



EFFECT OF DIFFERENT CONCENTRATION OF TABLE SALT AND IODINE TREATED SALT AGAINST 3RD AND 4TH INSTARS *Aedes aegypti* AND *Aedes albopictus* LARVAE IN LABORATORY

Myat Thandar Swe*, Maung Maung Mya**, Cho Cho Oo*, Tin Mar Yi Tun*, Ni Ni Win***, Yan Naung Maung Maung** and Kyaw Zin Thant**

*Yangon University of Distance Education,

**Department of Medical Research,

***Hpa-an University.

*Corresponding Author: Dr. Maung Maung Mya

Department of Medical Research.

Email ID: dr.mmgmya@gmail.com.

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ABSTRACT

The present study aimed to evaluate the larvicidal activity of different concentration of table salt and iodine salt on laboratory reared *Aedes aegypti* larvae from Dagon Myothit North Townships in Yangon Region from May 2017 to April 2018. Third and fourth instar larvae 50 each were exposed for 24 hours in different concentrations as 2.5, 2.0, 1.5, 1.0, 0.5, 0.25 gram of table salt and Iodine salt were prepared freshly by dissolving in 100ml each of tap water inside each 250ml plastic cups and also negative control test was done simultaneously of both salt solutions. The resulted found that Knockdown effect of *Ae. aegypti* and *Ae. albopictus* were not significantly difference at the highest concentration of 2.5g/100ml i.e. 64.80%, 65.20% in table salt and 66.00%, 67.20% in iodine salt respectively after 60 minute exposure period. Although after 24 hours exposure period the mortality of both 3rd and 4th instar larvae of *Ae. aegypti* and *Ae. albopictus* in same highest dilution were found 95-96% mortality in table salt and 100% mortality in iodine salt solution. Lethal Concentration for 50% mortality (LC₅₀) in both *Ae. aegypti* and *Ae. albopictus* was observed 1.0671g and 0.9930g for table salt and 0.6963g and 0.5240g for iodine salt solution and LC₉₀ values were found 2.4509g and 2.1375g for table salt and 1.4986g and 1.2268g for iodine salt solution. The findings of the present study revealed that 2.5g in 100 ml of iodine salt solution has strong larvicidal activity on both *Ae. aegypti* and *Ae. albopictus* mosquito larvae as a good source of preparations for mosquito control. Table salt and Iodinated salt are easily available and eco-friendly nature therefore these salts should be included as one of the tools to prevent Dengue fever/Dengue hemorrhage fever, Zika and Chikungunya infection from *Aedes* vector vie to destroy breeding place of *Ae. aegypti* and *Ae. albopictus* in household and per- household situations.

KEYWORDS: Table salt, iodine salt, LC₅₀, LC₉₀, *Aedes aegypti*, *Aedes albopictus*.

INTRODUCTION

Aedes aegypti and *Ae. albopictus* are vectors of four important human disease viruses: dengue fever (DF), dengue hemorrhage fever (DHF), yellow fever and Zika. It is estimated that dengue, a flu-like illness, is a risk to over 40% of the world's population. Dengue infection has expanded to other geographical areas due to changes in human ecology and behavior.^[1,2] Hundreds of thousands of cases of dengue and dengue hemorrhagic fever are reported each year in tropical regions of the Americas, Africa, Asia and Oceania.^[3] In 2013 dengue was estimated to be responsible for approximately 3.2 million severe cases and 9000 deaths, the majority occurring in lower middle income countries, and for 1.1 million disability adjusted life years (DALYs) globally.^[4]

