

EFFECTS OF RADIO FREQUENCY EXPOSURE ON HATCHING RATE AND DEVELOPMENT PERIOD OF *Ae. albopictus* (SKUSE) (DIPTERA: CULICIDAE)

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Abstract

Telecommunication technologies such as radio, cell phones, and television were developed to meet people's needs and have been widely used for decades. However, the invention relies on the transmission and reception of signals via radio (RF), which has increased concerns about the effects of RF fields on human health, including mosquito-borne diseases caused by exposure to these fields. To address these concerns, a study was conducted to evaluate the effects of exposure from RF on mosquitoes. The objective of this study is to determine the effects of exposure RF on hatching rate, development time, and egg morphology of the *Aedes* population. Under laboratory conditions, 50 *Ae. albopictus* eggs were exposed to three different exposures (control, low dose: 900 MHz and high dose: 18 GHz). The effects of exposure on hatching rate, development time and egg morphology were observed. This study revealed that higher doses of RF can shorten the hatching days of *Aedes* mosquitoes, but also slow down their development rate. In summary, this study provides important insights into the potential effects of exposure to RF on *Aedes* mosquito populations. Further research is needed to fully understand the effects of exposure RF on mosquito populations in natural environments and the potential public health implications. Bottom of Form

Keywords: Radio Frequency, *Ae. Albopictus*, Hatching Rate, Development Period, Egg Morphology

Introduction

Dengue fever (DF) is a mosquito-borne viral infection that is common in tropical and subtropical areas worldwide and has serious human health consequences (1-3). Dengue fever occurs in over 100 countries, with Southeast Asia among the most affected. It has become a major public health problem, infecting up to 400 million people each year, of whom approximately 100 million become ill as a result of infection and 40,000 die from severe dengue fever (1). According to Sairi et al. (2), dengue virus is transmitted to humans through the bite of infected female mosquitoes. The main vectors of dengue fever are *Aedes albopictus* (Skuse), also known as the "Asian tiger mosquito," and *Aedes aegypti* (Linnaeus). These mosquitoes can also transmit chikungunya, yellow fever, and Zika virus (3).

Dengue fever is classified as a common infectious disease in which three main components are related: the causative agent, the host, and the environment. Changes in environmental conditions can directly or indirectly affect mosquito ecology, such as larval habitat availability and suitability, development, and survival (4). Wireless communication between users is enabled by the increasing use of technological innovations such as wireless telecommunications that use radiofrequency electromagnetic fields (EMF) (RF) to transmit data. However, the widespread use of these technologies has raised concerns about the potential impact of emissions from RF-EMF on the environment, including mosquito-borne diseases, which are increasing with the growing use of wireless networks and devices. The concern is whether future variations of RF-EMF will have a greater

impact on *Aedes* populations and thus affect dengue fever transmission in urban areas. This is because little is known about the effects of radiation from RF-EMF on the hatching rate and development time of *Aedes* mosquito populations.

Exposure to RF - EMF can have serious consequences for mosquitoes. However, research on RF and its effects on *Aedes* mosquitoes have been conducted with limited resources (5). Few studies mentioned the effects of exposure on mosquito hatching rate and development time. Therefore, the results of the study will most likely benefit future research, especially that dealing with the effects of radiofrequency on *Aedes* mosquitoes. The objective of this study was to determine the effects of radiation from RF on the hatching rate and development time of the *Aedes* population. It also aimed to investigate the effects of exposure from RF on egg morphology.

Materials and Methods

Sample collection

The eggs of the laboratory strain were obtained from the Institute of Medical Research, while the eggs of the field strain were collected by placing ovitraps on the campus grounds. The laboratory experiment was conducted in a completely randomized 3 x 2 x 3 design (18 treatments). Two RF exposures (Low: 900 MHz and High: 18 GHz) served as independent variables for both the laboratory and field experiments. Each RF exposure was performed in triplicate to ensure the validity of the empirical data and the reliability of the accurate results.

