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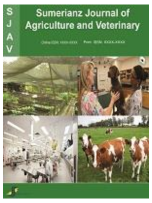

Original Article Open Access

Behavioural Responses and Mortality of *Clarias gariepinus* Juveniles Exposed to Acute Concentrations of Paraquat

Okey I. B.*
Department of Fisheries and Aquatic Science, Cross River University of Technology, Obubra campus, Cross River Nigeria
Email: piusbass@yahoo.com

Ayotunde E. O.
Department of Fisheries and Aquatic Science, Cross River University of Technology, Obubra campus, Cross River Nigeria

Patrick B. U.
Department of Fisheries and Aquatic Science, Cross River University of Technology, Obubra campus, Cross River Nigeria

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Abstract

Paraquat is the most common contact and non- selective herbicide for exterminating vegetative pest. Fish are ideal sentinels for detecting aquatic pollutants and are largely used as bio indicators of environmental pollution. This study is aimed to determine the behavioural changes, lethal concentrations (LCs) and mean lethal time (MLT) of paraquat exposed to *Clarias gariepinus*. A 96 hours renewable bioassay was conducted with various paraquat concentrations 0.0, 0.25, 0.50, 0.75, 1.0 and 1.25 mg/l. Behavioural changes and cumulative mortality were observed and recorded at 12, 24, 48, 72 and 96 hour. Behavioural changes such as air gulping, erratic swimming, loss of balance, excessive mucus secretion, discolouration and death were observed with severity increasing as concentration and duration of exposure increases. The LC_{50} values were decreased from 0.191mg/l (0.171 – 0.222) in 12 hour to 0.107mg/l (0.065 – 0.150) in 96 hour, while relative toxicity factor (TF) was increased from 1 to 1.79 times respectively. The 96hr MLT values were decreased from 91.18 hours (54.09-105.64) at the lowest concentration to 16.22 hours (9.06 – 25.15) at the highest concentration with relative toxicity time (RTT) increasing from 1 to 5.62 times. Herbicide should be apply with caution and studies on the sub lethal effects of paraquat on the haematological, biochemical and histological parameters of *C. gariepinus* juveniles will be necessary.

Keywords: *Clarias gariepinus*; Paraquat; Behavioural changes; Lethal concentration; Lethal time.

1. Introduction

Chemicals of agricultural or industrial origins have been reported to be source of contaminations to aquatic ecosystems by runoff and ground leaching through the area [1]. The use of herbicides in agricultural practices is on the increase, thereby causing ecological imbalance due to damage to non- target organisms [2]. According to Food and Agricultural Organisation FAO [3], approximately three million people are poisoned and 200,000 die each year around the world from pesticide poisoning most of which are from developing countries. Paraquat (1, 1-dimethyl-4, 4-bipyridinium dichloride) is one of the most common contact and non-selective herbicide for exterminating vegetative pests. It is used for controlling terrestrial weeds and aquatic plants in different countries and its presence is reported in many water bodies of the world [4, 5]. Due to availability, affordability and efficiency, its utilization has increased in recent years in Nigeria especially among the rural farmers. Although herbicides are designed to control plant pests by inhibiting photosynthesis but significantly large concentrations can be toxic to animal through necrosis [6]. Direct and indirect contamination of aquatic environment with pesticides may cause fish kills, reduce fish productivity and elevates concentration of undesirable chemicals in edible fish tissues [7]. It is imperative to find out the detrimental effects of pollutants especially herbicides on fish since they form important food chain. Fish are vital indicators of the effects of toxic compounds in aquatic toxicity [8], and are also important source of protein and essential fatty acids requirement in human diet. Fish are ideal sentinels for detecting and documenting aquatic pollutants and largely used as bio-indicators of environmental pollution, due to their ability to retain different dissolved xenobiotics that build up in the food chain whose continual presence in the aquatic environment can culminate in the death of aquatic organisms [9].

