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The Effectiveness of the Combination of Lethal Ovitrap Attractant of Local Organic Materials and Natural Larvicides in Dengue Hemorrhagic Fever Endemic Areas

Erlin Yustin Tatontos[1*], I Wayan Getas[1], Urip[1]

[1]Department of Medical Laboratory Technology, Poltekkes Kemenkes Mataram, West Nusa Tenggara, Indonesia

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Corresponding Author:
Erlin Yustin Tatontos
erlintatontos64@gmail.com

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Abstract: Dengue fever is a viral infection that is transmitted to humans through the bite of an infected mosquito. Dengue prevention and control depends on effective vector control measures, including lethalovitrap.The purpose of this study was to determine the effectiveness of mango leaf infusions (Mangifera indica), rambutan leaves (*Nephelium lappaceum*) and cashew leaf (Anacardium ocidentale) in lethal ovitrap a combination of ethanol extract of rambutan leaves (*Nephelium lappaceum*), srikaya seed water extract (Annona squomosa) and Baccilus thuringiensis var israelensis (Bti) in the endemic area of Mataram City Dengue Fever, This study was an experimental study that tested and analyzed the effectiveness of nine lethal ovitrap atractant combination. The population and sample of this study were Aedes aegypti mosquitoes female from colonization from endemic areas of DHF in Mataram City. The laboratory test results from nine effective lethal ovitrap combination attractants were Cashew leaf infusion and Rambutan leaf ethanol extract with trapped eggs of Aedes aegypti mosquitoes averaging 133 grains and no eggs hatching into larvae. Statistical analysis with ANOVA followed by the Tukey HSD post-Hoc test with a significant difference of <0.05 the effectiveness of the combination of lethal ovitrap attractants, especially Cashew leaf infusion and ethanol extract from rambutan leaves and Rambutan leaf infusion and rambutan leaf ethanol extract. The results of the field test were the percentage of the number of eggs trapped in combination of Cashew leaf infusion (41.6%) and Rambutan leaf infusion with ethanol extract of Rambutan leaves (58.4%).

Keywords: Effectiveness; Lethal ovitrap; Attractant

Introduction

Dengue hemorrhagic fever is a viral infection that is transmitted to humans through the bite of an infected mosquito (Madani et al., 2021). The main vectors that transmit this disease are the *Aedes aegypti mosquito* and, to a lesser extent, *Ae. albopictus* . Dengue fever is found in tropical and sub-tropical climates worldwide. Severe dengue fever is a major cause of serious illness and death in several Asian and Latin American countries (Guha-Sapir et al., 2005; Guzman et al., 2003; Miah et al., 2021). There is no specific treatment for dengue fever or severe fever. Vaccination is still under development. Prevention and control of dengue depends on effective vector control measures (WHO, 2021).

Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by a virus and spread by vectors, namely Aedes aegypti and Aedes albopictus mosquitoes (Guzman et al., 2010; Sofia et al., 2022; Sudarmaja et al., 2022). In Indonesia in 2021 there are 73,518 cases of DHF with a total of 705 deaths. Cases and deaths from DHF have decreased compared to 2020, namely 108,303 cases and 747 deaths (Kemenkes RI, 2021). The larva-free rate (ABJ) is the percentage of buildings where there are no mosquito larvae. When compared with the ABJ target, for 10 years Indonesia's ABJ has been below the target

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(<95%), namely in the range of 24.1-80.2% (Arisanti et al., 2021).

Dengue fever as an infectious disease is still a public health problem in NTB Province because of its fast spread, potential for death and all districts/cities have been infected with dengue fever. In 2020 there were 3,919 cases of DHF and decreased by 0.69 times to 2,719 cases in 2021 with 18 people dead (CFR: 0.7%) (Dikes Provinsi NTB, 2022). Placement of 2000 ovitraps in dengue endemic areas in Singapore can reduce the density of dengue vectors (Ginny Tan Ai-leen et al., 2000). In Brazil and Australia, dengue fever outbreaks were controlled by installing *lethal ovitraps* to reduce the density of *Aedes aegypti,* an environmentally friendly method (Perich et al., 2003; Long et al., 2015). In the city of Semarang, city of Salatiga and city of Makassar, it has been shown that the use of ovitrap attractants can reduce the density of dengue vectors (Ermayana et al., 2015; Pujiyanti et al., 2021).