Southeast Asia Region and Western Pacific Region in the world which bear nearly 75% of the current global disease burden due to dengue.^[5] DF and DHF are increasingly becoming serious public health problems in Myanmar especially among the 5-10 and 11-15 years old age groups and now noted 15 years above, a vast majority of the cases occur in 5-8 years old age group.^[6,7] The highest number of cases and deaths recorded were 9149 DHF cases and 55 deaths recorded across Myanmar.^[8] A severe outbreak of DHF occurred for the first time in Yangon in 1970.^[9] In Myanmar, the highest numbers of DHF cases were reported from Irrawaddy, Kachin, Magway, Mandalay, Mon, Rakkine, Sagaing, Tanintharyi and Yangon regions.^[10] *Aedes aegypti* is one of the world's most widely distributed mosquitoes and is of considerable medical importance as a vector of

dengue, Zika and yellow fever.^[11] Chemical and Synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air.^[12] Therefore, there is a need to find out the alternative ways for environmental safety, biodegradable, low cost and indigenous methods for vector control. Mosquito larvae can develop in fresh, salty and brackish water. The larvae of *Aedes aegypti* develop in fresh water. However, in laboratory studies, tolerance of this species for oviposition and hatching in brackish water was observed. Immature forms of *Ae. aegypti* have also been found developing in brackish water in coastal and plateau areas of southeastern areas of Brazil.^[13]

There is no proper drug or vaccine, for the control of dengue fever (DF) and dengue hemorrhagic fever (DHF) primarily, control of DF/DHF includes on controlling the mosquito vectors.^[14] The purpose of this study is to investigate the effects of different concentration of salt solution on survival of laboratory reared *Aedes* mosquito's larvae and to determine lethal concentration and death rate response to control the breeding of *Aedes* mosquitoes.

MATERIALS AND METHODS

Mosquito larvae collection

Aedes mosquito larvae were collected from different water storage containers from Dagon Myothit North Townships, Yangon Region from November 2017 to April 2018. All collected larvae were carried to DMR laboratory for larvicidal test with different concentration of table salt and iodine treated salt. *Aedes aegypti* and

Aedes albopictus mosquito larvae were reared in tap water which was carried from different study sites.

Species identification

Larvae and adult mosquitoes emerged from larva survey were identified by morphological methods.^[15]

Larvicidal testing procedure

Based on preliminary tests, further dilutions were prepared with same type of test water. Different concentrations as 2.5, 2.0, 1.5, 1.0, 0.5, 0.25 gram of table salt and Iodine salt were prepared freshly by dissolving in 100ml each of tap water inside each 250ml plastic cups and also negative control test was done simultaneously. Fifty (50) each *Aedes aegypti* and *Ae. albopictus* larvae were put into different concentrations of table salt and Iodine salt. Detail testing was done according to standard method.^[16,17] The exposure period of larvae were exposed 24 hours for each replication and concentration in laboratory at 26-30°C and 70 to 90% relative humidity. In the experiments, five replicates were carried out and Knockdown was checked and recorded after 60 minute of exposure periods and mortality was checked and recorded after 24 hours of exposure periods. Dead larvae were identified when the larvae failed to move after probing with a needle in the cervical region.

Data analysis plan

Data entry and processing was made using micro soft Excel software. Lethal Concentration LC₅₀ and LC₉₀ values were calculated by using dose-effect probit calculations.^[18,19]

RESULTS

Table 1: Knockdown effect of different concentrations of table salt and Iodine salt against 3rd and 4th instars *Aedes aegypti* and *Aedes albopictus* larvae.

Concentration (gm/100ml)	Total larvae	Table Salt		Iodine Salt	
		<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>
2.5	250	162 (64.80%)	163 (65.20%)	165 (66.00%)	168 (67.20%)
2.0	250	117 (46.80%)	115 (46.00%)	118 (47.20%)	116 (46.40%)
1.5	250	57 (22.80%)	67 26.80%	78 31.20%	74 29.60%
1.0	250	27 (10.80%)	32 (12.80%)	37 (14.80%)	25 (10.00%)
0.5	250	3 (1.20%)	2 (0.80%)	6 (2.40%)	1 (0.40%)
0.25	250	0	0	0	0
Control	250	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 1. shows that the highest knockdown effect was found 67.20% of *Aedes albopictus* and 66.00% of *Aedes aegypti* in 2.5g/100 concentrations of Iodine salt solutions and followed by 64.80% and 65.80% knockdown of both *Aedes aegypti* and *Aedes albopictus* larvae. Lowest knockdown was observed at the concentration of 0.5g/100ml of both salts.