Behaviour provides a unique perspective linking the physiology and ecology of an organism and its environment and allows the organism to adjust to external and internal stimuli to best meet the challenge of surviving in a changing environment [10]. Knowledge of acute toxicity of xenobiotics can be very helpful in predicting and preventing damage to aquatic life receiving waters as well as in regulating toxic waste discharge [11]. Many toxicants have been reported to alter the behaviour and physiology of aquatic organisms [12]. Several researchers have reported the following behavioural changes; air gulping, stunned positioning, skin peeling, aggression, excessive mucus secretion, motionless, respiratory distress, erratic swimming (fast and spiral movement) and death

*Corresponding Author

when they exposed *C. gariepinus* to acute concentrations of various xenobiotics [13-17]. *Clarias gariepinus* commonly known as African catfish belong to the family claridae geographically located in Africa, Brazil and Indonesia. They are highly esteemed group of fishes with popular delicacy relish throughout Africa and most cultured fish species due to its fast growth rate, high market value and can tolerate difficult aquatic condition [18]. This study investigated the acute toxicity of paraquat on *C. gariepinus* juveniles using behavioural characteristics and mortality.

2. Materials and Methods

Experimental fish: Two hundred (200) apparently healthy juveniles of *C. gariepinus* were bought from University of Calabar fish farm and transported to the wet laboratory of Fisheries Department CRUTECH, Obubra campus. The mean body weight and the length of the species were 38.26±1.20 (g) and 17.50±1.55 (cm) respectively. They were acclimated for 14 days during which they were fed with Coppen feed at 3% body weight twice daily and terminated 24 hours prior to the commencement of the experiment [19].

Chemical: Paraquat (1, 1-dimethyl-4, 4-bipyridinium dichloride) was procured from a local agro- chemical dealer shop at Ofatura – Adun, Obubra.

Acute toxicity bioassays were conducted in the Wet laboratory of the Department of Fisheries and Aquatic Science for a period of 96hrs. During acute bioassay 180 juveniles were introduced into 18 plastic tanks of 60 liters capacity each containing 20 liters of water and 10 fish in triplicates. The fish were randomly selected and stocked in the tanks containing the following concentration 0.25, 0.50, 0.75, 1.0, 1.25mg/l. The concentrations were obtained from a serial dilution of the stock solution of 200mg/l (2mL of paraquat in 10 litres liters of tap water). The test solution was not change (static) throughout the duration of the experiment (96hours). The behavioural changes such as erratic movement, air gulping, hyperactivity, loss of balance, excessive mucus secretion, discolouration, motionless and mortality were observed and recorded. The changes were ranked as, no visible sign (-), weak (+), moderate (++) and severe (+++) in 12, 24, 48, 72 and 96 hours. Dead fish was removed immediately to avoid contamination of the test solutions. Some water quality parameters such as dissolved oxygen, temperature, pH, conductivity and hardness were also monitored and recorded using the appropriate instrument and standard methods [20].

2.1. Statistical Analysis

Data obtained from the water quality parameters were subjected to simple descriptive statistic to determine the range and mean using SPSS version 20. Mortality data was used to determine the lethal concentrations (LCs) and mean lethal time (MLTs) values with their associated confidence limits using probit analysis method described by Finney [21].

3. Result

3.1. Water Quality Parameter of the Test Solutions

The result of the physicochemical parameters of the test solutions is presented in table 1. The result shows that slight changes with the range values of 4.74 to 5.12mg/l, 26.88 to 27.16^{0}C, 7.47 to 7.79, 37.61 to 39.32(mg/l) $CaCO_3$ in dissolved oxygen, temperature, pH and hardness respectively. The mean values of 4.93± 1.50 mg/l, 27.16±0.56^{0}C and 38.05 ± 0.55 (mg/l) $CaCO_3$ were also recorded for dissolved oxygen, temperature and hardness respectively and were within the optimal value required for the normal growth and culture of *C. gariepinus*.

Table-1. Water quality parameters of test solutions

Parameter	**Range**	**Mean ±SD**
Dissolved Oxygen (mg/l)	4.74 – 5.12	4.93 ± 1.50
Temperature (^{0}C)	26.88 – 28.13	27.16 ± 0.56
pH	7.47 – 7.79	6.68 ± 2.60
Conductivity (µS/cm)	165.96 – 167.23	166.15 ± 2.20
Hardness (mg/l) $CaCO_3$	37.61 – 39.32	38.05 ± 0.55

3.2. Behavioural Responses of *C. gariepinus* to Test Concentrations

The result of the behavioural changes observed in fish exposed to the various concentrations of Paraquat is presented in Table 2. The fish were observed to show air gulping, erratic swimming, loss of balance, mucus secretion, hyperventilation, discoloration, motionless, loss of balance and death. However, severity of these changes depends on the level of concentration and exposure time to the Paraquat. None of these abnormal physical changes was observed at the control (0.0mg/l). There was increase in weakness, motionless, discoloration, gulping and mucus secretion as concentration increase with exposure time.

