RESEARCH ARTICLE Open Access

Gema Lingkungan Kesehatan
Vol. 24, No. 3 (2026), pp 486-493
e-ISSN 2407-8948 p-ISSN 16933761
doi: https://doi.org/10.36568/gelinkes.v24i3.517 Journal Homepage: https://gelinkes.poltekkesdepkes-sby.ac.id/

Comparative Analysis of *Aedes spp*. Mosquito Oviposition Preferences for Various Natural Attractants in Ovitraps

Tatag Fajar Subekhi[1,2*], Nur Endah Wahyuningsih[1], Mursid Raharjo[1], Nurjazuli Nurjazuli[1], Sulistiyani Sulistiyani[1]

[1] Department of Environmental Health, Faculty of Public Health, Diponegoro University, Semarang, Indonesia
[2] Department of Environmental Health, Poltekkes Kemenkes Semarang, Semarang, Indonesia

Correspondence: tatagfajar02@gmail.com

Dengue fever remains a major public health problem in tropical countries, including Indonesia. Ovitraps are widely used as a simple and cost-effective tool for monitoring and controlling *Aedes* mosquito populations; however, their effectiveness depends on the type of attractant and trap material used. This study aims to evaluate various types of attractants for *Aedes* mosquito egg traps as part of surveillance efforts. The research method is a quasi-experimental study with a posttest-only control group design was conducted from August to October 2025 in Sokaraja Subdistrict, Banyumas Regency, Central Java, Indonesia. A total of 16 ovitraps consisting of different trap materials (glass, bamboo, coconut shell, and black plastic) and attractants (fermented palm sugar solution, fermented young coconut water, red chili infusion, and clean water as control) were deployed in 15 households. The attractants tested were fermented palm sugar solution (45%), fermented young coconut water (20%), and red chili infusion (15%), all prepared based on preliminary trials. Control treatments included egg traps filled with clean water and transparent glass egg traps to control for material-related effects. This study has received approval from the UNDIP ethics committee with NO. 280/EA/KEPK-FKM/2025. The results showed that egg counts were recorded from ovistrips after a five-day exposure period and analyzed using descriptive statistics and Mann–Whitney tests. Coconut shell ovitraps captured the highest mean number of eggs (67.58), followed by black plastic (26.07) and bamboo traps (20.83), while glass ovitraps showed the lowest mean (0.25). Among attractants, fermented young coconut water produced the highest mean egg count (70.05), followed by red chili infusion (24.38) and fermented palm sugar solution (14.35), whereas clean water showed the lowest value (5.95). Statistical analysis demonstrated significant differences among both ovitrap materials and attractant types ($p < 0.05$). These findings indicate that fermented young coconut water, combined with coconut shell traps, exerts a strong oviposition-attracting effect on *Aedes spp*. mosquitoes. This combination offers a potential, eco-friendly alternative method for optimizing community-based surveillance systems for dengue vectors.

Keywords: *Aedes* spp., Coconut shell trap, Dengue vector, Natural attractant, Ovitrap

INTRODUCTION

Environmental health is a core component of public health in Indonesia, as emphasized in Law No. 17 of 2023 and its implementing regulation, Government Regulation No. 28 of 2024, which highlight the importance of preventing environmentally mediated diseases through sustainable and community-based interventions (Government of Indonesia, 2024). In tropical countries, vector-borne diseases remain a major environmental health challenge, with dengue fever representing one of the most significant threats. Mosquitoes of the genus *Aedes* are the primary vectors of dengue virus transmission. Globally, dengue incidence has increased substantially over the past decade. In 2024 alone, more than 7.6 million cases were reported worldwide, accompanied by over 3,000 deaths, reflecting an accelerated global transmission pattern (World Health Organization, 2024). Indonesia remains highly affected, particularly during seasonal transitions. Central Java Province reported more than 17,000 dengue cases in 2024, with Banyumas Regency recording the highest burden. Within this regency, Sokaraja Subdistrict exhibited the highest incidence, underscoring its status as a priority area for vector control interventions (Banyumas District Health Office, 2024).

Conventional dengue vector control strategies, including chemical fogging and larvicidal application, have demonstrated limited long-term effectiveness and raise concerns regarding sustainability and environmental impact. Consequently, greater emphasis has been placed on physical–mechanical control methods, particularly ovitraps, which function by attracting gravid female *Aedes* mosquitoes to lay eggs in controlled breeding sites. Ovitraps are widely recognized as low-cost, simple, and effective tools for both entomological surveillance and population suppression, especially when integrated into community-based programs (Kristovel et al., 2025).

