ORIGINAL ARTICLE

An Experimental Study on the Effect of Radiofrequency and Temperature Exposure on Hatching Rate and Developmental Period of *Aedes aegypti* (Diptera: Culicidae)

Nazri Che Dom[1,2,3], Nik Muhammad Hanif Nik Abdull Halim[1], Nurulain Rabihah Rosdi[1], Rahmat Dapari[4], Nopadol Precha[5]

[1] Centre of Environmental Health & Safety Studies, Faculty of Health Sciences, Universiti Teknologi MARA, UITM Cawangan Selangor, 42300 Puncak Alam, Malaysia.
[2] Integrated Mosquito Research Group (I-MeRGe), Universiti Teknologi MARA, UITM Cawangan Selangor, 42300 Puncak Alam, Malaysia
[3] Institute for Biodiversity and Sustainable Development (IBSD), Universiti Teknologi MARA, Shah Alam, 40450, Selangor, Malaysia
[4] Department of Community Health, Universiti Putra Malaysia, 43400, Serdang, Malaysia.
[5] Department of Environmental Health and Technology, School of Public Health, Walailak University, Nakhon Si Thammarat, 80160, Thailand

ABSTRACT

Introduction: *Aedes aegypti* holds global significance as a disease-transmitting vector, particularly for various arthropod-borne viruses. Ongoing human-induced disturbances, such as habitat destruction, chemical pollution, urbanization, and climate change, have led to a shift in global insect communities. Recognized as a human impact factor, radiofrequency (RF) has been identified to influence the hatching rate and development of insects. This study addresses a research gap by investigating the effects of low RF exposure and temperature variations on *Aedes aegypti* populations, especially considering the increased reliance on RF technologies. The study's objective is to determine how RF and temperature exposure impact the hatching rate and development period of *Ae. aegypti*. **Material and Methods:** This study conducted under laboratory setup with 720 eggs were exposed to control and low RF exposure (900 MHz) with different temperature levels of 20°C, 25°C, 30°C, and 35°C. The study observed the effects on both hatching rate and development period. **Results:** The study discovered that eggs exposed to low RF hatched the most at 25°C. In comparison, the control group demonstrated the highest hatching rate at 30°C. The study found that at 20°C, the larval stage took the longest, while at 30°C, it was the shortest development. At 35°C, *Aedes aegypti* could not progress past the larval phase, resulting in a zero-emergence rate. **Conclusion:** This research sheds light on the potential effects of RF and temperature exposure on Aedes mosquito populations, emphasizing the need for further exploration in natural environments and understanding potential public health implications.
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Corresponding Author:
Nazri Che Dom, PhD
Email: nazricd@uitm.edu.my
Tel: +6096143465

INTRODUCTION

The focus of this research lies on the main carrier of dengue fever (DF), Aedes (Stegomyia) aegypti (Linnaeus) (Diptera: Culicidae), which is known as a vector of global concern. The Aedes genus encompasses over 950 species of mosquitoes, belonging to the order Diptera. *Ae. aegypti* inhabits tropical, subtropical, and temperate climates. *Ae. aegypti* is an anthropophilic insect that successfully colonizes urban and peri-urban, especially in highly populated areas (hotspots). It transmits major arthropod-borne viruses (arboviral) pathogens to humans, such as DF, yellow fever, zika, and chikungunya, which cause significant morbidity, fatality, and fluctuation in healthcare expenditure in second and third-class countries (1). Aedes mosquitoes go through a four-stage life cycle, which includes the egg, larval, pupal, and adult phases. After the eggs hatch, the development process takes place, and the duration can range from as short as seven days to several weeks

before the emergence of adult mosquitoes. *Ae. aegypti* dynamics were driven by temperature fluctuation and human activity. These factors potentially affect the hatching rate and mosquito's development especially on the *Ae. aegypti* (2).

