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Analysis of Aedes aegepty Larvicide Resistance to Starfruit Leaf Extract (Averrhoa bilimbi l.) as a Natural Larvicide

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Abstract

Eradication of Ae.aegypti can be done on its larvae using chemical, biological and physicalmethods, one of which is larvicide. Early detection of vector resistance to insecticides can be useful in determining the right insecticide to control vectors. People generally use insecticides from the synthetic pyrethroid group which are widely sold on the market in an effort to kill mosquitoes. This results in the resistance of the Aedes aegepty mosquito vector to synthetic insecticides, to prevent If resistance occurs, it needs to be treated with natural insecticides that come from plants, one of which is star fruit. The starfruit plant (Averrhoa bilimbi L.) The secondary compounds produced are alkaloids, saponins and flavonoids. Bioactive compounds found in plants can be used like synthetic insecticides. The aim of this research isto knowAedes aegepty larvicide resistance to starfruit leaf extract (Averrhoa bilimbi L.) as a natural larvicide.Type of pure experimental research (true experiment) with a "One Groups Pretest-Posttest Design" design, with a Completely Randomized Design (RAL).The research was conducted in July 2024. Samples of starfruit plants in Aceh Besar, samples of larvae from maintenance mosquitoes. The concentrations used were 800,900 and 1000 ppm with controls. Treat 4 repetitions, analysis of differences using anova test and resistance test. Research resultThe number of mosquito larvae that died was 32 at a concentration of 800 ppm to 98 at a concentration of 1000 ppm. The LC95 obtained is 5.007%. at a dose of 1000 ppm is included in the vulnerable category. Because mortality reaches 98%. Meanwhile, other doses are resistant with mortality below 95%. QThere was a significant difference in the average mortality of Aedes aegypti larvae from various dose variations.Natural Insecticide Starfruit leaves (*Averrhoa Bilimbi Linn*). So further LSD testing was carried out to determine the most effective dose.

Keywords : starfruit (Averrhoa bilimbi L.). resistance, aedes aegpty, larvae

Background

Aedes aegypti a mosquito that can act as a vector for various diseases, including Dengue Hemorrhagic Fever (DHF).[1]Dengue virus consists of serotypes DENV-1, 2, 3, 4, in humans Dengue virus infection can infect more than one serotype simultaneously.[2].Dengue virus transmission is influenced by interactions between the virus, vector mosquitoes, humans and environmental factors. The interaction of these factors will influence the survival of the Dengue virus in nature, making it difficult to control[3]. Aedes aegypti which is infected with the dengue virus and is resistant to the insecticides used in control will cause an increase in dengue cases and can even become an outbreak. The discovery of the Aedes aegypti vector with the level of resistance and the presence of the virus in the mosquito's body will determine the location where control carried out with synthetic insecticides is effective or not. Currently, eradicating Ae.aegypti is the main method used to eradicate dengue fever. Eradication of Ae.aegypti can be carried out on its larvae. Eradication of larvae can be done by chemical, biological and physical methods. One method of chemical eradication that can be carried out is larvicide.[4]

The types of insecticides commonly used in vector control include organophosphates, carbamates and pyrethroids.[5],[6]This type of insecticide is still used and is considered effective in controlling dengue fever. However, the impact of using these two organophosphate-based insecticides if done continuously is the danger of resistance of dengue vector mosquitoes. This is because the use of chemical insecticides in the long term with a high frequency per year will gradually suppress and select target insects (mosquito vectors) to become tolerant to and resistant to them. Resistance to malathion and temephos occurs when the vector cannot be killed by standard doses or manages to avoid contact with the insecticide through the phenomenon of evolution.[7]

However, after using organophosphate insecticides for a long time, mosquito resistance to organophosphates has been reported throughout the world. Persistence of the dengue virus in generations of infected mosquitoes through transovarial transmission has been reported in several studies. To reduce the negative impact of using chemical insecticides, it is necessary to look for other alternatives that are safer for the environment or humans. One way is to use natural insecticides. With this effort, it is hoped that the development of its life cycle will be hampered so that it cannot develop until adulthood.[5]

