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Behavioral, Biometric and Oxidative Stress-Induced Alterations in *Clarias gariepinus* Juveniles Exposed to Chlorpyrifos

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Chlorpyrifos is an organophosphate pesticide used to kill wide range of pest including insect and worms. The present study investigates the behavioral, biometric and oxidative stress alterations in *Clarias gariepinus* juveniles exposed to chlorpyrifos. The 96 h LC_{50} values of the pesticide estimated by probit analysis was 4.67 mg/L while the toxic unit was 21.41 indicating that the pesticide is very toxic. Fish were exposed to chlorpyrifos sublethal concentrations of 0.43 mg/L and 0.93 mg/L and the behavioral, biometric and oxidative stress parameters were determined on day 1, 5, 10, and 15. The results indicate changes in the behavior and hepatosomatic indices in *C. gariepinus* at different chlorpyrifos concentrations and time. There were concentration and duration dependent increase in lipid peroxidation, protein and glucose but mixed trends in catalase values.

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The use of chlopyrifos in the environment especially near aquatic ecosystem should be strictly monitored to avoid the hazards associated with its application.

Keywords: Pesticide; fish; toxicity; biochemistry; oxidative stress.

1. INTRODUCTION

The contamination of aquatic ecosystem by pesticides has gained increasing attention in recent decades [1]. The acute and chronic exposure, accumulation of these chemicals can result in tissue burdens that produce adverse effects not only in the exposed organism, but also organisms including human beings; therefore, it seems essential to study detrimental effects of such hazardous pollutants so as to formulate strategies for safe guarding aquatic organisms. For centuries, pesticides used in agricultural production have been recognized as having deleterious effects on aquatic organisms including fish. Pesticides are used to control pest of food crops, livestock and plantations, but due to their indiscriminate usage, water bodies like pond, river, and lakes, are continuously being polluted [2]. Aquatic ecosystem that runs through agricultural or industrial areas have high probability of being contaminated by run-off varieties of chemicals such as pesticides and other agricultural chemicals. These chemicals are released into the atmosphere through spray drift, post application, volatilization, and wind erosion of soil and find their way to water bodies and ultimately affect non-target organisms entering into the food chain causing physiological damage [1].

Chlorpyrifos is an organophosphate insecticide made up of white or colorless crystals. It has a slightly stinky odor, like rotten egg or garlic. Chlorpyrifos are used to control different kinds of insects, termites, mosquitoes and round worms. The main advantage of organophosphorus pesticides are their photo stability, high effectiveness even in low concentration and are easily degraded.

Fish is a highly nutritious source of protein which is fast and easily digested. It is much sought after by a broad cross section of the world's population particularly in developing countries. It is estimated that around 60% of people in many developing countries depend on fish for 30% of their animal protein supplies while almost 80% in most developed countries obtain 20% of their animal protein from fish [3]. Fish products are comparable to meat and dairy depending on method used in preservation and preparation. The protein content of most fish can be used as acid protein and improve all the protein quality of a mixed diet [3]. The fresh water African catfish *C. gariepnus* is prominent culture species recommended for toxicity studies because of its availability throughout the year, hardness, fast growth survival in shallow water and ability to survive during dry season due to the possession of accessing air breathing organs [4]. It can readily adapt to pond condition due to high quality of its flesh (i.e. presence of leathery skin) and its tolerance to crowded condition [5]. Environmental pollutants have been reported to accumulate in fish [6,7] and have threatened human health either directly or indirectly through the food chain. It thus becomes necessary to study the lethal toxicity and stress of the pesticide on catfish which would help in formulating the strategies for safe guarding aquatic organisms [8].

Toxic chemicals such as chlorpyrifos have contributed to the change in quality of water that affects fish and other aquatic organisms. The indiscriminate discharge of pesticides from agricultural water run-off and other sources into aquatic media affects non-target organism such as fish [9]. Therefore, it is necessary to determine the toxicity of these pesticides to make useful assessment of the level of damage to the non-target organisms.

