

EFFECTIVENESS OF CASSAVA FERMENTATION AS AN ATTRACTANT IN AEDES AEGYPTI MOSQUITO TRAP AT PT. X IN 2023

*Efektivitas Fermentasi Singkong (Manihot utilissima) Sebagai Atraktan
Perangkap Nyamuk Aedes Aegypti Di PT.X Tahun 2023*

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ABSTRAK

Nyamuk dapat menyebabkan dampak negatif apabila tidak dikendalikan, salah satunya penyakit yang ditularkan melalui vektor yaitu Demam Berdarah Dengue (DBD). Metode pengendalian nyamuk yang dapat digunakan salah satunya dengan pemasangan perangkap menggunakan atraktan dari fermentasi singkong. Pemasangan perangkap dapat menjadi alternatif karena lebih aman bagi lingkungan dan kesehatan manusia untuk mengendalikan vektor nyamuk *Aedes aegypti*. Tujuan penelitian ini yaitu untuk mengetahui konsentrasi fermentasi singkong yang paling efektif pada perangkap nyamuk *Aedes aegypti*. Jenis penelitian ini yaitu True experiment research skala laboratorium dengan metode Post-test Only with Control Design. Penelitian melakukan pengujian terhadap 3 variasi konsentrasi fermentasi perbandingan ragi dan singkong (gr) yaitu 1:50, 2:50, dan 3:50. Kontrol penelitian ini menggunakan perangkap nyamuk berisi akuades (tanpa fermentasi singkong). Sampel penelitian ini adalah nyamuk *Aedes aegypti* berumur 3 hari dengan teknik sampling purposive sampling. Berdasarkan hasil analisis data menggunakan One Way Anova diketahui bahwa terdapat perbedaan yang signifikan antara variasi konsentrasi fermentasi singkong terhadap nyamuk *Aedes aegypti* yang terperangkap, hal tersebut dikarenakan Nilai $P < \alpha$ ($0,000 < 0,05$). Konsentrasi fermentasi singkong yang paling efektif sebagai atraktan pada perangkap nyamuk *Aedes aegypti* adalah konsentrasi fermentasi singkong 3:50 dengan jumlah 88 ekor (14,67%). Disarankan penggunaan perangkap nyamuk dengan antaraktan konsentrasi fermentasi singkong 3:50.

Kata kunci: *Aedes aegypti*, atraktan, konsentrasi fermentasi, singkong

ABSTRACT

Mosquitoes can have negative impacts if left uncontrolled, one of which is vector-borne diseases such as Dengue Fever (DBD). One of the methods of mosquito control that can be used is the installation of traps using attractants from cassava fermentation. The installation of traps can be an alternative because it is safer for the environment and human health to control *Aedes aegypti* mosquito vectors. The purpose of this research was to determine the most effective concentration of cassava fermentation in *Aedes aegypti* mosquito traps. This type of research was a true experimental research in a laboratory scale with the Post-test Only with Control Design method. The research tested three variations of cassava fermentation concentrations, namely 1:50, 2:50, and 3:50, with different yeast doses. The control of this study used mosquito traps filled with distilled water (without cassava fermentation). The samples of this research were 3-day-old *Aedes aegypti* mosquitoes. Based on the results of data analysis using One-Way Anova, it is known that there was a significant difference between the variations of cassava fermentation concentrations on *Aedes aegypti* mosquito traps, this is because the P -value $< \alpha$ ($0.000 < 0.05$). The most effective concentration of cassava fermentation as an attractant in *Aedes aegypti* mosquito traps is the concentration of cassava fermentation 3:50, with a total of 88 mosquitoes (14.67%). It is recommended for further

research to investigate the effective variation of cassava fermentation concentrations up to 100% to attract mosquitoes into the mosquito trap device.

Keywords: *Aedes aegypti*, attractant, cassava concentration, fermentation

INTRODUCTION

Indonesia has many dengue fever endemic areas, one of which is West Bandung Regency, West Java. The Head of the Prevention and Control of Infectious Diseases Division of West Bandung Regency stated that four districts in West Bandung Regency (KBB) are endemic for dengue fever. These districts include Padalarang, Cikalongwetan, Lembang, and Batujajar, which consistently have high case numbers each year compared to other districts. Between January and June 2022, 957 residents were reported to have contracted dengue fever, and 10 deaths were recorded[1].

