Biolarvacide Leaves Powder of *Mimosa Pudica L.* on Mortality The Mosquito of *Culex quinquefasciatus*

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Track Record Article

Accepted: 21 June 2024
Revised: 03 April 2024
Published: 28 June 2024

How to cite :
Riswanda, J., Marisca, N., & Romadhan, M. F. (2024). Biolarvacide Leaves Powder of Mimosa Pudica L. on Mortality The Mosquito of Culex quinquefasciatus. *Contagion: Scientific Periodical Journal of Public Health and Coastal Health*, *6*(1), 715–725.

Abstract

This research aims to determine the potential of Mimosa pudica L. as a larvicide for the mosquito of Culex quinquefasciatus. This research used an experimental method with a Completely Randomized Design (CRD), consisting of 6 treatments and 4 replications. The treatments consisted of treatment 0 gr/100 ml (control -); abate 0.4 gr/100 ml (control +); treatment (P1) 0.1 gr/100 ml; treatment (P2) 0.2 gr/100 ml; treatment (P3) 0.3 gr/100 ml; treatment (P4) 0.4 gr/100 ml. The parameter observed in this study was the mortality rate larvae instar III of Culex quinquefasciatus. The analysis used in this research was One Way Anova using a software application, namely SPSS statistics 22. To find out the potential of Mimosa pudica L. on the mortality rate larvae instar III of Culex quinquefasciatus larvae, it can be seen from the significance value. If the significance value is less than 0.01, it means that the treatment has a very significant effect, then further tests will be carried out. The further test used is the Duncan test. From the results of research that have been calculated using the One Way Anova test, it is proven that there is an effect of giving Mimosa pudica L.leaf's powder on mortality larvae instar III of Culex quinquefasciatus. The effect that occurs can be seen in the significance value < 0.01, meaning that the leaves powder of Mimosa pudica L. has a very significant effect on the mortality larvae instar III of Culex quinquefasciatus. It can be concluded that the Mimosa pudica Lhad the potential as biolarvicide of Culex quinquefasciatus.

Keywords: Biolarvacide, Larvacide, Mimosa pudica L, Leaves Powder, Culex quinquefasciatus

INTRODUCTION

Larvicide is a pesticide slaying larvae (insects in development) (Molina et al., 2022). Eradication of larvae using larvicide is one of the best methods to control insects in the ecosystems. Unfortunately, synthetic larvicide is often used by society (Hou et al., 2020). The use of synthetic larvicide can cause insect resistance, environmental pollution, and pestiferous organisms, include humans. Consequently, biolarvicide should be used for diminishing the effects. Biolarvicide is the larvicide from the plant. It is felicitous to be developed due to its potential for controlling the vector of the disease. Its exertion is relatively safe and profitable (Wilson et al., 2020). The secondary metabolites in plants, such as flavonoids, terpenoids, and alkaloids have no impact to the environment, and others (Chang et al., 2020 ; Yeshi et al., 2022). Based on the references, many plants that have not been optimized yet functionally (Dai et al., 2020; Silvie et al., 2021; Kabtiyal et al., 2022).

Terpenoid and alkaloid of Nothopanax scutellarium have a role as biolarvicide of

Aedes aegypti (Zhou et al., 2021) .Moreover, the essential oil, terpenoid, alkaloid, flavonoid, and saponin, of Lansium domesticum, can slay the larvae of Aedes aegypti (Vitangcol et al., 2021). Alkaloid, saponin, and flavonoid as the toxic of larvae. Be in accordance, plants from Indonesia contain a lot of secondary metabolites which is effective to remove the insect's larvae. However, the researches do not exhibit yet its function as biolarvicide of Culex quinquefasciatus (Ananda & Rustam, 2023).

Culex quinquefasciatus is a vector filariasis (elephantiasis) disease. Filariasis is caused by 2 main species of filarial worms, namely Wuchereria bancrofti and Brugia malayi Its activities from 6 pm to 6 am so disturbing humans . The disease is caused by Wuchereria bancrofti. Its habitat is dirty water, which is filled with rotting plants and household waste or other impurities. Hence, biolarvicide is very needed to control the development of mosquito by using plants which often found in the environment, especially in Palembang, South Sumatra, and unknown yet another usefulness, such as Mimosa pudica L. (Marcos et al., 2023).

