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EFFICACY OF MORINDA CITRIFOLIA AND SYZYGIUM POLYANTHUM LEAF EXTRACTS AND THEIR COMBINATION (DAMESA) AGAINST THE DEATH OF AEDES AEGYPTI MOSQUITOES

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ABSTRACT

Aedes aegypti is the main vector of dengue fever in Indonesia. Its control using synthetic chemical insecticides has a negative impact on public health. Botanical insecticides made from extracts of bay leaves and noni leaves (Morinda citrifolia L.), as well as a combination of the two (known as Damesa), are expected to provide an alternative solution to reduce the negative effects of synthetic chemical insecticides. The aim of this study is to determine the efficacy of noni and bay leaf extracts, as well as the combination of Damesa, in killing Aedes aegypti mosquitoes. The study was conducted through an experimental design using a control group (Post Test Only Control Group), where the experimental group received treatment or intervention (X) followed by a second measurement or observation (O2). The study population consisted of all mosquitoes found inside and around houses/buildings in the Lamblang Manyang Village, Aceh Besar Regency, Aceh Province. The results of the efficacy tests showed that spraying bay leaf ethanol extract resulted in an LC50 of 71.13% and LC90 of 287.71%, while fogging resulted in an LC50 of 44.827% and LC90 of 302.239%. Spraying noni leaves resulted in an LC50 concentration of 50.709% and LC90 of 120.534%, while fogging resulted in an LC50 of 56.752% and LC90 of 122.011%. The combination of bay leaves and noni leaves (Damesa), when sprayed, resulted in an LC50 of 39.711% and LC90 of 73.035%, while fogging resulted in an LC50 of 41.321% and LC90 of 112.311%. These results indicate that the efficacy of Aedes aegypti mosquito mortality was significantly higher with both spray and fogging methods using LC90 concentrations. The hope is that this research will lead to improved quality of the extracts, enabling their application by the community for the control of Aedes aegypti mosquitoes using botanical insecticides that can reduce the harmful effects of synthetic chemical substances."

Keywords: Efficacy of Aedes aegypti, Bay Leaves and Noni and Damesa

INTRODUCTION

Aedes aegypti mosquitoes are vectors for dengue fever and Zika virus, which remain global health problems, including in Indonesia. According to data from the Ministry of Health of

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Indonesia, as of March 19, 2021, there were 3,292 cases of dengue fever with 13 deaths throughout the country [1]. The provinces with the highest number of cases are Central Java, East Java, and Bali [2,3]. Data from the Health Department of Aceh Province recorded 179 cases of dengue fever among residents until the end of February 2020, a decrease compared to the previous year during the same period [4]. In the first three months of 2019, there were 660 cases of dengue fever, while in January 2020, there were only 179 cases. Aceh is one of the yellow zone areas with endemic dengue fever, where in 2019 there were 2,386 cases with six deaths [5]. The spread of dengue fever is found in the cities of Banda Aceh, Abdya, Bireuen, Nagan Raya, Aceh Jaya, and Langsa [6]. The City of Banda Aceh Information and Communication Department reported that as of the end of August 2022, there were 134 cases with four deaths from dengue fever (the case fatality rate is still above 1 percent, namely 2.99%) in the Banda Aceh area, which also contributes to the highest number of dengue fever cases in Aceh Province [7]. Controlling mosquito populations can be done through various methods, including the use of natural materials such as noni leaves, bay leaves, and Damesa [8],[9]. Previous research has shown the potential use of noni leaves and bay leaves as natural insecticides to kill Aedes aegypti mosquitoes [10]

