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Acute toxicity study of Paraquat to *Clarias batrachus* (Linnaeus, 1758) at two different selected level of pH

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ABSTRACT

The aim of present investigation was to screen the acute toxicity of paraquat to the Clarias batrachus for 96 hours at two different selected level of pH (5.6 ±0.3 and 7.6 ±0.3) to determine the LC_{50} values for different concentrations by Probit analysis statistical method. However, the presumable safe concentration was also noticed and found to be too high as compared to safe dischargeable concentrations. The changes in behavioural pattern viz; imbalance and whirling movement, erratic swimming, vertical hanging for sometimes, reddish colour of the gills, abnormal opercular movement etc. were also noticed in the studied fishes during bioassay test. The LC_{50} values for two different selected pH were correlated and were found to be significant at 0.05 levels. Oneway ANOVA was also performed between Median lethal concentrations (LC_{50}'s) of Paraquat for 24, 48, 72 and 96 hrs to Clarias batrachus at both the pH of 5.6 ±0.3 (Group-1) and 7.6 ±0.3 (Group-2). Results exhibit that paraquat are highly toxic to freshwater fishes, Clarias batrachus since their LC_{50} values were noticed in ppm. This study has great importance from agricultural point of view since it helps to manage the aquatic animals, particularly fishes.

Key words: Toxicity, LC_{50} values, paraquat and *Clarias batrachus* .

INTRODUCTION

The intensive use of pesticides in agriculture and public health programme causes disturbance in the ecological balance of many non target organisms, particularly fishes [1].Since the ultimate sinks for agricultural and industrial pollutants are the aquatic bodies, therefore it has suffered from a global environmental problem, now a day [2]. Paraquat (N,N-dimethyl 4, 4-bipyridinium dichloride) is second widely used herbicides in agricultural system for weed control worldwide [3]. It is applied in cotton, sunflowers, soybeans, beans, potatoes and sugarcane field as a defoliant and desiccant [4]. It enters into aquatic ecosystem through rain water, and accumulated in aquatic animals, especially in fish [5].However, it has been reported that moderate sensitivity exhibited by fresh water fish species due to acute toxicity of paraquat dichloride [6]. It was found that paraquat is not bioconcentrated by aquatic animals and is assimilated quickly by plants or adsorbed to particulate matter in the water column [7, 8]. It has been found that indirect fish death may also take place by anoxia and is caused due to consumption of dissolved oxygen by

decaying weeds [9].There are many economically important plants and animals may be affected by using herbicides during aquaculture [8].

Therefore, looking to the environmental problems arises due Paraquat, the present investigation was aimed to screen the acute toxicity of paraquat to the *Clarias batrachus* at two different selected level of pH (5.6 ±0.2 and 7.6 ±0.2) to determine the LC_{50} values for different concentrations and time interval (for 96 hours).

MATERIALS AND METHODS

Experimental Fish-The experimental fish, *Clarias batrachus* were collected from local sources, were acclimatized separately in plastic tank of 250 liters capacity for 10 days and proper food supplied regularly. Healthy fishes of approximately equal sizes (10.80 ±1.20 cm) were selected for the bioassay tests.

Stock Preparation-Stock solution were prepared for Paraquat by using formula of $N_1V_1 = N_2V_2$, Where, N_1 = Concentration of available pesticide, V_1 = Volume of available pesticide, N_2 = Required concentration of pesticide to be prepared, V_2 = Volume of solution required for application. The solutions of different concentrations (in ppm) of selected pesticides were made by adding the stock solution into the measured diluents water with the help of micropipette. The series of different concentrations of Paraquat applied in the full scale static bioassay tests were based on the progressive bisection of intervals on a logarithmic scales [10].The static bioassay test (up to 96 hrs) for Paraquat to the experimental fish, *Clarias batrachus* were conducted separately in test container with 5 liter water capacity containing experimental water of two different selected pH of 5.6 ±0.3 and 7.6 ±0.3. The experimental water for two different pH were prepared by using solution of HCl/NaOH with the help of micropipette.

Preliminary or Screening Tests - The test range of Paraquat was taken between the highest and lowest concentrations at which most of the test fishes died or survived within a specified period of exposure, i.e. 24, 48, 72 and 96 hrs, was the basis for the full scale bioassay.

