Impact of container material on the development of *Aedes aegypti* larvae at different temperatures

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**ABSTRACT**

*Background & objectives:* *Aedes aegypti,* the primary vector of dengue generally breeds in intradomestic and peridomestic containers made up of different materials, i.e. plastic, iron, rubber, earthen material *etc.* The material of container is likely to affect the temperature of water in container with variation in environmental temperature. The present study was aimed to determine the effect of different container materials on larval development of *Ae. aegypti* at different temperatures.

*Methods:* Newly hatched I instar larvae (2–4 h old) were used in the study and experiments were conducted using three different containers made up of plastic, iron and earthen material. Three replicates for each type of container at 22, 26, 30, 34, 38, 40, and 42°C were placed in environmental chamber for the development of larvae.

*Results:* At temperatures >22°C, 50% pupation was completed in earthen pot within 4.3±0.6 to 6.3±0.6 days followed by plastic containers (5±0 to 8±0 days) and iron containers (6±0 to 9±0 days). Developmental time for 50% pupation in the three containers differed significantly (*p* < 0.05) at all the experimental temperature ranges. A significant variation was found in the temperature of environmental chamber and the temperature of water in three types of containers (*p* < 0.05). The difference in the temperatures of water in different containers resulted in significant variations in the developmental period of larvae. More than 35°C temperature of water was found inimical for pupal development.

*Interpretation & conclusion:* The results revealed the variation in temperature of water in different types of containers depending on the material of container, affecting duration of larval development. As the larval development was faster in earthen pot as compared to plastic and iron containers, community should be discouraged for storing the water in earthen pots. However, in view of containers of different materials used by the community in different temperature zones in the country, further studies are required for devising area-specific preventive measures for *Aedes* breeding.

**Key words** *Aedes aegypti;* container material; environmental temperature; larval development; water temperature

**INTRODUCTION**

Dengue is one of the most serious vector-borne diseases of public health importance in the world. It is caused by four serotypes of dengue virus (DEN 1–4) in India. *Aedes aegypti* is the principal vector of dengue in India with recent reports of *Ae. albopictus* also transmitting dengue viruses¹⁻². According to an estimate, about 40% of the world’s population is at risk of infection with dengue viruses³. Dengue is prevalent throughout India affecting most of the metropolitan cities and towns. During the year 2014, 40,571 cases of dengue with 137 deaths were reported from India⁴.

The biology of mosquito vectors is influenced by several factors of which temperature is one of the key factors. Effect of temperature on larval biology has been studied in different mosquito species including *Ae. aegypti*⁵⁻⁷. Temperature for rearing of mosquito larvae influences body size, fecundity and longevity of emerging adult mosquitoes⁸⁻¹⁰. Developmental time of larvae is also influenced by the temperature resulting in faster development at higher temperatures while slow development at lower temperatures¹¹.

*Ae. aegypti* is container breeder mosquito preferring intradomestic and peridomestic containers such as tank, cooler, cistern, bird pot, tyre, water storage jar, plastic cup *etc.*¹². All these containers are made up of different materials, i.e. plastic, iron, rubber, earthen material *etc.* The temperature of water in different containers is likely to vary depending upon the materials of the container. A significant variation between environment and water-container temperatures has been reported earlier¹³. While studying the effect of temperature on development of *Ae. aegypti* in Australia, Tun-Lin *et al.*⁶ found that the position of water containers kept in different places in field and larval food resources in nature affected larval developmental period.
and survival. No such studies have been undertaken in India where diverse water storage practices in different types of containers are quite prevalent. Therefore, the present study was planned and conducted under laboratory conditions. A clear understanding of how environmental condition of different water storage containers affects mosquito development may lead to the development/planning of more effective control strategy.

**MATERIAL & METHODS**

Eggs of *Ae. aegypti* were obtained from the insectary of the National Institute of Malaria Research, New Delhi on moist filter paper strips. The egg strips were placed in porcelain tray containing one litre of water and allowed to hatch for 24 – 48 h. Newly hatched I instar larvae (2–4 h old) were used in the study. Experiments were conducted using three different containers made up of plastic, iron and earthen material. The 500 ml of tap water kept for 24 h was added to each of the containers and 50 I instar larvae were pipetted into them. Three replicates for each type of container at 22, 26, 30, 34, 38, 40, and 42°C were placed in environmental chamber (Kaleidoscope Climatic Solutions, Bengaluru). Water temperature in each container was measured using portable digital thermometer every day at 1100 hrs. Larval food comprising of dog biscuits and fish food (60 : 40) was provided for larval feeding. The larval development in different containers was monitored after every 24 h and checked for mortality, if any. At the same time water and food in the containers was also changed. The difference in developmental time from larva to pupa was analysed by single factor ANOVA. Mortality rate and difference of temperature in water containers and environmental chamber was analysed by two factor ANOVA. All the statistical analyses were done with the help of MS-Excel 2007.

