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The Potential Test of the Mosquito Oviposition Preference Using Similar Subtracts: Colonized Water and Aides Larvae Extract

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Abstract

BACKGROUND: The research on ovitrap using attractants has been extensively developed, but studies on the use of similar substrates in the form of colonized water and Aedes larvae extract as attractants have not been widely published. Adding an attractant to the ovitrap can stimulate the sense of smell for mosquitoes to come to the place to lay their eggs. The use of ovitrap has recently begun to be developed because it is environmentally friendly.

AIM: The purpose of this study is to determine the attractants potential of colonized water and larvae extract as the oviposition preferences for Aedes Sp. to lay eggs in the ovitrap.

METHODS: The type of this research is a true experimental design, the design of the posttest-only control group design. Observations are made in the laboratory for nine repetitions.

RESULTS: Observational data show that the average number of eggs in ovitraps that uses the colonized water and larvae extract is higher than that of conventional/control water. The results of the Kruskal–Wallis test indicate that there is a significant difference in the average number of eggs in the colonized water attractant, larval extract, and conventional water (Asymp. Sig < 0.05).

CONCLUSION: The conclusion is obtained that similar substrate attractants in the form of larvae extract and Aedes colonization water have the potential to be Aedes sp oviposition preferences compared to conventional water. However, it is still necessary to conduct a field study so that it can be used as an environmentally friendly method of the surveillance and control of the vector transmitting dengue hemorrhagic fever.

Introduction

Dengue hemorrhagic fever (DHF) is still a major public health problem in all tropical and subtropical regions of the world [1]. Various efforts to control the dengue vector have been carried out, such as controlling adult mosquitoes (fogging) and larvicides. On the other hand, the use of these insecticides, however, has a potential to cause disturbance to the vector. Continuous use of insecticides can lead to vector resistance [2], [3], [4], in addition to having unwanted effects on natural enemies and non-target organisms as well as causing environmental damage [5], [6], [7].

The World Health Organization (WHO) has urged vector control programs to find and implement more environmentally friendly strategies, namely integrated, sustainable, and cost-effective vector management [8].

One of the environmentally friendly and cost-effective control efforts is the use of mosquito attractants (attractants) that are inserted into a trap called an ovitrap. The attractant materials that have been widely researched/used so far include chemicals [9], semiochemicals [10], [11], [12], as well as organic substances in the form of flowers and fruits [13] as well as water hyacinth infusion [14]. However, not many have used attractants from similar basic materials (similar substrates) in the form of colonized water and larvae extract.

Therefore, this study aims to examine the potential of similar materials to be used as attractants by combining locally available traps to attract mosquitoes in an effort to control the vector transmitting dengue hemorrhagic fever.

Materials and Methods

This research is a true experimental design using the posttest-only control group design. Repetitions were performed nine times as per Federer guidelines [15]. This research began in January 2020 and concluded in September in the same year.

Mosquito colony

The Aedes spp. used in this study was the result of colonization of Aedes larvae collected from the fields of Makassar City (S5009'24.6" E119025'35.0") and the Regency of Pangkajene and Islands (S4055'44.26104" E119041'24,86796") in 2020 by the author. Aedes larvae collected from the field were then reared to produce approximately 1000 Aedes eggs of F0 generation. Rearing was conducted in the insectarium of the Entomology Laboratory, Parasitology Section, Department of Medical Sciences, Hasanuddin University, Makassar, Indonesia.

Attractants for ovipositions

The colonization attractant (x1) was taken from the water from the colonization of Aedes spp. Aedes colonization was carried out in 3 black cast buckets containing 6 L of clean water each from the same source. Then in each bucket, 450−500 eggs of Aedes spp. are incubated. This method is a development carried out by Thavara, 2004 [16].

The larvae from the colonization were then used as an attractant for larval extract (×2). The attractant ×2 was created by taking 150 larvae instar 3−4, which was blended with 200 ml of water. Subsequently, the blend was mixed into a bucket containing 5800 ml of clean water. The extract attractant was used as much as three buckets in each repetition.

Laboratory bioassays

The oviposition test used 250–300 adult Aedes spp. for each repetition. When the mosquitoes have entered the age of 3–4 days, a blood feed process is carried out, then they are released in an insectarium measuring 3.20 × 2.60 × 2.0 m. In the intectarium, three groups of attractant ovitraps have been installed. Each group consists of X0 (conventional), 1 (colonization), and X2 (extract).

On the inside of each treated ovitrap container, white filter paper of the Whatman brand was installed with a length of 7 cm and a width of 6 cm as a place to place mosquito eggs based on the method of Lampman and Novak [17] as well as Allan and Kline [18]. A piece of cotton wool soaked in 10% sugar syrup was placed in a cage to feed adult mosquitoes.

After four days of oviposition, all oviposition containers were removed from the cage, and strips were removed and dried at room temperature (25–29°C). The number of eggs deposited in each strip was then counted using a stereomic microscope.