Full Scale Bioassay Test- It is based on test range of Paraquat obtained during preliminary exploratory test. The test container with 5 litre capacity, filled with 4 litre toxicant solution were placed in three rows and each container was labelled with the details of the experiment *viz*; date and time of the experiment, concentration, replicate number. The acclimatized experimental fish, *Clarias batrachus* of approximately equal sizes were transferred to these containers after about 30 minutes of the preparation of test solutions. The bioassays test for *Clarias batrachus* were carriod out for selected pesticides separately for both the selected pH. There are 10 acclimatized test fishes were taken in each experimental test containers and proper controls were run simultaneously. The test solutions were renewed and replaced after each 24 hrs by fresh toxicant solutions and the experiments were continued for a period of 96 hrs. The number of test fishes died in each concentration of toxicant solution were observed carefully and noticed at the time intervals of 24, 48, 72 and 96 hrs. The dead fishes were removed from the test solution regularly after knowing their exact mortality. The LC_{50} values and the 95 per cent confidence limits were calculated at different concentration and time intervals (24, 48, 72 and 96 hrs) for selected pesticides by Probit Analysis methods [11]. Whereas, Presumable safe/harmless and safe dischargeable concentrations of Paraquat for studied fish were calculated by using the formula of Hart *et al*, [12]. Any changes in behavioural response in the studied fishes were also noticed carefully during the course of bioassay.

RESULTS AND DISCUSSION

Median lethal concentrations (LC_{50}'s) of Paraquat to *Clarias batrachus* for 24, 48, 72 and 96 hrs at pH of 5.6 ±0.3 were noticed as 325.731, 290.152, 259.328 and 251.641 ppm respectively (Table -1),whereas these values were recorded as 437.064, 381.892, 351.788 and 325.424 ppm respectively at pH of 7.6±0.3 (Table -2).The safe or harmless and safe dischargeable concentrations of Paraquat were estimated as 77.562 and 1.059 ppm respectively at pH of 5.6 ±0.3 and; 100.146 and 1.069 ppm respectively at pH of 7.6±0.3 (Table -3). The safe concentration was reported too high as compared to safe dischargeable concentrations at both selected level of pH values (Table -3). The alteration in behavioural response such as erratic swimming, imbalance and whirling movement, vertical hanging for sometimes, reddish colour of the gills, abnormal opercular movement etc. were also noticed in the experimental fishes during bioassay test.The upper confidence limits, lower confidence limits and their confidence ratio (UCL/LCL) was also calculated for LC_{50}'s of Paraquat at both selected level of pH values and summarized in Table -1 and 2. T he LC_{50} values at both the selected level of pH were correlated and found to be significant at 0.05

levels. Oneway ANOVA was also performed between LC_{50} values of Paraquat for 24, 48, 72 and 96 hrs to *Clarias batrachus* at both the pH levels of 5.6 ±0.3 (Group-1) and 7.6 ±0.3 (Group-2) and SS, DF, MS and F values were calculated to compared it (Table-4).

Table 1: Median lethal concentrations (LC_{50}'s) of Paraquat (in ppm) for 24, 48, 72 and 96 hrs to *Clarias batrachus* at pH of 5.6 ±0.3

Duration (hrs)	LC_{50}'s of Paraquat (Group-1) (ppm)	UCL	LCL	R
24	325.731	417.643	288.124	1.449
48	290.152	341.086	247.113	1.380
72	259.328	289.226	217.036	1.332
96	251.641	277.074	215.118	1.288

UCL = Upper Confidence Limits; LCL = Lower Confidence limits; and
R = Confidence Ratio (UCL/LCL)

Table 2: Median lethal concentrations (LC_{50}'s) of Paraquat (in ppm) for 24, 48, 72 and 96 hrs to *Clarias batrachus* at pH of 7.6±0.3

Duration (hrs)	LC_{50}'s of Paraquat (Group-2) (ppm)	UCL	LCL	R
24	437.064	564.141	388.828	1.450
48	381.892	431.669	337.141	1.280
72	351.788	387.644	307.016	1.262
96	325.424	353.849	285.196	1.240

UCL = Upper Confidence Limits; LCL = Lower Confidence limits; and
R = Confidence Ratio (UCL/LCL)

Table 3: Safe or harmless and safe dischargeable concentrations of Paraquat (in ppm) for *Clarias batrachus* at pH of 5.6 ±0.3 and 7.6±0.3

Concentrations	pH 5.6±0.3	pH 7.6±0.3
Safe or harmless (as ppm)	77.562	100.146
Safe dischargeable (as ppm)	1.059	1.069

