THEORETICAL BIOLOGY FORUM

TBF: 01-05 (2024)
Research Article

An Experimental Assessment of acute toxicity of Sodium Chloride against *Aedes aegypti* L. larvae

Komalpreet Kaur Sandhu* and Sujata

Department of Zoology, Akal University, Talwandi Sabo, Bathinda, Punjab, 151302- India

ABSTRACT

The prevalence of dengue, one of the most important mosquito-borne diseases, has increased more than eight-fold during the past two decades. The primary and most prevalent mosquito vector, Aedes aegypti L., is responsible for spreading viruses that cause dengue and other harmful diseases. Control of vectors is of particular concern to block dengue transmission. Different chemical measures used for vector control caused various environmental issues, mosquito resistance, resurgence, and harmful impacts on non-target organisms increased attention towards cost-effective and environment-friendly control measures. Hence, the present study aimed to check the larvicidal potential of Sodium Chloride (NaCl) and examined its acute toxicity against 4th instar larvae of Ae. aegypti.Acute toxicity tests were conducted as per WHO guidelines. Different NaCl concentrations i.e., 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0% were prepared using pure NaCl along with distilled water. The Larvicidal activity was assessed and toxicity values were computed using the Log-Probit Method. Several morphological and behavioral alterations in treated Ae. aegypti larvae were also observed. Sixty percent larvae of Ae. aegypti died after exposure to 1.5% NaCl concentrations within 48 hours and larval mortality significantly increased up to ninety-seven and hundred percent after treatment with more than 2% NaCl solution within 24 and 48 hours respectively. Various ruptures were observed in the abdomen and anal papillae along with abnormal behavior in the treated larvae. Our study concluded that Sodium Chloride can be used as an effective and safe intervention in mosquito control programs due to its easy availability and less toxicity.

ARTICLE HISTORY
13 January 2024: Received
02 February 2024: Revised
09 March 2024: Accepted
05 April 2024: Available Online

DOI:
https://doi.org/10.61739/TBF.2024.13.1.01

CORRESPONDING AUTHOR:
Komalpreet Kaur Sandhu

Keywords- *Aedes aegypti; Bioassay; Larvicide; LC90; Sodium Chloride*

INTRODUCTION

Aedes aegypti L. is one of the medically important mosquito species acting as a key vector of viruses causing ailments such as Dengue, Zika, Chikungunya, and yellow fever. Originally restricted to Africa, it is widely distributed over tropical, sub-tropical, and temperate regions across the world [1]. Dengue Fever (DF) prevalence has been increasing globally in recent years. It is estimated that more than 3.9 billion people are suffering from dengue infections, out of which 96 million are confirmed cases with clinical symptoms [2 and 3]. The global burden of dengue has amplified over ten-fold with 5.2 million in 2019 from 5,05,430 cases in 2000[4].

In India, Dengue fever is pervasive in all states and a major cause of hospitalization. According to the recent statistics of the National Centre for Vector Borne Diseases Control, from 36 states/Union Territories of India, 193, 245 dengue cases have been reported in 2021, rising to 233, 251 with 303 deaths in 2022. The maximum outbreak was documented in West Bengal (67,271 cases) followed by Uttar Pradesh (19,821) and Bihar with 13,972 cases [5].

To date, there is no availability of anti-viral medications and vaccines against diseases, controlling and eliminating breeding sites of vector, *Ae. aegypti* is a crucial step forward for its suppression which ultimately lessens the global burden of Dengue and other *Aedes* borne diseases. Usually, traditional ways to manage the mosquito population and its preferred breeding habitats are environmental management, space spraying of insecticides, usage of Insecticide-treated nets (ITNs), Indoor residual sprays (IRS) and Long-lasting Insecticide-treated nets (LLINs) [6].

Utilization of biocontrol agents such as larvivores fishes has also become insufficient these days [7]. Hence, Current chemical control strategies are jeopardized because of their toxicity to humans and the environment as well as the establishment of resistance and resurgence among mosquitoes [8]. Thus, the development of novel and effective control tools is necessitated so that the present strategies are well optimized.

