***Int. J. Exp. Res. Rev.*, Vol. 5: 49-57 (2016)**

International Journal of Experimental Research and Review (IJERR)

ISSN: 2455-4855 (Online) ***Original Article***

Received: 2nd May, 2016; **Accepted:** 8th June, 2016; **Published:** 30th June, 2016

Acute toxicity of sodium chloride on different developmental stages (Egg, Spawn, Fry and Fingerlings) of *Labeo rohita* (Rohu)

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Abstract

The acute toxicity test of the sodium chloride (NaCl) was studied on different stages (egg, spawn, fry and fingerlings) of freshwater fish*, Labeo rohita,* Hamilton, through bioassay test. Different concentrations of sodium chloride salt solutions (0.5 – 10000, 1.2 - 20000, 0.75 - 11250 and 50-10000 ppm) were selected for toxicity studied of egg, spawn, fry and fingerlings respectively. Percentage of dead or damaged egg, spawn, fry and fingerling at 6, 12, 18, 24, 36, 48, 60, 72 and 96 hours were recorded for the calculation of LC_{50}. The LC_{50} values of NaCl of egg, spawn, fry and fingerling were ranged from 5561.04 -3.25, 3564 -357, 10001.87 - 750.23 and 9738 - 5206.95 ppm in 6 - 96 hours respectively. The fish exposed to the salt solutions showed erratic swimming and loss of balance in a dose dependent manner. The regression values and the 95% confidence limits of the LC_{50} values for each test were also calculated for different time periods. The increase in NaCl concentration in water increased the toxicity and reduced the duration to damage 50% of the eggs, spawn, fry and fingerling. Low hatchability, delayed hatching, poor survival observed in the test solutions >1000ppm concentration.

Keywords: Acute toxicity, bioassay, Labeo rohita, LC50, rohu, sodium chloride.

Introduction

Trace metals are natural components of the biosphere and some metals are essential for life but their excess concentrations become toxic. The chloride ions are naturally occurring, and therefore detection of increased levels of chloride in surface waters does not necessarily imply an anthropogenic source. Natural sources of chloride in aquatic systems include naturally-occurring saline lakes and groundwater discharges from saline aquifers. Aquatic organisms require chloride to maintain normal physiological functions but only at relatively steady concentrations to which the organism has adapted. Aquatic organisms exposed to excess or widely fluctuating chloride are vulnerable to survival, growth, and/or reproduction risks. Chloride occurs in the natural environment as salts of sodium (NaCl), potassium (KCl), calcium ($CaCl_2$) and magnesium ($MgCl_2$) (Nagpal et al., 2003; WHO, 2003). Chloride constitutes approximately 0.05% of the earth's outermost crust (lithosphere) (NRC, 1977). The sodium level in water bodies is quite variable. Wide ranges of seasonal fluctuations of sodium in freshwater bodies have been reported by a number of researchers (Khan and Siddqui, 1974; Goel et al.,1986; Khatavkar et al., 1990). Sodium chloride has many potential applications in fish production. High concentrations of sodium chloride have strong local effects in fish. In hypertonic solutions paralysis of the neuromuscular apparatus and serve damage to the gill epithelium of fish are observed (Metelev et al., 1983). The first symptoms of poisoning with sodium chloride and sulphate are observed after a short interval of time, namely arena swimming, which changes into various patterns of swimming and movement in jerks. Respiration is arrhythmic and gradually a condition similar to a narcotic state develops. The fish respond poorly to mechanical stimuli, sometimes turn on their sides, sometimes swim with the abdomen upward and subsequently succumb to paralytic phenomena and death. A characteristic symptom of poisoning with sodium salts is dark coloration of the body. The overall fish species were found to be quite tolerant of high chloride exposures for short (acute) durations. The fish exhibiting the higher sensitivity was the Fathead minnow (*Pimephales promelas)* with a 96h LC_{50} of 3386 ppm of Cl (Mount et al., 1997). The most chloride tolerant fish species was the Three-spined stickleback (*Gasterosteus aculeatus*) with a 96h LC_{50} of 10200 ppm of Cl (Garibay and Hall, 2004). Bioassay is necessary to determine the concentration of a toxicant, which may be allowed in receiving waters without adverse effects on the living resources (Standing Committee of Analysts, 1981; Ward and Parrish, 1982; Reish and Oshida, 1987).