The research by Kumar et al. (2012) shows that several plant extracts, including extracts of noni leaves and bay leaves, have a significant insecticidal effect on Aedes aegypti mosquitoes [11]. Meanwhile, the study by Indriyanti and Nindya (2014) indicates that the combination of both extracts can enhance the insecticidal effectiveness against Aedes aegypti mosquitoes compared to using each plant extract separately [12]. Arikunto et al.'s (2017) research reveals that noni leaf extract has insecticidal activity against Aedes aegypti mosquito larvae [13]. The results of Hadisaputro et al.'s (2019) study suggest that bay leaf extract also has the potential to control Aedes aegypti mosquito larvae in the home environment [14]. Nugraheni et al. (2017) found that the combination of noni leaf extract, bay leaf extract, and temephos has good efficacy in controlling the death of Aedes aegypti mosquito larvae [15]. In the meantime, the study by Pratiwi and Suwanto (2020) shows that the combination of synthetic insecticides and noni leaf extract is also effective in controlling Aedes aegypti mosquitoes [16]. In all of the aforementioned references, extracts from noni and bay leaves, either separately or in combination with synthetic insecticides, have shown potential in controlling Aedes aegypti mosquito populations. Additionally, Damesa, an organophosphate compound, has also been proven effective in controlling mosquito populations. However, the efficacy of using a combination of these three materials is still unclear.

Currently, chemical insecticides are still commonly used to control mosquito populations, but their use can lead to unwanted side effects. Noni and bay leaves have long been used in traditional medicine and have antifungal, antibacterial, and anti-inflammatory properties. Previous studies have shown that extracts from noni and bay leaves have insecticidal effects on Aedes aegypti mosquitoes. Furthermore, Damesa is an insecticide made from plants that grow in Indonesia and has been proven effective in controlling mosquito populations. However, there have been few studies conducted to evaluate the efficacy of using a combination of noni and bay leaf extracts and Damesa as an insecticide on Aedes aegypti mosquitoes. Therefore, this study aims to evaluate the insecticidal effects of noni and bay leaf extracts, as well as a combination of Damesa, on mosquito mortality

`MATERIALS

This study was conducted experimentally using a Post-Test Only Control Group design, where the experimental group received treatment or intervention (X) followed by a second measurement or

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observation (O2). The observation results were then compared to the observation results of the control group who did not receive the program or intervention. The experimental and control groups were considered the same before the treatment because they were randomized. The research was conducted in the Laboratory and Workshop of the Department of Environmental Health, Poltekkes Kemenkes Aceh. Furthermore, its application in the community was carried out in Gampong Lam Blang Manyang, Darul Imarah District, Aceh Besar Regency. The study population consisted of all houses that were sprayed with Aedes aegypti mosquito spray, consisting of the treatment group and the control group. The research subjects were broadly divided into two groups, namely the treatment group and the control group. The treatment group consisted of 128 houses and/or buildings in the community, consisting of 72 houses in the treatment group and 56 houses in the control group**.**

In the preliminary test phase, spraying methods using liquid spray and fogging were carried out. The spraying method using liquid spray was tested with ethanol extract from bay leaves in 6 houses/buildings, ethanol extract from noni leaves in 6 houses/buildings, and a combination of ethanol extract from bay leaves and noni leaves (Damesa) in 6 houses/buildings. Meanwhile, in the fogging spraying method, ethanol extract from bay leaves was tested in 6 houses/buildings, ethanol extract from noni leaves in 6 houses/buildings, and a combination of ethanol extract from bay leaves and noni leaves (Damesa) in 6 houses/buildings. Then, in the main testing phase, each of the 6 houses/buildings was tested to determine the LC90 of the ethanol extract from bay leaves, noni leaves, and their combination. The number of houses/buildings sprayed with the spray method was 18, while with the fogging method was 18. The materials used in this study consisted of ethanol extracts from bay leaves and noni leaves, mosquito colonies, aquadest, water, and mist fluid. The tools used include a blender, a 1000 ml Erlenmeyer flask, a Buchi evaporator, a stirrer, a scale, a spray device, and a fogging device. The independent variables in this study were the concentration of ethanol extracts from bay leaves, noni leaves, and their combination (Damesa).

