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Attractiveness test of attractants toward dengue virus vector (*Aedes aegypti*) into lethal mosquiTrap modifications (LMM)

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Abstract
Laboratory tests have been conducted to determine the ability of Lethal MosquiTRAP Modification (LMM). Modification is basically done to maximize the capture ability toward *Aedes aegypti* mosquitoes with the addition of black color, sticky material and attractants. Disposable plastic bottles were transformed to Lethal mosquiTRAP modification with the addition of attractant sources obtained from hay (*Oriza sativa*) and red chili (*Capsicum annum*) infusion and palm sugar (*Arenga pinnata*) fermentation. Aedes mosquitoes attracted towards different attractant sources are simultaneously killed directly in the trap. Response analysis of *Aedes aegypti* mosquitoes using different attractant sources was done. There is no significant difference ($p=0.984$) between different type of attractants, but, the attractant concentrations (10%, 30% and 60%) showed a significant difference ($p=0.00<0.05$) against number of mosquitoes caught in the trap. Statistical average of mosquitoes trapped showed that concentration of 30% was the best combination in the mosquiTRAP. Thus, expected from laboratory tests such mosquiTRAP can be installed in the environment as a dengue vector control measure.

Keywords: Lethal MosquiTRAP Modification, Attractants, *Aedes aegypti*

1. Introduction
Dengue fever (DF) has become an international health concern with worldwide cases reaching 50 million annually [1]. Margaret [2] estimated that approximately 50-100 million dengue infections occur each year, and almost half of the world population live in endemic countries. First cases of dengue fever in Indonesia were reported in 1968 of Surabaya and Jakarta city [3, 4]. The increasing cases of dengue fever are widespread in some rural and suburban areas, in addition to urban areas [5].
An alternative strategy to prevent dengue cases is to control spread of dengue vector [6]. The vector, female *Aedes aegypti* mosquitoes, transmit dengue virus from one human to the other, as horizontal transmission [7]. One of the dengue vector control methods, which is safe and getting a lot of attention in some countries, is using oviposition and adult mosquito traps [8, 9] with the development of a specific lure toward *Ae. aegypti* mosquito [10, 11, 12].
The Lethal MosquiTRAP Modifications (LMM) is a trap modified from potential disposable plastic that directly capture and kill *Ae. aegypti* mosquitoes. This trap is used to facilitate the identification and counting of mosquitoes trapped directly [13]. Modification traps made with the addition of three sources of attractants (hay infusion water, chili infusion water and sugar palm ferment), sticky surface and black cover of the trap were used in this study. Development of the LMM aims to generate traps with low cost, which are safe and able to control the spread of dengue virus vector.

2. Materials and Methods
Materials:
2.1 Lethal MosquiTRAP modifications (LMM)
MosquiTRAP consisted of a transparent plastic container with a height of 12 cm and a diameter of 9.5 cm. In the center of trap there is an attractant container that is loaded with 50 ml of attractant liquid. On the top of trap there is a hole through which the mosquito enter and respond to the smell of attractants. The overall outside of the trap is black. Double-tape is placed on the inside, lid and around the trap container as sticky surface to capture mosquitoes into the trap, as seen in Figure 1.

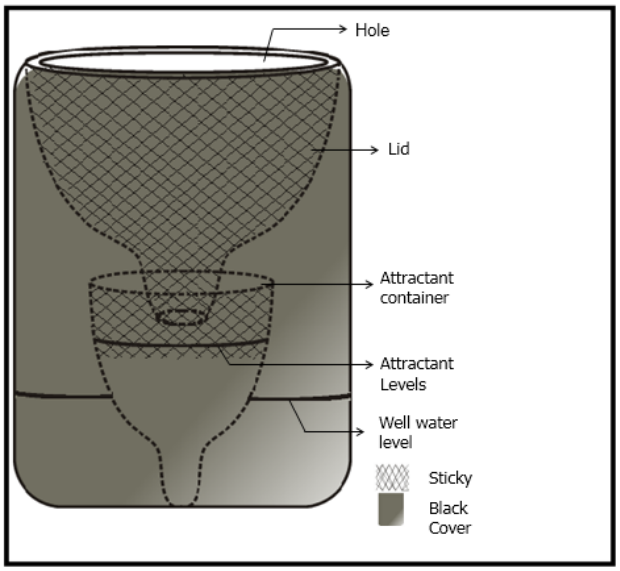

Fig 1: Lethal MosquiTRAP modification (LMM)

2.2 Attractant

Attractant sources (hay and red chili) [14] soaked for seven days and sugar palm (*Arenga pinnata*) fermented for two days were used. The attractants (hay and red chili) were obtained from 5 g of dry hay and 0.5 kg of fresh red chili that have been crushed, and then diluted with 500 ml of well water in different containers, whereas sugar palm attractant was obtained from the fresh sugar palm left for 2 days All the attractant were kept in a closed condition.

Three different concentrations, 10%, 30% and 60% of each attractant were placed in each trap with the aim to attract *Ae. aegypti* mosquitoes into the trap.

2.3 Mosquitoes

Eggs of Kepanjen, Malang East Java strain of *Ae. aegypti* (L.), which is sterile of dengue virus and have been maintained under conditions free from chemical contamination, were obtained from ITD (Institute of Tropical Diseases) laboratory of Surabaya. These eggs were allowed to hatch into larvae and then to develop to adult stage, which usually takes an average time approximately 12 days in the laboratory conditions (Animal Ecology and Diversity, Department of Biology, Brawijaya University of Malang).

