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Toxicity Test of Red-Shoot Leaves (*Syzygium myrtifolium* Walp.) Extract as Biolarvicide on Filariasis Vector Mortality

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Abstract: The Control of *Culex* sp. using synthetic larvicides is harmful to the environment and human health. Biolarvicide from red shoot leaves (*Syzygium myrtifolium*) is a safe alternative for the environment and human health. This study aims to determine the effect of *Syzygium myrtifolium* leaves extract with ethanol solvent and water solvent on larval mortality and the LC50 values of these extracts. ANOVA test results (α = 0.05) on the mortality of *Culex* sp. larvae exposed to the ethanol extract and aqueous extract of *S. myrtifolium* leaves consecutively showed F-calculated (287)>F-tabulated (3,11) and F-calculated (153)>F-tabulated (3, 11). The treatment of *S. myrtifolium* leaves extract with ethanol and water solvents had a significant effect on the mortality of *Culex* sp. larvae. The LC50 value of the ethanol extract and the aqueous extract of *S. myrtifolium* leaves are 1659 ppm and was 2053.7 ppm. It can be concluded that *S. myrtifolium* leaves extracts with ethanol solvent and water solvent have an effect on the mortality of *Culex* sp. larvae, but are not yet effective as biolarvicide.

Keywords: *Culex*; Larvicidal; Mortality; *Syzygium*

Introduction

One of the main issues in underdeveloped countries is vector-borne infection. More than a million individuals per year pass away from diseases carried by mosquitoes. Several mosquito-borne diseases including arboviruses, parasitic worms, and protozoa, affect more than 700 million people annually throughout the world, including Asia. Mosquitoes are the most well-known disease vectors (Dey et al., 2020). The common house mosquito *Culex pipiens* spreads several diseases such as West Nile virus, Sindbis virus, lymphatic filariasis, and Japanese encephalitis. Female *Culex pipiens* typically ingest vertebrate blood, including that of people and other animals, and as a result, spread infectious diseases from birds to humans and from humans to humans (Jang et al., 2020). Hidayati et al. (2020) also reported that mosquitoes from the Culex genus are the main vectors for filariasis in Indonesia.

Thousands of people die each year as a result of the diseases that *Culex pipiens* transmits. Furthermore, the majority of the diseases cannot be prevented in humans by vaccination. Consequently, pest management continues to be a crucial strategy in the management and avoidance of diseases caused by mosquitoes (Al-Solami, 2021). It is preferable to eradicate the larval stage since the adult mosquitoes would not emerge and so be unable to infect humans. Due to their dependence on water and the fact that their habitat may be easily treated, insecticides are targeted at mosquito larvae (Ashok & Babu, 2021). Endemic regions for mosquitos and are frequently found in accordance with environmental factors that can produce mosquito larvae, like the rainy season is currently in progress. This may result in standing water and river overflow, both of which may encourage the development of mosquito larvae (Hamid & Hamdin, 2023). The larval free rate can be used as a gauge for this prevention's effectiveness (Manulang et al., 2023). The eradication of mosquito nests is the most effective method of preventing the disease. It is anticipated that the existence of larvae designated by a larval-free number more than or equal

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to 95% will stop or lessen the disease's transmission (Nurdin et al., 2023).

Insect self-adaptation to insecticides may be accelerated by the application of synthetic organic pesticides at sub-lethal dosages. This characteristic will be carried over to the following generation, creating a new population that is resistant to a specific class of insecticide (Sembel, 2010; Moniharapon et al., 2023). Pesticide resistance, environmental pollution, and health concerns for people and non- target biota were all caused by the excessive use of synthetic insecticides combined with a complete lack of knowledge about the need to switch pesticides (Bosly, 2023). Insecticides are the largest class of pesticides and have different chemical contents, including organochlorine, organophosphate, kabamate, pyrethroid, and DEET (N, N-diethyl-m-toluamide). Organophosphates are insecticides with the highest level of toxicity to vertebrates (Kusumastuti, 2014). A wide spectrum of symptoms linked to cholinergic crises and impairment of hepatic and renal function are seen in organophosphate poisoning. Because the liver and kidneys play such important roles in the metabolism and excretion of diazinone, they are particularly vulnerable to its effects (Hendrawan et al., 2023).