Dengue fever cases can occur not only in residential areas, but also in public places such as offices and industries, as is the case at PT X. PT X is an industry located in an endemic area. This situation makes PT X have a high possibility of dengue fever cases occurring. Mosquitoes can cause negative impacts if not controlled, one of which is a vector-borne disease, namely Dengue Hemorrhagic Fever (DHF). In addition to health problems, the presence of mosquitoes can also cause stress and anxiety due to the disease, discomfort, and can hinder physical activity[2].

PT X is a company operating in the pharmaceutical sector. One of the environmental health issues identified in the industry is the presence of mosquito vectors in the non-production work environment. Mosquito control measures for workers have not been optimal. This is further supported by worker complaints about the large number of mosquitoes in non-production work areas, which disrupts work efficiency.

The results of a survey conducted at PT X regarding the resting rate of *Aedes aegypti* mosquitoes yielded the highest result, namely 8.20 resting mosquito density figures per hour. The standard value for *Aedes aegypti* mosquitoes is <0.025 resting mosquito density figures per hour[3], [4]. The results of the *Aedes aegypti* Mosquito Resting Rate survey do not meet the quality standards set out in the Minister of Health Regulation No. 50 of 2017 concerning Environmental Health Quality Standards and Health Requirements for Vectors and Disease-Carrying Animals and Their Control[4]. This could be caused by the environmental conditions of PT.X, which has many plants or bushes, which are places where *Aedes aegypti* mosquitoes rest.

Mosquito control efforts must be implemented to reduce the mosquito population to such a low level that their presence no longer poses a risk of disease transmission. Mosquito control is also carried out to improve human comfort in a given area.[5]One mosquito control method that can be used is setting traps that use attractants. This control method involves using attractants in traps placed in areas where mosquitoes are present.[6]The use of traps with this attractant can be a more environmentally friendly and safe alternative for human health to control the *Aedes.sp* mosquito vector[7].

Several studies have explored mosquito control using traps containing attractants. One example is research by Roni et al. (2022) on the use of fermented cassava, sugarcane juice, brown sugar, and banana peels as attractants in traps. Fermented cassava, weighing 50 grams, had the highest attraction rate for mosquitoes, with 18 mosquitoes being attracted[8]. The results of this study are in line with the research of Yunicho et al. (2020), which found that cassava fermentation was the most preferred attractant for mosquitoes compared to the sea shrimp-soaked water attractant, with 36 mosquitoes caught on the cassava fermentation attractant, while only 26 were caught on the shrimp-soaked water attractant[9].

This study will use yeast and cassava fermentation with a ratio of 1:50, 2:50, and 3:50 in gram units to determine the effectiveness of cassava fermentation with different yeast ratios as an attractant in *Aedes aegypti* mosquito traps. According to Berlian et al. (2016),

in their study, there were significant differences in the levels of alcohol and CO₂ produced from cassava fermentation with different yeast doses. The higher the yeast dose given, the higher the alcohol and CO₂ levels produced. This is because the higher the yeast dose given, the more yeast is produced. Yeast has an important role in the fermentation process by converting glucose into alcohol and CO₂[10].

Aedes sp. have sensilla on their antennae and palps to detect body odor, CO₂, heat, and moisture. Mosquitoes use their sense of smell as their primary means of detecting their hosts through chemical signals from humans. Mosquitoes use these signals to distinguish humans from other animals[11]. Therefore, the CO₂ produced from fermented cassava can be used as an attractant for mosquitoes and stored in mosquito traps.

The study will be conducted using yeast to cassava ratios of 1:50, 2:50, and 3:50. This differs from previous studies that focused on cassava weight, while this study focuses on the yeast dosage used and the resulting alcohol and CO₂ levels. This study aimed to determine the most effective concentration of cassava fermentation in trapping *Aedes aegypti* mosquitoes. The results of this study can be used as an alternative to chemical mosquito control and as an environmentally friendly way to reduce mosquito density.

