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Evaluating AedesTech Mosquito Home System (AMHS) Effectiveness on *Aedes* Mosquitoes

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ABSTRACT

Ovitrap deployment stands as a viable strategy for *Aedes* mosquito control. This study evaluated the efficacy of an autodissemination ovitrap called AedesTech Mosquito Home System (AMHS), which incorporates pyriproxyfen. The study encompassed laboratory trials. Within the laboratory trials, our investigations unfolded across two species of mosquitoes: *Aedes albopictus* and *Aedes aegypti*. Three distinct facets were explored in the laboratory trials: the influence of an attractant on the oviposition, the effect of trap positioning on oviposition, and the selection of oviposition sites. Our laboratory results indicated that the Mosquito Home Aqua (MHAQ) solution with attractant consistently attracted *Ae. aegypti* effectively (Welch's Analysis of Variance) F (2,68.66) =5.22, p=0.01). However, its efficacy with *Ae. albopictus* was suboptimal compared to other treatments (Two-way ANOVA, F=0.16, df=2, p>0.05), highlighting the need for considering additional attractants. Notably, the placement of AMHS exhibited no discernible impact on its attractiveness for both mosquito species (T-test, p>0.05), underscoring the flexibility in trap deployment. The occurrence of simultaneous oviposition choices within the same replicates hinted at the possibility that the existing attractant in MHAQ did not significantly influence oviposition (p> 0.05). Therefore, eliminating the attractant is suggested to reduce the cost of AMHS production. Overall, our investigation underscores the promising potential of AMHS for *Aedes* control, especially *Ae—aegypti* and substantiated by robust statistical evidence gleaned from this controlled laboratory study.

Keywords: *Aedes*, dengue, mosquito, ovitrap, pyriproxyfen

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INTRODUCTION

Entomological studies encompass laboratory and field testing and are indispensable for comprehensively assessing the effects of control methods on *Aedes* mosquitoes (Ferguson et al., 2008). Field testing, for example, can be employed to compare the efficacy of an ovitrap with other methods in identifying *Aedes* abundance (Gao et al., 2019). Meanwhile, a laboratory study, such as determining the most attractive colour of traps, can potentially influence the future design of the ovitrap (Khan et al., 2023).

Laboratory testing has many advantages for checking the efficacy of any methods in controlling *Aedes* mosquitoes. One of the advantages of laboratory testing is that it allows for the controlled selection of mosquito strains with a wide range of insecticide resistance phenotypes and genotypes (Thornton et al., 2020). Furthermore, laboratory testing can establish the concentration range that effectively kills *Aedes* mosquitoes at any stage of their development, unaffected by any uncontrolled factor such as rain (Reza & Ilmiawati, 2020). Laboratory testing is usually less laborious and time-consuming than field testing, as evidenced by a study in the Philippines where ten locations were used for field testing and conducted over a year (Gualberto & Demayo, 2022).

Numerous laboratory studies demonstrate the testing of various instruments and chemicals to control *Aedes* mosquitoes by exploiting their oviposition behaviour (Musunzaji et al., 2023; Snetselaar et al., 2014; Tawatsin et al., 2019). For example, an ovitrap using carpet shell extract as an attractant was proven to effectively draw in dengue vectors *Aedes albopictus* and *Aedes aegypti* for oviposition in a laboratory setting (Tawatsin et al., 2019). Another study showed that *Piper betle* L. essential oil concentration can act as a repellent against the oviposition of *Ae. aegypti* in a laboratory setting (Martianasari & Hamid, 2019). A separate study proved the attractiveness of banana infusion as a potential attractant for *Ae. aegypti* oviposition activity by assessing the number of eggs deposited after four days (Musunzaji et al., 2023).

This study utilised the AedesTech Mosquito Homes System (AMHS) trap, an ovitrap capitalising on *Aedes* mosquito oviposition behaviour. It utilises a 'lure and kill' strategy with an undisclosed mosquito lure agent (Lim, C. H, personal communication, September 22, 2020). The device includes an auto-dissemination feature that enables female mosquitoes to unintentionally spread the pyriproxyfen insecticide to other breeding sites (Man et al., 2020). Pyriproxyfen, which is an insect growth regulator that disrupts juvenile mosquito development by mimicking juvenile hormones, has been proven to change both the ethology and physical characteristics of *Ae. aegypti* (Campos et al., 2023; Fansiri et al., 2022; Fiaz et al., 2019).

Previous studies on AMHS in laboratory settings were limited. Only one study by Mohd Ngesom et al. (2021) explored the effects of different Mosquito Home Aqua Solution (MHAQ) dosage levels on *Ae. aegypti*, including their emergence, autodissemination

events, preference for MHAQ over water, direct impact on larvae, and effects on fecundity and fertility. MHAQ are the solution containing pyriproxyfen that was used with AMHS, and it was observed to cause a shrinkage in the wing length of *Ae. aegypti* mosquitoes (Mohd Ngesom et al., 2021). It is known that the wing measurement can be used as an indicator of body size (Yan et al., 2021). The body size is crucial because smaller mosquitoes lead to repeated hematophagy, elevating the risk of virus transmission through heightened interactions with humans (Tchouassi et al., 2022).

This study was performed to investigate the efficacy of AMHS traps under controlled laboratory conditions, bolstering the findings of the preceding research. This research primarily aimed to establish the effects of attractant on oviposition, optimal trap placement position, and oviposition selection in the AMHS in response to the attractant and variant of trap placements.

MATERIALS AND METHODS

This experimentation seeks to replicate outdoor settings with alterations made in accordance with the indoor setting under laboratory conditions with the methodology proposed by Roque and Eiras (2008) and the World Health Organization (WHO, 2018) with few alterations on the oviposition substrate and time of exposure. These studies encompassed two *Aedes* species, *Ae. aegypti* and *Aedes albopictus*. This inclusion was motivated by the prevalence of *Ae. aegypti* and *Ae. albopictus* in regions on Penang Island afflicted by dengue, as highlighted in the work of Hashim et al. (2019).

Ethical Approval

The procedures involving the use of rats for blood feeding in this study were approved by the Universiti Sains Malaysia Institutional Animal Care and Use Committee (USM IACUC) under animal ethics permission number USM/IACUC/2019/(117)(990).

Study Conditions

The testing was conducted in a spacious 30m^3 room size chamber in Laboratory 304A, Vector Control Research Unit (VCRU), Universiti Sains Malaysia. The laboratory contained two air-conditioned to control and maintain under the environment ambient between 25°C–29°C and 60%–100% humidity, providing an ideal setting condition for the experiment. The chamber featured 17 small opening windows around the walls as well as a door for entering the chamber. A small opening on the front door was used to release gravid females during the study, following Roque and Eiras (2008) and World Health Organization (WHO; 2018) methods. The researcher also used the front door to enter and exit the chamber before and after each replicate for setup and cleanup. During Study 1 and

Study 3, the researcher used the front door for OviTo linen collection and replacement at each time interval. To minimise disturbance, entry and exit were swift, with care taken to avoid direct contact with mosquitoes. Egg counting was performed outside the chamber. The chamber floor is white to enhance visibility and facilitate accurate counting of the mosquitoes, whether in the dead, knockdown, or alive state (Stupp et al., 2020).

AedesTech Mosquito Home System (AMHS) Trap

AedesTech Mosquito Home System (AMHS) traps were supplied by One Team Networks Sdn. Bhd. as an autodissemination trap (Figure 1). It comprises a black polyethene opaque bucket with dimensions of 19.70 cm (height) × 11.00 cm (bottom width) × 14.61 cm (top width) and features a plum-coloured lid. The Mosquito Home Aqua (MHAQ) solution also sponsored the trap, containing 400 ppm pyriproxyfen. Each trap was equipped with OviTo linen, a towel that allows mosquitoes to lay eggs and was used for data collection (Figure 2[a]). The dimensions of the OviTo linen are 7.5 cm in length and 17.5 cm in width (Figure 2[b]). The MHAQ solution bottle is centrally positioned and can be readily secured and detached from the bucket base. The flow of the MHAQ solution is facilitated by gravity.

