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OVITRAP MODIFICATION IN IMPROVING THE ABILITY OF AEDES SP. EGG TRAPPING IN BANJARBARU CITY

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Abstract

Introduction: *Dengue Hemorrhagic Fever (DHF) is a vector-borne disease that spreads through the bite of Aedes aegypti and Aedes albopictus. Until recently, vector control still becomes an essential step in breaking the dengue transmission chain. Besides of imago or larvicide method, the ovitrap way is also often used to trap the eggs of Aedes sp. This study aims to determine the trapping ability of modified ovitrap with various container colors and shrimp-paste attractant concentration levels.* ***Method:*** *This study was an experimental study using a post-test only design. This Research's object was Aedes sp eggs trapped in modified ovitrap at 20 research locations in Banjarbaru City. The data obtained were analyzed statistically using the Kruskal Wallis test. The Research used The Mann-Whitney test to perform a post-hoc analysis.* ***Result and Discussion****: Results have shown that differences in attractant concentration and color of ovitrap have a significant effect in attracting Aedes sp to lay eggs. Zero percent concentration (without attractants) has a substantial difference in trapping Aedes sp with 10, 20, and 30% concentrations. Colorless ovitrap is also significantly different from black and green ones in trapping Aedes sp eggs.* ***Conclusion:*** *The use of attractants with a 10% shrimp-paste concentration solution and a black ovitrap can be an alternative to control DHF vectors affordable and safer for the environment and humans.*

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) has become the primary vector-based infectious disease. The disease transmitted through the bites of the mosquitoes Aedes aegypti and Aedes albopictus has reportedly become a health problem for Indonesia's people for the past 45 years. Indonesia is the second country with the largest DHF cases among 30 endemic countries (1). In 2018, the Incidence Rate was reported to still reach 51.53 per 100,000 population with a death rate of 919 people (2). South Kalimantan Province is one of the provinces in Indonesia that is classified as prone to dengue cases, with an Incidence Rate (IR) reaching 56.10 per 100,000 thousand population and a Case Fatality Rate (CFR) of 0.63% (2). The highest cases occurred in Banjarbaru City and Balangan Regency. In Banjarbaru City itself, in 2019, the IR value was recorded at 55.95 per 100,000 population, far above the national standard (44 per 100,000 thousand population) with a Case Fatality Rate (CFR) of 2.1% (3).

The process of handling DHF in the City of Banjarbaru itself still depends on controlling the dengue vector. DHF vector control is the top way to do it as an effort to prevent and control DHF. The effort aims to break the dengue transmission chain because dengue antiviral drugs and vaccines have not been found to treat dengue (4-5). Dengue vector prevention and control efforts cannot rely on the use of insecticides such as temephos alone, considering that studies have shown a tendency for the emergence of vector resistance to temephos (6). Thus, efforts to prevent and control DHF must be combined with more environmentally oriented methods. During the dengue virus decreased activity period, systematic virus source eradication can be carried out by giving larvicide powder in a puddle of water that cannot be removed, covered, or stockpiled.

Efforts to suppress the dengue virus epidemic and prevent Extraordinary Events (KLB) emergency control measures to eradicate the Aedes aegypti massive and systematic population must be carried out. Control efforts are through insecticides and eliminating mosquito sources and habitats using variously integrated and integrated techniques (5). Several alternative methods have been carried out to control the dengue vector. The methods include community participation involvement, medicinal plant usage (7), climate information utilization (8), sterilizing male mosquitoes, spreading Toxorhyncites sp mosquito larvae, which act as predators of Aedes sp larvae (4,9). However, the results were less effective in reducing the dengue vector (10) until the Internet of Thing (11).

Another alternative technique is the mosquito trapping method, also known as ovitrap, which are simple tools used to detect the presence of Aedes aegypti and Aedes albopictus (10). The existence of ovitrap does not only function as a mosquito trap. However, it can also act as vector surveillance, such as control using the autocidal egg trap method (egg killer trap) (10,12). This method will yield maximum results if the potential number of larval habitat decreases, places multiple autocidal traps, or a combination of the two activities is carried out simultaneously. In various conditions, this method is believed to be more economical and capable of being a fast way to reduce female mosquitoes' natural density and act as a disturbance monitoring tool in a location. However, this method's application's success or failure is mostly determined by the number of tools installed and the tools attractiveness for female Aedes aegypti mosquitoes to make them lay their eggs (13).