METHODS

This research used a laboratory-scale True Experiment Research (TIR) design with a Post-Test Only with Control Design. The objective was to determine the most effective concentration of cassava fermentation in trapping *Aedes aegypti* mosquitoes. The study was conducted at the Environmental Health Laboratory of the Poltekkes Kemenkes Bandung to prepare yeast and cassava fermentation solutions and apply them at PT. X from March to May 2023.

Preparation begins with making a mosquito trap. The procedure for making the mosquito trap begins with preparing a used 1500 ml plastic bottle, then cutting it 18 cm from the bottom. The top of the bottle's opening is connected to a plastic mica shaped like a funnel with a 1.5 cm diameter hole. The goal is to allow mosquitoes measuring 4-13 mm to enter the trap and be trapped. Place the top of the bottle upside down again. The outside of the bottle is covered with black paper until the entire surface is covered. The goal is to attract *Aedes aegypti* mosquitoes, which tend to be attracted to dark colors.

The mosquito cage construction procedure, as shown in Figure 1, involves forming a 3.4 mm galvanized wire frame into a 50 x 50 cm square frame. Using a welding machine, combine the square frames to form a 50 x 50 cm cube. Cover each side with mosquito netting secured with thread, leaving about 50 cm of mosquito netting at the front for inserting your hand and a mosquito trap.

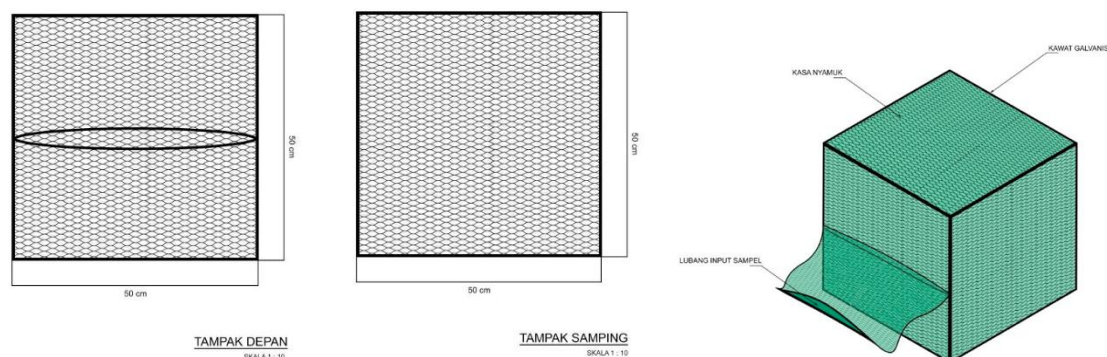


Figure 1. Mosquito Cage

The procedure for making cassava fermentation and yeast solution is done by peeling all the cassava skin. Wash the cassava until clean. Cut the cassava and weigh it to 50

grams. Steam the cassava for ± 30 minutes. Remove and cool the cassava in a sterile manner at room temperature for ± 1 hour. Puree the cassava using a blender. Put the pureed cassava into a 450 ml jar weighing 50 grams and add tape yeast weighing 1, 2, and 3 grams. Use a ratio of 1:50, 2:50, 3:50 in 200 ml of distilled water, then stir. Let the solution sit for 2 hours in a closed jar, this aims to allow the fermentation process to take place.[7], [8], [9], [12], [13].