Acute toxicity bioassays (LC_{50} and lethal concentration) are used to evaluate the toxicity of metals and it also helps to assess the potentials of various fish species to the toxicity of metals (Abdullah et al., 2007). In the present study an attempt has been made to determine the short term (upto 96 hrs) toxic effects of sodium chloride to the different developmental stages (egg, spawn, fry and fingerling) of *Labeo rohita*.

Material and Methods

Experimental design

The rohu (*Labeo rohita*) was selected as test organism. The egg, spawn, fry and fingerling of appropriate quality of rohu were selected for the experiment. Egg, spawn, fry and fingerlings were collected from the hatchery of Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar. The organisms were brought to the laboratory. The spawn, fry and fingerlings were acclimatized in the glass aquaria prior to experiments. 20 l glass aquaria were used for experiments. Before the experiment set all the glass aquaria were cleaned with laboratory detergents, then with 100% acetone and tap water. After each test, the containers were washed appropriately with acid to remove metals. Each of the test containers were provided with facilities of continuous aeration and covered with velon screen netting to prevent the test organisms from jumping out.

Selections of test concentrations were made following the APHA method (APHA, 1998). At least five different concentrations were taken in each experiment. While designing, minimum of five exposure (treatment) concentrations of a test substance, one control was also taken for bioassay test. In the present study the test organisms (rohu) were exposed to a wide range of sodium concentrations in different stages. The concentrations were as far as possible selected in logarithmic or geometric scale. For egg, spawn, fry and fingerling 0.5 - 10000 ppm, 1.2- 20000 ppm, 0.75- 11250 ppm and 50-10000ppm of NaCl were selected respectively. Four experiments were conducted for bioassay test. These experiments were egg bioassay (experiment-1), spawn bioassay (experiment-2), fry bioassay (experiment-3) and fingerling bioassay (experiment-4). Different concentrations of NaCl were used as the test solutions.

Percentage mortality was determined for each concentration of sodium chloride for the mortality study experiment. The data obtained from the experiments were processed by probit analysis (Finney, 1971; Reish and Oshida, 1987; Mohapatra and Rengarajan, 1995) for determination of LC_{50} values using SPSS software. The lethal concentrations were plotted against time in hours to get "Toxicity curve"(Seegert et al., 1979).

Results

Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals. No mortality was observed in control experiments. The fish mortality increased significantly when the concentration and the time of exposure to the metals increased. This test was conducted to gain preliminary information about the toxicity of sodium chloride. The toxicity of the sodium chloride on the rohu (egg, spawn, fry and fingerling) was assessed and LC_{50} was determined for 6, 12, 18, 24, 36, 48, 60, 72 and 96 hours by probit analysis. The 96-hr tests (LC_{50}) were performed to evaluate the sensitivity of (egg, spawn, fry and fingerlings) rohu to short term effects of Sodium chloride. Among all the developmental stages eggs are more sensitive to NaCl while fingerlings were less sensitive. The results of toxicity studies of NaCl expressed in terms of LC_{50} values obtained from probit analysis shown in figure 1-4.