Table 2: Mortality effect of different concentrations of table salt and Iodine salt against 3rd and 4th instars *Aedes aegypti* and *Aedes albopictus* larvae.

Concentration (gm/100ml)	Total larvae	Table Salt		Iodine Salt	
		<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>
2.5	250	238 (95.20%)	240 (96.00%)	250 (100.00 %)	250 (100.00%)
2.0	250	200 (80.00%)	212 (84.80%)	245 (98.00%)	248 (99.20%)
1.5	250	157 (62.80%)	187 (74.80%)	220 (88.00%)	228 (91.20%)
1.0	250	88 (35.20%)	110 (44.00%)	153 (61.20%)	195 (78.00%)
0.5	250	44 (17.60%)	47 (18.80%)	77 (30.80%)	101 (40.40%)
0.25	250	2 (0.80%)	3 (1.20%)	13 (5.20)	28 (11.20)
Control	250	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 2. Shows that 100% mortality of both *Ae. aegypti* and *Ae. albopictus* were found in 2.5g/100ml concentration of Iodine salt solution, although in same concentration of table salt solution the mortality rates were found 95.20% and 96.00% of both *Ae. aegypti* and

Ae. albopictus. Over 80% mortality of both *Ae. aegypti* and *Ae. albopictus* larvae were found at 2.0g/100 concentration of table salt and 1.5g/100ml concentration of Iodine salt solutions.

Table 3: Lethal concentration (LC) values of Table salt and Iodine salt against 3rd and 4th instars *Aedes aegypti* and *Aedes albopictus* larvae.

Lethal Concentration (LC)	Table salt		Iodine salt	
	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>
LC50	1.0671g	0.9930g	0.6963g	0.5240g
LC90	2.4509g	2.1375g	1.4986g	1.2268g
Chi Squire X ²	32.9146	10.0703	21.1874	22.7241
df	4	4	4	4
P value	0.05	0.05	0.05	0.05

LC₅₀= Lethal Concentration dose 50, LC₉₀= Lethal Concentration dose 90, df=degree of freedom

The doses of 50% mortality (LC₅₀) and 90% mortality (LC₉₀) values of table salt and Iodine salt against 3th and 4th instar *Aedes* larvae were shown in Table 3. The lowest dose for 50% mortality was found 0.5240g of iodine salt concentration against *Aedes albopictus* followed by 0.6963g of Iodine salt concentration against *Aedes aegypti* larvae. Table salt found highest amount of dose 1.0671g and 0.9930g concentration was need for 50% mortality of 3th and 4th instar both *Aedes* larvae.

The lowest dose (highest efficacy) for 90% mortality was found 1.2268g of iodine salt concentration against *Aedes albopictus* followed by 1.4986g of iodine salt against *Aedes aegypti* larvae. Table salt concentration found highest amount of dose was required as 2.4509g and 2.1375g concentration for 90% mortality of 3th and 4th instars larvae of *Aedes aegypti* and *Aedes albopictus* in 100ml water.

DISCUSSION

Aedes aegypti and *Ae. albopictus* are medically important vectors of DF, DHF and Zika causing major public health problems and burden of diseases in countries of the Southeast Asia Region, including Myanmar.^[20] *Aedes aegypti* and *Aedes albopictus* are presented in Myanmar but *Aedes aegypti* is a primary vector in urban areas and *Aedes albopictus* is a secondary role of dengue in rural areas in Myanmar.

