Reassessing *Aedes albopictus* oviposition: Influence of trap colour and water source

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ABSTRACT *Aedes* mosquitoes, the primary vectors of dengue, yellow fever, chikungunya, and Zika, remain a significant public health threat despite ongoing control efforts. Their adaptability to environmental changes has contributed to the continued rise in disease incidence. This study aims to evaluate and update the oviposition preferences of *Aedes albopictus* with respect to ovitraps colour and water sources across various sites at Universiti Sains Malaysia. The experiment tested five ovitraps colours: black, blue, green, orange, and red, alongside four water sources: rainwater, pond water, seasoned tap water, and distilled water. Over the study period, 2,640 eggs were collected, revealing significant differences (P<.05) in oviposition preferences for both ovitraps color (P = 0.012) and water source (P = 0.049). Black ovitraps attracted the highest number of eggs (672), followed by red (609), orange (410), blue (233), and green (190). Among the water sources, rainwater was most preferred (788 eggs), followed by pond water (725), seasoned tap water (630), and distilled water (497). Location 3 (School of Biological Sciences) recorded the highest egg count (972 eggs), although location differences were not statistically significant (P=0.434). Correlation analysis indicated a positive association between mosquito oviposition and water temperature (rs = 0.638; P = 0.047, P< .05). These findings highlight the importance of considering both ovitraps' colour and water source in mosquito surveillance and control strategies, as these factors significantly influence the oviposition behaviour of *Ae. albopictus*. The results contribute valuable insights into optimizing ovitrap designs and selecting suitable water sources for more effective mosquito management in urban and suburban environments.

KEYWORDS: *Aedes albopictus*, oviposition preferences, ovitrap colour, water sources, vector control strategies
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INTRODUCTION

The *Aedes albopictus* mosquito, also known as the Asian tiger mosquito, is a primary vector for several dangerous arboviruses, including dengue, chikungunya, yellow fever, and Zika. These diseases collectively pose a serious threat to global public health (WHO, 2024). Over the past few decades, the distribution of *Ae. albopictus* has expanded significantly, driven by its extraordinary adaptability to diverse environmental conditions. This adaptability allows it to thrive in both urban and suburban settings, often outcompeting native mosquito species (Tedjao *et al.*, 2020). The ongoing challenge is that despite various vector control efforts ranging from insecticide use to habitat modification mosquito-borne disease cases continue to rise. This trend is largely due to the mosquito's ability to adjust to environmental changes, complicating efforts to reduce their populations (Dusfour *et al.*, 2022).

Ovitraps, which are simple yet effective tools for monitoring and controlling mosquito populations, have become a key component in integrated vector management strategies. The effectiveness of these traps, however, is influenced by several factors, particularly the physical characteristics that attract female mosquitoes for egg-laying (Figurskey *et al.*, 2022). Among these characteristics, the color of the ovitrap and the quality of the water inside the trap are particularly important. Recent studies suggest that darker-colored traps, especially black, tend to attract more mosquitoes than lighter colors (David *et al.*, 2011). Similarly, the type of water used in the ovitraps whether it's rainwater, pond water, or treated water can influence the mosquito's choice for oviposition, with natural water sources (ponds, marshes, and rainwater) generally being more attractive (Dalpadado *et al.*, 2022).

In Malaysia and other Southeast Asian countries, several studies have examined *Aedes* mosquitoes' oviposition behaviour to enhance vector control strategies. For instance, research in Penang, Malaysia, indicated that *Ae. albopictus* strongly preferred oviposition in containers with dark-coloured walls, such as black and dark blue, over lighter-coloured containers (Jung *et al.*, 2021). Another study in Thailand found that *Ae. aegypti* and *Ae. albopictus* showed a preference for natural water sources, like rainwater and pond water, over artificially treated water (Dalpadado *et al.*, 2022). These findings are consistent with similar research across Southeast Asia, where the region's unique environmental conditions and rapid urbanization provide ideal breeding grounds for *Aedes* mosquitoes (Ferede *et al.*, 2018).