The increase in productivity, various ovitrap modifications are carried out with the help of attractants, which can influence mosquitoes' behavior in choosing the place to lay their eggs. Previous studies have shown differences in the number of mosquito egg catches between ovitrap using straw soaking water and ovitrap using plain water. The number of eggs trapped was more significant in ovitrap with straw soaked water than in plain water (14). However, the straw water immersion duration did not significantly affect the number of eggs captured (15).

Ovitrap can reduce larvae density, which impacts the effectiveness of dengue vector control (16). The essential elements in ovitrap are containers and attractants. Various containers such as plastic bottles, plastic cups, coconut shells, and bamboo have been tested for their effectiveness (17). As for attractant materials, generally use the straw soaking water (18), tape yeast water to shrimp immersion water (19). In this study, modifications were made to the materials and the attractant's concentration using shrimp-paste soaking water. Shrimp-paste is used with the consideration that it is easy to get the raw material freely. Also, South Kalimantan Province is a center for marine processing with one leading shrimp-paste (20).

This research is an experimental study with a randomized complete block design. The research was conducted in people's homes located in 20 sub-districts in the city of Banjarbaru. The selection of resident's houses was carried out by purposive sampling by taking one resident's house in each village to obtain a total sample of 20 houses. The ovitrap is placed both inside and outside the house in a place that is preferred by mosquitoes. Inside the house, the ovitrap was placed in a location close to a water reservoir. The outdoor ovitrap was placed in a shady plant area. In this study, researchers used a shrimp-paste solution to attract three types of concentration levels, namely 0, 10, 20, and 30%.

Meanwhile, for ovitrap, it uses a plastic material to modify two color variations, which is black and green. Colorless (transparent) ovitrap was used as a control. Researchers manipulated four variations of attractant concentration and three variations of ovitrap color with three repetitions so that a total sample of 720 samples was obtained. The eggs of Aedes sp mosquito caught from each ovitrap were counted using a counter and microscope or loop every day. That measure was conducted to make it easier to calculate the number of eggs caught. The data were processed and analyzed using the Kruskal Wallis statistical test, carried out by the Mann-Whitney test, to perform Post-Hoc analysis..

METHOD

The research was conducted using an experimental study with a randomized complete block design. The research was conducted in people's homes located in 20 sub-districts in the city of Banjarbaru. The selection of resident's houses was carried out by purposive sampling by taking one resident's house in each village to obtain a total sample of 20 houses. The ovitrap is placed both inside and outside the house in a place that is preferred by mosquitoes. Inside the house, the ovitrap was placed in a location close to a water reservoir. The outdoor ovitrap was placed based on the criteria for a place close to plants and shade. In this study, researchers used a shrimp-paste solution as an attractant with three types of concentration levels, namely 0, 10, 20, and 30%.

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RESULT

The Number of *Aedes sp.* Eggs Trapped

The experiment was carried out three times, and the overall average of eggs of *Aedes sp* trapped in all types of ovitrap was 4,858 eggs (see Table 1). Ovitrap with 10% attractant concentration was the most trapping concentration of mosquito eggs with 1,511 eggs with an average of 8.73 eggs and a maximum number of trapped eggs as many as 97 eggs. Ovitrap without attractant received the fewest eggs, 688 eggs, with an average of 6 eggs and a maximum number of catches of 52 eggs. Overall, the average number of mosquito eggs that were most trapped was on the ovitrap with black color, namely 2,253 eggs; the average was 12 eggs, and the maximum number of catches reached 108 eggs. Ovitrap without color (transparent) became the ovitrap that trapped the least amount of mosquito eggs, with only 1,069 eggs caught with an average of 6 eggs, and the maximum number of eggs trapped was only 44 eggs. In the ovitrap, which functioned as a control (without attractants and color), the total number of eggs trapped was 243, with an average of 6 eggs.

