COMPARING SHRIMP PASTE SOLUTION AND STRAW SOAKING WATER AS AN ATTRACTANT OF *AEDES* SP

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Abstract. The function of attractants plays a key role in future integrated vector management programs for mosquito control and supervision to reduce insecticide use. Tambakreja Village has the highest dengue cases in South Cilacap District, Cilacap Regency. This study aimed to determine which type of attractant was most effective in attracting mosquitoes to lay eggs in a field setting. The shrimp paste solution will be tested for its ability to attract mosquitoes by comparing its ability with that of straw soaking water (as a positive control) and groundwater (as a negative control). The study was conducted at 20 houses, selected by purposive sampling with inclusion criteria: no pets and the distance between houses ranged between 50 and 200 meters. The results showed there was difference in the number of eggs trapped based on the type of attractant (p=0.0001). The most effective attractants can be sorted as straw soaking water (18.23 eggs/ovitrap), shrimp paste solution (4.53 eggs/ovitrap), and groundwater (1.35 eggs/ovitrap). The vector mosquitoes that dominate in Tambakreja Village are *Aedes aegypti* (64.2%), *Ae. albopictus* (26.0%), and *Culex* sp (9.8%). Straw-soaking water attractants is still better in attracting *Aedes* sp. mosquitoes than shrimp paste solution.

Keywords: attractant, ovitrap, *Aedes* sp, shrimp paste

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INTRODUCTION

Dengue hemorrhagic fever (DHF) has become more common and is a global public health concern. The DHF poses a threat to 2.5-3 billion people worldwide and has the potential to spread throughout Indonesia (Wang *et al*, 2020). In 2019, the incidence of DHF in Indonesia was 51.53 per 100,000 people, higher than those in 2018 (24.75 per 100,000 populations) and in 2017 (26.1 per 100,000 populations) (MOH RI, 2020). Tambakreja Village has been a dengue fever endemic location since the previous decade, contributing to high dengue incidence in Cilacap Regency with an incidence rate (IR) of 150.4 per 100,000 inhabitants in 2020 (Cilacap District Health Office, 2021). The mosquito of the genus *Aedes* is a vector of DHF, transmitting the virus through biting (Ferreira-de-Lima and Lima-Camara, 2018). Insecticides are the most commonly used method to cut the transmission of dengue fever (Rather *et al*, 2017). However, long-term use of the insecticide may cause the mosquito resistance and may harm the environment (Msangi *et al*, 2020; Nkya *et al*, 2013). Alternatively, using an ovitrap has shown to decrease the mosquito density in several countries (Focks, 2003; James *et al*, 2022; Mackay *et al*, 2013). Previous research found that adding attractants to ovitraps improves mosquito control by reducing the density of mosquito as compared to ovitraps without attractants (Dwinata *et al*, 2015). To limit the future use of pesticides, adding these attractants to ovitraps is crucial to integrate vector management programs for mosquito control and surveillance.

Straw-soaking water is the attractant which is frequently used in previous studies. Soaked-straw releases ammonia, which causes odor and carbodioxide (CO_2) (Dwinata *et al*, 2015; Hidayati *et al*, 2017). Shrimp secretion is a natural components that can be used as attractants. The shrimp paste was chosen as the attractants because they are widely available, have CO_2-producing volatile components, and have a distinct aroma that attracts mosquitos (Gillies, 1980). These components have been tested in laboratory to attract mosquitos (Hasanah *et al*, 2017; Pratiwi *et al*, 2020; Thavara *et al*,

2004). While in the field, a 10.0% concentration of shrimp paste solution can catch a high number of mosquito eggs (Ambiya *et al*, 2020; Zubaidah *et al*, 2020).

Numerous studies have been conducted to control mosquito vectors using the ovitrap combining with various attractants. One of the ongoing studies is the utilization of natural compounds such as shrimp paste to eliminate *Aedes* sp. Natural attractants have no impact on animals, humans, or food sources (Geier *et al*, 1999). Therefore, the aim of this study was to compare shrimp paste solution with straw soaking water in order to see whether it could be more attractive to mosquitoes' egg laying in Tambakreja Village.

