-larvicide test solution used for testing has met WHO standards (WHO, 2005). Next, testing of the larvae was carried out on *Ae. Aegypti* by putting 25 instar three larvae into each test cup containing their respective test solutions, namely positive control, negative

Figure 1. The Process of Separating Essential Oils from Distillation Solvents With N-Hexane Solution
Image source: Researcher (2022)

control, and concentrations of 200, 400, 600, and 800 ppm. Then it was observed every 12 hours for 3×24 observations (3 HSP) of the larval death process, and the number of larvae was calculated on died *Ae. Aegypti*. Temperature and humidity measurements were carried out that affect the life of mosquito larvae. The same procedure is repeated on the second and third repetitions. To see the death of larvae of *Ae. Aegypti,* it is done by touching it with a stick, and if the larvae do not move, they are considered dead (WHO, 2005). Data analysis was carried out with a Statistical Test of Variance Analysis with RAL, followed by an ANOVA test. The ANOVA test cannot be performed because it is not eligible. Therefore, alternative tests were carried out, namely the Non-Parametric Kruskal-Wallis Test and the Spearman Analysis Test, to determine the degree of closeness of the relationship between the free variable and the bound variable.

Results and Discussion

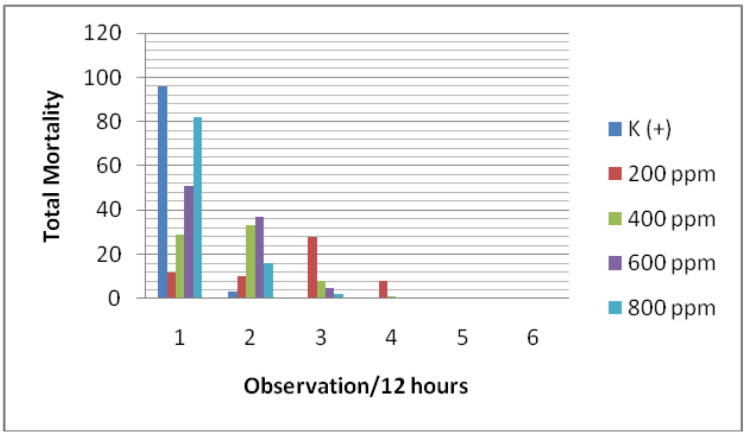
Based on the results of research that has been carried out on larvae of *Ae. Aegypti*, those which died with various concentrations of red ginger essential oil, namely 200 ppm, 400 ppm, 600 ppm, 800 ppm, and a positive control using temephos and a negative control using distilled water. Where the calculation and observation of the number of dead larvae was observed at an interval of 12 hours for 3 (three) observation days (HSP) with 4 repetitions. From the results of observation, the larvae of *Ae. Aegypti* shows a different pattern in the process of death. The first time the larvae are put in a test glass with various concentrations of red ginger essential oil, the larvae of *Ae. Aegypti* shows fluctuating active movement at concentrations of 200 ppm and 400 ppm. However, a very active movement is seen at essential oil concentrations of 600 ppm and 800 ppm and then returns to normal. At intervals of time, the larvae are seen to move stiffly, then weaken, fall to the bottom of the test glass, stand still, and die. Death is characterized by the larvae falling to the bottom of the test cup and not being able to move up and down again (WHO, 2005). The dead larvae look stiff, and the number of deaths increases as the concentration of the treatment increases. In the negative control (K-aquaades), all the larvae were still alive and active, and on two days of observation, it was seen that the larvae turned into pupae and then became mosquitoes. In the positive control (K+ temephos) in the first 12 hours of observation, there was 96% larval mortality. The larvae's movement is very rapid and active, followed by gradual weakening and death that occurs rapidly, and the larvae exhibit seizure behavior and eventually die.