Mimosa pudica L. contains secondary metabolites, like flavonoid, steroid, triterpenoid, and alkaloid. Mimosa pudica has alkaloid, flavonoid, terpenoid, sterol, tannin, and saponin. The compounds had found in the leaves, stem, and root (Giri et al., 2023). Based on the references, the compounds are suggested to play a role as biolarvicide of Culex quinquefasciatus. Nevertheless, the function of these plants is still not widely known by society. Therefore, this research needs to be done by using Mimosa pudica L. Based on phytochemical analysis, the leaves, stems and roots of Mimosa pudica L. plant positively contain various compounds such as alkaloids, flavonoids, tannins and saponins. The objective of this study was to find out the effect leaves powder of Mimosa pudica L.

Natural larvicide is a larvicide made from plants that contain poisonous substances to insects in the larval stage. The use of natural larvicides has no side effects on the environment, humans and does not cause resistance to insects. One of the plants that has the potential to be a source of natural larvicide is Mimosa pudica L. (Silvério et al., 2020).

Larvicide is a substance that can be used to break the life cycle of mosquitoes. Chemical control efforts are generally quite effective in breaking the life cycle of mosquito vectors. However, the use of synthetic larvicides can result in environmental pollution. Alkaloids, saponins and flavonoids can be used as larvicides because they are stomach poisons. Plants in Indonesia that have the potential to act as natural larvicides are plants that contain compounds such as alkaloids, flavonoids, terpenoids, tannins, sterols and saponins and essential oils were effective as natural larvicides (Kirar & Sehrawat, 2022).

Based on previous research, various secondary metabolites such as alkaloids, flavonoids, tannins and saponins can be used as mosquito larvicides. So, research will be carried out on Mimosa pudica L. which is thought to have potential as a larvicide for the Culex quinquefasciatus mosquito.

METHODS

The research was carried out in the natural science laboratory, UIN Raden Fatah Palembang. Larvae of Culex quinquefasciatus obtained was identified morphologically by using (Silva et al., 2021). The study was approved and reviewed by the Health Research Ethics Committee of Health Polytechnic Ministry of Health Palembang, No: 1248/KEPK/Adm 2/VIII/2023.

The method was an experimental with a completely randomized design (CRD) consisting of 6 treatments and 4 replications. The treatment consists of no treatment (control -); abate 0.4 gr/100 ml (control +); treatment (P1) 0.1 gr/100 ml; treatment (P2) 0.2 gr/100 ml; treatment (P3) 0.3 gr/100 ml; treatment (P4) 0.4 gr/100 ml. This treatment was obtained based on preliminary research. The tools used in this research were mortar, sieve, analytical balance, petri dish, gauze, stereo microscope, pH indicator, beaker, clock, spatula, rope, scissors, lab optics, dropper pipette, thermometer. The materials used were Mimosa pudica L), abate, sewer water and third instar Culexquinquefasciatus larvae (Stiani et al., 2022).

The plants used were derived from locations with an average ambient temperature of 29^{O}C and humidity of 87,5%. The leaves used are leaves that arenot too young and not too old. For the mimosa, all of its parts were used in this study. Dried samples were blended into powder, approximately 10 gr for each sample. Then, plant powder was experimented with larvae. As many as 10 Larva identified was prepared into 100 ml water volume. Leaves powder was given to test the potential of larvicide. Then, larval mortality was observed after 4 hours. The characteristics of dead larvae are immobile, smaller than normal size, stiff, and pale in color when compared to controls (Setlur et al., 2023). Data observed were analyzed by using ANOVA.

The research method used in this research was experimental research with a Comp(letely Randomized Design (CRD), consisting of 6 treatments and 4 replications. The treatment consists of no treatment (control -); abate 0.4 gr/100 ml (control +); treatment (P1) 0.1 gr/100 ml; treatment (P2) 0.2 gr/100 ml; treatment (P3) 0.3 gr/100 ml; treatment (P4) 0.4 gr/100 ml. This treatment was obtained based on preliminary research.

Research procedure

The procedures in this research are as follows:

1. Mosquito Larva Identification

a. Mosquito larvae were obtained from the sewers on Jln. Rawa Jaya, Palembang City, with water temperature conditions ranging between 28 oC-30 ‘ C and water pH ranging between 6-7.
b. Next, mosquito larvae were identified using a stereo microscope and laboratory optics, guided by the book Abdulazeez et al., (2019), to determine the type of mosquito larvae and their size.
c. Once the identification has been completed and it has been confirmed that the mosquito larvae obtained are Culex quinquefasciatus instar III type, then place the larvae into the control media and treatment media.