Biometric parameters like condition factor (CF) and hepatosomatic index (HSI) have been used to evaluate fish condition [10]. Sub lethal doses of chlorpyrifos can cause physiological and behavioral charges such as air gulping, erratic swimming, loss of equilibrium status, and this tends to reduce the population of fish in aquatic environment [11]. Chlopyrifos affects the nervous system by inhibiting the breakdown of acetylcholine (ACh), a neuro-transmitter. It binds to the active site of cholinesterase (ChE) enzymes, which prevent breakdown of ACh in the synaptic cleft, and thus results in accumulation of ACh in the synaptic cleft which may lead to neurotoxicity and eventually death of the organism. Aquatic organism appears to absorb chlorpyrifos directly from water rather than ingesting it with their diet or through

sediment exposure. Therefore, chlorpyrifos found in water run-off is likely as a result of soil bound chlorpyrifos eroding soil rather than from dissolved chlorpyrifos.

2. MATERIALS AND METHODS

2.1 Experimental Chemicals and Fish

The test pesticides used in the present study was commercial formulation of chlorpyrifos 20EC (trade name Durban, manufactured by Nantong Jinling Agricultural chemicals, supplied by West African Cotton Ltd) and purchased from local market. Three hundred (300) *Clarias gariepinus* juveniles were procured from Rogany Tourist fame Village in 500 L plastic aquaria tank and transported to our laboratory where they were acclimatized for two weeks. They were fed twice daily with Copen feed at 3% body weight. During the acclimation period, the fecal matter and other waste materials were siphoned off daily to reduce ammonia content in the water. Water was changed every alternate day during the acclimation period. Feeding was terminated 24 h before the commencement of the experiment in order to empty their stomach and avoid pollution of the water with faeces.

2.2 Range Finding Test

A range finding test was carried out to determine the concentration of the test solution for definitive test. This was determined by subjecting *C. gariepinus* juveniles to different concentrations of chlorpyrifos until the concentrations that will cause 100 and 0% mortalities are identified.

2.3 Acute Toxicity Test

The acute toxicity studies were conducted using 18 plastic tanks (60×30×30 cm) and a total of 180 fish specimens. The fish specimens were randomly divided into five treatment groups (A-F) and a control with each containing 30 fish. Groups A, B, C, D and E were exposed to 3.50, 4.50, 5.50, 6.50 and 7.5 mg/L Chlorpyrifos. Group F which is the control was exposed to only tap water. Each treatment group and control were further divided into three replicate groups of 10 fish each in plastic tanks containing 20 L of water each. The six -set up experiments ran simultaneously for 96 h after which the 96h LC_{50} was calculated using the probit analysis method [12] The safe level of pesticides was estimated by multiplying the 96h LC_{50} with different factor (AF): Committee on Water Quality Criteria [13], National Academy of Science/National Academy of Engineering (NAS/NAE) [14].

2.3.1 Computation of toxic unit

Toxic Unit = $100/LC_{50}$

2.3.2 Water quality parameters

Physiochemical properties of the test water such as pH, temperature, total hardness and conductivity were analyzed using standard methods. Water temperature was determined using Digital Thermometer (Model TDS-4TMA), pH by pH meter (MW 802 pH) and conductivity by digital conductivity water tester.

2.3.3 Determination of sublethal concentrations

The 96 h LC_{50} of chlorpyrifos in *C. gariepinus* was calculated to be 4.67 mg/L. Based on this value, two sublethal concentrations of 0.47 (1/10 LC_{50}) and 0.93 (1/5 LC_{50}) and a control were used for the sublethal exposure. Two experimental groups (A and B) and a control (C) were set up for the sublethal exposure. Set up A was exposed to 0.47 and B 0.93 mg/L chlorpyrifos. The control C was exposed to only tap water. Each of the exposure group and control contains 30 fish each. They were further replicated into three groups of 10 fish each and monitored for 15 days.

2.3.4 Determination of total protein and glucose

The total protein contents of liver and tissue homogenates were determined by the Folin-Phenol reaction method as described by Lowry et al. [15] while glucose level was analyzed using the method of Cooper and McDaniel [16]. The absorbance was taken at 680nm against the blank.

