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Behavioral Responses of African Freshwater Fish Clarias gariepinus to Different Concentrations of a Commercial Herbicide, 'Orizoplus' (Propanil/2,4-D)

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Abstract
The study was conducted to assess the behavioural responses of freshwater fish *Clarias gariepinus* subjected to different concentrations of a commercial herbicide, "Orizoplus (Propanil/2,4-D).The juveniles (20.3 ± 23.6g) were purchased from local sources and acclimatized for two weeks under laboratory conditions. During the acute toxicity bioassay, behavioural responses of fish such as convulsions, fin movement, hyperactivity, somersaulting activity and erratic swimming rate were observed in exposed groups whereas the control group maintained a normal behaviour. The physicochemical properties measured in the test solutions showed that water temperature varied slightly while PH was constant. Dissolved oxygen, conductivity and total dissolved solids showed some variations ranging from 2,78 ±6.08 mg/l, 49.2±59.6 µmhos, and 24.3 ±26.5 respectively. The fish in the highest concentration 140mg/l showed 100% mortality while the fish in the lower concentrations of 80mg/l and 60mg/l showed 0% mortality indicating that stress and eventual death of the fish is concentration dependent.
Keywords: *Clarias gariepinus*, Herbicide, Behavioral responses,Toxicity.

1 Introduction

One of the important factors contaminating the natural habitat is agricultural pesticides. Prior to the widespread use of chemical herbicides commonly known as weed killers, cultural controls such as mechanical control (including tillage and weeding) altering soil Ph, salinity or fertility levels were used to control weeds. However, with improved biotechnology the use of herbicides to control weeds has been recognized worldwide as part of agricultural practices to improve agricultural production. Unfortunately, the indiscriminate use of these herbicides, careless handling, accidental spillage or discharge of untreated effluents into natural water ways have harmful effects on the fish populations and other aquatic organisms and may contribute to long term effects in the environment (Nwani *et al.* 2010a). These agents used against pests, undesirable herbs and agricultural diseases were found to have adverse effects on the environment. As herbicides kill weeds so they can kill human beings. The world health organization (WHO 1992) reported that roughly 3 million cases of pesticides poisoning occur annually, resulting in 220,000 deaths worldwide. Many of these chemicals are mutagenic (Garaj-vrhovac and Zeljezic 2000; Kumar *et al.* 2009;Nwani *et al*. 2010b), linked to the development of cancers (Leiss and Savitz 1995) and may lead to developmental defects. The contamination of aquatic ecosystem by xenobiotics has gained increasing attention and several recent studies have demonstrated toxicity effects to fish under field and laboratory conditions (Abdul Farah *et al*. 2004; Pandy *et al*. 2005; Costa *et al*. 2008; Ayoola, 2008; Langiano, 2009; Kurma *et al*. 2009; Lushchak *et al*. 2009, Nwani *et al*. 2011). Chronic exposure and accumulation of these xenobiotics by aquatic biota can result in biochemical and tissue burdens that produce adverse effects not only on the exposed organisms but also in other organisms including human beings (IARC, 1993). Due to high water solubility, low persistence and extensive usage of these herbicides in the environment, exposure to non- target organisms is a source of concern. Therefore it is essential to study the lethal toxicity and stress of such environmental pollutants so as to formulate the strategies for safe guarding aquatic organisms. Neskovic *et al*. (1993) has shown that sub-lethal effects may occur at concentrations less than 2mgl-1 of attrazine during long term exposure with genotoxic, biochemical and histopathological alterations of fish tissues. Fish are directly exposed to aquatic pollutants and their behavioural responses are the most sensitive indication of potential toxic effects. Following these discoveries, all commercially – sold herbicides must be extensively tested prior to approval for sale and labelling by Environment protection agency. Due to the large number of herbicides in use, there is significant concern regarding their health effects. As a result of knowledge gaps in our understanding of ecological significance of genotoxicants in aquatic environment, there is a lack of consensus for an acceptable comprehensive decision-making framework, which could establish the roles of science and policy in formulating environment management principles, (Nagpure, 2007).

2. MATERIALS AND METHODS

2.1 Experimental Fish and Herbicides

300 juveniles (about 20g of Fresh water fish, *C. gariepinus* (Family: Clarridae) were purchased from local sources. The specimen were weighed in grams(g) using electronic weighing balance and the length in

centimetre(cm) determined using meter rule. The fishes were then acclimatized for two weeks under laboratory conditions. The fish samples were fed on a commercial pellet diet (3% of the body weight per day). The fecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. The herbicides-Orizoplus containing 360g propanil and 200g 2,4-D per litre manufactured by Proficol- Columbia and distributed by the Candel Company Limited 1koyi – Lagos, Nigeria, , a liquid herbicide for selective weed control was bought from the local market and used in the research.

