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POTENTIAL TESTING OF WASTE SKIN ONION (*Allium ascalonicum*) AS A LARVACIDE AGAINST THE DEATH OF MOSQUITO LARVAS *Culex* sp

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ABSTRAK

The purpose of this study was to determine the effective concentration of onion peel waste powder (*Allium ascalonicum*) and the effective LC_{50} of onion peel waste powder (*Allium ascalonicum*) as larvicides against the death of *Culex* sp mosquito larvae at an effective powder concentration of 0.025 gr/ml, 0.05 gr. /ml, 0.075 gr/ml and 0.1 gr/ml. This type of research is an experimental laboratory, carried out from August to September 2021 at the Microbiology Laboratory of D III Study Program Medical Laboratory Technology, Health Poltekkes, Aceh Ministry of Health. The sample of this study was 325 larvae of *Culex* sp Instar III mosquito larvae. The treatments consisted of 4 effective concentrations of onion peel powder 0.025 g/ml, 0.05 g/ml, 0.075 g/ml and 0.1 g/ml and a negative control (Water) with 3 repetitions. Each treatment group contained 25 larvae. The data obtained were analyzed using the formula for the percentage of mortality of *Culex* sp larvae and the Probit analysis test to calculate the LC_{50}. The results showed that the average larval mortality at a concentration of 0.025 gr/ml was 8 birds (32%), a concentration of 0.05 gr/ml was 9 birds (37%), a concentration of 0.075 gr/ml was 11 (44%), and the concentration of 0.1 g/ml is 16 animals (64%). While the negative control did not show death. And the LC_{50} value at 24 hours after treatment from the concentration of onion peel powder was 0.075%. Based on the results of the study, it can be concluded that onion peel powder (*Allium ascalonicum*) has the potential as a natural larvicide against the mortality of *Culex* sp larvae with LC_{50} obtained at an effective concentration of 0.075% powder.

Keywords: Onion peel powder, Larvicide, *Culex* sp.

INTRODUCTION

Diseases caused by mosquitoes continue to be the leading cause of illness and death worldwide. Malaria, filariasis, Japanese encephalitis, and dengue fever are well-defined diseases (parasitic and viral) transmitted by mosquitoes of the genera *Anopheles*, *Culex* and *Aedes* [1]. It is estimated that more than half of the world's human population is at risk of infection spread by mosquitoes [2]. Among these diseases, elephantiasis, which is caused by parasites of the genus Wuchereria and transmitted by infected mosquitoes of the genus *Culex*, and it continues to be a public health problem, especially in tropical and subtropical regions [3]. In addition, mosquitoes in the *Culex* genus have also been identified as the main vectors that transmit WNV (West Nile Virus) in several countries [4][5][6][7][8].

Various control efforts have been carried out in controlling vectors of diseases caused by mosquitoes, one of which is the use of chemical insecticides which are considered more effective in controlling vectors. The widespread and indiscriminate application of this insecticide in mosquito control causes various problems such as environmental pollution, insecticide resistance, and harmful effects on non-target organisms [9]. One of the other efforts is the eradication of mosquitoes by breaking the chain of mosquito distribution by eradicating mosquito nests and killing mosquito larvae. The use of abate insecticides as larvicides can be the most common way of controlling the growth of mosquito vectors. The use of abate for a long time will cause resistance or the larvae will be immune to abate (insecticide) [10].

Based on the above, it is necessary to look for other alternatives to synthetic insecticides such as plants that are effective, biodegradable, environmentally friendly, and inexpensive. About 2000 plant species have been known to produce secondary metabolites of value in biological pest control programs and only 344 plant species exhibit insecticidal activity against mosquitoes [9]. Plants are a rich source of bioactive chemical compounds with insecticidal properties [11]. Many studies have looked at the possibility of using plants as larvicides and growth inhibitors against mosquito larvae. And plants that may potentially be used as larvicides are onion peel.

The skin of the onion (*Allium ascalonicum*) is the outer part of the onion that has been fleshed. Shallot skin is often not used, but immediately thrown away because it is considered as waste. This red onion skin waste is commonly found in markets and restaurants. One of the studies regarding the use of onion peel waste as larvicides has been by Rahmayanti, et al., 2016 onion peel extract (*Allium cepa*) has the potential to cause the death of *Aedes aegypti* mosquito larvae [12], and there is also a study by Tutik, et al, 2020, namely extracts of onion peel is effective as a larvicide against *Aedes aegypti* mosquito larvae [13].

