

Effects of Ultrasound Waves on Immature Stages of *Aedes aegypti* (L) Mosquitoes

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Abstract The objective of this study was to evaluate the effect of ultrasound waves against dengue vector *Aedes aegypti*. The susceptibility tests were carried out to determine the egg hatchability and mortality rates of *Aedes aegypti* immatures. The ultrasonic bath device was used to test the effect of ultrasound waves on the dengue vector mosquito. Experiments were conducted at different series of time such as 30 S, 60 S, 90 S, 120 S, 150 S, 180 S and 200 S determine the percentage of mortality. The 100% larval mortality was observed in 180 seconds and 100% egg mortality observed in 150 seconds. The highest larval mortality was found in first instar larvae. These results suggest that ultrasound waves have more potential to kill the *Aedes aegypti* mosquitoes.

Keywords: *ultrasound waves, ultrasonic bath device, larvicidal activity, Aedes aegypti, egg hatchability*

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1. Introduction

The most crucial human health pest *Aedes aegypti* L. are established vector of arboviruses that causes diseases like dengue, yellow fever, and chikungunya [1]. *Ae. aegypti* can also transmit Ross River and Murray and Valley Encephalitis viruses [2]. Dengue vector control aims to reduce the amount of contact between humans and *Ae. aegypti*. Chemical insecticides, ecological management, biological control, bio chemicals, bed nets, repellents, sterile mosquito technology, and UV-irradiated control methods are recently available [3]. However, alternative strategies are still needed. Among them ultrasound waves may be a possible alternative to control immature stages of *Ae. aegypti* mosquitoes.

Ultrasound consists of acoustic wave and greater frequency limits [4]. The audibility of humans ranges from 1000 Hz to 4000 Hz. Most of the insects produce and identify acoustic signals for courtship, avoidance and alarm [5]. Foster and Lutes [6] reported that most of the mosquitoes are avoiding the ultrasonic sounds produced by bats. The prey has the ability to produce ultrasonic signals by predators [7]. Most of the studies have proved that ultrasonic signals are not effective to control insects preyed by bats [8]. The devices such as sonic, electronic and ultrasonic are produced world-wide as repel vector mosquitoes. In India mosquito control by ultrasonic devices still isn't getting significant attention. Therefore, the present study was carried out to evaluate the effectiveness ultra sounds produced by ultrasonic devices on eggs, larvae and pupae of *Ae. aegypti*.

2. Materials and Methods

2.1. Test Mosquitoes

Ae. aegypti egg cards were procured from the Centre for Research in Medical Entomology (CRME), ICMR Madurai and transported to the laboratory. The egg cards were placed in ion-free water and allowed to hatch out. The hatched larvae were transferred to enamel larval trays until adult emergence. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. After emergence, the adult mosquitoes were maintained separately in a net cage (45×35×45 cm³) of an insectary. Adult mosquitoes were provided with 10% sucrose solution and allowed to feed pigeon blood for a meal [9]. Mean room temperature of 27±2°C and relative humidity of 70 - 80% were maintained in insectary. Inside the cages a beaker was kept with moist filter paper for egg laying. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae on becoming pupae were collected, transferred to plastic bowls and keep the inside mosquito cage for adult emergence.

2.2. Ultrasonic Bath Device

Ultrasonic bath device contains the frequency ranges of 32 kHz to 38 kHz. Warm water was poured into the device until the water level reaches the lip of the tank. Ultrasonic device was operated by the timer dial to turn required time. The sonic button was pressed and heater became switched off.

2.3. Susceptibility Test

Ae. aegypti eggs, larvae and pupae were exposed to a container kept inside the device for 30 seconds in order to observe the effect of the ultrasonic wave. The experiment was conducted repeatedly at different series of time such as 30 seconds, 60 seconds, 90 seconds, 120 seconds, 150 seconds, 180 seconds and 200 seconds. Total number of eggs, larvae and pupae exposed to each concentration was 30 (n=30).

Ae. aegypti eggs' hatching percentage was calculated by the formula of

$$H = \frac{N_h}{N_i} \times 100 \quad (1)$$

Where,

H - Hatching percentage

N_h - Number of eggs hatched

N_i - Number of eggs introduced.