The use of insecticides, especially synthetic chemical larvicides, leaves residues that have a negative impact on the environment, so natural larvicidal research is developed to suppress the negative effects of chemical larvicides. However, natural larvicides, although proven effective, have weaknesses in use in the community, causing doubts due to appearance, smell, color, ease of use, application in mosquito breeding sites and availability of materials due to daily use (Pratiwi, 2012).

The results of research on rambutan leaf extract concentrations of 0.1% and 0.8% are effective as larvicides because they contain saponins (Asiah et al., 2009). The results of the *Lethal Concentration/*LC_{50} ethanol extract of Srikaya seeds (*Annona squamosa)* from Lombok was 25.37 ppm while the LC_{50} of Srikaya seed extract from Bali was 28.64 ppm (Romianingsih et al., 2015). The results of *lethal ovitrap research* with straw soaking as an attractant and natural insecticide, namely water extract of srikaya seeds, were effective in reducing the density of *Aedes aegypty mosquitoes* (Satoto et al., 2015).

The results of research on the infusion of cashew leaves (*Anacardium occidentale)* with a concentration of 50% can be an attractant that does not smell bad (E. Santos et al., 2010). Cashew leaves (*Anacardium occidentale)*, mango leaves (*Mangifera indica)* and rambutan leaves (*Nephelium lappaceum*) contain high levels of tannins which are thought to attract mosquitoes to lay eggs and do not smell bad. However, it is necessary to conduct research on the effectiveness of these attractants on *lethal ovitrap* using natural larvicides, namely water extract of Srikaya seeds and ethanol extract of Rambutan leaves and *Bacillus thuringiensis* which the program uses as a bioinsecticide.

Method

Experimental research in the laboratory using a completely randomized design to determine the effectiveness of a combination of lethal ovitrap attractants has been carried out on nine combinations of lethal ovitrap attractants. The population and sample of this study were adult female *Aedes aegypti mosquitoes* from eggs collected from residents' homes in the DHF endemic area of Mataram City and colonized in the Parasitology Laboratory of the Health Polytechnic of the Mataram Ministry of Health. Collection of mosquito eggs by installing *an ovitrap was* carried out in the Kamasan Environment, Monjok Village, which is the working area of the Mataram Health Center and is a DHF endemic area in Mataram City.

The attractant is made from the infusion of three types of local organic materials: mango leaves (*Mangifera indica L),* rambutan leaves *(Nephelium lappaceum)* and cashew leaves (*Anacardium occidentale L)*. 30 grams of each leaf were taken and then dried (24 hours, 100˚) placed in a bottle containing 2 liters of distilled water for seven days under anaerobic conditions, so that it was diluted to 50% in distilled water (Santos et al., 2010). The effectiveness test was carried out by comparing a combination of *lethal ovitrap* attractants made from local organic ingredients, namely mango leaves (*Mangifera indica L), rambutan* leaves *(Nephelium lappaceum)* and cashew leaves (*Anacardium occidentale L)* with organic larvicides, namely ethanol extract of rambutan leaves (*Nephelium lappaceum),* Srikaya seed water *(Annona squomosa)* and *Bacillus thuringiensis var israelensis (Bti),* distilled water as a control in the laboratory.

In laboratory tests, 25 *Aedes aegypti gravid mosquitoes were used* for each lethal ovitrap, a combination of infusion with three types of larvicides, namely the ethanol extract of rambutan leaves (*Nephelium lappaceum*) concentration of 0.8%, water extract of sugar apple seeds (*Annona squomosa)* concentration of 1 ml per test medium. and *Bacillus thuringiensis var israelensis (Bti)* concentration of 0.05 ml per test medium, volume of *lethal ovitrap test medium* 250 ml. The *lethal ovitrap test was* carried out by counting the number of trapped *Aedes aegypti mosquito eggs*, the number of hatched larvae and the number of dead larvae. Mosquitoes full of blood in theory will lay eggs ± 7 days and the eggs will hatch into larvae ± 2 days, but in this research turned out that on the 4th day they had laid eggs so observations were made for 9 days Furthermore, the most effective *lethal ovitrap attractant combination was* tested in the DHF endemic area of Mataram City.