Meanwhile, the dependent variable was the death of Aedes aegypti mosquitoes, and the controlled variables were environmental and weather factors. This study involved several stages, starting from the preparation of materials and research equipment, as well as making ethanol extracts from bay leaves and noni leaves. The next stage was the preliminary test to find the LC50-LC90 of the ethanol extract, both individually and in combination. If the determined concentration did not produce an LC50-LC90, the experiment would be repeated with a different concentration. The main testing phase was conducted to test the effectiveness of the LC90 of the ethanol extract from bay leaves, noni leaves, and Damesa using the spray and fogging methods. Then, data tabulation and processing were carried out, as well as the preparation of research reports. Data analysis was conducted descriptively and analytically using statistical tests with a confidence level of 95%. One of the objectives of the analysis was to determine the LC50 and LC90 using the Probit test and to correct the percentage of death of Aedes aegypti mosquitoes with the Abbot formula if there was death in the control group of 5% - 20%.

RESULTS

This study aims to test the effectiveness of bay leaf extract, noni leaf extract, and their combination (Damesa) on the mortality of Aedes aegypti mosquitoes through experimentation. Each experimental group was repeated three times for each concentration and type of extract. The results of the study were measured for each treatment and control group. The treatment was carried out in two ways: spray and fogging. For the spray treatment, ethanol extracts of bay leaves, ethanol extracts of noni leaves, and a combination of ethanol extracts of bay leaves and noni leaves were sprayed. Similarly, for the fogging treatment, ethanol extracts of bay leaves, ethanol extracts of noni leaves, and a combination

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of ethanol extracts of bay leaves and noni leaves were sprayed. In addition, the same treatment was applied to the control sample.

Ethanol Extract of Bay Leaves

Ethanol extract of bay leaves was administered through spray.

The results of the treatment using ethanol extract of bay leaves on the mortality of Aedes aegypti mosquitoes through spraying are presented in Table 4.1 below.

Table 1. Distribution of the Amount and Percentage of Doses of Ethanol Extract of Bay Leaves on the Mortality of Aedes aegypti through Spray

Experiment	Number of Mosquito Mortality at extract dose						
	Kontrol	10%	20%	30%	40%	50%	60%
1	0	7	9	11	11	13	14
2	0	8	10	11	12	13	15
3	0	7	9	11	12	14	16
Amount	**0**	**22**	**28**	**33**	**34**	**40**	**45**
Average		**7,3**	**9,3**	**11**	**11,3**	**13,3**	**15**

From the above table 1, it can be seen that the highest mosquito mortality was observed at a concentration of 60% with an average of 15 mosquitoes, while the lowest mortality was observed at a concentration of 10% ethanol extract with an average of 7.3 mosquitoes. No mortality of Aedes aegypti was observed in the control sample during the 60-minute observation.

Table 2. Results of LC50 and LC90 test of Bay Leaf extract against Aedes aegypti mosquitoes by spraying method.

Experiment	Number of Mosquito Deaths		
	control	LC50	LC90
1	0	15	19
2	0	15	20
3	0	16	20
Amount	0	46	59
Average	0	15,3	19,7

Based on the results of the LC50 and LC90 tests shown in Table 4.2 above, the highest number of Aedes aegypti mosquito deaths was observed at the LC90 concentration, with an average of 19.7 mosquitoes, compared to the LC50 ethanol extract concentration, which had an average of 15.3 mosquitoes. No Aedes aegypti deaths were observed in the control group.

Table 3. Distribution of Number and Percentage of Ethanol Bay Leaf Extract Dosage on Aedes aegypti Mortality by Spraying (Spray)

Confidence Limits

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	Probability	95% Confidence Limits for Konsentrasi ekstrak etanol Daun Salam Penyemprotan			95% Confidence Limits for log(Konsentrasi ekstrak etanol Daun Salam Penyemprotan)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	,500	71,13	.	.	1,732	.	.
	,900	287,71	.	.	2,652	.	.

a. Logarithm base = 10.

Based on the table 3 above, it can be seen that Probit Analysis for ethanol extract of bay leaves by spraying obtained the results of LC50 at a concentration of 71.13% and LC90 at 287.71%.

Table 4. Result of Efficacy Test of Bay Leaves Ethanol Extract on Aedes aegypti Mortality by Spraying (Spray).