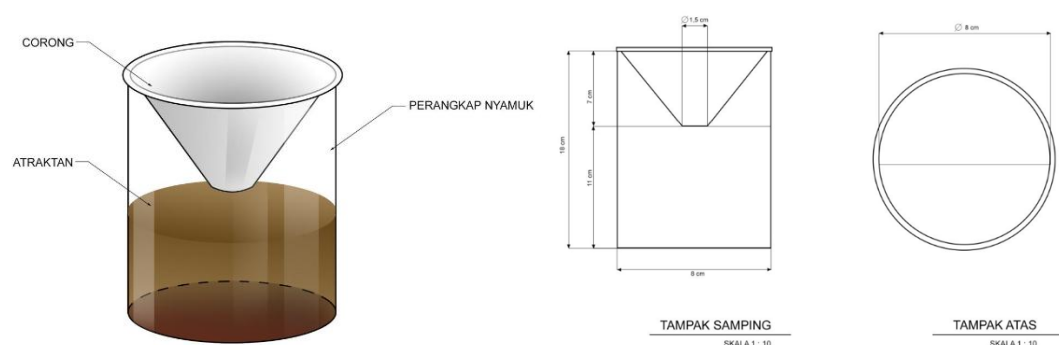
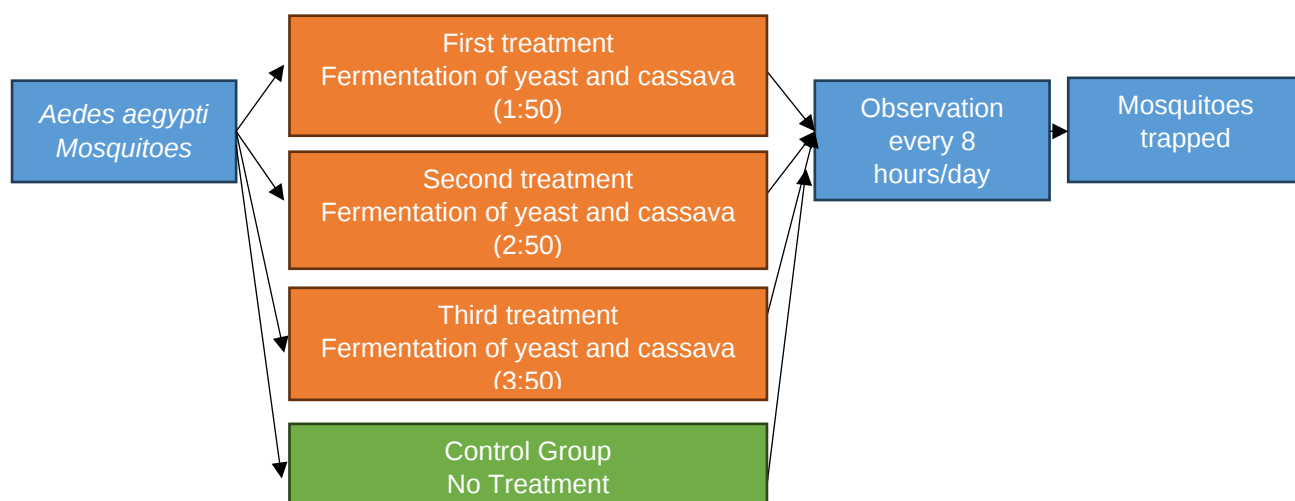


Figure 2. Yeast and cassava fermentation media

A pre-experiment was conducted to determine the attractant capacity of cassava fermentation with concentrations of 1:50, 2:50, and 3:50, and to determine whether the number of *Aedes aegypti* mosquitoes successfully trapped differed when observed for 1 day. The pre-experiment in this study was conducted by preparing 4 mosquito cages. 25 3-day-old *Aedes aegypti* mosquitoes were placed in each cage. Cassava fermentation solutions with concentrations of 1:50, 2:50, 3:50, and controls were placed in each plastic bottle. Mosquito traps containing cassava fermentation with concentrations of 1:50, 2:50, and 3:50, along with controls, were placed in each cage separately, so that each cage contained 1 mosquito trap. Turn on the Thermohygrometer and place it in the area where the research was conducted. Make observations, calculations, and records when entering the 8th, 16th, and 24th hour regarding the number of mosquitoes trapped as well as the temperature and humidity of the air.

The experiment in this study was conducted directly at PT.X by preparing 6 mosquito cages. Put 100 3-day-old *Aedes aegypti* mosquitoes into each mosquito cage. Cassava fermentation solution with concentrations of 1:50, 2:50, and 3:50 was put into each plastic bottle. Put the 3 treatments along with the control into the same mosquito cage. So, in 1 mosquito cage, there are 4 mosquito traps. Then, in 1 room, 6 repetitions were carried out in 1 time simultaneously. Turn on the Thermohygrometer and place it in the area where the study was conducted. Observe, count, and record the number of mosquitoes entering the mosquito trap each time the observation period is entered three times a day, namely at 16:00, 24:00, and 08:00. Recording was also carried out on temperature and humidity.

