EFFICACY TEST OF ETANOL EXTRACT OF TALLBOOD LEAVES *(Nicotiana tabacum L.)* AS A BIOLARVASIDA AGAINST *AEDES AEGYPTI* LARVAE.

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ABSTRAK

DBD merupakan penyakit infeksi yang diakibatkan masuknya virus dengue kedalam tubuh melalui gigitan nyamuk Aedes aegypti yang muncul sepanjang tahun. Perubahan iklim menyebabkan terjadinya perubahan curah hujan, suhu, kelembapan dan arah udara sehingga berpengaruh terhadap kesehatan, terutama dalam perkembangan vektor penyakit seperti nyamuk aedes. Daun tembakau bermanfaat sebagai larvasida karena memiliki kandungan alkanoid, saponin, polifenol, flavonoid, steroid, dan kuinon. Semakin tinggi konsentrasi ekstrak daun tembakau maka semakin tinggi pula efek larvasidanya dikarenakan nikotin atau alkaloid berfungsi sebagai racun yang dapat mempengaruhi sistem pencernaan dan sistem saraf larva sedangkan senyawa-senyawa lain dapat berfungsi merusak sistem pernafasan dan saluran cerna. Penelitian ini bertujuan mengidentifikasi potensi ekstrak etanol daun tembakau sebagai biolarvasida terhadap penyakit demam berdarah. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan masing-masing konsentrasi di uji bertingkat yaitu 12.5%, 25%, dan 50% untuk mrendapatkan analisa LC50,95 dan LT50,95 di analisis regresi probit dan linear regresi menggunakan aplikasi SPSS. Pada penelitian ini ada tiga kelompok perlakuan, masing-masing kelompok ada 3 kali pengulangan dengan konsentrasi masing-masing 12,5%, 25%, dan 50%. Setiap ulangan diamati dalam satuan menit selama 24 jam. Hasil yang diperoleh pada konsentrasi 50% tingkat kematian larva yang paling tinggi dengan persentase 30,7%, konsentrasi 25% dengan persentase 10,7%, selanjutnya pada konsentrasi 12,5% dengan persentase 0%.
Kata kunci: Demam Berdarah, Lethal Time dan Lethal konsentrasi, Aedes aegypti,

Biolarvasida, Mortalitas

ABSTRACT

DHF is an infectious disease caused by the entry of the dengue virus into the body through the bite of the Aedes aegypti mosquito that appears throughout the year. Climate change causes changes in rainfall, temperature, humidity and air direction that affect health, especially in the development of disease vectors such as the aedes mosquito. Tobacco leaves are useful as larvicides because they contain alkanoids, saponins, polyphenols, flavonoids, steroids, and quinones. The higher the concentration of tobacco leaf extract, the higher the larvicidal effect because nicotine or alkaloids function as a poison that can affect the digestive system and nervous system of larvae while other compounds can function to damage the

respiratory and gastrointestinal systems. This study aims to identify the potential of ethanol extract of tobacco leaves as a biolarvicide against dengue fever. This study used a completely randomized design (CRD) with each concentration tested in stages, namely 12.5%, 25%, and 50% to obtain LC50.95 and LT50.95 Probit regression and linear regression were analyzed using the SPSS application. In this study there were three treatment groups, each group had 3 repetitions with concentrations of 12.5%, 25%, and 50% respectively. Each replicate was observed in minutes for 24 hours. The results obtained at a concentration of 50% the highest larval mortality rate with a percentage of 30.7%, 25% concentration with a percentage of 10.7%, then at a concentration of 12.5% with a percentage of 0%.
Keywords: Dengue Fever, Lethal Time and Lethal concentration, Aedes aegypti, Biolarvicide, Mortality.

INTRODUCTION

Dengue hemorrhagic fever (DHF) is a public health problem in Indonesia due to the increasing number of patients and their geographical distribution, as well as the increasing incidence and population density throughout the year (R. Suci Bestari, 2020). Climate change causes changes in rainfall, temperature, and humidity. and air direction that affect health, especially the development of disease vectors such as the Aedes mosquito (Anita Dyah Listyarini and Erni Rosiyanti, 2021).