Synthetic pesticides and repellents are frequently used to control adult mosquitoes, but these treatments are pricy, ineffective, and have a significant environmental impact in addition to posing major risks to human health. Alternative natural insecticides and repellents are now highly regarded by customers for these reasons. They have proven to be environmentally safe, quickly biodegradable, barely hazardous to mammals, and effective at keeping out various mosquito species (Huong et al., 2020). The creation of natural insecticides is the best course of action at this time because the continuous use of chemical insecticides can lead to environmental pollution, the demise of various species, and the development of resistance in eradicated insects. Instead, natural insecticides must be used, which are relatively safe for humans and do not pollute the environment because they will soon vanish from the natural world (Moniharapon et al., 2023). Arunthirumeni et al. (2023) also stated that biologically derived pesticides are thought to be suitable for a sustainable environment.

Plants have many biological activities from their secondary metabolites production (Pehlivan et al., 2021). One of the plants currently used as traditional medicine is the red shoot plant (*Syzygium myrtifolium*). This plant contains flavonoids, phenols, and terpenoids, which have anti-tumour and anti-angiogenesis activities (Indriani et al., 2021). According to Maulidy et al. (2021), these substances are poisonous to insects. These substances are present in *Syzygium myrtifolium* leaves.

Syzygium myrtifolium can be extracted using a wide range of solvents, each of which has benefits and drawbacks, such as water and ethanol. The choice of ethanol as the extraction solvent was considered to provide many advantages over other organic solvents, which is relatively safer (less toxic) (Hikmawanti et al., 2021). The findings of the current literature review point to the necessity for additional research into the Toxicity Test of Red Shoots Leaves (*Syzygium myrtifolium* Walp.) Exctract as Biolarvicide on Filariasis Vector Mortality to determine the impact of aqueous extract and ethanolic extract of red shoots leaves on *Culex* sp. mortality.

Method

Research Design

The research design used was a completely randomized design with 10 treatments and 3 replications. The leaves of *Syzygium myrtifolium* from the Syiah Kuala District in Banda Aceh City were the primary component of this study.

The Process of Extracting the Leaves

Picking the leaves is the initial step. By counting the leaves from the shoots on each branch of the tree, the leaves that are taken are those that are in the fourth, fifth, and sixth orders. After that, the leaves were gathered and weighed to determine their wet weight, which came to 3 kg. Next, separate the leaves into those that can be used and those that should not be. According to the study by Indriani et al. (2021), *Syzygium myrtifolium* leaves were completely washed under running water and then drained. Wind energy acts to dry the leaves for two weeks, then the leaves are ground into a powder using a blender until obtained 500 grams of simplicia. The simplicia was sieved using a mesh sieve number 60 so that the powder size was homogeneous. The finely sifted powder was weighed as much as 500 g and then dissolved in distilled water with a ratio of 1:4 (500 mg in 2 liters of distilled water) and stored in a glass jar as a maceration container for 3×24 hours. The extract was filtered to separate the powder from the solution and then filtered using filter paper into another container until the final macerate was obtained. The final maserate was then evaporated using a rotary evaporator in the Pharmacy laboratory of Syiah Kuala University at a temperature of 500 ^{o}C at a speed of 150 rpm for 6 hours to obtain a 100% pure viscous extract. Phytochemical tests were carried out at the Chemistry Laboratory of

Syiah Kuala University with 3 grams of viscous extract. Preparation of water extract of *Syzygium myrtifolium* leaves was carried out in the same way, but water evaporation takes more time than ethanol.

The Process of Making Solution

Each pure extract was taken as much as 5 grams (5000 mg) to be diluted with 1 liter of water to obtain a stock solution with a concentration of 5000 ppm. The two stock solutions are stored in separate, named containers. The two stock solutions were then diluted with distilled water to obtain solutions with concentrations of 600 ppm, 1200 ppm, 1800 ppm and 2400 ppm. Each stock solution was stored in a glass bottle for use during the study.

Biolarvicidals Observations

The mosquito larvae used were 3rd instar larvae of *Culex* sp. in total of 300 tails developed by the Laboratory of Parasitology, Faculty of Veterinary Medicine, Syiah Kuala University. The larvae were acclimatized before being tested in a solution of *Syzygium myrtifolium* leaves extract. 3rd instar larvae of *Culex* sp. taken then transferred into a 250 ml plastic cup. Then, 10 mosquito larvae taken randomly were put into each plastic cup. Room temperature is measured using a thermometer. The plastic cup labeled P0 is filled with larvae habitat water, then the plastic cup labeled P9 is filled with abate. The next four plastic cups (P1, P2, P3, and P4) were filled with *Syzygium myrtifolioum* leaves extract with ethanol solvent and the other four plastic cups (P5, P6, P7, and P8) were filled with *Syzygium myrtifolioum* leaves extract with water solvent. Each extract concentration using ethanol and water solvents consisted of 600 ppm, 1200 ppm, 1800 ppm and 2400 ppm. The next stage observed and recorded the number of larvae that died every hour for 24 hours of observation. Dead larvae were recorded based on the time of death at each concentration. Each dead larva was transferred to a petri dish and then counted.

