Larvicidal Effect of Some Selected Salts Against the Dengue Vector Mosquito, Aedes aegypti (Diptera: Culicidae) in Bangladesh

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Abstract

Background The container breeding mosquito, *Aedes aegypti* is the major universal vector of dengue viruses capable of causing dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Vector control may have a potential role in reducing the morbidity and mortality of human by DHF/DSS. The purposes of this work were to evaluate the larvicidal effect of some salt solutions against the larvae of *Ae. aegypti*.

Methods Freshly collected larvae were transferred to the laboratory and reared using rainwater as a rearing medium with yeast granules as larval food. Five salts i.e. AgNO₃, HgCl₂, CdCl₂, CuSO₄, and CuCl₂ were tested to assess the larvicidal effect on both the 1st and 3rd instars larvae of *Ae. aegypti*. Serial concentrations (0, 1, 3, 5, 7 &10 ppm) of each salt were prepared by using distilled water as the solvent.

Results Silver nitrate (AgNO₃) was noted as the most effective larvicide followed by HgCl₂, CdCl₂, CuSO₄ and CuCl₂ against *Ae. aegypti* larvae. The LC₅₀ of AgNO₃ against 1st and 3rd instars larvae were 0.118 and 1.659 ppm, and the LC₉₀ were 2 and 3.347 ppm, respectively. The LT₅₀ of AgNO₃(1 ppm) against 1st and 3rd instars larvae were 0.575 and 30.42hrs, and the LT₉₀ was 6 and 67.49 hrs, respectively. All of the first instar larvae were killed and failed to pupate with every salt of each concentration (1-10 ppm) within 7 days. Whereas 3rd instar larvae were also unable to pupate entirely in AgNO₃ and HgCl₂ solutions but very few (5-36.66%) pupation was found at 7 ppm & higher concentrations of CdCl₂, CuSO₄, and CuCl₂ salts. CdCl₂, CuSO₄ and CuCl₂ prevented 66.66%, 57.14% and 50% adult emergences from pupae at 5 ppm concentration respectively. The order of decrease of toxicity for larval mortality was AgNO₃ > HgCl₂ > CdCl₂ > CuSO₄ > CuCl₂ and for prevention of pupation & adult emergence was AgNO₃ ≥ HgCl₂ > CdCl₂ > CuSO₄ > CuCl₂.

Conclusions AgNO₃ was found as a very good potential in the killing of *Aedes aegypti* larvae, prevention of pupae formation and adult emergence. Therefore, the results obtained could be considered a contribution to the search for eco-friendly larvicides of natural origin. Further studies are needed to understand the residual aquatic toxicity of this salt in the field.
Background

*Aedes aegypti* (L.) is the predominant vector of dengue; a mosquito-borne arbovirus. It is also a recognized vector of Zika, Chikungunya, and Yellow fever viruses. Like malaria, dengue virus had become a global threat [1–2]. Dengue fever (DF) is an acute and painful disease that is often not lethal but dengue hemorrhagic fever (DHF) may lead to Dengue shock syndrome (DSS), i.e. circulatory failure which is often lethal within 12 to 24 hrs. Dengue virus is transmitted almost wholly from man to mosquito and mosquito to man [3]. The dengue virus spread rapidly and the disease develops into pandemic proportion [4]. A recent study estimated that there were more people at risk of dengue infection calculating up to 3.97 billion those living in 128 countries [5]. Both the species of *Aedes* mosquito breed frequently in artificial containers such as water-storage vessels, flower vases, water accumulations, discarded tins, automobile tires, and blocked gutters. These also provide excellent larval habitat and adult resting sites [6]. Though dengue has been reported as an urban disease [7] in the recent past dengue infection and the vector mosquitoes have been detected in rural areas of Thailand and India [8–9]. Unplanned urbanization in cities and technological advancement in villages might have played a significant role in dengue transmission [10, 11].