Temperature is a crucial environmental factor that impacts the growth of mosquito populations, as proven by the stratification of mosquito ecology (3). Temperature is one of the most influential abiotic factors in ecology, physiology, behaviour and consequently, survival of *Ae. aegypti* (4). Specifically, higher, and prolonged exposure to elevated temperatures leads to the rapid development of *Ae. aegypti* embryos, accompanied by a faster mortality rate. According to the Malaysia Meteorological Department (MET), Malaysia's climate is distinguished by its constant temperature, high humidity, and copious rainfall. Mosquitoes exhibit increased oviposition activities in warmer climates because they can feed and digest more frequently and efficiently (5). Moreover, Aedes mosquito species have a survival temperature range of 15°C to 35°C, which is within the optimal temperature range for the *Ae. aegypti* mosquito. Warmer temperatures tend to shorten *Ae. aegypti* development time and increase their overall reproductive capacity. Extreme temperatures, on the other hand, can have a detrimental effect on the hatching rate and development of *Ae. aegypti*. Insects may experience desiccation, metabolic shifts, and reduced mobility due to the changing humidity levels that occur locally, daily, and seasonally (4).

The global alteration of insect communities has been caused by persistent human disturbances such as habitat destruction, chemical pollution, urbanization, and climate change (6). Radiofrequency (RF) is recognized as a contributing human impact factor on the hatching rate and development of insects. RF energy encompasses radio waves, microwaves, electromagnetic fields, and wireless signals. RF energy permeates our environment. It is utilized in diverse electronic devices and appliances, such as radio and TV broadcasting, satellite communications, microwave ovens, cell phones, radars, and industrial heating and sealing equipment (7). These instances illustrate just a portion of the wide array of uses for RF, which involves the transmission of energy or radiation through radio waves spanning frequencies from 100 kilohertz (kHz) to 300 gigahertz (GHz). (8). Wireless communication base stations are the predominant source of RF in outdoor settings. Humans and animals, particularly, insects are subjected to these electromagnetic fields, where their bodies absorb a portion of the radiation. The amount of radiation absorbed varies depending on the frequency, and it can notably escalate when a resonance effect happens either in the entire body or in specific parts of it (7). Additionally, there is concern about whether forthcoming fluctuations in RF a more significant influence on Aedes populations could affect DF transmission in urban settings. This is due to a lack of understanding about how RF exposure affects the hatching rate and development period of the Aedes mosquito population.

There has been insufficient research into the combined effect of two abiotic factors, specifically RF and temperature, on biological processes. The interplay between multiple variables can significantly influence outcomes (9). For example, adjusting temperature can directly affect the hatching rate and growth of *Ae. aegypti*. Therefore, it is important to evaluate the relationship between each independent variable (RF and temperature) and the dependent variable (hatching rate and developmental period). This study aims to investigate the effect of RF exposure and temperature variation on the hatching rate and development period of *Ae. aegypti*. The findings will provide valuable insights for developing methods to predict the impact of RF and temperature on *Ae. aegypti* hatching and development. Such information will benefit research and development teams, entomologists, and other researchers, serving as a reference for future studies. Ultimately, this study will contribute scientifically proven evidence that can be applied to real-world contexts.

MATERIALS AND METHODS

Sample Collection, Colonisation and Mosquito Cohort Establishment

The laboratory strains of *Ae. aegypti* eggs, specifically the [*Ae. aegypti* S7 strain], were sourced from the Department of Medical Entomology, Infectious Diseases Research Centre, Institute of Medical Research in Kuala Lumpur, Malaysia. The laboratory strains of *Ae. aegypti* eggs were sourced from the Department of Medical Entomology, Infectious Diseases Research Centre, Institute of Medical Research in Kuala Lumpur, Malaysia. They were bred and kept in controlled conditions at the vector insectary located at the Faculty of Health Sciences, Universiti Teknologi MARA (UiTM) in Puncak Alam Campus, Selangor. The insectary maintained a temperature of 29 ± 3°C, relative humidity of 75 ± 10%, and a 12-hour light-dark cycle. Larval growth was monitored daily, and TetraMin Plus Tropical Fish Flakes were provided as food to support larval development. Larvae were fed daily until they reached the pupation stage. The amount of food given to each larva increased gradually each day, ranging from 0.06 to 0.12 mg per larva during stages L1 to L2, 0.24 mg in stage L3, and 0.48 mg in stage L4. To maintain optimal conditions and prevent issues like foaming and volume loss, daily water changes were carried out. At the pupal stage, male and female mosquitoes were separated based on their size differences to obtain virgin mosquitoes. Around 50 pupae were sorted into groups and placed in containers with mesh covers, each containing 10 ml of dechlorinated water. For mating, 15 pairs of virgin adult mosquitoes were placed in standard rearing cages (30 ×

30 × 30 cm) with a 10% sucrose solution as their nutrient source. After a 72-hour mating period, a confined white mouse was introduced into the cage for 12 hours to provide a blood meal, ensuring the female mosquitoes were sexually mature and capable of egg-laying.