Plethora of scientific reports has shown that concentration of inorganic salts in an environmental habitat has a significant impact on oviposition, hatching, and development of mosquitoes which in turn influence their abundance and distribution [9]. In this regard, the most common inorganic salt i.e., Sodium Chloride (NaCl) in aquatic habitats is worth being explored. Some Government Health Ministries in Japan have also suggested the application of salt in possible mosquito habitats to kill their larvae [10]. It has been reported that saline water with more than 0.5% NaCl concentration act as an oviposition repellent in *Aedes albopictus* females [11]. However, the efficiency of salt as a larvicide, as well as the amount of salt required for mosquitoes' habitat eradication, is unknown. Therefore, we preliminary investigated the salinity in aquatic habitats in the Talwandi Sabo region of Bathinda city, Punjab, and then we conducted laboratory experiments to systematically investigate the toxicity effects of NaCl solution at various concentrations as a larvicide against *Ae. aegypti* with an aim that outcomes of the present study will aid in the development of relative control strategies for *Aedes* mosquitoes.

MATERIAL & METHODS

The present study was carried out in PG Research Laboratory in the Department of Zoology, Akal University (29.9718ºN, 75.0890ºE), Talwandi Sabo, Punjab, India from July to November 2022.

Collection and maintenance of Ae. aegypti larvae

Ae. aegypti larvae were collected from different fresh-water collections considered as natural breeding habitats of mosquitoes like earthen pots, roadside ditches, desert coolers, dishes under potted plants from gardens and plastic tanks and containers in and adjoining areas of Talwandi Sabo of District Bathinda, Punjab (India).

A standard dipping method was followed to collect the larval samples with the help of plastic droppers and kept in a plastic container of 500 ml capacity and transported to the Laboratory. Mosquito larvae were placed in mosquito-rearing enamel trays and fed with a mixture of Dog biscuits and yeast powder in ratio 3:1[12]. Mosquito-rearing enamel trays were observed daily for larvae feeding and water exchange to avoid scum formation. Collected Larvae were identified using common taxonomic keys based on their morphological characteristics [13 and 14]and A*e. aegypti* larvae separated from other mosquito larvae (if present) for further experimental purposes. A 50 ml Eppendorf tube was used to collect about 15 ml of habitat water from the center of each habitat, which was subsequently brought to the laboratory within 4 hours. The digital Salinity meter (Model 671) was used to measure the Sodium Chloride concentration in the water sample of each habitat.

Acute Larvicidal Toxicity test against Ae. aegypti larvae

The acute toxicity assays were performed against late 3^{rd} and early 4^{th} instar *Ae. aegypti* larvae following the standard protocol [15]. The extra pure form of inorganic salt i.e. Sodium Chloride (NaCl) was procured from Loba Chemie Private Limited, Mumbai, Maharashtra (India). The stock solutions were freshly prepared by dissolving 5.0g, 10.0g, 15.0g, 20.0g, 25.0g, and 30.0g of NaCl in 1000ml of deionized water. The larvicidal toxicity assays were conducted at concentrations of 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0% (w/v) of pure NaCl. The experimental set up was carried out in 300 ml plastic beakers using twenty-five larvae in three replicates of different concentrations of treatment. Along with the treatment set, A control set had also been run having dechlorinated water only. The plastic beakers were covered with muslin cloth and no food was provided during the acute toxicity testing. The larval mortality was observed after 3, 6, 12, 24, and 48 hours in treatment as well as in control sets. The concentration at which 100% larval mortality was observed within 24 hours was considered as effective concentration for the treatment set. Larvae were designated as dead when there was no specific response to different stimuli. Larvae that were unable to rise to the surface of the water (within 30-40 seconds) or shown no characteristic diving behaviors when the water was disturbed was termed moribund (on the verge of death). The percent and corrected larval mortality were calculated using the following formula [16].

$$\text{Percent larval mortality} = \frac{\text{Number of dead and moribund larvae}}{\text{Number of larvae taken initially}} \times 100$$

$$\text{Corrected larval mortality} = \frac{\text{Observed larval mortality intreatment set} - \text{Observed larval mortality in control set.}}{100 - \text{Larval mortality in the control set}} \times 100$$

Significant morphological changes were also observed in treated larvae and compared with control larvae. The whole experimental set-up was carried out in B.O.D. incubator at 26 ±2 °C.