Although there is extensive data on *Aedes* oviposition preferences, it is crucial to periodically reassess these behaviours. Rapid urbanization, climate change, and shifts in human activity can dramatically alter the environments where mosquitoes breed (Kolimenakis *et al.*, 2022). Additionally, *Ae. albopictus* is known for its ability to adapt quickly, which may result in changes to its oviposition preferences that render previous data outdated. For example, changes in water management practices or the introduction of new building materials could influence mosquito behaviour in ways that were not previously accounted for (Kolimenakis *et al.*, 2022). Therefore, updating our understanding of these preferences, particularly in specific locations like Universiti Sains Malaysia, is essential for developing effective and contextually relevant vector control strategies. Additionally, the data can serve as a valuable reference for future research, providing new ideas for improving targeted mosquito management in evolving environments. This study aims to evaluate and update the oviposition preferences of *Ae. albopictus* concerning ovitrap colour and water sources at various locations within Universiti Sains Malaysia**.**

METHODOLOGY

Study Area

The research was conducted at Universiti Sains Malaysia (USM) in Gelugor, Penang, Malaysia, between November 2023 and March 2024. The USM landscape features a blend of urban structures and green spaces, offering various potential breeding sites for *Aedes* mosquitoes. The geographical coordinates of USM are 5.3556°N latitude and 100.3025°E longitude. The region's tropical climate, characterized by high humidity and temperature, is conducive to mosquito breeding. Three specific locations were selected as study sites based on their accessibility and the likelihood of being utilized by *Aedes* mosquitoes for oviposition-Location 1, Cafe Bakti Siswa, Location 2, Cafe Cahaya Gemilang, Location 3, School of Biological Sciences (Figure 1).

Ovitrap Preparation and Placement

Five hundred mililitres (500 ml) tin cans were repurposed as ovitraps (Tang *et al.*, 2007). These cans were sourced from university cafés, cleaned with detergent, and sun-dried before being spray-painted in five different colors: black, blue, green, red, and orange. In the ovitrap color preference study, 15 ovitraps were placed at each location, totaling 45 cans, with 9 cans per color. At each site, the five differently colored ovitraps were arranged side by side at 5 cm intervals under shady areas, with three replicates per site. For the water source preference study, only black-painted ovitraps were used, with a total of 36 cans deployed in the field. Each ovitrap was equipped with a paddle, serving as a substrate for egg-laying by female *Aedes* mosquitoes (Barrera, 2022). The two study areas were within a 200-meter radius of each other. In both studies, the ovitraps were placed at 0700 hrs and left undisturbed for five consecutive days. This procedure was repeated twice a month from November 2023 to March 2024.