The incidence of Dengue Fever cases is related to the level of resistance status of Ae. aegypti mosquitoes as vectors. In locations with high cases of DHF, there has been a population of vectors that are resistant and tolerant to insecticides used for control.[8]. The use of insecticides is currently increasing in developing countries. Repeated use of one type of insecticide over a long period of time can lead to resistant target insect populations, resurgence, secondary pest explosions, and the buildup of insecticide residues in nature which can cause problems for humans, animals and agricultural products.[9]. Resistance mechanisms are divided into two types, namely, biochemical mechanisms, which are related to enzymatic functions in the body of the insect vector that are able to break down insecticide molecules into other non-toxic molecules (detoxification)

and behavioral resistance mechanisms, namely the vector naturally avoids contact with the insecticide.[10]

Resistance does not occur in a short time. Pesticide resistance develops after a selection process that lasts for many generations. Resistance is an evolutionary phenomenon caused by selection in insect pests that are treated with insecticides continuously. In nature, the allele frequency of susceptible individuals is greater than the allele frequency of resistant individuals, and the frequency of homozygous resistant (RR) alleles ranges from 10-2 to 10-13. Due to continuous selection, the number of susceptible individuals in a population decreases, leaving behind resistant individuals. These resistant individuals will mate with each other, producing resistant offspring. Populations that survive the initial pesticide application will increase the proportion of individuals that are resistant to the compound and pass this trait on to their offspring.[11]

Alkaloiddisrupts the nervous system of larvae by inhibiting the action of the enzyme acetylcholinesterase (AChE)[12].One of the plants ywho hasactive ingredients as natural larvicides, one of which is starfruit (Averrhoa bilimbi L.). The secondary compounds produced are alkaloids, saponins and flavonoids.[13].Bioactive compounds found in plants can be used like synthetic insecticides. The difference is that the active ingredients in botanical insecticides are synthesized by plants.[14],[15]. In Aceh Province, starfruit is a plant widely planted in every household, because the fruit is a fruit that is a typical mixture in every typical Acehnese cuisine. Getting it is very easy and the fruit is abundant.

Research by Nopianti et al, (2008) resulted in starfruit extract having an effect on the death rate of third instar Anopheles aconitus larvae with a dose of 4.5% resulting in 100%[16]. Oktavia's research (2012) also concluded that there was an effect of star fruit extract as an Aedes aegypti larvicide with a 3% dose resulting in 100% larval mortality.[13].The research was continued by Lisa (2015) withThe most effective concentration of starfruit (Averrhoa bilimbi L.) extract in granule form was 200 mg/100 ml with 100% larval mortality of all test larvae with an LC50 value of 91.677 mg and an LC90 value of 142.399 mg.[17]

The toxic compounds (poison) from starfruit extract are alkaloids, saponins, flavonoids and steroids. Starfruit leaves also contain flavonoid compounds, saponins, sulfur, formic acid, calcium oxalate, and potassium citrate. This study used Natural Insecticides Starfruit Leaves (Averrhoa

Bilimbi Linn), where the phytochemical content in starfruit leaves is expected to inhibit the resistance process of aedes aegypti mosquitoes. The aim of this research is to find outAedes aegepty larvicide resistance to starfruit leaf extract (Averrhoa bilimbi L.) as a natural larvicide

Research methods

Type of pure experimental research (true experiment) with a "One Groups Pretest-Posttest Design", an experimental design that uses a Completely Randomized Design (RAL).The research was carried out in July 2024. Location of sample collection for starfruit plants in Aceh Besar, maintenance and examination of samples was carried out at the Integrated Laboratory of the Health Polytechnic of the Ministry of Health of Aceh.The population in the study was aedes aegypti mosquitoes developed from mosquito rearing. Mosquito larvae were obtained from laboratory colonies.Center for Disease Vector and Reservoir Research and Development,Salatiga or FKH USK Laboratory and develop from eggs that have been resistedagainst organophosphate compoundsthen planted in the Aceh Ministry of Health Polytechnic laboratory, using mosquito eggskept in cages and allowed to mate and produce the first generation (F1).

The sample size for each treatment was 25 individuals, (WHO, 2005)[18]. The research sample size is the number of samples needed based on the number of repetitions, namely according to Supranto J (2000)[19], the equation used to calculate the number of repetitions is as follows:

$(t-1)(r-1) \geq 15$

Based on calculations, the number of repetitions obtained was 4 times for each test. The number of mosquitoes used for each repetition was 25.Extract making stageStarfruitstarting with election preparationsstarfruit leavesyang is obtained fromAceh Besar region, by sorting, namely selecting old leaves with dark green color and not damaged, not yellow spots. Making maceration with alcohol.Larval preparation is from eggs that have hatched into larvae but have not developed into mosquitoes. Wait a maximum of 21 days and are considered dead. Mosquitoes that were 7 days old were identified and then used as samples. Next are the identification stages for Ae.aegypti mosquito larvae.Susceptibility Test with WHO Susceptibility test kit standard. Susceptibility Status Criteria, The level of vector susceptibility is determined based on the percentage of death of test mosquitoes after a 24-hour observation/maintenance period (WHO 2016).