Statistical Analysis

Data was statistically analyzed by comparing the mortality data recorded from treated sets with control sets by using One way ANOVA and multiple comparison test (Duncan multiple range test) employing SPSS software version 16. However, if $p > 0.05$ in a variance homogeneity test, a Least-Significant Difference (LSD) was used for analysis. For calculating the LC_{50} and LC_{90}, the log concentration-mortality regression was conducted by log PROBIT analysis [17]. The level of significance was 0.05.

RESULTS:

NaCl concentration recorded from aquatic habitats.

A total of 12 habitats with freshwater collections in inland areas were studied, including a discarded earthen pot, a flowerpot, drainage ditches, impounded surface water, a discarded washing table, tanks, and tyres. All aquatic habitat water samples had very low NaCl concentrations i.e., less than 0.05%, The Salinity meter (Model 671) reported zero.

Acute Toxicity of Sodium Chloride to Aedes aegypti larvae

The development of 3rd-4th instar larvae of *Aedes aegypti* was significantly impacted by different concentrations of Sodium Chloride (NaCl). Considerable mortality was observed in 3rd-4th instar *Ae. Aegypti* larvae while exposing them to different NaCl concentrations i.e., 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (Table 1). The lowest mortality was observed in 0.5% concentration of NaCl after 3 hours of treatment (1.00±0.57%) but it was significantly higher than the larval mortality observed in the control set. When NaCl concentration reached 1.5%, the larvae were observed inactive within 24 hours and larval mortality rate reached nearly 60% (59.67±3.51%) after 48 hours of exposure. Meanwhile, if NaCl concentrations are 2.5% or more than 2.00%, the mortality of *Ae. aegypti* larvae presented 97.33±2.08% within 24 hours of exposure and reached 100% within 48 hours. If the concentration reached 3.00%, the larvae died at 3 hours and mortality could reach 100% within 12 hours. However, larval mortality is significantly increased with the increasing exposure as well as concentration of NaCl to *Ae. aegypti* larvae. Percent larval mortality values recorded in all treatment sets were statistically higher and significant as compared to those of control sets, which did not show any larval mortality.

Table 1. Effect of different concentrations of Sodium Chloride (NaCl) on mortality of third-fourth instar larvae of Aedes aegypti

Exposure Period	Per cent mortality (Mean±S.D) at Different NaCl concentrations (%) (n=25)						
	0.5	1.0	1.5	2.0	2.5	3.0	Control
3 hrs	$1.00±0.57^b$	$5.67±0.57^c$	$27.33±2.08^d$	$59.00±1.00^e$	$71.00±3.60^f$	$90.00±5.00^g$	$0.00±0.00^a$
6 hrs	$1.33±0.57^b$	$9.00±1.00^c$	$33.00±1.00^d$	$65.00±3.00^e$	$80.00±2.00^f$	$95.00±6.08^g$	$0.00±0.00^a$
12 hrs	$2.00±0.00^b$	$10.33±0.57^c$	$39.00±3.60^d$	$71.67±2.51^e$	$88.67±3.51^f$	$100.00±0.00^g$	$0.00±0.00^a$
24 hrs	$2.00±1.00^b$	$27.67±2.51^c$	$52.00±2.00^d$	$79.67±1.52^e$	$97.33±2.08^f$	-	$0.00±0.00^a$
48 hrs	$2.33±0.58^b$	$29.33±1.52^c$	$59.67±3.51^d$	$86.33±1.52^e$	$100.00±0.00^f$	-	$0.00±0.00^a$

- n represents number of larvae taken.
- Figures with different superscripts show significant difference ($p<0.05$) with respect to Control sets by using Duncan multiple range test (DMRT).