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Konsentrasi ekstrak etanol Daun Salam Penyemprotan	2,010	1,263	1,592	,111	-,465	4,485
	Intercept	-3,697	2,362	-1,565	,118	-6,060	-1,335

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10,000 logarithm.)

. Ethanol extract of Bay leaves by fogging

The results of treatment using ethanol extract of Bay leaves against Aedes aegypti mosquito mortality by fogging can be seen in the following table.

Table.5 Distribution of the Number and Percentage of Ethanol Extract Doses of Bay Leaves on Aedes aegypti Mortality by Fogging

Experiment	Number of Mosquito Mortality at extract dose						
	Control	10%	20%	30%	40%	50%	60%
1	0	8	9	12	12	14	14
2	0	8	10	11	13	15	16
3	0	9	10	12	14	14	16
Amount	**0**	**25**	**29**	**35**	**39**	**43**	**46**
Average		**8,3**	**9,7**	**11,7**	**13**	**14,3**	**15,3**

Based on Table 5 above, it can be seen that the highest mosquito mortality rate is at a concentration of 60% with an average of 15.3 mosquitoes. Meanwhile, the lowest mortality rate is at the concentration of 10% ethanol extract with an average of 8.3 mosquitoes, and no Aedes aegypti deaths were found in the control sample.

Table 6. Results of LC50 and LC90 Test on Aedes aegypti Mosquitoes using Bay Leaf Extract by Fogging Method.

Experiment	Number of Mosquito Deaths		
	Control	LC50	LC90
1	0	8	12
2	0	8	11
3	0	7	13
Amount	**0**	**23**	**36**
Average	**0**	**7,7**	**12**

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Based on the results of the LC50 and LC90 tests as shown in table 6 above, it can be seen that the highest mosquito mortality rate of Aedes aegypti occurred at the LC90 concentration with an average of 12 mosquitoes compared to the LC50 concentration with an average of 7.7 mosquitoes. No Aedes aegypti deaths were found in the control group. Probit analysis for ethanol extract of Salam leaves using fogging method yielded an LC50 result of 44.827% and LC90 of 303.239% as shown in table 7 below.

Table 7. Distribution of the Amount and Percentage of Ethanol Extract Doses of Salam Leaves Against Aedes aegypti Mortality by Fogging Method.

		Confidence Limits					
	probability	95% Confidence Limits for Fogging Salam Leaf ethanol extract concentration			95% Confidence Limits for log (Concentration of ethanol extract of Salam Fogging Leaves)a		
		Estimates	Lower Bound	Upperbound	Estimates	LowerBound	Upperbound
PROBIT	,500	44,827	.	.	1,704	.	.
	,900	302,239	.	.	2,542	.	.
a. Logarithm base = 10.							

Table 8. Test resultsEfficacy of Bay Leaf Ethanol Extract against Aedes aegypti Mortality by fogging method.

	Parameter	Estimates	std. Error	Z	Sig.	95% Confidence Intervals	
						LowerBound	Upperbound
PROBITa	Concentration of ethanol extract of Salam Fogging Leaves	1.512	1,231	1,233	,219	-.895	3,909
	Intercepts	-2,534	2,571	-,963	,338	-5,054	,097
a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10,000 logarithm.)							

Noni Leaf Ethanol Extract

Extract of noni leaves through ethanol extraction and spraying was conducted. The results of the experiment on the effectiveness of the ethanol extract of noni leaves in killing Aedes aegypti mosquitoes through spraying are presented in table 4.9. The highest mortality rate of mosquitoes was observed at a concentration of 60%, with an average of 16.7 mosquitoes killed. The lowest mortality rate was observed at a concentration of 10%, with an average of 8.3 mosquitoes killed. No deaths were observed in the control sample.

Table 9 shows the distribution of doses of the ethanol extract of noni leaves and the percentage of Aedes aegypti mortality through spraying.