Shallot skin has the potential to be developed as a biolarvicide for *Culex sp* mosquito larvae due to its phytochemical compounds. Based on the results of Prabowo & Noer's 2020 research, onion peel waste was proven positive for containing alkaloids, saponins, flavonoids and tannins [14]. And this is also in line with the results of research by Elsyana & Tutik, 2018, namely the ethanolic extract of onion skin contains phytochemical components of flavonoids, tannins, and saponins. It is suspected that these compounds can act as natural larvicides [15].

METHODS

1. Types of Research

The research to be carried out is experimental, which aims to find out a symptom or effect that arises, as a result of certain treatments [16]. The effect that we want to know is the larvicidal potential of onion peel powder (*Allium ascalonicum*) on the larvae of *Culex* sp.

2. Research Place and Time

Location for reseach is Gampong Beurawe Banda Aceh. The examination was continued at the Microbiology Laboratory of the D-III Medical Laboratory, Jalan Tgk. H. Mohd. Daud Beureueh No.168 A, Lampriet, Banda Aceh. This research was conducted from August to September 2021.

3. Population and Sample

The population used in this study was *Culex* sp instar III mosquito larvae. The sample in this study was taken based on the WHO reference in 2005, the sample used was 25 larvae with a concentration of 3 repetitions, with 3 treatment groups and 1 negative control group, so this study required a total of 325 larvae. The reason for the selection of third instar larvae is because this stage is considered sufficient to represent the condition of the larvae. The size of the third instar larvae is not too small so it is easy to observe and this larva is an active form of foraging so it is easy to identify, besides that the third instar larvae are research samples that become WHO standards. The 3rd instar larvae are 4-5 mm long, the chest spines are clear and the respiratory funnel (siphon) is blackish brown.

4. Making Onion Skin Waste Powder

1. Collected red onion skin waste, air-dried and then weighed as much as 500 grams.
2. Then mashed using a blender until it becomes powder
3. After grinding, the powder was weighed with a dose of 0.025 gram, 0.05 gram, 0.075 gram and 0.1 gram, respectively.

5. Testing the Concentration of Shallot Skin Powder to Test Larvae

1. Prepare 5 groups of containers (Beaker glass) where each group consists of 4 Beaker glass containers, each of which will be a place of experimentation.
2. In group I, onion peel powder was given with a concentration of 0.025 g/100 ml of water.
3. In group II, onion peel powder was given with a concentration of 0.05 g/100 ml of water.
4. In group III, onion peel powder was given with a concentration of 0.075 g/100 ml of water.
5. In group IV, onion peel powder was given with a concentration of 0.1 g/100 ml of water [17].
6. In group V, 100 ml of water was given.
7. Carefully inserted using a pipette 25 larvae of *Culex* larvae in each of these containers.
8. Observations were made after 24 hours of treatment, then recorded the number of dead larvae in each group

6. Data Collection

Data collection was obtained from the observation of larvicidal waste of onion peel powder at an effective concentration of 0.025 gr/ml, 0.05 gr/ml, 0.075 gr/ml and 0.1 gr/ml powder on the mortality of *Culex* sp larvae observed after 24 hours.

7. Data Analysis

The data obtained from the observations will be processed in the form of.

a. Percentage, with the formula:

$$\text{Percentage (\%)} = \frac{\text{Number of Dead Test larvae}}{\text{Total Number of Test Larvae}} \times 100\%$$

b. Probit Analysis Program

The number of dead larvae was analyzed using the Probit Analysis Program to obtain the LC_{50} value with a 95% confidence degree.

RESULTS AND DISCUSSION

Average Percentage of Larvae Mortality of *Culex* sp At Various Effective Concentrations Of Shallot Skin Powder (*Allium ascolonicum*)

Based on the research that has been done, the average percentage of mortality for the third instar larvae of *Culex* sp is obtained as shown in table 1 below.

Table 1. Average Percentage of Mortality of *Culex* sp Larva After Testing With Shallot Powder (*Allium ascolonicum*) In Various Concentrations For 24 Hours

No	Concentration of Shallot Peel Powder (%)	Number of test larvae (tails)	Repeat			Number of Larvae Deaths (tails)	Larvae Mortality Rate (tail)	Average Percentage of Larvae Mortality
			I	II	III			
1	0,025 gr/ml	25	8	7	9	24	8	32 %
2	0,05 gr/ml	25	9	9	10	28	9	37 %
3	0,075 gr/ml	25	11	12	10	33	11	44 %
4	0,1 gr/ml	25	14	15	20	49	16	64 %
5	Negative Control	25	0	0	0	0	0	0 %

Based on table 1 it can be seen that the average larval mortality at a concentration of 0.025 gr/ml is 8 birds (32%), a concentration of 0.05 gr/ml is 9 birds (37%), a concentration of 0.075 gr/ml is 11 (44%), and the concentration of 0.1 g/ml was 16 animals (64%). While the negative control did not show death.