Monitoring in this study was conducted three times over the course of one day. Mosquito traps were set up at 8:00 a.m. WIB. Monitoring was conducted at 8, 16, and 24 hours, namely at 4:00 p.m., 12:00 p.m., and 8:00 a.m., after the traps were set. The number of mosquitoes trapped, both dead and alive, was counted. If the trapped mosquito was dead, it was removed using tweezers. If the trapped mosquito was alive, the next step was to kill it. This was done by covering the top of the trap with plastic and then tying it with raffia rope to prevent water spillage. In this study, data analysis was carried out using the One-Way ANOVA and Post Hoc Tests, which aimed to determine the differences in variations in cassava fermentation concentration on the number of *Aedes aegypti* mosquitoes trapped.



RESULT

In the pre-experiment, the following results were obtained:

Table 1. Pre-Experimental Results: Number of *Aedes aegypti* Mosquitoes Trapped

The hour	Σ Sample	Number of Mosquitoes (tails)				Tempe rature	Humidity
		Control	Treatment 1	Treatment 2	Treatment 3		
0	25	0	0	0	0	23.4°C	91%
8		0	1	2	5	26.0°C	77%
16		1	2	3	5	23.2°C	99%
24		1	2	3	1	22.0°C	83%
Amount		2	5	8	11	-	-

Based on Table 1, the results of observations over 24 hours showed that the number of *Aedes aegypti* mosquitoes trapped at a cassava fermentation concentration of 1:50 was 5, at a cassava fermentation concentration of 2:50 was 8, and at a cassava fermentation concentration of 3:50 was 11. The results of temperature measurements carried out using a thermometer obtained a temperature between 22.0-26.0°C. The results of humidity measurements carried out using a hygrometer showed humidity between 77 - 99%. In experiments carried out on 17 - 18 May 2023, the following results were obtained:

Table 2. Experimental Results: Number of *Aedes aegypti* Mosquitoes Trapped

		Number of Mosquitoes(tail)			
Repetition	Σ Sample	Control	Treatment 1	Treatment 2	Treatment 3
			1	2	3
1	100	2	4	13	16
2		1	8	12	15
3		0	1	8	9
4		0	7	10	19
5		2	11	14	15
6		1	2	10	14
Amount		6	33	67	88
Minimum		0	1	8	9
Maximum		2	11	14	19
Percentage		1%	5.5%	11.17%	14.67%

Based on table 2, the results of observations over 24 hours showed that the number of *Aedes aegypti* mosquitoes trapped at a cassava fermentation concentration of 1:50

was 33, at a cassava fermentation concentration of 2:50 was 67, and at a cassava fermentation concentration of 3:50 was 88.

The following are the results of temperature and humidity measurements during the research:

Table 3. Temperature and Humidity Measurements

Repetition	Temperature During Treatment (°C)				Humidity During Treatment (%)			
	0 Hours	8 Hours	16 Hours	24 hours	0 Hours	8 Hours	16 Hours	24 hours
1 - 6	23.4	25.5	24.9	24.8	69	70	77	74

Based on table 3, the results of temperature and humidity measurements carried out 4 times in 24 hours using a Thermohygrometer obtained a temperature between 23.4-25.5°C and humidity between 69-77%. The application of mosquito traps containing fermented cassava attractant at the most effective concentration at PT. X, conducted on May 22-24, 2023, yielded the following results:

Table 4. Number of Aedes aegypti Mosquitoes Trapped in Mosquito Traps Containing Fermented Cassava Attractant at PT. X

Day th-	Number of Mosquitoes (tails)	Temperature			Humidity		
	Cassava Fermentation Concentration 3:50	08.00	12.00	16.00	08.00	12.00	16.00
1	17	23.1°C	28.3°C	27.5°C	61%	53%	51%
2	11	23.7°C	27.5°C	25.9°C	81%	50%	54%
3	5	22.5°C	27.2°C	26.5°C	75%	61%	70%
Total 33		-	-	-	-	-	-
Average 11		-	-	-	-	-	-

Based on Table 4, it is known that the cassava fermentation concentration of 3:50 on day 1 attracted 17 *Aedes aegypti* mosquitoes, 11 on day 2, and 5 on day 3. The results of temperature measurements carried out using a thermometer obtained temperatures between 23.1 and 28.3°C. The results of humidity measurements carried out using a hygrometer showed humidity between 50-81%.