Mosquito control is a worldwide problem due to their vector nature and the resurgence of much infectious disease [12]. The best way to keep a check on the larval population because breeding grounds are confined to limited stagnant water bodies. The major existing means of mosquito control all over the world is the employment of synthetic insecticides [13]. Several insecticides have been indiscriminately used against the mosquito larvae and as a result over the time they have developed resistance to a wide variety of chemicals used against them i.e. *Ae. aegypti* resistant to Malathion, Temephos, Permethrin, Propoxur [14–15] and Fenitrothion [16]. Though chemical controls require a short time and work quickly but have some adverse effects on man and the environment. Burdick et al. [13] observed that residual toxicity may be magnified biologically in the food chain. On the other hand, salts are generally safer, less hazardous and environmentally friendly than chemical pesticides. Therefore the present study was conducted to determine the effects of salt solutions on the larvae of *Ae. aegypti*. 
Methods
Mosquito rearing

The period from July to September has been reported as the peak dengue season in Bangladesh. Freshly collected Ae.aegypti larvae were brought to the laboratory and cleaned with distilled water. These larvae were reared using rainwater (previously stored) as a rearing medium at the IRES (Insect Rearing and Experimental Station), Department of Zoology, Jahangirnagar University, Savar, Dhaka at ambient temperature and relative humidity (28 ± 2 °C and 70 ± 5% RH). In each rearing container (10 x 10 x 5 cm³) about 500 ml rainwater was taken and 500 healthy larvae were released. Larval food (0.10 gm yeast granules) was regularly added to each rearing container. Each container along with the larvae was placed inside a rearing cage (30 x 30 x 30 cm³) made of iron wire and fine mesh size mosquito net to prevent egg-laying by other mosquitoes. To avoid fungal contamination the larvae were transferred from the old container to another at regular intervals. Inspections were made 6 hourly to measure water temperature, relative humidity, larval molting and wastage of food if any. Sometimes a surface film or scum food was removed with a spuit or a brush and yeast granules (˂0.02 gm/100 ml) were newly added to the rearing medium, if required. As the larvae developed, the pupae were picked with the help of a pipette and transferred to a glass jar (covered with gauze net). About 500 pupae were transferred to a new plastic bowl containing rainwater for adult emergence. No food was supplied for this non-feeding stage. Adult mosquitoes were identified morphologically under stereomicroscopes using taxonomic keys [17–21], within a few hours after sampling.

A ratio of 3 females to 1 male is preferable for mating in a cage. Therefore a batch of 100 males and 300 females were housed together in a cage (30 x 30 x 30 cm³) for about 5–6 days to mate. Cotton pads soaked with 10% glucose solutions were placed inside the cage for food supplement of the adults. Gravid females were then removed to another cage and a pigeon (Columba livia) was kept tight for about an hour on the roof of rearing cage for sucking blood meal by the adult mosquitoes. Five plastic cups (125 ml) filled with distilled water (2.5 cm depth) were lined with a 3 wide strip of filter paper were placed inside each cage for laying eggs. Eggs were removed at regular intervals and kept in air-dried condition for subsequent use. When required, those eggs were released in the
rainwater of a plastic bowl for hatching. During the period of hatching 0.02 gm, glucose or yeast granules were added in each 100 ml distilled water as larval food.

**Preparation of stock solutions**

Salt solutions of different concentrations were prepared through the process of serial dilution. Serial dilution is a method used to stepwise dilute substance into solution with constant dilution factor in each step. In this method, 0.1 gm salt was mixed with 100 ml water for preparing a stock solution of 1000 ppm. After that 1 ml, 0.7 ml, 0.5 ml, 0.3 ml and 0.1 ml of stock solution were mixed with 99, 99.3, 99.5, 99.7 and 99.9 ml of water respectively for preparing 10, 7, 5, 3 and 1 ppm salt solutions.