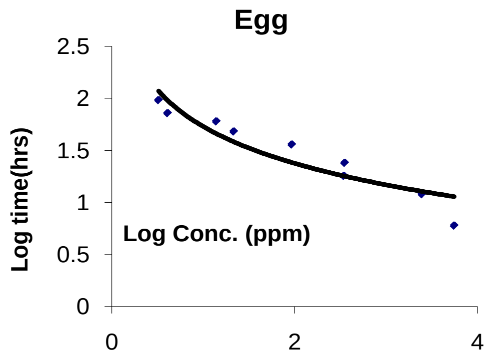

Figure 1. Toxicity curve of rohu eggs exposed to different lethal concentration of NaCl salt solution.

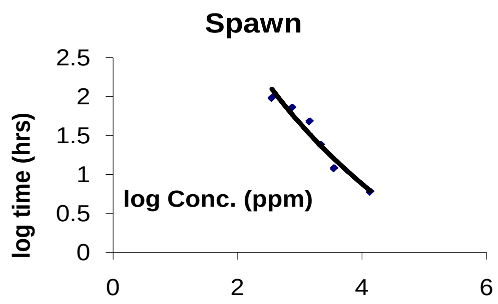

Figure 2. Toxicity curve of rohu spawn exposed to different lethal concentration of NaCl salt solution.

In case egg bioassay of rohu the LC_{50} value of sodium chloride concentration were 5561.04 ppm, 2471.946 ppm, 352. 96 ppm, 345.68 ppm, 93.23 ppm, 21.60 ppm, 13.93 ppm, 4.08 ppm and 3.25 ppm for 6 hrs, 12 hrs, 18 hrs, 24 hrs, 36 hrs, 48 hrs, 60 hrs, 72 hrs and 96 hrs of incubation respectively (Table-1).

Table 1. LC_{50} values of sodium chloride (NaCl) for rohu egg, spawn, fry and fingerling

Exposure periods (hr)	LC_{50} values of NaCl (ppm)			
	Egg	Spawn	Fry	Fingerling
6	5561.04	-	10001.87	9738
12	2471.94	3564	9407.13	8840.22
18	352.96	-	-	-
24	345.68	2184	1319.89	7016.65
36	93.23	-	-	-
48	21.60	1439	1258.72	7534.33
60	13.93	-	-	-
72	4.08	754	878.86	6048.93
96	3.25	357	750.23	5206.95

Table 2. LC_{50} values and toxicity of Sodium chloride (NaCl) on different stages of different fish species.

Species	Concentration (ppm)	Toxicity	Reference
Roach and Tench	10000-11000	Not toxic within 24 hrs	Metelev et al.,1983
	13000	Mortality after 1 day	Metelev et al.,1983
Carp (100-150g)	15000	Toxic after 6 hrs	Metelev et al.,1983
	13700	Toxic after 12 hrs	
	10 900	Toxic after 24 hrs	
	10800	Toxic after 48 hrs	
	10100	Toxic after 72 hrs	
	9000	Toxic after 96 hrs	
Trout(fry and fingerlings)	10000	Non-toxic for several hrs	Metelev et al.,1983
Catla catla	15600	6 hrs LC_{50}	Mohapatra, 1999
	13700	12 hrs LC_{50}	
	10900	24 hrs LC_{50}	
	10800	48 hrs LC_{50}	
	10100	72 hrs LC_{50}	
	9000	96 hrs LC_{50}	
Sarotherodon mossambicus	31300	24 hrs LC_{50}	Mohapatra, 1999
	30300	48 hrs LC_{50}	
	29700	72 hrs LC_{50}	
	27600	96 hrs LC_{50}	
Pimephales promelas (Fathead minnow)	4223	96 hrs LC_{50}	Mount et al., 1997; Birge et al., 1985