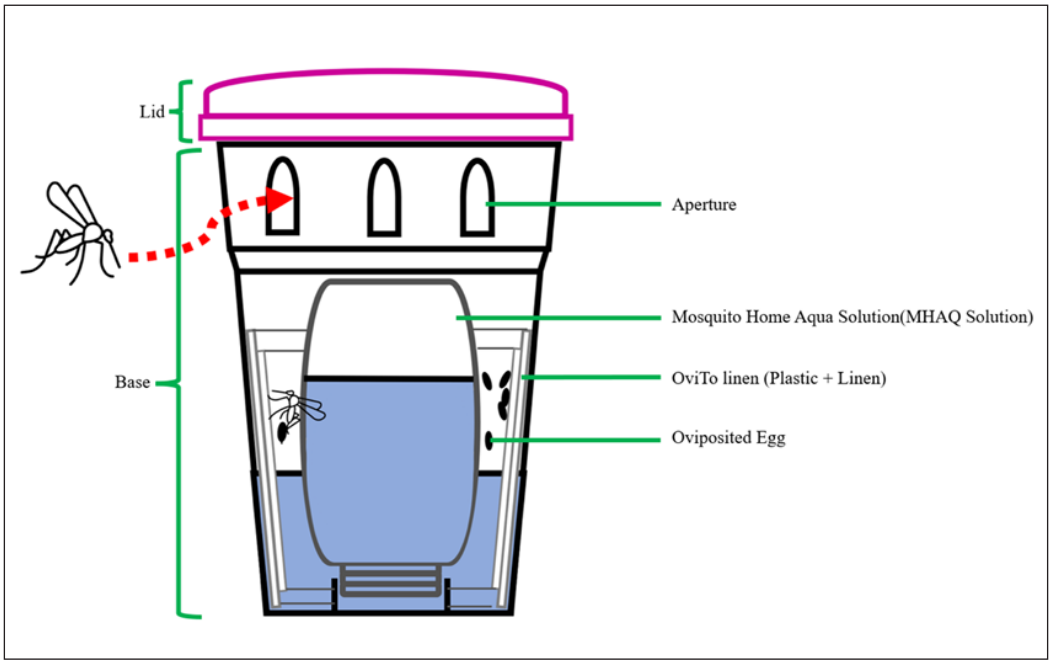

Figure 1. A concise visual representation showcasing the AedesTech Mosquito Home Trap equipped with OviTo Linen and MHAQ solution

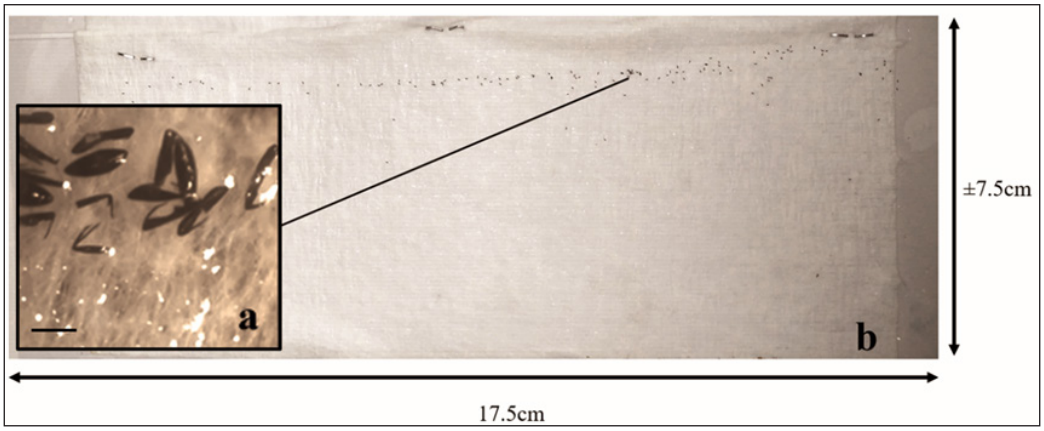

Figure 2. (a) Mosquito eggs attached to the OviTo linen (oviposition strip used in AedesTech Mosquito Home Trap) under a dissecting microscope. Scale bar = 500 µm. (b) Mosquito eggs attached to the OviTo linen and the linen's size

Gravid Female Mosquitoes

Six to eight-day-old gravid female *Ae. aegypti* and *Ae. albopictus* mosquitoes were prepared for the study by culturing eggs from a susceptible lab strain sourced from the Vector Control Research Unit (VCRU). The *Aedes* species was identified using the key by Rueda (2004). *Aedes* mosquito eggs were submerged in seasoned water trays and sorted according to species. They were kept in a controlled lab at 27 ± 2ºC with a 12:12 (L: D) light-dark cycle and 80%–90% humidity to ensure the successful hatching of larvae (Hogg & Hurd, 1997; WHO, 2018; Zuharah & Lester, 2010). A measured quantity of approximately one gram of larval nutrition, comprising a finely powdered amalgamation of dog biscuits, beef liver, yeast, and milk powder in a 2:1:1:1 ratio, was administered bi-daily (Ahbirami et al., 2014). The aqueous medium in the tray was renewed preceding each feeding session (Dieng et al., 2018, 2019).

Pupae were collected in 250 ml plastic containers filled with aged tap water and then transferred to collapsible breeding cages, each with a dimension of 30 cm^3 per layer and equipped with a screen mesh. The adults had continuous access to a 10% sugar solution (Dieng et al., 2017). Upon reaching six to eight days of adulthood, a rat was secured in a wire mesh and introduced into a breeding cage for an hour, allowing 100–200 female mosquitoes to feed on the rat (Buckner et al., 2017; WHO, 2018). The 50 selected females for each replicate were those who had taken their first blood meal after 48 hours to 96 hours before the experiment (WHO, 2018). Fully gravid females were identified by observing whitish eggs within their abdomens (Rebollar-Téllez et al., 1995; Santos et al., 2019).

Half of the gravid females were utilised to obtain eggs for the culture of the next generation, which was intended for use in upcoming replicates. These gravid females were

left in collapsible breeding cages and provided with an oviposition substrate comprising a piece of Smith Filter Papers 102 Qualitative and a black-coloured tin filled with 200 ml seasoned water (Maïga et al., 2017; Thavara et al., 2004; Yap et al., 1995).

Study Design

Three types of study were performed: (1) Effect of an attractant on female mosquitoes' oviposition, (2) Effect of trap position on female mosquitoes' oviposition, and (3) Oviposition selection by gravid females. There were three different treatments using the AedesTech Mosquito Home System (AMHS): (1) AMHS with Mosquito Home Aqua solution (MHAQ) containing an attractant, (2) AMHS with MHAQ without an attractant, and (3) a control group containing seasoned water only. All the solutions were used at a volume of 500 ml. The attractant consisted of the MHAQ provided by the One Team Network Sdn. Bhd. and the ingredients are unknown. However, the only information provided is that the attractant was derived from natural resources. The traps for this study were strategically positioned, ensuring a minimum distance of 1m between each other (Roque & Eiras, 2008; WHO, 2018).

The free-flying technique was utilised in all replicates with the gravid mosquitoes as a subject for testing following the study by Roque and Eiras (2008) and WHO (2018). A total of 50 gravid female mosquitoes were introduced into a room-sized chamber for each replication (WHO, 2018). The mosquitoes were released at the centre of the chamber from a 350 ml plastic container with a lid that was opened using a thread tied to the lid through the small opening attached to the front door. All the studies were run separately for triplicates. The data collection for all studies consisted of three distinct assessments: mean number of mosquito eggs, Hatching Index, and Emergence Rate. Manual counting for the eggs attached to the OviTo linen to determine the mean number of mosquitoes' eggs oviposited was performed thrice by two people using a magnifying glass (Gopalsamy et al., 2021). After all studies, all released mosquitoes were recaptured, and their status (alive, dead, gravid) was recorded. To ensure the validity of the assay, at least 50% of the released female mosquitoes were recaptured.

Study 1: Effect of An Attractant on Female Mosquitoes' Oviposition

In this study, we conducted an assessment within a room chamber, following the arrangement depicted in Figure 3(a). Two AMHS traps with MHAQ were placed at the horizontal position of east and west, whereas the resting place without MHAQ was placed vertically based on the study by Roque and Eiras (2008). Three treatments were run separately by replacing the treatment set with (1) AMHS with MHAQ (with an attractant), (2) AMHS with MHAQ (without an attractant), and (3) control (contained seasoned water). The AMHS traps were lined with the OviTo linen as a substrate for *Aedes* mosquito oviposition.

Subsequently, 50 gravid female mosquitoes were released freely through the small opening attached to the door. Following Roque & Eiras (2008), the OviTo linen in the AMHS trap was evaluated at 30, 60, 90, 120, 150, and 180 minutes to count deposited eggs. A researcher entered the chamber briefly at each interval to collect and replace the OviTo linen, except at the final 180-minute mark. Throughout the assessment, six replicates were carried out for each treatment. Before the next round of bioassays, the remaining mosquitos were removed. Notably, the assessment was carried out on *Ae. aegypti* and *Ae. albopictus* separately for all three treatments.