**Larvicidal testing procedure**

For experimenting with each of the salt solutions, thirty-six plastic cups (125 ml) were taken and cleaned with distilled water. They were air-dried and arranged in groups 1, 2, 3, 4, 5 and 6, where each of the groups having six cups. Each cup of the group 2, 3, 4, 5 and 6 was filled with 99.9, 99.7, 99.5 ml, 99.3 and 99 ml of rainwater respectively. Afterward, 0.1, 0.3, 0.5, 0.7 and 1 ml of the stock solutions of salt were added to each cup of groups 2, 3, 4, 5 and 6 respectively. Cups of group 1 were used as control i.e. no salt solutions were added. The first and third instars larvae were selected for the study, because of the easy differentiation of these two instars and separate sets of experiments were carried out for each of the instars. The fourth instar larvae were avoided for the possibility of immediate pupation. Thereby a batch of ten larvae of a particular instar was collected from the rearing container and released into each cup of the group 2, 3, 4, 5 and 6. An amount of 0.2 gm yeast granules were added regularly to each cup as larval food. All of the plastic cups along with larvae were placed inside the rearing cage to prevent any contamination and egg-laying by other mosquitoes. The cups of the experiment with a particular type of salt were kept in a separate cage. The larvae which showed no signs of motion were regarded as dead. Their number was counted and recorded at the time of each inspection. Inspections were made at 6 hours interval. To eliminate the impact of the decomposing larval food, it was removed with a spuit from each cup during the last inspection of the day. A little amount (< 0.02 gm/100 ml) of larval food was newly added to each cup if required. The mortality of larvae (after 24 hours), duration of larval instars, number of pupae, the
mortality of pupae and adult emergence were recorded.

Statistical Analysis

LC$_{50}$, LC$_{90}$, LT$_{50}$, LT$_{90}$. 95% confidence interval were generated through probit analysis using Statistical Package for Social Sciences (SPSS®) version 20 [22]. Mean mortality of larvae, standard error, % pupation, % pupal mortality and % adult emergence of mosquitoes were calculated using MS Excel, 2013.

Results

Effects on 1st and 3rd instar larvae

Data showing the LC$_{50}$ and LC$_{90}$ values along with a 95% confidence interval (CI) of different salts are presented in Table 1. The LC$_{50}$ and LC$_{90}$ values of salts against 1st instar larvae were ranged from 0.118 to 5.85 ppm and 2 to 11.50 ppm respectively. The LC$_{50}$ values of AgNO$_3$, HgCl$_2$, CdCl$_2$, CuSO$_4$ and CuCl$_2$ were 0.118, 0.81, 1.47, 1.99 and 5.85 ppm respectively, whereas the LC$_{90}$ values were 2, 4.063, 5.69, 7.314 and 11.50 ppm. AgNO$_3$ has the lowest LC$_{50}$ (0.118 ppm) and LC$_{90}$ (2 ppm) values whereas CuCl$_2$ has registered the highest LC$_{50}$ (5.85 ppm) and LC$_{90}$ (11.50 ppm) values among the five salts tested.

Results on the LT$_{50}$ and LT$_{90}$ values for 1st instar larvae at 1 ppm concentration of all salts were shown in Table 2. Among all salts lowest LT$_{50}$ (0.575 hrs) and LT$_{90}$ (6 hrs) values were found at 1 ppm concentration of AgNO$_3$. In contrast, higher LT$_{50}$ values were 6.861, 23.372, 28.392 and 37.144 hrs at 1 ppm concentration for HgCl$_2$, CdCl$_2$, CuSO$_4$, and CuCl$_2$ respectively, whereas the higher LT$_{90}$ values for the salts were 14.063, 140.982, 177.357 and 220.628hrs respectively. The LC$_{50}$ values of AgNO$_3$, HgCl$_2$, CdCl$_2$, CuSO$_4$ and CuCl$_2$ against 3rd instar larvae were 1.659, 1.842, 2.561, 3.292 and 7.84 ppm respectively, whereas the LC$_{90}$ values were 3.347, 4.215, 6.333, 8.075

| Name of salts | LC values (ppm) with 95% Confidence Limits against larval instar |
|---------------|---------------------------------------------------------------|
|               | 1st instar                                                  |
|               | LC$_{50}$          | LC$_{90}$          | LT$_{50}$          | LT$_{90}$          |
| AgNO$_3$      | 0.118             | 2.000             | 1.659 (1.554–1.767) | 3.347 (3.075–3.699) |
| HgCl$_2$      | 0.814 (.515 – 1.050) | 4.063 (3.145–6.424) | 1.842 (1.722–1.959) | 4.215 (3.885–4.642) |
| CdCl$_2$      | 1.469 (1.234–1.675) | 5.690 (4.698–7.494) | 2.561 (2.392–2.736) | 6.333 (5.621–7.370) |
| CuSO$_4$      | 1.997 (1.796–2.194) | 7.314 (6.005–9.793) | 3.292 (3.077–3.535) | 8.075 (6.959–9.875) |
| CuCl$_2$      | 5.850 (5.510–6.303) | 11.50 (10.078–13.641) | 7.84 (7.005–9.171) | 19.382 (15.311–26.852) |

*Data in parenthesis represents the 95% Confidence Limit of LC values against 1st and 3rd instar larvae.
and 19.382 ppm respectively (Table 1).