Table 1. The Number of Aedes sp. Eggs Caught Ovitrap in the 1st to 3rd Week Observation

Concentration of Attractants	Ovitrap Color	Min	Max	Average	Total	Std Deviation
10%	Green	0	97	8.28	497	12.74
	Black	1	54	10.15	548	12.32
	Colorless	0	44	7.90	466	8.72
Total		**0**	**97**	**8.73**	**1511**	**11.36**
20%	Green	1	55	8.93	402	10.40
	Black	0	108	15.77	820	22.21
	Colorless	0	13	4.89	186	3.78
Total		**0**	**108**	**10.43**	**1408**	**15.74**
30%	Green	0	110	13.21	515	21.91
	Black	0	86	12.49	562	14.72
	Colorless	0	17	4.70	174	3.64
Total		**0**	**110**	**10.34**	**1251**	**15.80**
Without Aktratan	Green	1	13	3.81	122	2.52
	Black	0	52	8.73	323	12.89
	Colorless	0	19	5.93	243	4.96
Total		**0**	**52**	**6.25**	**688**	**8.34**
Total	Green	0	110	8.73	1536	14.01
	Black	0	108	11.98	2253	16.36
	Colorless	0	44	6.11	1069	6.21
Grand Total		**0**	**110**	**9.01**	**4858**	**13.24**

Effect of Attractant Concentration and Ovitrap Colors on *Aedes sp.* Trapped Eggs

Based on the results of non-parametric statistical analysis using the *Kruskal-Wallis* test, with a significance level of 0.05 (p = 0.05) and a confidence level of 95%, the results obtained were p = 0.01 (p <0.05). This value (p) indicates that the attractant concentration has a significant effect on the ability of the ovitrap to attract *Aedes sp.* mosquitoes to lay eggs in the ovitrap (see Table 2). Whereas in Table 3, the p-value is less than 0.05, which means that there is a significant effect between the color of the ovitrap and the ability of ovitrap to trap the eggs of the mosquito *Aedes sp*. The difference in the number of trapped *Aedes sp.* eggs between the attractant concentration and ovitrap colors was based on the Post-Hoc analysis, as listed in Tables 4 and 5.

Table 2. Attractants Concentration Effect on Ovitrap Trapping Power

Concentration	Ovitrap Abundance With Eggs	Mean Rank	P
0%	106	219.58	0.010
10%	167	268.31	
20%	132	280.66	
30%	119	272.43	
Total	**524**		

Table 3. Effect of Color Usage on Ovitrap Trapping Power

Ovitrap Color	Ovitrap Abundance With Eggs	*Mean Rank*	*P*
Colorless	167	234.08	0.001
Green	177	255.62	
Black	180	295.64	
Total	**524**		

Table 4 shows that the difference in attractant concentrations in ovitrap significantly contributed to the ability of ovitraps to trap *Aedes sp.* eggs. Further tests using *Mann-Whitney* showed that the concentration of 0% (without attractant) was significantly different from the concentrations of 10, 20, and 30% in trapping *Aedes sp.* eggs. Meanwhile, 10% concentrations with 20 and 30% were not significantly different in trapping *Aedes sp.* eggs.

Table 4. Significance Test between Each Active Concentration in Ovitrap

Concentration	Concentration	P
0%	10%	0.008
	20%	0.002
	30%	0.011
10%	0%	0.008
	20%	0.481
	30%	0.796
20%	0%	0.002
	10%	0.481
	30%	0.687
30%	0%	0.011
	10%	0.796
	20%	0.687

Significance level 0, 05

Table 5. Significance Test between Ovitrap Colors

Color	Color	P
Colorless	Black	0,000
	Green	0.179
Black	Colorless	0,000
	Green	0.012
Green	Colorless	0.179
	Black	0.012

Significance level 0, 05

Table 5 shows a significance p-value = 0.001, which means that differences in the use of ovitrap colors can significantly affect the ability of ovitraps to trap *Aedes sp.* eggs. Further tests using *Mann-Whitney* showed that the colorless ovitrap (control) was significantly different from the black ovitrap and green ovitrap in trapping *Aedes sp* eggs. In contrast, the black and green on the ovitrap did not show a significant difference in the ovitrap ability to trap *Aedes sp.* eggs.

DISCUSSION

The Trend of Eggs Trapped in Ovitrap

The temperature at the time of the study was relatively high or hot due to dry season conditions, which resulted in higher air temperatures. Temperature affects humidity, and if the temperature is high, it will cause low humidity, which can be a contributing factor to mosquito breeding (21). The suitable range for mosquito breeding occurs in the temperature range between 20 and 30˚C (22), with optimal humidity for mosquito breeding in the range of 66 to 83% (21).

