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COMPARATIVE EFFECT OF AZO DYE AR-97 ON HATCHLINGS OF *LABEO ROHITA* AND *CIRRHINUS MRIGALA*

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ABSTRACT
When hatchlings were exposed after third day of hatching, 96h LC_{50} of AR-97 was worked out to be 49.85 mg L^{-1} and 93.22 mg L^{-1} for *L. rohita* and *C. mrigala* respectively. In higher concentration, hatchlings showed behavioral changes like restlessness, erratic movement, lateral swimming and hanging intensity. Percentage occurrence of abnormalities in hatchings increased markedly with an increasing concentration of the dye in both *L. rohita* and *C. mrigala*. Hatchlings of *L. rohita* were found to be more sensitive to dye as compared to *C. mrigala*. Mortality in hatchlings resulted from curving of body, fin folds, blockage of gills by precipitation of mucus and deposition of dye on fins. Percentage mortality was higher in *L. rohita* as compared to *C. mrigala*. SAR shows that dye is not safe for disposal without treatment.

INTRODUCTION

Azo dyes are important colorants having extensive application in textile, paper, leather, food stuff and cosmetic industries. Azo dyes are characterized by aromatic ring linked together by one or more azo bonds (-N=N-) and may contain many different substitutes such as sulfonic (-SO_3H), chloro (-Cl), methyl (-CH_3), nitro (-NO_2), amino (-NH_2), hydroxyl (-OH) and carboxyl (-COOH) group. Due to structural stability of azo dyes, these are less susceptible to oxidative catabolism and difficult to biodegrade. Azo dyes are highly soluble in water and persistent, once discharged in the natural environment (Bhullar and Sud, 2012). Azo dyes account for 70% of synthetic dyes used in textile and dyeing industries (Carliell *et al*., 1998). The dye manufacturing, food processing, printing, pharmaceutical, leather and textile industries produce a large volume of effluents. These effluents contain about fifteen percent of the total dyes used in the process and other products such as starch, surfactants, dispersants, oil, emulsifiers, caustic soda, antifoam etc (Tan *et al*., 2000). The release of dyes and untreated effluent from these industries in water bodies is of great concern because these are toxic to flora and fauna of water bodies. Fish being at the highest trophic level of aquatic food chain is maximally afflicted with azo dyes (Rana and Raizada, 1999). This in turn poses a threat to human beings on consumption. Dyes and untreated effluents are also mutagenic and carcinogenic (Khanna and Das, 1991). In addition, very low concentration of dye (less than 1 mg L^{-1}) can be highly visible in solution and interfere with penetration of light (Sloker and Le Marechal, 1998). Aniline, toulidine, benzidine, naphthalene are the cleavage products and impurities of azo dyes.

Acid Red (AR) - 97 dye is used for dying wool, nylon, silk, jute fiber, paper and leather. The present study was undertaken to compare the effect of dye AR-97 on hatchlings of *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.) as no report is available on the toxicity of AR-97. The objective of study was the determination of minimum lethal doses of dye and its effects on developing fishes which provides a sensitive measure to know the toxicity of dye and help in determining the levels of dye safe for disposal in natural aquifers. This study will provide a preliminary data on toxicity AR-97 for developing a decolorization and detoxification protocol for AR-97 before releasing in water bodies.

MATERIALS AND METHOD

Azo dye AR-97 used for this experiment was purchased from Punjab Rang Udhyog, a dye manufacturing unit, Amritsar, Punjab. This dye was selected for the present work because it is manufactured and used at large scale in textile industries. *L. rohita* and *C. mrigala* were used as test organism to estimate the toxicity of dye because both are important food fishes and widely found in India. Hatchlings of these fishes (sensitive developmental stage) were collected from Govt. Fish Farm Rajasansi, Amritsar. These were transported to laboratory in oxygen filled polythene bags. Hatchlings were exposed to dye after third day of hatching (after absorption of yolk sac).