Dengue affects about 3 billion people each year who live in high-risk areas (S. W. Handayani *et al.*, 2020). Health workers conduct preventive and health promotion efforts through health education on 3M Plus (i.e. drain and cover reservoirs, bury and dispose). Used goods, monitoring for the presence of larvae and environmental management continue to be carried out (M. Frsilia, 2020)(M. F. J. Mantik, 2021).

Vector control can be done physically, chemically, environmentally and biologically. Physical vector control uses physical materials to reduce the number of vectors, namely by changing the salinity or acidity (pH) of water bodies, setting traps, using electric beating devices, and others. Chemical vector control uses chemicals (pesticides) to reduce vector populations. The use of pesticides should be monitored and evaluated regularly. Environmental management for vector control involves two main approaches: environmental modification and environmental manipulation. In the case of environmental modification, breeding habitats are

filled, running water is managed, and waste is properly disposed of to ensure compliance with health standards. On the other hand, environmental manipulation entails regular and periodic removal of moss and drainage of clean water storage areas. Biological vector control methods utilize predatory organisms, venom-producing organisms (N. W. Septiani, 2017), and bioavicides derived from plant extracts (Maulana Sidik, 2020). Utilization of natural materials as bioavicides provides a reduced risk of harm compared to the use of chemical insecticides (W. H. Cahyati and S. Nuryanti, 2021).

Biolarvicides, which are derived from plants and easily decompose in the environment, water, and soil, serve as an environmentally friendly method of vector control. One such natural material is tobacco leaf, which contains alkanoids, saponins, polyphenols, flavonoids, steroids, and quinones that are effective as larvicides. The larvicidal effect is directly proportional to the concentration of tobacco leaf extract, as nicotine acts as a toxic substance that impacts the larvae's digestive and nervous systems, while other compounds target the respiratory and digestive systems (R. S. Bestari, 2020).

RESEARCH METHOD

Time and Place of Implementation

This study has started in August 2023 and is planned to be conducted until May 2024. Samples of Ae. aegypti larvae for larvicide efficacy testing were taken from clean water reservoirs in people's homes in Banda Aceh City. Species identification and efficacy testing will be carried out at the Parasitology Laboratory of the Faculty of Medicine, Abulyatama University.

Tobacco Leaf Extraction

Making the extraction starts with making tobacco leaf plant simplisia which is chopped and dried in an incubator at 37°C for 24 hours to reduce the water content. Then maceration is carried out, the simplisia is soaked in 96% ethanol for 72 hours and stored at temperatures below -10°C. The results of the soak were filtered for

evaporation which was carried out at the Chemistry Laboratory of Syiah Kuala University.

Testing of Tobacco Leaf Phytochemical Compounds

Phytochemical tests were carried out at the FKIP Chemistry Laboratory, Syiah Kuala University using reagents for each phytochemical compound.

Preparation of Tobacco Leaf Granulation

The method of making biolarvicidal wet granulation with the addition of ethanol as a solvent to obtain tobacco leaf extract. The results of the thick extract after the evaporation process are mixed with ingredients such as aqua bidestilata, lactose, magnesium strearat, polyvinyl pyropidol / PVP so that they bind together and form lumps, then molded using a 20 mesh sieve. Then an oven is carried out with the aim of evaporating the water content and organic solvents so that dry granulation is formed. The drying temperature was 400C for 8 hours.

Tobacco Leaf Efficacy Testing

Efficacy tests were conducted to obtain LT50.95 and LC50.95 values. The test used a completely randomized design (CRD) with each concentration tested in stages, namely 12.5%, 25%, and 50%. The test was conducted by placing 25 test larvae in 50 ml of extract concentration solution for each repetition. Observations of larval mortality began at the 10th, 20th, 30th, 40th, 50th, 60th, 2nd hour, 4th hour, 6th hour, 8th hour, and 24th hour after contact. If the control mortality is more than 10%, it will be corrected by Abbort's formula (M. E. Koraag, 2020).