Eggs of the laboratory strain of *Ae. albopictus* ($n = 50$) were exposed to the variability of RF for 24 hours as part of the pilot study. Juvenile development, including larval mortality, pupation, and adult hatching, was monitored to maximize the effects of RF exposure variation. In addition, the experimental phase was conducted with an *Ae. albopictus* field strain to determine the hatching rate (%) and developmental duration (days) of *Ae. albopictus*.

Chamber, antenna and detection setup

Figure 1 shows the exposure chamber, antenna, and detection setup used in the experiment. The RF exposure chamber is a large styrofoam box measuring 49.5 cm (W) X 62 cm (L) X 37 cm (H). To maximise the circulation of exposed RF radiation inside the chamber, the entire interior of the chamber was lined with aluminium foil. The top of the enclosure was cut to the same size as the RF antenna. The wideband horn antenna (LB -8180-NF, AinfoTM) has a frequency range of 0.8-18 GHz, a typical gain of 12 dB, linear polarisation, and a weight of about 1.5 kg. The antenna was used to transmit the generated RF wave to the subject. The wide bandwidth of the antenna allowed the use of a single antenna for a wide range of frequencies. The antenna was installed in the designated compartment as shown in Figure 1 and aimed directly at the intervention area.

In addition, an analog signal generator capable of generating a range from 9 kHz to 40 GHz (N5173B, KEYSIGHT EXG) was used as the source for the wave RF. RF This signal generator is connected by cable to a spectrum analyzer (N9923A, KEYSIGHT Fieldfox). A constant voltage DC with a power of 5 dBm was provided. Calibration of the setup was performed with the spectrum analyzer to confirm the RF detection provided by the antenna signal generator. Unfortunately, the spectrum analyzer used in the study can only detect radio frequencies up to 14 GHz, so the high exposure dose of 18 GHz used for the experiment could not be confirmed.

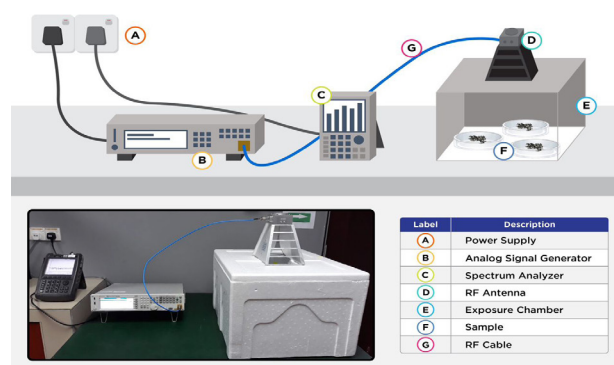


Figure 1: Design of exposure chamber was adopted with some modification from (5).

Radio frequency exposure variation

Fifty eggs of the *Ae. albopictus* strain were counted using a stereomicroscope or a dissecting microscope and then immersed in a plastic container with 200 ml of rainwater ($n = 50$) for 24 hours. At the end of the exposure period, the exposed eggs were cooled to room temperature, and the hatching process, larval development, and adult hatching were performed according to the exposure procedure after irradiation and preservation. This method was repeated with RF exposure parameters of 18 GHz for both laboratory and field strains of *Ae. albopictus*.

Post-radiation exposure procedure and maintenance

Aquatic phase development was examined daily at a temperature of 29 ± 3 °C, relative humidity of $75 \pm 10\%$ (RH), and a light/dark cycle of 12:12 hours. Observations of juvenile *Ae. albopictus*, including egg hatch rate, larval mortality, pupation rate, and adult emergence, were recorded and analyzed as part of the study. Temperature and rearing conditions were standardized to ambient temperature. A small pinch of TetraMin Plus Tropical Fish Flakes was added to the tank as soon as the eggs began to hatch. The larvae were fed appropriately and water clarity was maintained by regular water changes to promote optimal larval development.