Results and Discussion

Laboratory tests on the combination of *lethal ovitrap* attractants infused with mango leaves (*Mangifera indica*), rambutan leaves (*Nephelium lappaceum*) and cashew leaves (*Anacardium ocidentale*) with ethanol extract of rambutan leaves (*Nephelium lappaceum*), the results can be seen in Figure 1 and 2.

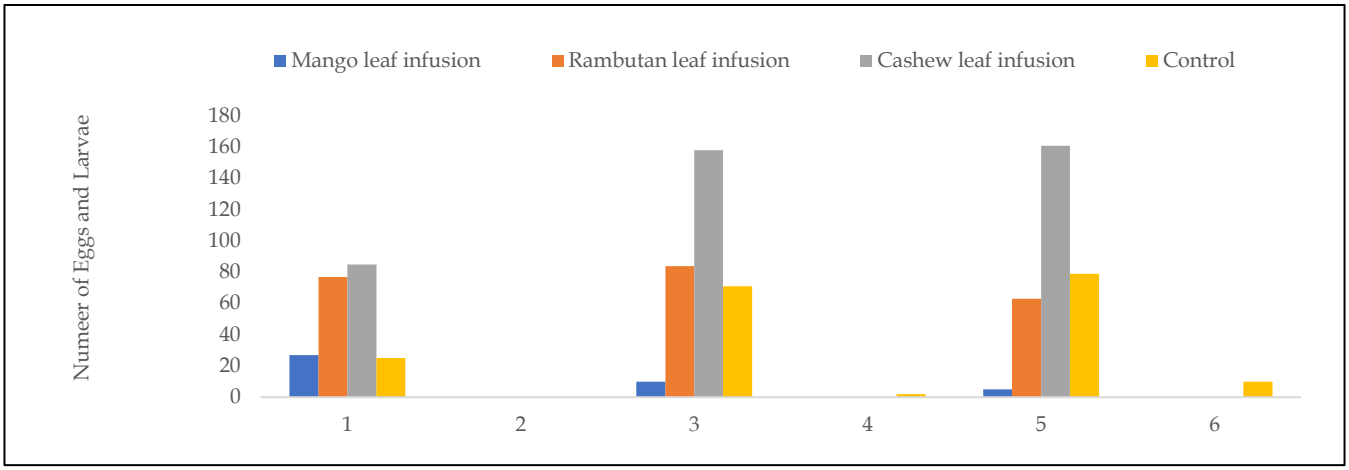

Figure 1. Diagrams test results for the combination of lethal ovitrap attractants infused with mango leaves (*Mangifera indica*), rambutan leaves (*Nephelium lappaceum*) and cashew leaves (*Anacardium ocidentale*) with ethanol extract of rambutan leaves (*Nephelium lappaceum*) in the laboratory (Note: Control: aquades, T: Eggs, L: Larvae)