MATERIALS AND METHODS

Study design

This field trial evaluation was conducted to test the ability of various attractants in attracting female mosquitoes to lay eggs. The field experiment was conducted at Tambakreja Village, Cilacap Regency. The study was conducted in 5 consecutive days. The first step in determining the number of ovitraps was determining the number of houses where ovitraps were placed. The Federer formula (Ihwan *et al*, 2018, Federer, 1966) was used to determine number of houses:

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

where t = the number of attractant types (in this study, it was 2)
r = minimum number of repetitions of ovitrap installation for an attractant types

This study used two types of attractants (t = 2) which were straw soaking water and shrimp paste solution, while groundwater was used as a control. Based on the calculation results, the minimum number of repetitions of ovitrap installation (r) was 16; for convenience, we rounded it up to 20, which also showed the number of houses that the ovitrap occupied. These 20 houses were included to minimize errors.

The total number of ovitraps installed obtained from the following formula:

$$\text{Total number of ovitraps installed} = r\,(t + \text{number of control group}) \times 2$$

where r = minimum number of repetitions of ovitrap (in this study, it was 2)
t = the number of attractant types (in this study, it was 2)

As the number of control group is this study = 1 (groundwater), the result of the calculation was that the total number of ovitraps needed was 120

The 120 ovitraps was placed in 20 houses because one house was placed with two inside and outside the house. Installing ovitraps inside and outside the houses was to increase the potential for catching mosquitoes. Purposive sampling was used to choose the twenty houses following the inclusion criteria: the distance between houses ranged between 50-200 meters and there were no pets in the houses.

Attractants preparation

Shrimp paste solution (investigational substance), straw-soaking water (positive control), and groundwater (negative control) were prepared as attractants in this study. The shrimp paste attractant was made from 10 grams of shrimp paste which was dissolved in 10 ml of water, filtered, and

diluted up to 10 times to produce a 10% shrimp paste solution concentration. The shrimp paste solution was a brown with a very pungent odor. The straw soaking water attractant was made from 100 grams of dry rice straw soaked in 10 liters of water for 7 days. The straw soaking water is yellowish and transparent. The source of groudwater in this study was from wells of local residence.

Ovitrapping

Ovitrap was made from plastic bottles which were painted in black. The ovitrap was filled with each investigational substance and controls, then placed inside and outside the house where the mosquito was usually found, such as damp and dark place. The air temperature, humidity, and pH of water at the beginning and end of the observation were recorded. Ovitrap was taken 5 days after installation to prevent mosquito eggs from hatching into larvae. If there was a positive egg overtop, the egg attachment media was transferred to a labeled-container.

Larvae identification

The microscopy identification of instar II – instar IV mosquito larvae was carried out at the Cilacap Class II KKP (Kantor Kesehatan Pelabuhan/ Port Health Office) Laboratory based on the *Aedes* Mosquito Identification Key Book published by the Indonesian Ministry of Health in 2017 (MOH RI, 2017). The number of mosquitoes' larvae, *Aedes aegypti*, *Ae. albopictus*, and other species, were counted and recorded.

Statistical analysis

Numbers and species of eggs laid in each attractant was recorded. Ovitrap index (OI) was calculated from the number of ovitraps with

mosquito eggs divided by the total number of ovitraps used. Kruskal-Wallis test was used when the numbers of eggs captured in each attractant were not normally distributed.

RESULTS

The average number of eggs found in the straw soaking water, shrimp paste solution, groundwater, were 18.23 eggs, 4.53 eggs, and 1.35 eggs, respectively. The straw-soaking water attractant yielded the most mosquito eggs (Table 1). The normality test revealed that the data were not normally distributed, and the Kruskal-Wallis test revealed that the number of mosquito eggs observed varied depending on the type of attractant (p-value = 0.0001, Table 1).