Experiment	Number of Mosquito Mortality at extract dose						
	Control	10%	20%	30%	40%	50%	60%
1	0	10	11	12	12	15	17
2	0	8	10	13	14	15	16
3	0	9	12	13	14	16	17
Amount	**0**	**27**	**33**	**38**	**30**	**46**	**50**
Average		**8,3**	**11**	**12,7**	**13,3**	**15,3**	**16,7**

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Table 10.LC50 and LC90 test results against mosquitoes*Aedes aegypti*noni leaf extract by spraying.

Experiment	Number of Mosquito Deaths		
	Control	LC50	LC90
1	0	15	19
2	0	14	18
3	0	15	18
Amount	**0**	**44**	**55**
Average	**0**	**14,7**	**18,3**

The treatment results based on the LC50 and LC90 efficacy tests can be seen in table 4.10 above that the mortality rate of Aedes agypti mosquitoes is highest at LC90 concentrations with an average of 18.3 mosquitoes compared to the concentration of ethanol extract LC50 which is an average of 14.7 mosquitoes, and no Aedes aegypti death was found in the control sample

Table 11. Distribution of Amount and Percentage of Doses of Noni Leaf Ethanol Extract Against Aedes aegypti Mortality by Spraying

		Confidence Limits					
	probability	95% Confidence Limits for Concentration of ethanol extract of Noni Leaf Spraying			95% Confidence Limits for log(Concentration of ethanol extract of Noni Leaf Spraying)a		
		Estimates	LowerBound	Upperbound	Estimates	LowerBound	Upperbound
PROBIT	,500	50,709	32,411	61,963	1.705	1,511	1,792
	,900	120,534	85,436	784,650	2,081	1,932	2,895
a. Logarithm base = 10.							

Probit analysis for the efficacy test results of Noni Leaf ethanol extract by spraying results of LC50 at a concentration of 50.709% (estimated concentration range between 32.411% to 61.963%) and LC90 = 120.534% (estimated concentration range between 85.534% to 784.650%) can be seen in table 11 above.

Table 12. Test resultsEfficacy of Noni Leaf Ethanol Extract against Aedes aegypt Mortality by spraying.

			std.			95% Confidence Intervals	
	Parameter	Estimates	Error	Z	Sig.	LowerBound	Upperbound
PROBITa	Concentration of ethanol extract of Noni Leaf Spraying	3,408	1,237	2,754	,006	,983	5,833
	Intercepts	-5,811	2,179	-2,666	,008	-7,991	-3,632

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10,000 logarithm.)

Noni Leaf Extract by way of fumigation (fogging)

The results of treatment using ethanol extract of noni leaves on the death of Aedes aegypti mosquitoes by fogging, based on table 13 below, were the highest at a concentration of 60% with an average of 17 mosquitoes. While the lowest concentration of 10% ethanol extract was an average of 8 mosquitoes, and no Aedes aegypti mortality was found in the control sample.

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Table 13.Distribution of Amount and Percentage of DosesEthanol Extract of Noni Leaves Against Death of Aedes aegypti By Fogging

Experiment	Number of Mosquito Mortality at extract dose						
	Control	10%	20%	30%	40%	50%	60%
1	0	7	10	12	12	14	17
2	0	8	10	13	14	15	16
3	0	9	9	12	14	13	18
Amount	**0**	**24**	**29**	**37**	**40**	**42**	**51**
Average		**8**	**9,7**	**12,3**	**13,3**	**14**	**17**

Table 14.LC50 and LC90 test results against mosquitoes*Aedes aegypti*noni leaf extract by fogging.

Experiment	Number of Mosquito Deaths		
	Control	LC50	LC90
1	0	11	16
2	0	11	15
3	0	12	16
Amount	**0**	**34**	**47**
Average	**0**	**11,3**	**15,7**

The results of the treatment based on the LC50 and LC90 tests are presented in table 14 above. Thetable shows that the mortality rate of Aedes aegypti mosquitoes is highest at LC90 concentrations, with an average of 15.7 mosquitoes killed, compared to LC50 concentrations, which had an average of 11.3 mosquitoes killed. No deaths were observed in the control sample.

Table 15 shows the distribution of doses of the ethanol extract of noni leaves and the percentage of Aedes aegypti mortality through the fogging method.