DISCUSSION

The results of the pre-experiment carried out with a yeast and cassava fermentation ratio of 3:50 showed that the number of mosquitoes trapped was 11 compared to other fermentation concentrations with temperatures and humidity between 22.0-26.°C and Humidity between 83 - 99%. While the results of the experiment that has been carried out for 1 day are known to show the number of *Aedes aegypti* mosquitoes trapped in mosquito traps containing a concentration of cassava fermentation 3:50 as many as 88 (14.67%), and mosquito traps without cassava fermentation (control) as many as 6 (1%). The results of temperature and humidity measurements obtained a temperature between 23.4-25.5°C and humidity between 69-77%. The results of the pre and experimental results obtained the same results with the concentration of yeast and cassava fermentation 3:50 can be a good attractant with the same temperature and range in mosquito bionomic conditions.

Based on the results of experimental observations in the 3rd repetition, it was found that the number of mosquitoes trapped was the least when compared to the other repetitions. This is thought to be due to the number of male mosquitoes in the mosquito cage being greater than the number of female mosquitoes. In the *Aedes aegypti* mosquito, only female mosquitoes suck blood, while male mosquitoes prefer fluids such as fruits, sugars, sweat, and plant fluids[6]. Female mosquitoes are thought to have

higher sensitivity compared to male mosquitoes, because female mosquitoes have sensitivity to CO₂ to detect their hosts to suck blood[11].

The mosquitoes used as samples in this study were all 3-day-old male and female *Aedes aegypti* mosquitoes. Mosquito traps using cassava fermentation attractant successfully caught more mosquitoes than those without cassava fermentation attractant (control). This cassava fermentation solution has the ability to act as an attractant because it produces CO₂[14]. This is proven by the number of *Aedes aegypti* mosquitoes trapped in mosquito traps containing attractants being greater than in standard mosquito traps that do not use attractants[13]. The increased mosquito population is due to the reaction between cassava and yeast during the fermentation process, which produces CO₂ and ethanol. Concentrated CO₂ is a chemical that can attract mosquitoes through their olfactory senses, making cassava fermentation a viable attractant[15].

Mosquitoes are attracted to the CO₂ produced by humans during their breathing process.[16] *Aedes aegypti* has sensilla on its antennae and palps to detect CO₂, which it uses to detect its host using chemical signals from humans. This is supported by research by Kawada et al. (2007), which found that combining black visual materials with CO₂ can increase the attractiveness of *Aedes aegypti* mosquitoes[17].

The same temperature during the pre- and experimental research produced results ranging between 24.8-25.5°C. Mosquitoes are cold-blooded insects, so their entire metabolic process depends on the temperature of their environment. Mosquitoes will become inactive and lose their ability to fly if the temperature drops[18]. Environmental factors such as air temperature, air humidity, and climate can affect the length or shortness of a mosquito's lifespan, so that in optimal air temperature and humidity conditions, *Aedes aegypti* mosquitoes can live longer.[19].

The activity and metabolism of *Aedes aegypti* mosquitoes can be directly influenced by environmental factors such as breeding sites, rainfall, air temperature, and humidity. *Aedes aegypti* mosquitoes require an average air temperature of around 25-27°C, and air humidity of around 70%-80%. The growth of *Aedes aegypti* mosquitoes will stop completely at temperatures below 10°C or more than 45°C[20].

The temperature that does not affect the death of the *Aedes aegypti* mosquito, if it is within a certain temperature range, is a temperature that is not too low (below 15°C) and the temperature is not too high (above 45°C)[21]. Temperature can also affect yeast growth. Yeast grows optimally at temperatures around 25°C to 30°C and will produce carbon dioxide[22]. Yeast can become inactivated if the temperature is too low or too high.