**RESULTS**

In order to record the impact of temperature on development from larvae to pupae, three aspects were observed, *i.e.* day of commencement of pupation, day of cessation of pupation and duration of 50% pupation (Table 1). Above 22 °C temperature, pupation commenced earlier in the earthen pots (4.3 ± 0.6 to 5.6 ± 0.6 days) followed by plastic (4.7 ± 0.6 to 7.3 ± 0.6 days) and iron container (5 ± 0 to 7.3 ± 0.6 days). Commencement of pupation did not differ significantly among different types of containers at different temperatures except at 26°C (*p* <0.05). In earthen pots, 50% pupation was achieved faster (4.3 ± 0.6 days) as compared to the plastic (5 ± 0 days) and iron containers (6 ± 0 days) at 34°C. At temperatures >22°C, 50% pupation in earthen pots was completed within 4.3 ± 0.6 to 6.3 ± 0.6 days whereas in iron containers it took 6 ± 0 to 9 ± 0 days and 5 ± 0 to 8 ± 0 days in plastic containers. Duration of 50% pupation in the three containers differed significantly (*p* <0.05) at all the selected temperature ranges. At 22°C the larvae took longest time to pupate (12.7 ± 0.6 to 15.7 ± 0.6 days) while shortest time in pupation was at 34°C (6 ± 0 to 7 ± 0 days) in all the containers. Except at 38°C, the difference in pupation time was significant (<0.05) among the three containers at all the selected temperature range . Out of the three containers, pupation did not occur in the iron containers at 38°C while at 40°C there was no pupation in any of the three types of containers. Larval development period at different temperatures was averaged for each of the containers, which was found shortest (6.4 days) in case of earthen pots followed by plastic containers (7.2 days) and iron containers (8.3 days) (Fig. 1)

A significant variation was found between the temperature of water in three types of containers and the temperature of environmental chamber (*p* < 0.05). The

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**Table 1. Impact of container material on development of *Aedes aegypti* larvae at different temperatures**

<table>
<thead>
<tr>
<th>Temperature of environmental chamber (°C)</th>
<th>Plastic container</th>
<th>Iron container</th>
<th>Earthen pot</th>
<th>p-value</th>
<th>Plastic container</th>
<th>Iron container</th>
<th>Earthen pot</th>
<th>p-value</th>
<th>Plastic container</th>
<th>Iron container</th>
<th>Earthen pot</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>10.3±0.6</td>
<td>9.7±0.6</td>
<td>9.3±0.6</td>
<td>0.177</td>
<td>12.3±0.6</td>
<td>11±1</td>
<td>10.3±0.6</td>
<td>0.042</td>
<td>15.7±0.6</td>
<td>12.7±0.6</td>
<td>13.7±0.6</td>
<td>0.001</td>
</tr>
<tr>
<td>26</td>
<td>7.3±0.6</td>
<td>7.3±0.6</td>
<td>5.6±0.6</td>
<td>0.018</td>
<td>8±0</td>
<td>9±0</td>
<td>6.3±0.6</td>
<td>0.0</td>
<td>9±0</td>
<td>10±1</td>
<td>7.6±0.6</td>
<td>0.014</td>
</tr>
<tr>
<td>30</td>
<td>5±0</td>
<td>5±0</td>
<td>4.6±0.6</td>
<td>0.421</td>
<td>5±0</td>
<td>6.3±0.6</td>
<td>5±0</td>
<td>0.003</td>
<td>6.3±0.6</td>
<td>8±0</td>
<td>6.6±0.6</td>
<td>0.010</td>
</tr>
<tr>
<td>34</td>
<td>4.7±0.6</td>
<td>5.3±0.6</td>
<td>4.3±0.6</td>
<td>0.177</td>
<td>5±0</td>
<td>6±0</td>
<td>4.3±0.6</td>
<td>0.002</td>
<td>6±0</td>
<td>7±0</td>
<td>6.3±0.6</td>
<td>0.027</td>
</tr>
<tr>
<td>38</td>
<td>5.6±0.6</td>
<td>NP</td>
<td>5±0</td>
<td>0.11</td>
<td>6±0</td>
<td>NP</td>
<td>5±0</td>
<td>0.0</td>
<td>8.3±0.6</td>
<td>NP</td>
<td>9±0</td>
<td>0.116</td>
</tr>
<tr>
<td>40</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>–</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
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<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>–</td>
</tr>
</tbody>
</table>

SD—Standard deviation; NP—No pupation.
In this study temperature of water in the different types of containers was found lower than the temperature of environmental chamber (Table 2). The difference in temperature between water and environmental chamber was highest in case of earthen pots (1.9–4.1°C) followed by plastic (1.2–3.3°C) and iron containers (0.8–2.5°C). The difference of environmental and water temperature was positively correlated with the rise in temperature of environmental chamber.

