EFFECTS OF SODIUM CHLORIDE CONCENTRATIONS ON LARVAE AND PUPAE OF *Aedes aegypti*

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ABSTRACT

Aedes aegypti is one of the commonest mosquitoes in the tropics and sub topics, feeding on human blood when exposed and is responsible for the transmission of urban yellow fever in Africa and out side Africa, it transmits dengue fever. Aedes aegypti are basically freshwater mosquito. The effect of different concentrations of saline solution on the survivorship of larvae and pupae of Aedes aegypti is studied to possibly find a control measure that will utilize waste water from our kitchen which contains some form of saline solution. Different grams of NaCl were measured and dissolved in 100 ml of water to get the different concentrations and the different larva were subsequently introduced into these solutions. Increase in salinity, brings about increase in mortality rate as well as increase in length of days for development. While there was no mortality in saline concentration between 0.1 - 1.0 % the length of days for development remains almost the same as in freshwater. But from 1.5%-5.0% saline concentration, mortality recorded was on the increase and rate of development was delayed taking about 4.5 days in pupa as against the normal 2.5 days on the average. However optimal mortality was recorded in the first larval instars having 100% mortality in 1.5% to 5.0%. Where as the pupal stage recorded the highest survivorship rate of 83.3% in all. Finally it was observed that Aedes aegypti even though a freshwater mosquito tolerated some level of salinity and at the same time can be controlled effectively with NaCl solution.

Keywords: Sodium chloride concentrations, Larvae, Pupae, *Aedes aegypti,* Mortality, Survivorship

INTRODUCTION

Mosquitoes are small two winged Diptera of the suborder Nematocera and found in the Family Culicidae (Imms, 1970). They are among the best known group of insect vectors. Mosquitoes under goes complete metamorphosis (egg, larva, pupa and adult) with the larvae having four larval instars. The metamorphic development of mosquitoes takes place in an aquatic in environment which can either be flowing water or stagnant water. The evolution of salt secreting rectal segment has enable some mosquito species including *Aedes* to tolerate salinities in excess seawater (Bradley and Philip, 1975; 1977).This enables them to exploit highly productive habitat. *Aedes aegypti* is one of the commonest mosquitoes of the tropics and sub topics, feeding on man inside houses. Aedes is responsible for the transmission of urban yellow fever in Africa and out side Africa it transmits dengue fever. According to Bradley (1987), *Aedes* are known as a salt marsh mosquito because it inhabits estuaries and salt marshes where salinities fluctuate from essentially freshwater to seawater following evaporation. *Aedes aegypti* are found primarily in small bodies of water like Cans tins, discarded tires, roof gutters, broken bottles, flower pots etc. (Gillet, 1972). Longevity of mosquitoes is dependent upon temperature thus that of *Aedes aegypti* is normally between 15 – 20 days but may be as short as 11 days. The survival of adulthood *Aedes aegypti* has obvious fitness consequences. Environmental influence on aspect of growth and development are also important in determine this fitness. Mosquitoes are characterized by rapid completion of cycle and this rapid development allows them to complete more generation and experience explosive population growths during favorable breeding season. In insects generally, females are larger than the male in terms of body size, and this size helps them to reproduce as many offspring as possible, thus, there is a trade off maturity existing. These phenotypic traits such as growth rates and body mass are affected by genes and environmental interactions like salinities, density, food supply, physical size and shape of larval habitat (Trips and Horsfall, 1969; McGinnis and Brust, 1983; Clark *et al.,* 2004). Mosquito larvae are found in variety of aquatic habitats including freshwater and saline environment of diverse composition. Work done by Bradley (1987) revealed that mosquitoes have three distinct osmoregulatory strategies which are freshwater osmoregulator, euryhaline osmoregultor and euryhaline osmocomfomer.

Two of these strategies according to Reinsert (2000) were observed in closely related genera within the tribe of Aedinii (*Aedes ochlerotatus*) which was a sub genus of *Aedes* until recently. Also, *Aedes natronius* larva found in Crater Lake in Uganda had a salinity equivalence of 3.9 % sodium chloride. In low Salinity water the larvae of *Aedes* are hyperosmotic to the medium but in a higher concentration they are hyposmotic. These larvae can adapt to concentration that extend over a 500 fold rang without changing the concentration of the major ions in the haemolymph more than twofold. The larvae of *Aedes* response to rising external salinities by increasing their drinking rate several fold. This seems to be the only way of obtaining water to compensate for the loss to the concentrated medium but drinking also means ingesting of a heavy load of dissolved ions (Nielsen, 1995; Patrick *et al*., 2002). Therefore the objective of this research is to determine the survivorship of the *Aedes aegypti* larvae and pupae in different concentration of sodium chloride, and also to find a possible local and inexpensive means of controlling this insect vector in our environment.