Based on Table 1 below, it can be seen that after the administration of red ginger essential oil, observed for 3 observation days (HSP), indicates that each concentration of red ginger essential oil can kill larvae of *Ae. Aegypti*. The results of the tests that have been carried out on the larvae of *Ae. Aegypti.* By using red ginger essential oil at concentrations of 200, 400, 600, and 800 ppm, the total mortality was 58%, 71%, 93%, and 100%. From the total larval mortality of *Ae. Aegypti*, the lowest is at a concentration of 200 ppm, which reached 58%, and the highest total death is at a concentration of 800 ppm, which was 100%. Positive control using temephos showed total larval mortality of *Ae. Aegypti,* which reached 100%, and in the

Table 1. Total *Ae. Aegypti Larval Mortality* During 3 Observation Days (HSP)

No.	Concentration of Red Ginger Essential Oil	Total larval mortality *of Ae. Aegypti*					
		Repetition				Total Deaths (Tail)	Total Percentage of Deaths (%)
		1	2	3	4		
1	200 ppm	12	15	14	17	58	58
2	400 ppm	20	16	18	17	71	71
3.	600 ppm	25	24	22	22	93	93
4.	800 ppm	25	25	25	25	100	100
5.	(+) Controls	25	25	25	25	100	100
6.	Control (-)	0	0	0	0	0	0

Primary data for 2022



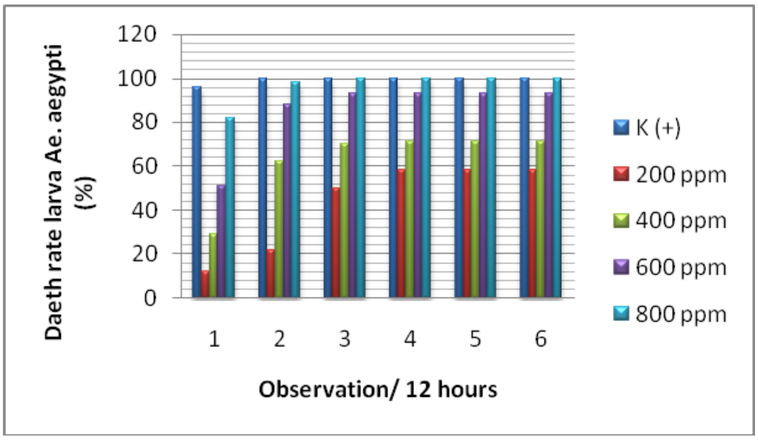
Primary data for 2022

Figure 2. Number of *Ae. Aegypti Larval Deaths* at Various Concentrations Every 12 Hours of Observation

negative control using aqueducts showed no death in the larvae of *Ae. aegypti.*

Based on Figure 2, the number of *Ae. aegypti larval deaths* at various concentrations of red ginger essential oil were observed every 12 hours of observation. In the first 12 hours of observation for each concentration, there was a high number of *Ae. Aegypti* larval deaths, as many as 12, 29, 51, and 82, respectively. At concentrations of 800 ppm and 600 ppm, the highest number of deaths, namely 82 and 51, occurred in the first 12 hours of observation. Meanwhile, at concentrations of 400 ppm and 200 ppm, the highest number of deaths of 33 and 28, occurred in 24 hours and 36 hours of observation. In the positive control (K+) of Temephos, there were 100 deaths of *Ae. Aegypti larvae* in the first 12 hours of observation. Whereas negative control (K-) distilled water did not cause larval death at all. Effective mortality at concentrations of 600 ppm and 800 ppm occurred at the first 12 hours of observation, while concentrations of 400 and 200 ppm were effective at 24 hours and 36 hours, respectively. This shows that the higher the concentration, the greater the number of larval deaths, along with the increase in the faster the death time as well.

The average mortality of larvae of *Ae. Aegypti* observed every 12 hours for 3 observation days (HSP) in Figure 3 above, at 1 HSP, the average mortality of larvae at 12



Primary data for 2022

Figure 3: Average Mortality of *Ae. Aegypti* Larvae at Each Biolarvicide Concentration Red Ginger Essential Oil Every 12 Hours of Observation

hours of observation with red ginger essential oil treatment at a concentration of 200 ppm of 12%, a concentration of 400 ppm of 29%, a concentration of 600 ppm of 51% and a concentration of 800 ppm of 82%. In 2 HSPs, at 36 hours of observation, the average mortality with red ginger essential oil treatment was 50 ppm concentration, 400 ppm concentration was 70%, 600 ppm concentration was 93%, and 800 ppm concentration was 100%. In 3 HSPs, the average mortality of larvae with red ginger essential oil treatment was 58 ppm concentration, 400 ppm concentration was 71%, 600 ppm concentration was 93%, and 800 ppm concentration was 100%. In positive cocks, the average mortality of larvae was 100%, while in negative controls, larval mortality did not occur (0%). The higher the concentration of red ginger essential oil, the higher the percentage of larval mortality of *Ae. Aegypti*.