Mortality rate of *Ae. aegypti* larvae in different containers is shown in Fig. 2. At 38°C temperature, the larval mortality in earthen pots, plastic and iron containers was found 43.3, 50 and 100% respectively. At 40°C, 100% mortality was observed in all types of containers. Overall, lowest mortality rate was recorded in the earthen pots except at 22°C temperature. Mortality rate differed significantly (*p* < 0.01) among different types of containers and across all temperature treatments. Two way interactions between temperature and container materials were also significant for mortality rate (*p* <0.01).

**Table 2.** Observed variation in water temperature of different containers with respect to environmental temperature

<table>
<thead>
<tr>
<th>Temperature of environmental chamber (°C)</th>
<th>Temperature of water in different containers (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plastic containers</td>
</tr>
<tr>
<td>22</td>
<td>20.8</td>
</tr>
<tr>
<td>26</td>
<td>24.2</td>
</tr>
<tr>
<td>28</td>
<td>25.9</td>
</tr>
<tr>
<td>30</td>
<td>27.6</td>
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<tr>
<td>34</td>
<td>31.3</td>
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<tr>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>40</td>
<td>36.7</td>
</tr>
<tr>
<td>42</td>
<td>38.7</td>
</tr>
</tbody>
</table>

In the present study the optimum temperature of water for larval development was found to be ranging from 23.6 to 32.2°C as delayed development was observed at 35°C in all the containers. Larval development was arrested leading to 100% larval mortality beyond this temperature. As per the results of the study, at temperature >32.2°C, *Ae. aegypti* larvae experienced stress due to increase in temperature that might have caused abnormalities at the cellular level in larvae. Higher temperature causes faster growth resulting in less nutrient reserves.
which leads to failure in moulting\textsuperscript{16–17}. Moreover, cell damage occurs due to heat that might have resulted in 100\% mortality at temperature $>35^\circ$C\textsuperscript{18}. Our results are in conformity with that of Bar-Zeev\textsuperscript{3}, who reported no larval development of \textit{Ae. aegypti} at 36.1$^\circ$C temperature of water. However, the results are different from a study conducted in Trinidad by Hemme \textit{et al}\textsuperscript{19}, where \textit{Ae. aegypti} larvae were found surviving in water storage drum of steel at a temperature of 36$^\circ$C. This may be due to the difference in study conditions as the present study was undertaken with constant temperature in environmental chamber, while the earlier study was conducted in field conditions with diurnal variation of temperature in water storage drums.

In one of the study undertaken recently in Delhi, Singh \textit{et al}\textsuperscript{20} reported that water filled earthen pots kept for birds are the second most preferred breeding habitats for \textit{Aedes} mosquitoes. Similar observations were also made by Kumari \textit{et al}\textsuperscript{15}; and Das and Hazra\textsuperscript{21}. Use of earthen pots may serve as very potent container for the breeding of \textit{Ae. aegypti} due to faster development of larvae. Therefore, to reduce larval habitats for \textit{Aedes} breeding, the use of earthen pots should be discouraged. In Delhi and other states of India, it is a common practice to store water in drums and containers made up of plastic. These containers are one of the major sources of \textit{Aedes} breeding. If iron containers are used instead of plastic particularly during summers when temperature reaches $>34^\circ$C, development of \textit{Aedes} larvae will be hampered resulting in lower density of \textit{Aedes} mosquito.

CONCLUSION

This study underscores the importance of container material as breeding ground for in developmental time and survival of larvae of \textit{Aedes aegypti}. The results provide evidence of the variation in temperature of water experienced by mosquito larvae in different types of containers depending on the material of container. As the development of \textit{Ae. aegypti} was found more conducive in earthen pots as compared to plastic and iron containers, community should be discouraged to store water in earthen pots. Keeping in view, the variety of containers used in different temperature zones of the country, further studies may be undertaken with other container materials for better understanding of \textit{Aedes} larval development in nature and to adopt better control strategies.

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