All the *Aedes* eggs laid on the Ovito linen throughout this study were checked for the mean number of eggs attached to OviTo linen, hatching and emergence index. The culture was separated between replicates, treatments, times, and *Aedes* species. After tallying the egg counts on the OviTo linen for all replicates at each time and counting for mean eggs oviposited, each OviTo linen was submerged in a tray containing seasoned water separately and was cultured following the methods in

Gravid Female Mosquitoes

The hatched larvae were counted, and the Hatching Index (HI) was determined using Equation 1. Subsequently, these larvae were nurtured until adulthood to calculate the Emergence Rate (ER) using Equation 2. The HI were counted based on Yazan et al. (2020):

$$\text{Hatching Index} = \frac{\text{No of egg that hatched}}{\text{Total no. of egg counted}} \times 100\% \text{Hatching Index} = \frac{\text{No of egg that hatched}}{\text{Total no. of egg counted}} \times 100\% \quad [1]$$

The ER were calculated and modified using percentage of emergence based on Gualberto and Demayo (2022):

$$\%\ \text{Emergence} = \frac{\text{No. of adults emerge}}{\text{Total no. eggs counted}} \times 100\% \%\ \text{Emergence} = \frac{\text{No. of adults emerge}}{\text{Total no. eggs counted}} \times 100\% \quad [2]$$

Study 2: Effect of Trap Position on Female Mosquitoes' Oviposition

A stratified random design was employed for trap placement in the study to minimise factors like position biases. Within the room-sized chamber (Figure 3 [b]), traps were positioned in two separate positions: horizontal (West to East) and vertical (North to South), according to the study by Roque and Eiras (2008).

The assessment utilised two AMHS traps with a MHAQ solution containing an attractant and two additional resting place AMHS traps for each position set (Figure 3 [b]). The study was run separately for two treatments with the same setting: AMHS with MHAQ (without an attractant) and control (with seasoned water only).

Each trial involved releasing 50 gravid female mosquitoes into the chamber cage. Each replicate was conducted for 17 hours, starting at 16:00 hours, and the traps were monitored the following morning at 9:00 am, which was aligned with the WHO protocol (WHO, 2018). This timing aimed to maximise heat stress and align with the biting cycle of *Aedes* mosquitoes. The OviTo linen was evaluated for the number of eggs present. Three replications were done for each position. The assessment was carried out separately for *Ae. aegypti* and *Ae. albopictus*.

In Study 2, procedures akin to Study 1 were followed, involving inspecting *Aedes* eggs on the OviTo linen for hatching and emergence. Cultures were segregated by replicates, treatments, position, and *Aedes* species, omitting a time-based culture. Following egg counts for each replicate to calculate the mean number of eggs oviposited, linens were individually immersed in a tray with seasoned water, per the methods outlined in *Gravid Female Mosquitoes*. Hatched larvae were calculated to determine the HI using Equation 1, followed by their maturation to adulthood for calculating the ER using Equation 2.

Study 3: Oviposition Selection by Gravid Females

This investigation was carried out to assess oviposition selection influenced by the presence of an attractant and the variation in positions of traps for *Aedes* mosquito oviposition. This part of the assessment has two types of variants for trap placement to minimise factors like position biases using a stratified random design. Two types of AMHS traps with and without attractants were placed in the chamber across each other, as shown in Figure 3(c). The assessment utilised two AMHS traps with MHAQ solution containing an attractant, along with two additional AMHS containing no attractant were used for each variant setting.

Fifty fully gravid female mosquitoes were released into the room chamber through the opening at the chamber door. Based on Roque and Eiras (2008), the OviTo linen in the AMHS trap was inspected for egg deposition at 30, 60, 90, 120, 150, and 180-minute intervals. To switch the linen, a researcher briefly accessed the chamber at each time point, excluding the 180-minute mark. Entry and exit were rapid to reduce disturbance, and egg counting was performed outside the chamber. Each variant was replicated three times. The assessment was carried out separately for *Ae. aegypti* and *Ae. albopictus*. Upon completing the Variant 1 setting assessment, the methods were repeated by replacing all the trap placements according to the Variant 2 setting.

Aedes eggs attached on the OviTo linen were counted to the mean number of eggs oviposited according to each replicate. Then, we assessed the HI and ER of *Aedes* eggs on

Ovito linen as in Studies 1 and 2. *Aedes* eggs were cultured on OviTo linens considering replicates, variants, treatments, times, and *Aedes* species. This culturing followed the methods outlined in *Gravid Female Mosquit.*

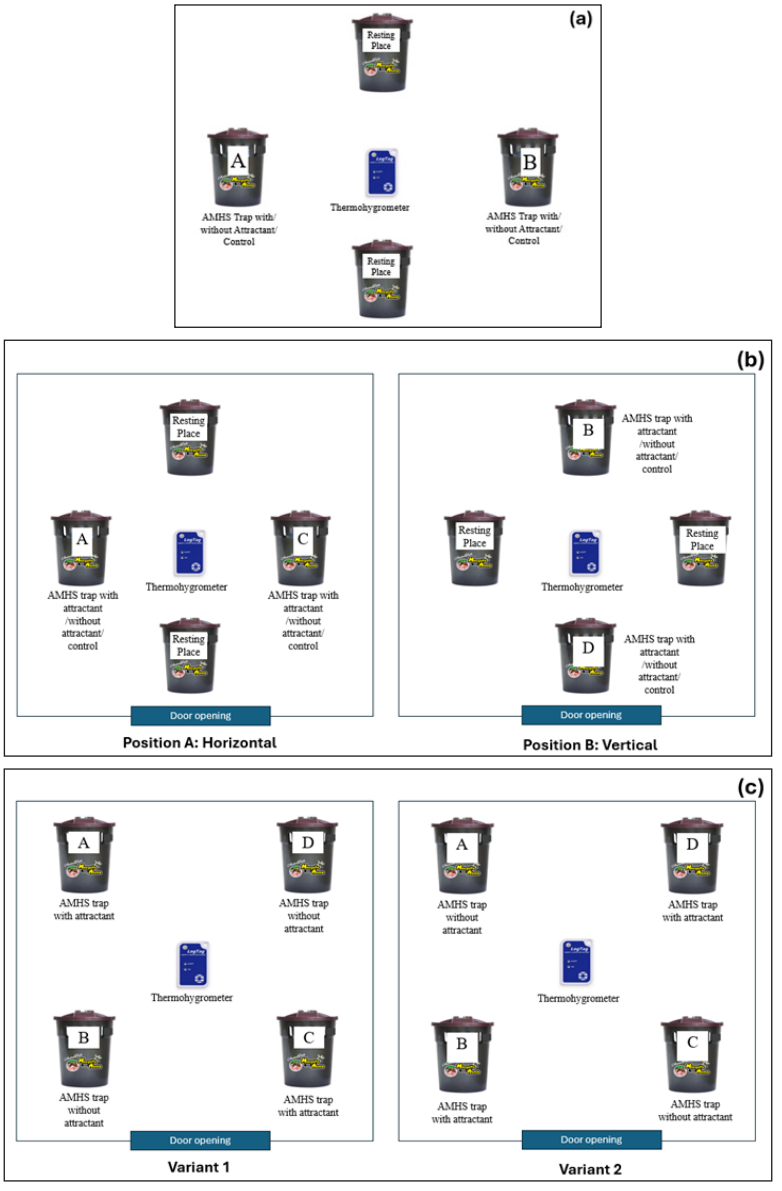

Figure 3. (a) The position of traps in the room chamber during Study 1. The study was run separately using three treatments: Aedes Mosquito Home System (AMHS) without an attractant, AMHS with an attractant, and control. (b) Treatment traps and resting traps with the thermohygrometer were placed in the middle of the room chamber for Study 2. The study was conducted separately using three treatments: AMHS without an attractant, AMHS with an attractant, and a control group in two positions. (c) The placement of treatment traps containing an attractant and without an attractant in two different variant settings in Study 3

Laboratory Condition

Temperature and humidity were carefully monitored using the Log Tag Analyzer®. Optimal conditions were maintained throughout the trials, with the temperature at around 27°C ± 2°C and the relative humidity between 60% and 80%. Additionally, a balanced 12-hour light-dark cycle (12L:12D) was implemented. These controlled conditions allowed for consistent and reliable observations and measurements during the study, minimising the influence of external factors.