In this study, plastic ovitrap was used because different research results indicated that plastic is the most common container material used by the community and has high potential as a place for mosquito breeding (23-24). The slightly rough plastic surface allows mosquitoes to be well-positioned to lay eggs. Many factors support female mosquitoes in laying their eggs, including color, shape, size, type, water quality, and food availability (25).

The most number of trapped *Aedes sp.* eggs occurred in the first week of observation and continuously decreased until the third week. This phenomenon occurs because there are at least two main indications. First, the *Aedes sp*. population at the research location decreases because the mosquito regeneration process is disrupted due to ovitrap usage. The *Aedes sp* existing population cannot continue the regeneration process optimally because of the egg trap (ovitrap). The presence of ovitrap itself can significantly withstand the hatching rate of mosquito eggs (26).

Second, observations are made when the dry season arrived so that naturally, the *Aedes sp.* population decreases along with the decrease in breeding places. The decrease in rainfall and its frequency also reduces the number of natural and artificial clean water reservoirs scattered around the settlements. This condition can be considered a natural mosquito population control process (27) because the higher the temperature and the lower the humidity, the lower the oviposition level (28).

The mean values of air and room temperature at the study location ranged from 26.4 to 35.5˚C, with a mean temperature of 32.5˚C. Air humidity ranges from 51-82%, and the average is 63.7%. This temperature range provides ideal conditions for mosquito breeding. In general, mosquitoes will lay their eggs at temperatures around 20 to 30˚C (22). Optimal humidity for the reproduction and development of mosquitoes alone ranges from 70 to 89.5% (29). Specifically, at a temperature of 35˚C and relative humidity of 60%, mosquitoes' oviposition level will decrease to an average of 54.53 ± 4.81 eggs. Whereas at a temperature of 25˚C and relative humidity of 80%, mosquitoes' potential to breed reaches an average of 99.08 ± 3.56 eggs (22).

In a temperature range of 25-30˚C, accompanied by inundation points, it is believed to encourage an increase in the sufficient reproductive number from the DHF vector to 10 days. This condition will then lead to an outbreak (30). The *Aedes sp*. itself adapts to the mechanism quite rapidly to environmental changes. In the face of unfavorable environmental conditions (early dry season) and limited breeding places, gravid female mosquitoes will try to find a place to lay eggs until the mosquitoes find ovitrap in residential areas (31).

Attractant Concentration and Ovitrap Trapping Ability

Attractants are materials that attract insects, both chemically and visually (32). Attractants can be obtained from various natural materials such as fruit and vegetable waste, corn stalks, to rice stalks (33). While attractants derived from chemicals generally come from CO_2, ammonia compounds, lactic acid, octanol, and fatty acids (34). These substances or compounds are derived from organic materials and the results of the metabolic processes of living things, including humans. CO_2, lactic acid, and octanol are good attractants for mosquitoes. This attraction is because the aroma of fatty acids produced can be detected effectively up to a distance of 7 to 30 meters; even in some conditions, it can reach 60 meters (35).

The addition of the shrimp-paste immersed water attractant is indicated to have increased the attractiveness of the ovitrap to the *Aedes sp.* to lay their eggs in it. Although there was a fluctuation in the mean of trapped Aedes sp mosquito larvae, the highest average always occurred in ovitrap with a 10% concentration of shrimp-paste immersion water. It happens because the shrimp-paste soaking water contains residual protein and chemical compounds derived from shrimp, both in gas and liquid form, which is preferred by *Aedes sp*. The content of shrimp-paste contains ammonia, which is produced from fermentation. This condition causes the sharp aroma of shrimp-paste and stimulates the detection senses.

The content of CO_2, octanol, and lactic acid itself is an excellent attractant for mosquitoes. The aroma of fatty acids produced from normal skin flora will be effectively detected at 7 to 30 meters distances and reach 60 meters for some species (36-37). *Ammonia* compounds are the most preferred attractants for *Aedes sp.* (38). The shrimp-paste immersion solution itself contains relatively high ammonia levels to attract and influence female mosquitoes to choose a place to lay their eggs. These compounds are produced from the fermentation process of organic substances or are the excretion of metabolic processes (39). Another possibility is that there are compounds, substances, or other attractive materials in the shrimp-paste bathwater that are not found in ordinary water content. These findings are in line with studies that state that ovitrap with CO_2 solution contained in shrimp-paste can attract and trap *Aedes aegypti* compared to traps with direct water straw solution (40).