DATA ANALYSIS

LC50.95 and LT50.95 were analyzed by probit regression and linear regression using SPSS application. Granule evaluation was analyzed univariately and interpreted in tabular form.

RESULTS AND DISCUSSION

Phytochemical Test Results

After phytochemical tests on tobacco leaves, there are Alkaloids, Saponins, Flavonoids, Steroids, Quinones, Polyphenols.

Efficacy Test Results

Table 1. Average and Percentage Mortality of Aedes Aegypti Larvae at Various Concentrations of Ethanol Extract of Tobacco Leaf

Concentration (%)	Treatment	Sample	Average and Percentage of Larval Mortality after Tobacco Leaf Extract at Minute												Mean Mortality at 1440th Minute ± SD	Percentage of Death at 1440th Minute
			10	20	30	40	50	60	120	180	240	360	720	1440		
12.5	1	25	0	0	0	0	0	0	0	0	0	0	0	0	0 ± 0	0%
	2	25	0	0	0	0	0	0	0	0	0	0	0	0		
	3	25	0	0	0	0	0	0	0	0	0	0	0	0		
Quantity			0	0	0	0	0	0	0	0	0	0	0	0		
Average Deaths Per Time			0	0	0	0	0	0	0	0	0	0	0	0		
25	1	25	0	0	0	0	0	0	1	2	2	2	2	2	3,00 ± 0,58	10,7%
	2	25	0	0	0	0	0	0	0	0	0	2	2	3		
	3	25	0	0	0	0	0	0	0	0	1	2	2	3		
Quantity			0	0	0	0	0	0	1	2	3	6	6	8		
Average Deaths Per Time			0	0	0	0	0	0	0,33	0,67	1,00	2,00	2,00	2,67		
50	1	25	0	0	0	0	0	0	0	2	2	3	5	6	7,67 ± 2,08	30,7%
	2	25	0	0	0	0	0	0	0	1	2	5	7	10		
	3	25	0	0	0	1	1	1	1	1	1	2	4	7		
Quantity			0	0	0	1	1	1	1	4	5	10	16	23		
Average Deaths Per Time			0	0	0	0,33	0,33	0,33	0,33	1,33	1,67	3,33	5,33	7,67		

which was repeated three times with concentrations of 12.5%, 25%, and 50%. Observations were recorded every minute for 24 hours. The highest larval mortality rate was found in the 50% concentration with a percentage of 30.7%. Furthermore, the 25% concentration produced a mortality rate of 10.7%, while the 12.5% concentration showed no mortality at all with a percentage of 0%.

LINEAR REGRESSION ANALYSIS RESULTS

Classical Assumption Test

The assumption test results have been met, so the probit data generated in this study can be continued with a simple linear regression test.

Table 2. Regression test results of the effect of concentration on probit

Concentration (%)	Millilitres (ml)	Mortality	Mortality (%)	Probit	P_value	Regression Equation	R^2
12,5	0,625	0	0	0.0000	0,004	Y=-0,369+10,675x	0,846
12,5	0,625	0	0	0.0000			
12,5	0,625	0	0	0.0000			
25	1,25	2	8	3.5949			

25	1,25	3	12	3.8250
25	1,25	3	12	3.8250
50	2,5	6	24	4.2937
50	2,5	10	40	4.7467
50	2,5	7	28	4.4172

Based on Table 2, the use of ethanol extract of tobacco leaves proved to be effective as a larvicide against dengue hemorrhagic fever (DHF) vectors. Statistical analysis showed a significant value of less than 0.05 and an effect size of 0.846, indicating that the extract had a positive impact on larval mortality, resulting in an impressive increase of 84.6%. The derived model represented by the equation Y=3.688+0.107x showed that as the concentration of the extract increased by one unit, the percentage of larvicidal mortality also increased. These findings indicate that the ethanol extract of tobacco leaves exhibits acute toxicity and is classified as highly toxic in the strong category.