Table-2. Behavioural Patterns displayed by *Clarias gariepinus* Juveniles Exposed to Various Concentrations of Paraquat

Concentration (mg/l)	Behavioural pattern	Duration of exposure (hr)				
		12	24	48	72	96
0.0	Air gasping	-	-	-	-	-
	Erratic swimming	-	-	-	-	-
	Loss of balance	-	-	-	-	-
	Hyperactivity	-	-	-	-	-
	Excessive mucus secretion	-	-	-	-	-
	Skin discolouration	-	-	-	-	-
	Motionless	-	-	-	-	-
	Death	-	-	-	-	-
0.25	Air gasping	-	-	-	+	+
	Erratic swimming	-	-	-	-	+
	Loss of balance	-	-	-	-	-
	Hyperactivity	-	-	-	-	+
	Excessive mucus secretion	-	-	-	-	-
	Skin discolouration	-	-	-	-	-
	Motionless	-	-	-	-	+
	Death	-	-	-	+	+
0.50	Air gasping	-	+	+	++	++
	Erratic swimming	-	+	++	++	+++
	Loss of balance	-	-	+	++	+++
	Hyperactivity	-	+	++	+++	+++
	Excessive mucus secretion	-	-	+	++	++
	Skin discolouration	-	-	-	+	+
	Motionless	-	-	+	++	++
	Death	-	+	++	++	++
0.75	Air gasping	+	++	++	+++	+++
	Erratic swimming	+	+	++	+++	+++
	Loss of balance	-	+	++	+++	+++
	Hyperactivity	+	++	+++	+++	+++
	Excessive mucus secretion	+	+	++	++	++
	Skin discolouration	-	+	++	++	+++
	Motionless	-	-	+	+	++
	Death	+	++	++	+++	+++
1.0	Air gasping	+	++	+++	+++	+++
	Erratic swimming	++	++	++	+++	+++
	Loss of balance	+	++	++	+++	+++
	Hyperactivity	+	++	+++	+++	+++
	Excessive mucus secretion	++	+++	+++	+++	+++
	Skin discolouration	+	++	+++	+++	+++
	Motionless	+	+	++	+++	+++
	Death	+	++	+++	+++	+++
1.25	Air gasping	++	+++	+++	+++	+++
	Erratic swimming	+++	+++	+++	+++	+++
	Loss of balance	+	++	+++	+++	+++
	Hyperactivity	++	+++	+++	+++	+++
	Excessive mucus secretion	+	++	+++	+++	+++
	Skin discolouration	+	+++	+++	+++	+++
	Motionless	+	++	+++	+++	+++
	Death	++	+++	+++	+++	+++

No visible sign (-), weak (+), moderate (++), severe (+++)

3.3. Lethal Concentration (LC) and Mean Lethal Time (MLT) and Associated 95% Confidence Limit

The lethal concentrations (LCs) of paraquat on the fish expressed as percentage cumulative mortality at 10 (LC_{10}), 50 (LC_{50}) and 95% (LC_{95}), safe concentration (that will not kill the fish) and relative toxicity factor (potency of a given concentration) 12, 24, 48, 72 and 96 hours is presented in Table 3. The result revealed that the LC values the respective duration increased within the given time but decrease with increase in duration of exposure. The 12hrLC values increase from 0.092mg/l, to 0.318ml/l for LC_{10} and LC_{95} respectively while the LC_{50} values decrease from 0.191, 0.148, 0.138, 0.125 and 0.107ml/l for 12, 24, 48, 72 and 96hours respectively. While the values of safe concentrations decreased from 0.019mg/l at 12 hours to 0.011mg/l, those of the relative toxicity factor increased from 1 to 1.79 at 12 and 96hours respectively. The later implies that the concentration that will kill 50% of the *C.*

gariepinus juveniles at 96hours (0.107mg/l) was 1.79 times less toxic (lower in concentration) than that of 12hours (0.191ml/l).