For acute toxicity test, 10 hatchlings were exposed to various

concentrations of dye in 1 liter jars along with two replicate. Tap water was used as control and diluent after dechlorination. Experimental water was renewed after every 24 hours. Mean room temperature was 30 ± 3ºC. Mortality was recorded after 24, 48 and 96h of exposure. Behavior of hatchlings was also recorded. Dead hatchlings were removed immediately from jar to avoid asphyxiation of other test organisms.

The toxicity of dye was evaluated with the help of static bioassay tests for 96h, according to method recommended by American Public Health Association (APHA, 1985). The concentrations at which 50% of test animals were able to survive (96h LC_{50}) were calculated by subjecting the data to probit analysis (Finney, 1971). Safe application rate (SAR) was calculated by using following formula given by Basak and Konar (1977):

Safe Application Rate = $LC_0 \times LC_{50} / LC_{100}$

RESULTS AND DISCUSSION

The 96h LC_{50} value along with 95% fiducial limits, SAR, regression equation and statistical values for hatchlings of *L. rohita* and *C. mrigala* exposed to AR-97 are given in Table 1. Percentage mortalities after 96h for *L. rohita* and *C. mrigala* are represented in Fig. 1. The 96h LC_{50} value for hatchlings of *L. rohita* exposed to AR-97 after third day of hatching was 49.85 mg L^{-1}. For *L. rohita*, 100% mortality was found in 90 mg L^{-1} concentration of dye after 96 hours of exposure. In all the concentrations above 90 mg L^{-1}, all hatchlings of *L. rohita* died within 72 hours of exposure. 100% survival was found up to 4 mg/L^{-1} concentration of dye. The 96 hours LC_{50} value for hatchlings of *C. mrigala* exposed to dye after third day of hatching was 93.22 mg L^{-1}. For *C. mrigala*, 100% mortality was found in 140 mg L^{-1} and 100% survival up to 35 mg L^{-1} concentrations of dye.

The toxicity of AR-97 may be attributed to its rapid uptake and accumulation by the tissues. Anderson *et al.* (1974) reported that entry of naphthalene compound and the subsequent histopathological disturbances in the sensitive, metabolically active tissue like liver produces a metabolic stress. Tonogai *et al.* (1980) also revealed the acute toxicity of organic nitrogenous compounds and dyes to fish after their passage through cell membrane of fish and accumulation in the body. Percentage mortality in both *L. rohita* and *C. mrigala* was found

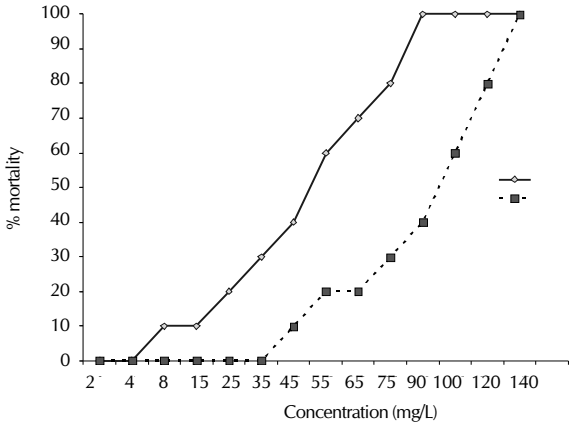

Figure 1. Percentage mortality after 96h at various concentrations of dye for hatching *L. rohita* and *C. mrigala*

to be increased with increasing concentration of dye and time of exposure. Wright (1976) evaluated the mortality rate of malachite green exposed eggs and fry of largemouth bass, *Micropterus salmonides* and found that two-fold increase in malachite green concentration resulted in more than 20 times increase in mortality rate of eggs and fries. Ugwu *et al.* 2011 also found an increase in mortality in fingerlings of *Clarias gariepinus* with increasing hours of exposure.

