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

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Abstract: 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 humans by DHF/DSS. The purposes of this work were to evaluate the larvicidal effect of some salt solutions against the larvae of *Ae. aegypti*. 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. $AgNO_3$, $HgCl_2$, $CdCl_2$, $CuSO_4$ and $CuCl_2$ 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 and 10 ppm) of each salt were prepared by using distilled water as the solvent. Silver nitrate ($AgNO_3$) was noted as the most effective larvicide followed by $HgCl_2$, $CdCl_2$, $CuSO_4$ and $CuCl_2$ against *Ae. Aegypti* larvae. The LC_{50} of $AgNO_3$ against 1st and 3rd instars larvae were 0.118 and 1.659 ppm and the LC_{90} were 2 and 3.347 ppm, respectively. The LT_{50} of $AgNO_3$ (1ppm) against 1st and 3rd instars larvae were 0.575 and 30.42 h and the LT_{90} was 6 and 67.49 h, 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 $AgNO_3$ and $HgCl_2$ solutions but very few (5-36.66%) pupation was found at 7 ppm and higher concentrations of $CdCl_2$, $CuSo_4$ and $CuCl_2$ salts. $CdCl_2$, $CuSo_4$ and $CuCl_2$ 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 $AgNO_3$>$HgCl_2$>$CdCl_2$>$CuSO_4$>$CuCl_2$ and for prevention of pupation & adult emergence was $AgNO_3$ =$HgCl_2$>$CdCl_2$>$CuSO_4$>$CuCl_2$. $AgNO_3$ 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.

Key words: *Aedes aegypti* · Larvicidal Effect · Organic Salts

INTRODUCTION

Aedes aegypti (L.) is the predominant vector of dengue; a mosquito-borne arbovirus [1]. It is also a recognized vector of Zika, Chikungunya and Yellow fever viruses. Like malaria, dengue virus had become a global threat [2, 3, 4]. 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 [5]. The dengue virus spread rapidly and the disease develops into pandemic proportion [6]. 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 [7]. 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 [8]. Though dengue has been reported as an urban disease [9] in the

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recent past dengue infection and the vector mosquitoes have been detected in rural areas of Thailand and India [10, 11]. Unplanned urbanization in cities and technological advancement in villages might have played a significant role in dengue transmission [12, 13].

WHO described the mosquitoes as public enemy no.1[14]. Mosquito control is a worldwide problem due to their vector nature and the revival of various infectious diseases.The control of dengue transmission can be achieved by keeping in view the different strategies against immature stages of *Stegomyia aegypti* (=*Ae. aegypti*). Application of control measures during these stages would increase the mortality of this vector before it reaches an adult stage [15]. 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 [16]. 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 [17, 18] and Fenitrothion [19]. Though chemical controls require a short time and work quickly but have some adverse effects on man and the environment. Burdick *et al*. [16] 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*.

MATERIALS AND 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 (10x10x5 cm^3) about 500 ml rainwater was taken and 500 healthy larvae were released. Larval food (0.10 g yeast granules) was regularly added to each rearing container. Each container along with the larvae was placed inside a rearing cage (30x30x30 cm^3) 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 g/100ml) 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 [20-24], 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 (30x30x30 cm^3) 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 rain water of a plastic bowl for hatching. During the period of hatching 0.02g, glucose or yeast granules were added in each 100ml 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 100ml water for preparing a stock solution of 1000ppm. After that 1ml, 0.7ml, 0.5ml, 0.3ml and 0.1ml of stock solution were mixed with 99, 99.3, 99.5, 99.7 and 99.9ml of water respectively for preparing 10, 7, 5, 3 and 1ppm 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 were filled with 99.9, 99.7, 99.5ml, 99.3 and 99ml 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 (<0.02gm/100ml) 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 [25]. Mean mortality of larvae, standard error, % pupation, % pupal mortality and percent of adult emergence of mosquitoes were calculated using MS Excel, 2013.

RESULTS

Effects of Salts 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 5.85ppm 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 5.85ppm 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} (0.118ppm) and LC_{90} (2ppm) values whereas $CuCl_2$ has registered the highest LC_{50} (5.85ppm) and LC_{90} (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*

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

Table 2: Lethal time of 1st and 3rd instar larvae (*Ae. aegypti*) for different concentrations of salt solutions

		Lethal time (LT) against each Concentration of a salt				
Name of salts	LT Values	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.