Statistical Analysis

The data analysis for this study was performed utilising Statistical Package for the Social Sciences (SPSS) Version 25. All the data collected, including the mean number of eggs, HI, and ER, were subjected to the Shapiro-Wilk test to assess their distribution characteristics. All data were log-transformed using (ln(x+1)) to achieve normal distribution. Subsequently, a two-way analysis of variance (ANOVA) was independently conducted for the following data, as shown in Table 1. Then, the data were further analysed using Tukey's HSD multiple comparison test.

Table 1
List of data that were analysed using Two-way Analysis of Variance (ANOVA) in this chapter

Study	Species	Factor (s)		
		Dependent	**Fixed**	
1	*Aedes albopictus*	The mean number of eggs oviposited	Time	Treatment
		Hatching Index		
		Emergence Rate		
	Aedes aegypti	Emergence Rate		
2	*Aedes aegypti*	Hatching Index	Position	Treatment
		Emergence Rate		
3	*Aedes albopictus*	Hatching Index	Time	Variant_ Treatment
		Emergence Rate		
	Aedes aegypti	The mean number of eggs oviposited		
		Hatching Index		
		Emergence Rate		

Meanwhile, Welch's ANOVA was employed for analysis since some data did not meet homogeneity assumptions. The data underwent log transformation using (ln(x+1)) to achieve a normal distribution. Subsequently, ANOVA analyses that included Welch as an option in the statistics selection were conducted for this study. Following this, post-

hoc analysis using Games-Howell multiple comparisons was performed for datasets with more than two groups.

This study also employed independent *t*-tests for Study 2 to compare the mean numbers of eggs oviposited and the position of the trap. Initially, the data normality was confirmed using the Shapiro-Wilk test. Independent *t*-tests were then used to evaluate the influence of trap position on oviposition by gravid mosquitoes. These tests compared mean egg numbers in horizontal and vertical positions for *Ae. albopictus*.

RESULTS

Study 1: Effect of an Attractant on Female Mosquitoes' Oviposition

Effect of an Attractant on Female Mosquitoes' Oviposition, Hatching Index, and Emergence Rate in Aedes albopictus

Initially, the MHAQ with attractant had a higher mean number of eggs (2.17) compared to the MHAQ without attractant (0.42) and the control (0.75), as shown in Figure 4(a). However, this trend was inconsistent over subsequent checks, as depicted in Figure 4(a). This part uncovered the impact of the attractant in the MHAQ on *Ae. albopictus* oviposition and revealed no significant effect (Two-way ANOVA, F=0.16, df=2, p>0.05). This finding implies that the presence of an attractant has no discernible impact on oviposition in preference of *Ae. albopictus*.

Further analysis of the three-hour trials across six time points using two-way ANOVA also indicated no distinct trend in the mean number of eggs oviposited by *Ae. albopictus*. Showing that *Ae. albopictus* did not exhibit any time preferences for oviposition. The analysis highlighted that time did not significantly impact the results as the p-value was at the threshold and not less than 0.05, confirming these observations (Two-way ANOVA, F=2.29, df=5, p=0.05). Similarly, the treatments within each time point showed no difference (Two-way ANOVA, F=0.84, df=10, p>0.05). These findings collectively suggest that neither time nor treatment, individually or in combination, significantly influences the oviposition preference of *Ae. albopictus*.

The HI and ER data did not exceed 0.2% for all times and treatments. The highest recorded values were 0.19% for HI (observed during 60 minutes in MHAQ without attractant) and 0.08% for ER (noted during 120 minutes in the control). Most remaining data consistently recorded 0.00% for both HI and ER across all times and treatments, showing no variation.

Following these, we concluded that the presence of attractant in the MHAQ solution did not result in any significant differences in the HI and ER across different times (Two-way ANOVA, HI: F=0.53, df=5, p>0.05; ER: F=0.41, df=5, p>0.05), treatments (Two-way ANOVA, HI: F=0.47, df=2, p>0.05; ER: F=0.89, df=5, p>0.05), or when comparing

treatments within each time (Two-way ANOVA, HI: F=1.68, df=10, p>0.05; ER: F=1.07, df=10, p>0.05).

Effect of an Attractant on Female Mosquitoes' Oviposition, Hatching Index, and Emergence Rate in Aedes aegypti

Regarding treatment preferences alone, without considering time, *Ae. aegypti* demonstrated a significant inclination toward MHAQ with an attractant (28.65) compared to MHAQ without an attractant (5.29) (Welch's ANOVA F (2, 68.66) = 5.22, p = 0.01). However, this preference did not extend to the control (12.80). This shows that the presence of an attractant in MHAQ does affect the oviposition of *Ae. aegypti*.

When considering both timing and treatment, *Ae. aegypti* exhibited its highest mean oviposition rate at the 30-min mark, depositing an impressive 111.00 eggs in MHAQ with attractant, surpassing counts in both MHAQ without attractant (0.92) and the control (0.42) (Figure 4[b]). These results were significant according to Welch's ANOVA analysis (F [2,5.46] =7.48, p=0.03), but further checking due to homogenous violation with Games-Howell's Post-hoc Test showed no significance between the treatments (p>0.05). However, the preference of *Ae. aegypti* for MHAQ with an attractant (7.42) shifted to the control (37.92) at the 60-minute mark (Welch's ANOVA, F (2,9.01) =7.48, p=0.33) (Figure 4[b]). Subsequent observations revealed fluctuating preferences between MHAQ with the attractant and the control with no significant preference shown (Welch's ANOVA, p>0.05). Suggesting no preference time for the *Ae. aegypti* for ovipositing.

In this part, we researched the impact of treatment on the HI of *Ae. aegypti*. The HI of *Ae. aegypti* in MHAQ without attractant (0.10%) was significantly higher than in MHAQ with attractant (0.01%) and the control (0.04%) (Welch's ANOVA analysis; F (2, 51.23) = 3.69, p=0.03).

We also investigated the HI of *Ae. aegypti* over time in comparison to each treatment—the data for these metrics consistently registered values below 0.20%. Notably, the highest HI values were observed at 120 min, with a reading of only 0.19% in MHAQ without attractant, while the remaining data ranged from 0.00% to 0.17%. This pattern suggests that time does not exert a significant effect on the HI of *Ae. aegypti* in any treatments. This observation was further supported by Welch's ANOVA analysis for each treatment within each time, which indicated that time has no statistically significant impact on the HI of *Ae. aegypti* across all treatments (30 min: F (2, 6.67) = 1.54, p=0.28; 60 mins: F (2,7.78) = 0.64, p=0.56; 90 mins: F (2, 9.51) = 0.16, p=0.85; 120 mins: F (2, 7.48) = 0.77, p=0.50; 150 mins: F (2,8.89) = 0.29, p=0.76; 180 mins: F (2, 9.40) = 0.18, p=0.84).

The highest recorded ER in *Ae. aegypti* was 0.12% in MHAQ without attractant during the 120 min. It implies that approximately 99.88% of the larvae were inhibited from progressing into adult mosquitoes, indicating a notably low ER across all periods. Conversely, the lowest recorded ER was 0.00%, signifying that none of the hatched

larvae emerged as adults. Notably, there were no obvious fluctuations in the recorded ER values. This observation is reinforced by the result of Two-way ANOVA, which indicates that neither time nor treatment significantly influences the ER (Two-way ANOVA, ER: F=0.95, df=5, p>0.05), treatments (Two-way ANOVA, ER: F=2.72, df=2, p>0.05), nor when comparing treatments within each time (Two-way ANOVA, ER: F=0.68, df=10, p>0.05). It underscores the conclusion that neither time nor treatment or a combination of both exerts a substantial impact on the ER of *Ae. aegypti*.

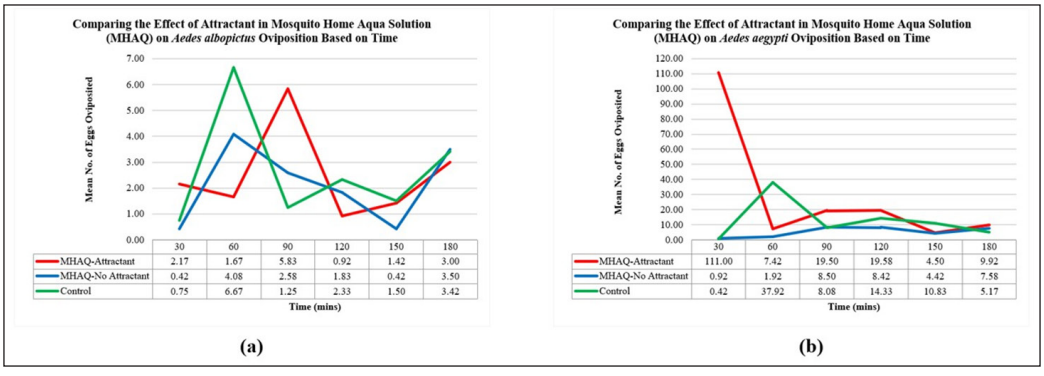

Figure 4. (a) The comparison of the oviposition of *Aedes albopictus* in all treatments at each time. (b) The comparison of the oviposition of *Aedes aegypti* in all treatments at each time

Study 2: Effect of Trap Position on Female Mosquitoes' Oviposition

Effect of the Position of Trap, Hatching Index, and Emergence Rate in Aedes albopictus

Within the control group, the *Ae. albopictus* egg oviposition data in the horizontal position were significantly higher than those in the vertical position (T-test, F=1.47, df=4, p=0.01). Specifically, the mean number of eggs oviposited in the horizontal position stood at 408, a significant contrast to the 105 recorded in the vertical position, as shown in Figure 5(a). This disparity indicates that the horizontal position yielded approximately four times as many eggs as the vertical position in control.