MATERIALS AND METHODS

Viable eggs of *Aedes aegypti* was collected and soak in plastic basin containing 5000 ml of ordinary tap water and kept in the laboratory. After 24 hours the eggs hatched. The first larval instars were collected with droplet pipette and introduced into 100 ml beakers containing different concentration of NaCl solutions (0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.5, 4.0, 4.5 and 5.0 g/l). In each of these NaCl solution beakers, 15 larvae were introduced. The mouth of the beakers was covered with mosquito net and fastened with rubber bands to avoid the entrance / escape of mosquito. The set up was done in three replicates and monitored twice daily for the developmental stages. Effects like mortality, survivorship and rate of developments on various larval instars were observed and recorded. In the case of total mortality, the next stage was taken from the reservoir and re-introduced back into the next stage. For the pupae, fourth larval instar of this mosquito was placed in ordinary tap water and allowed to pupate. After two days, the pupae was placed in 100 mls beakers containing different concentrations of NaCl solution and monitored in triplicates for mortality, survivorship and rate of developments of various larval instars. For each of this setup, a control experiment having 0 g/l sodium chloride concentration was mounted. All mosquitoes were adequately fed.

RESULTS

In saline concentrations between 0.1 and 0.5 there was no mortality. But in saline concentrations 1.0, 6.7 % mortality was recorded which in fact was very low. The rate of development still remains the same as in the normal water. But from saline concentrations 1.5 - 5.0, they all died. Total mortality recorded was 67.2 % and total survivorship was 24.4 %. No mortality was recorded in saline concentrations 0.1 – 1.0 (Table 1). When the second larval instar was introduced into saline concentration 1.5, only two survived and the

Table 1: Survivorship, mortality and development duration of first and second larval instars of *Aedes aegypti*

NaCl Concentration	Larva		Mortality		Average rate of Development
	Initial No	Survived	No	%	
		First larval instars			
0.0	15	14	1	6.7	1
0.1	15	15	0	0	1
0.5	15	15	0	0	1
1.0	15	14	1	6.7	1
1.5	15	0	15	100	-
2.0	15	0	15	100	-
2.5	15	0	15	100	-
3.0	15	0	15	100	-
3.5	15	0	15	100	-
4.0	15	0	15	100	-
4.5	15	0	15	100	-
5.0	15	0	15	100	-
Total	165	44/24.4	121	67.2	
		Second larval instars			
0.1	15	15	0	0	1
0.5	15	15	0	0	1
1.0	15	15	0	0	1
1.5	15	0	13	8.6	2
2.0	15	0	15	100	-
2.5	15	0	15	100	-
3.0	15	0	15	100	-
3.5	15	0	15	100	-
4.0	15	0	15	100	-
4.5	15	0	15	100	-
5.0	15	0	15	100	-
Total	165	47/26.1	118	65.5	
Control	15	15	0	0	1

rest died. The rate of larval development was delayed. Total mortality here was very high (65.5%) with total survivorship of 26.1% (Table 2). It was noted that from 1.5% - 3.5%, Mortality gradually increased from 66.7 to 93.3 and finally 100% from 4.0% to 5.0 %. Rate of development equally increased from 1 which is normal to 2.5. It was also observed that as the total morality was decreasing (57.7%) survivorship was increasing (34.0%). Survivorship continues to increase from 0.1% to 4.5% NaCl concentrations. While mortality remained at 0 % in 0.1% to 1.0%, and was 100% in 5.0% concentration. As this success in survivorship was celebrated, it was noted that the rate of development was greatly retarded.

Just like in the first larval instars, the pupae recorded no mortality in 0.1% and 0.5% concentrations and development rate remained normal as in control (Table 3). From 1.0 %, survivorship increased greatly with a total of 83.5% and rate of development increased as high as 4.5 %. It was observed that the highest mortality percentage was recorded in 3.5% and 4.0% concentrations. Mortality generally was low; total mortality percentage was 7.8%.