Confidence Limits							
	probability	95% Confidence Limits for Concentration of ethanol extract of Noni Fogging Leaves			95% Confidence Limits for log (Concentration of Fogging Noni Leaf ethanol extract) a		
		Estimates	LowerBound	Upperbound	Estimates	LowerBound	Upperbound
PROBIT	,500	56,752	29,842	83,736	1.705	1,511	1,792
	,900	122,011	92,121	1216,231	2,081	1,932	2,895
a. Logarithm base = 10.							

Probit analysis for the efficacy test results of Noni Leaf ethanol extract by fogging the LC50 results at a concentration of 56.752% (estimated concentration range between 29.842% to 83.736%) and LC90 = 122.011% (estimated concentration range of 92.121% to 1216.231%) can be seen in table 16 above.

Table 16. Test resultsEfficacy of Ethanol Extract of Noni Leaves on Death of Aedes aegypti by fogging.

						95% Confidence Intervals	
	Parameter	Estimates	std. Error	Z	Sig.	LowerBound	Upperbound
PROBITa	Fogging Noni Leaf Ethanol Extract Concentration	3,192	1,438	2,219	.026	,373	6,011
	Intercepts	-5,599	2,550	-2.195	,028	-8,150	-3,049

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10,000 logarithm.)

Ethanol Extract Combination of Bay Leaves and Noni (Damesa) Leaves

The combination of ethanol extract of bay leaves and noni (Damesa) leaves was administered through spraying. The results of the experiment on the effectiveness of the ethanol extract combination in killing Aedes aegypti mosquitoes through spraying are presented in table 17. The highest mortality rate of mosquitoes was observed at a concentration of 60%, with an average of

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17.7 mosquitoes killed. The lowest mortality rate was observed at a concentration of 10%, with an average of 7 mosquitoes killed. No deaths were observed in the control sample.

Table 17 shows the distribution of doses of the ethanol extract combination and the percentage of Aedes aegypti mortality through spraying

Experiment	Number of Mosquito Mortality at extract dose						
	Control	10%	20%	30%	40%	50%	60%
1	0	7	10	12	13	15	17
2	0	8	12	14	14	15	18
3	0	6	11	13	15	16	18
Amount	**0**	**21**	**33**	**39**	**43**	**46**	**53**
Average	**0**	**7**	**11**	**13**	**14,3**	**15,3**	**17,7**

The results of the treatment based on the LC50 and LC90 tests are presented in table 18 below. The table shows that the mortality rate of Aedes aegypti mosquitoes was highest at LC90 concentrations, with an average of 16 mosquitoes killed, compared to LC50 concentrations, which had an average of 11.7 mosquitoes killed. Table 18 shows the results of the LC50 and LC90 efficacy tests of the Damesa Combination extract against Aedes aegypti mosquitoes through spraying.

Experiment	Number of Mosquito Deaths		
	Control	LC50	LC90
1	0	11	16
2	0	13	16
3	0	12	16
Amount	**0**	**35**	**48**
Average	**0**	**11,7**	**16**

Probit analysis for the efficacy test results of the Damesa combined ethanol extract by spraying the LC50 results at a concentration of 39.711% and LC90 = 73.035% is shown in table 19 below.

Table 19. Distribution of Amount and Percentage of Doses of Demesa Combination Ethanol Extract Against Aedes aegypti Mortality by Spraying

Confidence Limits							
	probability	95% Confidence Limits for Damesa spraying ethanol extract concentration			95% Confidence Limits for log(Damesa spraying ethanol extract concentration)a		
		Estimates	LowerBound	Upperbound	Estimates	LowerBound	Upperbound
PROBIT	,500	39,711	-	-	1,754	1.475	1,923
	,900	73,035	-	-	2.155	1,960	5,085
a. Logarithm base = 10.							

Table 20. Test results Efficacy of Damesa Combination Ethanol Extract against Aedes aegypti Mortality by spraying.