2. Manufacturing Stage Powder of *Mimosa pudica L*

The stages of making millet leaves powder of Mimosa pudica L are as follows:

a. dried indoors the leaves of Mimosa pudica L for 30 days.
b. Mash the leaves of Mimosa pudica L using a mortar.
c. Then filter the puree leves of Mimosa pudica L using a sieve to get a really fine powder.
d. After that, the powder of Mimosa pudica L was weighed using an analytical balance according to the treatment concentration, namely 0.1 gr; 0.2 gr; 0.3 gr and 0.4 gr.

3. Testing Phase the powder of Mimosa pudica L Against Mooqito larvae Instar III of Culex quinquefasciatus

The stages of testing the leaves powder of Mimosa pudica L against larvae instar III of Culex quinquefasciatus are :

a. Prepare the treatment media (24 beakers each containing 100 ml of sewer water).
b. Then measure the pH and temperature of the water.
c. Then enter the mosquito larvae Culex quinquefasciatus10 larvae instars III in each treatment medium.
d. Then add the powder of the mollusk leaves of Mimosa pudica L into the 4 treatment media (0.1 gr/100 ml sewer water; 0.2 gr/100 ml sewer water; 0.3 gr/100 ml sewer water; 0.4 gr/100 ml sewer water).
e. Then cover the treatment media and control media using gauze.
f. Then observe the treatment media for 8 hours.
g. Larvae that no longer move (declared dead) (Abebe Teshome, Berhanu Erko , Lemu Golassa , Gedeon Yohannes , Seth R. Irish , Sarah Zohdy and Sisay Dugassa, 2023).

Data analysis

The analysis used in this research is One Way Anova using a software application, namely SPSS statistics 22. According to Anagha S Setlur, Chandrashekar K, Ritwija Bhattacharjee, Jitendra Kumar & Vidya Niranjan (2024), the data obtained was analyzed using analysis of variance. To determine the potential of Mimosa pudica L. on the mortality rate mosquito larvae,instar III of Culex quinquefasciatus, it is determined using the F test. If the test results Fcount > Ftable with a confidence level of 0.05, it means that the treatment has a significant effect, whereas if it is 0.01, it means that the treatment has a very strong effect. real, then further tests will be carried out. The further test used is the Duncan test.

RESULTS

The results showed that the mortality of larvae. It showed the discrepancy in the percentage of Culex. quinquefasciatus' larval mortality. The percentage of Culex quinquefasciatus'larval mortality in each treatment can be seen in Table 1.

Table 1. Results of One Way Anova Test Number Mortality larvae Instar III of culex quinquefasciatus treated with Leaves Powder's Mimosa pudica L

Diversity Source	Sum of Squares	Degrees of Freedom	Middle Square	F_{Count}	Sig.	F_{Table} (1 %)
Treatment	261,333	5	52,267	209,067	,000	4.25
Error	4,500	18	,250			
Total	265,833	23				

The results of the One Way Anova analysis in table 1 show that the mollusc powder Putri Malu (Mimosa pudica L) has a very significant effect of sig < 0.01 (0.00 < 0.01) or Fcount> Ftable (209.067 > 4.25) on the mortality larvae instar III of Culex quinquefasciatus . So H1 is accepted and H0 is rejected. Therefore, to see the effect between treatments, further tests were carried out which can be seen in table 2.

Table 2. Results of Further Tests (Duncan Test) Number of Mortality Larvae Instar III of Culex quinquefasciatus treated with Leaves Powder's Mimosa pudica L

Further testing	Treatment	N	Subset for alpha = 0.01			
			1	2	3	4
Duncan Test	Control (-)	4	.00			
	P1	4		4.50		
	P2	4		5.25		
	P3	4			6.50	
	P4	4				9.50
	Control (+)	4				9.75
	Sig.		1,000	,048	1,000	,489

Duncan's further test results in table 2 show that there are differences in the effects between control treatment (-), control treatment (+), treatment 1 (P1), treatment 2 (P2), treatment 3 (P3) and treatment 4 (P4). In the control treatment (+) using abate powder, the number of mortality larvae instar III of Culex. quinquefasciatus was 9.75 and in treatment 4 (P4) with The highest concentration leaves powder's of Mimosa pudica L, namely 0.4 grams, showed that the number mortality larvae instar III of Culex. quinquefasciatus was 9.5.

The results of observations in this study showed that there was an effect of leaves powder's of Mimosa pudica L. as a larvicide for larvae instar III of Culex quinquefasciatus. The results of the research show that there are differences in the average number of larval deaths in each treatment which can be seen in Figure 1 below.