2.3.5 Determination of Hepatosomatic Indices (HSI) and Condition Factor (CF)

The indices HSI and CF were calculated according to White and Fletcher [17].

$$\mathrm{HSI} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100$$

$$\mathrm{CF} = \frac{\text{Body weight (g)}}{\text{Fork Lenght}^3\ \text{(cm)}}$$

2.3.6 Determination of catalase activity (CAT)

CAT was determined according to the method described by Aebi [18]. 10% homogenate was prepared in 0.9% NaCl and centrifuged at 1500rpm at 15 minutes. The supernatant was used for the analysis. 25µl of sample and a typical reaction mixture containing 1ml 50Nm Potassium phosphate buffer (7.4) was added to the mixture. The reaction was then initiated with the addition of dichromate acetic acid and 1ml of H_2O.

2.3.7 Determination of lipid peroxidation (LPO)

Lipid peroxidation (LPO) was determined by estimating the thiobarbituric acid reactive substance as described by Sharma and Krishna-Murti [19].

2.4 Statistical Analysis

The data obtain were analyzed with statistical package SPSS (Version 17) which was used to determine the LC_{50}. These data were subjected to the one way analysis of variance (ANOVA) and Duncan multiple range test to find the significant differences at 5% probability level and separation of means for further analysis of result.

3. RESULTS

3.1 Specification and Toxicity of Chlorpyrifos

The specifications of the chlorpyrifos insecticide used in the experiment are contained in Table 1. The 96 h LC_{50} of chlorpyrifos in *C. gariepinus* was calculated to be 4.67 mg/L while the toxic unit was 21.41.

3.2 Water Quality Parameters

The results of the water quality parameters such as pH, temperature and dissolved oxygen are presented in Table 2. The water quality parameter in various treatment levels (Table 2) showed that there were no significant difference between the water parameters in the treatments and control.

3.3 Behavioral Responses of *Clarias gariepinus*

The behavioral characteristics of *C. gariepinus* exposed to different chlorpyrifos concentrations are provided in Table 3. There were changes in the behavioral characteristics of *C. gariepinus* juveniles within the exposure period (24, 48, 72 and 96 h). The behavioral characteristics ranged from none to strong reactions of *C. gariepinus* in response to different chlorpyrifos concentrations and exposure periods.

3.4 Estimate of the Safe Levels

The estimates of the safe levels of chlorpyrifos after 96 h exposure to *C. gariepinus* determined following the estimates of some authorities and institutions are shown in Table 4.

3.5 Hepatosomatic Indices of *C. gariepinus*

The HSI values in *C. gariepinus* exposed to chlorpyrifos is shown in Table 5. There was no significant difference in HSI in *C. gariepinus* exposed to 0.43 chlorpyrifos throughout the duration of the study. However, there was duration dependent significance in HSI in *C. gariepinus* exposed to 0.97 mg/L chlorpyrifos. There were no significant differences in HSI between the two concentrations of the insecticide compared to the control.

3.6 Protein and Glucose Levels

The values of protein and glucose in the muscles of *C. gariepinus* exposed to chlorpyrifos concentrations are presented in Table 6. The protein values in both chlorpyrifos concentrations and the control values were the same on day 1 but significantly higher than the control on day 5 at 0.97, while the values in the 0.43 and 0.97 chlorpyrifos concentrations were significantly higher than the control on days 10 and 15.

Table 1. Specification of test toxicant chlorpyrifos

Pesticide name	Cas Reg. No.	Supplier	Grade	Chemical name	Alternative name
Chlorpyrifos	2921-88-2	West African Cotton Ltd	Commercial Formulation	Chlorpyrifos	Durban

Table 2. Physicochemical analysis of test water

Concentration (mg/L)	pH	Temperature (℃)	Dissolved oxygen (mg/L)
00 (Control)	7.52±0.02	25.06±0.01	5.5±0.02
0.43	7.65±0.12	24.44±0.04	5.6±0.04
0.97	7.80±0.70	26.02±0.7	5.3±0.03

Table 3. Behavioral responses of *C. gariepinus* juveniles exposed to different concentrations of chlorpyrifos at 24, 48. 72 and 96 h duration

Duration (h)	Concentration (mg/L)	Hyperactivity	Equilibrium status	Swimming rate	Fin movement	Jerky movement	Air gulping	Erratic movement
24	0	+	+	+++	+++	-	-	+
	0.43	+	+++	++	+++	++	+	+
	0.97	++	+++	+++	+++	+	++	+
48	0	-	-		+++	+	++	++
	0.43	+	++	++	+	+	++	+
	0.97	+++	+++	++	+	+	++	+
72	0	-	-	++	+++	-	-	-
	0.43	++	+++	+++	++	++	++	+
	0.97	+++	++	++	++	+	++	+
96	0	-	-	+++	++	+	-	-
	0.43	+++	++	+++	+	++	++	+
	0.97	+++	++	++	+	++	+	+