After the blood meal, a designated area for egg-laying was set up within the cage. This involved placing plastic containers (measuring 9 cm in height and 7.5 cm in diameter) filled with 100 ml of water and filter paper as the substrate. The egg-containing strips were collected and replaced daily for a week. The process of larval development, adult mating, and egg-laying continued until a sufficient number of F2-generation mosquitoes were obtained. Only these mosquitoes were used to assess whether the desired traits remained consistent or showed unexpected changes (10). After being collected, the eggs were allowed to undergo embryonation for two days. Following this, they were briefly dried on paper towels for 30 seconds and then stored in a humid environment for an additional day.

Instead of completely drying, the eggs were dried at rearing temperatures until they were slightly damp. They were then stored in sealed bags with moist absorbent cotton to maintain high humidity and prevent them from drying out. For this study, F2 eggs were used after being stored for two to three weeks. The age of egg batches can potentially affect their hatching response, so eggs of inconsistent ages were excluded. To conduct a single triplicate experiment investigating the impact of RF exposure and temperature on egg hatching rate and development period in *Ae. aegypti* mosquitoes, 720 eggs from the laboratory were required.

Configuration of the Exposure Chamber and Antenna

In research involving radiofrequency (RF) exposure, it is essential to establish a controlled experimental setup to accurately assess the effects on biological subjects. This study utilized a comprehensive experimental setup, which includes a large polystyrene foam box coated uniformly in white paint to enhance visibility. The configuration comprises seven main components: a power supply, an analogue signal generator, a spectrum analyzer, an RF antenna, an exposure chamber, a sample, and RF cables. A wideband antenna (A-Info, LB8180-NF model) with a frequency range of 0.8 to 18 GHz and an average gain of 12 dB was employed to transmit RF waves to the test subjects. This single antenna could cover various frequencies due to its wide bandwidth and was strategically positioned in a designated compartment, directed towards the intervention site. The RF wave source consisted of a wideband function generator (Keysight, model N5173B EXG X-Series), capable of producing high-frequency signals from 9 kHz to 40 GHz. Furthermore, a super ultra-wideband amplifier was connected to the signal generator via a coaxial cable with SMA connectors to enhance the signal strength. The amplifier was then linked to the antenna through another coaxial cable, with both cables terminating in SMA-to-N connectors (25).

The RF Exposure Protocols

To ensure consistency, F2 generation eggs of the *Ae. aegypti* strain was randomly assigned to the experimental group, eliminating any potential influence of egg age. Before exposure to RF, the eggs were carefully examined for any defects using an Olympus, model Courtesy, UK Ltd stereomicroscope. Then, they were placed on filter paper inside 16.5cm × 7.5cm × 10cm plastic containers with lids and stored at room temperature. Approximately 360 mosquito eggs were subjected to low RF (900 MHz) exposure for 24 hours in specialized chambers, with the RF exposure process repeated in triplicate to validate and ensure the reliability of results for the laboratory strains of *Ae. aegypti* mosquitoes. These repetitions were conducted on the same day. Meanwhile, duplicate procedures were performed without RF exposure to serve as the control group. A single RF exposure was also conducted on the *Aedes aegypti* eggs to evaluate mosquito development.

Temperature Variation Exposure

After being exposed to RF exposure at 900 MHz, and unexposed (control), the eggs of the laboratory strains *Ae. aegypti* were completely assessed to identify the combined effect of the variation in temperature exposure under control laboratory conditions. The investigations were conducted in a humidified incubator (FRIOCELL®, MMM C1111082, Мьnchen, Germany). The eggs were submerged in a plastic container containing 300 ml of deionized water (n = 30) and placed inside a humidified incubator at 20°C. They had a photoperiod set to 12:12 (light: dark), which included 1 hour of dawn and 1 hour of darkness. RH was maintained under control and set at 75 ±10% throughout all experiments based on insectarium conditions. A thermo-hygrometer was set in each chamber to monitor and ensure that there was sufficient temperature and relative humidity for each temperature tested. This technique must be repeated, utilising thermic exposure conditions of 25°C, 30°C, and 35°C with a 5°C interval.