On the sixth or seventh day, pupae began to hatch, but they usually do not hatch for 24 hours; therefore, they were left in the container until the next day to allow time for separation of pupae and larvae. Plastic pipettes were used to transfer pupae to a labeled plastic cup containing clean water and a mesh cover during the pupation process. Feeding of the larvae was maintained, but once pupation was observed, the amount of food was to be reduced to avoid excessive contamination and foaming. Adults were fed 10% sucrose, and on the second day after adult hatching, adults of *Ae. albopictus* were aspirated with a suction cup and placed in a labeled universal bottle. After collecting the adults, this bottle was placed in the refrigerator at 4°C for two hours to kill the adults.

Data analysis

The effects of anatomical changes in *Ae. albopictus* following early exposure to RF were measured and recorded in a Microsoft Excel spreadsheet. Larvae of the first larval stage were counted 24 hours after egg hatching. Larval development time is the period between egg hatching and adult emergence. The percentage of eggs hatched was calculated as follows: the number of eggs hatched divided by the original number of eggs multiplied by 100. In this study, the hatching rate (%) and development time (days) of *Ae. albopictus* were monitored (6). The purpose of this assay was to determine if *Ae. albopictus* undergoes a structural change after being exposed to RF during its juvenile phase (eggs).

The egg morphology of *Ae. albopictus* was examined by viewing the egg under an inverted microscope. Egg length was calculated based on the distance between the anterior and posterior poles (7). Egg width was measured at the widest point (7, 8). Egg index was calculated using the ratio of length to width (8), and surface area was automatically generated from Image J. Paired samples t-test was used to examine the significance relationship between differential dose exposure and egg morphology. The variable database was analyzed using IBM SPSS Statistics (version 28, copyright IBM Corporation).

Results

Egg weight

The relationship between RF radiation exposure and egg weight of mosquitoes were summarized in Table 1. The study found that eggs from laboratory strains were heavier than eggs from field strains when exposed to RF radiation at 18 GHz and 900 MHz. The results showed that the eggs of the laboratory strain were heavier than those of the field strain at the highest RF dose, 18 GHz (8.66 ± 1.01 µg and 6.49 ± 0.68 µg, respectively). Moreover, exposure at a low RF dose, 900 MHz, showed the same results, with the laboratory strains having a heavier weight than the field strains (6.67 ± 1.22 µg and 6.21 ± 1.76 µg, respectively). In contrast, the unexposed eggs of the field strains had a higher average weight than the eggs of the laboratory strains (7.44 ± 0.78 µg and 5.46 ± 1.81 µg, respectively).

Table 1: Average weight (µg) ± standard deviation (SD) for each dose exposure of laboratory and field strain of *Ae. albopictus* eggs

Dose	Strain	I	II	III	Average (µg)
Control	Laboratory	3.47	5.89	7.01	5.46 ± 1.81
	Field	8.07	7.67	6.57	7.44 ± 0.78
900 MHz	Laboratory	5.45	6.66	7.89	6.67 ± 1.22
	Field	6.80	4.23	7.60	6.21 ± 1.76
18 GHz	Laboratory	9.80	8.33	7.86	8.66 ± 1.01
	Field	6.34	7.23	5.89	6.49 ± 0.68

* In every experiment for each dose, a total of 30 egg has been used

Morphometric analysis

Table 2 shows the effects of RF radiation exposure on the egg morphology of mosquitoes. The study revealed that the eggs of the laboratory strains exposed to RF radiation at 900 MHz and 18 GHz had greater lengths, widths, egg numbers and areas compared to the eggs of the field strains at the same exposure. On the other hand, the field strain eggs exposed at 900 MHz and 18 GHz had smaller dimensions with lengths of 164.61 ± 11.89 µm, widths of 49.69 ± 14.42 µm, egg counts of 3.66 ± 1.43 µm, and areas of 42.16 ± 3.00 µm, and lengths of 164.85 ± 7.16 µm, widths of 57.06 ± 7.02 µm, egg counts of 2.93 ± 0.37 µm, and areas of 42.58 ± 1.86 µm. However, for the unexposed eggs (control), the field strain performed better than the laboratory strain, with length, width, egg count, and area of the control strain being 166.71 ± 6.34 µm, 59.91 ± 5.52 µm, 2.81 ± 0.30 µm, and 43.64 ± 1.72 µm, respectively. It is important to note that these results are based on one specific study and that further research is needed to confirm these results and to understand the mechanisms behind the observed effects. In addition, it is important to consider the potential implications of these results in the context of efforts to control mosquito-borne diseases.

