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The Effectiveness of Pineapple Peel Extract (*Ananas comosus L. merr*) as Natural Larvicides of *culex sp.* Larvae

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ABSTRACT

Culex sp. is known to be a type of vector for filariasis. A filarial worm is the cause. The most common method of controlling insects is with synthetic insecticides. Synthetic compounds are hazardous for both the environment and exposed users. The development of environmentally friendly and readily biodegradable pesticides is therefore essential. The study aimed to determine how well pineapple extracts worked as natural larvicides for *Culex* larvae. The Aceh Health Polytechnic Laboratory served as the site of the investigation. This type of research is experimental research, using a sample of 625 *Culex* larvae. Each treatment contained 25 larvae, performed five repetitions, and had an observation time of 12 hours. Extract concentrations were 2%, 4%, 6%, and 8%. The analysis employed the T-test to compare each extract, the Least Significant Difference (LSD) test to identify significant differences, and the ANOVA test to ascertain the difference in treatment averages. The average concentration of pineapple peel extract varied in their effects on *Culex* larvae mortality, according to the study's findings. Larvae were killed in 77.6% of cases by an 8% concentration of pineapple peel extract. Results showed that the extracts of pineapple, with an average of 7.40 larvae from pineapple peel. *Culex* larvae can be killed more effectively with pineapple peel extract.

Keywords: *Culex sp.*, pineapple peel, larvicide

INTRODUCTION

Culex mosquitoes are known to be vectors for the spread of malaria, elephantiasis fever, and arboviruses in poultry. This particular genus of mosquitoes inhabits the human environment. In addition, numerous studies have demonstrated that, in addition to their ability to endanger human life through bites, several species of this mosquito are also disease carriers.(1)

The development of dangerous organisms, particularly *Culex sp.* larvae, found in unclean channels like murky ponds, ditches, and puddles with poor water quality, has been aided by a lack of public awareness regarding environmental cleanliness issues. To control mosquito larvae, the community has employed a variety of techniques, such as chemical (by adding larvicide to water reservoirs), mechanical (by closing water reservoirs), and biological (by keeping fish that consume larvae) methods. There are multiple steps involved in controlling mosquito vectors. The first step is to decrease the number of mosquitoes by eliminating mosquito breeding grounds, using insecticides to kill adult or larval mosquitoes,

and preventing mosquito bites to prevent diseases brought on by mosquito bites.(2)

One of the world's tropical nations, Indonesia offers ideal temperatures and humidity levels for insect survival. Because they spread disease, mosquitoes are one insect that can be dangerous to people. Mosquitoes are responsible for several diseases, including filariasis, which the *Culex sp.* mosquito spreads.(3)

Culex sp. is an animal that is a vector for transmitting various diseases. *Culex sp.* is a type of mosquito vector known as the house mosquito. Several types of diseases can be transmitted through *Culex sp.*, such as *lymphatic filariasis*, often known as *elephantiasis*, *Japanese Encephalitis* (JE) or inflammatory brain disease that attacks animals and humans, *Saint Louis Encephalitis*, which is a disease that attacks the central nervous system in humans. *Filariasis* (*elephantiasis*) is a disease caused by filarial worms (*Brugia malayi*, *Wuchereria bancrofti*, and *Brugia timori*). Through mosquito bites, humans can contract the filarial parasite from infected mosquitoes; *Culex sp*. is one such mosquito type.(4)

Last year, the prevalence of filariasis or elephantiasis among Indonesians of all ages was 1.5%. This figure results from examining a weighted sample (n) of 877,531 people. Regarding elephantiasis prevalence, Central Papua has the highest national rate at 4.8%. Central Kalimantan and Papua have similar rates at 2.7% and Bangka Belitung at 2.5%, respectively, followed by Mountain Papua at 2.4%. In contrast, the prevalence in Aceh is 2.1%.(5)

Filariasis worms are the cause of the infectious disease elephantiasis. This illness is known as chronic because it can result in acute or mild swelling. Any body part, including the hands, feet, and face, may have swell lymph nodes.(6)