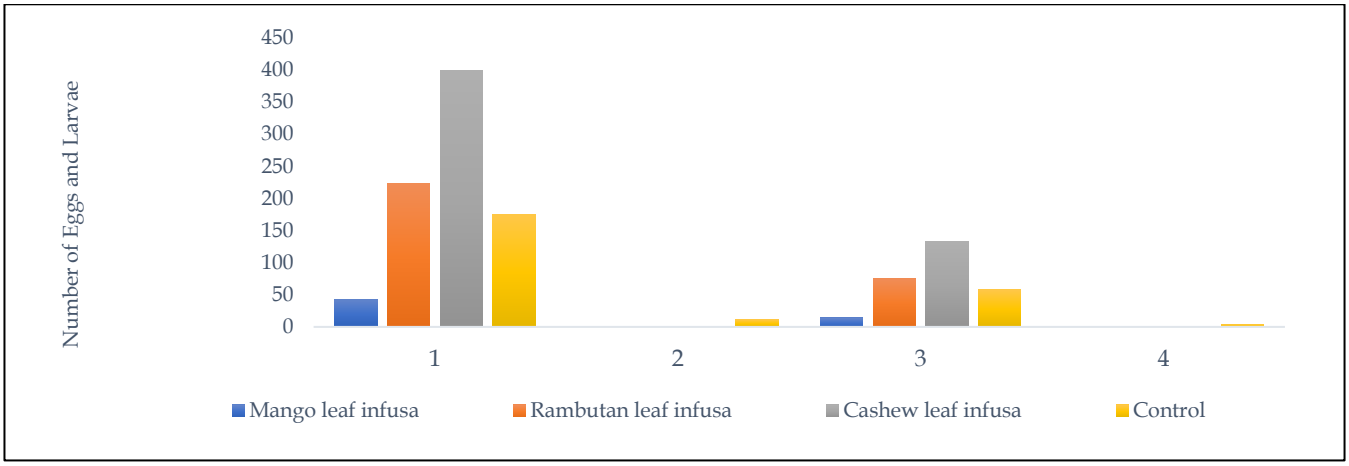

Figure 2. Average digram of test results for the combination of lethal ovitrap attractants infused with mango leaves (*Mangifera indica*), rambutan leaves (*Nephelium lappaceum*) and cashew leaves (*Anacardium ocidentale*) with ethanol extract of rambutan leaves (*Nephelium lappaceum*) in the laboratory

The results of the laboratory test for the combination of *lethal ovitrap* attractant infusion with ethanol extract of Rambutan leaves showed that the highest average trapped eggs were 133 eggs in Cashew leaf infusion, then infusion of Rambutan leaves with 75 items and lowest infusion of Mango leaves with 14 items, while control distilled water was 58 items. while the average control larvae are only 4 tails. Laboratory test combination of *lethal ovitrap* attractants infused with mango leaves (*Mangifera indica*), rambutan leaves (*Nephelium lappaceum*) and cashew leaves (*Anacardium ocidentale*) with water extract of Srikaya seeds (*Annona squomosa*).

The results of laboratory tests on the combination of *lethal ovitrap* attractant infusion with water extract of Srikaya seeds (*Annona squomosa*) showed that the highest average number of *Aedes aegypti* trapped eggs was 2 in the rambutan leaf infusion, then 1 cashew leaf infusion and in the infusion of mango leaves and eggs were not found in the control, while larvae were not found in all treatments. Lethal ovitrap laboratory tests combined infusion of mango leaves (*Mangifera indica*), rambutan leaves (*Nephelium lappaceum*) and cashew leaves (*Anacardium ocidentale*) with *Bacillus thuringiensis var israelensis (Bti)*, the results are presented in the form of a diagram as follows.