Table 2: Egg morphology for laboratory strain and field strain of *Ae. albopictus* for each different RF exposure

Dose	Strain	Length (µm)	Width (µm)	Egg index (µm)	Area (µm ²)
Control	Laboratory	165.31 ± 8.37	50.30 ± 11.77	3.49 ± 1.00	43.97 ± 2.23
	Field	166.71 ± 6.34	59.91 ± 5.52	2.81 ± 0.30	43.64 ± 1.72
900 MHz	Laboratory	171.44 ± 12.11	44.11 ± 10.24	4.06 ± 0.88	46.13 ± 3.28
	Field	164.61 ± 11.89	49.69 ± 14.42	3.66 ± 1.43	42.16 ± 3.00
18 GHz	Laboratory	181.55 ± 15.53	47.60 ± 8.77	3.90 ± 0.62	49.39 ± 4.22
	Field	164.85 ± 7.16	57.06 ± 7.02	2.93 ± 0.37	42.58 ± 1.86

* All the values are in mean ± standard deviation (SD)

Table 3 shows the results of a statistical analysis of morphometric measurements of *Ae. albopictus* eggs exposed to different frequencies of microwave radiation. The study used a paired t-test to determine if there was a significant difference between the morphometric parameters and the exposure dose. The results show that

there was no significant difference between the field strain eggs and all parameters assessed ($p > 0.05$). However, a significant difference was found for the laboratory strain eggs when comparing the length and area between the laboratory strain control and the 18 GHz exposure ($p < 0.05$).

Table 3: Morphometric analysis for the dose exposure, unexposed (control), 900 MHz and 18 GHz

Strain	Variables	Dose	t	df	p-value
Laboratory	Length (μm)	Control and 900 MHz	-1.228	8	0.254
		Control and 18 GHz	-2.803	8	0.023*
		900 and 18GHz	-1.158	8	0.280
	Width (μm)	Control and 900 MHz	1.073	8	0.315
		Control and 18 GHz	0.477	8	0.646
		900 and 18GHz	-0.900	8	0.395
	Egg index (μm)	Control and 900 MHz	-1.071	8	0.315
		Control and 18 GHz	-0.939	8	0.375
		900 and 18GHz	0.467	8	0.653
	Area (μm^2)	Control and 900 MHz	-1.612	8	0.146
		Control and 18 GHz	-3.396	8	0.009*
		900 and 18GHz	-1.386	8	0.203
Field	Length (μm)	Control and 900 MHz	0.366	8	0.724
		Control and 18 GHz	0.576	8	0.581
		900 and 18GHz	-0.063	8	0.951
	Width (μm)	Control and 900 MHz	1.761	8	0.116
		Control and 18 GHz	0.897	8	0.396
		900 and 18GHz	-1.385	8	0.203
	Egg index (μm)	Control and 900 MHz	-1.617	8	0.145
		Control and 18 GHz	-0.739	8	0.481
		900 and 18GHz	1.511	8	0.169
	Area (μm^2)	Control and 900 MHz	0.997	8	0.348
		Control and 18 GHz	1.213	8	0.260
		900 and 18GHz	-0.445	8	0.668

Note: (*) significantly different at $p < 0.05$

Hatching rate

Figure 2 shows the average hatching rate of laboratory eggs exposed to different frequencies of microwave radiation. The results show that eggs exposed at low RF (900 MHz) had the highest hatching rate of 8%, followed by unexposed eggs (control) with a hatching rate of 6%. On the other hand, eggs irradiated at high RF (18 GHz) had the lowest hatching rate of 4.67%.