Table 4: Oneway ANOVA between Median lethal concentrations (LC_{50}'s) of Paraquat (in ppm) for 24, 48, 72 and 96 hrs to *Clarias batrachus* at both the pH of 5.6±0.3 (Group-1) and 7.6±0.3 (Group-2)

Source of Variance	Sum of Squares (SS)	DF	Mean Square (MS)	F
Between Groups	6892.345	3	2297.448	94.697
Within Groups	.000	0		
Total	6892.345	3		

Results indicates that Paraquat is highly toxic to the *Clarias batrachus* as their 96 hours LC_{50} values were noticed 251.641 and 325.424 ppm at both the selected level of pH. It has been reported that Paraquat is generally more toxic to early developmental stages than to juveniles or adults and its toxicity based upon formulations (more toxic in formulations with wetting agents as compared to without it) and also more toxicity was noticed to aquatic fauna in soft water as compared to hard water [8].It was also documented that indirect fish death may also occur due to anoxia, later is may be caused by consumption of dissolved oxygen by decaying weeds [9]. However, there are 1 mg/L concentration of parquat seem to be improve the oxygen concentration in aquatic ecosystem because it inhibit nitrate production due to restriction of bacterial nitrification [13, 14]. According to Haley [15] paraquat was found to be toxic to eggs of three species of snail vectors of bilharzias viz; *Bulinus truncatas, Biomphalaria alexandrina, Lymnaea calliaudi*, but not to their adults at effective herbicidal concentrations. Whereas, newly hatched snails were found to be most sensitive. Further, Seiyaboh *et al*. [16] conducted a comparative study of the acute toxicity of paraquat dichloride on blood plasma indices of *Clarias gariepinus* and reported the increased values of haemoglobin. PCV, WBC and platelets with increasing concentration of the toxicant. It has been reported that paraquat exposure might produces adverse effect on haematological parameters of fish which leads to anemia, the later may affect normal growth, reproduction, immunity and survival of fish in natural environment and also during aquaculture conditions [17]. Results of the present investigation are also in support of previous researchers in terms of LC_{50} values and; alteration in behavioural responses *viz*; [18, 19, 20].

However, Banaee *et al*. [21] have studied the effect of sub-lethal toxicity of paraquat on the pathology of gill, liver, and spleen tissues in gourami fish (*Trichogaster trichopterus*) and also determined the LC_{50} values as 7.16±0.69, 4.46±0.43, 2.19±0.27 and 1.41±0.17 mg/l of paraquat for 24, 48, 72 and 96 hours, respectively. These authors noticed the behaviour of exposed fish as erratic swimming and became lethargic. Further, alteration in in gills structure were also reported such as hypertrophy, increased epithelium of gill filament, edema and secondary gill lamella by these authors. It has been noticed that the hepatosomatic index was decreasing with increasing

concentration of paraquat for 24 hours exposure on nile Tilapia (*Oreochromis niloticus*) and also air gulping and erratic swimming, molting, loss of reflex, haemorrhage and loss of scales were observed on exposure of this herbicides [20]. The cell proliferation, lamellar fusion, lamellar cell hyperplasia, and epithelial lifting vacuolation of hepatocytes and necrosis in liver and; respiratory stress, erratic swimming and instant death of fish were observed in African Catfish (*Clarias gariepinus*) juvenile on 96 h (LC50 value of was 1.75mg/l) of exposure of Paraquat dichloride [19]. It has been also reported that the behaviour and morphology of *Clarias gariepinus* were affected on the 96 hours exposure of paraquat, LC_{50} value was recorded as 27.46 mg/L [22]. Further, a significant decreases (P<0.05) in the mean values of hemoglobin, red blood cells, packed cell volume and cellular hemoglobin concentration whereas, the levels of WBC, glucose, aspartate aminotransferase, and alanine aminotransferase significantly increased (P<0.05) with decline in protein levels, has been noticed [22]. However, various literature cited by prominent researcher in this field, such as [23, 24, 25, 26, 27, 28], are also important in terms of toxic effect of herbicides and should be considered from aquaculture point of view.

From present investigation, it is clear that Paraquat is highly toxic to the *Clarias batrachus*, therefore it is suggested to the users, be careful regarding concentration of this herbicides while using it in both natural as well as artificial aquatic environment in order to control weeds.

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