The performance of ovitraps is strongly influenced by the type of attractant used. Organic attractants derived from fermentation or infusion processes release volatile compounds such as carbon dioxide (CO_2), ammonia, lactic acid, and octenol, which stimulate mosquito olfactory receptors and mimic natural oviposition cues (Saleh et al., 2023). Fermented sugar solutions, fermented young coconut water, and red chili infusions have each been reported to exhibit attractive properties toward *Aedes* mosquitoes through the production of these compounds (Gustina et al., 2025; Lala et al., 2018; Suyudi et al., 2018). However, the effectiveness of these attractants varies according to environmental conditions, mosquito behavior, and local ecological contexts.

These three types of natural attractants utilize volatile compounds produced through fermentation or soaking processes to lure Aedes mosquitoes. Their use offers an ecologically sound and sustainable alternative for mosquito population control. This research is significant as it provides scientific data to support more effective community-based vector control efforts. Furthermore, selecting effective attractants can reduce operational costs a common challenge in implementing local-level vector control programs.

A preliminary study was conducted on using fermented young coconut water to trap Aedes aegypti mosquitoes at concentrations of 0%, 15%, 25%, and 35%. The results indicated that the 15% concentration was the most effective at trapping mosquitoes, suggesting that fermented young coconut water has potential as an alternative mosquito attractant medium. Despite this potential, fermented young coconut water remains underutilized by the public, largely because people are unaware of the specific compounds within it that attract mosquitoes (Lala et al., 2018). Subsequent research investigated the effectiveness of attractants derived from red chili (Capsicum annuum) soak water (15% concentration), rice straw (Oryza sativa) (10% concentration), and jengkol (Pithecellobium lobatum) pod powder against Aedes aegypti (Suyudi et al., 2018). The current study builds upon this previous work by testing fermented palm sugar solution (45%), fermented young coconut water (20%), and red chili infusion (15%), with all concentrations determined based on preliminary trials. This study aims to identify which attractant combination exerts the strongest pull on *Aedes spp*. mosquitoes.

Despite the growing body of research on ovitrap-based surveillance, direct comparative studies evaluating multiple natural attractants under identical field conditions in Indonesia remain limited. Moreover, few studies have focused on high-incidence areas where locally available and low-cost materials could be optimized for large-scale community implementation.

Therefore, this study aims to evaluate and compare the effectiveness of three locally available natural attractants—fermented palm sugar solution, fermented young coconut water, and red chili infusion—for use in **Aedes** mosquito egg traps for surveillance purposes. The study seeks to identify the most potent attractant for *Aedes* egg traps in endemic field settings. By identifying the most effective attractant under these conditions, the research aims to provide empirical evidence for a practical, economical, and eco-friendly larval monitoring tool to support local, community-based vector surveillance programs.

METHODS

Study Design and Setting

This study employed a quasi-experimental design using a posttest-only control group approach to evaluate the effectiveness of different natural attractants in ovitraps. The study was conducted from August to October 2025 in Sokaraja Subdistrict, Banyumas Regency, Central Java, Indonesia, an area identified as dengue-endemic with the highest reported dengue incidence in the regency in 2024. This study has received approval from the UNDIP Ethics Committee (No. 280/EA/KEPK-FKM/2025).

Study Population and Sampling

The study population consisted of households located in dengue-endemic areas of Sokaraja Subdistrict. A total of 15 households were selected using predefined inclusion and exclusion criteria. Inclusion criteria included households located in endemic areas, including those with a history of dengue cases, while households whose occupants declined ovitrap placement were excluded. The inclusion criteria for this study were the homes of Dengue Hemorrhagic Fever (DHF) patients in endemic areas of Sokaraja District and other homes within endemic areas of Sokaraja District. Conversely, the exclusion criteria were homeowners whether of DHF patients or others in the endemic areas of Sokaraja District—who did not consent to the placement of ovitraps.