Table 4 shows the minimum and maximum days of hatching for the eggs of the laboratory strains exposed to RF and for the unexposed eggs. The results show that unexposed eggs (control) have a minimum number of hatching days on day 0 and a maximum on day 14. For eggs exposed to RF, hatching days at a low dose of 900 MHz (minimum: day 1; maximum: day 7) and a high dose of 18 GHz (minimum: day 0; maximum: day 3) differ from those of the control group.

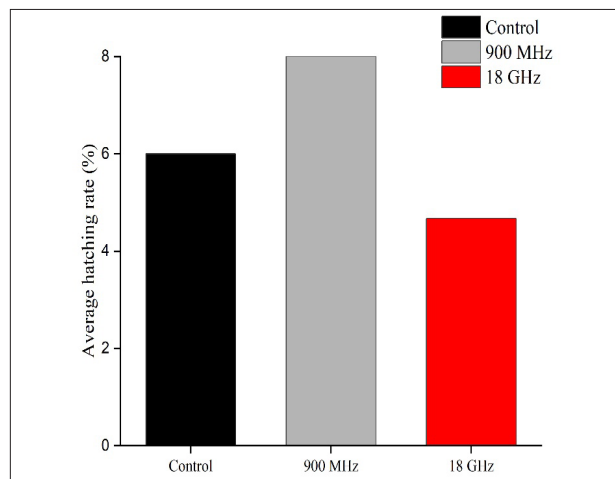


Figure 2: Average hatching rate (%) of laboratory strain eggs.

Table 4: Minimum and maximum days of hatching

Dose	Experiment	Hatching period		Analysis days of hatching
		First day	Last day	
Control	I	DAY 0	DAY 14	Min = DAY 0 Max = DAY 14
	II	DAY 0		
	III	DAY 1	DAY 4	
900 MHz	I	DAY 1		Min = DAY 1 Max = DAY 7
	II	DAY 1	DAY 4	
	III	DAY 2	DAY 7	
18 GHz	I	DAY 1	DAY 3	Min = DAY 0 Max = DAY 3
	II	DAY 1		
	III	DAY 0		

The study found that the median days to hatch were shortest at the high dose of 18 GHz at 1.5 days, followed by the low dose of 900 MHz at 3.5 days, while the unexposed eggs took 7 days to hatch. Figure 3, a boxplot, shows that the higher the dose of RF exposure, the faster the mosquito eggs hatch. Studies have shown that exposure to radiofrequency radiation (RF) can affect mosquito egg hatching. In general, the higher the intensity of RF exposure, the greater the effect on hatching rate. However, it should be noted that the effects of RF radiation on mosquito hatching may vary depending on the specific frequency and duration of exposure. It is also important to keep in mind that the studies were conducted under laboratory conditions and the results could be different under other environmental conditions.

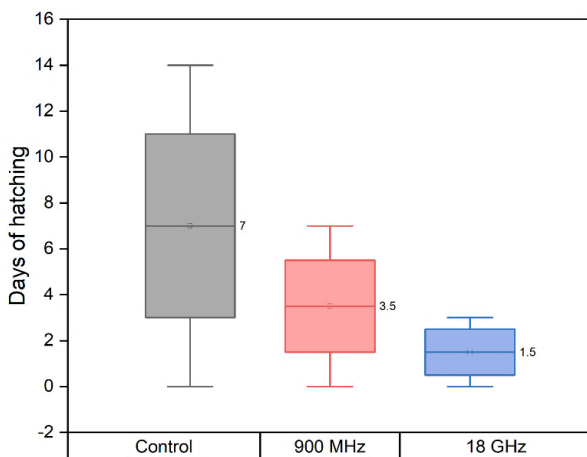


Figure 3: Days of hatching for each exposure.

Development period

Figure 4 illustrates the developmental trend of *Ae. albopictus* from larval stage to adult emergence after exposure to various doses of RF. The study found that the larval stage lasted the longest at the high dose, 8 days,

compared to 6 days at the control and low dose. Also, during pupal development, both the control and low-dose pupae hatched on the seventh day, while the high-dose pupae hatched on the ninth day. However, adulthood occurred more slowly. The low dose of 900 MHz appeared on day 10, while the control and high dose of 18 GHz appeared on day 12.