Based on Figure 3, it shows that in the treatment of the concentration of red ginger essential oil, the concentration of 200 ppm causes mortality of *Ae. Aegypti* larvae occurred until the 2nd day of HSP at the 48th hour, with the concentration of 400 ppm, 600 ppm, and 800 ppm, the average mortality of *Ae. Aegypti* larvae occurred until the 2nd day of HSP at the 36th hour. For positive control, larval mortality has occurred since the first 12 hours of observation, while in negative control, larval mortality does not occur after 3 HSP. This shows that red ginger essential oil can work from 1 HSP and is most effective on day 1 HSP, and then decreases. From the results of observations of the phase development of *Ae. aegypti* larvae at the time of testing, some of the non-dead larvae turned into pupae, some even became mosquitoes, especially at concentrations of 200 ppm and 400 ppm. For the concentrations of 600 ppm and 800 ppm, no changes in larval development to pupa were found. Environmental conditions such as water temperature, air humidity, and water pH are the reasons for the larvae to survive when tested with red ginger essential oil. From the results of observations that have been made, the water temperature ranges from 240 °C- 250 °C, and the indoor air humidity during normal research is around 69.8% - 72.3%

From the results of the Kolmogorov-Smirnov Test Statistics, a P-value of 0.001< 0.05 was obtained, meaning that the distribution of data from each group was abnormal. The Variance test obtained a P-value of 0.001< 0.05, concluding that the data variance was unequal or abnormal. So that the ANOVA test cannot be used, even though the data transformation has been repeated several times, and the results are fixed. Therefore, the Kruskal-Wallis Non-Parametric Test is used as an alternative to the ANOVA test. From the results of the Kruskal-Wallis test, a P-value of 0.001 < 0.05 was obtained, this value showed that there was a significant difference in the mortality of *Ae. Aegypti* larvae with a difference in the concentration of red ginger essential oil. In the

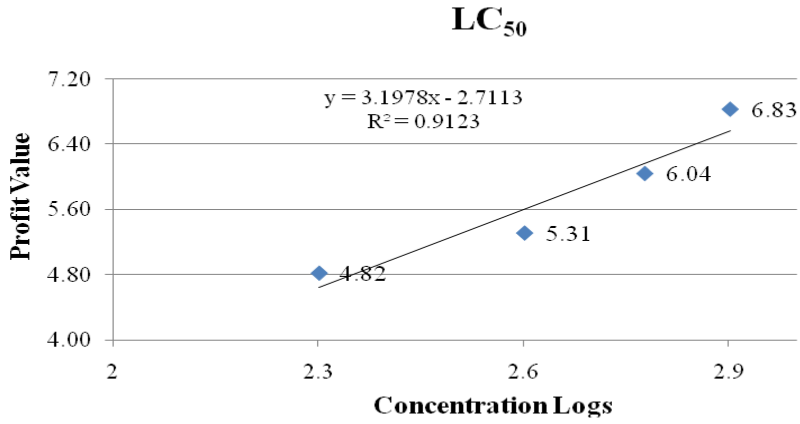


Figure 4. Deadly Concentration of 50 (LC_{50}) Biolarvicide of Red Ginger Essential Oil

Non-Parametric Analysis correlation test, a Sig Spearman (2-tailed) value of 0.001 < 0.05 was obtained, which showed an influence between the increase in the concentration of red ginger essential oil on the number of larval deaths. The strength of the correlation is shown by the interpretation of "moderate" with a value of 0.503**. This is consistent with the observed trend that higher concentrations of red ginger essential oil lead to increased larval mortality, accompanied by faster death times.