2.2 Range Finding Test:
The range finding test was carried out prior to the definitive test to determine the concentration of the test solutions. The test water with the different herbicide concentrations were changed after every 48hr by replacing with fresh Orizoplus, solutions in order to counterbalance decreasing herbicide concentrations. Five concentrations of the herbicide was prepared from the original solution using the formula described by Solbe (1995). The concentrations of the trial test were prepared by pipetting different volumes of the original concentration of the herbicide into 10 L of water in five static tanks at a time to make five different solutions. The purpose of the trial test was to determine the range of concentration to be used for the definitive test.

2.3 Definitive Test
Based on the range finding test five concentrations (140, 120, 100, 80, and 60mg/l) of orizoplus were prepared and tested on the *Clarias gariepinus* juveniles for the definitive test. In the definitive test a set of ten acclimated fish specimens were randomly exposed to each static tank containing the different concentrations of Orizoplus. The experiments were set in triplicate. The fish specimens were exposed to the aforementioned test concentrations of Orizoplus in semi-static systems with the change of test water after every 48hr of exposure. The exposure was continued up to 96 hrs. Some fishes were maintained in tap water to serve as negative control. The test media was changed every 24hours. This was done in an attempt to maintain a constant concentration of the test media to which the animals were exposed and to prevent excessive accumulation of toxic metabolites

2.4 Determination of the Physicochemical Properties of the Test Water;
The physicochemical characterization of the water used for fish bioassay was carried out using standard methods (APHA, 2005). Water temperature reading was taken by the use of a thermometer and the PH measured using and the pH meter. The total dissolved oxygen level, the conductivity and tota2.3l dissolved solutes of the water were also measured using digital Hanna instruments.

2.5 Behavioural Responses
During the acute toxicity bioassay experiment, behavioral responses of fish such as convulsions, equilibrium status, fin movement, hyperactivity, somersaulting activity and swimming rate were observed in exposed as well as the control group as suggested by Rand (1985).

3. DATA PRESENTATION AND STATISTICAL ANALYSIS
Statistical analysis were performed with the SPSS 17.1 Computer program (SPPS INC. Chicago IL, USA). The patterns of variations (in the physicochemical properties of the test water) due to the exposure time and treatments and their interactions were studied by a one way analysis of variance (ANOVA), p-values less than 0.01 were considered to be statistically significant.

4. RESULTS
4.1 Physicochemical characteristics of the test water
The physicochemical characteristics of the test water are presented in table 1 below. The water temperature varied from 24. 30 to 26.500c. The pH was constant at the value of 9.00. The dissolved oxygen, conductivity and total dissolved solids showed some variations ranging from 2.78 ±6.08 mg/l, 49.2±59.6 µmhos, and 24.3 ±26.5 respectively.

Table1. Physicochemical properties of the Test Water

S/N	Characteristics	Unit	Mean	Range
1	Water Temperature	0C	25.72	24.30-26.50
2	PH	-	9.00	9.00-9.00
3	Dissolved oxygen	mg/l	4.32	2.78-6 .08
4	T. D. S	(µshos/cm)	25.72	24.30-26.50
5	Conductivity	µM/cm	52.20	49.2-54.00

4.2 The behavioural response and the mortality rate of the fish
The fish in the control experiment showed no abnormal behaviour throughout the exposure time and no mortality

was recorded in it. However, at the beginning of the definitive test the fish in the higher concentrations 140 and 120mg/l exhited somersaulting activity, jumping out of the static tank, erratic swimming, swimming upside down and with time settled at the bottom of the tank and all died before the end of the exposure period. In other concentrations the fish were seen to come to the water surface to gulp air, convulse from time to time with high rate fin and opercula movement. Some of the fish died and some survived to the end of the exposure period of 96hrs. Table.7 Shows a summery of the mortality rate of the fish at various concentrations and durations.

Table 2. Impact of Orizoplus Herbicide on the Behavioral parameters of *Clarias gariepinus* at various Durations and Concentrations

EXPOSURE DURATION (hrs) 12

T. C mgL-1	Hyperactivity	Equilibrium status	Swimming rate	Convulsion	Somersaulting activity	Fin movement	Opercula movement
60mg/l	+	-	+	-	-	++	+
80mg/L	+	-	+	-	+	++	+
100mg L	++	+-	+	-	++	++	++
120mgL	+++	++	+	+	+++	+++	+++
140mgL	+++	+++	+	+	+++	+++	+++

Keys:
- None, Mild = +; ++ Moderate; +++ Strong,TC = Toxicant concentration

Table 3. Impact of Orizoplus Herbicide on the Behavioral parameters of *Clarias gariepinus* at various Durations and Concentrations

EXPOSURE DURATION (hrs) 24

T. C mgL-1	Hyperactivity	Equilibrium status	Swimming rate	Convulsion	Somersaulting activity	FM movement	Operculum movement
60mg/l	+	+	+++	-	-	+++	+++
80mg/L	+	+	++	-	+	+++	+++
100mg/ L	++	+	+	-	++	+	++
120mg/L	+++	+	+	-	+++	+	-
140mg/L	+++	_	_	_	+++	+	+

Keys:
- None; Mild = +; ++ Moderate; +++ Strong; TC = Toxicant concentration

Table 4. Impact of Orizoplus Herbicide on the Behavioral parameters of *Clarias gariepinus* at various Durations and Concentrations