Key None -, + = mild, ++ moderate, +++ = strong

Table 4. Estimates of safe levels of chlorpyrifos after 96 h of exposure duration of *Clarias gariepinus*

Pesticide	96 h LC_{50} (mg/L)	Method	AF	Safe level (mg/L)
Chlorpyrifos	4.67	Hart et al. [20]*	-	3.75×10^{-1}
		Sprague (1971)	0.1	4.67×10^{-1}
		CWQC [13]	0.01	4.67×10^{-2}
		NAS/NAE [14]	0.1 – 0.00001	4.67×10^{-1} – 4.67×10^{-5}
		CCREM [21]	0.05	2.34×10^{-1}
		IJC [22]	5 % LC_{50}	2.34×10^{-1}

**C = 48h LC_{50} x 0.03/S^2, where C = presumable harmless concentration and S = 24 h LC_{50}/48h LC_{50}*

Table 5. Hepatosomatic indices of *C. gariepinus* juveniles after exposure to chlorpyrifos

Exposure (Days)	Chlorpyrifos (mg/L)		
	Control	0.43	0.97
1	0.91 ± 0.02^{a1}	0.86 ± 0.01^{a1}	0.75 ± 0.03^{a1}
5	1.12 ± 0.02^{a1}	1.09 ± 0.02^{a1}	1.29 ± 0.01^{b1}
10	0.89 ± 0.03^{a1}	1.22 ± 0.02^{a1}	1.36 ± 0.01^{b2}
15	0.80 ± 0.01^{a1}	0.88 ± 0.02^{a1}	1.18 ± 0.02^{b1}

Values with different alphabetic (lowercase) superscripts differ significantly (p <0.05) between different exposure periods within the same concentration. Values with different numeric superscripts differ significantly (p< 0.05) between different concentrations within the same exposure duration

3.7 Lipid Peroxidation and Catalase Activities

The values of lipid peroxidation and catalase in *C. gariepinus* exposed to chlorpyrifos are presented in Table 7. There were concentration and duration dependent significant increase in LPO compared to the control but mixed trends in the values of CAT during the exposure period.

Table 6. Mean values of protein and glucose levels in the muscle of *Clarias gariepinus* exposed to sublethal concentrations of chlorpyrifos

		Exposure Duration (Days)			
Parameter	**Concentration (mg/L)**	**1**	**5**	**10**	**15**
Protein (mg/100g)	Control	32.11±0.04^{a1}	33.72±0.55^{a1}	34.11±2.04^{a1}	28.78±4.36^{a1}
	0.43	32.06±2.12^{a1}	35.14±1.78^{a1}	59.74±5.38^{b2}	57.28±8.40^{b2}
	0.97	34.12±4.16^{a1}	38.95±0.02^{a2}	53.35±8.73^{b2}	113.23±19.63^{3}
Glucose (mg/100g)	Control	53.63±1.42^{a1}	51.79±0.14^{a1}	50.10±2.10^{a1}	54.12±0.91^{a1}
	0.43	54.00±0.98^{a1}	57.89±4.04^{a2}	52.14±0.53^{a1}	73.68±0.10^{b2}
	0.97	57.89±3.40^{a2}	65.20±1.11^{b3}	73.68±0.00^{02}	84.21±4.19^{a3}

Values with different alphabetic (lowercase) superscripts differ significantly (p <0.05) between different exposure periods within the same concentration. Values with different numeric superscripts differ significantly (p< 0.05) between different concentrations within the same exposure duration

Table 7. Activities of lipid peroxidation and catalase in the liver of *C. gariepinus* exposed to sublethal concentrations of chlorpyrifos for 1, 5, 10 and 15 days