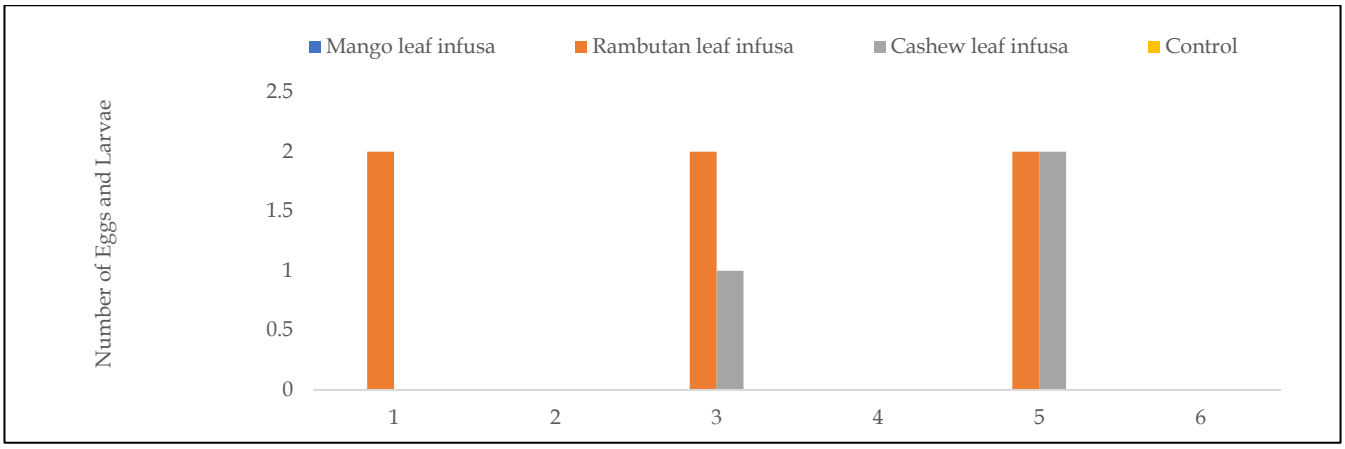

Figure 3. Test results for *lethal ovitrap* attractant combinations infused with mango leaves (*Mangifera indica*), rambutan leaves (*Nephelium lappaceum*) and cashew leaves (*Anacardium ocidentale*) with water extract of Srikaya seeds (*Annona squomosa*) in the laboratory

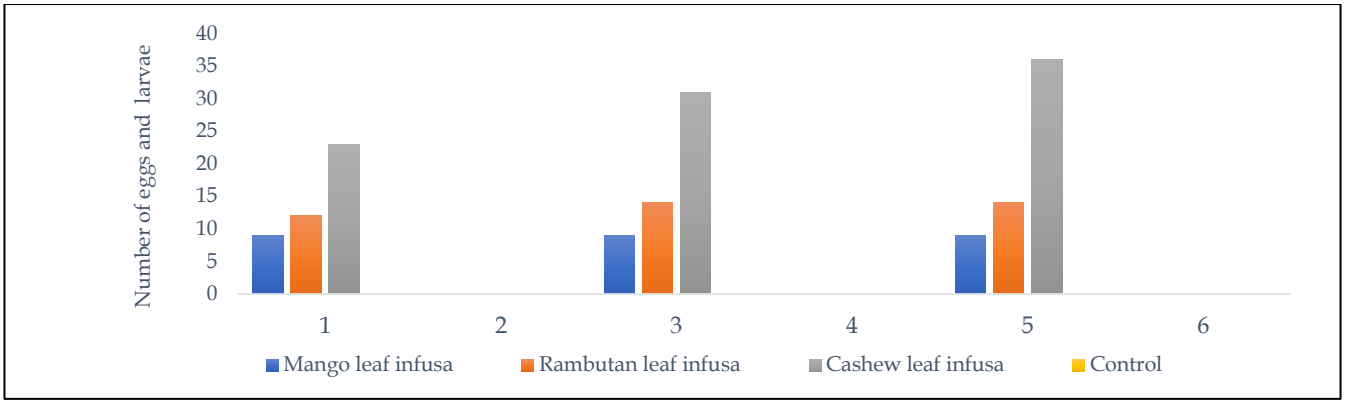

Figure 4. Digram of test results for the combination of *lethal ovitrap* attractants infused with mango leaves (*Mangifera indica*), rambutan leaves (*Nephelium lappaceum*) and cashew leaves (*Anacardium ocidentale*) with *Bacillus thuringiensis var israelensis (*Bti) in the laboratory