Ovitrap Types and Attractants

A total of 16 ovitraps were deployed at each study location, consisting of three ovitrap materials—dark plastic cups, bamboo containers, and coconut shells—each combined with three natural attractants. The attractants tested were fermented palm sugar solution (45%), fermented young coconut water (20%), and red chili infusion (15%), all prepared based on prior pilot testing. Control treatments included ovitraps filled with clean water and transparent glass ovitraps to control for material-related effects. The specific combinations and concentrations of attractants used here were developed based on prior research aimed at identifying the types and concentrations that exert the strongest attraction on Aedes aegypti mosquitoes. These combinations and concentrations were selected following a preliminary study on the use of fermented young coconut water to trap Aedes aegypti mosquitoes, testing concentrations of 0%, 15%, 25%, and 35%. The preliminary results indicated that the 15% concentration was the most effective at trapping mosquitoes, suggesting that fermented young coconut water has potential as an alternative mosquito attractant medium. Despite its potential as an attractant, fermented young coconut water remains underutilized by the public, largely because people are unaware of the specific compounds within it that attract mosquitoes. Subsequent research has focused on determining the effectiveness of other attractants specifically red chili (Capsicum annuum) infusion at 15%, rice straw (Oryza sativa) infusion at 10%, and jengkol (Pithecellobium lobatum) pod powder against Aedes aegypti mosquitoes.

Experimental Procedure

Ovitraps were installed indoors at locations characterized by low light, high humidity, and regular human activity, conditions favorable for *Aedes* oviposition. The traps were arranged in a linear configuration with equal spacing of 10 cm, and their positions were randomized daily to minimize location-related bias, as illustrated in Figure 1. Each treatment combination was exposed for a five-day period and replicated 15 times. At the end of each exposure period, ovistrips were collected and the number of *Aedes* spp. eggs was recorded. The number of replications for the study was determined using Federer's formula. The calculation indicated a minimum of 2.87 replications, which was rounded up to 3. To ensure more reliable results and minimize experimental error, the number of replications was increased to 15—meaning 15 units of analysis were assigned to each treatment. Consequently, each treatment combination was replicated 15 times, yielding data that is more representative for statistical analysis.

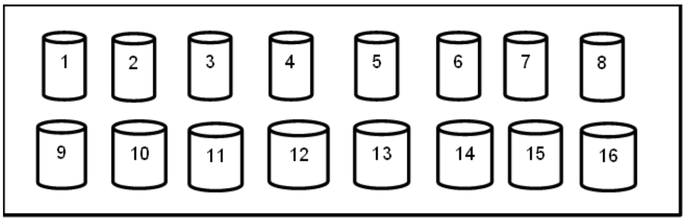

Figure 1. Experimental layout and randomization of ovitrap placement.

Environmental Measurements

Environmental parameters at each ovitrap location including air temperature (°C), relative humidity (%), and light intensity (lux)—were measured using calibrated thermohygrometers and lux meters at the time of ovitrap placement to control for potential confounding environmental effects.

Data Collection and Variables

The primary outcome variable was the number of *Aedes* spp. eggs collected per ovitrap. Independent variables included attractant type, ovitrap material, and environmental conditions. Secondary outcomes included the ovitrap index, calculated as the proportion of positive ovitraps. This study was limited to identifying the quantity of eggs where a higher count indicated greater attractiveness of the tested attractants without identifying the specific source species (i.e., distinguishing between Aedes aegypti and other Aedes species), as doing so would have required further laboratory analysis. Similarly, regarding the ovitrap index, this study did not report ovitrap positivity rates, as the focus was solely on counting the eggs associated with each attractant used.

Statistical Analysis

Data were processed through editing, coding, and tabulation prior to analysis. Univariate analysis was conducted to describe the distribution of egg counts and environmental conditions, including measures of central tendency and dispersion. Prior to inferential analysis, data normality was assessed using appropriate normality tests. As the data were found to be not normally distributed, nonparametric statistical methods were applied. Bivariate analysis was conducted using the Kruskal–Wallis test to determine differences in egg counts across different attractant types and ovitrap materials. When statistically significant differences were identified ($p < 0.05$), further pairwise comparisons were performed using the Mann–Whitney U test as a post hoc analysis.

RESULTS AND DISCUSSION

Distribution of *Aedes* spp. Eggs by Ovitrap and Attractant Type

The distribution of *Aedes* spp. eggs captured by different ovitrap materials and attractant types is presented in Table 1. The highest number of eggs was observed in ovitraps containing fermented young coconut water attractant (4203 eggs), particularly in coconut shell ovitraps (2818 eggs). In contrast, clean water attractant produced the lowest egg counts (357 eggs). Among ovitrap materials, coconut shell traps recorded the highest total egg counts (4055 eggs), followed by black plastic (1564 eggs) and bamboo traps (1250 eggs), while glass ovitraps captured only 15 eggs.

Table 1.