		Exposure (Days)			
Parameter	**Concentration (mg/L)**	**1**	**5**	**10**	**15**
LPO	Control	31.50±2.50^{a1}	24.30±0.20^{a1}	28.40±1.00^{a1}	24.00±0.00^{a1}
(mg/ml)	0.43	23.10±0.80^{a1}	35.45±1.40^{a1}	38.48±0.40^{b2}	39.09±3.80^{b2}
	0.97	22.12±4.20^{a1}	47.45±3.55^{b3}	49.52±0.18^{b3}	49.69±2.20^{03}
CAT	Control	0.30±0.00^{a1}	0.36±0.00^{a1}	0.42±0.12^{a1}	0.30±0.00^{a1}
	0.43	0.36±0.12^{a1}	0.36±0.00^{a2}	0.18±0.00^{b2}	0.30±0.00^{b2}
(mg/100g)	0.97	0.60±0.06^{b2}	0.36±0.00^{b3}	0.72±0.24^{02}	0.36±0.00^{a3}

Values with different alphabetic (lowercase) superscripts differ significantly (p <0.05) between different exposure periods within the same concentration. Values with different numeric superscripts differ significantly (p< 0.05) between different concentrations within the same exposure duration

4. DISCUSSION

There were no significant differences in the water parameters in the treatments and control hence, the water quality did not bring about mortality of experimental fish, because they were within the standard range for aquaculture [20,23]. The present study showed that exposure of the juvenile catfish to higher chlorpyrifos increases mortality and decrease survival rates at different levels of concentration. This is in line with the report from Nwani et al. [24,25] that fish and other aquatic organisms are harmed by pesticides contaminated water. Behavioral changes are sensitive indicators of the toxic effects of pollutants in fishes [26]. Chlorpyrifos concentrations of 0.43 mgl^{-1} and 0.97 mgl^{-1} caused behavioral changes in fin and opercula movements, loss of equilibrium, swimming behavior, jerky movements, gulping of air, skin discolorations and subsequently death. The behavioral alterations as observed in the present study with chlorpyrifos are consistent with the results obtained when fish were exposed to pesticides [27] and other herbicides like glyphosate [28] and fluazifop-p-butyl [2]. The LC_{50} at 96 h was found to be 4.67mg/L and indicates that chlorpyrifos is toxic to *C. gariepinus.* The safe level obtained for chlorpyrifos in the present study varied from 2.34 x 10^{-1} to 4.67 x 10^{-5}. The variations in the safe levels as obtained by different researchers have been a serious concern due to the difficulty in correlating laboratory values to real field data [2]. Hepatosomatic indices (HSI) help to determine the health conditions of the fish in the environment [29]. Exposure to higher chlorpyrifos concentrations elevated the HIS in *C. gariepinus*. Similar to our results, HSI values were elevated

in *C. gariepinus* exposed to different pollutants [30,10].

Exposure to pesticides such as chlorpyrifos may lead to oxidative stress that may cause injuries to biomolecules including lipids. The peroxidation of lipids is basically damaging because the formation of lipid peroxidation (LPO) products generate reactive oxygen species [31]. There was increase in LPO in *C. gariepinus* exposed to chlorpyrifos. Blahova et al. [24] reported an increased level of LPO in *Danio rerio* (Zebrafish) after exposure to atrazine. Catalase (CAT) provides line of defense against ROS. It converts hydrogen peroxides to water and molecular oxygen [25]. Our results indicate mixed trends in CAT activities in *C. gariepinus* exposed to chlorpyrifos. Similar to our findings, some researchers obtained variations in CAT activities in fish after exposure to [1,32,2].

Assessments of protein and glucose contents are considered as diagnostic tools to determine the physiological status of the cell. There were mixed trends in protein and glucose values in the present study which may be attributed to the effect of chlorpyrifos on muscle cells of the fish [27]. Similar to our results, Anih et al. [2] reported mixed trends in protein and glucose values in *C. gariepinus* exposed to fluazifop-p-butyl. Okpe et al. [10] however reported the decrease in both protein and glucose levels in *C. gariepinus* exposed to antipsychotic drug chlorpromazine.

5. CONCLUSION

The wide use of pesticides in the environment especially in agriculture has raised concern about their potential toxic effects in human and other animals. The results of the present study indicates that chlorpyrifos is toxic and resulted in physiological changes in exposed *C. gariepinus.* The use of pesticides in the environment must be closely monitored to avoid eco-toxicological effects on non-target organisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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