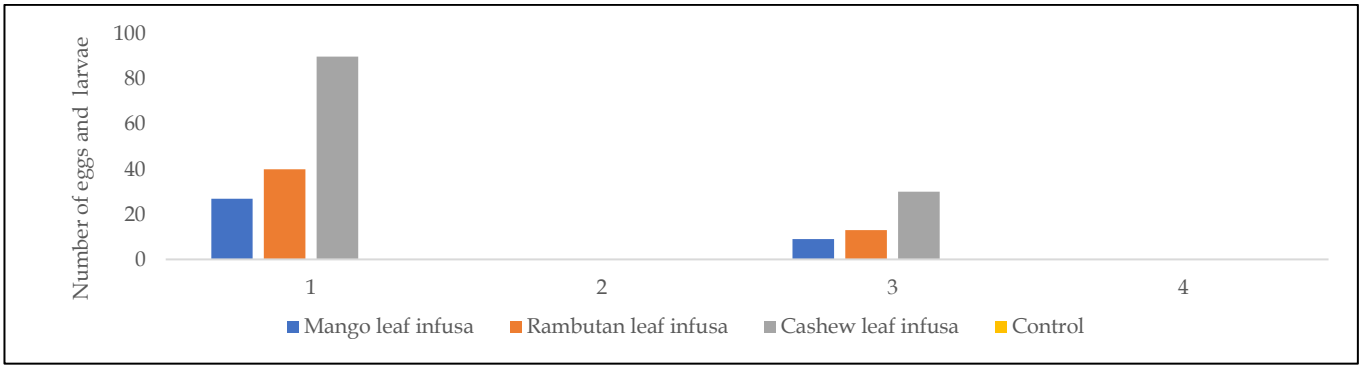

Figure 5. Average results of the analysis of combination tests of *lethal ovitrap* attractants infused with mango leaves (*Mangifera indica*), rambutan leaves (*Nephelium lappaceum*) and cashew leaves (*Anacardium ocidentale*) with *Bacillus thuringiensis var israelensis (*Bti) in the laboratory

Comparison of the effectiveness of the combined *lethal ovitrap* attractant infused with mango leaves (*Mangifera indica*), rambutan leaves (*Nephelium lappaceum*) and cashew leaves (*Anacardium ocidentale*) with ethanol extract of rambutan leaves (*Nephelium lappaceum*), water extract of Srikaya seeds (*Annona squomosa)* and Baccilus *thuringiensis var israelensis (Bti)* can be seen in table 4 as follows. The results of the effectiveness test on the *lethal ovitrap* attractant combination with all types of infusions including control of the percentage of trapped eggs were 6.9% mango leaf infusion, 26.9% rambutan leaf infusion, 48.9% cashew leaf infusion and 17% control (aquades). 3% while there were only 4 larvae in the control. The percentage of

mosquito eggs trapped in the combined *lethal ovitrap* attractant with organic larvicide was 83.6% ethanol extract of rambutan leaves, 0.9% water extract of srikaya seeds and 15.5% *Bti*.

Table 1. Results of the Effectiveness Test of the Combination of *Lethal Ovitrap* Attractants Infused with Mango Leaves (*Mangifera indica*), Rambutan Leaves (*Nephelium lappaceum*) and Cashew Leaves (*Anacardium ocidentale*) with Ethanol Extract of Rambutan Leaves (*Nephelium lappaceum*), Water Extract of Srikaya Seeds (*Annona squomosa)* and *Bacillus thuringiensis var israelensis (Bti)* in the Laboratory

Infusa type	The average number of eggs and larvae of Ae. aegypti on lethal ovitraps												Amount egg Ae aegypti	%
	Leaf ethanol extract rambutans				Seed water extract Srikaya				Bti					
	T	%	L	%	T	%	L	%	T	%	L	%		
Mango leaf infusa	14	5	0	0	0	0	0	0	9	17.3	0	0	23	6.9
Rambutan leaf infusa	75	28.8	0	0	2	66.7	0	0	13	25	0	0	90	26.9
Cashew leaf infusa	133	47.5	0	0	1	33.3	0	0	30	57.7	0	0	164	48.9
Control	58	20.7	4	100	0	0	0	0	0	0	0	0	58	17.3
Amount	280	100	4	100	3	100	0	0	52	100	0	0	335	100
Average	70		1		1				13				84	
Percentage (%)	83.6				0.9				15.5				100	

The results of the ANOVA test effectiveness of the *lethal ovitrap* attractant combination were significantly different. = 0.000 (< 0.05). The *post hoc test* with *Tukey HSD* continued the effectiveness of the *lethal ovitrap* attractant combination which was significantly different compared to the effectiveness of other combinations, namely the combination of Cashew Leaf infusion with Rambutan Leaf ethanol extract and the combination of Rambutan Leaf infusion with Rambutan Leaf ethanol extract. These results are illustrated through the graph in Figure 6.