Table 3. Regression test results of the effect of time on probit

TIME	Probit		
	Group 1	Group 2	Group 3
10	0	0	0
20	0	0	0
30	0	0	0
40	0	0	2.7738
50	0	0	2.7738
60	0	0	2.7738
120	0	2.7738	2.7738
180	0	3.0569	3.3351
240	0	3.2493	3.4037
360	0	3.5949	3.8877
720	0	3.5949	4.2039
1440	0	3.7184	4.4928
P-Value	-	0,017	0,027
Regression Equation	-	Y=0,901+0,003x	Y=1,866+0,002x
R^2	-	0,671	0,632

Based on table 3. Ethanol extract of tobacco leaves is effective as a larvicide against Dengue Fever vectors with sig value <0.05. In treatment group 1, there were no

larvicidal deaths so no prediction model was available. In addition, the effect size was 0.671 (45.0%) in treatment group 2 and 0.632 (39.9%) in treatment group 3. This means that the ethanol extract of tobacco leaves has a positive effect on larval mortality and is in the potent category at all times, so the longer it is used, the higher the larvicide mortality rate. The model obtained in treatment group 2 is Y=0.901+0.003x, and in treatment group 3 is Y=1.866+0.002x, which means that if one unit of time is added, the percentage of larvicide mortality will also increase. From this point of view, the ethanol extract of tobacco leaves has acute toxicity and falls into the highly toxic category in the highly toxic standard.

Table 4. Results of Probit Analysis of Lethal Time (LT) 50.95 and Lethal Concentration (LC) 50,95 for Ethanol Extract of Tobacco Leaf

CONCENTRATION %	PVALUE	LT	LC
12,5	P1= 0,000	LT 50: 0 LT 95**:** 0	
25	P2= 0,000	LT 50: 1322.169136 LT 95**:** 2583.339379	LC 50: 0.279603 LC 95: 0.415715
50	P3= 0,000	LT 50: 409.909224 LT 95: 764.164805	

Based on Table 4. It can be seen from the three treatment groups that during the 24-hour experiment, treatment group 1 with a concentration of 12.5% obtained the estimated value of LT 50 = 0 and LT 95 = 0. In addition, at a concentration of 25 in treatment group 2, the value of % LT50 = 1322.169136 and LT95 = 2583.339379 means that it takes ± 55 hours to kill 50% of 75 larvae and ± 107 hours

to kill 95% of larvae. In treatment group 3 with a concentration of 50%, the value of LT50 = 409.909224 and LT95 = 764.164805 means that it takes ±17 hours to kill 50% of 75 larvae, and ±17 hours to kill 95% of the larvae. It took ±31 hours. Find out what concentration is needed to kill 50% of the larvae, i.e. 27.9%, 95%, 41.5%. Therefore, it can be concluded that the high and low percentage of concentration used will affect larval mortality. Statistical test results obtained based on time and concentration are significant with a confidence level of p (0.05) as seen from the p value <0.05.

CONCLUSIONS

Ethanol extract of tobacco leaves is proven to have potential as a bioavicide, with compounds that cause the death of Aedes aegypti mosquito larvae. Based on data analysis of concentrations of 12.5%, 25% and 50% in this study, it was found that the highest larval mortality rate was at a concentration of 50% with a percentage of 30.7%, and at a concentration of 25% the percentage was 10.7%, then the concentration of 12.5% the percentage was 0%. Therefore, the most effective concentration to kill Aedes aegypti larvae is 50% with a mortality rate of 30.7%. In this study, there was a Lethal time with the highest mortality rate of Aedes aegypti larvae at a concentration of 50%. The values obtained are LT50 = 409.909224 and LT95 = 764.164805 which takes ±17 hours to kill 50 larvae. % of 75 larvae, and it takes ±31 hours to kill 95% of larvae. To find out what concentration is needed to kill 50% of the larvae, it is 27.9%, and 95% is 41.5%. Therefore, the higher the concentration, the higher the larval mortality and the shorter the time required.

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