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Hay Infusion is More Effective than The Yeast and Palm Sugar Combination as an Ovitrap Attractant of *Aedes Aegypti* Mosquitoes

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ABSTRACT

The *Aedes aegypti* mosquito is widely known as a vector for Dengue Hemorrhagic Fever (DHF). Mosquito population can be controlled by using oviposition traps (ovitrap). Attractants can be used to attract mosquitoes to lay their eggs. This study aims to compare the effectiveness of *Ae. aegypti* mosquitoes ovitrap attractant between hay infusion, the yeast-palm sugar combination and ovitrap standard without attractant. The research method was an experimental research posttest only control group design. The results showed that the highest number of eggs during the 4 days of observation was in the hay infusion with an average number of eggs 253,44±58,445 followed by ovitrap without attractant and palm sugar with the number of eggs 31,89±25,300 and 89,78±39,499 respectively sequentially. There was a significant difference in the mean number of eggs between 10% hay infusion and 10% yeast and palm sugar combination and water with p=0.000. The conclusion of this study is that hay infusion attractant is more effective than yeast and palm sugar combination.

Keywords: *Aedes aegypti*, Yeast, Hay Infusion, Ovitrap, Palm Sugar.

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is caused by the dengue virus which is transmitted by the *Aedes aegypti's* bite. This disease is usually found in tropical regions such as Indonesia, especially after the rainy season. Based on data from the Ministry of Health of the Republic of Indonesia, in 2019 there were 13,683 confirmed cases of DHF with 132 cases of them dying in Indonesia. In 2017, there were 4499 cases of dengue fever in the province of Bali with 13 deaths.[1]

There are 4 stages in mosquito life cycle, which is egg, larva, pupa, and adult mosquito. *Ae. aegypti* generally breeds in water reservoirs (manmade containers).[2] Larvae that have hatched will experience growth and metamorphosis into adult mosquitoes. *Ae. aegypti* eggs can survive for 2-3 months without being submerged in water.[3] This causes the population of *Ae. aegypti* increases after the rainy season.[4]

Placement of mosquito eggs (oviposition) can be controlled by using ovitrap. Mosquito eggs collected in the ovitrap will be cleaned regularly to prevent the eggs from hatching.[5,6] This action will reduce the mosquito population by breaking their life cycle. Ovitrap can be used together with attractants to increase their effectiveness, several types of attractants have been studied and their effectiveness compared.

Oviposition can be increased by using attractants. Attractants can be made from natural or synthetic materials.[7] The attractant that has proven to be effective is hay infusion, this attractant is easy to find

and has been proven effective as an ovitrap material.

Research on hay infusion has been carried out with various concentrations of 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, and 10%. In this study, it was found that hay infusion with a concentration of 10% had the highest number of eggs. Data from this study showed that the hay infusion consistently obtained a higher number of eggs than the distilled water attractant used as a control.[8] Apart from hay infusion, another material that can undergo a fermentation process and has potential as an attractant is yeast solution.[9,10]

Yeast is an ingredient that is easy to find in everyday life. The yeast fermentation process uses a microbe with the scientific name *Saccharomyces cerevisiae*. This microbe is very popular and has been widely used in fermented products such as wine, beer, and bread, and tape making. Under aerobic conditions, yeast can oxidize pyruvate to produce energy and produce water and carbon dioxide.[9] Carbon dioxide compounds stimulate the mosquito's olfactory nerves and invite mosquitoes to lay their eggs there.

MATERIALS & METHODS

This experimental research uses the posttest only control group design method. This research was conducted at the Laboratory of Parasitology, Faculty of Medicine, Udayana University. This research was approved by the Research Ethics Commission of the Faculty of Medicine, Udayana University.

The material used in this study were ovitrap made form black colored glass with diameter of 10 cm.

Ovitrap were divided into 3 groups, first group ovitrap standard (water only), second group ovitrap with 10% hay infusion, and the third group ovitrap with the yeast and palm sugar combination. The first group was hay infusion with a concentration of 10% made by soaking hay in a tightly closed bucket for 7 days. The attractant of baker's yeast (Fermipan®) and 10% palm sugar combination is made by mixing yeast and palm sugar that has been melted into water with a concentration of 10%. Each ovitrap contains 150 ml of solution. Each ovitrap will be given filter paper as a place to lay eggs for mosquitoes. The filter paper was changed every day at the same hour.