### Table 2

Lethal time of 1st and 3rd instar larvae (*Ae. aegypti*) for different concentrations of salt solutions

| Name of salts | LT values | Lethal time (LT) against each Concentration of a salt |
|---------------|-----------|-----------------------------------------------------|
|               |           | 1 ppm | 3 ppm | 5 ppm | 7 ppm | 10 ppm |
| **AgNO₃**     | LT₅₀      | 0.58(30.42) | 0.58(3.11) | 0.58(2.14) | 0.58(1.32) | 0.58(1.31) |
|               | LT₉₀      | 6.00(67.49) | 6.00(11.28) | 6.00(6.83) | 6.00(5.32) | 6.00(3.48) |
| **HgCl₂**     | LT₅₀      | 6.86(33.27) | 3.17(6.29) | 2.86(4.18) | 2.32(2.26) | 2.32(1.99) |
|               | LT₉₀      | 14.06(73.99) | 12.04(24.55) | 7.52(20.64) | 3.26(8.90) | 3.26(7.73) |
| **CdCl₂**     | LT₅₀      | 23.37(62.07) | 9.49(10.55) | 7.49(10.31) | 4.97(6.13) | 3.68(3.69) |
|               | LT₉₀      | 140.98(149.36) | 31.79(36.57) | 17.89(33.39) | 12.20(220.59) | 10.00(10.74) |
| **CuSO₄**     | LT₅₀      | 28.39(82.53) | 11.15(21.96) | 7.99(18.42) | 5.57(9.75) | 3.57(5.32) |
|               | LT₉₀      | 177.36(213.17) | 46.41(36.90) | 31.22(35.37) | 17.02(25.16) | 10.92(13.34) |
| **CuCl₂**     | LT₅₀      | 37.11(30.14) | 21.88(30.14) | 13.21(22.65) | 10.45(16.54) | 5.64(13.61) |
|               | LT₉₀      | 220.63(57.09) | 165.96(57.09) | 60.57(43.90) | 40.69(42.16) | 13.94(38.79) |

*Data in parenthesis represents the lethal time of 3rd instar larvae against respective concentration.*

Among all tested salts effective lowest LT₅₀ (30.421hrs) and LT₉₀ (67.492hrs) values were found at 1 ppm concentration of AgNO₃ solution, on the other hand, the highest LT₅₀ (82.529) and LT₉₀ (213.173) values were found at 1 ppm concentration of CuSO₄ solution (Table 2). The higher LT₅₀ values (33.269, 62.07& 82.529hrs) and LT₉₀ values (73.99, 149.364 & 213.173hrs) were found at 1 ppm concentration of HgCl₂, CdCl₂, and CuSO₄ respectively. Furthermore, it was observed that out of five salts, AgNO₃ showed the lowest LC₅₀ and LC₉₀ values and the lowest LT₅₀ & LT₉₀ values at each concentration.

**Effects on pupation and adult emergence**

It was noted that 100% 1st instar larvae died at each concentration (1-10 ppm) of every salt within 7 days. All larvae have died within 9, 6 and 3 hours when exposed to 1 ppm, 3 ppm and 5 ppm solutions of AgNO₃ respectively. On the other hand, 46%, 93% and 100% 3rd instar larvae were killed within 24, 24 and 9 hours when exposed to 1, 3 and 10 ppm solutions of AgNO₃ respectively. No pupation and adult emergence was also observed from 3rd instar larvae even at 7 and 10 ppm concentrations of CdCl₂, CuSO₄ and CuCl₂ salt solutions (Table 3).
Table 3