Nevertheless, chemical control with synthetic pesticides has remained the primary method of controlling mosquitoes to date. Using commercially available chemical anti-mosquito medications to deter and stop mosquito growth is common among Indonesians. Chemical use, however, can have harmful effects on non-target insects, the environment, and people. Excessive and continuous use of synthetic pesticides can negatively impact the health of the surrounding environment, such as the environment becoming polluted, increasing resistance to target insects, increasing the mortality of non-target animals, and leaving certain residual substances that are difficult to decompose in nature.(7)

Consequently, it is preferable to use plants as a larvicide since they are more ecologically friendly. One benefit of using natural larvicides is that they degrade quickly in the presence of sunlight, air, humidity, and other natural elements, which lowers the possibility of soil and water contamination. The use of natural larvicides allows for their application in human life because of their low toxicity to mammals. The material chosen as a larvicide must be safe for humans or other organisms, readily available, and anticipated to improve human health.(8) Many people have been using herbal plants as natural pesticides and as alternatives to synthetic ones. Kaffir lime (*Citrus hystrix*) peel and pineapple (*Ananas comosus L. merr*) peel are two examples of natural ingredients. Each portion of the pineapple (*Ananas comosus L. merr*) peel and the kaffir lime (*Citrus hystrix*) peel contain active substances called tannins and saponins that can kill and stop *Culex sp.* larvae from growing.(9)

According to a journal that has researched the content of pineapple peel on the death of *Culex sp.* larvae, the death of *Culex sp.* larvae is due to the presence of active substances or natural ingredients in pineapple (*Ananas comosus L. merr*) peel, which can be a larvicide, one of which contains active compounds in the form of *saponins*, *tannins*, and *flavonoids*. By entering the larvae's body through the respiratory system, *flavonoids* act as respiratory poisons, damaging the respiratory system and causing nerve damage, which prevents the larvae from

breathing. In order to reduce the activity of digestive enzymes and interfere with food absorption, *tannin* and saponin compounds act as stomach poisons that enter the larvae's body through the digestive tract. As a result, scientists wish to compare pineapple (*Ananas comosus L. merr*) peel to other plants.(10).(3)

MATERIALS & METHODS

Using a *Completely Randomised Design* (CRD) with five treatments and five repetitions, this study employed an experimental design type. To conduct a comparative test of the efficacy of pineapple (*Ananas comosus L. merr*) peel extract with concentrations of 2%, 4%, 6%, and 8% as a natural larvicide to exterminate *Culex sp.* larvae, the *Completely Randomised Design* (CRD) is the most straightforward. The subjects in this study were pineapple (*Ananas comosus L. merr*) peel extract with a total amount of 100 ml pineapple peel extract (for five repetitions at a dose with one control). The study's objects were the 1,250 *Culex sp.* instar III mosquito larvae (6-7 days old) collected from the ditch for five repetitions at four dose variations with one control. Instar III was selected as the test phase due to its larger size, complete organs (body organs), and relative environmental stability compared to instars I and II. In addition, third-stage larvae have a considerable amount of time to develop into pupae and imago (adult mosquitoes), and they are physically resistant to mechanical factors during larval transfer. There are 25 larvae in each sample block and 25 larvae in each control block.(11)

Federer's formula was applied in the following manner to ascertain the number of repetitions in this study:

$$(\boldsymbol{t-1})(\boldsymbol{r-1}) \geq \mathbf{15}$$

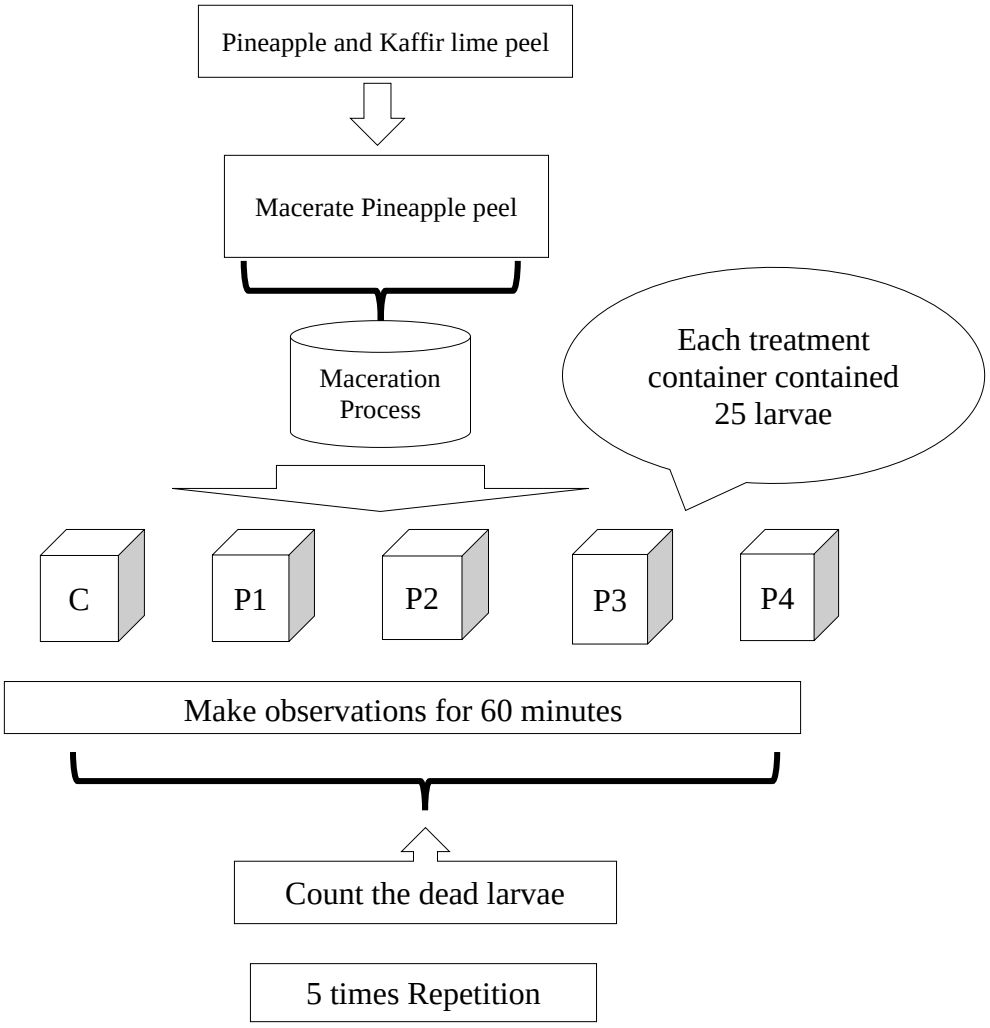
Explanation:
r = is the number of repetitions
t = is the number of treatment groups

The number of repetitions that can be performed can be determined using Federer's formula, which is as follows:
(t-1) (r-1) ≥15, (5-1) (r-1) ≥ 15. 4r- 1 ≥ 15. 4r ≥ 15+4. r ≥ 19/4. r ≥ 4,75 (Using 5 times repetition). The number of treatment repetitions was performed five times, according to the computation results above, meaning that the total sample size that will be utilised in this study is:Number of larvae x Number of repetitions x Number of treatments. Consequently, the number of larvae needed, as determined by the formula, namely:
25 x 5 x 5 = 625 larvae (for pineapple peel extract)
25 x 5 x 5 = 625 larvae (for Kaffir Lime peel extract)
Total = 1.250 *Culex sp.* larvae
The extract was added to a treatment container containing 25 larvae at 2% per 100 millilitres of water. The extract was added to a treatment container containing 25 larvae in treatment II at 4% per 100 millilitres of water. Treatment III involved pouring the extract into a treatment container containing 25 larvae at a rate of 6% per 100 millilitres of water. The extract was added to a treatment container containing 25 larvae in treatment IV at 8% per 100 millilitres of water. Additionally, 25 *Culex sp.* larvae were given as the extract-free control. The study was conducted at the integrated health polytechnic laboratory of the Aceh Ministry of Health. Pineapple (*Ananas comosus L. merr*) peel extract diluted with water from the well (WHO). Using the formula, use the dilution method to get 100 ml concentrations of 2%, 4%, 6%, and 8%.(12)