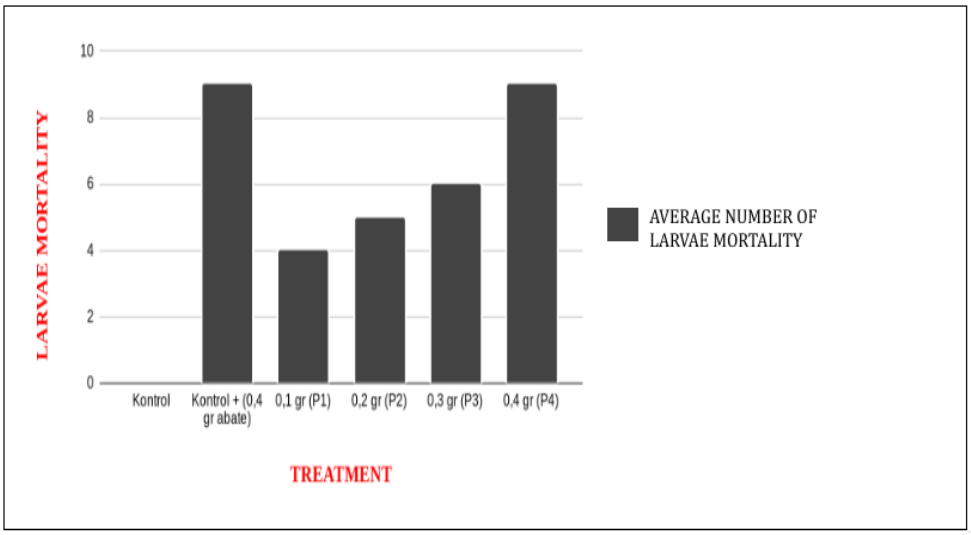

Figure 1. Average number mortality larvae Instar III of Culex quinquefasciatus

Figure 1 represented that the enhancement in larval mortality of Culex. quinquefasciatus. This improvement revealed that the powder used in this study had an impact on mortality. Based on these improvements and changes, analysis of variance was carried out. Analysis of variance pointed out that the powder of plants has very significant leverage ($P < 0,05$) to the larval mortality of Culex. quinquefasciatus. It meant that the powder used in this study could be used as biolarvicide of Culex. quinquefasciatus.

DISCUSSION

Based on the data obtained from the results of statistical tests regarding the number mortality larvae instar III of Culex quinquefasciatus and calculated using One Way Anova analysis which can be seen in table 1, the leaves powder Mimosa pudica L. has a very significant effect of sig < 0.01 (0.00 < 0.01) or $F_{count} > F_{table}$ (209.067 > 4.25) on mortality

larvae instar III of Culex quinquefasciatus. So H1 is accepted and H0 is rejected.

By therefore, The influence between treatments was carried out by Duncan's further test which can be seen in table 2, showing that there are differences in the influence between treatments control (-), treatmentcontrol (+), treatment 1 (P1), treatment 2 (P2), treatment 3 (P3) and treatment 4 (P4). In the control treatment (+) using abate powder the average number of deaths of third instar Culex quinquefasciatus mosquito larvae was 9.75 and in treatment 4 (P4) with the highest concentration of Putri Malu powder (Mimosa pudica L), namely 0.4 g, the average the number of deaths of third instar Culex quinquefasciatus mosquito larvae was 9.5. This can be seen in Figure 1 that there was an increase in the number of deaths of third instar Culex quinquefasciatus mosquito larvae in each treatment .

The higher the concentration of mollusc Leaves powddeer of Mimosa pudica L given to the treatment, the higher the larval death rate. In treatment 4 using 0.4 grams of mollusc leaves powder of Mimosa pudica L. caused the most larvae to die and the average death rate was almost the same as the control + treatment which used abate. Abate is an organophosphate class insecticide. Which Leaves powder used in the study, specifically Mimosa pudica L, a very significant clout on larval mortality of Culex quinquefasciatus. This leverage could be seen in the P3. However, the percentage at P4 revealed that all larva had died. Larval mortality is caused by the secondary metabolites found in plant powder, such as flavonoid, saponin, tannin, and alkaloid (Tia & Small, 2023). Based on observations after 4 hours, it was seen that the larva was immobile, smaller than normal size, stiff, and pale in color when compared to controls. This situation indicated that the larva had died. It was suggested to be caused by secondary metabolites in plants powder that was used in this study, such as flavonoids, saponins, tannins, and alkaloids. The compounds from the plant's powder used to bind to water and its contacts to larvae.