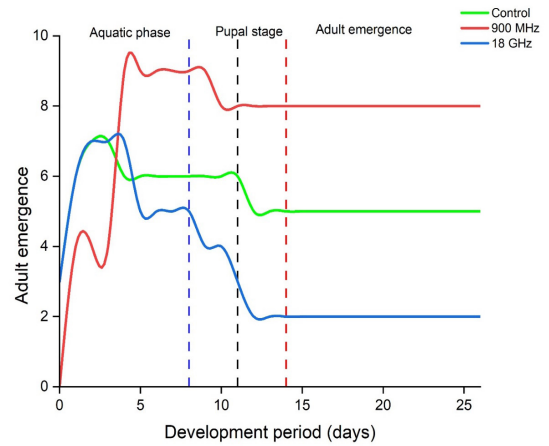


Figure 4: Development period of *Ae. albopictus* exposed to RF at different doses (Control, Low: 900 MHz, High: 18 GHz).

It can be seen that the group with the low dose of 900 MHz had the highest rate of adult mosquitoes at 5.33%, while the control group had 3.33% and the group with the high dose of 18 GHz had the lowest rate at 1.33% (Table 5).

Table 5: Emergence rate for laboratory strain eggs

Experiment	Dose	Adult	Emergence rate (%)
I	Control	1	3.33
II		1	
III		3	
I	900 MHz	3	5.33
II		4	
III		1	
I	18 GHz	1	1.33
II		0	
III		1	

Discussion

The study found that the average weight of *Ae. albopictus* eggs exposed at 900 MHz and 18 GHz was heavier than that of control eggs, but the average weight of control field strain eggs was heavier than that of field strain eggs exposed at 900 MHz and 18 GHz. The study suggests that

the higher exposure dose may have affected the average weight of the eggs. The results of the study could not be linked to previous research because there is limited information on the average egg weight of mosquitoes and the previous studies involved a different species, *Ae. aegypti*, and focused on resistance to desiccation. It is possible that there is an interaction between RF exposure and environmental factors that affects the average weight of eggs. One possible explanation is that RF exposure affects the physiology of field strain hens, making them less able to absorb and process nutrients from their environment. This could result in lighter eggs overall, even though radiation from RF increases egg weight. However, it is also possible that the control field strain eggs are heavier due to other environmental factors that are not present in the field strain hens that were exposed to RF radiation. For example, the control field strain could have access to more nutritious feed or more exercise space, both of which could contribute to higher egg weight. The interaction between RF and environmental factors is complex and can be influenced by a variety of factors. Further research would be needed to determine the exact mechanisms. This study may provide a basis for future research on the average egg weight of mosquitoes.

In summary, this study found that the length of eggs from laboratory strains exposed to radiation RF was greater than that of unexposed (control) eggs from laboratory strains, but for eggs from field strains, the unexposed eggs had greater length measurements. The egg length for laboratory and field strains showed no significant difference, but the comparison between unexposed and 18 GHz irradiated eggs from laboratory strains showed a significant difference. The results of this study differ from previous studies on the length of *Ae. albopictus* eggs (8). In this study, it was found that the length of laboratory strain eggs exposed to RF radiation was greater than that of unexposed (control) laboratory strain eggs, but for field strain eggs, the unexposed eggs had greater length measurements than the exposed eggs. The length of *Ae. albopictus* eggs found in this study was smaller than those reported in previous studies, with mean lengths of 509.3 μm and 524.52 μm (8, 9). The study also found that there was no significant difference in egg length between laboratory and field strains, but there was a significant difference when comparing unexposed and 18 GHz eggs from laboratory strains (9). These results differ from those of previous studies and may indicate a possible effect of radiation RF on egg length in laboratory strains of *Ae. albopictus* (9).