EXPOSURE DURATION (hrs) 48

T. C mgL-1	Hyperactivity	Equilibrium status	Swimming rate	Convulsion	Somersaulting activity	FM movement	Operculum movement
60mg/l	+	+	+++	+	+	+++	+++
80mg/L	+	+	+++	+	+	+++	+++
100mg/ L	++	+	++	+	+	+++	++
120mg/L	+++	+	+	+	+	++	+
140mg/L	+++	+	-	_	-	-	

Keys:
- None, Mild = +; ++ Moderate; +++ Strong; TC = Toxicant concentration

Table 5. Impact of Orizoplus Herbicide on the Behavioral parameters of *Clarias gariepinus* at Various Concentrations and Durations

EXPOSURE DURATION (hrs) 72

T. C mgL-1	Hyperactivity	Equilibrium status	Swimming rate	Convulsion	Somersaulting activity	FM movement	Operculum movement
60mg/l	+	+	+++	-	-	+++	+++
80mg/L	+	+	+++	-	-	+++	+++
100mg/ L	+	+	++	+	-	++	++
120mg/L	++	+	+	+	++	++	++
140mg/L	-	-	-	-	-	-	-

Keys:
- = None; + = Mild ; ++ = Moderate; +++ = Strong; TC = Toxicant concentration

Table 6. Impact of Orizoplus Herbicide on the Behavioral parameters of *Clarias gariepinus* at Various Concentrations and Durations 96hrs

EXPOSURE DURATION (hrs) 96

T. C mgL-1	**Hyperactivity**	**Equilibrium status**	**Swimming rate**	**Convulsion**	**Somersaulting activity**	**Fin movement**	**Operculum movement**
60mg/l	+	++	+++	-	-	+++	+++
80mg/L	+	++	+++	-	-	+++	+++
100mg/ L	+	+	++	+	-	++	++
120mg/L	+	+	+	+	++	+	++
140mg/L	-	-	-	_	-	-	-

Keys - = None, + = Mild; ++ = Moderate; +++ Strong; TC = Toxicant concentration

4. 2. 1 Mortality

The fish in the highest concentration 140mg/l showed 100% mortality while the fish in the lower concentrations of 80mg/l and 60mg/l showed 0% mortality while those in 100mg/l and 120mgl/ showed 50% and 80% mortality respectively.

Table:7 Mean Mortality rate of *C. gariepinus* in different conc. Of Orizoplus at different duration

Conc. Mg/l	Exposure time(hrs)					
0	24	48	72	96	Total	%
60	-	-	-	-	0	0
80	-	-	-	-	0	0
100	2	1	1	2	5	50
120	5	1	2	-	8	80
140	4	5	2	-	10	100

Key: - = None

5. Discussion and Conclusion

5.1. Discussion

The physicochemical properties measured in the test solutions showed that water temperature varied slightly while the PH was constant. The dissolved oxygen, conductivity and total dissolved solids showed some variations ranging from 2.78±6.08 mg/l, 49.2±59.6 µmhos, and 24.3 ±26.5 respectively. The fish in the control experiment showed no abnormal behaviour throughout the exposure time and no mortality was recorded in it. However, at the beginning of the definitive test the fish in the higher concentrations 140 and 120mg/l exhibited somersaulting activity, jumping out of the static tank, erratic swimming, swimming upside down and with time settled at the bottom of the tank and all died before the end of the exposure period. In other concentrations the fish were seen to come to the water surface to gulp air, convulse from time to time with high rate fin and opercula movement. Some of the fish died and some survived to the end of the exposure period of 96hrs.The fish in the highest concentration 140mg/l showed 100% mortality while the fish in the lower concentrations of 80mg/l and 60mg/l showed 0% mortality indicating that stress and eventual death of the fish is concentration dependent. This is in agreement with the work of Omoniyi *et al.* (2002), Rahman *et al.* (2002), Aguiwo and Ayoola (2008) who reported several abnormal behaviours such as incessant jumping and gulping of air, restlessness, surface to bottom movement, sudden quick movement and resting at the bottom. He also observed that the fish became inactive at higher concentrations with increasing time of exposure to toxicant which is a normal observation in acute and chronic toxicity test (Kulakkattolick and Kramer 1997). He further observed that the highest concentration of the toxicant resulted in the highest mortality rate which he described as normal, that in all toxicant threshold is reached above which there is no drastic survival of animal. Below the threshold, animal is in a tolerance zone, above the tolerance zone is the zone of resistance. Ferguson (1989), attributed erratic swimming and surface fairly frequent movement to the hyper-contraction of the muscles due to cholinesterase inhibition while Atalla, *et al.(*1997), attributed such changes to the extraordinary need for oxygene due to thick coating of the gills with profuse mucus together with congestion and hyper-plastic epithelium of the secondary lamella.

5.1.1 Conclusion:

This study reveals that Orizoplus (Propanil/2,4.D) herbicide is toxic to fish especially the juveniles and causes behavioural changes and eventual death of the fish, therefore it's use near aquatic environment should be discouraged.

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