Statistical analysis

The average of Aedes spp. eggs obtained from each observation was carried out by a different test. Different tests using ANOVA test or Kruskal–Wallis test. A normality test was conducted before the difference test. SPPS for windows version 24.00 was used to analyze the data.

Results

Based on the results of observations on day 4 after the release of gravid mosquitoes in the insectraium, the average number of eggs on the filter paper that stuck to the ovitrap varied. Calculations on the fourth day were carried out to avoid disturbances in the oviposition process. At the time of egg counting, no larvae were found in each ovitrap group, either using ×1, ×2, or ×0 attractants.

Based on the results of the calculation, the total average of eggs in all repetitions was 312.62 (Table 1).

Table 1: The value of the number of *Aedes* spp. eggs in each experimental attractant

Attractant	N	Mean	Minimum	Maximum
Conventional	27	197.77	24	420
Colonization water	27	364.66	109	971
Larvae extract	27	375.40	65	658
Total	81	312.62	24	971

Table 1 shows that the observations of 9 times of replication, overall found the average number of eggs of Aedes spp. 312.62. The average number of eggs in ovitrap using colonized water attractant and larvae extract were 364.66 eggs and 375.40 Aedes eggs, respectively, while the control was only 197.77 eggs. Average eggs per repetition are shown in Table 2.

Table 2 shows that, based on observations from nine repetitions, more ovitraps used a similar substrate attractant (Colonized water and larvae extract) than conventional/control attractants. However, after the normality test was carried out, the air colonization variable showed a value of 0.009 below the value of Asyimp sig. 0.05, so that to find out the average difference, it was continued with the Kruskal–Wallis test.

Based on the results of the Kruskal–Wallis test, the Asymp value obtained was Sig. < 0.000. This result states that there is a significant difference in the average number of mosquito eggs in each attractant. The average number of eggs in each repetition was higher in the attractant of the same substrate compared to the controlled one (Table 2).

The much more detailed graph of the average number of eggs found at each stage of the experiment can be seen in the following Figure 1:

Table 2: Average Aedes Spp. eggs for each attractant in each replication experiment

Attractant	Average eggs on repetition									Mean	df	Sig*
	1	2	3	4	5	6	7	8	9			
Conventional	206,3	164,7	216,0	73,7	216,3	362,6	199,7	104,7	236,0	197,7	2	0.000
C. water	376,3	232,3	455,7	236,0	325,3	723,3	293,3	196,0	443,7	364,7		
L. extract	475,7	224,3	471,0	193,0	560,7	633,7	250,3	212,0	358,0	375,4		
Total	352,8	207,1	380,9	167,6	367,4	573,2	247,8	170,9	345,9	312,6		

*Kruskal–Wallis Test.

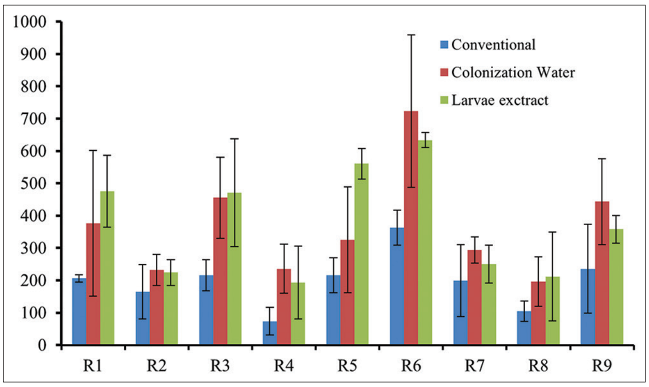

Figure 1: Graph of Aedes spp.'s eggs mean on conventional attractants, colonized water, and larvae extract for each repetition

Discussion

The selection of oviposition sites for various mosquito species is strongly influenced by visual cues and chemical stimuli. Chemical factors that affect oviposition are caused by the presence of chemosensory. Chemosensory can be olfactory and gustatory, as well as the influence of both [19]. Different chemical cues can cause different oviposition behavior. The oviposition preference behavior begins with an initial orientation toward a potential site and culminates in egg laying [14], [18]. The presence of the same larval species and other semiochemical influences on the site of oviposition indicate suitable conditions for their reproduction [20].

According to Mawardi, the interest of mosquitoes in choosing the sites of oviposition is also influenced by the content of substances/compounds in the water medium (Mawardi and Busra, 2019). In addition, the interest of mosquitoes in choosing a site to lay their eggs is also influenced by the color of the container (Meyer, 2006). Aedes mosquitoes prefer dark places to light places to rest. This is because mosquitoes are phototaxis negative [21], [22], [23]. Furthermore, water from natural breeding sites such as ponds (Qjullin *et al.*, 1965) and/or infusion of organic matter such as straw attracts gravid female mosquitoes for the oviposition in these sites [24].