In the context of MHAQ with attractant, the mean number of eggs deposited in the horizontal position (96) was observed to be higher than that in the vertical position (55) (Figure 5[a]). However, despite this difference, no statistically significant variations were found in egg deposition between the horizontal and vertical positions within MHAQ with an attractant (t-test, F=3.43, df=4, p=0.22). Furthermore, there were no statistically significant differences in egg deposition between the vertical and horizontal positions in MHAQ with non-attractant (T-test, F=0.35, df=4, p=0.51). Thus, the oviposition of *Ae. albopictus* in MHAQ remains unaffected by suggesting a particular position, regardless of the presence or absence of an attractant.

In this part, we explored the impact of various treatments on the HI and ER of *Ae. albopictus*. Notably, *Ae. albopictus*'s HI in MHAQ without attractant (0.35%) significantly exceeded that in MHAQ with attractant (0.01%) and the control group (0.01%). The same situation occurred with the ER of *Ae. albopictus.* Welch ANOVA analyses confirmed the statistical significance of these differences for both HI (F [2, 8.76] = 11.63, p = 0.003) and ER (F (2, 8.96) = 17.23, p = 0.001). These findings suggest that attractant presence does not impact HI and ER in *Ae. Albopictus,* reducing these entomological parameters.

Subsequently, an investigation into the positional impact on the HI and ER of *Ae. albopictus* was undertaken. The recorded data spanned from 0.00% to 0.50% for HI and 0.00% to 0.24% for ER. In the MHAQ with attractant, both horizontal and vertical positions yielded identical HI results (0.01%) (Welch's Analysis, F (1, 4.00) = 0.54, p = 1.00). No significant difference was observed in terms of position for ER in the MHAQ with attractant, with the horizontal position marginally higher at approximately 0.01% compared to the vertical position (0.00%) (Welch's ANOVA, F (1, 2.63) = 0.310, p = 0.62). These findings indicate no observable contrast in the Hatching Index and Emergence Rate readings of *Ae. albopictus* when positioned vertically or horizontally in any treatment.

Effect of the Position of Trap, Hatching Index, and Emergence Rate in Aedes aegyptii

The data on the mean number of *Ae*. *aegypti* eggs oviposited in all treatments did not show a significant difference between both horizontal and vertical positions (t-test, p>0.05). As illustrated in Figure 5(b), although the non-attractant treatment exhibited a higher mean number of eggs collected at the vertical position (367) compared to the horizontal (94), this difference was not statistically significant (t-test, F=3.24, df=4, p= 0.28). It implies no inclination towards any specific position within the various treatments.

The positional orientation, treatment variations, and their combined influence on the HI and ER in *Ae. aegypti* were thoroughly examined. Results from the Two-way ANOVA revealed no significant effects on HI (Position: F=0.89, df=1, p>0.05; Treatment: F=1.07, df=2, p>0.05; Combination: F=0.15, df=2, p>0.05) or ER (Position: F=0.51, df=1, p>0.05; Treatment: F=2.04, df=2, p>0.05; Combination: F=0.16, df=2, p>0.05) based on position, treatment, or their combination.

The HI demonstrated a range from 0.01% to 0.15%, with the highest value recorded at 0.15% in the horizontal position within MHAQ without attractant. Similarly, the peak value for ER was observed in the horizontal position in the control group (0.13%), while the lowest was noted in the vertical position in MHAQ with an attractant (0.00%). In conclusion, neither the position, treatment type nor their interaction significantly influences the HI and ER in *Ae. aegypti*.

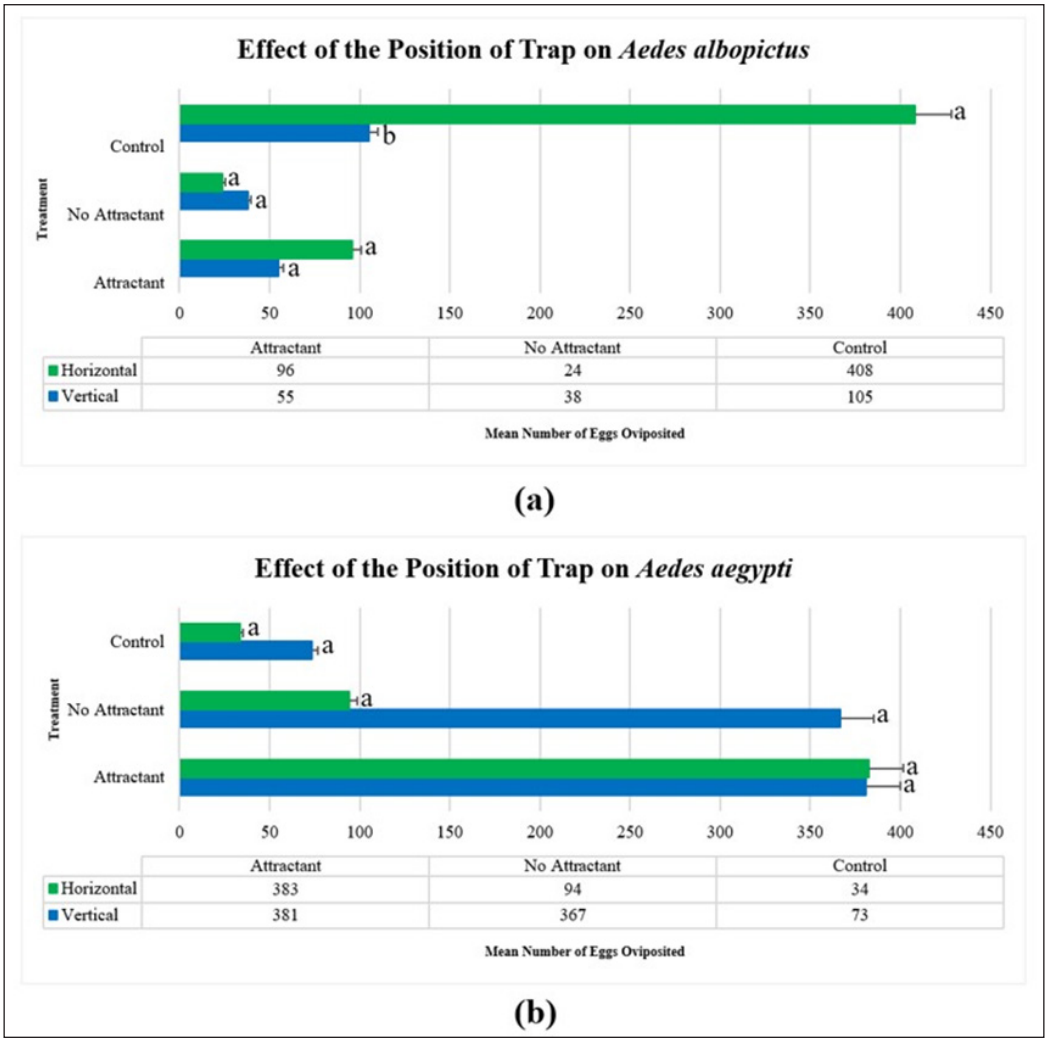

Figure 5. (a) The effects of trap position in each treatment on *Aedes albopictus* oviposition. (b) The effects of the position of the trap in each treatment on *Aedes aegypti*
Note. The same small letter shows no significant differences within the position of trap placement

Study 3: Oviposition Selection by Gravid Females

Oviposition Selection by Gravid Females, Hatching Index, and Emergence Rate in Aedes albopictuS