The study was replicated 9 times with each replication using 20 female mosquitoes that already ingested blood placed in cages. Three ovitrap from each group were placed in each cage.

To observe the number of eggs, this study was carried out for 4 days, the data was obtained based on the number of eggs trapped in the ovitrap. Data calculation was carried out using One-way ANOVA statistical analysis.

RESULT

The experiment was comparing the hay infusion as well as a yeast and palm sugar combination. Based on the number of eggs from each ovitrap, it was found that hay infusion had the highest number of eggs compared to other ovitraps. The research data showed that the highest number of eggs was on the fourth day in hay infusion (Figure 1). The mean for each ovitrap on the number of eggs for 4 days was 253.44 ± 58.445 eggs for hay infusion, 31.89 ± 25.300 eggs for yeast and palm sugar combination, and 89.78 ± 39.499 eggs for water (Table 1). The number of eggs with hay infusion, yeast and palm sugar combination, and water for 4 days respectively was 2281 eggs, 287 eggs and 808 eggs.

The number of eggs on the first and second day did not meet the requirements, so the researchers did not use these data in the analysis. Analysis was carried out on the number of eggs obtained on the third day, fourth day, and the accumulated number of eggs for 4 days. Based on the normality test using Shapiro Wilk, it was found that the data were normally distributed.

1. Third Day

The results showed that there was a significant difference between the attractant

of hay infusion and yeast and palm sugar combination with more eggs in hay infusion and mean difference of 60.111 with p-value of 0.044 ($p<0.05$). Based on these results, it can be concluded that hay infusion is more effective than yeast and palm sugar combination from the average number of eggs per solution, namely 82.44 ± 57.763 for hay infusion, and 22.33 ± 22.216 for yeast and palm sugar combination.

2. Fourth Day

The hay infusion is significantly different from the yeast and palm sugar combination with more eggs in hay infusion and mean difference of 137.556 with p-value of 0.002 ($p<0.05$). The hay infusion was significantly different from water with more eggs in hay infusion and mean difference of 106.778 with p-value of 0.000 ($p<0.05$). Based on these results, it can be concluded that hay infusion is more effective than yeast and palm sugar combination and water from the average number of eggs in each solution, namely 147.11 ± 61.124 for hay infusion, 9.56 ± 10.150 for yeast and palm sugar combination, and 40.33 ± 32.261 for water attractant.

3. Total 4 Days

The hay infusion is significantly different from yeast and palm sugar combination with more eggs in hay infusion and mean difference of 221.556 with p-value of 0.000 ($p<0.05$). The hay infusion was significantly different from water with more eggs in hay infusion and mean difference of 163.667 with p-value of 0.000 ($p<0.05$). Water has significantly different from yeast and palm sugar combination with more eggs in water and mean difference of 57.789 with p-valuer of 0.009 ($p<0.05$). Based on these results, it was proven that the most effective ovitrap attractant was hay infusion, followed by water, and yeast and palm sugar combination from the average number of eggs for each solution, namely 253.44 ± 58.445 for hay infusion, 31.89 ±25,300 for yeast and palm sugar combination, and 89,78±39,499 for water attractants.

Table 1. Mean and standard deviation of collected *Ae. aegypti* eggs for 4 days

Attractant	Third Day $\bar{x}$ ±SD	Fourth Day $\bar{x}$ ±SD	Total 4 days $\bar{x}$ ±SD
Water	48.56±35,214	40,33±32,261	89,78±39,499
Hay	82,44±57,763	147,11±61,124	253,44±58,445
Yeast and Sugar	22,33±22,216	9,56±10,150	31,89±25,300