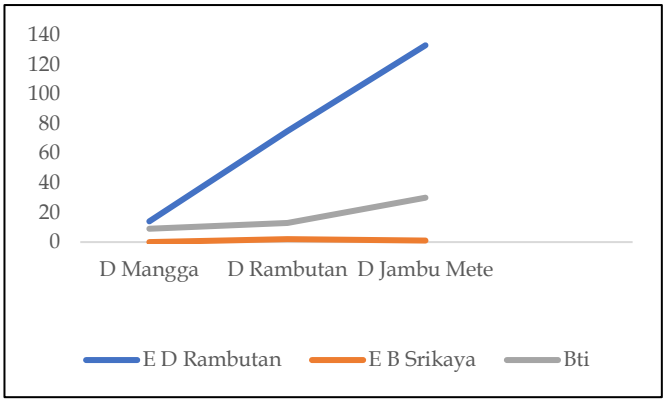

Figure 6. Graph of the effect of the *lethalovitrap combination*

The results of the field test combination of *lethal ovitrap* attractant ethanol extract of Rambutan leaves with cashew leaf infusion and rambutan leaf infusion in figure 7. Field test results on the effectiveness of the *lethal ovitrap* attractant combination of ethanol extract of rambutan leaves with 41.6% cashew leaf infusion and 58.4% rambutan leaf infusion. *Lethal ovitrap* attractant is one of the ways to control Dengue Hemorrhagic Fever which can reduce morbidity rates, such as in Brazil and Australia controlling Dengue Fever outbreaks by installing *lethal ovitrap* to reduce the density of *Aedes aegypti,* an environmentally friendly method (Perich et al., 2003; Long et al., 2015). In the city of Semarang, city of Salatiga and city of Makassar, it has been shown that the use of ovitrap attractants can reduce the density of dengue vectors (Barrera, 2022; Brouazin et al., 2022; Djiappi-Tchamen et al., 2022; Ermayana et al., 2015).

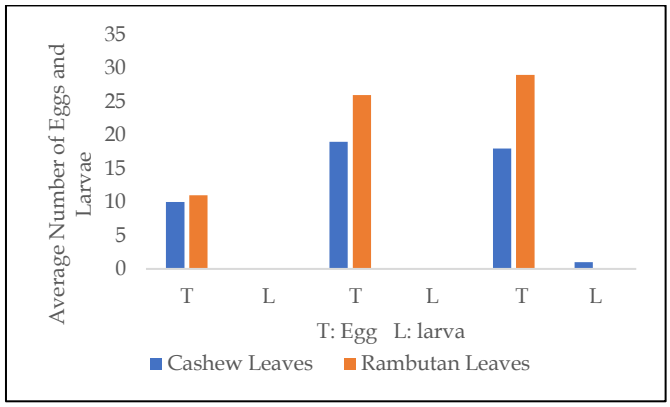

Figure 7. Field test results of *lethal ovitrap* attractant combinations of ethanol extract of rambutan leaves with cashew leaf infusion and rambutan leaf infusion in the Kamasan Environment, Monjok Village, Mataram City

The attractants so far used in ovitrap generally have a foul smell, such as soaking straw, soaking herbs, soaking shrimp shells because they produce ammonia, lactic acid and CO_2 which can attract mosquitoes to lay their eggs. Because it smells bad, people are reluctant to install ovitrap inside the house which is the habitat of the *Aedes aegypti mosquito*. The results of Santos et al's research, 2010 Cashew leaf infusion is able to become an attractant without a bad smell. Mosquito attractant *Ae. aegypti* to lay eggs requires appropriate larvicide so that it can kill mosquito larvae that will hatch from mosquito eggs in ovitrap. Larvicides added to ovitrap are

generally chemicals that can endanger the health of humans and non-target animals. Research results by Aisyah et al. (2009) the ethanol extract of rambutan leaves can be used as a biological/organic larvicide, as well as aqueous extract of Srikaya seeds (Satoto et al., 2015). Therefore, in this study, local organic larvicides were used, namely the ethanol extract of Rambutan leaves and water extract of Srikaya seeds, the ingredients of which are commonly found in the city of Mataram, as well as *Bacillus thuringiensis var israelensis (bti)* which has been used in the P2P program for Health Services in the Province of West Nusa Tenggara.

