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Impact of three different types of water media on the life stages of *Aedes aegypti* under laboratory conditions

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Abstract

Water plays a crucial role in a mosquito’s life cycle as it includes an aquatic phase. Female mosquitoes lay their eggs in different water bodies and the immature stages successfully develop into adults depending on the water quality. In this research work, we studied the effects of three different types of water media (deionized, distilled, and tap water) on the developmental stages and quality parameters of *A. aegypti*. The study was aimed to find more affordable ways to improve mass-rearing protocol toward the implementation of sterile insect techniques. In this experiment, a suitable rearing media for *A. aegypti* mosquito in laboratory conditions was selected by assessing the mosquitoes' tolerance to variations in water quality with respect to electrical conductivity (EC), dissolved oxygen (DO), total dissolved solids (TDS), and pH. During this study, pupal production rate, time to pupation, adult emergence rate, male and female pupal body size and adult longevity were examined. According to the obtained data, laboratory used tap water is moderately hard and has no noticeable effect on the growth and quality of *A aegypti* mosquito species. These results might be helpful in cost effective rearing of *A. aegypti* using tap water for the successful implementation of sterile insect technique. Considering that reverse osmosis or deionized water is costly in Bangladesh and the country is struggling with the ongoing challenges of *Aedes* mosquito-borne diseases.

Keywords: *A. aegypti*, mass rearing, sterile insect technique, rearing media

Introduction

All organisms' growth is greatly influenced by the physico-chemical characteristics of their surroundings [1]. Mosquitoes, in particular, undergo an aquatic phase in their life cycle, relying on water bodies for oviposition, larval and pupal stages, making water a crucial factor for their overall reproductive success and adult traits [2] [3]. Numerous studies on insect taxa demonstrate how physico-chemical characteristics of breeding grounds affect mosquito abundance, with individual species showing preferences for water qualities. For instance, *A. aegypti* typically lays its eggs in artificial containers with rain water. Several studies have indicated that variables like as dissolved oxygen, pH, temperature, conductivity, and vegetation all have a significant impact on the number of mosquito larvae that belong to distinct species [4] [5] [6] [7]. Around the world, scientists are raising insects for research and to control pests. Rearing mosquitoes is a complicated task because it involves carefully checking water quality, the number of larvae, their food, and the environment [8]. While many insectaries utilize deionized or reverse osmosis water for mosquito rearing, regions in arid zones often resort to alternative water sources, including tap water, surface water, groundwater, and desalinated water [9]. To evaluate the quality of water, indicators including salinity, total dissolved solids, electrical conductivity, and water hardness are frequently used [10] [11].

A significant portion of the global population resides in regions where mosquitoes are prevalent, leading to a substantial worldwide burden of mosquito-borne diseases [12]. *A. aegypti* and *A. albopictus* identified as invasive species, continue to expand their geographical range and are highly effective in transmitting various viruses that cause severe diseases such as dengue, chikungunya, yellow fever, West Nile fever, and Zika [13]. They are spreading to new areas and happening more often in both rich and poor countries [14]. In places where the diseases are usually found, the time when they can be transmitted is getting longer [15]. Sometimes, the diseases come back in areas where they hadn't been for a while. Due to limited availability of commercially viable vaccines and antiviral treatments, controlling *Aedes* spp. populations becomes pivotal in preventing disease transmission [16]. Utilizing the sterile insect technique (SIT) as part of integrated pest management strategies to reduce mosquito-borne illnesses has gained renewed attention globally after the Zika epidemic in the Americas at the end of 2015 [17] [18] [19]. The SIT is reliant on mass rearing of mosquitoes in large quantities. This process demands a substantial amount of water [20] [21]. However, securing a sufficient and reliable water supply remains challenging, particularly in arid regions with inadequate environmental protection and treatment methods, and especially in developing countries where access to pure drinking water is limited [22]. Globally, sterile *Aedes* mosquitoes are being mass-reared for release as part of disease control programs. Even though researchers are working hard to provide ideal raising circumstances and standard operating procedures, differences in water quality between nations and seasons have been noted [23] [24] [25] [26]. Since there are variations in the mass-rearing outputs in many of the Food and Agriculture Organization (FAO) and International Atomic Energy Agency (IAEA) Member States, it is important to investigate how water quality influences mosquito growth, productivity, and adult quality [27]. In this research work, a comparative study was done to evaluate the respective responses of *Aedes aegypti* life stages when reared in three different types of water. Deionized, distilled and tap water were used for rearing as well as some important physico-chemical parameters of three different types of water (electric conductivity, dissolve oxygen, total dissolve solids, and pH) were accurately measured.