Experimental Design

The study utilized a completely randomized factorial 4 × 2 × 3 design, resulting in a total of 24 treatments. The independent variables included two levels of exposure duration: a 24-hour exposure and a control group with no exposure. The study also considered variations in *Ae. aegypti* populations separately, which might be influenced by exposure duration. To ensure the strength of the findings, the entire experiment was replicated three times on different occasions. Each combination of the independent variables represented a unique treatment condition, resulting in a total of 24 treatments. By using a completely randomized design, treatments were allocated to experimental units randomly and

impartially, enhancing the trustworthiness and accuracy of the results.

This study provided a methodical and controlled investigation into the effects of exposure duration and population variation on the *Ae. aegypti* population, enhancing the comprehensiveness and reliability of the findings. Approximately 30 eggs from the laboratory strains of *Ae. aegypti* mosquitoes were used for each replication, categorized into two groups: unexposed (control) and exposed to 900 MHz RF. Daily monitoring of larval development during the aquatic phase was carried out. The study observed and measured various factors, including egg hatching rate, larval mortality, pupation rate, pupation duration, and adult emergence. Additionally, a small amount of TetraMin Plus Tropical Fish Flakes was added to the tank when the eggs started to hatch. The larvae were fed daily throughout all stages until they reached the pupation stage. Each day, larvae in stages L1 to L2 were provided with 0.06 to 0.12 mg of food per larva, while those in stage L3 received 0.24 mg, and those in stage L4 were given 0.48 mg. Furthermore, the pans were checked daily at the same time, and plastic pipettes were used to remove debris. Daily water replenishment was also carried out to maintain consistent levels due to evaporation.

Typically, the first pupae emerge around day six to seven, but they're intentionally left undisturbed in the tank for over 24 hours to aid in larvae separation. During the pupation process, pupae are moved to labelled plastic cups with clean water and covered with mesh using plastic pipettes. While larvae still require feeding, the food quantity is reduced to avoid excessive contamination and foaming. Adult mosquitoes are primarily fed a diet containing 10% sucrose. On the second day after hatching, adult *Ae. aegypti* mosquitoes are collected using a suction cup and placed in labelled universal bottles. These bottles are then stored in a refrigerator at 4°C for 2 hours to end the mosquitoes' lifespans.

Data Analysis

IBM SPSS Statistics (version 28, copyright IBM Corporation) was utilized to analyze the database of variables comprehensively. This analysis will determine whether there are statistically significant changes in the egg-hatching rate (as a percentage) and the development period (in days) of *Aedes aegypti* mosquitoes between the same temperature but different exposure groups, the control group, and the low radiofrequency group, 900 MHz RF. If applicable, a parametric analysis independent t-test was conducted to assess if there is a significant relationship between the two variables being studied. In addition, a parametric one-way analysis of variance (ANOVA) also is executed to examine the effects, specifically within the same RF exposure and control, but under diverse temperature conditions (20°C, 25°C, 30°C, and 35°C). If the results of the one-way ANOVA indicated a statistically significant difference between temperature variations, Tukey's honestly significant difference (HSD) or Dunnet T-3 post hoc tests were conducted. These tests identified specific groups that differed significantly from each other. The study examined and discussed the t-test study and one-way ANOVA, using a significance value of $p < 0.05$. It aimed to explore potential structural and life cycle changes in *Ae. aegypti* mosquitoes due to intense RF exposure and temperature variation during their immature phase (eggs).