| Name of Salts | Concentrations (ppm) | Larval mortality (%) | Pupation (%) | Pupal mortality (%) | Adult Emergence (%) |
|---------------|----------------------|----------------------|--------------|---------------------|---------------------|
| CdCl₂         | 1                    | 78.34                | 21.66        | 53.84               | 46.16               |
|               | 3                    | 88.34                | 11.66        | 57.14               | 42.86               |
|               | 5                    | 95                   | 5            | 66.66               | 33.33               |
|               | 7                    | 100                  | 0            | 0                   | 0                   |
| CuSO₄         | 1                    | 73.34                | 26.66        | 50                  | 50                  |
|               | 3                    | 86.67                | 15           | 55.55               | 44.45               |
|               | 5                    | 88.34                | 11.66        | 57.14               | 42.86               |
|               | 7                    | 100                  | 0            | 0                   | 0                   |
| CuCl₂         | 1                    | 63.34                | 36.66        | 36.36               | 63.64               |
|               | 3                    | 81.67                | 18.33        | 45.45               | 54.55               |
|               | 5                    | 86.67                | 13.33        | 50                  | 50                  |
|               | 7                    | 100                  | 0            | 0                   | 0                   |
| AgNO₃         | 1                    | 100                  | 0            | 0                   | 0                   |
| HgCl₂         | 1                    | 100                  | 0            | 0                   | 0                   |

Table 4

| Name of Salts | Lethal concentrations (LC₉₀) on larval stage | Lethal time (LT₉₀) on larval stage | Development of 3rd instar larvae | Order of toxicity |
|---------------|---------------------------------------------|-----------------------------------|----------------------------------|-------------------|
| AgNO₃         | 2 ppm                                       | 3.347                             | < 6 ppm                         | 1                 |
| HgCl₂         | 4.063                                       | 4.215                             | 12.039                          | 2                 |
| CdCl₂         | 5.690                                       | 6.333                             | 31.792                          | 3                 |
| CuSO₄         | 7.314                                       | 8.075                             | 46.412                          | 4                 |
| CuCl₂         | 11.50                                       | 19.382                            | 165.962                         | 5                 |

Discussion

The killing efficiency of salt solutions on the larvae of mosquito might be due to its impact on the cells of the anal gill. Similar larvicidal efficacy was reported by Wigglesworth [23] that the cells of anal gills in Ae. argenteus (Poir.) become swollen, perhaps due to the diffusion of the hypertonic NaCl into the cells which were caused by the difference of the concentration between hemolymph and external medium. He also stated that the concentration of NaCl in the cells rises above that in the hemolymph and water from the latter moves into the cells by osmosis. This difference of osmotic pressure between body fluids and the external medium is the main factor to swell up of cells. MacFie [24] also found that the destructive action of a salt solution of 2% or higher on the larvae of Stegomyia fasciata (= Ae. aegypti) is due to the hypertonicity of the solution. Bradley [25] stated that only 5% of all extant species of the family Culicidae are capable of surviving in saltwater and those species of Aedes do not possess an additional segment of the rectum cannot survive for not eliminating excess ions. During the present study, it was also observed that anal gills of Aedes larvae become swollen by absorbing salts solution and then larvae have died. It was noted that all salt solutions were able to kill 1st instar larvae of Ae. aegypti at a lower concentration. It is pertinent to mention here the findings of Suzuki [26] who found that LC₅₀ of AgNO₃ and HgCl₂
were 1.7 ppm (0.00017%) and 1 ppm (0.0001%) respectively against fourth instar larvae of *Culex pipiens pallens*. In the present study, it was noted that AgNO₃ kills 60% of 3rd instar larvae at 1 ppm (0.0001%) concentration and when concentration increased to 3 ppm (.0003%) mortality also increased to 98.3%. Riaz *et al.* [27] reported that a 10% NaCl solution killed the majority of larvae of *Ae. aegypti* in the laboratory and 100% mortality were achieved within the minimum time when exposed to a 20% salt solution. MacFie [28] reported that undiluted seawater killed larvae of *Stegomyia fasciata* within 2 to 4 hrs and 50% or more diluted seawater caused death after 24 hrs. Wigglesworth [29] conducted experiments with sodium chloride and found good results at 1.25% solution against *Aedes* species within 4-7days. Wigglesworth [30] also stated that 1% of NaCl is effective to kill larvae within a week and mortality increased with the increase of salt concentrations. Suzuki [26] studied the relation of required time to death of 50% of larvae (TD₅₀) to the concentration of the salts and calculated the order of decreasing toxicity of the heavy metal salts. He also stated that TD₅₀ increases in parallel to the decreases in salt concentration. High larval toxicity of AgNO₃ against *Aedes aegypti* larvae was found in the present study. Nearly similar result were found by Suzuki [26] in Japan.