In higher concentration of dye, hatchlings of both the fishes showed behavioral changes like restlessness, jerky movements, lateral swimming and finally the hanging intensity. Raj *et al.* (1987) reported similar behavioral changes in of the catfish, *Mystus keletius* on exposure to distillery effluent and textile mill effluent. Pathan *et al.* (2009) also observed same behavior in *Rusbora daniconius* exposed to paper mill effluent for 24, 48, 72 and 96h. Behavioral parameters can serve as good biomarkers of pollution even without scarification of animals (Shukla and Tripahi, 2010). These symptoms may have been the consequences of failure of lateral acuistic system of neuromast system as reported by Verma *et al.* (1978) in *L. rohita*, *Notopterus notopterus*, *Colisa fasciatus* and *Saccobranchus fossilis* on exposure to textile waste. Percentage occurrence of behavioral changes was more in *L. rohita* as compared to *C. mrigala*.

Hatchlings that died showed curving of body and tail, fin-fold (resulting in lack of balance), opened mouth (indicating difficulties in breathing) and deposition of dye on fins. Curvature of the body axis is due to damage in the vertebral column (Jezierska *et al.*, 2000). Azo dyes and their precursors are well known carcinogens and mutagens (Khanna and Das, 1991). Therefore it leads to development of such abnormalities in the hatchlings of both the fishes in present study. *L. rohita* hatchings started showing abnormalities in and above 4 mg/L concentration of dye while *C. mrigala* hatchings showed abnormalities in and above 35 mg L^{-1} concentrations of dye. Percentage occurrence of abnormalities increased with increasing concentration of the dye for both the test fishes. A fall in fish respiration due to stress on exposure to AR-97 may also be responsible for the mortality as we observed dye deposition on body and gills of hatchlings. Similarly Dange and Masurekar (1984) have reported a fall in fish respiration on exposure to naphthalene. Fall in fish respiration resulted in respiratory distress in hatchlings of *L. rohita* and *C. mrigala*. Ross *et al.* (1985) observed respiratory distress in rainbow trout, *Oncorhynchus mykis* on exposure of malachite green. Due to stress, hatchlings secrete mucus that cause blockage of gills. Blockage of gills because of precipitation of mucus leading to a decrease in respiratory surface area may be the reason for observed mortality (Verma *et al.*, 1978). It seems that AR-97 has high affinity to gills which is responsible to depressing the function of gill. This make fish suffer from anoxemia as indicated by opened mouth of died hatclings. Tonogai *et al.* (1980) revealed that fish suffer from anoxemia and die due to depression of gill function when exposed to methylene blue and rose bengale.

In present experiment, 96 hours LC_{50} value of dye was lower for *L. rohita* as compared to *C. mrigala*. Percentage occurrence of behavioral changes and mortality was higher in hatchlings of *L. rohita* than *C. mrigala*. This clearly indicates that *L. rohita*

Table1: Statistical values of 96h static bioassay for hatchlings of *L. rohita* and *C. mrigala*.

Hatchlings	LC_{50} (mg/L^{-1})	Variance	S.E.#	Fiducial Range		χ^2	SAR*	Regression equation
				Upper	Lower			
L. rohita	49.85	0.0143	0.1196	85.76	29.12	0.2095	0.5864	Y = 2.010X + 1.58
C.mrigala	93.22	0.0067	0.0819	134.94	63.65	0.2224	23.305	Y = 4.403X-3.672

SE#- Standard Error; SAR*- Safe Application Rate

is more sensitive than *C. mrigala* to dye AR-97. These results are supported by findings of Mahmood (2003) who reported *L. rohita* to be more sensitive than *C. mrigala* to lead. Kumar and Gupta (2006) also found higher sensitivity of *L. rohita* as compared to *C. mrigala* when exposed to mercury. SAR of dye was 0.5864 mg L^{-1} for *L. rohita* and 23.305 mg L^{-1} for *C. mrigala*. This shows that dye is not safe for disposal in the concentrations higher than calculated SAR. So, our study indicates the need for development of relevant techniques to make of dye decolorized and safe for disposal in aquifer.

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