The results of this laboratory research bioassay have shown the effect of chemical cues from a similar substrate attractant from the Aedes mosquito to oviposition as the Kruskal–Wallis Test has been carried out. The value of the attractant groping variable Asymp. Sig. obtained is 0.000. This effect is assumed to be due to the presence of residual Aedes mosquito enzymes in the colonized water attractant and larval extract used in the test media. Sutrisno mentioned the remaining enzymes as mosquito pheromones (Sutrisno, 2018). This enzyme is one of the substances that can stimulate the smell of mosquitoes, so the mosquitoes will approach and rest to lay their eggs around the media.

The advantage of this research is that it combines the use of attractants with bucket containers as ovitrap. The bucket container used is a local potential that is very easy to find in the community. The size and color of the container as the new generation ovitrap of Auto-cidal Gravid Ovitrap (AGO) [25], which is then recommended by the CDC based on the results of a review by Johnson *et al*., 2017 [26].

Laboratory bioassay results show that the average number of Aedes eggs fluctuates, and the value of each recipe is different, and sometimes, the average value is quite high. However, the average number of eggs in the attractant of similar substrates always shows a higher percentage than the control/conventional one (Graph 1). In the repetitions of R1, R3, R5, and R8, the average Aedes spp. eggs are higher in attractant extract larvae. Meanwhile, in the repetitions of R2, R4, and R7, the average number of eggs is more in the colonized water attractant. While the control for each repetition of the average egg is always below the amount of the colonized water attractant and larvae extract. The existence of an average that is not constant/fluctuating is due to the uncontrolled number of gravid mosquitoes released in the attractant bioassay test. There is a difference in the number of female gravid released in the insectarium at each repetition, and the range of the number of mosquitoes released is 250−270 including males and females. However, the number of gravid female Aedes mosquitoes is unknown. However, the average number of eggs in conventional/control attractants at each repetition is always less than with similar substrate attractants (colonization or extract). Based on the Kruskal–Wallis test, it shows a significant difference in the average eggs of the three attractants used.

Based on the information above, the advantage of this research is that it has combined the color of the container used as ovitrap, with an environmentally friendly attractant. The container used is local potential which is very easy to find in local communities. While the attractants used are easy to obtain.

The difference in the response of Aedes spp. oviposition to similar and conventional substrate attractants may be due to differences in volatile infusion (odor) released from the ovitrap container. The odor released by the colonized water attractant and larvae extract is more favored by Aedes spp. This is supported by the research of Wong *et al*., 2011, that the choice of mosquito nesting sites is influenced by the presence of similar larvae and pupae in the container [27].

In addition, many studies related to water attractant media that can attract mosquitoes to lay eggs on ovitraps that use attractants have been found, such as using polluted water attractants (Wurisastutui, 2013), proving the attraction of using water media mixed with cow dung. As for the clean water media (control), the Aedes mosquitoes lay the least number of eggs.

Furthermore, the influence that causes mosquitoes to be attracted to laying eggs is the presence of media using black buckets. It is known that one of the characteristics of mosquitoes is to like dark places to rest or lay eggs. This is in accordance with the research by Made Agus Nurjana, (2017), who has found that the color that mosquitoes like the most is black. Photoreceptor organs in compound eyes (ommatidium) can distinguish colors (Meyer, 2006), several studies have shown that Aedes mosquitoes, especially females, prefer dark-colored objects to light ones, both for resting and laying eggs.

In general, humid and undisturbed room conditions favor mosquito oviposition. When a site is approached, visual and smelling cues are used to evaluate the quality of the site for oviposition. The chemical ecology of mosquito oviposition is a source of additional information on various aspects of mosquito egg-laying behavior. It can take days, weeks, or even months for the gravid female mosquito to find a suitable oviposition site. Delayed oviposition can affect disease transmission by mosquitoes. From this explanation, it shows the extraordinary diversity of mosquito egg-laying behavior. Mosquitoes will ensure that the eggs are deposited into a microenvironment conducive to successful larval development and the emergence of the next generation of mosquitoes.

Based on the information stated above, the advantage of this research is that it has combined the color of the container used as ovitrap, with an environmentally friendly attractant. The container used is local potential which is very easy to find in local communities. While the attractants used are easy to obtain.

Any further research related to oviposition attractants is still very much needed, especially field research. Since vector control is oriented toward mosquito eggs, it is considered a long-term, cheap, and effective control.

Conclusion

Similar substrates (colonized water and larvae extract) have great potential as an environmentally friendly oviposition preference attractant. These findings suggest that similar substrates can be used as future attractants in responding to the challenge of mosquito resistance in disease vector control so far. However, field research is needed to strengthen this potential.

Ethical Clearance

Ethical approval has been obtained from the Ethical Commission of Health Research, Faculty of Public Health, Hasanuddin University, with protocol number 4432/UN4.14.1/TP.01.02/2021.

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