RESULTS

Effects on the Hatching Rate of *Aedes aegypti*

Figure 1 illustrates the combined effect between 900 MHz RF exposure and temperature variation on the egg hatching rate (%) of *Ae. aegypti*. The graphical representation for the exposed group depicts a bell-shaped trend, signifying an increasing hatching rate from 20°C to 25°C, followed by a decline from 30°C to 35°C. The control group indicated a rise in the hatching rate from 20°C to 30°C, followed by a decreasing pattern at 35°C. Specifically, the findings indicate that the RF exposure group at 25°C exhibited the highest hatching rate of 46.67 ± 23.34%, while the RF exposure group at 35°C demonstrated the lowest hatching rate of 5.56 ± 1.93% compared to the other combination. A significant difference was demonstrated in the hatching rate of the combined effect between the RF exposure group and temperature variation ($F(3, 8) = [5.312]$, $p = 0.03$) (Table 1). On the other hand, the control group at 30°C indicated the highest hatching rate of 43.34 ± 30.567% while the control group at 20°C indicated the lowest hatching rate of 15.55 ± 3.85% ($F(3, 8) = [1.564]$, $p = 0.27$). Table 2 reveals the comparative analysis of the combined effect between the RF exposure group and also for the control group. At 20°C and 30°C, the control group of *Ae. aegypti* consistently exhibited a higher hatching rate than those exposed to RF. Surprisingly, the control group exhibited a significantly higher hatching rate compared to the RF exposure group and 35°C combination. ($t(4) = 7.905$, $p = 0.01$). This observed significant difference suggests a temperature-dependent effect on the hatching rate of *Aedes aegypti* mosquito. The specific conditions and biological responses at 35°C may have contributed to the detectable influence of temperature on the hatching rate of *Ae. aegypti* rather than other factors. However, at the temperature of 25°C, the hatching rate for the RF exposure group surpasses the control group. This indicates a preference for exposed *Ae. aegypti* eggs to hatch at 25°C, essentially room temperature with the combination of RF exposure while similar effects may not have been observed at other temperatures.

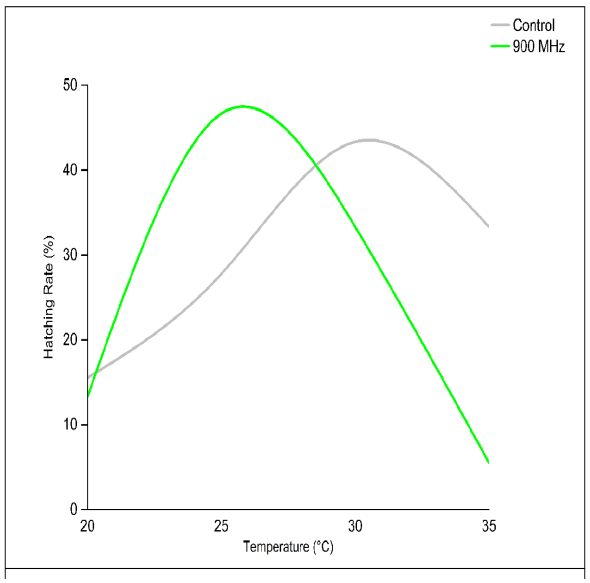

Figure 1 : Hatching rate (%) of *Ae. aegypti* for each control and RF exposure (900 MHz) with temperature variation

Table I : Statistical comparison of hatching rate (%) of *Ae. aegypti* for each control and RF exposure (900 MHz) group with temperature variation

Exposure	df		F-value	p-value
	Between group	Within group		
Control	3	8	1.564	0.27
RF exposure (900 MHz)	3	8	5.321	0.03**

Note: **Significant difference at p < 0.05.

Table II : Statistical comparison of hatching rate (%) of *Ae. aegypti* between control and RF exposure (900 MHz) group for each temperature

Temperature	95% Confidence Interval		df	t	p-value
	Lower bound	Upper bound			
20°C	-5.94606	10.38606	4	0.755	0.49
25°C	-57.91369	20.13369	4	-1.344	0.52
30°C	-44.75224	64.75224	4	0.507	0.64
35°C	18.02261	37.53739	4	0.507	0.01**

Note: **Significant difference at p < 0.05.

Effects on the developmental period of *Aedes aegypti*

Figure 2 displays the developmental period (days) of immature *Ae. aegypti* mosquitoes from aquatic phase until adult emergence when subjected to 900 MHz RF exposure and temperature variations of 20°C, 25°C, 30°C and 35°C. This depiction provides valuable insights into the temporal dynamics of the development of *Ae. aegypti* in response to the combination of RF exposure and temperature. Specifically, Figure 2A demonstrates that the control group at 20°C exhibited a significantly shorter larval period of 5.67±0.76 days compared to the RF exposure group of 8.67 ± 0.76 days ($t(4) = -4.811$, $p = 0.01$). In terms of the adult emergence period, it was observed that the RF exposure group exhibited a slightly longer period of 13.17 ± 0.29 days compared to the control group of 12.83 ± 0.29 days at the same temperature condition. On the other hand, a different pattern was observed at the temperature of 25°C. Figure 2B illustrates that the RF exposure group exhibits a slightly shorter period for both larval and adult emergence stage of 3.33 ± 0.29 days and 8.83 ± 0.76 days compared to the control group of 3.50 ± 1.52 days and 9.17 ± 0.57 days respectively. As depicted in Figure 2C, at 30°C, the control group demonstrated a shorter larval period of 2.83 ± 0.76 days while 3.00 ± 0.50 days for the RF exposure group. Surprisingly, the control group reached the adult phase slightly slower period of 7.67 ± 1.26 days compared to the RF exposure group of 7.33 ± 1.04 days. In contrast at 35°C, the RF exposure group exhibited a slightly shorter period of the larval stage of 3.50 ± 0.50 days compared to the control group of 4.83 ± 0.76 days. Intriguingly, *Ae. aegypti* subjected to this temperature fails to progress beyond the larval phase, halting development before reaching the pupal and adulthood stages for both the RF exposure group and the control group as depicted in Figure 2D. The detailed thorough statistical study of the differences in *Ae. aegypti* developmental period and how they are contrasted between various temperature conditions between RF exposure and control group as demonstrated in Table 3.