Data Analysis

The quantitative data were analyzed statistically using the ANOVA test which has a 95% confidence level (α = 0.05) (Thomatou et al., 2013). Duncan Multiple Range Test (DMRT), and probit regression analysis on both extracts separately between ethanol extract and aqueous extract of *Syzygium myrtifolium* leaves to determine the value of Lethal Concentration 50 (LC_{50}).

Result and Discussion

Figure 1 shows the mortality rate of *Culex* sp. larvae different in each treatment for 24 hours. Larval death began to occur at a concentration of 600 ppm. At a concentration of 0 ppm (negative control), no test animals died, with a mortality rate of 0%. The concentration of 100 ppm abate (positive control) kills all larvae with a mortality rate of 100%. The concentration of 2400 ppm was able to kill 77% of the test larvae which was the highest percentage. This figure shows that the concentration of 2400 ppm had the best ability to kill larvae among all concentrations of the ethanol extract of *Syzygium myrtifolium* leaves used. The concentration of *Syzygium myrtifolium* leaves extract is directly proportional to the death of the larvae. The higher the concentration of *Syzygium myrtifolium* leaves extract, the number of *Culex* sp. larvae the dead are also increasing.

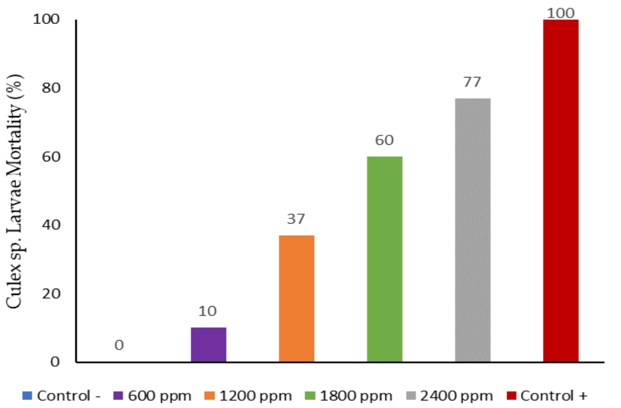

Figure 1. The average mortality of *Culex* sp. larvae for 24 hours due to the influence of the ethanol extract of *Syzygium myrtifolium* leaves

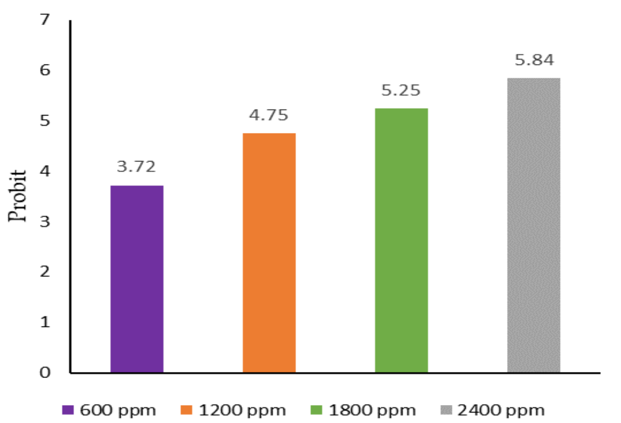

Figure 2. Probit values at various concentrations of ethanol extract of *Syzygium myrtifolium* leaves

The ability of *Syzygium myrtifolium* leaves extract to kill *Culex* sp. also analyzed using probit regression analysis so that the LC_{50} value is known, namely the concentration value of the test substance needed to kill *Culex* sp. larvae as much as 50%. The Ministry of Agriculture's pesticide commission stated that larvicides were said to be effective if they had an LC_{50} value below 1000 ppm. The results of the probit regression analysis showed that the concentration of the ethanol extract of *Syzygium myrtifolium* leaves which caused 50% death (LC_{50}) was 1659 ppm. So, the ethanol extract of *Syzygium myrtifolium* leaves is not yet effective as a larvicide because it has an LC_{50} value above 1000 ppm. The probit value of each concentration of *Syzygium myrtifolium* leaves extract can be observed in Figure 2.

In the study of the effect of *Syzygium myrtifolium* leaves extract on the mortality of *Culex* sp. as a biolarvicide the value obtained from the probit regression analysis can be seen in Figure 2. The correlation between variable X (concentration) and variable Y (probit value) shows a positive relationship with a very strong interpretation.