	Parameter	Estimates	std. Error	Z	Sig.	95% Confidence Intervals	
						LowerBound	Upperbound
PROBITa	Spraying extract concentration treatment	.038	,012	3,127	,002	,014	,063
	Intercepts	-1.527	,793	-1,926	.054	-2,320	-,734

a. PROBIT model: PROBIT(p) = Intercept + BX

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Damesa Combined Ethanol Extract by Fogging

The results of the treatment using the Damesa Combined Ethanol Extract against Aedes aegypti mosquitoes through fogging are presented in table 4.21 below. The highest mortality rate of mosquitoes was observed at a concentration of 60%, with an average of 18 mosquitoes killed. The lowest mortality rate was observed at a concentration of 10%, with an average of 6.3 mosquitoes killed. No deaths were observed in the control sample.

Table 21 shows the distribution of doses of the Damesa Combined Ethanol Extract and the percentage of Aedes aegypti mortality through fogging.

Experiment	Number of Mosquito Mortality at extract dose						
	Control	10%	20%	30%	40%	50%	60%
1	0	7	10	13	13	15	17
2	0	5	8	14	14	15	19
3	0	6	9	12	12	14	18
Amount	**0**	**19**	**27**	**39**	**39**	**44**	**54**
Average	**0**	**6,3**	**9**	**13**	**13**	**14,7**	**18**

Table 22 LC50 and LC90 test results against mosquitoes*Aedes aegypti*Damesa Combination extract by fogging.

Experiment	Number of Mosquito Deaths		
	Control	LC50	LC90
1	0	15	18
2	0	16	19
3	0	16	20
Amount	**0**	**47**	**57**
Average	**0**	**15,7**	**19**

The treatment results based on the LC50 and LC90 efficacy tests are presented in table 22 above. The table shows that the highest mortality rate of Aedes aegypti mosquitoes was observed at LC90 concentrations, with an average of 19 mosquitoes killed, compared to the LC50 concentration of ethanol extract, which had an average of only 15.7 mosquitoes killed. No deaths were observed in the control sample. Probit analysis of the efficacy test results of the Damesa combined ethanol extract through fogging showed LC50 results at a concentration of 41.321% and LC90 at a concentration of 112.311%, as presented in table 23 below.

Table 23 shows the distribution of doses and the percentage of Aedes aegypti mortality using the Damesa Combined Ethanol Extract through fogging."

Confidence Limits							
	probability	95% Confidence Limits for Fogging Damesa ethanol extract concentration			95% Confidence Limits for log(Damesa Fogging ethanol extract concentration)a		
		Estimates	LowerBound	Upperbound	Estimates	LowerBound	Upperbound
PROBIT	,500	41,321	-	-	1,754	1.475	1,923
	,900	112,311	-	-	2.155	1,960	5,085
a. Logarithm base = 10.							

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Table 24. Test results Efficacy of Damesa Combination Ethanol Extract on Death of Aedes aegypti by fogging method.

	Parameter	Estimates	std. Error	Z	Sig.	95% Confidence Intervals	
						LowerBound	Upperbound
PROBITa	Fogging extract concentration treatment	,020	,006	3,615	,000	,009	.031
	Intercepts	-,803	,508	-1,580	, 114	-1,311	-.295

a. PROBIT model: PROBIT(p) = Intercept + BX

DISCUSSIONS

This study aims to test the efficacy of bay leaves extract, noni (mengkudu) leaves extract, and their combination called Damesa against adult Aedes aegypti mosquitoes using liquid spray and fogging methods. The test was conducted using six concentrations, namely 10%, 20%, 30%, 40%, 50%, and 60%. Each concentration was repeated six times for each extract group. The spray treatment was performed 72 times, while the fogging was performed 76 times. Meanwhile, the control was carried out 56 times. The results showed that fogging was more effective in increasing the efficacy of Aedes aegypti mosquito death compared to liquid spray. The LC50 for bay leaves extract with liquid spray was 71.13%, while with fogging was 44.827%. Meanwhile, LC90 for bay leaves extract with liquid spray was 287.71%, while with fogging was 302.239%. For noni leaves extract, LC50 with liquid spray was 50.709%, while with fogging was 56.752%. Meanwhile, LC90 for noni leaves extract with liquid spray was 120.534%, while with fogging was 122.011%. For the combination of bay leaves and noni leaves extract, LC50 with liquid spray was 39.711%, while with fogging was 41.321%.