There were no pupation and adult emergence from 3rd instar larvae in the solution of AgNO₃ and HgCl₂. Though very few pupations (5-36.66%) were found in other three salt solutions they could not develop into adult or died or might be lengthened its time of emergence. Our results support the Pappas *et al.* [31] from Peru who reported that less than 50% of the larvae of *Culiseta inornata* reached the pupal stage at sodium chloride concentration above 0.01 m.

**Conclusion**

During the present study, it was recorded that all of the tested salt solutions were effective in killing off both the tested (1st & 3rd ) larval instar of *Ae. aegypti* and the AgNO₃ showed the highest efficacy. The LC₅₀ & LC₉₀ values of AgNO₃ were 0.118 & 2.0 ppm against 1st instar and 1.659 & 3.347 ppm against 3rd instar larvae respectively. The lowest concentration (1 ppm) of low effective salt CuCl₂ also killed 90% larvae within 220.628 hrs (Table 2). The LC₅₀ and LC₉₀ values of each salt were lower for 1st instar than 3rd instar. Among five salts AgNO₃ has the lowest LT₅₀ and LT₉₀ values were 0.575 and 6 hrs for 1st instar whereas 30.421 and 67.492 hrs for 3rd instar respectively. AgNO₃ and HgCl₂ successfully prevented pupation and adult emergence of both instars (1st & 3rd )
larvae. These promising results could be useful in the search for a more effective and eco-friendly larvicidal product against \textit{Ae. aegypti}, especially in the areas where mosquitoes are highly resistant to chemical insecticides. However, the findings of this laboratory-based study need to be evaluated in the field conditions. Besides, further investigation regarding the residual toxicity on non-target organisms’ extremely important and requiring attention soon.

Declarations

\textbf{Ethics approval and consent to participate}

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

\textbf{Competing interests}

The authors declare that they have no competing interests.

\textbf{Authors' contributions}

KB and AJH designed the study. MZHI have done the laboratory and fieldwork. MZHI computed data entry and analysis. MZHI, KB, and AJH collaborated to write the manuscript. All authors read and approved the final manuscript.

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\textbf{References}
1. Pinheiro PF, Corber SJ: Global situation of dengue and dengue hemorrhagic fever and its emergence in the Americas. *Worl.Healt.Stat.Q* 1997, 50:161-169.

2. Gubler DJ. Resurgent vector borne disease as a global health problem. *Emerg Infect Dis.* 1998;4:442-50.

3. Rudnick A, Lim TW: Dengue fever studies in Malaysia. *Lim, T. W, editor, Bull. No. 23, Ins. Med. Res.*, Malaysia 1986, 162 pp.

4. Jelinek T. Dengue Fever in International Travelers. *Clin Infect Dis.* 2000;31:1447.

5. Brady OJ, Gething PW, Bhatt S. Refining the Global Spatial Limits of Dengue Virus Transmission by Evidence-Based Consensus. *Nature.* 2013;496(7446):5047.

6. Curtis CF: Control of disease vectors in the community. *Wolf Publishing, London* 1991. 233 pp.

7. Gubler DJ. : Cities spawn epidemic dengue viruses. *Nat Med.* 2004;10:129-30.

8. Mammen MP, Pimgate C, Koenraadt CJ, Rothman AL, Aldstadt J, Nisalak A. : Spatial and temporal clustering of dengue virus transmission in Thai villages. *PLOS Med.* 2008;5(11):205.