Lepomis macrochirus (Bluegill sunfish)	5272	96 hrs LC_{50}	Birge et al., 1985; Trama, 1954
Cyprinella leedsi (Bannerfin shiner)	6070	96 hrs LC_{50}	Environ, 2009
Oncorhynchus mykiss (Rainbow trout)	8634	96 hrs LC_{50}	Elphick et al., 2011; Vosyliene et al., 2006
Gambusia affinis (Mosquito fish)	9099	96 hrs LC_{50}	Al-Daham & Bhatti, 1977
Gasterosteus aculeatus (Three spined stickleback)	10200	96 hrs LC_{50}	Garibay & Hall, 2004
Anguilla rostrata (American eel)	13012	96 hrs LC_{50}	Hinton and Eversol, 1979
Labeo rohita (Egg)	5561.04	6 hrs LC_{50}	Present study
	2472.94	12 hrs LC_{50}	
	352.96	18 hrs LC_{50}	
	345.68	24 hrs LC_{50}	
	93.23	36 hrs LC_{50}	
	21.6	48 hrs LC_{50}	
	13.93	60 hrs LC_{50}	
	4.08	72 hrs LC_{50}	
	3.25	96 hrs LC_{50}	
Labeo rohita (spawn)	3564	12 hrs LC_{50}	Present study
	2184	24 hrs LC_{50}	
	1439	48 hrs LC_{50}	
	754	72 hrs LC_{50}	
	357	96 hrs LC_{50}	
Labeo rohita (fry)	10001.87	6 hrs LC_{50}	Present study
	9407.13	12 hrs LC_{50}	
	1319.89	24 hrs LC_{50}	
	1258.72	48 hrs LC_{50}	
	878.86	72 hrs LC_{50}	
	750.23	96 hrs LC_{50}	
Labeo rohita (Fingerling)	9738	6 hrs LC_{50}	Present study
	8840.22	12 hrs LC_{50}	
	7016.65	24 hrs LC_{50}	
	7534.33	48 hrs LC_{50}	
	6048.93	72 hrs LC_{50}	
	5206.95	96 hrs LC_{50}	

In case of spawn bioassay the LC_{50} values of sodium chloride were 3564 ppm, 2184 ppm, 1439 ppm, 754 ppm and 357 ppm after 12 hrs, 24 hrs, 48 hrs, 72 hrs and 96 hrs of exposure respectively (Table-1).

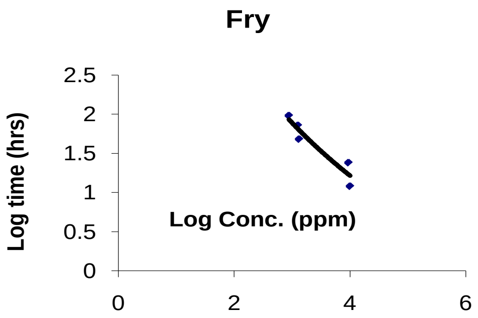

Figure 3. Toxicity curve of rohu fry exposed to different lethal concentrations of NaCl salt solution.

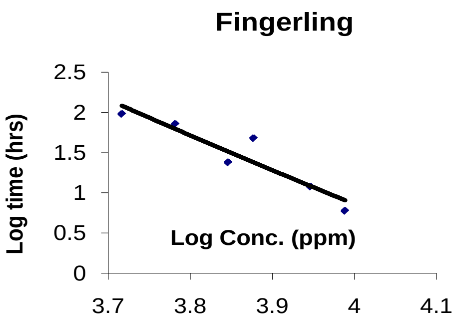

Figure 4. Toxicity curve of rohu fingerlings exposed to different lethal concentrations of NaCl salt solutions.

In case of fry the LC_{50} values of sodium chloride were 10001.87 ppm, 9407.13 ppm, 1319.89 ppm, 1258.72 ppm, 878.86 ppm and 750.23 ppm after 6 hrs, 12 hrs, 24 hrs, 48 hrs, 72 hrs and after 96 hrs of exposure respectively (Table-1).

Likewise in case of rohu fingerling the LC_{50} values of sodium chloride were 9738 ppm, 8840.22 ppm, 7016.65 ppm, 7534.33 ppm, 6048.93 ppm and 5206.95 ppm after 6hrs, 12 hrs, 24 hrs, 48 hrs, 72 hrs and 96 hrs of exposure respectively (Table-1).