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 (3ppm) regarding LC_{90} (ppm), LT_{90}, percent pupation and percent adult emergence of the 1st and 3rd instar larvae of *Ae. aegypti*

	Lethal concentrations (LC_{90}) on larval stage		Lethal time (LT_{90}) on larval stage		Development of 3rd instar larvae		
Name of Salts	1st instar	3rd instar	1st instar	3rd instar	% pupation	% Adult emergence	Order of toxicity
$AgNO_3$	2.00	3.347	<6.00	11.278	0	0	1
$HgCl_2$	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

Results on the LT_{50} and LT_{90} values for 1st instar larvae at 1ppm concentration of all salts were shown in Table 2. Among all salts lowest LT_{50} (0.575 h) and LT_{90} (6 h) values were found at 1ppm concentration of $AgNO_3$. In contrast, higher LT_{50} values were 6.861, 23.372, 28.392 and 37.144 h at 1ppm 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 220.628h 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 7.84ppm respectively, whereas the LC_{90} values were 3.347, 4.215, 6.333, 8.075 and 19.382ppm respectively (Table 1).

Among all tested salts effective lowest LT_{50} (30.421h) and LT_{90} (67.492h) values were found at 1ppm concentration of $AgNO_3$ solution, on the other hand, the highest LT_{50} (82.529) and LT_{90}(213.173) values were found at 1 ppm concentration of $CuSO_4$ solution (Table 2). The higher LT_{50} values (33.269, 62.07 & 82.529h) and LT_{90} values (73.99, 149.364 & 213.173h) were found at 1 ppm concentration of $HgCl_2$, $CdCl_2$ and $CuSO_4$ respectively. Furthermore, it was observed that out of five salts, $AgNO_3$ showed the lowest LC_{50} and LC_{90} values and the lowest LT_{50} & LT_{90} values at each concentration.

Effects of Salts on Pupation and Adult Emergence: It was noted that 100% 1st instar larvae died at each concentration (1-10ppm) of every salt within 7 days. All larvae have died within 9, 6 and 3 hours when exposed to 1ppm, 3ppm and 5ppm solutions of $AgNO_3$ 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 10ppm solutions of $AgNO_3$ respectively. No pupation and adult emergence was also observed from 3rd instar larvae even at 7 and 10 ppm concentrations of $CdCl_2$, $CuSO_4$ and $CuCl_2$ salt solutions (Table 3).

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 [26] 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 [27] 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 [28] 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 [29] who found that LC_{50} of $AgNO_3$ and $HgCl_2$ were 1.7ppm (0.00017%) and 1ppm (0.0001%) respectively against fourth instar larvae of *Culex pipiens pallens*.

In the present study, it was noted that $AgNO_3$ kills 60% of 3rd instar larvae at 1ppm (0.0001%) concentration and when concentration increased to 3 ppm (.0003%) mortality also increased to 98.3%. Riaz *et al*. [30] 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 [31] reported that undiluted seawater killed larvae of *Stegomyia fasciata* within 2 to 4 h and 50% or more diluted seawater caused death after 24 h. Wigglesworth [32] conducted experiments with sodium chloride and found good results at 1.25% solution against *Aedes* species within 4-7days. Wigglesworth [33] also stated that 1% of NaCl is effective to kill larvae within a week and mortality increased with the increase of salt concentrations. Suzuki [29] studied the relation of the required time to death of 50% of larvae (TD_{50}) to the concentration of the salts and calculated the order of decreasing toxicity of the heavy metal salts. He also stated that TD_{50} increases in parallel to the decreases in salt concentration. High larval toxicity of $AgNO_3$ against *Ae. aegypti* larvae was found in the present study. A nearly similar result was found by Suzuki [29] in Japan.

There were no pupation and adult emergence from 3rd instar larvae in the solution of $AgNO_3$ and $HgCl_2$. 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.* [34] from Peru who reported that less than 50% of the larvae of *Culiseta inornata* reached the pupal stage at sodium chloride concentration above 0.01m.

CONCLUSION

During the present study, it was recorded that all of the tested salt solutions were effective in killing both the tested (1st & 3rd) larval instar of *Ae. aegypti* and the $AgNO_3$ showed the highest efficacy. The LC_{50} & LC_{90} values of $AgNO_3$ were 0.118 & 2.0ppm against 1st instar and 1.659 & 3.347ppm against 3rd instar larvae respectively. The lowest concentration (1ppm) of low effective salt $CuCl_2$ also killed 90% larvae within 220.628 h (Table 2). The LC_{50} and LC_{90} values of each salt were lower for 1st instar than 3rd instar. Among five salts $AgNO_3$ has the lowest LT_{50} and LT_{90}values were 0.575 and 6 h for 1st instar whereas 30.421and 67.492 h for 3rd instar respectively. $AgNO_3$ and $HgCl_2$ 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.

Authors' Contributions: KB and AJH designed the study. MZHI have done the laboratory and field work. 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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