1. test mosquito mortality ≥ 98% is declared susceptible
2. mosquito death test 90 - <98 % is suspected of being resistant
3. while mortality < 90% is resistant

The larvicide test in this study was carried out based on standard methods[18]. The extract was made into several concentrations: 800, 900, 1000 mg/L using PDAM water as a solvent as much as 1000 ml per plastic container. Negative control used PDAM water. Then, 25 third instar Ae.aegypti larvae were placed in each container and observed for 24 hours. Larval mortality was evaluated at the end of 24 h exposure.Persen mosquito larvae were counted at 15, 30, 45, 60, 24 hours. Criteriaa efficacy is taken based on time of death 50% and 95% of the number of test mosquito larvae were calculated from data that had been corrected for the mortality and paralysis of test mosquitoes in the control[20].

Data obtained from simplex lattice design calculations (predictions) are compared with actual test data.analyzed with one-way Analysis of Variance (ANOVA). If the results of the ANOVA test are significant, namely p<@ then proceed with the Last Significant Difference (LSD) test.[21]. The LC50-24 hour value for each solvent was determined using probit (probability unit) analysis.

Results

Observation results during the research carried out 4 repetitions of treatment against Aedes aegypti larvae for 24 hours based on the dose level used withNatural Insecticide Starfruit leaves (*Averrhoa Bilimbi Linn*) in killing mosquito larvae*Aedes aegpty*the average results of the number of deaths of Aedes larvae were obtained aegypti in Table 1:

Table 1. Distribution of Number of Eggsmosquito*Aedes aegpty*that does not hatch AgainstUseNatural Insecticide Starfruit leaves (*Averrhoa Bilimbi Linn*).

Starfruit Extract Dosage	Number of Larva Samples	observation and handling time				observation and handling time				observation and handling time				observation and handling time				observation and handling time			
		15 minutes				30 minutes				45 minutes				60 minutes				24 hours			
		I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Dosage 800 ppm	100	1	1	1	1	2	2	2	2	4	4	4	4	5	6	5	5	8	8	8	8
Dosage 900 ppm	100	2	1	3	1	3	4	2	4	5	5	3	4	7	7	7	6	11	12	11	12
Dosage 1000 ppm	100	8	10	11	10	13	13	13	14	15	15	15	17	21	20	21	20	24	25	25	24
Control (-)	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control (+)	100	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25

Based on the data obtained in Table 1, inNatural Insecticide Starfruit leaves (*Averrhoa Bilimbi Linn*).The number of mosquito larvae that die at each concentration increases the number of larval deaths.The graph of the percentage of eggs that do not hatch can be seen in Figure 1 below:

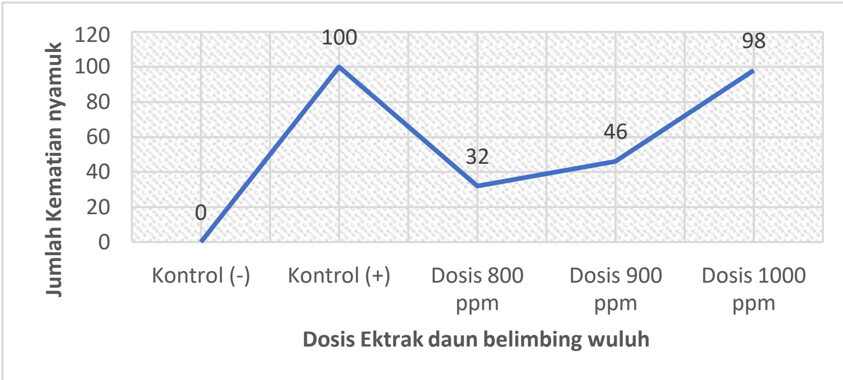

Figure 1. Graph of Concentration Response to Mortality of Aedes aegypti Larvae at Various Concentrations after 24 Hours of Treatment

In the picture, you can see an increase in the number of dead mosquito larvae, namely 32 at a concentration of 800 ppm to 98 at a concentration of 1000 ppm. The probit results for good concentration are in Table 2 below:

Table 2. Lethal Concentration Values and Confidence Limits of Natural InsecticidesStarfruit leaves (*Averrhoa Bilimbi Linn*).on the death of Aedes aegypti larvae after 24 hours of treatment

Point	Exposure concentration	95% Confidence Limits consentration	
		Lower Bound	Upper Bound
LC1	-0.52250	-6,847	,660
LC2	-0.14293	-5,595	,896
LC3	0.09788	-4,804	1,048
LC4	0.27904	-4,209	1,165
LC5	0.42640	-3,728	1,260
LC10	0.93227	-2,089	1,605
LC20	1.54483	-,180	2,097
LC30	1.98653	1,019	2,630
LC40	2.36395	1,742	3,387
LC50	2.71671	2,166	4,346
LC60	3.06947	2,475	5,419
LC70	3.44689	2,755	6,619
LC80	3.88858	3,053	8,053
LC90	4,50114	3,444	10,063

LC95	5.00701	3,758	11,733
LC96	5,15437	3,848	12,220
LC97	5,33553	3,959	12,820
LC98	5.57635	4,105	13,617
LC99	5.95592	4,334	14,876

Location of LC95
Exposure concentration: The concentration obtained

Based on Table 3, it can be concluded that the LC95 value obtained is 5.007%. The lower limit value is 3.758% and the upper limit is 11.733%. The resistance test results are

Table 3. Bioassay test results of Ae. aegypti mosquito larvae against Natural InsecticidesStarfruit leaves (*Averrhoa Bilimbi Linn*).

Starfruit Extract Concentrate	Number of Mosquito Larvae Samples Exposed	Σ Mosquito larvae die from 24 hour exposure	%	Resistance Status
Dosage 800 ppm	100	32	32	Resistant
Dosage 900 ppm	100	46	46	Resistant
Dosage 1000 ppm	100	98	98	Prone to
Control (-)	100	0	0	Resistant
Control (+)	100	100	100	Resistant

From Table 3, it can be seen that the dose of 1000 ppm is in the susceptible category. Because mortality reaches 98%. Meanwhile, other doses are resistant with mortality below 95%.The larval mortality data was then processed using ANOVA to determine whether the treatment with varying concentrations. Natural Insecticide Starfruit leaves (*Averrhoa Bilimbi Linn*) different will produce different numbers of dead mosquito larvae. The test results for each are presented in Table 4:

Table 4. Anova test results are averagedead mosquito larvaewith differences in dosage and Natural Insecticide Starfruit leaves (*Averrhoa Bilimbi Linn*)

Natural Insecticide Starfruit leaves (*Averrhoa Bilimbi Linn*)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		P Value
					Lower Bound	Upper Bound	
800ppm	4	15,0000	12.38278	6.19139	-4,7038	34,7038	0.012
900 ppm	4	20.7500	17,32772	8.66386	-6,8223	48,3223	
1000ppm	4	63,0000	25.17936	12.58968	22.9340	103.0660	
Total	12	32,9167	28.21817	8.14588	14,9877	50,8456	

In Table 4, the results of the Anova test obtained a p value of 0.012 ($p < 0.05$), meaning that there was a significant difference in the average mortality of Aedes aegypti larvae from various dose variations.Natural Insecticide Starfruit leaves (*Averrhoa Bilimbi Linn*). So a follow-up LSD test was carried out to determine the most effective dose. The LSD test results are presented in Table 5 below:

Table 5. LSD Test Results on Averageunhatched mosquito eggs*Aedes aegypti*with differences in dosage and Natural Insecticide Starfruit leaves (*Averrhoa Bilimbi Linn*)

(i) Dosage	(J) Dosage	Mean Difference (IJ)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
800ppm	900 ppm	-5,75000	13.46343	0.679	-36,2064	24,7064
	1000ppm	-48.00000*	13.46343	0.006	-78.4564	-17,5436
900 ppm	800ppm	5.75000	13.46343	0.679	-24,7064	36,2064
	1000ppm	-42.25000*	13.46343	0.012	-72,7064	-11.7936
1000ppm	800ppm	48,00000*	13.46343	0.006	17.5436	78,4564
	900 ppm	42,25000*	13.46343	0.012	11.7936	72,7064

**. The mean difference is significant at the 0.05 level.*

Table 5 above shows that there is no significance for Natural Insecticides. Starfruit leaves (*Averrhoa Bilimbi Linn*)doses of 900 ppp and 800 ppm, with a p value = > alpha value of 0.05, . Meanwhile, in other concentrations, the p value < alpha value is 0.05, and the 95% CL value also shows the same difference between the upper and lower limits.