Discussion

In this study the median lethal concentration (LC_{50}) of sodium chloride on rohu decreased gradually with the increase in exposure time from 6 to 96 hours. It is accepted that salinity influenced juvenile mullet growth and fish reared at salinity 24‰ grew better than those maintained in freshwater (salinity 0‰) (Lisboa, Viviana, et al*.,* 2015). In case of 96-hour egg bioassay, the LC_{50} values of sodium chloride decreased from 6 hrs to 96 hrs with values ranging between 5561.04 - 3.25 ppm. In case of rohu spawn the sodium chloride toxicity was found 3564-357 ppm from 6 hrs to 96 hrs of exposure and in case of fry it was 10001.87 - 750.23 ppm. In fingerling bioassay the LC_{50} values were in between 9738 - 5206.95 ppm for 6 hrs to 96 hrs of exposures. In another experiment studied by Iqbal et al., (2012) that better growth performance in terms of average weight gain(g) and average length gain (cm) was seen in treatment containing highest salinity level (4000 ppm) while the lowest was observed in control group. Better food conversion ratio (FCR) was found in 1600 ppm salinity level and increased with increased salinity levels. Feed intake was lowest at the lowest level of salinity i.e., 800 ppm and linearly increased with increasing levels of salinity. It is accepted that salinity is a key factor in controlling growth in tilapia that shows better performance in brackish water (Boeuf and Payan, 2001; Vonck et al., 1998). Sparks et al., (2003) reported that seawater (SW) rearing of tilapia accelerated its growth. According to Metelev et al., 1983, 15000 ppm of sodium chloride was toxic for carp (100-150g) after 6 hrs; 13700 ppm after 12 hrs; 10 900 ppm after 24 hrs; 10800 ppm after 48 hrs; 10100 ppm after 72 hrs and 9000 ppm after

96 hrs. According to them 10000 – 11000 ppm NaCl salt solution was not toxic within 24hrs, but at 13000 ppm mortality happened after 1 day for roach and tench. They also observed 10000 ppm NaCl was non- toxic for several hours on fry and fingerlings of trout. According to Mohapatra (1999) the LC_{50} value of NaCl were 15600, 13700, 10900, 10800, 10100 and 9000 ppm at 6, 12, 24, 48, 72 and 96 hrs of exposure respectively on *Catla catla* and another experiment on *Sarotherodon mossambicus,* the LC_{50} concentration of NaCl were found 31300, 30300, 29700 and 27600 ppm after 24, 48, 72 and 96 hrs of exposure respectively. The toxicity values in the present study for rohu were found less than the values obtained by Mohapatra, 1999, because of larval and seed stages. The toxicity tolerance values may vary in different size, weight and age groups of the organism in a stable environmental condition. Metal ions and their complex exhibit widening toxicity to the organism that ranges from sub-lethal to lethal depending upon the time of exposure and the prevailing condition in the ambient water (Goel, 1997).

These toxicity values cannot be compared directly with the available results of other workers, but, a comparative statement is given in Table 2. Metelev et al., (1983) reviewed that symptoms of magnesium poisoning in fish are similar to those with sodium salts.

Conclusion

Early larval stages are the most crucial and vulnerable one in the life cycle of fish. Hence, the rate of survival in each stage depends on the maintenance of water quality parameters. It is essential to know the content of sodium chloride in fish pond water as it is one of the important water quality parameter for fish culture. From the findings of this experiment it was concluded that the median lethal concentrations (LC_{50}) of sodium chloride on different stages of rohu (egg to fingerling stages) decreased gradually with the increase in exposure time from 6 to 96 hours.

Acknowledgements

The authors acknowledge to the DBT (GoI) for fund supporting and the Director Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, for providing facilities for experimental work. We also acknowledge to Dr. B. K. Das (PI of the DBT project).

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