Humidity during the study ranged from 70-77% in the field, while in the pre-experiment, it ranged from 77-99% in the laboratory. Air humidity affects mosquito flight distance, breeding rate, lifespan, biting habits, and resting. Optimal air humidity can extend mosquito lifespan and increase their reproductive rate, ultimately leading to an increase in mosquito populations. This can also impact the risk of disease transmission from infected mosquitoes to humans[19].

Air humidity affects the reproduction rate and lifespan of mosquitoes. Mosquitoes have a respiratory system consisting of air tubes (trachea) and holes in the body wall (spiracles). Low air humidity can cause water to evaporate from the mosquito's body, ultimately leading to dehydration[23]. Low humidity will cause mosquitoes to dry out, while too high humidity can affect their metabolism. The humidity level that can support mosquito life ranges from 60% to 89%[21]. It was concluded that the indoor air humidity at the time of the study was suitable for mosquito life and had no effect on the mortality rate in the study results.

This study was conducted to determine the differences in the concentration variations of cassava fermentation 1:50, 2:50, and 3:50 on trapped *Aedes aegypti* mosquitoes. Based on the analysis using the one-way ANOVA test with a significance level of 5%,

the results obtained showed a significant difference in the concentration variations of cassava fermentation (1:50, 2:50, 3:50, and control) on trapped *Aedes aegypti* mosquitoes. This was due to the significant value $<\alpha$ ($0.000 < 0.05$). A significant value smaller than the significance level indicates a significant difference in the trapped *Aedes aegypti* mosquitoes[24].

Based on the results of further analysis using Post-Hoc LSD with a significance level of 5%, the results obtained were a significant difference between the concentration of cassava fermentation 1:50 with the concentration of cassava fermentation 2:50 and the concentration of cassava fermentation 3:50. There was a significant difference between the concentration of cassava fermentation 2:50 with the concentration of cassava fermentation 3:50. Based on the results of the analysis using the Post-Hoc Test, it showed that between the variations in cassava fermentation concentration had a significant value <0.05 , which means that the concentration of cassava fermentation between variations had a significant difference in the trapped *Aedes aegypti* mosquitoes.

This significant difference occurs because the three variations in fermentation attractant concentration contain starch and produce CO_2 gas after the fermentation solution is mixed with yeast. This produces a pungent aroma that attracts mosquitoes to the trap.[8] However, with different CO_2 levels due to the influence of the yeast dosage used in the fermentation process[10]. The most effective concentration of cassava fermentation in trapping *Aedes aegypti* mosquitoes was a 3:50 concentration, compared to 2:50 concentrations, 1:50 concentrations, and the control. This is because the higher the yeast dose, the higher the CO_2 content to attract mosquitoes.

There are significant differences in the alcohol and CO_2 levels produced during cassava fermentation with different yeast dosages. The alcohol and CO_2 levels tend to increase with increasing yeast dosage. This is because the higher the yeast dosage, the greater the number of yeasts involved in the fermentation process. These yeasts play a crucial role in converting glucose into alcohol and CO_2 [10], therefore, in line with this research, the higher the dose of yeast used, the higher the CO_2 content that will attract mosquitoes to enter the mosquito trap.

Limitations in this study include the lack of CO_2 levels in the cassava fermentation solution that has been made as an attractant. Measuring the CO_2 levels in the cassava solution can determine mosquito attraction based on the CO_2 levels produced. The concentration used in the mosquito trap media was found to be that the higher the weight of yeast used, the higher the mosquito attraction to the attractant media.

CONCLUSION

The number of *Aedes aegypti* mosquitoes trapped in a concentration of cassava fermentation of 1:50 was 33, a concentration of cassava fermentation of 2:50 was 67, and a concentration of cassava fermentation of 3:50 was able to attract 88 *Aedes aegypti* mosquitoes. The most effective concentration of cassava fermentation used as an attractant in *Aedes aegypti* mosquito traps is a concentration of cassava fermentation of 3:50, with an effectiveness presentation of 14.67%. It is recommended to use mosquito traps with natural attractant media using yeast and cassava fermentation of 3:50 as an environmentally friendly mosquito control with easy and inexpensive application.

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