PROBIT analysis showed that 24 hours 50% Lethal Concentrations (LC_{50}) and 90% lethal concentrations (LC_{90}) were 1.322 and 2.274%, 1.254 and 2.059% respectively after 48 hours as shown in Table 2. The bracketed values in Table 2 represent lower and higher fiducial limits. Pearson Chi-square (χ^2) goodness of fit test showed $p>0.15$, indicating this model fitted data well. During the present investigation, 2.5% NaCl concentration was considered to be the most effective concentration as there is 100% larval mortality was observed within 48 hours of treatment.

Table 2: Median and 90% lethal NaCl concentrations (%) estimated for third-fourth instar larvae of Aedes aegypti.

Time period	Within 24 hours	After 48 hours
LC_{50}	1.322 (1.227-1.413)	1.254 (1.165-1.338)
LC_{90}	2.274 (2.092-2.526)	2.059 (1.903-2.270)
Slope	5.437±0.443	5.949±0.493
χ^2	9.342	8.382
P	0.533	0.799

Various morphological deformities and abnormalities in behavior have been seen when 3rd-4th instar *Ae. Aegypti* larvae were exposed to effective NaCl concentration i.e., 2.5%. The ruptures were observed in the abdominal segments along with darkening of the body (Melanization, Figure 1a). The Anal gills were also damaged after treatment (Figure 1b). The abnormal restlessness and excitation, altered motion, and strong self-bite of anal papillae and mouth parts leading to the formation of circular or ring-like structures was also noted in the first three to six hours of treatment (Figure1b).

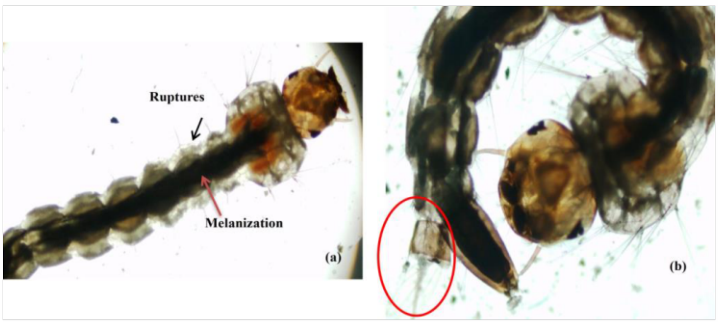

Figure 1: Morphological destructions observed in *Aedes aegypti* larvae after treatment with effective Concentration of Sodium Chloride (NaCl) 40X.
a) Ruptured abdominal segments
b) Damaged anal gills and bitingb).

DISCUSSION

We found that *Ae. aegypti* larvae showed high sensibility with an increase in exposure time as well as NaCl concentration. Our results showed consistency with earlier research findings which demonstrated that NaCl exposure can kill *Ae. aegypti* larvae[18]. The larval mortality in 2.0% NaCl concentration was 79.67±1.52% while in 2.5% concentration, the mortality rate reached up to nearly 100% (97.33±2.08) within 24 hours. Some studies demonstrated that the majority of *Aedes aegypti* larvae in the laboratory were killed after treatment with 10% NaCl concentration[19]. The acute toxicity of sodium chloride to first and fourth instar *Aedes albopictus* larvae and concluded that *Ae. albopictus* first instar larvae were more susceptible to NaCl than fourth instar[10] but our results indicated that the 3rd-4th instar larvae could be more susceptible to NaCl concentrations, possibly as a result of cumulative toxicity brought on by prolonged exposure to the Sodium Chloride solution which is at par with the recent studies conducted at China[9]. Our experiments showed that in 24 hours, LC50 and LC90 of NaCl concentration on 3rd-4th instar larvae was 2.27 and 1.32% respectively which is similar to previous studies[10]. These toxicity values suggested that if 2.27% concentration of NaCl is applied in larval habitats like used tyres, and flower pots, 90% of larvae would be killed within one day.