OI is usually used to determine the density and tendency of mosquitoes to lay eggs inside or outside the house. Overall, as seen in Table 2 that the highest OI is observed in straw-soaking water attractants both inside the house (OI 32.5%) and outside the house (OI 30%). At the same time, OI of the shrimp paste solution attractant was 10.8% outside the house and 0.0% inside the house. In ovitrap filled with groundwater, the OI outside the house was 10%, and inside the house is 0.0%.

Table 1
Number of eggs in ovitraps

Type of attractant	Number of eggs		p-value
	Total	Average	
Straw soaking water	1,458	18.23	0.0001
Shrimp paste solution	362	4.53	
Groundwater	108	1.35	

Table 2
Ovitrap index calculation

Type of attractant	Ovitrap index (%)		Non-insect target* (%)
	Outside the house	Inside the house	
Straw soaking water	30.0	32.5	0.0
Shrimp paste solution	10.8	0.0	29.9
Groundwater	10.0	0.0	0.0

*Such as flies

These results showed that OIs in Tambakreja Village were at Levels 2 to 3, which were considered in the low range according to Food and Environmental Hygiene Department (FEHD, 2022). Moreover, the shrimp paste (29.9% of ovitraps) attracted non-target insects, including flies and ants.

In Table 3, larvae identification from hatched eggs revealed three species; *Aedes aegypti* (63.3%), *Aedes albopictus* (26.6%), and *Culex* sp (10,1%). The three species were identified in the straw-soaked water attractant; in the shrimp paste solution, only *Ae. Aegypti* was found; and in groundwater mosquito, larvae *Ae. aegypti* and *Ae. albopictus* were found.

DISCUSSION

This study was carried out in March, 2022 so it was hot and dry. During the study, the average temperature was 32-32.8 ^{o}C with the humidity of 63%-68.7%. This can affect the activity of laying mosquito eggs related to the effectiveness of the attractant (Zubaidah *et al*, 2020). This study found that the number of trapped mosquito eggs varied depending on the type of attractant (p=0.0001). The most effective attractant was straw-soaking

Table 3
Number of mosquitos' larvae identified in each attractant

Mosquito species and location	Number of mosquitos' larvae observed in different attractants			
	Straw soaking water	Shrimp paste solution	Groundwater	Total *n* (%)
Aedes aegypti	512	233	19	764 (63.3)
Inside the house	299	0	0	
Outside the house	213	233	19	
Ae. albopictus	262	0	59	321 (26.6)
Inside the house	18	0	0	
Outside the house	244	0	59	
Culex sp	122	0	0	122 (10.1)
Inside the house	0	0	0	
Outside the house	122	0	0	

water (positive control). Straw-soaking water was used as a positive control because it is well studied and considered a standard attractant (Iossi *et al*, 2018). Despite being standard, some studies demonstrated that straw-soaking water has insignificant effects on *Aedes* sp and *Culex* sp (Cahyati *et al*, 2019).

The result suggested that straw-soaking water more effectively attracted mosquitos than shrimp paste. However, it was not in line with previous studies (Hajimi *et al*, 2017; Sunu, 2017) by which is possibly caused by different parameters. The number of trapped eggs in ovitraps was likely to yield different results than the number of adult mosquitoes trapped in mosquito traps. High nutrition is one of the considerations in the use of shrimp paste solution attractants (Hajimi *et al*, 2017). There is a very strong correlation between the nutritional content in mosquito breeding places and the density of larvae (Dianti *et al*, 2019).