Forty *Ae. aegypti* mosquitoes were placed in the release cage and left for 24 hours without treatment (acclimatization process). Male mosquitoes were allowed to feed honey with at a concentration of 10% and female mosquitoes were kept starved before treatment [15]. During the acclimatization process and treatment, temperature and humidity was maintained in a stable condition, so it can be assumed that only traps were responsible for the *Ae. aegypti* responses.

Methods: Effectiveness of laboratory tests was carried out in four cages, each cage provided with different type and concentration of attractant traps.

Mechanism of Lethal MosquiTRAP Modifications (LMM)

Forty mosquitoes were released in the release cage through acclimation (24 h), each cage placed a mosquiTRAP with different concentrations (10%, 30% and 60%) and control (no attractant). Each treatment was done under same condition with three replicates for each type of attractant.

Release cages were connected with four other trap cage which were loaded with LMM. Mosquitoes contained in the release cage will respond to the attractant derived from the LMM, the stimulus from the LMM approach mosquitoes entering each cage traps to be trapped in to the LMM. Identification of mosquitoes trapped was done by opening the top of the trap.

2.4. Data analysis

All results obtained from the experiments were presented as mean ± standard deviation. All statistical analyses were performed using SPSS version 17 statistical software for Windows. The data were analyzed using one way variance (ANOVA) and continued with Duncan test (α=0.05).

3. Results and Discussions

The number of mosquitoes trapped in the mosquiTRAP with control, 10%, 30% and 60% concentrations of hay and chili infusion and sugar palm ferment respectively are shown in Table 1. Increasing concentrations of each attractant increased the average no. of mosquito trapped inside mosquiTRAP. The observation was conducted on daily basis up to four days (96 hours) in laboratory conditions. Based on time, the total responses of mosquitoes toward attractant increased. However the pattern indicated a decrease in mosquito attraction on daily basis up to 96 hours of observation into the trap. Results of statistical analysis showed no significant effect of the type of attractant (P = 0.984> 0.05), but there is a significant effect of each concentration (*P <0.05*).

Table 1: Responses of *Ae. aegypti* mosquitoes toward in different types and concentrations of attractant

Attractant	Concentration	Time (H)				Mean mosquito responses	±SD
		24	48	72	96		
Hay infusion	Control	3.33	1.00	1.33	0.00	1.42[a]	±1.21
	10%	2.00	2.33	2.00	0.00	1.58 [a]	±0.92
	30%	2.67	4.00	3.33	0.33	2.58 [ab]	±1.38
	60%	6.33	6.00	4.67	0.67	4,42 [b]	±2.25
Red chili infusion	Control	1.67	3.00	0.67	1.00	1.59[a]	±1.03
	10%	2.00	2.33	2.00	0.33	1.67 [a]	±0.90
	30%	2.67	3.67	1.67	1.00	2.25 [ab]	±1.17
	60%	5.00	7.00	3.67	2.33	4.50 [b]	±1.99
Sugar palm ferment	Control	3.00	1.33	1.33	0.00	1.42[a]	±1.23
	10%	4.33	4.33	4.00	1.00	3.42 [ab]	±1.62
	30%	4,33	4,00	3.33	0.33	3.00 [ab]	±1.83
	60%	3.67	3.00	1.00	0.67	2.09 [ab]	±1.48

Mean in the same column with different letters were significant different at *P<0.05* level by one way ANOVA followed by Tukey's test

Different concentrations showed different effect on average number of mosquitoes trapped into mosquiTRAP. Treatment with the attractant concentration of 10% showed no significant difference between the control treatment ($p=1.000>0.05$), However, the concentration of 60% showed different effect to the control treatment ($p=0.042<0.05$) and the 10% concentration ($p=0.044<0.05$), but there was no significant difference in the effects of 30% with 60% concentration ($p=0.137>0.05$).

Ae. aegypti mosquitoes showed the same pattern with each attractant type. Table 1 shows that the average number of *Ae. aegypti* mosquitoes attracted in to the trap declined for four days which may be because the attractant continues to evaporate until a certain time limit so that given stimulus is reduced against *Ae. aegypti* receptor [17]. Research concerned with *Ae. aegypti* receptors [15, 16] have shown that, *Aedes* mosquito is capable of responding to several types of attractants such as lactic acid, ammonia, octenol and CO_2 already evidenced by the discovery of olfactory receptor neurons (ORNs) as the detection of chemical vapors caused by the attractant.

The statistics indicate, the overall type of attractant does not have a significant difference ($P= 0.984>0.05$), but there are significant different of overall attractant concentration ($P= .00 <0.05$). Control treatment had the same influence at a 10% concentration, while 30% and 60% having an effect different from the control treatment and 10%. Between 30% and 60% concentrations there is no different influence on the average mosquitoes trapped [18], it indicates that the attractant with a concentration of 30% has the ability to attraction mosquitoes are quite good, because the concentration is less than 60% already have the same capabilities and more optimal compared with 10% in attracting mosquitoes into the trap.

4. Conclusions

Based on the data analysis there is no significant difference in attractiveness effect of the three types of attractants. All types of attractants have the same ability to lure female *Ae. aegypti* mosquitos. Whereas attractant concentration showed a significant difference and the concentration of 30% has the most effective capability of every type of attractant to lure female *Ae. aegypti* mosquitos in modification mosquito traps.

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