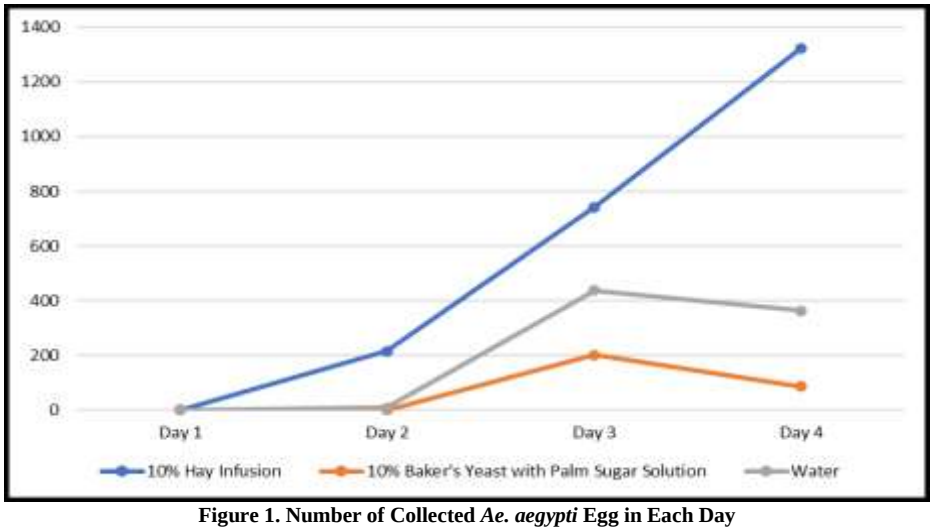

Figure 1. Number of Collected *Ae. aegypti* Egg in Each Day

DISCUSSION

In this study, it was found that the number of eggs in a hay infusion was higher than that in baker's yeast and palm sugar combination. A 10% hay infusion solution has been shown to be effective as an ovitrap attractant for *Ae. aegypti*, this statement can also be seen in other studies that also compared hay infusion with other types of attractants. In a study conducted by Wahidah *et al*. who compared hay infusion with tiger prawn infusion and tapai's yeast

infusion, it was found that hay infusion had significantly more effective results when compared with tiger prawn infusion and tapai's yeast infusion.[11] A study conducted by Polson *et al*. which comparing hay infusion with water also supports that hay infusion has significantly more effective results when compared to water.[12] Chadee *et al*. conducted a test on the concentration of hay infusion by comparing 11 ovitraps where each ovitrap had a different concentration of hay from 0% to 100% concentration. The results found that hay infusion with a concentration of 10% had the most effective results compared to other concentrations.[8]

Hay infusion was chosen to be used as an attractant because hay is an easy material to find, besides that in the hay fermentation process it will produce compounds such as carbon dioxide (CO2), ammonia gas, and octenol. These substances have beneficial effects for use against mosquitoes because they can stimulate the mosquito's olfactory nerves. *Ae. aegypti* mosquitoes have a chemotactile sensory organ that can detect these substances, the chemicals in the hay infusion will stimulate mosquitoes to land and lay eggs in the solution that has been provided.

In general, the compounds CO2, octenol, and ammonia are the reasons why mosquitoes are attracted to lay their eggs in certain places, therefore the hay infusion can be used as an attractant for mosquito ovitrap.[8] Similar compounds also can be found in yeast fermentation, therefore researchers tested the difference of effectiveness between hay infusion and yeast and palm sugar combination an attractant for *Ae. aegypti*.[13]

In the results of the study, the researchers found that in a solution of yeast and palm sugar combination, the number of mosquito eggs was lower than the control using water. These results contradict with research conducted by Widya *et al*. where in that study in a baker's yeast with 20% sugar and water solution of tapai's yeast accompanied by 20% sugar, there were more mosquito eggs than controls.[14] Likewise, in a research conducted by Sazali *et al*., it was shown that a 10% concentration of palm sugar solution produced better results than the hay infusion[15]. Researchers suspect this difference is due to differences in the composition and content of palm sugar used in this study that is different from previous studies.

CONCLUSION

This research found that hay infusion proved to be the most effective compared to the other solutions.

Suggestions for further research can use research on the concentration of baker's yeast and the use palm sugar with different concentrations and compositions.

Declaration by Authors

Ethical Approval: This research has obtained ethical clearance and approved by the Research Ethics Commission of the Faculty of Medicine, Udayana University.

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Conflict of Interest: The authors declare no conflict of interest.

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