9. Tewari SC, Thenmozhi V, Katholi CR, Manavalan R, Munirathinam A, Gajanana A. Dengue vector prevalence and virus infection in a rural area in south India. *Trop Med Int Health.* 2004;9:499-507.

10. Pongsumpun P, Garcia Lopez D, Favier C, Torres L, Llosa J, Dubois MA. : Dynamics of dengue epidemics in urban contexts. *Trop Med Int Health.* 2008;13:1180-7.

11. Samuel PP, Thenmozhi V, Tyagi BK. : A focal outbreak of dengue fever in a rural area of Tamil Nadu. *Indian J Med Res.* 2007;125:179-81.

12. Khan JK: Effect of the crude extract of different plant on the larvae of Mosquito, say (Diptera: Culicidae), M.Sc. thesis, Department of Zoology, Jahangirnagar University, Savar, Bangladesh. 1999, iii + 69.

13. Burdick GE, Arris EJ, Dean HJ, Walker TM, Skea J, Colby D. *TransAmer Fish Soc.* 1964;73:127.

14. Brown AWA. *Insecticide resistance in mosquitoes: A pragmatic review.* *J Am Mosq C.*
5. Lee HL, Lime W.: Are-evaluation of the susceptibility of field-collected *Aedes* to temephos in Malaysia. J Mosq Borne Dis Bull. 1989;6:91–5.

6. Wattanachai P, Rielrangboonya P, Boonyabuncha S, Phunurai P.: Susceptibility of *Aedes aegypti* to organo phosphorus compound in Thailand. Mosq Borne Dis Bull. 1994;12:27.

7. Barraud PJ. The fauna of British India, Including Ceylon and Burma, Diptera Vol 5 Family – Culicidae. London: *Tribes Megarhinini and Culicini* Taylor and Francis; 1934.

8. Mattingly PF. Contributions to the mosquito fauna of Southeast Asia. XII. Illustrated keys to the genera of mosquitoes (Diptera:Culicidae). Contributions of the American Entomological Institute. 1971;7(4):1–8.

9. Rattanarithikul R. A guide to the genera of mosquitoes (Diptera: Culicidae) of Thailand with illustrated keys, biological notes and preservation and monitoring techniques. Mosquito systematics. 1982;14:139–208.

10. Amerasinghe FP. Illustrated keys to the genera of mosquitoes (Diptera: Culicidae) in sri Lanka. J Natn Sci Coun Sri Lanka. 1995;23(4):183–211.

11. Rueda LM. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue virus transmission. Zootaxa 2004, 589(1):1–60.

12. SPSS® v 20. SPSS for Windows. Spss Inc. Chicago, IL 2007.

13. Wigglesworth VB. The effect of salts on the anal gills of the mosquito larva. J Exp Biol. 1933a;10:1–15.

14. MacFie JWS. A note on the action of common salt and sea water on *stegomyia fasciata*. (= *Aedes aegypti*). Bull Ent Res. 1914;4:339-44.

15. Bradley TJ: The role of physiological capacity, morphology and phylogeny in determining habitat use in mosquitoes. In ecological morphology: integrative organismal biology (ed. P. C. wainwright and S. M. Reilly), *Chicago, IL*: University of Chicago Press 1994, 303–318 pp.
6. Suzuki K: The toxic influence of heavy metal salts upon mosquito larvae. Jour. Fac. Sci. Hokkaido Univ. Ser 1959, VI, Zool.14.

7. Riaz MA, Riaz A, Baqir M, Ijaz B. The effect of different NaCl concentration on the survival of Aedes aegypti larvae in Wahga Town Lahore. J Basic Appl Chem. 2013;2(4):12–5.

8. MacFie JWS. The effects of saline solutions and sea water on stegomyia fasciata. Ann Trop Med Parasit. 1922;15:277–80.

9. Wigglesworth MA. The adaptation of mosquito larvae to salt water. J Exp Biol. 1993;X (I):27–37.

10. Wigglesworth VB. The effect of salts on the anal gills of the mosquito larva. JExp Biol. 1933;10:1-15.

11. Pappas LG, Pappas CD. Laboratory studies on the significance of NaCl as an oviposition different in Culiseta inornata. Mos News. 1983;43(2):153–5.

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