

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 30.42 hrs, 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 $70 \pm 5\%$ RH). In each rearing container ($10 \times 10 \times 5$ 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 ($30 \times 30 \times 30$ 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 sput 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 ($30 \times 30 \times 30$ 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 sput 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

LC₅₀, LC₉₀, LT₅₀, LT₉₀, 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 LC₅₀ and LC₉₀ values along with a 95% confidence interval (CI) of different salts are presented in Table 1. The LC₅₀ and LC₉₀ values of salts against 1st instar larvae were ranged from 0.118 to 5.85 ppm and 2 to 11.50 ppm respectively. The LC₅₀ values of AgNO₃, HgCl₂, CdCl₂, CuSO₄ and CuCl₂ were 0.118, 0.81, 1.47, 1.99 and 5.85 ppm respectively, whereas the LC₉₀ values were 2, 4.063, 5.69, 7.314 and 11.50 ppm. AgNO₃ has the lowest LC₅₀ (0.118 ppm) and LC₉₀ (2 ppm) values whereas CuCl₂ has registered the highest LC₅₀ (5.85 ppm) and LC₉₀ (11.50 ppm) values among the five salts tested.

Table 1

Lethal concentrations (LC) at 95% Confidence Interval of the salt solutions against 1st and 3rd instar larvae of *Ae. aegypti*

Name of salts	LC values (ppm) with 95% Confidence Limits against larval instar			
	1st instar		3rd instar	
	LC ₅₀	LC ₉₀	LC ₅₀	LC 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.				

Results on the LT₅₀ and LT₉₀ values for 1st instar larvae at 1 ppm concentration of all salts were shown in Table 2. Among all salts lowest LT₅₀ (0.575 hrs) and LT₉₀ (6 hrs) values were found at 1 ppm concentration of AgNO₃. In contrast, higher LT₅₀ values were 6.861, 23.372, 28.392 and 37.144 hrs at 1 ppm concentration for HgCl₂, CdCl₂, CuSO₄, and CuCl₂ respectively, whereas the higher LT₉₀ values for the salts were 14.063, 140.982, 177.357 and 220.628hrs respectively. The LC₅₀ values of AgNO₃, HgCl₂, CdCl₂, CuSO₄ and CuCl₂ against 3rd instar larvae were 1.659, 1.842, 2.561, 3.292 and 7.84 ppm respectively, whereas the LC₉₀ values were 3.347, 4.215, 6.333, 8.075 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
AgNO ₃	LT ₅₀	0.58(30.42)	0.58(3.11)	0.58(2.14)	0.58(1.32)	0.58(1.31)
	LT ₉₀	6.00(67.49)	6.0(11.28)	6.00(6.83)	6.00(5.32)	6.00(3.48)
HgCl ₂	LT ₅₀	6.86(33.27)	3.17(6.29)	2.86(4.18)	2.32(2.26)	2.32(1.99)
	LT ₉₀	14.06(73.99)	12.04(24.55)	7.52(20.64)	3.26(8.90)	3.26(7.73)
CdCl ₂	LT ₅₀	23.37(62.07)	9.49(10.55)	7.49(10.31)	4.97(6.13)	3.68(3.69)
	LT ₉₀	140.98(149.36)	31.79(36.57)	17.89(33.39)	12.20(220.59)	10.00(10.74)
CuSO ₄	LT ₅₀	28.39(82.53)	11.15(21.96)	7.99(18.42)	5.57(9.75)	3.57(5.32)
	LT ₉₀	177.36(213.17)	46.41(36.90)	31.22(35.37)	17.02(25.16)	10.92(13.34)
CuCl ₂	LT ₅₀	37.11(30.14)	21.88(30.14)	13.21(22.65)	10.45 (16.54)	5.64(13.61)
	LT ₉₀	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
Effect of salts on pupation, pupal mortality and adult emergence from the 3rd instar larvae of *Ae. aegypti*

Name of Salts	Concentrations (ppm)	Larval mortality (%)	Pupation (%)	Pupal mortality (%)	Adult Emergence (%)
CdCl ₂	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
CuSO ₄	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
CuCl ₂	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
AgNO ₃	1	100	0	0	0
HgCl ₂	1	100	0	0	0

Table 4

Order of toxicity of salt solutions (3 ppm) regarding LC₉₀ (ppm), LT₉₀, percent pupation, and percent adult emergence of the 1st and 3rd instar larvae of *Ae. aegypti*

Name of Salts	Lethal concentrations (LC ₉₀) on larval stage		Lethal time (LT ₉₀) on larval stage		Development of 3rd instar larvae		Order of toxicity
	1st instar	3rd instar	1st instar	3rd instar	%pupation	% Adult emergence	
	AgNO ₃	2.00	3.347	< 6.00	11.278	0	
HgCl ₂	4.063	4.215	12.039	24.553	0	0	2
CdCl ₂	5.690	6.333	31.792	36.572	11.66	42.86	3
CuSO ₄	7.314	8.075	46.412	36.903	15	44.45	4
CuCl ₂	11.50	19.382	165.962	57.096	18.33	54.55	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 4-7 days. 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 *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

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.

Competing interests

The authors declare that they have no competing interests.

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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