Methodology

Mosquito colony and rearing conditions

The *A. aegypti* colony was maintained in a controlled environment in the laboratory of the Insect Biotechnology Division (IBD), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka, for over 50 generations, The temperature was kept at 27 ± 1◦C, the relative humidity (RH) was 70 ± 10%, and the photoperiod was 12 hours of light and 12 hours of dark. Every day, adults provided with a fresh cotton ball soaked in a 10% glucose solution. Adult females were supplied chicken using a membrane feeding system. In each cage for egg laying, a white paper strip (21 x 7 cm) was put in a 250 ml beaker filled with 200 ml of distilled water as an oviposition substrate. Every day, egg sheets were taken out, and each egg was hand counted manually as previously described [28].

Physico chemical analysis of rearing water

Deionized, distilled and tap water were used for rearing. Deionized water was produced by using Milli-Q Water Purification System (Millipore SAS, Molsheim, France). Physico chemical analysis of three types of water was studied in the using Hanna Instrument Multipara meter (HI 5321, HI 5421, HI2211)). Electric conductivity, Dissolve oxygen, Total dissolve solids, and Ph were determined.

Assessment of the Effects of Water Treatments on Larval Development and Adult Quality

Egg sheets were submerged in three different types of water for three hours and the larvae hatched during this time were used for experiments. About 1000 first-instar larvae were counted using larvae dispensing system (Orinno Technology, Singapore) and shifted to plastic rearing trays (38 cm x 26 cm x 1.5 cm) with 667 ml of tap water ensuring the density of 1.5 larvae per ml water. An amount of 0.7g powdered aquarium fish feed (Super Nova, Perfect Companion Group Co. Ltd., Thailand, nutritional composition: protein 20%, fat 3%, fiber 7%, moisture10%, calcium 0.7% and phosphorus 0.7%) was supplied daily up to the pupation time. Pupae were collected, recorded and transferred to water in 50 ml beakers. Three replicates were performed for each water treatment and the experiment was carried out twice. Larvae were checked daily for pupation, and pupae were collected and counted on a daily basis. For all experimental water treatments, we recorded: (i) pupal production rate, time to pupation and adult emergence rate (ii) male and female pupal body size (iii) Adult longevity. Photograph of pupal body size was taken under a dissecting microscope (Leica DM004x light microscope). 30 male pupae from each rearing media were placed into 30 different plastic cup with the top being closed with a mosquito net for each water treatment. As soon as the adult emerges the water was removed, and 10% sugar solution was provided every day using a cotton ball. Mortality was recorded for each day; dead males were removed regularly until all mosquitoes are demised. From the data, longevity was averaged and calculated.

Statistical Analysis

Chi square test was conducted to find the statistical significance in the proportion of conversion. A one-way ANOVA was conducted to find significance of adult mosquitos' longevity for three different water treatments. For numerical analysis, a 3rd order polynomial was fitted with the conversion data with least square approximation to find the emergence time for 50% conversion (ET_{50}) for three different water treatments. All analyses were conducted by using MATLAB 2022.

Results

Electrical Conductivity, Dissolve oxygen, TDS and pH of the Rearing Media

Electrical conductivity, dissolved oxygen, total dissolved solids and pH of the rearing media are presented in Table 1. EC ranged from 2.58 to 126 µs/cm, Water hardness or TDS were notably different between water media, ranging from 1.65 to 80.48ppm. Using the World Health Organization's (WHO) recommended classification system for water hardness [29], our rearing media can be classified as soft (Deionized and Distilled water), moderately hard water (Tap water).

Table 1: Measured EC, DO, TDS and pH of the rearing media used for *Ae. aegypti* mosquito in the present experiment

Water Types	Electric Conductivity (EC)	Dissolved Oxygen (DO)	Total Dissolved Solids (TDS)	pH
Deionized water	2.883 µs/cm	4.24 mg/L	1.84 ppm	7.63
Distilled water	2.582 µs/cm	5.64 mg/L	1.65 ppm	7.28
Tap water	126 µs/cm	3.92 mg/L	80.48 ppm	7.15

Effects of Water Treatments on Pupal production rate, Time to pupation and Adult emergence

Fig. 1 shows the cumulative conversion from 1st instar larvae to pupae during successive 10 days of observation. Highest number of converted pupae is reported for deionized water (n= 2894 out of 3000). The lowest number of converted pupae is reported for distilled water (n= 2078 out of 3000). The rate of conversion has been found by taking the first derivative of the cumulative conversion curve. The maximum rate of conversions for tap water, distilled water, and deionized water were 320 (day 2), 197 (day 2), and 339 (day 3) respectively.