Determination of Lethal Concentration 50 (LC_{50})

LC_{50} is the concentration that can cause death by 50% of the total larvae of the test. The LC_{50} value of the mortality of *Culex* sp larvae at various observation times was obtained by performing the Probit test. According to WHO (2005), the maximum percentage concentration in larvicide research is 1% [18]. The LC_{50} of the onion peel powder concentration is 0.075%. As shown in the table and graph (Figure 2).

Table 2. Determination of LC_{50} Concentration Based on Probit Analysis

	95% Confidence Limits for Consentration		
Probability	**Estimate**	**Lower Bound**	**Upper Bound**
,01	-,13535	-,31496	-,07063
,02	-,11069	-,26823	-,05376
,03	-,09504	-,23860	-,04304
,04	-,08327	-,21632	-,03497
,05	-,07369	-,19821	-,02838
,06	-,06554	-,18281	-,02277
,07	-,05840	-,16931	-,01784
,08	-,05200	-,15723	-,01342
,09	-,04618	-,14626	-,00939
,10	-,04083	-,13616	-,00568
,15	-,01865	-,09447	,00982
,20	-,00102	-,06154	,02234
,25	,01410	-,03357	,03336

95% Confidence Limits for Consentration			
Probability	**Estimate**	**Lower Bound**	**Upper Bound**
,30	,02768	-,00889	,04370
,35	,04026	,01320	,05405
,40	,05220	,03271	,06534
,45	,06375	,04904	,07880
,50	,07512	,06201	,09514
,55	,08649	,07265	,11383
,60	,09804	,08216	,13411
,65	,10998	,09130	,15575
,70	,12257	,10055	,17895
,75	,13615	,11028	,20424
,80	,15127	,12093	,23257
,85	,16889	,13322	,26574
,90	,19107	,14856	,30759
,91	,19643	,15225	,31771
,92	,20225	,15625	,32871
,93	,20864	,16065	,34081
,94	,21579	,16555	,35433
,95	,22394	,17114	,36976
,96	,23351	,17770	,38789
,97	,24529	,18576	,41019
,98	,26093	,19645	,43985
,99	,28560	,21329	,48661

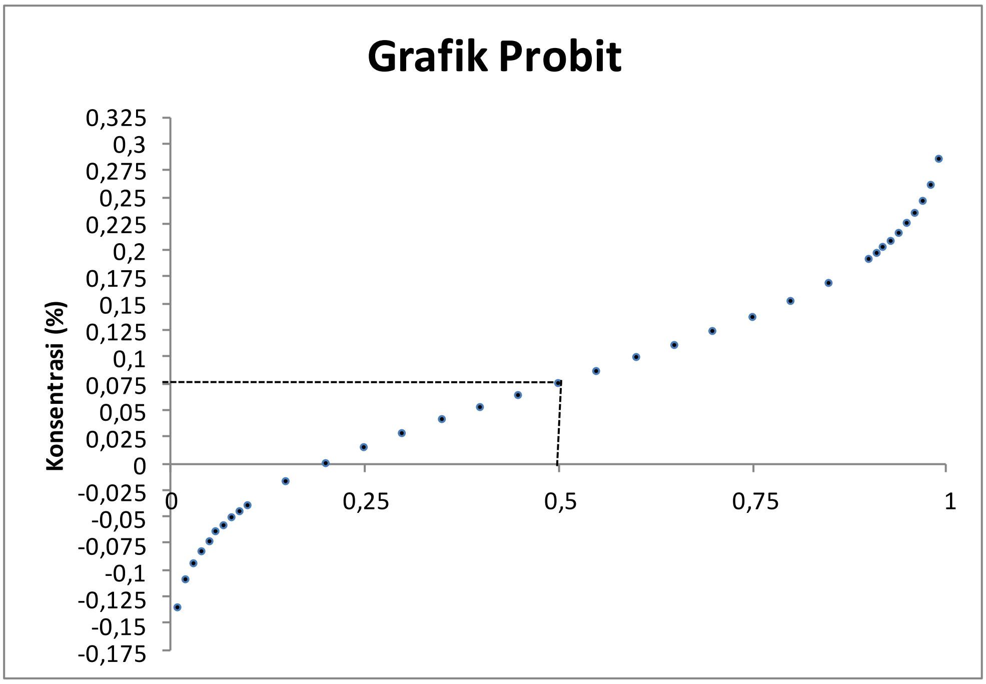

Figure 1. Graph of LC_{50} Value

Effectiveness Test

Based on the results of research on the potential test of onion skin (*Allium ascolonicum*) on the mortality of *Culex sp* mosquito larvae, it was seen that there was a difference in the number of dead larvae between the treatment groups as shown in table 1. Biolarvicidal activity was observed in third instar mosquito larvae 24 hours after administration of onion peel powder concentration at room temperature. Tests were carried out with concentrations of 0.025 gr/ml, 0.05 gr/ml, 0.075 gr/ml, and 0.1 gr/ml. Variations in mortality of *Culex sp* mosquito larvae increased with increasing concentration of onion peel powder (Table 1).