Ovicidal activity was assessed in terms of Egg Mortality Rate (EMR) using the given formula

$$EMR = \frac{N_{nh}}{N_i} \times 100 \quad (2)$$

Where,

EMR - Egg Mortality Rate

N_{nh} - Number of eggs not hatched

N_i - Number of eggs introduced.

3. Results

Ae. aegypti egg' hatching percentage and ovicidal rate on different time (seconds) series are presented in Table 1, and Figure 1 respectively. The effect of egg mortality was dependent on allowed time of the device. *Ae. aegyptis*' eggs were exposed to time ranges of 30, 60, 90,120, 150, 180, and 200 respectively.

Table 1. ULTRASOUND WAVES ON *AE. AEGYPTI* EGGS

| <i>Aedes aegypti</i> immature stage | Time (Seconds) | Hatchability(%) | Egg Mortality Rate (%) |
|-------------------------------------|----------------|-----------------|------------------------|
| Eggs | 30 | 46.7 | 53.3 |
| | 60 | 36.7 | 63.3 |
| | 90 | 23.3 | 76.7 |
| | 120 | 16.7 | 83.3 |
| | 150 | 3.3 | 96.7 |
| | 180 | 0 | 100 |
| | 200 | 0 | 100 |

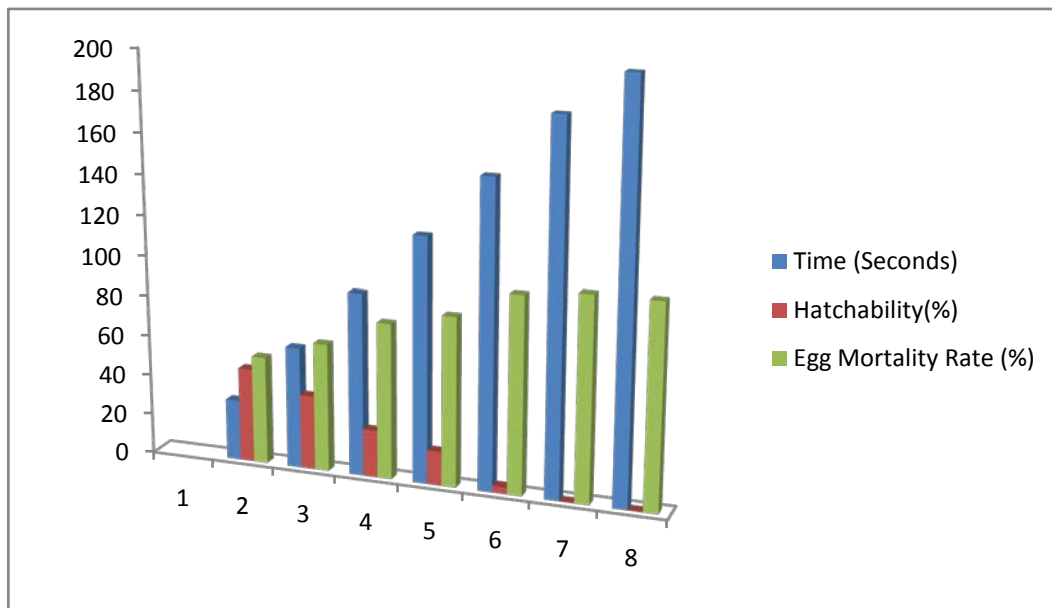


Figure 1. Ultra sound waves against *Ae. aegypti* eggs

Table 2. ULTRASOUND WAVES ON *AE. AEGYPTI* LARVAE AND PUPAE

| Time (Seconds) | Larval Mortality Rate (%) | | | | |
|----------------|---------------------------|------------------|-------------------|------------------|-------|
| | I Instar larvae | II Instar larvae | III Instar larvae | IV Instar larvae | Pupae |
| 30 | 46.7 | 43.3 | 36.7 | 23.3 | 16.7 |
| 60 | 56.7 | 53.3 | 46.7 | 46.7 | 23.3 |
| 90 | 76.7 | 63.3 | 53.3 | 53.3 | 36.7 |
| 120 | 83.3 | 76.7 | 76.7 | 63.3 | 53.3 |
| 150 | 90 | 90 | 83.3 | 83.3 | 76.7 |
| 180 | 100 | 100 | 100 | 96.7 | 96.7 |
| 200 | 100 | 100 | 100 | 100 | 100 |