The effectiveness of the lethal ovitrap attractant combination in this study was based on the number of *Ae. aegypti* trapped, the number of mosquito eggs that hatch into larvae and the number of dead larvae. The results of research in the laboratory, the effectiveness of the lethal ovitrap attractant combination only found trapped mosquito eggs because no eggs hatched into larvae and no larvae died after seven days of observation according to the mosquito life cycle. The analysis of the effectiveness of the lethal ovitrap attractant combination is done by comparing the average number of trapped mosquito eggs.

The mean number of mosquito eggs trapped in each combination of lethal ovitrap attractants which were significantly different were combinations of cashew leaf infusion and rambutan leaf ethanol extract and rambutan leaf infusion and rambutan leaf ethanol extract. The results of the study show that the chemical content in mango leaves, rambutan leaves and cashew leaves that attract female mosquitoes to lay eggs is tannins. Meanwhile, saponins which act as larvicides in rambutan leaves destroy red blood cells through a hemolysis reaction, are toxic to cold-blooded animals including insects so that mosquito eggs do not hatch or die immediately upon hatching (Asiah et al., 2009).

The number of mosquito eggs in the *lethal ovitrap combination* of Srikaya seed water extract with infusion of mango leaves, rambutan leaves and cashew leaves compared to the ethanol extract of rambutan leaves from the results of the study had no effect. Srikaya seed extract contains two compounds of the annonain group consisting of annonain and squamosin which act as larvicides (Al Kazman et al., 2022; George et al., 2005; Nenotek et al., 2022). The results of research by Salim et al. (2015) ovitrap with straw soaking attractant and larvicide water extract of Srikaya seeds can attract *Ae. aegypti* lays eggs, but has no effect on egg hatchability.

The combination *of lethal ovitrap attractants infused* with mango leaves, rambutan leaves and cashew leaves with organic larvicide *Bacillus thuringiensis var israelensis (bti)* had the least effect on the number of trapped eggs. *Bti* has been used as a larvicide in malaria control, but is now being produced as a dengue vector larvicide and is already being used in disease control programs. The results of research on grass infusion with *Bti* affected the number of trapped eggs proportional to the concentration of the infusion and was effective in killing larvae for 15 days (Santos et al., 2003).

The field test in the Kamasan Environment, Monjok Village, used two combinations of *lethal ovitrap* effective attractants in the laboratory, namely cashew leaf infusion and rambutan leaf ethanol extract as well as rambutan leaf infusion and rambutan leaf ethanol extract. The field test results of the combination of the two lethal ovitraps did not differ much, on the other hand the percentage of trapped mosquitoes was higher in the combination of rambutan leaf infusion and rambutan leaf ethanol extract.

There are many cashew and rambutan plants in Indonesia, especially in the city of Mataram, so the potential for developing this infusion is very wide open. The way to make it is easy, people can apply it for their own home needs, namely by soaking dry leaves in *an aerobic state* for seven days. Until 2017, the larvae-free rate in Mataram City was 90%, so it is still a DHF endemic area. Likewise, the results of community service examination of larvae in the Monjok Sub-district were 70% indicating that the distribution and density of the DHF vector, namely *Ae. aegypti* has spread and does not meet WHO standards ≥ 95%.

Efforts to control *Ae. aegypti* with a combination of *lethal* ovitrap attractant infused with rambutan leaves and cashew leaves and larvicide made from local organic ingredients, namely rambutan leaf extract, can be used as an alternative for biological control. Vector control using natural materials is widely available in the city of Mataram to support the Integrated Vector Control (PVT) program which is safe, rational, effective and sustainable (Permenkes RI, 2010; Cameron et al., 2013)

Conclusion

Cashew leaf infusion and rambutan leaf infusion are the most effective attractants in attracting mosquitoes to lay eggs compared to mango leaf infusion. The most effective organic larvicidal rambutan leaf ethanol extract is combined with cashew leaf infusion attractant and rambutan leaf infusion to attract mosquitoes to keep laying eggs and the eggs do not hatch into larvae compared to aqueous extracts of Srikaya seeds and *Bacillus thuringiensis var israelensis*.

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