According to the graph in Figure 6(a), the mean number of eggs oviposited by *Ae. albopictus* in MHAQ with attractant was higher at all time points, except at 180 min, compared to MHAQ without attractant. This trend was observed for both Variant 1 (red line) and Variant 2 (red dashed line) in the attractant treatments versus Variant 1 (blue line) and Variant 2 (blue dashed line) in the non-attractant treatments. However, when we

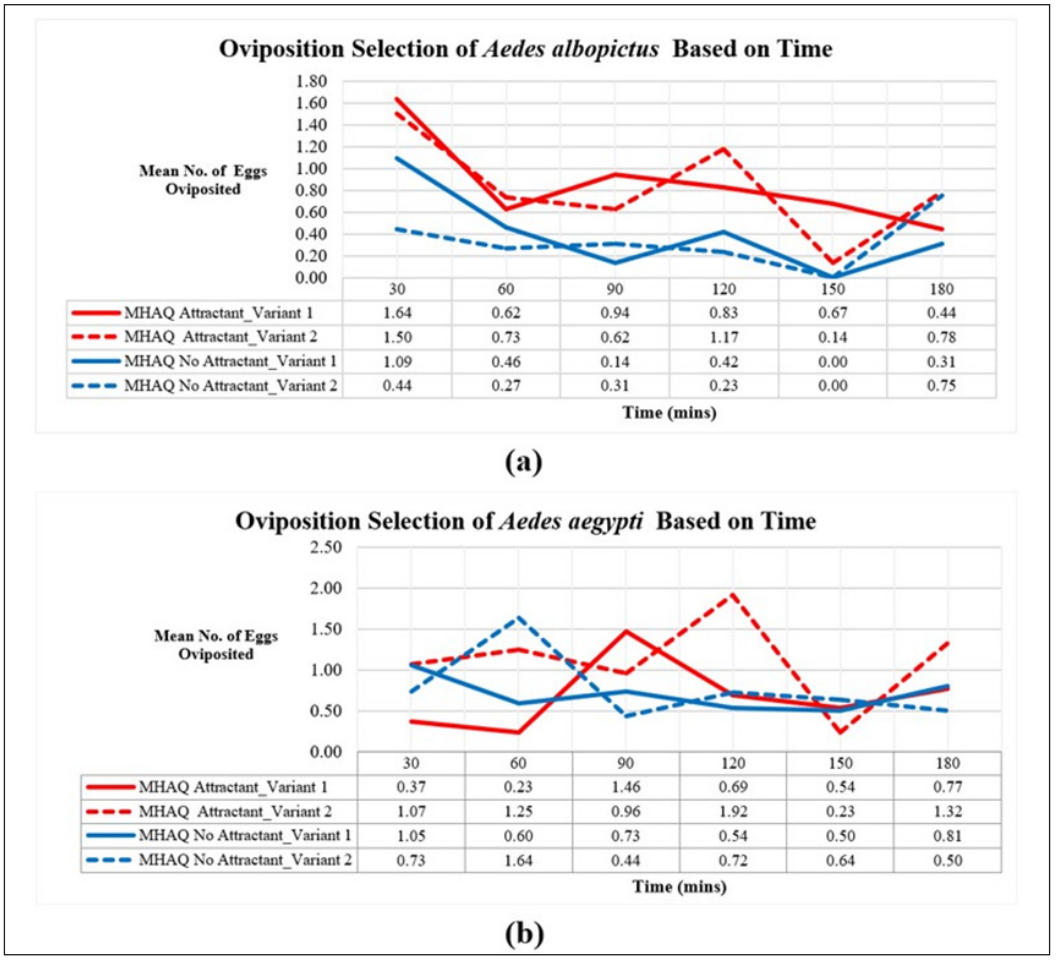

Figure 6. (a) Oviposition selection of *Aedes albopictus* based on time for MHAQ for different variants. (b) Oviposition selection of *Aedes aegypti* based on time on two variant types in MHAQ

differentiated according to the treatment and variant, there was no significant difference in the oviposition selection in *Ae. albopictus* as detailed in Table 2 (Welch ANOVA, F (3, 37.24) = 3.07, p>0.05). Suggesting there is no preference site for oviposition over the treatment and variant in *Ae. albopictus*.

However, when comparing the oviposition selection based on time, the mean number of eggs oviposited within the first 30 min was higher in comparison to the rest of the observation points (p<0.05). Specifically, the mean number of eggs laid within the first 30 min (1.17) was significantly greater than the count at 150 min (0.20) shown in Table 2. The Welch ANOVA supported these results (F (5, 30.42) = 3.36, p = 0.02). Thus, *Ae. albopictus* prefers ovipositing in the initial half-hour of trap introduction compared to the 150-min mark.

Table 2
Comparison of mean number of eggs oviposited by Aedes albopictus *using Welch's ANOVA analysis according to treatment and time in Study 3*

Treatment	**Mean no. of eggs oviposited**
MHAQ with attractant_Variant 1	0.86 [a]
MHAQ with attractant_Variant 2	0.83 [a]
MHAQ without attractant_Variant 1	0.40 [a]
MHAQ without attractant_Variant 2	0.33 [a]
Mean ± SE	0.60 ± 0.08
Time (min)	Mean no. of eggs oviposited
30	1.17 [ad]
60	0.52 [ac]
90	0.50 [ac]
120	0.66 [ac]
150	0.20 [bc]
180	0.57 [ac]
Mean ± SE	0.60 ± 0.08

Note.
*Welch's ANOVA was run for a mean number of eggs oviposited by *Ae. albopictus* with time as a factor. Then, the analysis was repeated with treatment as a factor
**The same small letter shows no significant differences within the treatment/time of the trap within a column. ***Significant result with $p<0.05$

The HI recorded ranged from 0.00% to the highest recorded at 0.14%, as indicated in Table 3. The peak Hatching Index (HI) occurred at 30 min in variant 1 with an attractant, 90 min in variant 2, and 120 min in variant 2 without an attractant. However, the HI in *Ae. albopictus* were not affected by the time (Two-way ANOVA, HI: $F=0.58$, $df=5$, $p>0.05$) treatment (Two-way ANOVA, HI: $F=0.70$, $df=3$, $p>0.05$) and the combination of both (Two-way ANOVA, HI: $F=1.31$, $df=15$, $p>0.05$).

Correspondingly, the ER of *Ae. albopictus* exhibited a similar range in line with the HI. These findings suggest that inhibition of *Ae. albopictus* adults emerged with the highest inhibition values, reaching 100.00% observed in almost all treatments and times, except for a few instances (six data points), as listed in Table 3. However, ER in *Ae. albopictus* were not affected by the time (ER: $F=0.56$, $df=5$, $p>0.05$), treatment (ER= $F=0.58$, $df=3$, $p>0.05$) and the combination of both (ER: $F=1.22$, $df=15$, $p>0.05$). In conclusion, the HI and ER of *Ae. albopictus* were unhindered by the time, and the treatment was introduced despite the presence of a high percentage of inhibitions.

Table 3
Comparison of the hatching index and emergence rate according to time and treatment for Aedes albopictus *and* Aedes aegypti *in Study 3*

Time (mins)	Treatment	Species			
		Aedes albopictus		Aedes. aegypti	
		Hatching index (%)	Emergence rate (%)	Hatching index (%)	Emergence rate (%)
30	Variant 1 with Attractant	0.14 [a]	0.09 [a]	0.00 [a]	0.00 [a]
	Variant 1 without Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
	Variant 2 with Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
	Variant 2 without Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
60	Variant 1 with Attractant	0.02 [a]	0.02 [a]	0.14 [a]	0.00 [a]
	Variant 1 without Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
	Variant 2 with Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
	Variant 2 without Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
90	Variant 1 with Attractant	0.00 [a]	0.00 [a]	0.14 [a]	0.00 [a]
	Variant 1 without Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
	Variant 2 with Attractant	0.14 [a]	0.13 [a]	0.00 [a]	0.00 [a]
	Variant 2 without Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
120	Variant 1 with Attractant	0.02 [a]	0.02 [a]	0.00 [a]	0.00 [a]
	Variant 1 without Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
	Variant 2 with Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
	Variant 2 without Attractant	0.14 [a]	0.14 [a]	0.00 [a]	0.00 [a]
150	Variant 1 with Attractant	0.00 [a]	0.00 [a]	0.07 [a]	**0.07** [a]
	Variant 1 without Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
	Variant 2 with Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
	Variant 2 without Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
180	Variant 1 with Attractant	0.05 [a]	0.05 [a]	0.00 [a]	0.00 [a]
	Variant 1 without Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
	Variant 2 with Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
	Variant 2 without Attractant	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
Mean ± SE		0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00