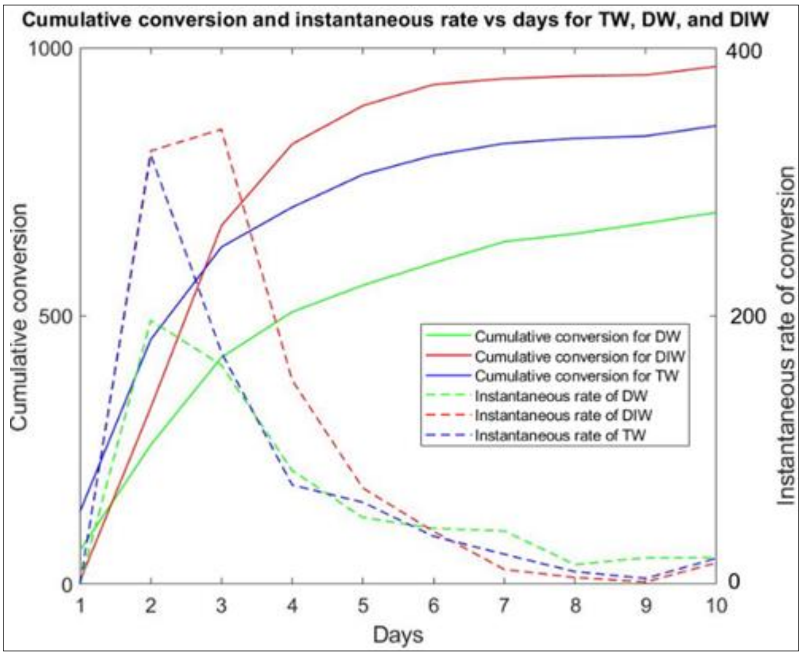

Fig 1: Cumulative and instantaneous rate of conversion of pupae vs days for tap water, distilled water, and deionized water

A chi-square test with 2 degrees of freedom showed that the conversion rate was significantly different for three types of water body used in this experiment ($\chi^2= 134.17$, df=2, $p< 0.01$).

To find the 50% emergence time (the time required for converting larvae to 50% pupae), the cumulative curves were fitted with 3rd order polynomial in Fig. 2. The emergence time for tap water, distilled water, and deionized water were 2.36, 3.94, and 2.44 days accordingly.

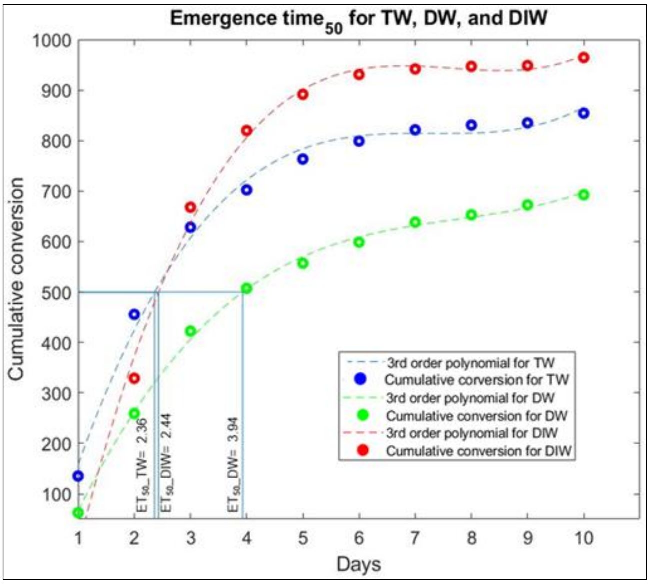

Fig 2: The 50% pupal emergence time for tap water, Distilled water, deionized water

Table 2: Mean time to pupation and adult emergence rate (Mean±SD)

Water Types	Time to pupation (Days)	Adult emergence rate (Percentage)
Deionized water	7±0	98.33%
Distilled water	8 ±0.47	95.84%
Tap water	7± 0.27	96.67%

Effects of Water Treatments on Pupal size

The t-test was performed between male and female pupae on the group of 10 (randomly extracted) from each of the water bodies to observe the effect of water treatment on their size. Only significant difference in male pupal size was found in distilled water vs deionized water, where the male pupal size was higher in distilled water than deionized water. No significant difference in size was also found among female pupae. Table 3, 4, and 5 showed the mean along with standard deviation and the t-statistics respectively on pupal size for different pair of water bodies.