The result showed that the values of the 96hrMLT increase within given concentration but decrease as the concentration increases (Table 4). At the lowest concentration (0.25mg/l) of paraquat, 91.18 and 156.43 hours will be required to kill 50 and 95% of *C. gariepinus* juveniles respectively whereas 16.42 and 66.07 hours is required to kill the same percentages of the fish at the highest concentration (1.25mg/l). However this trend decrease with increasing level of concentrations as indicated by the values of the 96hrsMLT$_{50}$ from 91.18 to 16.22 hours in 0.25 and 1.25mg/l respectively in this study. The relative toxicity time (RTT) of 96hrsMLT$_{50}$ increased from 1 to 5.62 at lowest (0.25mg/l) and the highest (1.25mg/l) concentrations respectively. This implies that the lowest concentration (0.25mg/l) is 5.62 times more toxic than the highest concentration (1.25mg/l) at 50% mortality. The smaller the concentration of a substance required to kill a given percentage of fish the more toxic the substance.

Table-3. Lethal concentrations and associated 95% confidence limit of paraquat on *C. gariepinus* juveniles for 96hours

Exposure Time (hr)	**Lethal Concentration at 95% Confidence Limit**			**Safe Concentration.**	**Toxicity Factor**
	LC$_{10}$	**LC$_{50}$**	**LC$_{95}$**		
12	0.092 (0.061– 0.112)	0.191 (0.171– 0.222)	0.318 (0.272– 0.405)	0.019	1
24	0.044 (-0.133– 0.089)	0.148 (0.108– 0.232)	0.281 (0.210– 0.622)	0.015	1.29
48	0.030 (-0.186 – 0.079)	0.138 (0.093– 0.221)	0.276 (0.203– 0.652)	0.014	1.38
72	0.010 (0.245 – 0.064)	0.125 (0.075– 0.201)	0.273 (0.204 – 0.696)	0.013	1.53
96	0.004 (0.161 – 0.045)	0.107 (0.065– 0.150)	0.249 (0.190– 0.451)	0.011	1.79

T.F= Toxicity factor =LC$_{50}$ value at 12hrs ÷ LC$_{50}$ value of any other periods; safe concentration = LC$_{50}$ value ÷ 100

Table-4. Mean lethal time (MLT) and associated 95% confidence limit of *C. gariepinus* juveniles exposed to paraquat for 96hours

Concentration. (mg/l)	**Mean Lethal Time (MLT) and associated 95% Confidence Limit**		**Relative Toxicity Time (RTT)**
	MLT$_{50}$	MLT$_{95}$	
0.25	91.18 (54.09– 105.64)	156.43 (149.13– 206.14)	1
0.50	43.45 (33.19– 144.36)	115.55 (168.64– 275.36)	2.10
0.75	37.13 (21.08– 76.21)	98.18 (61.20 – 129.56)	2.46
1.0	24.17 (13.07– 31.15)	83.26 (61.19– 104.41)	3.77
1.25	16.22 (9.06– 25.15)	66.25 (42.19– 88.45)	5.62

RTT= Toxicity factor =MLT$_{50}$ value at lowest concentration (0.25mg/l) ÷ MLT$_{50}$ value of any other concentration