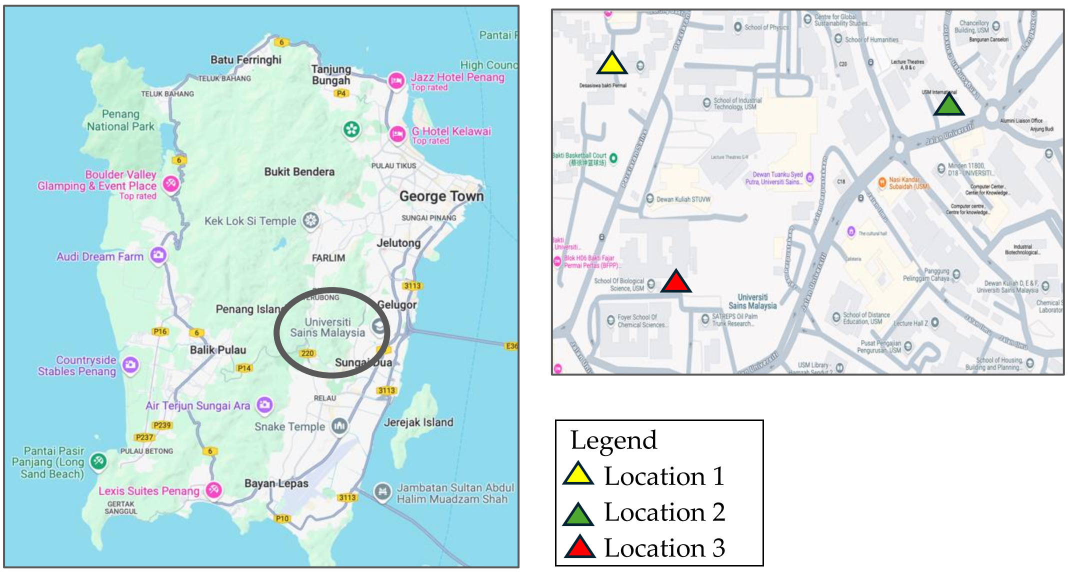

Figure 1. Sampling locations at three different areas at USM, Pulau Pinang

Collection of Water Source

Four types of water sources were utilized in this study to simulate the varying conditions that mosquitoes might encounter in natural and urban environments: distilled water, collected from the laboratory at the School of Biological Sciences, USM; seasoned tap water, collected from the USM campus and left to stand for a few days to allow for dichlorination (the removal of chlorine); pond water, collected from Harapan Lake within the university; and rainwater, collected during rainfall events on the university campus. A total of 36 black-painted ovitraps were used in this study.

Sample Processing and Identification

After the 5-day period, the ovitraps were collected and taken back to the laboratory for sample processing. The paddles were carefully removed and examined under a stereo microscope to count the eggs. Any larvae found in the ovitraps were filtered out and transferred into petri dishes using a pipette (Ahmad-Azri *et al.*, 2019). The larvae were then placed on glass slides, covered with cover slips, and identified to the species level using identification keys that focus on the comb scales of the 8th abdominal segment (OECD, 2018). For example, *Ae. aegypti* can be distinguished by the presence of a medial spine without stout subapical spines, which are absent in *Ae. albopictus* (Nelson, 1986). The contents of each overtip, including the paddle, were transferred into labeled plastic cups, covered with plastic lid, and monitored for larval development. Collected eggs were reared in the laboratory for one week until they hatched into larvae. Fish food was provided to support larval growth, and the Larval 3 and Larval 4 stages were observed under a light microscope for species identification. These procedures were repeated for all three sampling sites.

Mosquito Culturing

To culture the mosquito larvae, the paddles from the ovitraps were soaked in seasoned water placed in plastic cups. After three days, the water was sieved to collect any larvae or pupae present. The larvae were then transferred into labeled glass bottles and fed small amounts of fish pellets to support their growth. Once the larvae reached the third or fourth instar stage, they were collected and prepared for species identification using the methods described earlier (Ahmad-Azri *et al.*, 2019).

Statistical Analysis

Data gathered from the field and laboratory analyses were recorded and analyzed using IBM SPSS Statistics Version 27. For each site, average values of temperature, pH, precipitation, and humidity were calculated. To assess the preferences of *Aedes* mosquitoes for various ovitrap colors and water sources, and to evaluate the influence of physical factors on oviposition, several statistical tests were conducted. A One-Way ANOVA was performed to quantify the number of eggs laid by *Ae. albopictus*. The Kruskal-Wallis H test and Spearman correlation were used to compare temperature and humidity means. A p-value of less than 0.05 was considered statistically significant, indicating a meaningful difference or relationship within the data (Njila *et al*., 2023).

RESULT AND DISCUSSION

A total of 2,114 eggs were collected from ovitraps of various colours across three localities, revealing distinct preferences among the mosquitoes as shown in Figure 2. The black ovitraps attracted the highest number of eggs, totalling 672 (32%). This was followed by the red ovitraps, which accounted for 609 eggs (29%), orange with 410 eggs (19%), blue with 233 eggs (11%), and green with the least, 190 eggs (9%). In summary, black ovitraps are the most effective in attracting *Ae. albopictus* mosquitoes, regardless of location. This suggests that the black colour overtip is a significant factor in the oviposition behaviour of these mosquitoes. Red and orange ovitraps also show some level of attractiveness, but not as consistently as black. Blue and green ovitraps are the least attractive, indicating that these colours are less effective for mosquito egg-laying. Additionally, a one-way ANOVA with Bootstrap analysis, due to the non-normal distribution of data, indicated significant differences in oviposition preferences across the different colours (Welch's F (4,60) = 3.980, $P = 0.012$), confirming that *Ae. albopictus* demonstrated a clear preference for certain colours.