Distribution of *Aedes* spp. egg counts across ovitrap materials and attractant types

Ovitrap / Attractant	Glass	Coconut shell	Bamboo	Black plastic	Total
	N	N	N	N	N
Clean water	0	205	101	51	357
Red chili infusion	5	664	405	389	1463
Fermented young coconut water	10	2818	521	854	4203
Fermented palm sugar	0	368	223	270	861
Total	15	4055	1250	1564	6884

These findings are consistent with previous studies showing that organic infusion-based attractants are more effective than clean water. A study by Barrera et al. reported that infusions of organic materials such as leaves, grass, and plant waste significantly increased the number of Aedes aegypti eggs compared to plain water controls because they produced volatile compounds that attract gravid mosquitoes (Musunzaji et al., 2023). Similar results were also found by Melo et al., who showed that the use of organic infusions in ovitraps significantly increased the sensitivity of vector surveillance in various field settings (Melo et al., 2020). Another study by David et al also confirmed that physical characteristics of containers such as dark color, rough texture, and moist conditions increase oviposition preference because they resemble natural habitats such as tree holes or organic containers (David et al., 2023).

Comparison of Egg Counts among Ovitrap Types

Descriptive statistical analysis revealed clear differences in ovitrap performance (Table 2). Coconut shell ovitraps recorded the highest mean egg count (mean = 67.58), followed by black plastic (mean = 26.07) and bamboo ovitraps (mean = 20.83). Glass ovitraps exhibited the lowest mean egg count (mean = 0.25). The wide confidence interval observed in coconut shell ovitraps reflects substantial variability but also highlights their superior egg-trapping potential relative to other materials.

Table 2.

Comparison of *Aedes* spp. egg counts captured by different ovitrap materials

Ovitrap material	N	Mean rank	Mean	Std. deviation	Min	Max	p-value
Glass	60	43.03	0.25	0.895	0	4	<0.001
Coconut shell	60	168.74	67.58	102.54	0	485	
Bamboo	60	130.48	20.83	20.23	0	88	
Black plastic	60	139.76	26.07	36.91	0	264	

Descriptive statistical differences in ovitrap performance indicate that container material significantly influences the oviposition behavior of *Aedes spp*. Coconut shell ovitraps, which produced the highest mean egg count, likely offer more favorable ecological conditions due to their dark color, rough texture, and organic composition that supports microbial growth and moisture retention, thereby enhancing chemical and visual cues for gravid females (Prasad et al., 2023). This finding is consistent with studies demonstrating that *Aedes aegypti* preferentially selects dark, organic, and rough-surfaced containers that mimic natural breeding habitats such as tree holes and leaf axils (Bentley & Day, 1989; Wong et al., 2011). In contrast, the extremely low egg counts observed in glass ovitraps can be attributed to their transparent and smooth surface, which lacks both visual contrast and semiochemical stimuli, making them less attractive for oviposition.

Differences in descriptive statistics regarding ovitrap performance in this study suggest that container material may influence the egg-laying behavior of *Aedes spp*. Specifically, containers with certain characteristics appeared to yield higher egg counts compared to others. These findings provide a basis for the hypothesis that visual factors and container material composition contribute to mosquito oviposition preferences.

This hypothesis aligns with previous research reporting that artificial or reflective containers tend to be less preferred than dark-colored substrates, which are thought to serve as important visual cues for egg-laying site selection (Ritchie et al., 2003). Furthermore, studies indicate that the presence of organic matter and microbial activity in natural containers can generate volatile compounds such as geosmin and fatty acid derivatives that potentially influence ovitrap attractiveness (Melo et al., 2020; Ponnusamy et al., 2008). Consequently, it is plausible that a combination of visual and chemical cues plays a role in determining mosquito behavioral responses.

The relatively similar performance of black plastic and bamboo ovitraps in this study suggests that dark color may be a dominant factor in attracting mosquitoes for oviposition. However, there are indications that additional characteristics such as organic content and microhabitat stability, as observed in coconut shells may further enhance ovitrap attractiveness. This is reflected in the tendency toward higher egg counts in such containers, despite the relatively wider data variation indicated by the confidence intervals.

Nevertheless, these findings should be interpreted with caution. As this study was not designed to directly test underlying mechanisms, the observed relationships remain associative in nature. Therefore, further research employing more controlled experimental approaches is required to confirm hypotheses regarding the roles of visual, chemical, and microbiological factors in *Aedes spp*. oviposition preferences and to establish the underlying causal relationships.