4. Discussion

The apparently close values of the water quality parameters in this study is an indication that there never contributed to the changes observed in the behaviour of the exposed fish. The values fall within the normal range of water quality for aquaculture [22]. This was in agreement with the studies of Ayanda, *et al.* [11], Hassan, *et al.* [14] and Nwamba, *et al.* [16]. Behavioural patterns are the most sensitive indicators of potential toxic effects in fishes and are frequently use in toxicity test to determine safe lethal concentration of xenobiotics [6, 23]. *Clarias gariepinus* juveniles on the various concentrations of paraquat were stressed progressively with time before death in this study. The stressful behaviour includes loss of balance, erratic swimming, air gulping, sudden quick movement, and excessive secretion of mucus, motionless, discolouration and general hypersensitivity. No such pattern was observed for fish in the control tanks. The observed behavioural pattern in this study are consistent with previous reports with some herbicides [7, 11, 14, 16]. Besch [24] reported that fish exposed to toxicants is characterized by the behavioural phases such as contact (high excitability in a moment), exertion (fast swimming, leaping and attempts to jump out of the toxicant), equilibrium (instability) and death (lack of response to external stimulus). Nwanna, *et al.* [25] also reported that fish exposed to toxicants usually exhibits some behavioral changes such as increased opercular rate, erratic swimming, mucus secretion and gulping for air before death. The stressful behaviour of clariids which tend to show the toxic effect has been reported on exposure to various xenobiotics such as detergent, Dichlorvos, glyphoshate and paraquat respectively [11, 16, 26]. The behavioural score showed stronger with increase the concentration such as aggression, stunted posture, erratic swimming and more frequently move at the bottom. Increased mucus secretion in fish exposed to toxicants is a defensive response by which fish attempts to reduce the entrance of toxicant through the skin and gill surfaces [27]. Behavioural responses of fish to most toxicants and differences in reaction time have been observed to be due to the effect of chemicals, their concentrations, species, size and specific environmental conditions [28]. These stressful behaviours exhibited by the fish before dead may be as a result of respiratory impairment due to active ingredient of the chemical component of the paraquat on the tissues and general metabolism of the exposed fish. Literatures have reported that increase utilization of energy substances (carbohydrate, protein and lipids) when fish is under xenobiotic leads to stress [29, 30]. Fatigue due to exhaustion may have accounted for the death recorded in the exposed fish. The mortality rate was concentration and time dependent, suggesting that the degree of exhaustion due to depleted energy sources may have been raised by increasing the concentration and exposure durations. This finding corroborates the works of several researchers using various toxicants on *C. gariepinus* [16, 31, 32].

The value of 96hrsLC_{50} of 0.107mg/l reported in this study is higher than the 0.07mg/l earlier reported by Ayanda, *et al.* [11], for paraquat but much lower than those reported for dichlorvos (17.21mg/l), glyphosate (0.53mg/l) and diazinon (7.3mg/l) by Nwamba, *et al.* [16], and Ayanda, *et al.* [11] respectively on clariid species. Hassan, *et al.* [14] reported a much higher LC_{50} value of 26.07mg/l of paraquat on common carp, *Cyprinus carpio* indicating they are most tolerant to paraquat than *C. gariepinus*. The lower the LC_{50} value of a toxicant the more toxic the substance. The differences in the LC_{50} values recorded in this study and that of others who use paraquat could be attributed to a number of factors including species, water quality and age of fish. Neibor and Richardson [33] reported that the level of toxicity of any pesticide depends on its bioaccumulation, the different chemistries of the compound forming the pesticide and the reactions of the organisms receiving the toxicant. World Health Organisation, [34] reported that LC_{50} values depend on fish species and the test conditions as well as herbicide formulations. Toxicity of pesticides to organisms is affected by the strains of species, size, age, sex, temperature, water quality and formulation of the test chemicals [35]. The decrease in the LC_{50} values, safe concentrations and increase in the toxicity factor with increase in duration of exposure in this study is in agreement with the reports of others researchers investigating acute toxicity of various chemicals on fishes [16, 32].

The mean lethal time (MLT) values for the species give an insight into the pattern of death of fishes with duration of exposure under acute toxicity of toxicants. The decline in 96hrMLT_{50} values and increase in the relative toxicity time with increasing concentrations of paraquat was in conformity with the works several researchers [32, 36]. This implies that increase in concentration will decrease the time require to kill 50% of the juveniles which will however increase within a given concentration. Mortality is therefore affected by both concentration and duration of exposure.

5. Conclusion

The study demonstrated that paraquat showed some changes on the behavioural pattern of *C. gariepinus* juveniles. Stressful behaviour pattern showed severity with increasing concentration with no sign of abnormality displayed by fish in the control tanks. The low 96hrLC_{50} value (0.107mg/l) is an indication that paraquat is highly toxic to *C. gariepinus* juveniles. Whereas the safe concentrations were decreasing the toxicity factor was increasing with duration of exposure. The 96hrMLT_{50} for the various concentration decreases while the relative toxicity time (RTT) increase with increasing concentration of paraquat. Studies on the sublethal effects of paraquat on the haematology, biochemistry and histology on *C. gariepinus* juveniles will be necessary.

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