DISCUSSION

Aedes aegypti used in this research was the freshwater type and this was evident as their immature stage only tolerates low range of salinity.

Table 2: Mortality, survivorship percentage and development duration of third and fourth larval instars of *Aedes aegypti*

NaCl Concentration	Larva		Mortality		Average rate of Development
	Initial No	Survived	No	%	
		Third larval instars			
0.1	15	15	0	0	2
0.5	15	15	0	0	2
1.0	15	15	0	0	2
1.5	15	6	9	5.45	2.5
2.0	15	4	11	6.66	2.5
2.5	15	4	11	6.66	2.5
3.0	15	3	12	7.27	3
3.5	15	2	13	7.87	3
4.0	15	1	14	8.48	3
4.5	15	1	14	8.48	3
5.0	15	0	15	9.09	2
Total	165	66/40.0	99	59.96	
Control	15	15	0	0	1
		Fourth larval instars			
0.1	15	15	0	0	1
0.5	15	15	0	0	1
1.0	15	15	0	0	1
1.5	15	0	13	7.87	2
2.0	15	0	15	9.09	-
2.5	15	0	15	9.09	-
3.0	15	0	15	9.09	-
3.5	15	0	15	9.09	-
4.0	15	0	15	9.09	-
4.5	15	0	15	9.09	-
5.0	15	0	15	9.09	-
Total	165	45/27.2	118	71.5	
Control	15	15	0	0	1

Table 3: Mortality, survivorship percentage and development duration of pupae of *Aedes aegypti*

NaCl Conc	No of Larva	No of Survived /%	No of Mortality	% mortality	Ave. rate of Dev.
0.1	15	15	0	0	1
0.5	15	15	0	0	1
1.0	15	15	0	0	1
1.5	15	0	13	7.87	2
2.0	15	0	15	9.09	-
2.5	15	0	15	9.09	-
3.0	15	0	15	9.09	-
3.5	15	0	15	9.09	-
4.0	15	0	15	9.09	-
4.5	15	0	15	9.09	-
5.0	15	0	15	9.09	-
Total	165	45/27.3	118	71.5	
Control	15	15	0	0	1

Any increase above 1.5%, hampered survival particularly at the first larval instar. In low salinity, the larvae of *Aedes* were hypertonic to the medium but in higher concentration they are hypotonic (Nielsen, 1995). The first larval instar was able to survive in NaCl concentration as low as 0.1%-1.0% but all died at higher concentrations from 2.0% - 5.0%. This high mortality discerned at the very first larval instar in concentrations between 1.5% - 5.0% agreed with Pelizza *et al*. (2007) which reported higher mortality in younger instars than in older ones and this also accounts for why the second, third and fourth larval instar were able to survive in such higher concentrations when they were later re-introduced. Associated with this increase in saline concentration is the physiology of hypertonic haemolymph which may be responsible for the delay in rate of development as observed, and is in line with Lee and Hong (1985) where development is delayed at high salinities. Extra time and energy spent in trying to excrete the extra salt absorbed may also contribute to the slow rate of development as uptake of salt occur principally through the anal papillae and some through the abdomen, therefore salt solution in excess of 1.5% will pose an irresolvable difficulties to the fragile osmotic balance of the larvae particularly the first larval instar where we had 100% mortality and causes delay in the later stages or even death in concentrations as high as delay in the 5.0%.Again not only dose high salinity effects Aedes larva in terms of rate of development, the body size is greatly reduced, or retarded.

This was in perfect agreement with Song and Brown (2002), and Clark *et al*. (2004) who reported that high salinity was of a great stress on *Aedes* species. From the result, degree of saline concentration has little effect on the pupae probably because of the pupal skin .It was observed that despite the delay in the various concentrations of saline solutions pupation still occurred. Survivorship was also very high, this goes to prove that may be as adult such saline concentrations may not have any effect on the mosquito. In conclusion, in Nsukka and its environs it was observed that stagnant water bodies, pounds and gutters are rich with various species of mosquito larvae including Aedes. This means that the adult gravid female of *Aedes* may not be selective of its ovopositing site like the female of *Anopheles*. Therefore, as a way of control, waste water from our kitchen which always contain some concentration of sodium chloride solution when sieved and poured into these stagnant water bodies like gutters and pounds around our houses will to some extent if not 100% wipe or make it impossible for the larval stages of *Aedes* to survive particularly the first instar which is most susceptible to salinity even in concentrations as low as 1.5%.

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