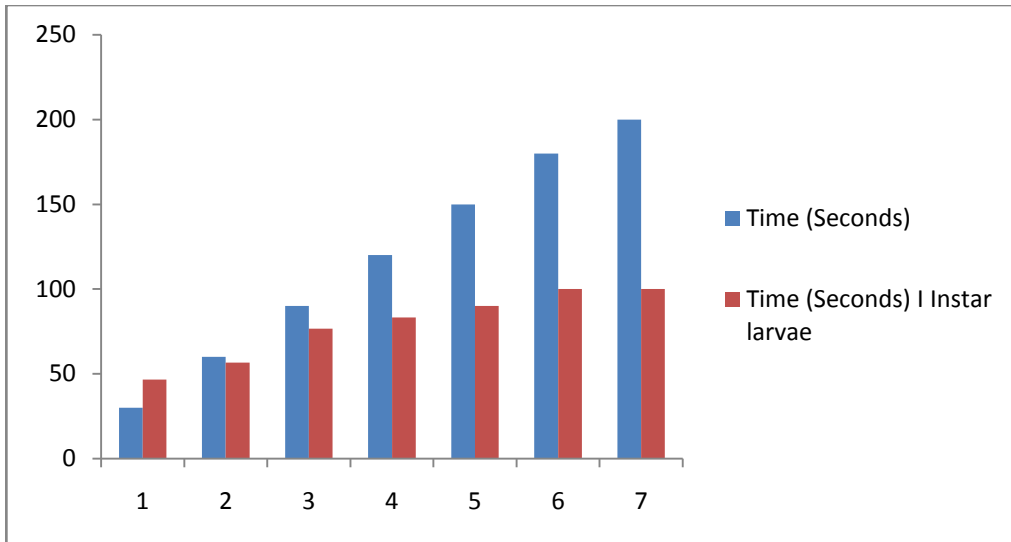


Figure 2. Ultra sound waves against *Ae. aegypti* I Instar larvae

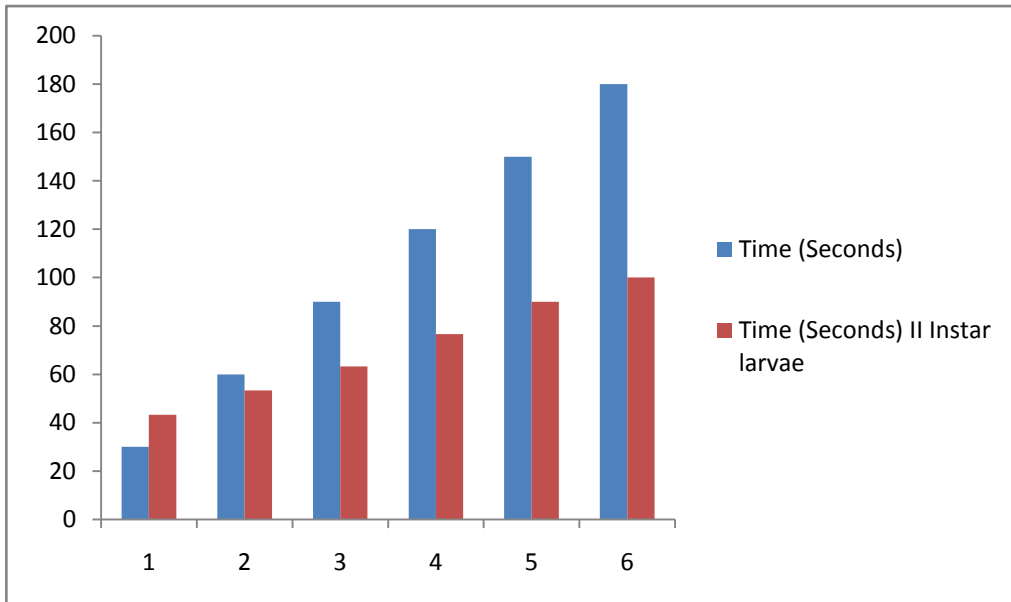


Figure 3. Ultra sound waves against *Ae. aegypti* II Instar larvae

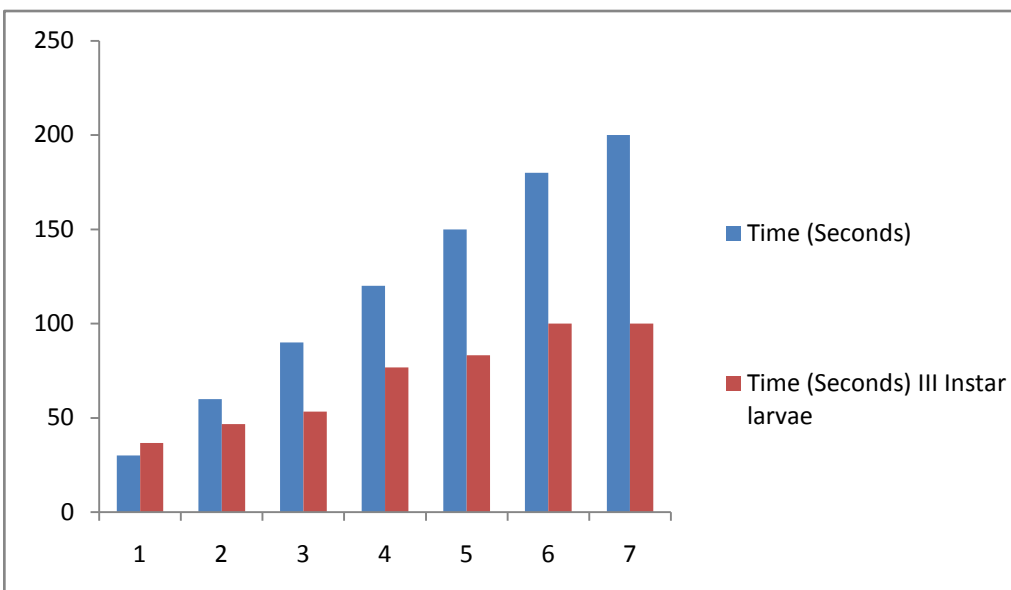


Figure 4. Ultra sound waves against *Ae. aegypti* III Instar larvae

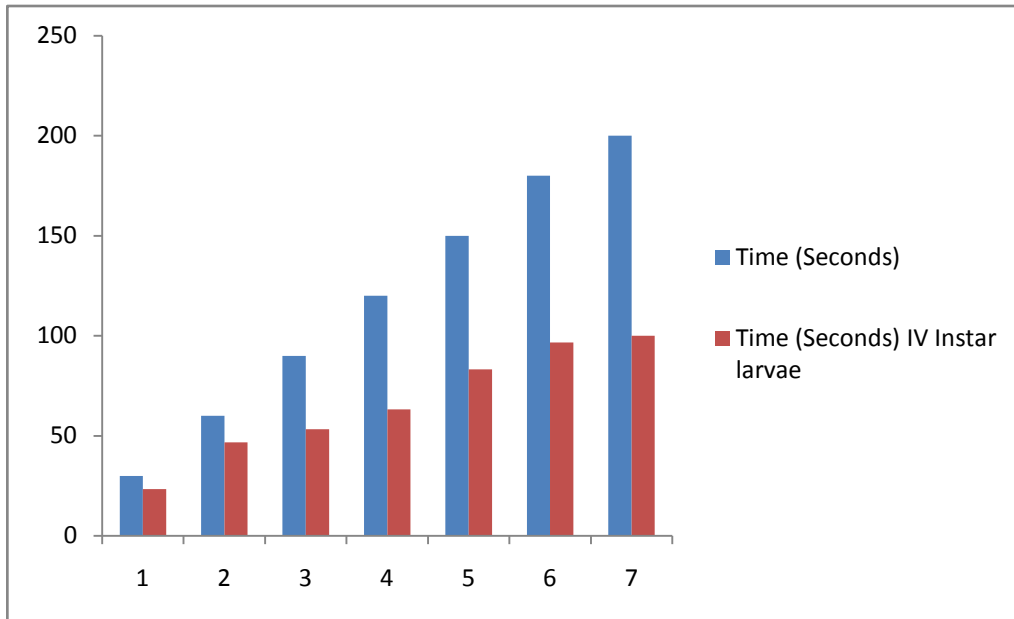


Figure 5. Ultra sound waves against *Ae. aegypti* IV Instar larvae

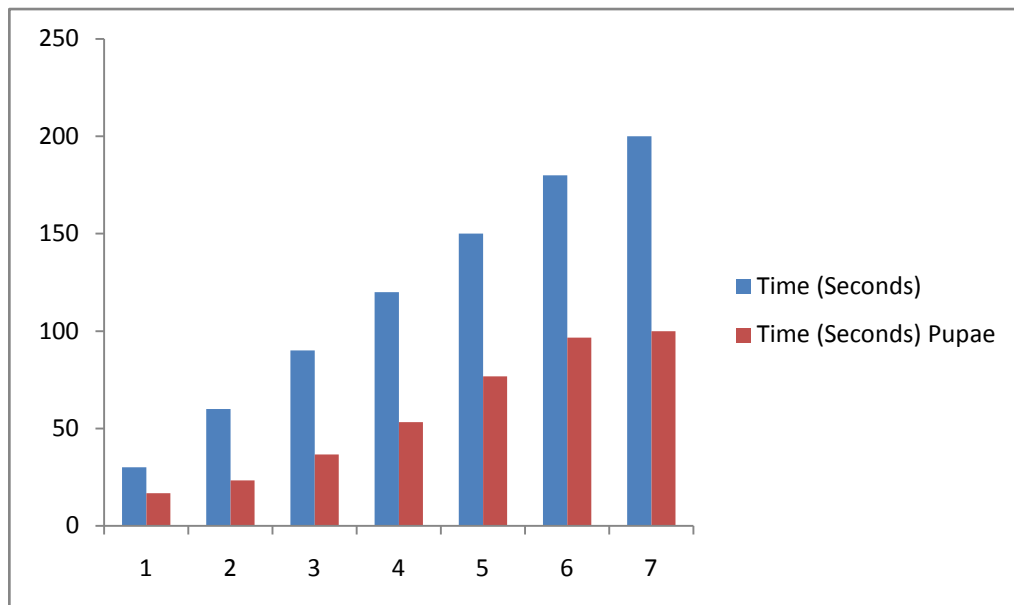


Figure 6. Ultra sound waves against *Ae. aegypti* pupae

Ae. aegypti larval and pupal mortality rates are presented in Table 2 and Figure 2 to Figure 6 respectively. The mortality rate of first instar larvae in each time series was 46.7, 56.7, 76.7, 83.3, 90, 100 and 100 respectively. The mortality rate of second instar larvae in each time series was 43.3, 53.3, 63.3, 76.7, 90, 100 and 100 respectively. The mortality rate of third instar larvae in each time series was 36.7, 46.7, 53.3, 