The larvae of several mosquito species are tolerant to salinity, including some species of the genus *Aedes*.^[21] Observations related to *Ae. aegypti* have indicated that the species occurs in salt concentrations of up to 16%, mainly in abandoned vessels^[22] and, especially in Brazil, in vessels still in use but stored in marinas.^[23] In the laboratory, larvae of this species exposed to different salt concentrations are able to survive until a maximum concentration of 14% is reached and the adults that emerge are smaller than the average size of adults that emerge in fresh water.^[24]

In the present study, laboratory reared Dagon Myothit North Township strains of *Ae. aegypti* and *Ae. albopictus* larvae have some tolerance to salt solution because the knockdown effect was found low, i.e. 61.80% and 65.20% in table salt, 66.00% and 67.20% knockdowns in iodinated salt solutions were the highest concentrations (2.5g/100ml). Although mortality of both larvae was observed higher in same concentration of both salt solutions in laboratory within 24 hours. Study showed that table salt and iodine salt concentrations of 2.5g in 100ml water killed the larvae of *Ae. aegypti* and *Ae. albopictus* in the laboratory. The mortality of both larvae were found 95- 96% in 2.5g table salt solution and 100% mortality in same concentration of iodine salt solution within 24 hours. Although Mukhopadhyay *et al.*,^[25] revealed that 95.42% of the *Ae. aegypti* larvae were died within 36hours in 1.50% salt solution and 50 to 90%

larvae die within 19 to 29 hours as well as 81.81% died within 60 hours. When *Aedes aegypti* larvae exposed to distilled water or high NaCl media, the pH level sources a significant decrease in hemolymph Na⁺ and Cl⁻ levels in species of *Aedes* mosquitoes but the hemolymph ion levels were not affected.^[24,26] Same result has been found in the concentrations of 10%, 15% and 20% NaCl salt solutions on the larvae of *Aedes aegypti* mosquitoes. Riaz *et al.*,^[27] observed that NaCl salt concentration 10% in water would kill the majority of larvae of *Aedes aegypti* in the laboratory. The finding of the both researchers are similar with our findings in 2.0g and 2.5g/100 ml table and iodine salt solutions would kill the majority on laboratory reared *Ae. aegypti* and *Ae. albopictus* larva in laboratory after a long time (24hours) period. Pappas *et al.*,^[28] from Peru reported that less than 50% of the larvae of *Culiseta inornata* reached the pupal stage at above 0.01M NaCl concentration. Another study found that 4.58% of *Ae. Aegypti* larvae transferred to pupae in the 1.50% NaCl concentration. Although present study not found larvae were transferred to pupae in the concentration of 2g and 2.5g of both table salt and iodinated salt solutions within 24 hours. According to LC₅₀ and LC₉₀ values of Iodine salt found highly effective to control both *Aedes* larvae. The lethal dose for 50% mortality (LC₅₀) and 90% mortality (LC₉₀) of iodine salt was found 0.5240g and 1.2268g against *Aedes albopictus* and 0.6963g and 1.4986g table salt against *Aedes aegypti* larvae in 100ml water. Although study of same iodine salt trail was given to observe death rate of immature stage of *Ae. Aegypti* in the same concentration solution (1.00%) with analytical reagent of sodium chloride which results showed nearly similar results with solution of common salt.^[29] In the present study 100% mortality of both *Ae. aegypti* and *Ae. albopictus* were found in the laboratory trial. It may be due to the fact that hypertonic solutions of NaCl penetrate the gill surface very rapidly and cause marked swelling of the cells of the protoplasmic lining in *A. aegypti*.^[30]

CONCLUSION

In the present study, researchers found that both 3rd and 4th instar larvae of *Ae. aegypti* and *Ae. albopictus* mosquitoes from Dagon Myothit North Township, Yangon Region were 100% die in 2.5g table salt and iodine salt in 100ml water within 24hours in laboratory. Table salt and Iodine salt are easily available and eco-friendly nature therefore these salts should be included as one of the tools to prevent Dengue fever/Dengue haemorrhagic fever, Zika and Chikungunya infection vie to destroy breeding place of *Ae. aegypti* and *Ae. albopictus* as well as other mosquitoes in household containers.

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