Comparison of Egg Counts among Attractant Types

Descriptive statistics showed that fermented young coconut water had the highest mean egg count (mean = 70.05), followed by red chili infusion (mean = 24.38) and fermented palm sugar solution (mean = 14.35). Clean water exhibited the lowest mean egg count (mean = 5.95). These results indicate a clear gradient in attractant effectiveness, with fermented organic substrates outperforming the control medium (Table 3).

Table 3.

Descriptive Statistics and Bivariate Analysis of *Aedes* spp. Egg Counts by Attractant Type

Attractant Type	N	Mean rank	Mean	Std. deviation	Min	Max	p-value
Clean Water	70.75	5.95	15.17	0	88	70.75	**<0.001**
Red Chili Infusion	139.56	24.38	20.47	0	81	139.56	
Fermented Young Coconut Water	157.93	70.05	106.72	0	485	157.93	
Fermented Palm Sugar	113.77	14.35	13.62	0	52	113.77	

Descriptive differences in egg counts across attractant types indicate that chemical composition plays a decisive role in *Aedes* oviposition behavior. Fermented young coconut water, which produced the highest mean egg count, likely generates a complex mixture of volatile organic compounds (VOCs) such as CO_2, organic acids, and microbial metabolites that strongly stimulate olfactory receptors of gravid females. This is consistent with recent evidence showing that oviposition site selection in *Aedes aegypti* is highly regulated by VOCs derived from microbial activity and organic decomposition, which act as key cues indicating suitable larval habitats (Khan et al., 2023). Studies have also demonstrated that organic infusions significantly increase egg-laying responses compared to clean water due to the presence of bioactive compounds produced during fermentation (Mosquera et al., 2023). Furthermore, recent experimental findings confirm that bacteria-associated volatiles and symbiotic microbial communities can directly mediate oviposition preference through odorant receptor pathways, enhancing egg eposition in organically enriched media (Zhao et al., 2024). In contrast, clean water lacks these chemical signals, resulting in minimal oviposition activity. Overall, the observed gradient reinforces that fermentation-based attractants provide stronger ecological and chemical stimuli, making them more effective for ovitrap optimization in vector surveillance and control.

Bivariate Analysis of Ovitrap Types

Pairwise comparisons using the Mann–Whitney test revealed statistically significant differences between glass ovitraps and all other ovitrap types ($p < 0.01$). Coconut shell ovitraps were significantly more effective than bamboo and glass ovitraps, while no statistically significant difference was observed between bamboo and black plastic ovitraps ($p = 0.508$). Overall, coconut shell ovitraps demonstrated the highest effectiveness in trapping *Aedes* eggs (Table 4).

Tabel 4.

Pairwise Comparison of Ovitrap Types Using Mann–Whitney Test

Comparison	Mean Rank Group 1	Mean Rank Group 2	p-value	Interpretation
Glass vs Coconut shell	43.03	168.74	<0.001	Significant
Glass vs Bamboo	43.03	130.48	<0.001	Significant
Glass vs Black plastic	43.03	139.76	<0.001	Significant
Coconut shell vs Bamboo	168.74	130.48	<0.05	Significant
Coconut shell vs Black plastic	168.74	139.76	<0.05	Significant
Bamboo vs Black plastic	130.48	139.76	0.508	Not significant

Pairwise analysis using the Mann–Whitney test confirms that ovitrap material significantly influences *Aedes* oviposition, with coconut shell traps demonstrating superior effectiveness compared to bamboo and glass, while bamboo and black plastic show comparable performance. This pattern aligns with recent evidence indicating that oviposition preference is strongly driven by the physicochemical properties of the container, including surface texture, moisture retention, and organic composition, which collectively create favorable microhabitats for egg deposition (Momen et al., 2025). Studies have further shown that natural substrates such as coconut shells support higher oviposition due to their ability to enhance microbial activity and water quality parameters, thereby increasing attractiveness to gravid females (Weerakoon et al., 2021). Conversely, materials lacking these characteristics, such as glass, tend to be significantly less preferred. Additionally, recent field-based research confirms that variations in container material can lead to statistically significant differences in egg counts, reinforcing the role of substrate characteristics as a key determinant of oviposition behavior (Ranasinghe et al., 2025). Overall, these findings support the conclusion that coconut shell ovitraps provide the most optimal conditions for *Aedes* egg-laying and are therefore more effective for vector surveillance.