Discussion

The research results showed that extracts from the starfruit plant were able to kill aedes larvae and this was due to the inhibitory effect of natural alkaloids on acetylcholinesterase in Ae.

Aegypti using molecular docking studies. Research on the use of A. bilimbi plants as natural insecticides includes research by Etik A. Rohmah et al.[22]which aims to determine the activity of primary and secondary metabolites from A. bilimbi on the death of Ae. Aegypti and midgut histopathology. There is a Review of Plant-Base Bioinsecticides for Mosquito Control: Impacton Insecticide Resistance and Disease Transmission by Meryem ¸S et al.[23]which reviews developing safer and more effective mosquito control strategies with botanical bioinsecticides, one of which is plants containing alkaloids, seen how it works and its potential impacts and inhibits resistance. Research by Oktavia, A. et al., who looked at the effectiveness of Starfruit Extract on Aedes Mosquito Larvae Mortality[13]. AndEvi, (2005) who researched insecticidesVegetables are synthesized by plants[14],Nopianti et al., (2008)'s research aims to see eEffectiveness of Star Fruit to kill Anopheles aconitus Instar III larvae.[16]. Oktavia's research (2012) also examined the effect of starfruit extract as an Ae. aegypti larvicide with the results of the dose[13].Lisa's research (2015) withresearched the concentration of starfruit extract (A. bilimbi.) in granule form on the death of Ae.Aegepty larvae[17].In this study, we only looked at larval mortality mortality in mosquitoes that were susceptible to the use of star fruit extract.

Based on the research results[24] mentions that alkaloids can also be used as insecticides. Alkaloids in fresh leaves or fruits taste bitter on the tongue, alkaloids are in the form of salts so they can degrade cell walls, enter and damage cells. Saponins are a group of triterpennoid compounds that can also be used as insecticides. Saponins are found in plants that are then consumed by insects, have a working mechanism that can reduce the activity of digestive enzymes and food absorption, so that saponins act as stomach poisons. Flavonoids are phenol compounds as antimicrobials, antivirals, antifungals and work against insects.

Flavonoid compounds are polar compounds because they have a number of bound sugars, therefore flavonoids tend to dissolve in polar solvents. Flavonoid compounds are divided into several types, each type of flavonoid has a different polarity depending on the number and position of the hydroxyl group of each type of flavonoid so that this will affect the solubility of flavonoids in solvents.[25]..[26],[27]. The high total flavonoids in matoa leaf extract with methanol solvent explains that the characteristics of the flavonoid compounds in matoa leaf extract have the same polarity as methanol, so that matoa leaf extract with methanol solvent produces the highest flavonoid compound content.

Different alkaloids were selected as ligands and their docking ability with Acetylcholinesterase 1 (AChE1) receptor in Ae. AegyptI was performed by AutoDock.[28], further research by Kaihena, M et al., research on the use of ethanol extract of betel leaves (Piper betle L.) on the mortality of Anopheles sp and Culex larvae focused on alkaloid compounds in inhibiting the activity of the AchE enzyme.[29]Wijaya's research (2023) examined the effects of flavonoid, alkaloid, steroid, tannin, and saponin content in C. hystrix plants, on AChE levels in aedes larvae, also found research by Samal, R. (2022) which aims to see the role of metabolic detoxification and target site insensitivity in causing resistance in Ae. aegypti larvae to organophosphate and carbamate insecticides in resistance events. The ace-1 gene encodes the synthesis of the enzyme acetylcholinesterase.[30],[31].

Conclusion

The more dosesStarfruit Leaves (*Averrhoa Bilimbi Linn*) then the more larvaedead mosquitoes.There is a significant influence on the averagemosquito larvae*Aedes aegypti*from various doses of extractStarfruit Leaves (*Averrhoa Bilimbi Linn*), AndFrom the table, the results obtained at a dose of 1000 ppm are included in the vulnerable category because mortality reaches 98%.Starfruit Leaves (*Averrhoa Bilimbi Linn*) can be used as a natural larvicide in an effort to kill Aedes aegypti mosquito larvae. It can be used as an alternative to synthetic insecticides which have been used to reduce resistance rates.

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