76.7, 83.3, 100 and 100 respectively. The mortality rate of fourth instar larvae in each time series was 23.3, 46.7, 53.3, 76.7, 63.3, 83.3 and 96.7 respectively. The mortality rate of pupae in each time series was 16.7, 23.3, 36.7, 53.3, 76.7, 96.7 and 100 respectively.

4. Discussion and Conclusions

Many studies used ultrasonic devices with the frequency ranges of 2–60 kHz against *Aedes aegypti*,

Culex pipiens and *Anopheles quadriannulatus* mosquitoes. The present study was comparable to other reports. Ahmad *et al.* [10] proved that the significant differences found in mosquitoes within the ultrasound chamber. Hadi *et al.* [11] proved that the usage of ultrasound was existed to be effective in knock down and expelling *Ae. Aegypti*. The effects of the ultrasound device in different frequencies tested from designing steps had on the knockdown and expel rate of the mosquitoes with the fan. Also found the complimentary approach in the use of designing devices used for the control of *Anopheles gambiae* (s.l.)

After the breeding male mosquito becomes a natural enemy of female mosquitoes. On the first day of emergence, the female mosquito does not bite humans, but after mating get blood. Female mosquito does not allow males to mate once again and avoid the males. Females identify the presence of males with the ultrasounds developed by the males. In the present study an ultrasound

bath device had 38 kHz ultra sound waves, it may imitate male mosquito. This can repel female mosquitoes. The ultrasound can create stress on the *Ae. aegypti* immature and arrest their further development. Ultrasound scanning is mostly used in hospitals. Echocardiogram also makes an ultrasound to take images of the heart's functions. As per the scientific facts, ultrasound will not cause any human health issues. Therefore the ultrasounds can be used as one of the dengue vector control strategies.

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