Effectively kills mosquito larvae or other water insects that are often used by the public. This further strengthens the suspicion that the mollusk leaves powder of Mimosa pudica L. has great potential as a larvicide for the Culex quinquefasciatus mosquito larvae (Alhag et al., 2021).

Flavonoid acts as an inhibitor of the respiratory system of Culex quinquefasciatus larvae. It interrupts the siphon membrane. It goes into the larval body through the siphon and devastates the siphon membrane by interfering with the phospholipid- binding so that the membrane ruptures (Hao et al., 2024). The breakage can obstruct absorption the oxygen. Besides, flavonoid inhibits the enzyme of ATPase which causes insufficient energy so that the larvae undergo the paralysis of the respiratory organs (Ahmed et al., 2021). Consequently, the

larva will difficultto adopt oxygen and death. This mechanism was expected to cause the larva to turn pale.

Besides flavonoids, there are several active compounds contained in the powder used in this study, such as saponin and tannin. Saponin has a role as the digestive toxic for the larva (Babin et al., 2023). Water-soluble saponin enters the body of the larva through the mouth and comes into the digestive tract (foregut, midgut, and hindgut). However, in the midgut, it only has a peritrophic membrane that protects the cells from the friction of particles (Nile et al., 2020; Divekar et al., 2022) (Hussain et al., 2019; Jampilek, Kos, & Kralova, 2019). Saponin wrecks this membrane. Saponin has strong quality as the surfactant. Surfactant is the molecules that can dissolve with lipid and water (Rasool et al., 2023). Mechanism of saponin as the toxic can penetrate the peritrophic membrane and binding to lipids until the epithelium of midgutbreakage. This causes the absorption of nutrients hampered and the larvae will lack nutrients. As well as saponin, tannin also had a role as the digestive toxic for the larvae.

Tannin is the polyphenol that can form complex compounds with proteins. Formation of tannin and protein due to hydrogen bonds. The mechanism of action of tannin as the poison occurs in the midgut which is an organ that absorbs nutrients and secretes digestive enzymes (Aristri et al., 2023). The digestive enzymes, that is protease enzyme, are inhibited by tannin so that the role of the protease enzyme in catalyzing proteins into amino acids as the nutrient intake needed for growth is disrupted. Protein deposition by tannin causes the protease enzyme cannot break down the protein so that the work of the enzyme is inhibited. This retardation can disrupt metabolism and larvae will lack nutrients. If this process occurs continuously, it will cause larval mortality marked by the larvae was smaller than normal size.

Alkaloid has the mechanism to degrade the exoskeleton by dissolving the chitin layer. It is a waxy material as a source of carbon and nitrogen for the growth of larvae after molting**.** The disruption of exoskeleton causes the molting process (Baskar et al., 2020). It perhaps caused the larvae to become stiff. This mechanism still needs further research.

According González & Vallejo (2023) to the more concentrated the concentration of the solution, the more toxic substances contained in the treatment of the mollusk leaves powder of Mimosa pudica L., so that more poison is consumed and the larvae die higher. According to (Stiani et al., 2022) the percentage of larval mortality in each treatment increases as the concentration increases. This effect indicates that the mollusk leves powder of Mimosa pudica L. has the potential to act as a larvicide. In this study, the larvae were said to be dead if they no longer moved, the larvae settled at the bottom of the water, the larvae's bodies were soft and

limp. The death of the mosquito larvae is thought to be due to the presence of alkaloids, flavonoids, tannins and saponins from the powder of the mollusk leves powder of Mimosa pudica L. which dissolves in water.

These toxic compounds enter the body of C. quinquefasciatus instar III mosquito larvae through the exoskeleton which acts as a contact poison and through the mouth which acts as a stomach poison (Yeshi et al., 2022).

CONCLUSIONS

Based on the research results, it can be concluded that the higher the concentration of mollusc leaves powder of Mimosa pudica L. given to the treatment, the higher the larval death rate. In treatment 4 using 0.4 grams of mollusc leaves powder of Mimosa pudica L caused the most larvae to die and the average death rate. The mollusk leaves Powder of Mimosa pudica L. has the potential to act as a larvicide on mortality for instar III Culex quinquefasciatus.

ACKNOWLEDGMENT

The author would like thank to staf, chief and all parties the integrated laboratory UIN Raden Fatah Palembang for their assistance in this research.

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