In addition, the study found that egg width was smaller in exposed eggs from laboratory and field strains compared with unexposed eggs, contradicting previous studies (7, 8). However, the egg index (ratio of egg length to egg width) of exposed eggs was larger in both strains, and the area of exposed eggs of the laboratory strain was larger than that of control eggs. The area of exposed eggs of the field strain was smaller than that of the control eggs. These results

cannot be compared with previous studies because there are currently no other studies measuring egg area (9).

The study also found that irradiating mosquito eggs with RF affected the hatching rate of the eggs. Eggs exposed to a high RF radiation of 18 GHz hatched least often, but also the fastest. Eggs exposed to a low dose of 900 MHz had the highest average hatch rate, but took a moderate amount of time to hatch. The control group, i.e., the unexposed eggs, had a moderate hatching rate and took the longest time to hatch. The study concluded that exposure RF had a direct effect on average hatch rate and hatch days. High-dose exposure resulted in faster hatching days, followed by low-dose exposure and no exposure. The study found that the high-dose RF exposure (18 GHz) resulted in faster hatching days than the low-dose exposure (900 MHz) and the unexposed control group. This is consistent with the results of other studies, which found that hatching rates decreased significantly with increasing radiation exposure (10, 11). The researchers also found in the study that there was no significant difference in induced sterility between pupal irradiation at 40 Gy and 60 Gy. Adult females from irradiated pupae had significantly lower egg counts and hatching rates after mating with normal males than the control group. Both egg number and hatching rate were positively correlated with radiation dose. The study also found that cell phone radiation significantly reduced hatching and may affect pupal development (12).

Exposure to different doses of RF radiation altered the developmental duration of a given species, with the highest dose (18 GHz) having the longest developmental time for each stage and the low dose (900 MHz) and control having comparable developmental times. Nevertheless, the low dose resulted in the greatest adult emergence rate, followed by the control and the high dose. These results are consistent with previous studies on the effects of microwave exposure EMF on the growth of *Ae. albopictus*. Based on the findings by Atli and Ünlü H (13) discovered that exposure to microwaves EMF caused a delay in average pupation time but had no effect on pupation rate. According to Ernawan et al. (14), pupation rate decreased with increasing irradiation dose, but the difference between control and 70 Gy treatment was not statistically significant. Similarly, Aldridge et al. (15) discovered that pupal irradiation dose had no statistically significant effect on the proportion of successfully hatched adults. Ranathunge et al. (10) discovered that pupal hatching rates were not significantly different from control rates across all age groups and irradiation doses.

The results of this study have potential implications for mosquito control strategies, mosquito-borne diseases, and public health. Mosquitoes are vectors of many deadly diseases, including dengue fever, chikungunya, and Zika virus, and controlling their populations is an important public health goal. If irradiation with RF can affect the hatching and development rates of *Aedes* mosquitoes, it may be possible to use RF radiation as a control method. However, before such a method can be

used, it is important to fully understand the effects of RF on mosquito populations in the natural environment. In addition, it is important to consider the potential unintended consequences of using RF radiation as a control method, such as the development of resistance in mosquito populations or unintended harm to non-target species.

Conclusion

Exposure to radiofrequency radiation has been shown to alter the hatching and development of *Ae. albopictus* mosquitoes, possibly reducing their numbers and preventing dengue fever transmission. Further studies are needed to understand the full extent of these effects and how they can be used to control dengue vectors. These studies are essential to understand the potential of radiofrequency exposure (RF) as a strategy to minimise the spread of dengue disease and to select the most effective use of this knowledge in control measures.

This research discovered that higher exposure to RF can reduce the number of days *Aedes* mosquitoes take to hatch, but also limit their growth rate. This information can be used to understand the effects of RF exposure on *Aedes* mosquito populations and provide more insight into population dynamics. However, results may vary depending on the type of RF exposure and the mosquito species studied. Further research is needed to understand the potential hazards and long-term effects on other living organisms.

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Competing interests

The authors declare that they have no competing interests.

Ethical clearance

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). The approval duration was July 2022 until June 2023.

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