Table 3: Tap water vs. Distilled water

Pupae	Tap Water (Mean ± SD)	Distilled Water (Mean ± SD)	p-value
Male	3.99±0.328 mm	4.22±0.332 mm	0.18
Female	4.73±0.316 mm	5.04±0.353 mm	0.07

Table 4: Distilled water vs. Deionized water

Pupae	Deionized Water (Mean ± SD)	Distilled Water (Mean ± SD)	p-value
Male	3.92±0.22 mm	4.22±0.332 mm	0.01*
Female	4.70±0.352 mm	5.04±0.353 mm	0.11

*$p<0.05$

Table 5: Deionized water vs. Tap water

Pupae	Deionized Water (Mean ± SD)	Tap Water (Mean ± SD)	p-value
Male	3.92±0.22 mm	3.99±0.328 mm	0.66
Female	4.70±0.352 mm	4.73±0.316 mm	0.85

Effects of Water Treatments on Adult Longevity

Figure 3 shows the mean number of alive adult male *A. aegypti* mosquitoes as recorded during the time span from 1 to 20 days. As shown in the figure, there is no clear difference in the pattern of the time of series of alive mosquito at three different water treatments. The one-way ANOVA shows no significant difference of the overall longevity profile at three water treatments (p= 0.6).

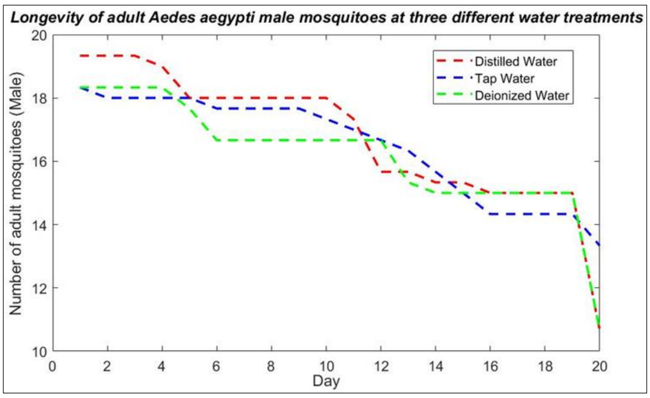

Fig 3: Longevity of male adult *Aedes aegypti mosquitoes at three different water treatments*

Discussion

For mass rearing of mosquito to support SIT programs, water availability and quality are crucial requirements. Depending on a variety of conditions, including climate, physical topography, and human activity, each species has a preferred body of water for oviposition. Breeding areas might be artificial or natural, different in size, situated in running or still water, with shade or sunshine, long-term or short-term [30] [31]. In the current study, the mosquitoes' tolerance to variations in water quality with regard to electrical conductivity, dissolved oxygen, total dissolved solids, and pH was evaluated in order to choose an appropriate rearing medium for *A. aegypti* mosquitoes in laboratory circumstances. Pupal production rate, time to pupation, adult emergence rate, male and female pupal body size, and adult longevity were all investigated in this study.

The results of this study indicated that there is almost no difference in the pupal conversion rate between tap and deionized water (Figure 1,2), which is contrary to the findings of the study conducted by W. Mamai *et al*. (2021). The cause might be that while our tap water is moderately hard, W. Mamai *et al*. used hard tap water. In terms of time to pupation or the rate of adult emergence there was no difference for these three water treatment methods (Table 2). According to W. Mamai *et al*. (2021), when growing *A. aegypti*, there was no discernible difference between the time to pupation or emergence rates using tap water and reverse osmosis water in different ratio. Nevertheless, D. Foko *et al*. (2007) found that when *A. gambiae* were reared in tap, spring and pond water, larval developmental period and adult recovery rate were considerably different. They discovered that, in comparison to pond and spring water, tap water has an unusually prolonged developmental period [32].

No significance difference was found for adult longevity in these three types of water. W. Mamai *et al.*, (2021) also found the similar result by using tap water and reverse osmosis water in different ratio for *A. aegypti*. According to their study, the longevity of males and females A. aegypti was not affected negatively by the water hardness level In addition, W. Mamai *et al.*, (2017) investigated that neither egg hatching nor larval development time or survival were affected by the use of dirty, re-used, larval-rearing water instead of clean dechlorinated water for *Anopheles arabiensis.*

Conclusion

Aquatic species have a characteristic range that they can withstand, and the composition of the water can play a significant role in their development and survival. Water quality is crucial for the mass rearing of mosquitoes since their larval and pupal stages are aquatic. This study was conducted to ascertain whether tap water can be an appropriate medium for rearing *A. aegypti* mosquitoes in laboratory settings in Bangladesh by assessing the tolerance of it to variations in water quality in relation to electrical conductivity, dissolved oxygen, total dissolved solids, and pH. According to data from this study, tap water in Bangladesh is moderately hard and has no noticeable effect on the growth and quality of *A aegypt*i mosquito species. In fact, the findings demonstrated that when *A. aegypti* was reared with tap water, the quality parameters needed for the SIT implementation program were sufficient. While reverse osmosis water is the best option but when it comes to low-cost techniques for better mass-rearing in preparation for SIT application, tap water works well in Bangladesh. These findings might have a significant impact on how the SIT is implemented in places where reverse osmosis water is expensive or sparse.

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