In probit analysis, adjusted to the parameters of larvicide effectiveness according to WHO in 2005, the larvicide concentration is considered effective if it can cause the death of test larvae between 10-95%, which will later be used to determine the lethal concentration (LC) value (WHO, 2005). Based on the graph of the bio-larvicide probit value of red ginger essential oil on larvae of *Ae. Aegypti,* in this study, shows an increase in larval mortality from a concentration of 200 ppm to 800 ppm, with an LC_{50} value of 257.89 ppm for 3 HSPs. This means that the bio-larvicide concentration of red ginger essential oil is 257.89 ppm and is most effective in influencing death in larvae of *Ae. Aegypti* based on LC_{50} (Figure 4)

From the results of the Kruskal-Wallis Non-Parametric test, a P-value of 0.001<0.05 was obtained. This value means that there is a significant difference in the mortality of *Ae. Aegypti* larvae between treatment groups with different mean values in soaking time for 3 HSPs. This is evidenced by the results of observation of the death of various concentrations of treatment, it can be seen that there is an increase in the mortality of *Ae. Aegypti* larvae as the concentration of red ginger essential oil increases. This is strengthened from the results of the study in Figure 2 on the number of deaths and Figure 3 on the average mortality of *Ae. Aegypti* larvae are increasing along with the increasing concentration of red ginger essential oil. This can happen because the higher the concentration, the more toxic active compounds contained in red ginger essential oil accumulate in the body of *Ae. Aegypti* larvae, so that more larvae die.

The same conclusion but different results were also obtained in Kosini's research on the effects of the extract *Gnidia kassiana* (Thymeleaceae) on *Callosobruchus maculatus,* which indicates that more and more toxic compounds are being absorbed by the larvae *C. maculatus* will slowing down the development and accelerating the death of the larvae by the melanization of the cuticle, which results in the disruption of the endocrine system due to the secondary metabolite compounds contained in the extract *Gnidia kassiana* (Kosini & Nukenine, 2017). In addition, in the research that we have done previously on bio-insecticides of cattilage extract (*Polygala paniculata*) in mosquito control of *Ae. Aegypti* with the concentrations of 10%, 15%, 20%, and 25%, the higher concentration of *P. Paniculata* extract, there will be more *Ae. Aegypti* mosquitoes died. At a concentration of 10%, it can only kill *Ae. Aegypti* mosquitoes as much as 52%, while the concentration of 25% kills more *Ae. Aegypti mosquitoes,* i.e., 100% within 60 minutes, every 5 minutes of observation (Wahyuni *et al.*, 2022). Likewise, with research on vegetable insecticides of basil leaf extract (*Ocimum basil*) against the death of *Ae. Aegypti*, get more and more *Ae. Aegypti* mosquitoes absorbing the toxin compounds of basil leaf extract, the more mosquitoes die, and the longer the exposure, the higher the toxicity level (Wahyuni & Yulianto, 2018).

The influence of larvicides to kill insects by the soaking method depends on the shape, how it enters the insect's body, the type of substance contained, the concentration dose, and the duration of exposure (Vasantha-Srinivasan *et al.*, 2016; Pineda-Cortel *et al.*, 2019). In this study, it can be seen in Figure 2 that at 12 hours of soaking observation in a concentration of 800 ppm, 82% of the Ae. *Aegypti* larvae can be killed, and the total larvae that died during the 3 HSPs was 100%. This number is the largest and fastest number in killing *Ae. Aegypti* larvae, compared to other concentrations. Another thing that needs to be considered during the testing process in this study is water temperature and air humidity, because one of the causes of *Ae. Aegypti* larvae survive even after immersion has been carried out, which is caused by the temperature of the water and the humidity of the air where the test was carried out. From the results of observations that have been made, the average water temperature when testing red ginger essential oil on *Ae. Aegypti* larvae are around 24^0C- 25^0C and normal indoor air humidity, which is around 69.8% - 72.3%. Conditions for mosquito breeding of *Ae. Aegypti,* that is, at a conducive humidity between 60 – 80%. In this study, the temperature did not affect the death of the *Ae. Aegypti* larvae, because the temperature between 24^0C- 25^0C is the optimal temperature. It is also explained by Nikookar that water temperature and pH are environmental factors that can affect the growth of larvae, and the optimum temperature for mosquito growth is 20°C to 25°C, and pH 6–8 (Nikookar *et al.*, 2017).

Larval death process of *Ae. Aegypti* in this study is influenced by compounds contained in red ginger essential oil, namely Sesquiterpene compounds consisting of three substances, namely: (Zingiberene, Zingiberol, Farnesene), which play a role in providing aroma (smell), and Gingerol, Shogaol, and Paradol as compounds that provide flavor (Stoner, 2013). The mechanism of action of this substance enters the body of *Ae. Aegypti* larvae through the surface of the body (contact toxins), the respiratory system (respiratory toxins), and through the mouth and digestive tract (stomach toxins). This is reinforced by several opinions that state that the level of larvicide toxicity to kill larvae depends on the form of larvicide, how it enters the larva's body, the size and arrangement of the larva's body, as well as the stage and habitat. Larvicides enter the larva's body in three ways, namely through the surface of the body, through the mouth and digestive tract, and through the respiratory system (da Botas *et al.*, 2017; Husna *et al.*, 2020; Rohmah *et al.*, 2020).