$$v1.m1 = v2.m2$$

Explanation: V1 = Initial volume, M1 = Main solution concentration, V2 = Volume after dilution, M2 = Dilution concentration
Preparation of 2% concentration in 2 ml at 100% concentration. Initial parameter:
M1= 100%, M2= 2%, V2= 100 ml
Asked: V1=….?

Solution:
V1. M1 = V2. M2
V1.100% = 100 ml. 2% V1.100% = 200 ml% V1= 200 ml% /100% V1= 2 ml
The research's instruments include a blender, knife, sieve, tray, spoon, jar, analytical balance, funnel, *rotary evaporator*, 2000 ml *beaker glass*, glass, and *stirrer*. The tools used for research are test containers, *counters*, *thermometers*, and *hygrometers*. The materials used in the research were pineapple peel and kaffir lime peel, *Culex* instar III larvae, distilled water, 95% ethanol, filter paper, 96% ethanol, *aluminium foil* abate, rubber bands, and water. The following image illustrates the research's flow:



Explanation:
K = Control
P1 = 2% Extract
P2 = 4% Extract
P3 = 6% Extract
P4 = 8% Extract

Figure 1: Research Flow

STATISTICAL ANALYSIS

The data were examined using the one-way ANOVA test, which is the most straightforward method for comparing treatment means and is frequently employed to pineapple (*Ananas comosus L. merr*) peel

extract as natural insecticides for killing *Culex sp.* larvae. When a difference is found, the concentrations of pineapple (*Ananas comosus L. merr*) peel extract on the mortality of *Culex sp.* larvae are determined using LSD (*Least Significance Difference*).

RESULT

The research period for testing the effectiveness of pineapple (*Ananas comosus L. merr*) peel extract on the death of *Culex sp.* larvae was carried out for 12 hours to see the effect of the extract. Table 1 below displays the outcomes of the pineapple (*Ananas comosus L. merr*) peel extract test.

Table 1 Number of deaths of *Culex sp.* larvae in each treatment of Pineapple (*Ananas comosus L. merr*) Peel extract

Pineapple Peel Concentration	Total Larvae	Repetition					Total	Average	%
		1	2	3	4	5			
Control	25	0	0	0	0	0	0	0,00	0.00
2%	25	1	2	2	1	0	6	1,21	4,8
4%	25	0	6	7	3	1	17	3,60	13,6
6%	25	13	4	19	17	11	64	12,80	51,2
8%	25	19	13	25	22	18	97	19,40	77,6

Following testing with four treatments and five repetitions, Table 1 illustrates the impact of pineapple peel extract on *Culex sp.* larvae mortality. A total of 25 *Culex sp.* larvae per treatment and repetition died due to the use of pineapple peel extract at doses of 2%, 4%, 6%, and 8% in each repetition. In contrast, the control group did not use pineapple peel extract. There were variations in the quantity of dead larvae in each treatment. This result demonstrates the ability of pineapple peel extract to kill *Culex sp.* larvae.