In our present study, when *Ae. aegypti* larvae exposed to effective concentration of Sodium Chloride showed abnormal behaviour and various ruptures were seen in their cuticle. Various deformities and ruptures in head, thorax and abdominal segments of *Ae. aegypti* larvae were reported in recent studies after treating with hybrid as well as eucalyptus oil based nanoemulsions(Sandhu *et al.*, 2023; Sandhu and Vashishat, 2022) Larvae of *Ae. aegypti* and *Culex quinquefasciatus* showed same vibrating movements and various paralytic symptoms after treating with the metabolites of fungal isolates as larvicidal agents and acted as neurobehavioral toxicant[22] which is at par to the present study.

In recent decades, *Aedes* spp. has expanded well along the edges of their distribution due to the drastic change in climatic like Global warming, unplanned urbanization and increasing population mobility[23]. To achieve sustainable vector management, the integrated vector management concept and its effective application is the need of the hour. For villages, cities, and even national governments, vector control is still expensive, particularly in developing countries. The current evaluation of the toxicity effects of NaCl solution at various concentrations showed that ≥2.0% NaCl has toxic effects against *Ae. Aegypti* which can be used as a low-cost, ecologically friendly insecticide to manage vector populations. Thus, it is worthwhile to use cheap techniques such the use of saltwater and NaCl solutions with concentrations of ≥2.0% for vector management in a variety of application scenarios.

Nowadays, In Punjab, Bathinda city is hitting at first position in Dengue cases after floods with 376 cases in the month of September 2023[24]. Therefore, we recommend that 2.0% NaCl is quite enough for indoor use and residents can add edible salt in flowerpots or other aquatic habitats for suppression of *Aedes* larvae. It should be highlighted that further research is necessary due to the possibility that *Aedes* populations may have different salinity tolerance as per their habitat, which could impact the effective insecticide concentration of NaCl. Therefore, the present study suggests that Sodium Chloride (NaCl) Solutions with concentrations of ≥2.0% can be used as a cheap larvicide for *Ae. aegypti* in their habitats. Furthermore, the technological procedures of using NaCl as an insecticide or in combination with other larvicides to help in controlling vector mosquitoes and prevent dengue epidemics, still need further investigations.

CONCLUSION

The present study concluded that Sodium Chloride can be used as an effective and safe intervention in mosquito control programs that target different immature stages of *Ae. aegypti* due to its easy availability and less toxicity. So, Sodium Chloride could be developed and used as an alternative to synthetic insecticides for the management of *Ae. aegypti* population to manage the spreading of dengue in the future.

ACKNOWLEDGEMENTS

The authors are grateful to the Honourable Vice Chancellor, Prof. Gurmail Singh, Akal University, Talwandi Sabo, and The Kalgidhar Trust for providing all the necessary facilities to carry out this research.

REFERENCES

1. Powell RJ, Gloria-Soria A, Kotsakiozi P. Recent history of *Aedes aegypti*: vector genomics and epidemiology records. Biosci. 2018; 68: 854–860.

2. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012; 6: e1760.

3. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013: 496; 504–507.

4. World Health Organization (WHO). Dengue and severe dengue. 2022. Available at: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue.

5. National Vector Borne Disease Control Programme. Dengue cases and deaths in the country since. 2022. Online. Available at: https://nvbdcp.gov.in/index4.php lang=1&level=0&linkid=431&lid=3715 (Accessed on June 05, 2023).

6. Dulacha D, Were V, Oyugi E, Kiptui R, Owiny M, Boru W et al. Reduction in malaria burden following the introduction of indoor residual spraying in areas protected by long-lasting insecticidal nets in Western Kenya, 2016–2018. PLoS One. 2022; 17: e0266736.