Note:
*Two-way ANOVA was run separately for the hatching index and emergence rate with time and treatment as factors
**Analysis was run separately according to species of mosquitoes
***The same small letter shows no significant differences within the position of trap placement
***Significant result with $p<0.05$

Oviposition Selection by Gravid Females, Hatching Index, and Emergence Rate in Aedes aegypti

Figure 6(b) illustrates the oviposition selection behaviour of *Ae. aegypti*, revealing no specific trend throughout the conducted experiment. Upon the initial introduction of the traps, MHAQ with attractant-Variant 2 (1.07) exhibited the highest mean number of eggs oviposited, followed by MHAQ without attractant-Variant 1 (1.05), MHAQ with attractant-Variant 1 (0.37), and MHAQ without attractant-Variant 2 (0.73) (Table 4). Subsequently,

Table 4
Comparison of the mean number of eggs oviposited according to time and treatment for Aedes aegypti *in Study 3*

Time (min)	Treatment	Mean no. of eggs oviposited
30	Variant 1 with Attractant	0.37 [a]
	Variant 1 without Attractant	1.05 [a]
	Variant 2 with Attractant	1.07 [a]
	Variant 2 without Attractant	0.73 [a]
60	Variant 1 with Attractant	0.23 [a]
	Variant 1 without Attractant	0.60 [a]
	Variant 2 with Attractant	1.25 [a]
	Variant 2 without Attractant	1.64 [a]
90	Variant 1 with Attractant	1.46 [a]
	Variant 1 without Attractant	0.73 [a]
	Variant 2 with Attractant	0.96 [a]
	Variant 2 without Attractant	0.44 [a]
120	Variant 1 with Attractant	0.69 [a]
	Variant 1 without Attractant	0.54 [a]
	Variant 2 with Attractant	1.92 [a]
	Variant 2 without Attractant	0.72 [a]
150	Variant 1 with Attractant	0.54 [a]
	Variant 1 without Attractant	0.50 [a]
	Variant 2 with Attractant	0.23 [a]
	Variant 2 without Attractant	0.64 [a]
180	Variant 1 with Attractant	0.77 [a]
	Variant 1 without Attractant	0.81 [a]
	Variant 2 with Attractant	1.32 [a]
	Variant 2 without Attractant	0.50 [a]
Mean ± SE		0.82 ± 0.10

Note:
* Two-way ANOVA was run separately for the Hatching Index and Emergence Rate with time and treatment as factors
**The same small letter shows no significant differences within the time/treatment of the trap within a column
***Significant result with $p<0.05$

the highest mean number of oviposited eggs alternated between MHAQ without attractant-Variant 2, MHAQ with attractant-Variant 1, and MHAQ with attractant-Variant 1 in the following time intervals. Analysis of these data suggests that there is no specific preference for oviposition in *Ae. aegypti* concerning time (Two-way ANOVA, F=0.57, df=5, p>0.05), treatment (Two-way ANOVA, F=1.16, df=3, p>0.05), or treatment within each time (Two-way ANOVA, F=0.90, df=15, p>0.05. Consequently, we conclude that neither time, treatment, nor their combinations significantly affect the oviposition selection in *Ae. aegypti*.

The recorded range of HI spans from 0.00% to 0.14% in Variant 1, with an attractant at 60 min and 90 min (Table 3). These results indicate a range of inhibitions for the conversion of *Ae. aegypti* eggs to larvae varying from 100% to 99.86%. As evaluated through Two-way ANOVA, the time factor did not significantly influence the HI in *Ae. aegypti* (HI: F=0.64, df=5, p>0.05). Similarly, the treatment introduced also failed to yield a significant effect (HI: F=2.82, df=3, p=0.05), and the combination of both factors exhibited no substantial impact (HI: F=0.64, df=15, p>0.05).

Simultaneously, the ER of *Ae. aegypti* displayed a range from 0.00% to 0.07%, resulting in the inhibition of *Ae. aegypti* emergence as adults, ranging from 100% to 99.93%. Surprisingly, in *Ae. aegypti,* all larvae failed to reach adulthood except for variant 1, with attractant in 150 min at 0.07%, as listed in Table 3. Emergence Rate (ER) in *Ae. aegypti* showed no significant differences in times (ER: F=1.00, df=5, p>0.05), treatments (ER: F=1.00, df=3, p>0.05) and time x treatments (ER: F=1.00, df=15, p>0.05). In summary, the time and the administered treatment showed no significant impact on the HI and ER of *Ae. aegypti*.

DISCUSSION

Examining ovitrap efficacy is a well-established practice in numerous studies (Ritchie et al., 2014; Tawatsin et al., 2019; Withanage et al., 2020). This study specifically investigates the efficacy of the AMHS, employing the MHAQ-containing PPF. The findings indicate a significant preference for *Ae. aegypti* oviposition in the MHAQ with attractant compared to the MHAQ without attractant. However, this preference lacks statistical significance compared to the control, despite a higher oviposition rate in the MHAQ with attractant compared to other treatments. It unveils the specific preferences of *Ae. aegypti* towards the attractant, evident in the mean egg count of 28.65 eggs, almost six times more than that of MHAQ without attractant, which recorded 5.29 eggs. In contrast, *Ae. albopictus* did not exhibit any significant preference for oviposition in either treatment. It appears that MHAQ with attractant is less attractive to *Ae. albopictus*. These outcomes contribute valuable insights into species-specific attractiveness, which addresses a notable gap in prior laboratory evaluations (Mohd Ngesom et al., 2021; Yazan et al., 2020).

An attractant can be described as a substance or factor attracting mosquitoes toward a specific location, such as an AMHS trap (Mwingira et al., 2020). In this study, the manufacturer undisclosed the attractant used. Attractants strategically utilised the communication methods of either the same or different species, which involved using semiochemicals (El-Ghany, 2020). Pheromones are a distinctive category of semiochemicals that play a crucial role in linking communication with individuals of the same species (Rizvi et al., 2021). In the context of *Aedes* mosquitoes, an attractant which is a sex pheromone named heptacosane, has been demonstrated to enhance the sterile insect technique (Wang et al., 2023). Remarkably, several attractants have proven effective in ovitraps, including infusions of *Leucaena leucocephala* (Barreto et al., 2020; Ridha et al., 2020).

Due to the lower attractiveness of attractant in the product towards *Ae. albopictus* than *Ae. aegypti*, our results spark an interest in the manufacturer's focus on attracting *Ae. albopictus* over *Ae. aegypti* for MHAQ's future development (Lim Chee Hwa, personal communication 2023). Towards the MHAQ, several lures could be used to tackle the low level of attractiveness of *Ae. albopictus*. Studies have identified effective attractants for *Ae. albopictus*, including sodium chloride solution (0 to 2.0% dilution), lactic acid bacteria infusion, and a 2-Hydroxyethylcellulose-based hydrogel formulation (Friuli et al., 2022; Guo et al., 2022; Suria et al., 2022).

Interestingly, the rationale behind *Ae. aegypti's* preference for AMHS traps over *Ae. albopictus* may stem from a species-specific response to MHAQ concentration, as hypothesised in a study on volatile organic compounds (VOC) influence on both species' sensory perception (Hutcheson et al., 2022). Unlike *Ae. albopictus*, which can sense and perceive any concentration range of the VOCs, *Ae. aegypti* appears more restricted, favouring only a specific concentration range. Outside this concentration level, both heightened and reduced concentrations are not effectively sensed by *Ae. aegypti* (Hutcheson et al., 2022). Alternatively, *Ae. albopictus* required more days for egg maturity than *Ae. aegypti* (Tsunoda et al., 2020). In this study, both species were used for experimentation mostly after a 48-hour blood feeding session, shorter than the durations used in the experiment, which is 96 hours for *Ae. albopictus* and 72 hours for *Ae. aegypti*. Thus, the number of eggs laid by these species is affected due to the suspected incomplete egg maturation.

By comparing each treatment's position for both species, MHAQ with attractant indicated flexibility in room-size cage positioning for evaluating gravid females' responses. No significant differences were observed in the present study, which aligned with cautionary notes on position biases by Roque and Eiras (2008). Daily repositioning of ovitraps in laboratory studies is a common practice to minimise bias, as observed in studies involving *Culex* spp. and *Anopheles* spp. (Borel et al., 2021). Similarly, a prior AMHS laboratory study also took meticulous precautions by regularly altering the ovitrap's position (Mohd Ngesom et al., 2021).