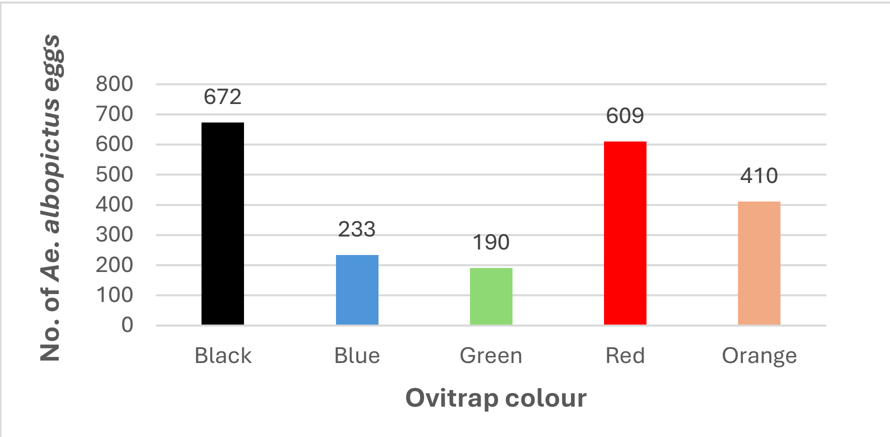

Figure 2. Bar chart showing the total number of *Ae. albopictus* eggs laid in ovitraps of different colors

On the other hand, Table 1 shows the distribution of eggs laid across three different locations. The Chi-Squared test yielded a value of 30.83 with a p-value of 0.000151. This indicates that there is a statistically significant difference in the number of eggs laid between the different locations. The significant p-value suggests that the distribution of eggs laid is not uniform across the locations. This could imply that certain locations are more favorable for egg-laying, possibly due to environmental factors, availability of resources, or other location-specific conditions. Environmental conditions play a crucial role in egg-laying behavior (Yu *et al*., 2021). Factors such as temperature, humidity, and light exposure can significantly influence where insects choose to lay their eggs. Certain locations may offer optimal conditions that promote egg survival and development (Waldock *et al*., 2013). The availability of food and suitable oviposition sites places where eggs are laid is crucial. Areas rich in resources are more likely to attract egg-laying insects, as the presence of food and proper nesting sites increases the likelihood of larval survival (Khallaf & Knaden, 2020).

Table 1. Distribution of eggs laid by *Ae. albopictus* in ovitraps of different colours colours across various locations.

Ovitrap colours	Total egg laid			Chi-squared test χ^2
	Location 1	Location 2	Location 3	
Black	114	250	308	30.83 *P*=0.000151 (*P*< .05)
Blue	72	72	89	
Green	51	60	79	
Red	126	207	276	
Orange	109	119	182	
TOTAL	472	708	934	

The presence of predators, competitors, or disturbances can deter insects from laying eggs in certain locations. Insects generally prefer sites that provide protection from predators and harsh environmental conditions for egg-laying (Yu *et al.*, 2021). Human activities and population growth can affect mosquito habitats and breeding patterns, particularly for some mosquito-borne disease vectors. For example, pesticides can spread into ponds, killing fish that usually eat mosquito larvae. Without these natural predators, mosquito numbers can increase (Khallaf & Knaden, 2020).

In addition to colour, the study evaluated the influence of different water sources on the oviposition preferences of *Ae. albopictus*. A total of 2,640 eggs were distributed across seasoned tap water, distilled water, rainwater, and pond water, each contributing distinctly to the overall egg count. Rainwater attracted the most eggs laid, with 788 (29.85%) collected, followed by pond water with 725 eggs (27.46%), seasoned tap water with 630 eggs (23.86%), and distilled water with 497 eggs (18.83%) (Figure 3). A significant difference was observed in the mean number of *Aedes* eggs across these water sources (P = 0.049).