Bivariate Analysis of Attractant Types

Bivariate analysis indicated that all tested attractants resulted in significantly higher egg counts compared to clean water ($p < 0.001$). Fermented young coconut water was significantly more effective than red chili infusion and fermented palm sugar solution. Red chili infusion also showed significantly higher effectiveness than fermented palm sugar solution. These results establish fermented young coconut water as the most effective attractant among those evaluated (Table 5).

Table 5.

Bivariate Analysis of Attractant Types

Comparison	Mean Rank Group 1	Mean Rank Group 2	p-value	Interpretation
Clean water vs Fermented palm sugar	70.75	113.77	<0.001	Significant
Clean water vs Red chili infusion	70.75	139.56	<0.001	Significant
Clean water vs Fermented young coconut water	70.75	157.93	<0.001	Significant
Fermented palm sugar vs Red chili infusion	113.77	139.56	<0.05	Significant
Fermented palm sugar vs Fermented young coconut water	113.77	157.93	<0.001	Significant
Red chili infusion vs Fermented young coconut water	139.56	157.93	<0.05	Significant

Bivariate analysis demonstrates that all organic attractants significantly increased *Aedes* egg counts compared to clean water, with fermented young coconut water showing the highest effectiveness, followed by red chili infusion and fermented palm sugar solution. These findings suggest that the increased attractiveness may be linked to the presence of volatile organic compounds (VOCs) produced during organic decomposition and microbial activity. VOCs are thought to act as olfactory cues influencing the oviposition site preferences of gravid female mosquitoes (Khan et al., 2023). Consistent with this, previous studies have reported that complex mixtures of VOCs such as aldehydes, fatty acids, and microbial metabolites can enhance oviposition stimulation in a concentration dependent manner. Consequently, media enriched with organic matter are considered more attractive than clean water (Mulatier et al., 2022).

Furthermore, it is hypothesized that the more complex microbial profiles and chemical compositions of organic substrates trigger behavioral responses in mosquitoes through specific sensory detection mechanisms. However, these mechanisms were not definitively established in the present study, and further research is required to confirm the underlying causal relationships. Therefore, the superior performance of fermented young coconut water likely reflects a higher level of biochemical complexity, which provides a stronger ecological signal for the selection of oviposition sites. The limitations of this study include the small scope, the relatively short observation period, the absence of species identification, the failure to report ovitrap indices, the lack of chemical characterization of the mosquito attractants, the absence of environmental covariate analysis, and the inability to assess the impact on mosquito population density or the incidence of dengue fever.

CONCLUSIONS

This study demonstrates that both ovitrap material and attractant type significantly influence the oviposition behavior of *Aedes* spp. mosquitoes in dengue-endemic environments. Among the ovitrap materials tested, coconut shell ovitraps showed the highest effectiveness in capturing mosquito eggs, followed by black plastic and bamboo ovitraps, while glass ovitraps exhibited the lowest performance. Similarly, among the attractants evaluated, fermented young coconut water produced the highest mean number of eggs compared with red chili infusion, fermented palm sugar solution, and clean water as the control. Statistical analysis confirmed significant differences among both ovitrap materials and attractant types, indicating that natural substrates and fermentation-based attractants provide stronger oviposition cues for *Aedes* mosquitoes. Overall, These findings demonstrate that fermented young coconut water, combined with coconut shell traps, exerts a strong oviposition-attracting effect on *Aedes* spp. mosquitoes. This combination offers a potential, eco-friendly alternative method for optimizing community-based dengue vector surveillance systems in Sokaraja District, Banyumas Regency, Central Java. The practical implications of this study indicate that the use of coconut shells and fermented young coconut water provides a cost-effective, environmentally friendly, and locally accessible approach capable of enhancing community participation in dengue surveillance programs. This translational aspect represents a key strength of the study.

SUGGESTION

Based on the findings of this study, several recommendations can be proposed. First, the use of mosquito egg traps made from natural materials—such as coconut shells combined with fermented young coconut water as an attractant—can serve as an alternative method for dengue surveillance and control; this approach is cost-effective, environmentally friendly, and locally accessible, potentially boosting community participation in dengue surveillance programs. Second, public health authorities and community health centers are encouraged to promote the use of simple and locally available ovitrap technologies as part of integrated vector management programs to improve community participation in dengue prevention. Third, further studies are recommended to evaluate the long-term effectiveness of these ovitrap combinations, including their impact on reducing mosquito population density and dengue transmission risk. Future research may also explore other potential natural attractants, different ovitrap designs, and environmental factors that may influence oviposition behavior of *Aedes* mosquitoes in various ecological settings.

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