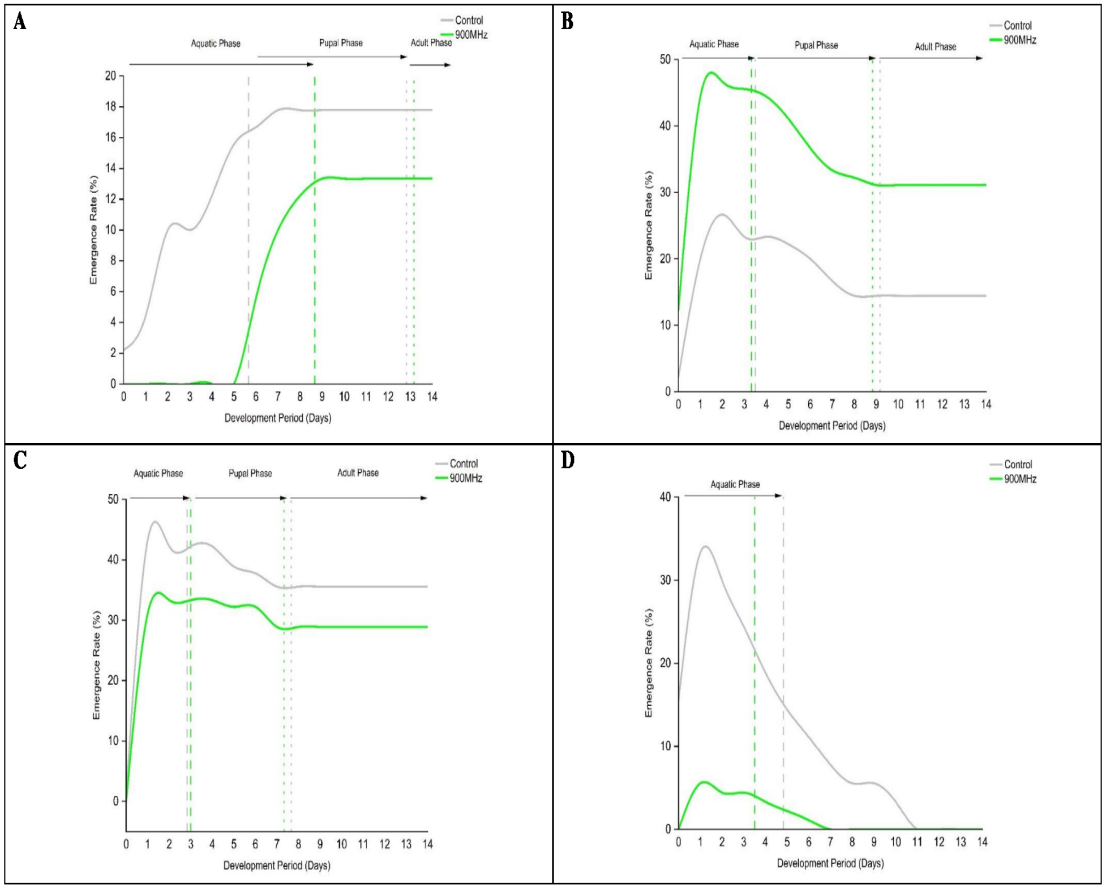

Figure 2 : Development trend (days) of *Ae. aegypti* for each control and RF exposure (900 MHz) with temperature variation

Table III : Statistical comparison of larval period (days) and adult emergence of *Ae. aegypti* between control and RF exposure (900 MHz) for each temperature