From the observations, behavior, and condition of the larvae of *Ae. Aegypti* When soaking with red ginger essential oil, it looks restless and moves actively, fluctuating at concentrations of 200 ppm and 400 ppm, but moves very restlessly and is very active at a concentration of 600 ppm, especially at 800 ppm. At intervals, it can be seen that the movement of the larvae looks stiff like a seizure, followed by weakening movements, falling to the bottom of the test glass, and dying. Larval death of *Ae. Aegypti* is due to the content of red ginger, namely zingiberene, zingiberol, curcumene, and farnesene, which have a sharp taste and aroma (Stoner, 2013). Zingiberen, zingiberol, curcumene, and farnesene indirectly function as fumigants that evaporate into gases. Fumigants are volatile insecticides that become gases and enter the body of insects through the surface of the body and respiratory system, namely the trachea, and are distributed throughout the body, causing death (Husna *et al.*, 2020).

Zingiberen, which is one of the compounds in red ginger essential oil, enters the larvae of *Ae. Aegypti* through the surface of the body, which works as a contact poison. This compound plays a role in causing larvae of *Ae. Aegypti* cannot eat because they cannot recognize food. This may be due to the influence of zingiberen, which is in contact with the larva's body, which interferes with nerves, especially the olfactory organs that function to recognize food. As stated by Moon and Lee that Zingiberen acts as a receptor that activates anti-eating signals in the insect's central nerves, which inhibits the work of the olfactory organs (olfactory), resulting in insects being unable to smell and recognize the presence of food around them. In addition, zingiberen can cause damage to the digestive tract to reduce the larvae's feeding activity, which causes the larvae to become weak and die slowly (Moon *et al.,* 2018). Zingiberen can also be released into larval cells so that it interferes with the process of absorption of food juice and the process of cell transport. With damage to the cytoplasmic membrane, it will cause the compound contained in red ginger, namely zingiberen, to easily penetrate the larva's body to cause damage to the membrane tissue and interfering with the physiological function of the larva's body (Boekoesoe & Ahmad, 2022). In addition to zingiberen, the content of essential oil compounds from red ginger plays a role in causing the death of larvae of *Ae. Aegypti* Zingiberol is also a contact poison, acting as a receptor that activates anti-feeding signals in the central nervous system of insects (Moon *et al.,* 2018; Husna *et al.*, 2020). Zingiberen and zingiberol are the most likely to cause larvae *Ae. Aegypti* The movement looks like it's stiff, then weakens and eventually dies.

In addition to zingiberen and zingiberol, red ginger essential oils also contain gingerol and shagaol. In red ginger, gingerol, shogaol, and paradol are compounds that give a spicy taste (Stoner, 2013). Shogaol is effective in suppressing intestinal contractions and is an antitussive, 6-Shogaol is a dehydrator of gingerol (Mao *et al.*, 2019). Effect of gingerol on larval mortality of *Ae. Aegypti* in this study is a stomach poison that is ingested. Gingerol actively damages the outer membrane and cytoplasmic membrane of the digestive *Ae. Aegypti* larvae cause the lysis of the larval cell membrane so that the absorption of food is disrupted, and finally, the larvae limp and die. The same opinion, according to Mao, is that the lysis of the cell membrane will result in its permeability being disrupted, so that there is a leakage of the cytoplasmic membrane because of the breakdown of phospholipid molecules due to H+ ions. This damaged cytoplasmic membrane will cause toxic compounds to freely penetrate the insect's body, so that physiological, hormonal work, and digestive system disorders occur, which eventually lead to death (Mao *et al.*, 2019). Likewise, Syukur's opinion in his research on Properties of red ginger fraction (*Zingiber officinale* Roscoe var. rubrum) as an insecticide against *Aedes aegypti* and Boekoesoe in his research on extracts of *Zingiber officinale Rosc* as a natural insecticide against larvae of *Aedes aegypti* (Syukur *et al.*, 2018; Boekoesoe & Ahmad, 2022).