Straw-soaking water and shrimp paste solution, emit CO_2 and have a distinctive odor that can attract more mosquitoes. The odor, such as ammonia, radiates from the straw-soaking water and shrimp paste solution (Astuti and Nusa, 2011; Zubaidah *et al*, 2020). Naturally, CO_2 emissions guide mosquitoes to their prey; thus, the higher the CO_2 is, the more attractive it is to mosquitos (Soleh *et al*, 2018; Sari *et al*, 2017). The findings suggested that straw-soaking water was more appealing to the mosquitos than the shrimp paste solution. The shrimp paste solution contains ammonia and carbon dioxide, which affect the olfactory nerve of *Ae. aegypti* (Dianti *et al*, 2019). In this study, the organic matter content of each attractant was unknown. Ammonia levels of 0.720 mg/l indicate a contribution to the growth and development of *Ae. aegypti* larvae, ammonia levels of 120 mg/l are very attractive to female mosquitoes laying eggs, and ammonia levels of more than 300 mg/l can be a mosquito repellent (Dianti *et al*, 2019; Weinzierl *et al*, 2005)

The presence of non-target insects in the ovitraps, such as flies and ants, avoid mosquitos from laying eggs in the ovitraps (Mulatier *et al*, 2022; Oliva *et al*, 2021) and this is what was observed in the ovitraps containing shrimp paste solution in this study. In addition, the distinctive odor of these solutions also attracts non-target insects (Mullin *et al*, 2020). The high ammonia level in the shrimp paste solution compared to the straw-soaking water causes the odor to be unacceptable to mosquitos but acceptable to other insects (van Schoor *et al*, 2020). These findings contradicted with Zubaidah who claimed higher ammonia levels attract mosquitos (Zubaidah *et al*, 2020). Groundwater, selected as a negative control, has appropriate properties preferred by *Aedes* sp for laying eggs but lacks other compounds that attract mosquitoes.

The number of eggs captured in ovitraps outside the house was higher than in ovitraps inside the house. This finding followed Ramadhani and Trisnawati (2014) who reported that OI outside would be higher than inside for all treatment conditions. OI outside the houses in Tambakreja

is considered at the Level 2 or low according to FEHD (FEHD, 2022). The OI describes the density of adult mosquitoes that may be used as a vector monitoring tool for *Aedes* sp and knowing the existence of mosquitoes in an area acts as an indicator for the level of vulnerability of the area (Codeço *et al*, 2015). The result of this study suggested that the risk factor remained low in Tambakreja Village. A study by Ermayana *et al* (2015) stated that attractants from shrimp were more effectively used to reduce the density of larvae and eggs of *Aedes* sp outdoors in areas with high and low endemicity.

This study identified larvae of *Ae. aegypti* (64.2%), *Ae. albopictus* (26.0%), and *Culex* sp (9.8%). The findings were comparable with previous study conducted by Wahidah *et al* (2016) in Bulusan Village, Tembalang District, who reported a higher percentage of *Ae. aegypti* than *Ae. albopictus*. The reason why *Ae. aegypti* was more commonly found in these locations was because the conditions in these areas supported the life of *Ae. aegypti*, consistent with the study of Pramestuti and Djati (2013). Moreover, *Ae. aegypti* mosquito is more agile, and has a higher reproductive rate, higher growth rate, and a greater survival rate than the *Ae. albopictus* (Ramadhani and Trisnawati, 2014). This study also identified *Culex* sp in the study location, which was in line with previous study by Weitzel *et al* (2015) that *Culex* mosquitoes can be found in open rainwater buckets, sewage-contaminated discharges, and sewer drains.

In summary, there was a difference in the number of eggs based on the type of attractant (p-value = 0.0001). The straw-soaked water (positive control) is more efficient at attracting mosquitoes to lay eggs in the ovitraps than other solutions. Tambakreja Village has an OI score of 11.43% which is classified as Level 2 (low). Vector mosquito larvae identified included *Aedes aegypti* (63.3%), *Ae. albopictus* (26.6%), and *Culex* sp. (10.1%). Straw soaking in water is still more effective than shrimp paste solution at luring *Aedes* mosquitoes. This finding highlights the possible of using shrimp paste, when the straw is not available in certain areas.

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CONFLICT OF INTEREST DISCLOSURE

There is no conflict of interest.

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