Temperature	Larval period (days)							Adult emergence period (days)						
	Control	RF Exposure	95% Confidence Interval		df	t	p-value	Control	RF Exposure	95% Confidence Interval		df	t	p-value
			Lower bound	Upper bound						Lower bound	Upper bound			
20°C	5.67 ± 0.76	8.67 ± 0.76	-4.73	-1.27	4	-4.81	0.01**	12.83 ± 0.29	13.17 ± 0.29	-0.98	0.32	4	1.41	0.23
25°C	3.50 ± 1.32	3.33 ± 0.29	-2.00	2.33	4	0.21	0.84	9.17 ± 0.58	8.83 ± 0.76	-1.20	1.86	4	0.60	0.58
30°C	2.83 ± 0.76	3.00 ± 0.50	-1.62	1.29	4	-0.32	0.77	7.67 ± 1.26	7.33 ± 1.04	-2.28	2.95	4	0.35	0.74
35°C	4.83 ± 0.76	3.50 ± 0.50	-0.12	2.79	4	2.53	0.07	0.00	0.00	-	-	-	-	-

Note: **Significant difference at p < 0.05.

Interestingly, the findings demonstrated a significant difference ($p < 0.05$) for both larval and adult emergence periods between each exposure and control group. Specifically, for the larval period, the results indicate that the combination of RF exposure with 30°C exhibited the shortest period (mean: 3.00±0.50 days, p =. 0.77) while the combination with RF exposure at 20°C demonstrated the longest period (mean: 8.67±0.76 days, p =0.01) compared to other combinations. Similarly, for the control group, the shortest period was also observed at 30°C (mean: 2.83±0.76 days, p = 0.77), while the longest was at 20°C (mean: 5.67±0.76 days, p = 0.01). In terms of the adult emergence period, the findings indicate a declining trend in the emergence rate from the aquatic phase at 20°C, 25°C, 30°C, and 35°C for both the RF exposure and control groups. Again, the combination of RF exposure with 30°C exhibited the shortest emergence period (mean: 7.33±1.04 days, p = 0.74), while the combination with 20°C demonstrated the longest (mean: 13.17±0.29 days, p = 0.23). Notably, at 35°C, the emergence rate reached zero, with no larvae surviving in either the RF exposure or control groups ($p < 0.05$), indicating that temperature significantly impacts the duration of adult emergence. This absence of adults at 35°C highlights the critical role of temperature in the development of *Ae. aegypti* at this specific life stage.

DISCUSSION

The research findings indicate that exposing mosquito eggs to higher temperatures influences hatching rates, consistent with (11) where eggs from warmer regions exhibited higher hatching rates under elevated temperatures. Furthermore, the study revealed that RF exposure (900 MHz) resulted in a slower hatching period compared to the unexposed control group. This finding is supported by studies conducted by (12) and (13) demonstrating a significant decrease in hatching rates with increased radiation exposure. In this investigation, it was observed that the larval stage duration was prolonged at 20°C, while at 30°C, the larval period was notably abbreviated. These findings align with prior studies supporting the notion that higher temperatures generally contribute to shorter development periods (14-16). However, it is important to note that temperatures surpassing the thermal optimum can have adverse effects on species performance, particularly in terms of immature survival.

The current study is consistent with the findings of Sukiato et al, and Nosrat et al, as elevated temperatures were associated with a significant increase in the likelihood of low *Ae. aegypti* pupal abundance (2,3). This aligns with the broader understanding that heat stress can impact the developmental stages of mosquitoes. Temperature appears to play a more dominant role in shaping pupation periods, with RF exposure exacerbating temperature-induced delays in pupae commencement. At 35°C, *Ae. aegypti* fail to progress beyond the larval phase, with the emergence rate reaching zero, indicating a halt in development before reaching the pupal and adulthood stages. This is in line with the previous study by (16) as the larvae manage to only reach until 3rd instar. Highest adult emergence rates are observed for both unexposed (control) and exposed eggs at 20°C. This is inconsistent with the study provided by (17) and (18). In addition to temperature and radiofrequency exposure, it is crucial to delve into the broader ecological factors shaping mosquito development. The egg hatching rate and development period of *Ae. aegypti* are influenced by a myriad of factors. Notably, studies by (19) and (20) underscore the substantial influence of dark and shaded areas on mosquito life cycles. These findings emphasize the importance of considering the microenvironments where mosquitoes breed and develop. Moreover, few researcher propose that factors such as rainfall and humidity also contribute significantly to altering the overall development of Aedes mosquitoes (21-22). The interplay between climatic conditions and mosquito life stages highlights the complexity of their ecological dynamics. Furthermore, Rahman et al and Roche et al insights into the preference of *Ae. aegypti* mosquitoes for urban areas introduce an additional layer of complexity (23-24). Urban environments may provide unique conditions that impact mosquito behaviour and development differently than in non-urban settings. Understanding these urban-related variations is crucial for comprehensive insights into the ecology of *Ae. aegypti* mosquitoes.