Furthermore, this study also calculated the LC_{50} value, which is intended to measure the lethal concentration of red ginger essential oil against larvae of *Ae. Aegypti.* Based on the calculation using the probit analysis method, the LC_{50} value was obtained as 257.89 ppm. The test results showed that red ginger essential oil had larvicide potential against larvae of *Ae. Aegypti*, with an LC value$_{50}$ of 257.89 ppm. LC Value$_{50}$ when compared to the lowest concentration in this study, which is 200 ppm, there is a small difference of 57.89 ppm. From these results, it can be concluded that the concentration of 257.89 ppm of red ginger essential oil in this study is the most effective in killing 50% of the larvae, because it provides an effective concentration of 257.89 ppm, which can provide a death effect of 50% on the larvae of *Ae. Aegypti*. Larval death *Ae. Aegypti* is due to its inability to detoxify the essential oil compounds of red ginger that enter the body (Figure 4).

The results of this study are different from Akon's research on essential oil activities of *Ocimum basil* and *Cymbopogon citratus* in mosquito control of *Anopheles funestus,* as Antimalarial was carried out at concentrations of 250 ppm, 200 ppm, 150 ppm, 100 ppm, and 50 ppm during 24 hours of observation. The results showed that essential oils of *O. basilicum* and *C. citratus* have insecticidal and anti-plasmodial potential on Larvae of *Anopheles funestus* with LC_{50} by 35.5 ppm and 34.6 ppm (Akono Ntonga *et al.*, 2014). Furthermore, Najar reported that the larvicide potential of essential oils *S. dorisiana* and *S. sclarea* LC_{50} ranges from 71.08 to 559.77 ppm (Najar *et al.*, 2020). The results of Pandiyan's research found that the LC_{50} of *Syzygium aromaticum* essential oil is effective against *Ae. Aegypti* 66.90 mgL^{-}

[1], essential oil *Illicium verum,* shows larvicide potential against *Ae. Aegypti* at LC_{50} 41.30 mgL^{-1} and essential oils *Trachyspermum ammi* also show larvicide potential against *Ae. Aegypti* with LC_{50} by 39.48 mgL^{-1} (Pandiyan *et al.,* 2019).

From the results of this study, it can be concluded that the active substances contained in red ginger essential oil have an effect and are effective in killing the *Ae. Aegypti* larvae. The most effective concentration is 257.89 ppm in killing 50% of the larvae (LC_{50}). The positive control in this study is intended to compare the quality of the concentration of red ginger essential oil, whether it is the same as the positive control, namely, abate powder containing temephos, which has been used in the community so far. Results obtained in the first 12 hours of observation showed 100% larval mortality of *Ae. aegypti.* While negative control with aqueducts was also carried out to compare its effectiveness with red ginger essential oil, it turned out that there was no *Ae. Aegypti* larvae that died after every 12 hours of observation. Therefore, red ginger essential oil is effective and a potential biolarvicide in mosquito control of *Ae. Aegypti* because environmentally friendly, safe for other living things, not cause resistance to *Ae. Aegypti* mosquitoes, has high economic value, and easily accessible in different parts of the world, so that it can be used as an alternative to chemical larvicides (Pandiyan *et al.,* 2019). In addition to having various pharmacological activities (Zhang *et al.*, 2022), antioxidant and anti-cancer (Ghasemzadeh *et al.,* 2016), Antibacterial (Sivasothy *et al.*, 2011), Immunomodulatory activity, Antihypertensive, Antihyperlipidemic, Antihyperuricemic, Antimicrobial, and Cytotoxic (Zhang *et al.*, 2022).

Conclusion

The active substances contained in red ginger essential oil (*Zingiber officinale* Var. Rubrum) Effective and effective in killing *Ae. Aegypti* larvae. The most effective concentration is 257.89 ppm in killing 50% of the larvae (LC_{50}). Therefore, Red Ginger Essential Oil Potential to be a biolarvicide for controlling *Ae. Aegypti* because not so toxic that it is safe for humans, does not leave residue in the environment, and will not cause resistance to Aegypti mosquitoes. Red ginger essential oil (*Zingiber officinale* Var. Rubrum) is effectively used as a natural biolarvicide in mosquito control of *Ae. Aegypti.* So it is expected to reduce dengue fever cases.

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Top of Form

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