7. Katzelnick LC, Coloma J, Harris E. Dengue: Knowledge gaps, unmet needs, and research priorities. Lancet Infect Dis. 2017;17: e88–e100.

8. Foko LPK, Meva FE, Moukoko CEE, Ntoumba AA, Ekoko WE et al. Green-synthesized metal nanoparticles for mosquito control: A systematic review about their toxicity on non-target organisms. Acta Trop. 2021; 214: 105792. doi:10.1016/j.actatropica.2020.105792.

9. Guo X, Zhou S, Wu J, Zhang X, Wang Y, Li Z et al. An Experimental Evaluation of Toxicity Effects of Sodium Chloride on Oviposition, Hatching and Larval Development of *Aedes albopictus*. Pathogens. 2022;11: 262. https://doi.org/10.3390/ pathogens11020262.

10. Jinguji H, Fujiwara Y, Ohtsu K, Shin M, Morimoto M. Acute toxicity of sodium chloride to first and fourth instar *Aedes albopictus* larvae. Med Entomol Zool. 2021*vv*: 199-204.

11. Jinguji H, Fujiwara Y, Ohtsu K, Morimoto M. Effects of Sodium Chloride on Oviposition Behavior of *Aedes albopictus*. J Am Mosq Control Assoc. 2020; 36: 253–256.

12. Mavundza EJ, Maharaj R, Chukwujekwujekwu JC et al. Larvicidal activity against *Anopheles arabiensis* of 10 South African plants that are traditionally used as mosquito repellents. S Afr J Bot. 2013; 88: 86–89.

13. Becker N, Petric D, Zgomba M, Boase C, Dahl C, *et al.* Mosquitoes and their Control, 2nd Ed. 2010; pp. 9-40, Springer Publication.

14. Bar A, Andrew J. Morphology and Morphometry of *Aedes aegypti* Adult Mosquito. Annu Res Rev Biol. 2013; 3: 52-69.

15. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. WHO. 2005. WHO/CDS/WHOPES/GCDPP/2005.13-39.

16. Abbott WS. A method of computing the effectiveness of insecticides. J Ecol Entomol. 1925; 18: 265–267.

17. Finney DJ. Probit analysis, III Edn. Cambridge: Cambridge University Press 1971; pp. 68-72.

18. Mukhopadhyay AK, Tamizharasu W, Satya BP, Chandra G, Hati AK 2010. Effect of common salt on laboratory reared immature stages of *Aedes aegypti* (L). Asian Pac J Trop Med. 2010; 3: 173–175.

16. 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. 2023;2: 12-15.

20. Sandhu KK, Vashishat N, Kocher DK. Potential larvicidal bioefficacy of copper sulfide-based hybrid nanoemulsions of eucalyptus oil against *Aedes aegypti* (Linnaeus). J Vector Borne Dis. 2023; 60: 79-87.

21. Sandhu KK, Vashishat N. Nanoemulsified *Eucalyptus globulus* essential oil against mosquito *Aedes aegypti*. Indian J Entomol. 2022; 84: 290–295.

22. Ragavendran C, Manigandan V, Kamaraj C, Balasubramani G, Prakash J S, Perumal P, et al. Larvicidal, histopathological, antibacterial activity of indigenous *Fungus penicillium* sp. against *Aedes aegypti* L. and *Culex quinquefasciatus* (Say) (Diptera: Culicidae) and its acetylcholinesterase inhibition and toxicity assessment of zebrafish (*Danio rerio*). Front Microbiol. 2019; 10: 427.

23. Kraemer MUG, Reiner RC, Brady OJ, Messina JP, Gilbert M, Pigott DM, et al. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. Nat Microbiol. 2019; 4: 854–863.

24. https://www.tribuneindia.com/news/punjab/at-372-dengue-cases-kapurthala-2nd-worst-hit- district-after-bathinda-543125.