Employing a multifaceted approach in studying the ecology of mosquitoes involves considering various factors that influence their behaviour and development. By examining ecological elements such as temperature, radiofrequency exposure, urbanization, and the impact of different environments, researchers can gain nuanced insights into the intricacies of mosquito life cycles. For a more comprehensive understanding that aligns with real-world conditions, field strain, representing mosquitoes from their natural habitats, becomes essential. Studying how mosquitoes from the field respond to these ecological factors offers a more realistic perspective on their behaviour and adaptation strategies. This comprehensive approach not only contributes to our knowledge of mosquito ecology but also lays the foundation for developing targeted and effective strategies for mosquito control. Understanding how mosquitoes interact with their environment enables researchers to design interventions that are more closely aligned with the complex dynamics of natural ecosystems. Ultimately, this knowledge aids in the development of more informed and impactful measures to manage mosquito populations in ways that are both environmentally responsible and effective in reducing potential public health risks.

While this study provides significant insights into the effects of temperature and RF exposure on *Ae. aegypti* development, several limitations must be acknowledged. Conducted in a controlled laboratory environment, the research may not accurately reflect the complex ecological interactions present in natural habitats, as factors such as predation, competition, and varying microclimatic conditions were not considered. This narrow focus could restrict our understanding of the broader effects of temperature and RF exposure on mosquito development and survival. To address these limitations and deepen our understanding of *Ae. aegypti* development biology, future research should include field studies to evaluate how natural populations respond to temperature variations and RF exposure in their habitats. Expanding the range of temperatures and RF frequencies investigated would help identify critical thresholds and interactions affecting development stages. Additionally, incorporating other ecological factors, such as rainfall, humidity, and habitat types, could elucidate the complex dynamics influencing mosquito life cycles. Long-term exposure experiments would allow researchers to assess the cumulative effects of these stressors on life history traits. Finally, examining different genetic strains of *Ae. aegypti*, including field-collected strains, could provide insights into the variability in responses to environmental conditions. By addressing these areas, future research can build upon the findings of this study, leading to a more comprehensive understanding of *Ae. aegypti* development and informing effective mosquito management strategies.

CONCLUSION

In conclusion, the investigation into *Ae. aegypti*'s response to radiofrequency (RF) exposure and temperature variations has uncovered intricate nuances in its life cycle. The study reveals temperature-sensitive hatching rates, varied developmental durations, and a nuanced reaction to RF exposure. The bell-shaped trend in hatching rates and the observed differences in developmental stages underscore the complexity of *Ae. aegypti*'s responses. Notably, the study identifies significant temperature and RF sensitivity at 35°C, emphasizing the importance of environmental conditions in shaping the developmental dynamics of this mosquito species. Due to the limited research on the potential ecological effects of RF, it is imperative to expand studies in this domain, facilitating comparisons and enhancing our understanding of the complex interactions between ecosystems and the environment.

Based on the study's outcomes, it is recommended to conduct more detailed molecular and physiological investigations to unravel the specific mechanisms governing *Ae. aegypti*'s response to RF exposure and temperature variations. Additionally, field studies under real-world environmental conditions would provide valuable insights into the broader ecological impact. Long-term effects of RF exposure and comprehensive exploration of population dynamics should be prioritized for a more holistic understanding. Standardized experimental designs and protocols across studies would enhance comparability, facilitating a more comprehensive assessment of the ecological consequences of RF exposure on *Ae. aegypti*. Lastly, expanding the sample size in future studies would contribute to the robustness and generalizability of the findings.

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ETHICAL CONSIDERATIONS

The study was approved by the Committee on Animal Research and Ethics (UiTM CARE) under the reference number UiTM CARE: 377/2022 (13th May 2022).

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