Through repositioning, we confirmed that the position does not contribute to the attractiveness or deterrence of the AMHS trap as the bias has been minimised, as mentioned in Borel et al. (2021) and Eiras et al. (2021). It facilitates future users of AMHS traps to position the traps flexibly when creating floor plans for deployment. For example, users can place them alongside one side of the corridor or arrange them in pairs on both sides. Consequently, expenses can be minimised by reducing the number of traps used by planning the least number of traps that should be used as the trap can be placed adaptably without any concern on positioning. Economic efficiency is crucial when executing methods for mosquito control (Hustedt, 2020; WHO, 2012).

Building on this understanding, a previous laboratory study conducted in Brazil explored spatial orientation and found no significant difference in oviposition rates when gravid mosquitoes were introduced into four types of placements. However, a notable preference emerged when considering the vertical position, with a higher propensity for oviposition observed at point C compared to point D (Roque & Eiras, 2008). In a separate study, *Ae. aegypti* was revealed to distinctly favoured ovipositing in ovitrap placed in the corner as opposed to the central position, leading to the collection of more than 85.00% of eggs in the corner position (de Jesus et al., 2020).

In our study, the position of the traps at the horizontal or vertical position has less impact on the oviposition of both mosquito species, *Ae. albopictus* and *Ae. aegypti*. In the case of a favourable position in any treatment, more precautions will be needed for the subsequent study. One possible explanation for any favourable outcome could be the slightly higher temperature and humidity experienced during testing in the horizontal position, which has been proven to impact the oviposition of both *Ae. albopictus* and *Ae. aegypti* (Thongsripong et al., 2023). It only requires a one-degree Celsius rise, which can induce a more than fourfold increase in the oviposition of *Ae. aegypti*, while another study predicts a 50% or more rise with the same temperature increase (do Nascimento et al., 2022; Gimenez et al., 2020). These results were also supported by a field study conducted in Parana State, Brazil, which demonstrated that the rise in temperature has a significant impact on the oviposition rate of *Aedes* spp. (Souza et al., 2022).

Notably, when given a choice between two types of treatment selections, both *Aedes* species showed no preference over MHAQ with attractant or without attractant in terms of oviposition sites. Despite the presence of an attractant, the lack of significant differences in the mean number of eggs oviposited between the two treatments in both variants suggests that the attractant used in MHAQ may not be influential for oviposition. Thus, it opens the possibility for its elimination to reduce costs, aligning it with consumer preferences for lower-priced alternatives. Notably, farmers in Besur Village, Lamongan, Indonesia, have already shifted away from costlier chemical pesticides, opting for more economical biological pesticides (Afandhi, 2020). Another reason that could explain the lack of a subtle

effect of the attractant may be attributed to the colour of the AMHS trap itself, which is black. Research has shown that black-coloured ovitraps were preferred over red or other colours tested (Marin et al., 2020; Tsunoda et al., 2020). Besides, the cylindrical design of the AMHS, paired with the incorporation of OviTo linen, could potentially enhance its overall attractiveness. It was aligned with a study that showed the attractiveness of a tube-shaped ovitrap lined with a propagation towel has a higher oviposited egg in comparison to paddles or styrofoam pieces (Velo et al., 2016). However, more studies must be conducted to find a more attractive substance in MHAQ.

Another potential factor contributing to the absence of specific selection by *Aedes* mosquitoes when presented with two treatment selections is the competition among breeding sites. In Study 3, four oviposition traps were compared to two in Study 1 and Study 2 in each replicate. This increase resulted in a higher number of available oviposition sites, leading to heightened competition among the traps. The competition of breeding sites emerges as a potential concern, highlighted in several field studies (Brisco et al., 2023; de Resende et al., 2013; Moura et al., 2020).

In Study 3, *Ae. albopictus* exhibited a higher number of eggs laid in the traps at the early test time of 30 min. This situation might be attributed to a fading attraction to the breeding site over time. This observation aligns with research on a species of *Aedes* mosquito, indicating a heightened oviposition within the initial introduction to the breeding site followed by a subsequent decline. While there is no recent data depicting the interplay between the introduction time to the ovitrap and the mean number of eggs laid, previous studies only highlighted the correlation between the mean egg count and the ovitrap design, attractiveness, or efficacy of insecticides compared to water (McGaughey & Knight, 1967; Mohd Ngesom et al., 2021; Parker et al., 2017; Tawatsin et al., 2019).

A human entering the chamber during OviTo linen collection in Study 1 and Study 3 could impact mosquito oviposition behaviour, as it may disturb the mosquitoes. However, this method remains the most direct way to measure treatment efficiency over time. Potential disturbances include mosquitoes flying away or altering their flight patterns. For example, non-blood-fed female *Ae. aegypti* has been shown to fly more vertically in response to human presence (Poh et al., 2017). However, this concern is mitigated in our study, as the *Ae. aegypti* used were blood-fed. Meanwhile, a study has shown that *Ae. albopictus* mosquitoes aged 10–15 days are sensitive to human scent (Drago et al., 2021). Given that our study utilised mosquitoes aged six to eight days, this issue is irrelevant.

Lima-Camara et al. (2014) found *Ae. aegypti* and *Ae. albopictus* females exhibit reduced locomotion after insemination and blood-feeding compared to unmated, unfed females. Since our study used blood-fed and after-inseminated mosquitoes, their reduced locomotion further lessens the impact of human presence. Additionally, a study noted that blood-fed female *Ae. aegypti* mosquitoes have reduced sensitivity to human odours important for

host-seeking but increased sensitivity to odours that help locate egg oviposition sites (Chen et al., 2019). Thus, human presence is less concerning in our study, as blood-feeding may have already diminished their sensitivity to human odours.

Even though the impact of human presence seems minimal, several precautions were still taken to further minimise potential disturbances during chamber entry. A rapid entry and exit practice was employed, with the researcher quickly replacing the OviTo linen and conducting counts outside the chamber. Only one researcher, who handled all chamber entries for every replicate, wore full protective gear, including a long-sleeved lab coat, long pants, gloves, closed shoes, long socks, a face mask, and a fully covered head, with only the eyes and forehead exposed. The lab coat blends with the floor and is light in colour, making it less attractive to mosquitoes (Benz et al., 2024). This helped reduce both visual and olfactory attraction. Precautions were also taken to gently brush off any mosquitoes before exiting. Multiple replications were conducted to average out variability, as replication is crucial for ensuring scientific reliability (National Academies of Sciences Engineering and Medicine, 2019).

In the context of hatching and adult inhibition observed across all studies, the Mosquito Home Aqua Solution (MHAQ) with attractant notably impacted both *Ae. albopictus* and *Ae. aegypti*, with the highest recorded inhibition, reaching 100.00% for both species. This observation aligns with prior investigations on pyriproxyfen intervention, underscoring the significance of species-specific dosage requirements and asserting the cross-species efficacy of an insecticide effective against both *Ae. albopictus* and *Ae. aegypti* as well (Gómez et al., 2011). Moreover, these findings regarding the effectiveness of MHAQ-containing pyriproxyfen (PPF) in impeding adult emergence are supported by earlier research associated with the AMHS (Harburguer et al., 2016; Iyaloo et al., 2021; Mohd Ngesom et al., 2021; Yazan et al., 2020).

Another factor that reduces adults' hatching index and emergence rate is using OviTo linen in the traps. *Aedes* spp.'s inherent ability to hatch in water is a key element in the control traps (Prameswarie et al., 2023). The rapid 24-hour hatching capability and the possibility of pre-submersion hatching on OviTo linen, influenced by moisture, could explain the situation (Ninditya et al., 2020). *Aedes* spp. exhibit breeding adaptability in minimal water, but OviTo linen, despite retaining moisture, lacks sufficient volume of water for larvae survival, prompting premature hatching and subsequent mortality due to inadequate water volume (Dharmamuthuraja et al., 2023; Owolabi & Bagbe, 2019; Ratnasari et al., 2020).

Overall, this investigation accentuates the necessity for ongoing exploration and optimisation of attractants within the AMHS, especially for *Ae. albopictus*. It also emphasises the efficacy of the MHAQ with attractants in inhibiting the hatching and emergence of both species, providing pivotal insights essential for refining effective mosquito control strategies.

CONCLUSION

This study examines the effectiveness of the AMHS with MHAQ in attracting and controlling *Ae. albopictus* and *Ae. aegypti*. While MHAQ consistently entices *Ae. aegypti*, its performance with *Ae. albopictus* is suboptimal, prompting consideration of additional attractants. Simultaneous oviposition choices in the same replicates suggest that the current attractant in MHAQ may not influence oviposition, raising the possibility of cost-effective elimination. The positioning of the AMHS does not affect its attractiveness, indicating flexibility in deploying the trap. This research offers nuanced insights for optimising ovitrap efficacy in comprehensive mosquito control strategies.

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