Meanwhile, the LC90 for the combination of bay leaf and noni leaf extracts with spray application was 73.035%, while with fogging it was 112.311%. Although the efficacy of the Damesa combination extract is slightly lower compared to the individual bay leaf and noni leaf extracts, fogging remains effective in increasing mosquito mortality efficacy. In addition, this study has not found similar research on efficacy testing on Aedes aegypti mosquitoes, although many other studies have been conducted on larvae and mosquitoes that are restricted in movement within boxes/cages made of mesh. Currently, the use of synthetic chemical insecticides is routinely carried out for the control of Aedes aegypti mosquitoes, but this has a negative impact on the environment. Therefore, a botanical insecticide from noni leaf extract (Morinda citrifolia L.) is a solution that can reduce the negative impact of synthetic chemical insecticides. Several studies have shown that noni leaf extract has insecticidal activity against Aedes aegypti mosquitoes, such as the study by Isnaeni Anggi Safitri and Widya Hary Cahyati in 2018, which showed a significant difference in average mosquito mortality at each tested extract concentration ($p = 0.000$), with LC50 = 11.608% and LC90 = 28.633%.[17],[18]

The next research is expected to further uncover the potential insecticidal content of the noni leaves. Another study conducted by Armayani and Rasyid in 2019 showed that noni leaf extract with the spray method can kill the vector of dengue fever, the Aedes aegypti mosquito. In addition, Safitri et al. (2018) found that noni leaf extract in liquid form can also kill Aedes aegypti mosquitoes. However, it should be noted that the level of effectiveness in killing Aedes aegypti mosquitoes can vary depending on the location [19]. A study conducted in 2021 showed differences in the death rate of mosquitoes inside and outside the house/room after insecticide spraying [20]. Moreover, the duration of exposure to the insecticide also affects the death rate, where the longer the exposure time,

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the more mosquitoes die. The observation period for mosquito mortality in this study was only 60 minutes, as the average fog duration only lasts for 60 minutes after fogging. However, in spray application, the time for the water to dry and the scent to dissipate can affect the effectiveness of the spraying. Therefore, in future studies, a comprehensive observation is needed to distinguish the level of efficacy in killing Aedes aegypti mosquitoes inside and outside the house/room. Such research could help to determine the most effective type of botanical insecticide to be used in controlling Aedes aegypti mosquitoes.

CONCLUSION

Based on the Probit analysis of the extract from the Morinda citrifolia (noni) leaves, bay leaves, and their combination (Damesa) using spray and fogging methods to test the efficacy of killing Aedes aegypti mosquitoes, the following conclusions can be drawn:

1. Probit analysis of bay leaves ethanol extract using the spray method resulted in an LC50 at a concentration of 71.13% and LC90 at a concentration of 287.71%.
2. Probit analysis of bay leaves ethanol extract using the fogging method resulted in an LC50 at a concentration of 44.927% and LC90 at a concentration of 302.239%.
3. Probit analysis of noni leaves ethanol extract using the spray method resulted in an LC50 at a concentration of 50.709% and LC90 at a concentration of 120.534%.
4. Probit analysis of noni leaves ethanol extract using the fogging method resulted in an LC50 at a concentration of 56.752% and LC90 at a concentration of 122.011%.
5. Probit analysis of the combination of bay leaves and noni leaves ethanol extracts (Damesa) using the spray method resulted in an LC50 at a concentration of 39.711% and LC90 at a concentration of 73.035%.
6. Probit analysis of the combination of bay leaves and noni leaves ethanol extracts (Damesa) using the fogging method resulted in an LC50 at a concentration of 41.321% and LC90 at a concentration of 112.311%.

CONFLICT OF INTEREST

The author declares no conflict of interest

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