A dosage of 8% pineapple peel extract resulted in the most significant number of larvae perished following administration, totalling 97 *Culex sp.* larvae over five repetitions. No larval deaths were in the control group, but the lowest number of dead larvae six was recorded at a dose of 2% pineapple peel extract. Use the ANOVA statistical test to demonstrate how pineapple peel extract affects larval mortality; the statistical results are in the following table:

Table 2 Anova Statistical Results on Pineapple (*Ananas comosus L. merr*) Peel Extract Against Death of *Culex sp.* Larvae

Variable	Average	Std. deviation	CI 95% value	P value
Control	0.0	0,00	0,00 - 0,00	0,00
2% concentration	1,20	0,83	0,16 - 2,24	
2% concentration	3,60	2,88	0,02 - 7,18	
2% concentration	12,80	5,84	5,54 - 20,06	
2% concentration	19,40	4,50	13,18 - 24,99	

A 99% confidence level can demonstrate that variations in the concentration of pineapple (*Ananas comosus L. merr*) peel extract impact the mortality of *Culex sp.* larvae. The table above demonstrates that the sig. value (p-value) < H0, specifically 0.00 < 0.01; therefore, rejected H0. After knowing the effect of differences in extract concentration, then proceed with the LSD (*Least Significance Different*) test to find out which pairs of each group of concentration data have significant differences in average. The following table shows the results of LSD:

Table 3 LSD Statistical Test Results on Pineapple (*Ananas comosus L. merr*) Peel Extract Against the Death of *Culex sp.* Larvae

LSD Responses						
Group (I)	**Treatment (J)**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**CI 99% value**	
					Lower bound	**Upper bound**
Control	2%	-1,2	2,25	**0,6**	-7,61	5,21
	4%	-3,6	2,25	**0,12**	-10,01	2,81
	6%	-12,80*	2,25	**0**	-19,21	-6,39
	8%	-19,40*	2,25	**0**	-25,81	-12,99
2%	Control	1,2	2,25	**0,6**	-5,21	7,61
	4%	-2,4	2,25	**0,3**	-8,81	4,01
	6%	-11,60*	2,25	**0**	-18,01	-5,19
	8%	-18,20*	2,25	**0**	-24,61	-11,79
4%	Control	3,6	2,25	**0,12**	-2,81	10,01
	2%	2, 60	2,25	**0,3**	-4,01	8,81
	6%	-9,20*	2,25	**0**	-15,61	-2,79
	8%	-15,80*	2,25	**0**	-22,21	-9,39
6%	Control	12,80*	2,25	**0**	6,39	19,21
	2%	11,60*	2,25	**0**	5,19	18,01
	4%	9,20*	2,25	**0**	2,79	15,61
	8%	-6,60*	2,25	**0**	-13,01	-0,19
8%	Control	19,40*	2,25	**0**	12,99	25,81
	2%	18,20*	2,25	**0**	11,79	24,61
	4%	15,80*	2,25	**0**	9,39	22,21
	6%	6,60*	2,25	**0**	0,19	13,01

Explanation:
(*) = There is a difference

The table above shows the sig. value (p-value) of each pair of pineapple peel extract concentrations, with the sig. value (p-value) of each pair of pineapple peel extract concentrations on the death of *Culex sp.* larvae. Therefore, with a 99% confidence level, it shows that pineapple peel extract concentrations starting from 6% can make a real difference in the death of *Culex sp.* larvae. However, concentrations of 2% and 4% have not made a real difference in the death of *Culex sp.* larvae.

DISCUSSION

This test showed that the pineapple (*Ananas comosus L. merr*) peel extract caused the death of *Culex sp.* larvae at doses of 2%, 4%, 6% and 8%, but in the control sample, no larvae died. The ANOVA test results showed that the control and treatment samples differed. These findings suggest that the amount of pineapple (*Ananas comosus L. merr*) peel extract impacts the mortality of *Culex sp.* larvae.

From the results of previous research, the percentage of death of *Culex sp.* larvae after administration of pineapple peel extract at different concentration levels, namely at a concentration of 1%, with a death result of 72.5%, at a concentration of 2% with a death result of 82.5%, at a concentration of 3 % with a mortality result of 87.5%, and a concentration of 4% it produces a mortality rate of 97.5%. From the results of this research, the percentage of death of *Culex sp.* mosquito larvae after administration of pineapple peel extract at different concentration levels, namely at a concentration level of 2% with a death percentage of 4.8%, at a concentration level of 4% with a death percentage of 13.6%, at a concentration level of 6% with a death percentage of 51.2% and at a concentration level of 8% with a death percentage of 77.6%. There was a significant effect of the dose of pineapple (*Ananas comosus L. merr*) peel extract on the death of *Culex sp.* larvae for 4 hours, 6 hours, and 12 hours at dose levels of 6% and 8%, according to the statistical test results, which showed that the p-value < 0.00, hence rejecting H_0. It is possible to use 8% pineapple peel extract as