Culex sp larvae used in this study were 3rd instar larvae, because in this instar the defense system is stronger than 1st and 2nd instars. Thus, it is assumed that a dose capable of killing 3rd instar larvae is also capable of killing 1st and 2nd instar larvae [19]. The 3rd instar larvae are 4-5 mm long, the chest spines are clear and the respiratory funnel (siphon) is blackish brown. The use of third instar mosquito larvae is because the third instar stage has better resistance than the first and second instars so that it gives good test results.

The dose of powder with the most larval mortality was at a concentration of 0.1 g/ml which was 64% of larval mortality. This proves that the higher the concentration and the longer the exposure time, the higher the mortality. The mechanism of action of larvicides in killing larvae is that they enter through contact with the skin. It is then applied directly through the insect's integument (cuticle), trachea or sensory glands and other organs associated with the cuticle [20]. Larval death is also caused by the inability of larvae to detoxify toxic compounds that enter their bodies [21].

The larvicidal activity observed in onion peel powder (*Allium ascalonicum*) can be explained as the action or influence of phytochemical components: flavonoids, tannins, and saponins. Flavonoids have a key role as a stress response mechanism in plants [22]. The adaptive role of flavonoids in plant self-protection against bacterial, fungal, and viral diseases and insects is beginning to gain importance in understanding plant defense [1]. Based on the observations, the test larvae showed symptoms of instability which were indicated by up and down movements on the test medium, while in the control, the larvae showed resting conditions on the surface forming a certain angle.

Larvae are said to be dead if there is no further movement, are at the bottom of the water and do not come to the surface and their bodies look pale. This is in accordance with the theory put forward by Dinata (2009), that flavonoids are toxic and inhibit eating larvae. Flavonoid compounds can also damage the cytoplasmic membrane, causing leakage of important metabolites and inactivating enzyme systems [23]. This results in phospholipids unable to maintain the shape of the cytoplasmic membrane as a result the cytoplasmic membrane will leak and the larvae will experience growth inhibition and even die [24].

Meanwhile, tannins can reduce the intensity of eating which results in the disruption of insect growth [25]. Another active compound contained in the skin of the onion is saponins. Saponins can reduce the surface tension of the mucous membrane of the digestive tract of larvae so that the walls of the digestive tract of larvae become corrosive and eventually damaged. Saponins are found in various types of plants with the highest concentration in certain parts [26]. Saponins are known to have various biological properties, namely having membrane permeability, hemolytic, antioxidant, anti-inflammatory, immunostimulant and anticarcinogenic activities, saponins affect feed intake, growth and reproduction in animals, and saponins can also be used as fungicides, molluscicides and pesticides, as well as against several bacteria and viruses [27].

Lethal Concentration 50 (LC_{50})

The LC value chosen in this study is LC_{50}. LC_{50} is a large concentration that can cause death by 50% of the total larvae of the test. This is because for research on the killing power of an insecticide, the concentration level of the insecticide is considered to have good killing power and is not harmful to the environment if it reaches LC_{50}. LC values below LC_{50} are categorized as having low killing power, and LC values above LC_{50} are categorized as having effective killing power. However, for insecticides capable of reaching LC above LC_{50}, testing is required to determine the level of safety for environmental sustainability [28].

In this study, the LC_{50} value 24 hours after treatment from the concentration of onion peel powder was 0.075%. This was due to the longer the test larvae were exposed to, the more concentration of shallot skin powder that entered the larva's body, and the faster the larvae would die. The efficacy of insecticides to kill insects depends on the form, the way into the insect's body, the type of chemical, concentration, and the amount (dose) of the insecticide [29].

CONCLUSION

Based on the results of the research on the larvicidal potential of onion peel powder (*Allium ascalonicum*) on *Culex sp* mosquito larvae, it can be concluded that:

1) The concentration of onion peel powder (*Allium ascalonicum*) has the potential to be a larvicide against the larvae of *Culex sp.*
2) The concentration of onion peel powder (*Allium ascalonicum*) affects the mortality rate of larvae, the higher the concentration
3) The higher the mortality rate of the test larvae.
4) The concentration of onion peel powder (*Allium ascalonicum*) that killed the most *Culex sp* larvae was 0.1 g/ml, which could kill 64% of larvae.
5) The LC_{50} value of the concentration of onion peel powder (*Allium ascalonicum*) against *Culex sp* larvae was obtained at 0.075%

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