Ovitrap Color Modification and Ovitrap Trap Ability

Aedes sp. is equipped with two organs, namely chemoreceptors and mechanoreceptors. These two organs can detect the place or point of laying eggs, food sources, identification of mosquitoes, distinguishing enemies (predators), and finding the opposite sex. The mosquito's compound eye (ommatidium) also has photoreceptor organs that can distinguish colors (41). From various studies, it is known that the female mosquito *Aedes sp.* prefers dark objects to light ones to rest or lay eggs (oviposition) (42). Bright or clear colors can make light sources, both from artificial light and sunlight, penetrate and illuminate the container's contents. These conditions affect the response of the mosquito photoreceptor organs (43).

The observation area's condition is at an altitude of 0-500 meters above sea level (asl) and has an outdoor environment covered with shrubs and tree vegetation. These conditions are ideal conditions for the *Aedes sp.* (*albopictus*) mosquito to rest and wait for their eggs' maturation process. Meanwhile, the observation house conditions were detected by many dark and humid spots, making it an ideal and safe place for *Aedes sp.* to lay their eggs.

It was then confirmed by the success of the black ovitrap in trapping the most mosquito eggs (2,253 eggs) while the colorless ovitrap trapped the fewest mosquito eggs (1,069 eggs). At the observation time, the air temperature ranged from 26.4–35.5 ˚C with humidity ranging from 51–82%. Humidity alone, together with temperature and rainfall, has become a factor influencing the achievement of a 100% ovitrap index, especially in lowlands (44). The *Kruskall-Wallis* test showed that differences in ovitrap colors significantly affected trapping Aedes sp mosquitoes to lay eggs in the ovitrap. Further tests using *Mann-Whitney* showed that colorless ovitrap (control) had a significantly different ability to trap *Aedes sp.* eggs than black or green ovitrap. Meanwhile, the use of black and green on the ovitrap, both of them did not show a significant difference in trapping *Aedes sp.*

This finding is in line with studies in Bengkulu and Sri Lanka, which showed that female *Aedes aegypti* mosquitoes were more likely to lay their eggs in dark containers (45-46). It is also in line with research that shows a tendency for mosquitoes to prefer laying eggs in black containers compared to other colors (41). Likewise, research conducted in the Banyumas region shows that *Aedes albopictus* prefer black plastic bottles or cups to other materials (23).

Several studies have shown that dark containers hold more mosquito eggs, which is inversely related to light-colored containers (47). Dark colors can provide a sense of security and calm for *Aedes sp.* when laying their eggs so that more eggs are placed in water reservoirs (48). It is reinforced by the research results, which states that bright colors and light exposure can reduce larvae's density and become *Aedes aegypti* mosquito repellents (49).

In contrast to research conducted in Banten, it is stated that there is no relationship between the presence of mosquito larvae and the container colors (50). This finding contradicts the results of this study. This difference is thought to be due to differences in the study area's treatment and geographic conditions, where mosquito eggs will be found more in highland than in lowland locations (51).

The method of controlling mosquito populations using the ovitrap method itself is quite effective in Malaysia and Puerto Rico with *polyacrylamide* (PAM). This PAM method is also known as the plant gel method, which functions as an ovitrap substrate (50,52). Carpet shell molluscicide rinses (*Paphia undulata*) and giant tiger prawns (*Penaeus monodon*) have also shown that the *Aedes albopictus* prefers them over other attractants in terms of egg-laying. With the attractants and larvicides combined, the mosquito's control effort in a sustainable manner will be well-synergized (53).

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CONCLUSION

The difference in the concentration of ovitrap attractant had a significant effect on the ability of the ovitrap to trap *Aedes sp.* eggs, where the attractant with a concentration level of 10% shrimp-paste solution was able to attract *Aedes sp.* mosquitoes to lay more eggs in the ovitrap. Meanwhile, from the ovitrap color, black was the color that trapped *Aedes sp.* eggs the most. The use of attractants from soaking shrimp-paste water with a 10% concentration and combined with black ovitrap can be a choice to control the dengue hemorrhagic fever vector is affordable, safe for the environment and humans, and is sustainable.

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