a reference for the most effective dose because it is the highest dose that kills *Culex sp.* larvae. This outcome is because pineapple peel typically has a distinct bitter flavour, a strong odour, and a mildly spicy taste that may be able to kill *Culex sp.* larvae.

In addition to environmental factors like humidity and temperature that affect the sensitisation of *Culex sp.* larvae, trauma experienced during the removal process from the sewer may also impact each larva's condition. Research limitations could also cause differences in the number of larval deaths in this study because a reaction occurred while administering the extract to each container.(13)

The active ingredients or secondary metabolites in pineapple (*Ananas comosus L. merr*) peel extract can kill *Culex sp.* larvae. These active ingredients include flavonoids, tannins, and saponins. Flavonoids are respiratory toxins that enter the larvae organ through the respiratory system. They damage the respiratory system and cause nerve damage, which prevents the larvae from breathing. Tanin and saponin compounds act as stomach poisons that enter the larvae's body through the digestive tract. They decrease the activity of digestive enzymes and interfere with food absorption.(12)

There was more than one factor contributing to the variation in the number of larvae that perished from each dose; the higher the extract dose, the more viscous the extract and the chemical compounds it contained, which may have contributed to the higher number of larval deaths. The influence of additional factors, such as the distinct immune systems of each larva, is another reason. Additional elements that affect each larva's condition include the possibility of trauma during the removal process from the sewer and environmental factors like humidity and temperature that can affect how sensitive *Culex sp.* larvae become.

Flavonoid compounds, such as potent respiratory poisons or inhibitors, can block mosquitoes' respiratory tracts. By entering the mosquito's respiratory tract and causing the nerves and respiratory muscles to atrophy, *Flavonoid* compounds render the insect incapable of breathing and ultimately result in its death.(14) The larva's digestive tract mucous membrane is irritated by saponin compounds. The larvae also get a bitter taste from saponin, which can make them less hungry and ultimately kill them. Furthermore, saponins have the potential to harm the wax covering that shields the insect's exterior, which could result in significant fluid loss and death.(15)

Through their mechanism of action, *alkaloids* function as gastric poison and prevent larvae from absorbing food. *Alkaloids* can inhibit the work of the acetylcholine enzyme, resulting in a buildup of acetylcholine and disruption of the impulse transmission system to muscle cells. Convulsions will occur, followed by paralysis and death for the larvae.(16)

When converting amino acids, *tannin* compounds reduce the activity of the protease enzyme. The larva may be denied food, and its cellular metabolic functions may be interfered with. In addition, tannins bind digestive system proteins that are essential for larval development. If this persists, mosquito larvae will perish.(17)

Based on the research results, it shows that pineapple peel extract and kaffir lime peel extract can be used as insecticides to kill *Culex sp.* larvae. The higher the extract dose, the more effective it kills the larvae. Observations for 12 hours showed differences in larval mortality at each dose. Pineapple peel extract at a dose of 8% was more effective against the death of *Culex sp.* larvae.

CONCLUSION

Based on the results of this research, the following conclusions emerge: The average number of *Culex sp.* larvae that died at a 2% concentration of pineapple peel extract was 1.20 larvae, 3.60 larvae at a 4% concentration, 12.80 larvae at a 6% concentration, and 19.40 larvae at an 8% concentration. Pineapple peel extract was more effective than kaffir lime peel extract,

as evidenced by the average death rate of 7.40 *Culex sp.* larvae versus 6.80 *Culex sp.* larvae for the former. An extract concentration of 6% killed *Culex sp.* larvae, but concentrations of 2% and 4% were ineffective.

Declaration by Authors

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