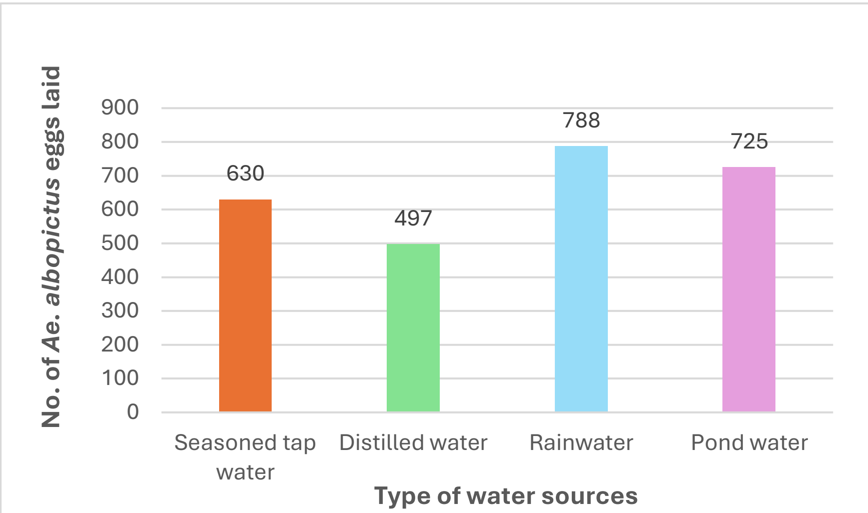

Figure 3. Total number of *Ae. albopictus* eggs laid in ovitrap of different water sources

Table 2 presents the mean values of parameters across different water sources. The highest mean water temperature was recorded in seasoned tap water, with an average of 27.53°C ± 0.21, while the lowest was in rainwater, averaging 27.21°C ± 0.23. Statistical analysis of Kruskal-Wallis H test showed no significant variation in water temperature among the different water sources (P = 0.549). Whilst, the highest mean pH value was observed in pond water, at 7.46 ± 0.07, with rainwater displaying the lowest mean pH of 6.68 ± 0.14. Spearman's correlation test shows a positive correlation with a significant difference of a water temperature, 27.21 ± 0.23 °C (rs = 0.638; *P* = 0.047, *P*< .05). However, there was no correlation with a pH.

Table 2. Mean values of parameters in different water sources

Water sources	Temperature (°C)	pH
Seasoned tap water	27.53 ± 0.21	7.36 ± 0.47
Distilled water	27.35 ± 0.23	6.89 ± 0.27
Rainwater	27.21 ± 0.23	6.68 ± 0.14
Pond water	27.38 ± 0.11	7.46 ± 0.07

Additionally, the water source also plays a critical role in oviposition, with rainwater being the most preferred, followed by pond water, seasoned tap water, and distilled water (Njila *et al.*, 2022). The high organic matter content in rainwater and pond water likely provides essential resources for mosquito larval development, making these water types more attractive for oviposition (Zhang *et al.*, 2022; Guillemette *et al.*, 2023). Conversely, the presence of chlorine in tap water and the lack of nutrients in distilled water may deter mosquitoes from laying eggs in these environments. The study's findings underscore the adaptability of *Ae. albopictus* to different breeding conditions, with the species showing a higher oviposition rate in various water sources compared to *Ae. aegypti*, possibly due to its superior adaptability and aggressive larval behaviour (Paupy *et al.*, 2009; O'Neal & Juliano, 2013). These insights are crucial for developing targeted mosquito control strategies, particularly in managing breeding sites in urban and suburban environments.

CONCLUSION

This study highlights the significant influence of ovitrap colour and water source on the oviposition preferences of *Aedes albopictus*. Black and red ovitraps were most effective in attracting gravid females, with black slightly preferred, while rainwater was the most favoured water source, likely due to its higher organic content which support larval development. These results emphasize the importance of optimizing ovitrap design for mosquito surveillance and control. Given the adaptability of *Ae. albopictus* to varying environmental conditions, future research should explore the impact of factors like light intensity and humidity, test additional colours and water sources, and assess regional differences to develop more effective mosquito management strategies.

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