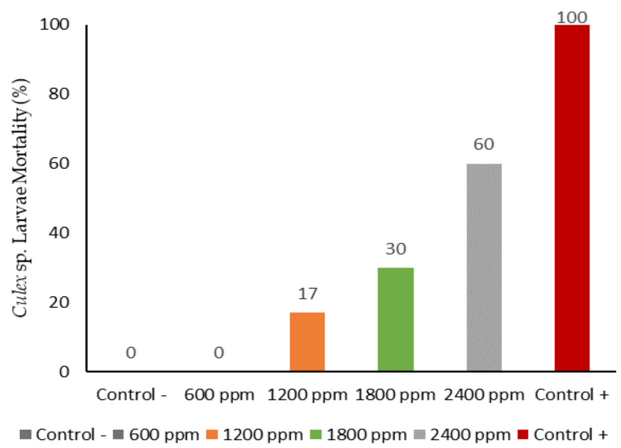

Figure 3. Average mortality of *Culex* sp. for 24 hours due to the influence of *Syzygium myrtifolium* extract with water solvent

Figure 3 shows the mortality rate of *Culex* sp. larvae different in each treatment for 24 hours. Larval death began to occur at a concentration of 1200 ppm. At concentrations of 0 ppm (negative control) and 600 ppm no test animals died, with a mortality rate of 0%. The concentration of 100 ppm abate (positive control) kills all larvae with a mortality rate of 100%. The concentration of 2400 ppm was able to kill 60% of the test larvae which was the highest percentage. This figure shows that the concentration of 2400 ppm had the best ability to kill larvae among all the concentrations of the aqueous extract of *Syzygium myrtifolium* leaves used. The concentration of *Syzygium myrtifolium* leaves extract is directly proportional to the death of the larvae. The higher the concentration of *Syzygium myrtifolium* leaves extract, the number of *Culex* sp. larvae the dead are also increasing.

In the study of the effect of *Syzygium myrtifolium* leaves extract on the mortality of *Culex* sp. as a biolarvicide the value obtained from the probit regression analysis can be seen in Figure 4. The correlation between variable X (concentration) and variable Y (probit value) shows a negative relationship with a very strong interpretation.

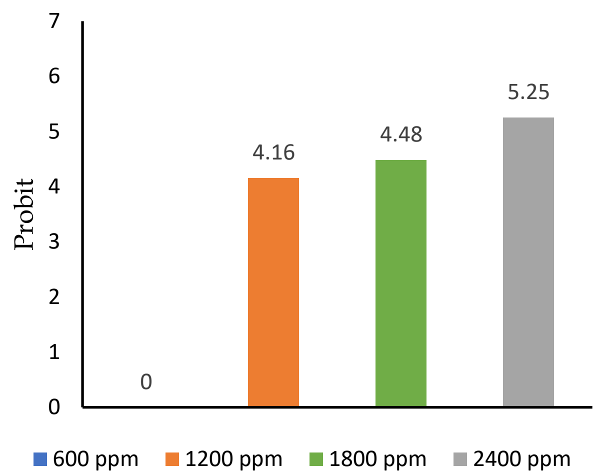

Figure 4. Probit values at various concentrations of aqueous extract of *Syzygium myrtifolium* leaves

The increase in larval mortality was caused by an increase in the concentration of the extract. The toxicity of the extract at each concentration has a different impact. Low concentrations have low toxic levels, causing low larval mortality. Conversely, at high concentrations the toxic levels are also high so that the mortality of the larvae is also high. The relationship between concentration and larval mortality is directly proportional. The higher the concentration used, the more larvae will die. In addition to concentration, the type of solvent used in making the extract also affects larval mortality. The mortality rate of larvae exposed to ethanol extract of *Syzygium myrtifolium* leaves was higher than that of water extract of *Syzygium myrtifolium* leaves. The length of exposure to the extract also affects the death of the larvae. The longer the larvae are exposed to the extract, the higher the mortality of the larvae.

According to Mahyoub et al. (2016), the relatively small variations in the range of effective concentrations seen in these investigations may be attributed to variations in the strains, the biological reaction of the larvae under investigation, the drug formulation, or the experimental setup. To find out how the environment affects these insecticides' larvicidal efficacy in field control operations, long-term follow-up researches are required. The explanation could stem from Cx. pipiens's varying susceptibility to various active substances.

Those active substances percentages are vary based on the maceration process.

The maceration process is utilized because it is simple, doesn't require heating, and allows for the extraction of numerous chemicals despite its lengthy duration and lack of heating. Natural materials are also less likely to be harmed or degraded (Khairi et al., 2023). Water is an organic solvent that is polar and can dissolve bioactive compounds, including alkaloids, phenolics, tannins and alkaloids. Water can extract other components that are polar, non-polar and other semi-polar (Senduk et al., 2020). The high volume of water found in ethanol had an impact on the polarity of the three concentrations of ethanol employed in this investigation. The higher its degree of polarity absolute ethanol, the more water it contains. A group of chemicals having a larger polarity can be extracted using solvents with high polarity. This made it possible for non-phenolic polar molecules like proteins and carbohydrates to dissolve during the extraction process, increasing the extraction yields (Hikmawanti et al., 2021). Aliyu et al. (2022) also stated that ethanolic extract of mosquitocidal potential had the highest efficacy on mosquito repellency compared to another solvent.

This statement is reinforced based on the results of phytochemical tests that have been done in Chemistry Laboratorium in Syiah Kuala University. *Syzygium myrtifolium* leaves extract using 70% ethanol and *Syzygium myrtifolium* leaves extract using water as the solvents contains the same phytochemical compounds, namely alkaloids (dragendrof and wagner), saponins, tannins, flavonoids, polyphenols and triterpenoids. Rumalolas et al. (2023) stated that Wagner, Meyer, and Dragendorf reactants were the three reactants used in the identification of alkaloid compounds. When a brownish-white precipitate appears, the Dragendorf reactant has a good outcome.

Saponins have hemolytic and membrane-permeabilizing effects. They damage the larvae's cuticle membrane, disrupting it, and ultimately killing the larvae. They cause increased death rates and decreased diet intake, which causes insect pests to lose weight, grow more slowly, experience growth instabilities, and have fewer offspring. The mechanism of action is caused by saponins reducing their desire for food or causing gastrointestinal issues due to molting issues or its harmful effects on cells (Ekpoma et al., 2022). Yuliany et al. (2020) added that saponins can cause hemolysis in larvae. This statement is supported by Noer et al. (2023), which stated that active substances called saponins have a surface that resembles that of a detergent; this surface damages the permeability of the microbial plasma membrane.

The finding of tannins in S. *Myrtifolium* extract is supported by Muthmainnah (2017) which also stated that Tannins are bioactive substances that fall under the phenolic chemical class and have the property of dissolving in water and polar solvents. Tannins adhere to essential proteins required for development, preventing an insect from further metabolizing nutrients and using protein. A few of tannins' most well-known toxicities include increased cytoplasmic vacuolation, a lack of cytoplasmic restrictions, the appearance of apical vesicles and the release of cytoplasmic components from the cell, increased intercellular space, and the separation of cells from the basement membrane, which are symptoms of being exposed to a toxic substance (Ekpoma et al., 2022). According to Yuliany et al. (2020), tannins are able to inhibit digestive enzyme activity and metabolic disorders in larvae.

Flavonoids work by entering the body of the larvae through the respiratory system which results in damage to the respiratory system and paralysis of the nerves of the larvae. The larvae die because it is difficult to breathe (Maulidy et al., 2021). According to Yuliany et al. (2020), flavonoids reduce the growth rate of mosquitoes by inhibiting the work of endocrine enzymes and preventing the release of digestive enzymes. Triterpenoids also interfere with the respiratory system in larvae.

The presence of active substances in the form of tannins, flavonoids, alkaloids, and saponins lowers the surface tension of the cell wall and damages the permeability of the membrane, causing leakage of proteins and enzymes inside the cell (Anarkhis et al., 2023). Plants create secondary metabolites to interact with their surroundings and protect themselves against disease and pests. Among these secondary metabolites that may prevent insect growth include terpenoids, alkaloids, flavonoids, saponins, and tannins (Astuti et al., 2023). This study is also in line with research by Moniharapon et al. (2023), stated that tannins, flavonoids, and saponins are chemical substances function as respiratory poisons or inhibitors to kill mosquitoes.

Conclusion

The conclusions obtained from this study that *S. myrtifolium* leaves extracts with ethanol solvent and water solvent have an effect on the mortality of *Culex* sp. larvae, but are not yet effective as biolarvicide.

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Author Contributions

Conceptualization, Supriatno, Riska, D. Syafrianti; methodology, Supriatno, Riska, D. Syafrianti, F.A. Ulhusna; validation, Supriatno, D. Syafrianti, H. Rahmatan, Y.I.M. Nur; investigation, Riska; resources, Riska; writing—original draft preparation, Riska; writing—review and editing, Supriatno, D. Syafrianti; supervision, Supriatno and Riska; project administration, Riska; funding acquisition, Supriatno. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors have declared no conflict of interest.

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